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Laser-based double beam absorption detector for high-performance liquid chromatography

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ABSTRACT

The performance of a new double-beam absorption detector based on an all-electronic noise canceler was tested under HPLC conditions. The test compounds were chosen to be 2'-Cl-4-dimethylaminoazobenzene and 4'-Cl-4-dimethylaminoazobenzene since they have absorption peaks in both the UV and the visible region. The major properties of the detector are discussed. The detector is linear through almost 4 orders of magnitude (10^{-5}–10^{-9} M). The deviation between consecutive measurements is about 3% and the absorption noise level is $2 \cdot 10^{-6}$ AU, which is lower by one order of magnitude compared to commercial absorption detectors.

INTRODUCTION

Liquid chromatography (HPLC) is already firmly established as a powerful technique for the separation, quantitation and identification of chemical components in liquid solutions. Much emphasis was put in developing new schemes for detection [1,2]. During the last decade mass spectrometry and Fourier transform infrared spectroscopy (FT-IR) coupled with HPLC have been perfected for qualitative analysis [3,4]. The use of laser excitation in fluorescence analysis has reduced the limit of detection (LOD) sometimes by 2–3 orders of magnitude compared to absorption detection and has opened up new possibilities for ultrasensitive measurements [5,6]. The major disadvantage of fluorescence detection is that not all compounds of interest fluoresce under HPLC conditions.

UV–visible absorption is by far the most widely used detection method. For conventional LC conditions typical detectability with commer-

cial absorption detectors is $1 \cdot 10^{-4}$ AU [7]. With the most modern state-of-the-art system the minimum noise level is about $2 \cdot 10^{-5}$ AU using a single wavelength [8,9]. The same equivalent noise level was obtained by using laser-induced photoacoustic spectroscopy (LIPAS) [10,11]. The LOD can be reduced even more by utilizing thermal lens calorimetry [12]. However, the thermal lens effect increases the observed signal but does not reduce the noise. In fact, because lasers are used and because of spatial filtering, noise is actually increased. So, thermal lens calorimetry can be enhanced further if intensity noise can be suppressed.

Utilizing a single laser beam as a light source in absorption detectors leads to limited performance because the intensity stability of a typical laser is barely above one part in 10^3 [13]. A typical approach to overcome this problem is to design a double-beam detector. In a conventional double-beam absorption detector, the beam is split into signal and reference beams. The two photocurrents or voltages are either subtracted from each other or divided. For subtraction an extremely fine adjustment is needed while divi-

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sion suffers from the poor performance of a typical analog divider [14]. Recently Haller and Hobbs [15,16] have proposed a new double-beam absorption detection scheme. It is based on an all-electronic noise canceler which theoretically can offer shot-noise-limited performance. The calculated noise equivalent absorption for 1 Hz bandwidth for their experimental conditions was $4.2 \cdot 10^{-8}$. However they did not demonstrate absorption measurements down to the detection limit. Based on averaging and filtering scans at 1000 Hz they determined the noise level of their system to be $3 \cdot 10^{-7}$ (equivalent absorption units). This technique was recently applied to sensitive absorption measurements in capillary electrophoresis (CE) [17], with a factor of 25 improvement in LOD compared to commercial detectors.

In the present paper the performance of the all-electronic noise canceler absorption detector under HPLC conditions is evaluated. We have tested the stability, linearity and LOD of the detector. Comparison is made with state-of-the-art commercial instruments. The two isomers of chloro-4-dimethylaminoazobenzene (DAAB) were chosen because they have absorption in both the UV and visible regions [10]. No attempt was made to optimize the chromatographic conditions. The main thrust is to demonstrate the performance of the new detector under HPLC conditions. The differences between the performance of the all-electronic noise canceler under CE and HPLC conditions are also discussed.

