Broadband Cavity Enhanced Absorption Spectroscopy as a Detector for HPLC

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The first demonstration of a cavity enhanced absorption spectroscopy (CEAS) based technique, applied to HPLC detection is reported. Broadband cavity enhanced absorption spectroscopy (BBCEAS) has been used for detection in a HPLC system (HPLC-BBCEAS). Measurements were made on the dyes rhodamine 6G and rhodamine B between 450 and 600 nm. The sensitivity of the measurements as determined by the minimum detectable change in the absorption coefficient, \(a_{\text{min}}\), were 2.9 \(\times\) \(10^{-5}\) cm\(^{-1}\) at 527 nm and 1.9 \(\times\) \(10^{-5}\) cm\(^{-1}\) at 556 nm, the peak absorption wavelengths of rhodamine 6G and rhodamine B, respectively. The limits of detection (LOD) for the two dyes were 426 and 271 pM, respectively. The LOD of the HPLC-BBCEAS setup was found to be between 54 and 77 times lower than with a Perkin-Elmer HPLC (series 200) comprising a 200EP photodiode array detector. The sensitivity of the developed setup also compared favorably with the previous single wavelength HPLC-CRDS studies while using a considerably lower cost experimental setup and simpler experimental methodology. The use of BBCEAS detection also allowed the discrimination following an isotropic HPLC separation of the nearly co-eluting dyes rhodamine 6G and rhodamine B.

Separation techniques are widely used for the analysis of complex mixtures. HPLC is a particularly important technique that is used in many applications including quality assurance and quality control. The most widely used detection technique for HPLC is UV–visible absorption spectroscopy. This is partly because many compounds have an absorption spectrum in this part of the electromagnetic spectrum; also, the technique provides an absolute measurement and is relatively simple and reasonably sensitive. However, the sensitivity is poor when compared to detection techniques such as fluorescence or mass spectrometry which have detection limits several orders of magnitude lower but are either not as widely applicable (fluorescence) or are considerably more expensive (mass spectrometry). Over the last two decades optical cavity techniques based initially on cavity ring down absorption spectroscopy (CRDS) and later on the experimentally simpler variants, cavity enhanced absorption spectroscopy (CEAS)\(^4\) and broadband CEAS (BBCEAS)\(^5\) have led to a considerable improvement in the sensitivity of absorption spectroscopy through an increase in the effective path length of measurement. Most previous cavity based studies have reported measurements on gas phase species, as the scattering and absorption losses are significantly lower than measurements for liquid and solid phase species, and thus greater number of passes through the sample can be achieved. In principle however, there are more species of interest for study in the liquid phase. Given the importance of HPLC as an analytical tool, the application of cavity based techniques to potentially enhance the sensitivity of UV–visible detection is desirable. Most of the few liquid phase cavity studies reported to date have involved the use of CRDS as a detector for HPLC systems.\(^5\)–\(^11\)

CRDS requires the measurement of ring down times typically on the high nanosecond to microsecond time scale and hence appropriate fast detection systems. These along with the common use of pulsed laser systems leads to CRDS setups being relatively expensive and experimentally complex. However, the technique provides a means for directly measuring the absorption coefficient (\(\alpha\)) 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.\(^9\)–\(^11\) 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. Previous HPLC studies with CRDS detection have involved measurements at a single wavelength which emulates HPLC UV–visible detectors operating at a single wavelength. This

