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OVERALL CHARACTERISTICS OF A LIQUID CHROMATOGRAPHIC DETECTION SYSTEM USING A SILICON PHOTODIODE ARRAY

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SUMMARY

The use of a silicon photodiode array as a multi-wavelength detector which allows the recording of complete UV spectra for each component in the effluent from liquid chromatography is described. This detector gives noise levels of the order of $6 \cdot 10^{-5}$ absorbance units in the UV range, and a linear calibration curve for 0.5–1000 ng biphenyl has been obtained.

INTRODUCTION

Several types of rapid scanning spectrophotometers for instantaneous recording of the spectra of eluted components have recently been introduced into liquid chromatographic (LC) systems. Denton *et al.*[1] employed an oscillating mirror rapid scanning spectrophotometer. Saitoh and Suzuki[2] designed a spectrophotometer to scan the 200–800 nm range in 375 msec. Rapid scanning spectrophotometers employing a silicon target vidicon have also been reported[3–5].

The recent availability of inexpensive silicon photodiode arrays has enabled the use of these devices in multi-wavelength UV detectors for LC. Applications of such arrays in LC have been reported by Dessy and co-workers[6,7] and Grushka and co-workers[8,9]. However, further work seems to be necessary to evaluate the overall system for use in high-performance liquid chromatography (HPLC).

The purpose of this study was to determine the overall performance characteristics of an LC system consisting of a reversed-phase column and a diode array detector (Reticon RL 1024C/17 or RL 512 S) and to demonstrate its ability to give lower noise levels of $6 \cdot 10^{-5}$ absorbance units (AU) at 250 nm.

EXPERIMENTAL

Optics

A single-beam spectrophotometer consisting of a light source, optics and a photodiode array detector was constructed as shown in Fig. 1. A deuterium lamp (L 613-03, Hamamatsu TV. Co.) was used as the light source. Light from this lamp is collimated, then passes through a flow cell (volume 8 μl, path length 1 mm) and forms

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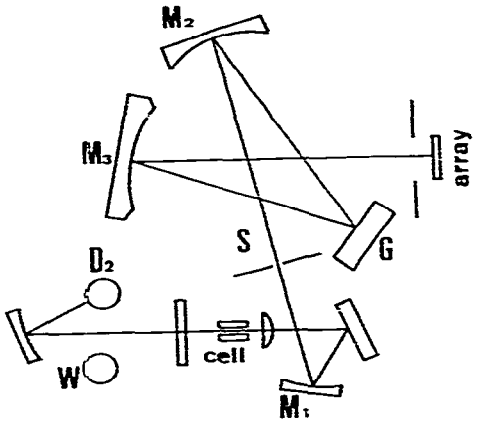

Fig. 1. Optical diagram. D_2 = Deuterium lamp; W = tungsten lamp; M_1, M_2, M_3 = mirrors; G = diffraction grating; S = slit.

an image on the entrance slit(s) of the polychrometer (Shimadzu Seisakusho Ltd.). An entrance slit width of 0.11 mm was used. A Bausch & Lomb grating G (600 lines per mm for the RL 1024 C/17 or 300 lines per mm for 512 S) was used. The wavelength range covered by each array is 220 nm. This range is focused on the sensing area of the array by a concave mirror M_3 (F/5.3). The RL 1024 C 17 array has 1024 pixcels (pitch 1 mil, aperture 17 mil), the RL 512 S array has 512 pixcels (pitch 1 mil, aperture 100 mil). The wavelength range focused on the sensing area can be adjusted within 200–800 nm region by mechanical rotation of the grating. The resolution of the polychrometer, measured by observing a line spectrum emitted from a pen Ray lamp, was 2.5 nm.

Liquid chromatograph

The liquid chromatograph consisted of a Model SF-0396-57 pump (Milton Roy), an injection port (Kyowa Seimitsu) permitting direct syringe injection on top of the column and a flow cell volume 8 μl. The column (150 × 4.6 mm I.D.) was packed with octadecyl silica (Zorbax ODS, particle diameter 5–6 μm). The mobile phase was methanol–water (90:10), flow-rate 1.0 ml/min or 0.5 ml/min.