EXPERIMENTAL

Optical setup

The double-beam laser absorption detector for HPLC measurements is shown in Fig. 1. We have used an Ar ion laser (Cyonics 2211-30SL) as a light source operating at 488 nm. The 8-mW partially polarized laser beam was split using a broad-band (200–700 nm) beam splitter (Newport-BK7). The reflected beam was again reflected by a mirror (Newport), focused by a 300-mm focal length quartz lens (Melles-Griot) and directed to the reference photodiode. The
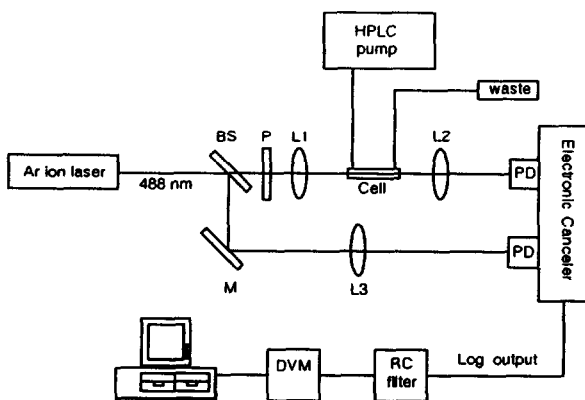

Fig. 1. Double-beam absorption detection system for HPLC. The instrument consists of three major components: a detection system (Ar ion laser and the all-electronic noise canceler), a separation system (HPLC pump and 1-cm length commercial absorption cell) and data acquisition system (A/D interface and computer). BS = Beam splitter; P = polarizer; L1 = 70-mm focal length lens; L2 = 50-mm focal length lens; M = mirror; L3 = 300-mm focal length lens; PD = photodiode; RC = resistor–capacitor; DVM = voltmeter. For details see text.

transmitted beam passed through a polarizer (Glan-Thompson) and was focused by a 70-mm focal length quartz lens (Melles-Griot) into the absorption cell. The flow cell is a commercial HPLC absorption cell taken directly from an Altex instrument (Model 153). After passing the cell the diverged beam was refocused by a 50-mm focal length quartz lens (Melles-Griot) and fell on the signal photodiode. The ratio between the reference and signal beam intensities was determined by the angle of rotation of the polarizer. For our experimental conditions a reference/signal ratio of 1.5 was maintained in order to minimize the log output to a typical offset voltage of 20 mV. The measurements were carried out in a dark box to prevent noise contributions due to the exposure to room light.

Electronic canceler and data acquisition

The double-beam absorption measurements were carried out utilizing the log output of the all-electronic noise canceler that was recently developed by Haller and Hobbs. A full description of the device is given in refs. 15 and 16. The

circuit of the noise canceler is shown in Fig. 2. Two photodiodes (BPW34, Siemens) were used as the signal and reference beam detectors. The bipolar junction transistor differential pair (Q1, Q2) (Analog Devices MAT-04) split the current. A PNP bipolar transistor (2N3906) was used to prevent the capacitance of the signal photodiode from loading the summing junction of the operational amplifier A1 (Motorola, Op-27) and causing instability. The operational amplifier converts the photocurrent to a voltage. The second operational amplifier A2 integrates the output voltage of A1 and adjusts the current splitting ratio of Q1/Q2 to keep that voltage at 0 V. The output voltage of A1 is the linear output of the circuit. The circuit has another output from A2 which is related to the log ratio of the signal and reference photocurrents and provides a straightforward method for absorption measurements. The circuit was driven by ±12 V batteries. The driving voltages were further
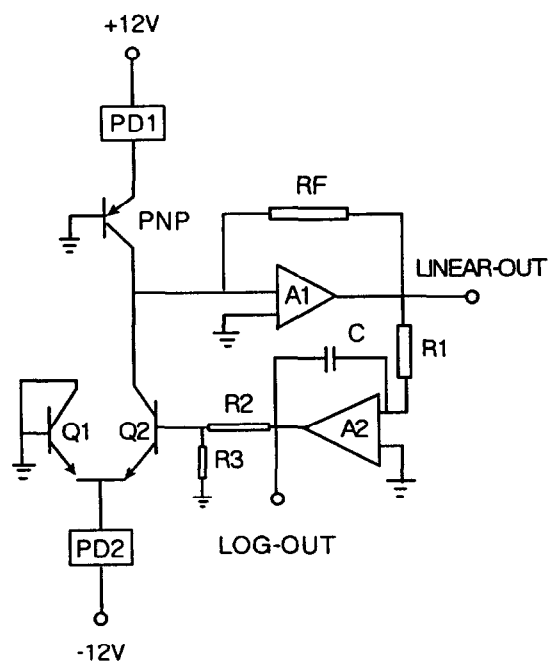