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EXPERIMENTAL SECTION

The HPLC-BBCEAS experimental setup is shown in Figure 1. The collimated beam from a 1 W white LED (Luxeon O star) was coupled into a 5 cm optical cavity formed by two plano-concave R ≥ 0.99 mirrors, diameter = 25 mm, radius of curvature (roc) = −50 cm, with a bandwidth of ~420–670 nm (Layertec, Germany). The 1 cm path length HPLC cell (Starna, U.K.) had quartz windows with a 3 mm diameter circular aperture and a nominal volume of 70 µL. The HPLC cell was coupled to a Genesis C18 (4 µm particle size 150 mm × 4.6 mm; Alltech Associates, Inc.) column which was attached to a pump and degasser (Knauer) and injection port with a 20 µL loop. The mobile phase used was 85% acetonitrile (CH₃CN) HPLC grade (Fisher Scientific, U.K.), 14.9% HPLC water (H₂O) (Fisher Scientific, U.K.), and 0.1% formic acid (HCOOH) (Fisher Scientific, U.K.), under isocratic elution at a flow rate of 1 mL min⁻¹. The test samples were injected through the injector port, ensuring minimum disturbance to the optimum alignment. The light leaking out of the cavity was focused by a 50 mm focal length lens onto the entrance of a 600 µm diameter, 1 m length, 0.22 numerical aperture quartz fiber (Thorlabs U.K.). This was connected to the entrance slit of the spectrograph by an SMA905 fiber connection. The output of the cavity was monitored by a compact CCD spectrograph (Avantes AVS2000), operating between 200 and 850 nm with an optical resolution of 1.5 nm. Spectral data was acquired with the aid of the “Avasoft” program. After introduction of the analyte through the injection port, data acquisition was manually started for each run. These data were then continuously acquired at a sampling rate of approximately one sample point every 3 s and streamed to the PC for 3 min for each run on the system. The sampling rate arose from the combination of the spectral integration time of ~240 ms and the 10 consecutive spectra recorded for each point. Performance limitation on data throughput was introduced by the hard disk buffer on the PC and resulted in a timing error of about ±0.2 s between each spectrum. These data were then processed using a custom built macro in Excel. Figure 2 shows representative data collected; each chromatogram consisted of up to 75 data points which were used to manually extract the peak absorbance of the analyte from its full wavelength profile. The inset in Figure 2 shows representative absorbance profiles for rhodamine 6G collected at their respective sampling time over the wavelength range 450–600 nm.

Choice of Analytes. Two analytes, rhodamine 6G (Sigma - Aldrich, U.K.) and rhodamine B (Sigma - Aldrich, U.K.), were chosen for separation by HPLC. Figure 3 shows the chemical structure and visible spectra of the two analytes in the solvent mixture used as mobile phase. Rhodamine 6G has peak absorbance at 527 nm while that of rhodamine B is 556 nm. These peaks are shifted from 530 and 543 nm, respectively, when dissolved in ethanol. The rationale for the choice of the two analytes was that both were chemically similar species but with distinctive visible spectra which would allow demonstration of the advantage of multiplex detection following HPLC.


Figure 2. Representative chromatogram of rhodamine 6G obtained using the HPLC-BBCEAS setup. The inset shows full absorption profiles obtained at the peak of the chromatogram at selected concentrations.

Figure 3. Structural formulas and visible spectra of rhodamine 6G and rhodamine B dissolved in the mobile phase solvent mixture.

Standard HPLC Measurements. A Perkin-Elmer series 200 HPLC instrument, comprising a PE 200 Quaternary Pump and Genesis C18 (4 µm particle size 150 mm × 4.6 mm) column (Alltech Associates, Inc.), was used for comparative measurements with our HPLC-BBCEAS system. Samples solutions (20 µL) were chromatographed with a (85% CH3CN: 14.9% H2O: 0.1% HCOOH) mobile phase with an isocratic elution for 5 min at a flow rate of 1 mL min⁻¹. After separation of the rhodamine dyes by the column, analysis by UV–visible absorption spectroscopy, in the range 200–900 nm in a 1 cm path length HPLC cuvette was performed using a series 200EP photodiode array detector. Spectral data were collected at a sample rate of ~1 s⁻¹. The operation of the HPLC system was controlled by Perkin-Elmer’s TotalChrom Chromatography Data Systems software, while these spectral data generated by the Series 200EP PDA Detector were processed by the IRIS Spectral Processing Software.

Experimental Methodology. One disadvantage of CEAS based techniques is that, unlike CRDS experiments, a cannot be directly calculated and instead must be obtained through a separate calibration. For the HPLC-BBCEAS experiments reported in this study the calibration and the experimental methodology could be performed in a straightforward manner by first determining the cavity enhancement factor (CEF) or the number of passes made within the cavity. Once the effective path length had been calculated, the minimum detectable change in the absorption coefficient (a min), the standard measure of the sensitivity of cavity based experiments, could also be calculated.