Data acquisition system

System I. This system was used to evaluate the noise level of a single pixcel. It is a conventional fixed-wavelength detection system with which the desired wavelength can arbitrarily be selected within the 220 nm range by presetting a 10 bit digital switch. The system consists of the array (RL 1024 C/17 or 512 S), an evaluation circuit board (RL 103 for RL 1024 C/17, RC 1024 S for RL 512 S), a control logic section for selecting the pixcel number and an analogue interface section for recording the chromatogram. The last two sections are shown in Fig. 2. A clock pulse, generated by the evaluation circuit board, was fed to an asynchronous binary counter (SN 74197 × 4), and each bit of the counter was connected in parallel with a 10-bit preset switch. As soon as it is cleared by the start pulse provided by the board, the counter begins to count the clock pulses. When the counts reach a preset value, the

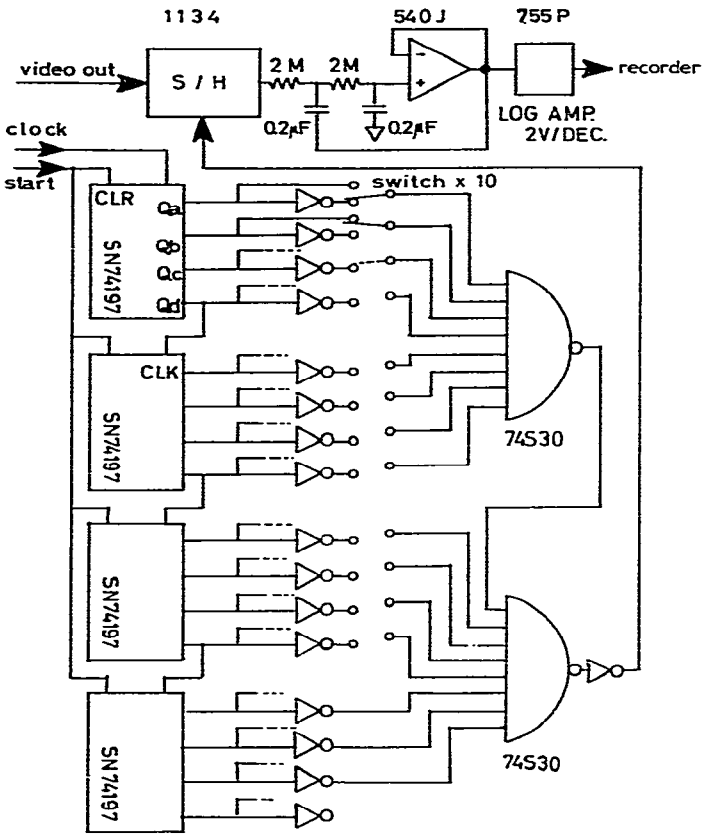

Fig. 2. Control logic and analogue interface for system I. M = MΩ; DEC. = decade; CLR = clear; CLK = clock; Qa, Qb, Qc, Qd = bit of counter.

control logic, which comprises switches and NAND gates (74530 × 2), is operated and a sampling pulse is transferred to an analogue sample/hold module (Analog Devices, SHA 1134) whose input is the spectral signal (video-out) from the array. The video-out signal of a certain pixcel, which is sampled and held during each scan, is recorded as a conventional chromatogram at the fixed wavelength after passing through a secondary Butterworth low-pass filter having a time constant of 0.4 or 1.0 sec and a logarithmic converter (Analog Devices, 755 P). If further control logic is installed in the counter, it is also possible to display the signals at additional wavelengths by using a multi-pen recorder.