Fig. 2. Schematic diagram for an all-electronic noise canceler. PD1 = Signal photodiode; PD2 = reference photodiode; PNP = transistor; Q1, Q2 = differential pair of biopolar junction transistors; A1, A2 = operational amplifiers; R1 (1 kΩ), R2 (1 kΩ), R3 (24 Ω), RF (20 kΩ) resistors; C = 2.2 μF unpolarized capacitor.

stabilized by voltage regulators for the positive (LM7812, National Semiconductors) and for the negative (LM7912, National Semiconductors) voltages. The circuit was put in a metal box that was carefully grounded in order to shield the circuit from environmental noise. The log output voltage was passed through an RC filter which consists of a 10 kΩ resistor and a 10 μF unpolarized capacitor to increase the time constant of the circuit to 100 ms. The filtered output was connected to an analog voltmeter, DVM (Keithley 177), which was used to amplify the log output voltage. The amplified voltage was acquired at 5 Hz by an IBM-PC compatible computer equipped with an A/D board (Chromperfect, Justice Innovation).

The shot-noise limit measurements of the conventional absorption detectors were taken using a photodiode (Hamamatsu). The instruments were ISCO-3850, ISCO-3140, and Spectra-Physics SpectraPhoresis 1000TM. The three are CE absorption detectors that are based on deuterium and tungsten lamps as light sources.

Chromatography

An HPLC pump (LKB-2150) was operated at a pressure of 75 bar and delivered 1 ml/min with ±1% flow-rate accuracy. This pump has a pulsating flow of approximately 0.5 Hz. A 3-μm adsorbosphere column (100 mm × 4.6 mm I.D.) (Alltech) and an injection valve with a loop of 50 μl were used. The cell volume was 7.5 μl and the pathlength was 1 cm. All of the connections and tubing used in the HPLC system were stainless steel of 0.25 mm I.D. to minimize dead volume.

Reagents

HPLC-grade methanol (Fisher Scientific) was used as the eluting solvent without further purification. The solvent was degassed before use by a sonicator operated under vacuum for 15 min. The two isomers of chloro-4-dimethylaminoazobenzene, 2'-Cl-DAAB and 4'-Cl-DAAB (Tokyo Kasei Co.) were used as received. The stock solutions of dyes were prepared and diluted just before use and were kept covered with Al-foil to prevent photodecomposition due to exposure to room light. The absorption spectra

of these dye solutions were measured with a
photodiode array spectrometer (Hewlett-Pack-
ard HP8452A).

RESULTS AND DISCUSSION

UV–visible spectrum of Cl-4-DAAB

The absorption spectrum of 2'-Cl-4-DAAB in
methanol is shown in Fig. 3. This spectrum is
similar to that of 4'-Cl-4-DAAB. The molar
absorptivities of 2'-Cl-4-DAAB were calculated
(using Beer's law) and found to be 7575 1 mol^{-1}
cm^{-1} at 488 nm and 9500 1 mol^{-1} cm^{-1} at 254
nm. These results deviate by less than 5% from
the results obtained by Oda and Sawada [10] for
the same compounds.

Separation of Cl-4-DAAB isomers

We were able to separate the two Cl-4-DAAB
isomers following the procedure that was sug-
gested by Oda and Sawada [10], which is based
on using methanol as the solvent. The absorption
peaks were detected by utilizing the all-elec-
tronic noise canceler double-beam system. A
typical chromatogram of the Cl-4-DAAB isomer
mixture is shown in Fig. 4. The retention times
are 2.18 min for 2'-Cl-4-DAAB and 2.79 min for
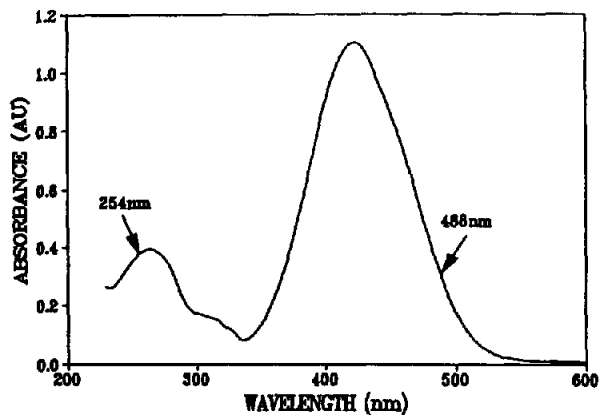4'-Cl-4-DAAB. The two peaks are clearly
baseline separated.