The methodology to calculate the CEF and a min has been described in detail previously¹² and is only briefly outlined here. The CEF value at a particular wavelength could be calculated from the single pass absorbance at cavity concentrations divided by the cavity absorbance. The value of a 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 × 1 cm).

The limit of detection (LOD) for an analyte could be calculated in two ways. The usual method for HPLC 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 3σ min by 2.303ε.

RESULTS

HPLC-BBCEAS measurements have been performed in a 1 cm cuvette for the dyes rhodamine 6G and rhodamine B, at their peak absorption wavelength, using a white LED and a cavity formed by two high reflectivity R ≥ 0.99 mirrors. The first series of measurements were made on each dye passing individually through the HPLC-BBCEAS system, to test the performance of the setup. These measurements are summarized in Table 1 which lists important figures of merit obtained from these data such as the cavity enhancement factor (CEF), the LOD obtained both from a linear regression through a plot of concentration versus absorbance and also the 3σ noise on a spectrum. The LOD values refer to injected concentrations. Chromatographic broadening by the column means the actual detected concentrations in the cuvette were between eight and 10-fold lower. The sensitivity of the measurement is given by the listed a min values. The LOD values obtained from the HPLC-BBCEAS measurements are also compared with measurements from the Perkin-Elmer HPLC system.

The determination of the LOD for rhodamine 6G and rhodamine B required plots of maximum absorbance versus injected con-

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**Table 1. Summary of the Results Obtained in Terms of Analyte Used, the Wavelength of Measurement, the CEF value, the Minimum Detectable Change in Absorption a min, the LOD of the Analyte, and the Molar Extinction Coefficient ε at the Wavelength of Measurement**

<table>
<thead>
<tr>
<th>Analyte Used</th>
<th>Wavelength of Measurement</th>
<th>CEF value</th>
<th>a min (cm⁻¹)</th>
<th>LOD BBCEAS (nM)</th>
<th>LOD Perkin-Elmer (nM)</th>
<th>ε (M⁻¹ cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhodamine 6G</td>
<td>(527 nm)</td>
<td>40</td>
<td>2.9 × 10⁻⁵</td>
<td>2.1</td>
<td>0.43</td>
<td>90558</td>
</tr>
<tr>
<td>Rhodamine B</td>
<td>(556 nm)</td>
<td>45</td>
<td>1.9 × 10⁻⁵</td>
<td>2.1</td>
<td>0.27</td>
<td>92676</td>
</tr>
</tbody>
</table>
To simulate conditions under which multiplex detection was obtained previously from our studies on a 2 mm cuvette placed in a cavity with $R \approx 0.99$ mirrors. Given that the setup of the optical detection is essentially the same aside from a longer 1 cm path length cuvette, these values are to be expected. The slight difference in CEF values between rhodamine 6G and rhodamine B probably arises as a result of the variation in reflectivity of the cavity mirrors with wavelength. The CEF values are limited by scattering losses from the cuvette of $\sim 1 \times 10^{-2}$ per pass. This value implies that the CEF values for the current experimental setup cannot be improved significantly by simply using higher reflectivity mirrors and that the $R \approx 0.99$ mirrors used in this study are close to the optimum mirror reflectivity.

The small difference in the $\alpha_{\text{min}}$ values for rhodamine 6G and rhodamine B of $2.9 \times 10^{-5}$ cm$^{-1}$ and $1.9 \times 10^{-5}$ cm$^{-1}$, respectively, is probably a result of the higher photon flux at 556 nm which allowed a shorter integration time to be used on the detector and thus lead to lower levels of dark noise on the rhodamine B spectra and consequently smaller $\alpha_{\text{min}}$ values for rhodamine B. Figure 6 shows the wavelength profile of the white LED used in this study and also the red LED used in our previous study. The $\alpha_{\text{min}}$ values can also be compared with our earlier measurements in a 2 mm cell using essentially the same experimental setup. The lowest $\alpha_{\text{min}}$ value obtained was $5.1 \times 10^{-5}$ cm$^{-1}$ for measurements on brilliant blue-R at 630 nm using a red LED and the $R \approx 0.99$ mirror set. One would expect on the basis of simple scaling that the $\alpha_{\text{min}}$ value would be five times smaller, that is, $\sim 1 \times 10^{-5}$ cm$^{-1}$ rather than the obtained value of $1.9 \times 10^{-5}$ cm$^{-1}$. The reason for the discrepancy is most probably because the red LED used previously had a significantly higher photon flux per nanometer than the white LED ($\sim 5$ mW/nm versus $0.5$ mW/nm) and thus could be used with a shorter CCD integration time of 10 ms, leading to lower dark noise levels and a smaller $\alpha_{\text{min}}$ value.