System II. This system is interfaced to a microcomputer and can be used as an on-line multi-wavelength detection system. It is expected to have a lower noise level than system I because of the lack of analogue interface (logarithmic amplifiers, sample-hold amplifiers, etc.). As shown in Fig. 3, it consists of a microcomputer LSI-11 with a 27 k-word RAM, a cartridge disk, an analogue-to-digital (A/D) converter (Datel 12 bit ADC-EH12B), a digital-to-analogue converter and a timing circuit section, which is shown in Fig. 4. The last section consisting of random logic is

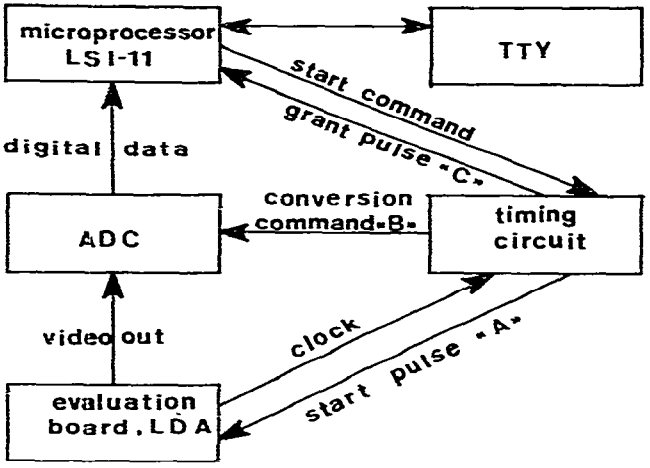

Fig. 3. Schematic diagram of system II. ADC = Analogue-to-digital converter; LDA = linear diode array; TTY = teletypewriter.

used to generate three kinds of pulses for commanding the array scanning (pulse A), the A/D conversion (pulse B) and the data acquisition by the computer (pulse C).

The whole system is initiated by a starting pulse generated by the software. This pulse from the computer is synchronized with the clock pulse from the board in the timing circuit by using two flip-flops and is fed back to the board as pulse A. After the array scanning is started by pulse A, the boxcar type video-out signal of each pixcel, which is pushed out of the array by the clock pulse, is converted into digital

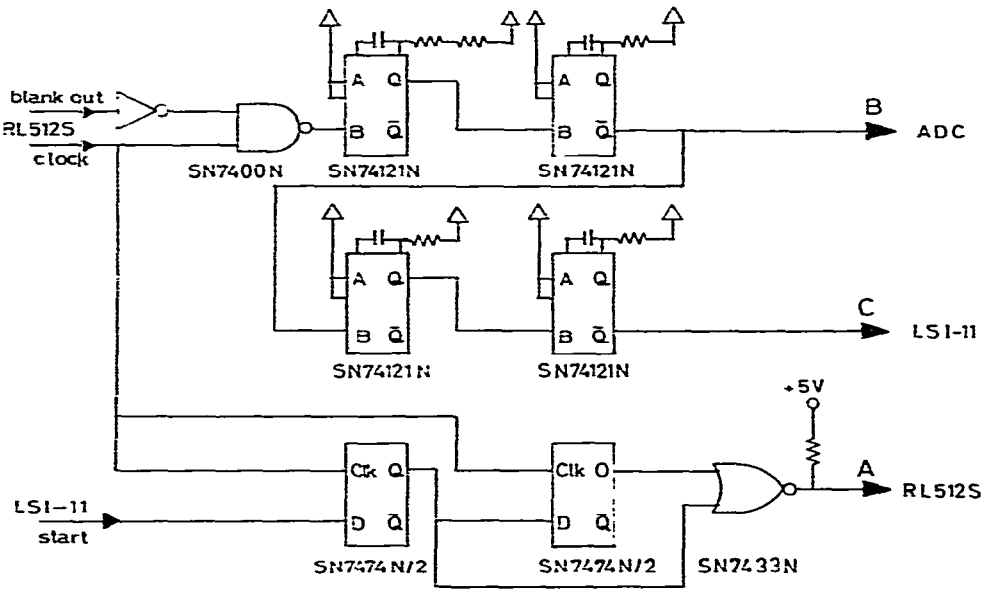

Fig. 4. Timing circuit. A, B, C, see text; Q = output of flip-flop in monostable multi-vibrator; $\bar{Q}$ = not Q; Clk = clock.