Fig. 3. Absorption spectrum of 2'-Cl-4-DAAB in methanol.
The concentration is $4 \cdot 10^{-5}$ M and the cell pathlength is 1
cm. The molar absorptivities are found to be $7.575 \cdot 10^3$ 1
mol^{-1} cm^{-1} for 488 nm and $9.5 \cdot 10^3$ 1 mol^{-1} cm^{-1} for 254
nm.
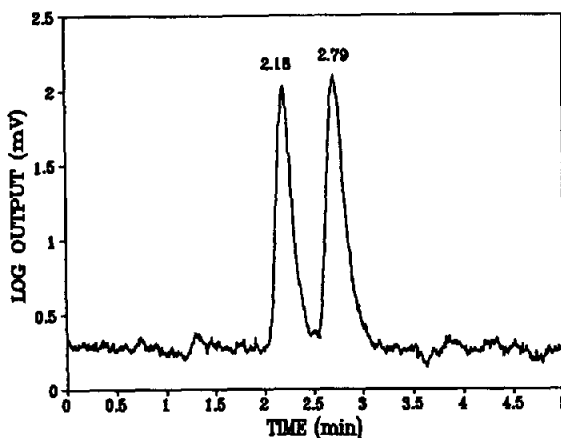

Fig. 4. Separation of a Cl-4-DAAB isomer mixture. The
concentration of each isomer is $1 \cdot 10^{-7}$ M. A 3-μm adsorbo-
sphere column (100 mm × 4.6 mm I.D.) and an injection
valve with 50-μl loop were used. The cell volume is 7.5 μl
and the pathlength is 1 cm.

Detection performance

The major advantage of the all-electronic
canceler over a conventional double-beam detec-
tor is the ability to adjust the reference photo-
current so that an identical unmodulated copy of
the signal photocurrent is subtracted. This
unique property of the device led to perfect
noise cancellation down to almost shot-noise
limited performance. As was mentioned previ-
ously [15,16] the log output of the electronic
canceler $V_{\log}$ was used to perform the absorption
measurements. The dependence of the log out-
put voltage on the ratio between the signal and
reference photodiode currents is given in the
following expression:

$$V_{\log} = -\ln (i_{\mathrm{ref}}/i_{\mathrm{sig}} - 1) \qquad (1)$$

It is the maximum linear output voltage that was
taken from operational amplifier A1 of the
circuit when the reference beam is blocked. For
2 mW beam power at the signal photodiode $V_{\max}$
was measured to be 4.5 V. i_{ref} and i_{sig} are the
photocurrents of the reference and signal photo-
diodes. The voltage difference $\Delta V_{\log}$ due to the
presence of absorption is therefore:

$$\Delta V = (2 - V_0) \ln(i_0/i) \qquad (2)$$

where subscript 0 refers to the photocurrents in the absence of absorption. According to Beer's law:

$$\log (i_0/i) = \varepsilon bc \tag{3}$$

where ε is the molar absorptivity of the sample, b is the pathlength and c is the sample concentration. The log output voltage difference can then be expressed as:

$$\Delta V = (2 - V_0) \ln 10 \varepsilon^{bc} \tag{4}$$

It can be seen that the concentration of the analyte can be directly evaluated from eqn. 4. In order to define the linear dynamic range of the double-beam absorption detector a concentration *versus* peak height calibration curve for 2'-Cl-4-DAAB in our HPLC system was constructed. The solution concentrations were from $5 \cdot 10^{-9}$ M to $5 \cdot 10^{-5}$ M. Each peak height was an average from three consecutive injections. The deviation from the average was about 3%. In order to confirm eqn. 4 a plot of log (peak height) as a function of log (concentration) is shown in Fig. 5. The slope of this curve is 0.94 with a correlation coefficient r^2 of 0.997. It can be seen that the deviations from the linear curve are larger at lower concentrations, where signal-to-noise ratios (S/N) are poorer.
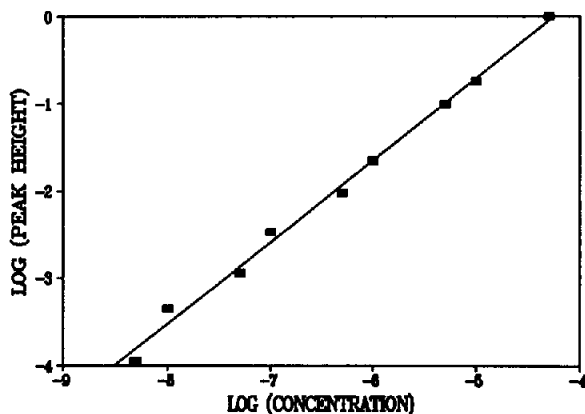
Limit of detection

Due to optical losses caused by the optical components and the absorption cell the signal beam power at the detector was measured to be 2 mW. The photon flux of a 2-mW Ar ion laser beam that is operated at 488 nm is $N = 5 \cdot 10^{15}$ s^{-1}. For a measurement time of 1 s and a unit quantum efficiency the calculated S/N is $N^{1/2}/N = 1.4 \cdot 10^{-8}$. The noise-equivalent absorption should be $\sqrt{2}$ higher, *i.e.* $= 2 \cdot 10^{-8}$. The theoretical voltage noise level $\sigma V_{\log}$ for our HPLC system can be derived using the following expression:

$$\sigma V_{\log} = \sqrt{2}[\exp(V_{\log}/V_{\max}) + 1)](2e/i_{\mathrm{sig}})^{1/2} \tag{5}$$

where e is the electron charge ($1.6 \cdot 10^{-19}$ Coulomb). Since $V_{\log}/V_{\max} \approx 0$, $\sigma V_{\log}$ is given as:

$$\sigma V_{\log} = 4(e/i_{\mathrm{sig}})^{1/2} \tag{6}$$

For the measured 220 μA signal current $\sigma V_{\log} = 1.07 \cdot 10^{-7}$ V/$\sqrt{\mathrm{Hz}}$. With 5 Hz bandwidth the minimum noise level should be 0.24 μV. According to eqn. 4 and assuming a signal-to-noise ratio of 5 the theoretical limit of detection for 2'-Cl-4-DAAB ($\varepsilon = 7.575 \cdot 10^3$) is $1.5 \cdot 10^{-11}$ M (signal level of 1.2 μV).

A typical chromatogram of 2'-Cl-4-DAAB which demonstrates the performance of the all-electronic canceler at the detection limit is shown in Fig. 6. The peak height is about 100 μV and

Fig. 5. Plot of log (peak height) as a function of log (concentration) for 2'-Cl-4-DAAB. The concentration range is 10^{-5}–10^{-9} M. Each data point is an average of three consecutive measurements. The best-fit line has a slope of 0.94 and a correlation coefficient $r^2 = 0.997$.
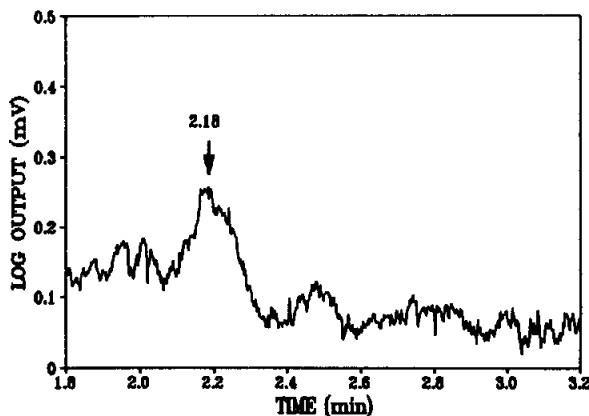

Fig. 6. Chromatogram of ultra-low concentration of 2'-Cl-4-DAAB. The peak height is 100 μV with a signal-to-noise ratio of 5. The concentration at the detector is $1.1 \cdot 10^{-9}$ M (50 pg injected).