The LOD values for the two analytes have been measured by two means, first from the calculated $\alpha_{\text{min}}$ value and also from an error weighted linear regression through a plot of absorbance versus concentration. There are significant differences between the LOD values obtained by the two methods, with the values from the regression method being substantially worse, even though they were obtained from three replicate measurements. This discrepancy was due to the timing error between successive sample points on the chromatogram, introduced by the limitations of the hard disk buffer on the PC. As mentioned previously this results in an error of approximately $\pm 0.2$ s between successive sample points. This means that, for replicate measurements, the peak absorbance will differ as a result of the timing jitter between sample points. By comparison, data collected from the commercial Perkin-Elmer HPLC instrument, which sampled at a faster rate of around 1 sample per second, showed no detectable timing jitter between sample points. Consequently the LODs obtained by the spectral method are probably a more accurate reflection of what is possible with the HPLC-BBCEAS setup, while the LODs obtained by the regression method highlight what is obtainable for replicate measurements with the current experimental limitations. Comparing the HPLC-BBCEAS LODs obtained by the regression method with those from the commercial Perkin-Elmer instrument, it can be seen that our values are between
10 and 11 times lower. If the LODs from the spectral method are seen as a fairer indication of what is obtainable from HPLC-BBCEAS, then the values from the current study are between 54 and 77 times better.

Comparison of HPLC-BBCEAS with Previous HPLC-CRDS Measurements. Table 2 compares some of the figures of merit of our HPLC-BBCEAS with previous HPLC-CRDS studies. The sensitivity of the HPLC-CRDS measurements is usually quoted in terms of the baseline noise on the measurement in absorbance units. However, for our measurements we cannot easily determine the sensitivity in these units and instead quote the sensitivity in terms of $R_{\text{min}}$ in cm$^{-1}$ units. It is possible to convert the absorbance unit values of the HPLC-CRDS measurements to $R_{\text{min}}$ values by converting to ln (log$_e$) units and then dividing by the path length of the cell in centimeters. Care is taken that the comparison is made with the root mean squared (rms) 1σ noise value rather than the peak-to-peak 3σ noise value, which is often stated. Table 2 also lists the base path length of the measurement, the wavelength of measurement, and the reflectivity of the cavity mirrors used in the studies.

The earliest HPLC-CRDS studies were performed in the research group of Zare et al.$^6,7$ These utilized a custom built HPLC cell which had the cell windows at the correct Brewster's angle at both the air/glass and glass/liquid interfaces to minimize reflection losses. The base path length of the cell was only 300 µm which allowed very small cell volumes to be used. The sensitivity of the experiments were very high considering the short base path length, reflecting the extremely low losses at the cell interfaces. However, it can be argued that the experimental complexity was also high given the requirement for the expensive custom built HPLC cell and fast detection schemes. The remaining four previous HPLC-CRDS studies have all been performed by Ariese et al.$^8-11$ The first three experiments utilized a simple 2 mm path length HPLC cell which had been constructed by using a rubber spacer with a 12 µL elliptical hole to separate compact high reflectivity cavity mirrors which were in direct contact with the analyte.$^8-10$ The first two studies were performed at 532 nm while the last study made measurements at 457 and 355 nm. The use of high reflectivity mirrors and the lack of interface losses resulted in low values of $R_{\text{min}}$ for the measurement at 532 and 457 nm ($1.0 \times 10^{-5}$ cm$^{-1}$). The measurement at 355 nm had a significantly worse $R_{\text{min}}$ value of $5.0 \times 10^{-5}$ cm$^{-1}$ as the fabrication of high reflectivity mirrors becomes increasingly more difficult at UV wavelengths. However, the applicability and usefulness of the technique also increases the further into the UV that measurements can be made, as a large fraction of potential analytes have strong absorptions in the 200–300 nm range. The final study is the one most similar to ours as it utilized a 1 cm path length HPLC cell to make measurements at 355 and 273 nm.$^{11}$ A cell had been “superpolished” to reduce scattering losses and when placed in the cavity ($R \geq 0.9995$ mirrors) this allowed $\sim 500$ passes at 355 nm with a blank eluent filled cuvette.