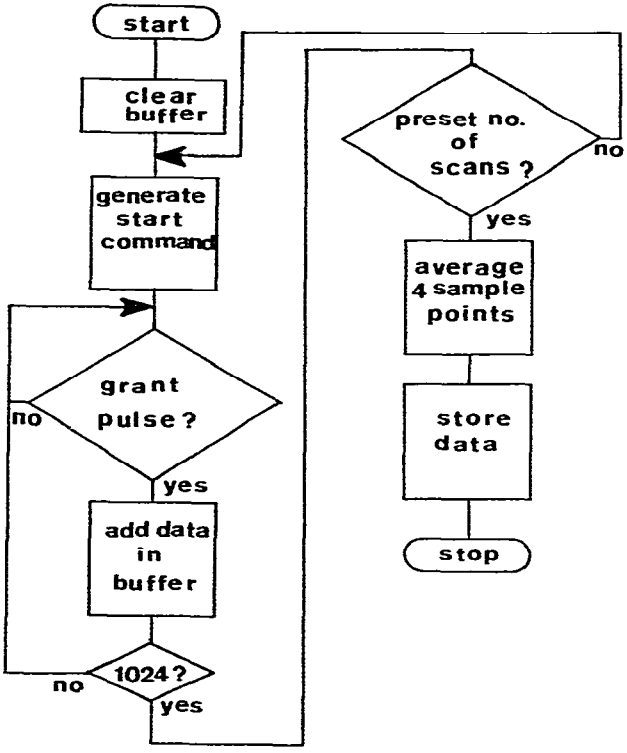

Fig. 5. Flow chart for data acquisition.

data and stored in the computer memory. The command pulse for the ADC (pulse B) and the grant pulse to the computer for data acquisition (pulse C) were produced by use of four monostable multi-vibrators in the timing circuit. Pulse B is generated by delaying the clock pulse from the board for a certain period required to stabilize the video-out signal of a single pexcel. Pulse C is generated by delaying the clock pulse for a certain period required for the completion of the A/D conversion. These two pulses are generated only for the scanning period by performing NAND operation between the clock pulse and the blanking pulse which blanks out the video signal of the board. Therefore, after a starting pulse has been sent to the timing circuit, the computer can store the 512 or 1024 digital data from all pixcels by sensing the grant pulse from the timing circuit.

The program for system II is composed of two parts; (i) acquisition of the sample data, S, the reference data, R, and the dark current data, D; (ii) floating point calculation (8 bit exponent and 24 bit mantissa) of the absorbance by using the expression log $[(R-D)/(S-D)]$, and display of the pseudo three-dimensional chromatogram. The flow chart for the data acquisition is shown in Fig. 5. Because the single-beam type polychrometer is used, a spectrum is obtained with the light source blocked and this spectrum is subtracted from all subsequent spectra to correct for the finite dark current of the array. The reference spectrum is the video-out signal when the mobile phase passes through the flow cell. The initial variables specified by the program are the sampling rate, the exposure time of the array, which is the interval

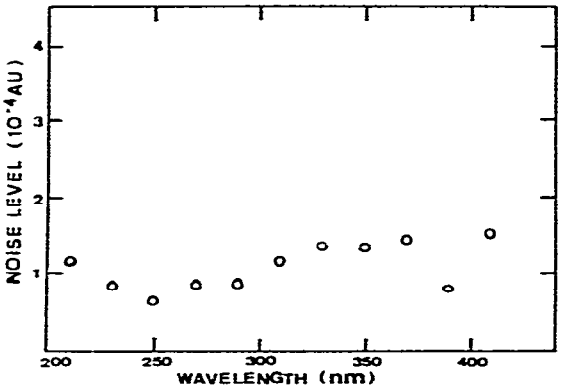

Fig. 6. Noise level of the array detector (512 S).

between two starting pulses, the number of accumulations, the starting point of the sampled video-out and the number of terms for quadratic/cubic least squares smoothing.

The processed data are displayed in various forms, *i.e.*, a plot of the absorbance at any single wavelength *vs.* elution time as in a conventional chromatogram, the spectrum of the eluting component at the selected time, the pseudo three-dimensional chromatogram (time *vs.* wavelength *vs.* absorbance) and the total integrated absorbance at all wavelengths *vs.* time.

RESULTS AND DISCUSSION

Noise level at a single pixcel

To determine the noise level of a single pixcel, the noise on the baseline, recorded by system I, was calibrated in absorbance units (AU) by using a metal wire net having a known absorbance of 0.66 AU as a reference. The noise level of a spectrophotometer generally depends on the slit width (0.11 mm in this work), the source intensity and the exposure time. Here, only the exposure time is variable.