the signal-to-noise ratio is 5. The concentration of the injected solution was $3.75 \cdot 10^{-9}$ M. The concentration at the detector was $1.1 \cdot 10^{-9}$ M due to a dilution factor of 3.3. This signal level deviates by less than 15% from the predicted concentration according to eqn. 4 which is $1.3 \cdot 10^{-9}$ M. The root mean square (rms) noise level is 20 μV. In terms of absolute absorption the 100 μV signal is related to an absorption peak height of $9 \cdot 10^{-6}$ AU while the 20 μV noise level is related to $2 \cdot 10^{-6}$ AU. This measurement implies that the sample mass detected is about 50 pg (injected). However, the LOD is still 100 times worse than the theoretical limit. This is partly due to the fact that the quantum efficiency of the photodiode is not unity. Also, theoretical performance is not achieved due to excess laser noise and environmental perturbations such as mechanic vibrations and flow disturbance.

In order to have a better comparison between our new detector and commercial systems we can use the predicted performance of commercial detectors based on the manufacturers' specifications. We also can estimate the shot-noise limit of commercial detectors that utilize a lamp as a light source. This estimation is based on actual measurements of the light power at the detector in several commercial instruments. The measurements were taken by placing a photodiode (Hamamatsu, S1227) into the pathlength of the detector. Based on the photodiode response curve and the wavelength dependence of the photodiode response the average power at the detector was found to be around $1 \cdot 10^{-4}$ mW for all three instruments tested. Assuming a measurement time of 1 s and a unit quantum efficiency this power corresponds to a photon flux of $1.25 \cdot 10^{11}$ s^{-1}. The shot-noise limit is thus $3 \cdot 10^{-6}$ and the equivalent absorption noise level is around $4.2 \cdot 10^{-6}$ AU. A typical state-of-the-art commercial detector is limited to a noise level of $2 \cdot 10^{-5}$ AU which is 5 times higher than the theoretical shot-noise limit of the lamp. The fact that the quantum efficiency is not unity and that there is a flow cell in the light path explains why commercial detectors do not approach the shot-noise limit. We were not able to replace the circuitry in a commercial detector with the all-electronic noise canceler because the beam size

there is substantially larger than the active area of the photodiodes. A major redesign of the optics would be necessary to allow a direct comparison.

Although the cost, fixed wavelength coverage and relatively short lifetimes of typical continuous wave (cw) lasers are disadvantages, when a lower noise level is needed, an absorption detector that utilizes a laser as a light source must be used. The equivalent absorption noise level of our detector is ca. $2 \cdot 10^{-6}$ AU, and it is still far from the full theoretical potential by almost two orders of magnitude. Further improvement can probably be achieved by using a low-pass narrow-band preamplifier between the log output and the input to the computer, by using more rigid mechanical mounts, by additional electronic shielding, and by narrowing the feedback bandwidth to 1 Hz. Even with the present performance we were able to decrease the minimum noise level and the detection limit to the 10^{-6} range. To the best of our knowledge this is an improvement by one order of magnitude compared to any commercial absorption detector. It should also be noted that since the molar absorptivity of DAAB at 254 nm is $9.5 \cdot 10^3$ l mol^{-1} cm^{-1} the detection limit of a commercial detector at a signal-to-noise ratio of 5 would be $1 \cdot 10^{-8}$ M, which is still poorer than in our detector. Other comparisons of LOD with those in the literature should always take into account the dilution factors on column and the molar absorptivities.

It is interesting to compare the performance of the all-electronic noise canceler in our HPLC system to its performance in a CE system [17]. While the HPLC system suffers from pump pulsation noise and from flow gradients due to the fact that a massive volume of liquid phases through the absorption cell, the CE system suffers from high voltage interference and from mechanic vibrations of the thin capillary. Although the two systems present different problems there are only minor differences in the detector performance, which amounts to a factor of 2 better (AU) under HPLC conditions. The CE detector however is 25 times better than the corresponding commercial system because the use of a laser increased the effective absorption

path there by a factor of 5. Our experiments under both CE [17] and HPLC conditions demonstrate that the all-electronic noise canceler is a powerful device that comes close to the performance of a fluorescence detector. This fact make the double-beam absorption detector suitable for a variety of future applications, particularly if UV lasers are used.

ACKNOWLEDGEMENT

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