The results obtained in our study compare well with the previous measurements. The sensitivity of our HPLC-BBCEAS technique as determined by the best $R_{\text{min}}$ value of $1.9 \times 10^{-5}$ cm$^{-1}$ is toward the lower end of the values obtained from the six previous HPLC-CRDS. The value of $R_{\text{min}}$ is inversely proportional to the base path length and at 1 cm the path length used in this study is the same as van der Sneppen et al.$^{11}$ This cell was used because it is the standard path length used in most commercial HPLC systems and makes for ease of direct comparison. The $R_{\text{min}}$ values in our study have been obtained...
using relatively low reflectivity $R \geq 0.99$ mirrors and generating only 45 passes. The previous studies have all used $R > 0.999$ mirrors and have typically in excess of 1000 passes. The flow cell volume of 70 $\mu$L is among the highest when compared to the previous studies and in general, smaller flow cell volumes will result in less chromatographic broadening. Given that a similar cell volume is used for our HPLC-BBCEAS and a standard HPLC system then the chromatographic broadening would be expected to be similar and allow more direct comparison between the two approaches. The novelty of our work lies in the demonstration of the first application of CEAS detection to an HPLC system, and we demonstrate in particular the use of the broadband approach. The major advantages of the BBCEAS approach over more conventional CRDS is that information can be obtained over a wider range of wavelengths, and the experimental setup is relatively simple and of lower cost. The low cost and the simplicity of the experimental methodology and data analysis allow the BBCEAS approach to be highly applicable as a detector for HPLC.

Our HPLC-BBCEAS measurements on rhodamine 6G and rhodamine B were all recorded between 450 and 600 nm, while the previous HPLC-CRDS studies only made a measurement at a single wavelength. This means any analyte which absorbs in the detection range of the measurement can be studied rather than only those which absorb at the single wavelength of measurement of the HPLC-CRDS studies.

Our studies show that HPLC-BBCEAS can be used to distinguish between two nearly co-eluting analytes. This type of measurement would be difficult using single wavelength detection. The two analytes chosen for this experiment (rhodamine 6G and rhodamine B) are chemically similar and hence would be expected to have very similar retention times in an HPLC separation. The shapes of the visible spectra are also very similar but with different peak absorption wavelengths. If the two analytes co-eluted, one would expect the visible spectrum of each point around the peak of the chromatogram to have the same shape and show two maxima corresponding to the peak absorption of the two dyes. The studies on a mixture of rhodamine 6G and rhodamine B show that they nearly co-elute under the experimental conditions used. The chromatogram (Figure 4), obtained from plotting the absorbance at 541 nm as a function of time, shows a single peak which given the absence of any other information would indicate the presence of a single compound. HPLC-BBCEAS provides an absorption spectrum for each chromatogram point and the inset to Figure 4 shows the absorption spectrum between 450 and 600 nm for selected time points across the chromatogram peak. From this, one can see that the shape of the absorption spectrum changes markedly across the chromatogram peak. The time evolution of the absorption spectra allows the order of elution for the 2 analytes to be determined and is consistent with rhodamine 6G eluting slightly faster than rhodamine B. These data can also be represented in the form of a contour plot as shown in Figure 5. The $y$ axis shows the wavelength scale while the $x$ axis represents the time scale. The intensity of any $x,y$ point is represented by a color coded scale. This type of plot also allows nearly co-eluting analytes to be discriminated. If the two dyes co-eluted then the peak intensity decrease would be parallel to the $x$ axis. In Figure 5, however, at increasing times the peak intensity shifts to slightly longer wavelengths indicating the presence of a more slowly eluting analyte.

**Further Work.** Although the performance of our HPLC-BBCEAS system compares favorably with a standard Perkin-Elmer system and also the previous HPLC-CRDS studies, the sensitivity could be improved by a number of modifications. The discrepancy between LODs obtained by the spectral method and the regression method highlighted the problem of excessive timing jitter between sample points limiting the precision of replicate measurements. This is simply addressed by use of a faster PC with a larger hard disk buffer. The experimental sensitivity is mainly limited by the dark noise produced by the uncooled CCD detector, as was the case for our earlier BBCEAS experiments. The dark noise could be reduced by using a spectograph with a more expensive thermoelectrically cooled detector. Alternatively a more powerful light source could be used as this would shorten the integration times needed, resulting in lower levels of dark noise from the CCD detector. The current measurements have been made in the wavelength range 450–600 nm and in fact usable spectral data to 700 nm has been recorded. The applicability of the technique would, however, be greatly increased if measurements could be extended into the UV as most common analytes have strong absorptions in the UV region from 200 to 400 nm. The technical challenges involve finding suitable high intensity broadband light sources which operate in the UV and also broadband high reflectivity dielectric mirrors which function in the UV. Recent advances have resulted in high intensity LEDs which operate at wavelengths as short as 250 nm; these, however, only have typical bandwidths of 10–20 nm, which means that several different LEDs would be needed to cover the UV wavelength range. High intensity xenon arc lamps similar to the

<table>
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<th>study</th>
<th>technique</th>
<th>mirror reflectivity</th>
<th>base path length (cm)</th>
<th>wavelength (nm)</th>
<th>$c_{\text{min}}$ (cm$^{-3}$)</th>
<th>LOD (M)</th>
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<td>this work</td>
<td>BBCEAS</td>
<td>0.99</td>
<td>1</td>
<td>450–600</td>
<td>$1.9 \times 10^{-5}$</td>
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<td>488</td>
<td>$2.6 \times 10^{-6}$</td>
<td>1 $\times 10^{-7}$</td>
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<td>$2.5 \times 10^{-4}$</td>
<td>9.2 $\times 10^{-8}$</td>
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<td>0.2</td>
<td>532</td>
<td>$1.6 \times 10^{-4}$</td>
<td>2.5 $\times 10^{-9}$</td>
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<td>CRDS</td>
<td>0.2</td>
<td>532</td>
<td>$1.0 \times 10^{-5}$</td>
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<td>457</td>
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<td>7.5 $\times 10^{-8}$</td>
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<tr>
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<td>355</td>
<td>$3.0 \times 10^{-5}$</td>
<td>4 $\times 10^{-8}$</td>
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<td>van der Sneppen et al.</td>
<td>CRDS</td>
<td>1</td>
<td>273</td>
<td>$1.0 \times 10^{-3}$</td>
<td>5 $\times 10^{-7}$</td>
<td></td>
</tr>
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</table>

one used by Fielder et al. in their studies\textsuperscript{15} offer a source of broadband radiation down to about 200 nm, at the expense of greater cost, size, and power consumption. High reflectivity mirrors also tend to be more difficult to manufacture for operation in the UV. Nevertheless mirror manufacturers are currently able to produce custom broadband UV mirrors which would cover the range 250–400 nm with $R \geq 0.99$ reflectivity. Consequently, the application of BBCEAS to HPLC detection at UV wavelengths looks both feasible and promising.

**CONCLUSIONS**

The first reported application of a CEAS based detection method attached to an HPLC system has been demonstrated by this study. Furthermore the use of HPLC-BBCEAS has allowed the absorption spectrum of the analyte in the range 450–600 nm to be recorded for every point on the chromatogram. The experimental methodology is very similar to that used in standard HPLC systems and offers a number of advantages over the previous single wavelength HPLC-CRDS studies. The overall cost of the experimental setup is also very low when compared to the HPLC-CRDS studies. Experiments have been performed on the dyes rhodamine 6G and rhodamine B and comparisons have been made with a standard HPLC system as well as the previous HPLC-CRDS studies. The LOD values for the two analytes obtained by a spectral method indicate that our HPLC-BBCEAS setup is between 54 and 77 times more sensitive than a standard HPLC system. A comparison of the $\alpha_{\text{min}}$ values with the previous HPLC-CRDS studies showed that our measurements were among the most sensitive. Finally experiments were performed on a mixture of the two dyes, which nearly co-eluted under the conditions used. The use of BBCEAS detection allowed the presence of the two dyes to be determined even though the chromatogram consisted of a single peak.

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