The noise level of the RL 512 S measured with the cell at an exposure time of 30 msec (clock pulse 33 kHz) is shown in Fig. 6. It is seen that the noise level is $6 \cdot 10^{-5}$ AU at 250 nm. The present noise level of $0.6 \cdot 10^{-4}$–$1.4 \cdot 10^{-4}$ AU in the UV range is better

TABLE I

NOISE LEVELS AT THE INPUT TERMINAL OF THE LOGARITHM AMPLIFIERS *VS.* WAVELENGTH

Wavelength (nm)	*Input voltage (V)*	*Noise of input voltage (mV)*
210	1.15	0.30
230	2.38	0.31
270	1.70	0.30
310	1.37	0.28
350	0.88	0.31
370	0.83	0.31
410	0.72	0.32

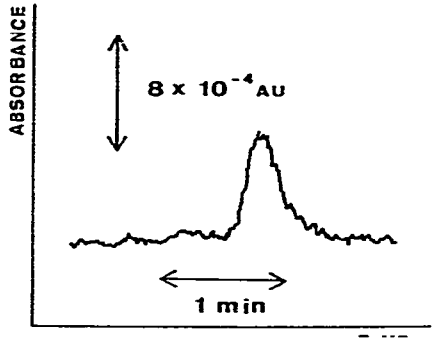

Fig. 7. Chromatogram at 230 nm of biphenyl (1 ng) measured by system I. LC conditions: Zorbax ODS, methanol–water (90:10), flow-rate 0.5 ml/min.

than those of $1.3 \cdot 10^{-4}$–$2.0 \cdot 10^{-4}$ AU in commercially available fixed-wavelength UV detectors for HPLC. On the other hand, the noise level of the RL 1024 C/17 can be ten times larger than that of the RL 512 S.

Some improvement in the noise level was achieved by decreasing the exposure time and increasing the number of accumulations. Such a dependence on integration time means that the source of the noise is predominantly in the detection system and not in the light source. Furthermore, it appears that the source of noise in the detection system is not in the array itself but in the electronic circuits. First, the noise levels observed at exposure times of 30 msec and 250 msec have the same waveforms (frequency) and amplitudes. Secondly, the noise in AU is correlated with wavelength, as shown in Fig. 6, but the noise in the input of the logarithmic converter at various wavelengths is constant regardless of input voltage as shown in Table I. These results

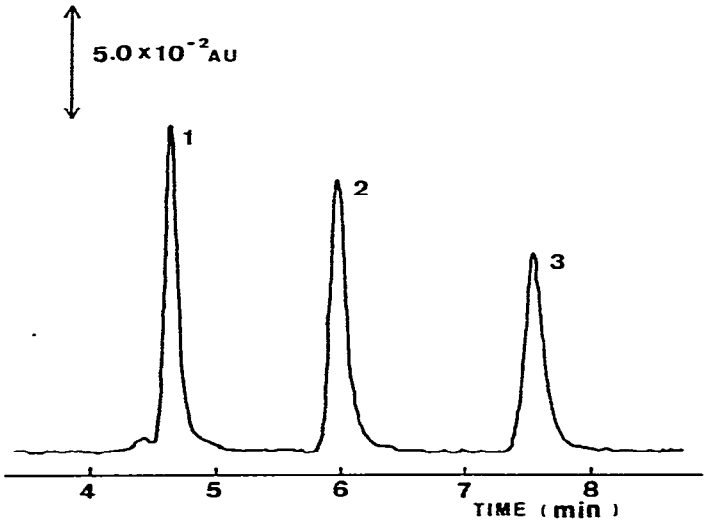

Fig. 8. Chromatogram at 230 nm of a mixture of acenaphthene (peak 1, 200 ng), acenaphthylene (peak 2, 100 ng) and fluoranthene (peak 3, 100 ng). LC conditions: Zorbax ODS, methanol–water (90:10), flow-rate 1.0 ml/min.

seem to indicate that the noise is associated not with the photodiode itself but with the electronic circuit of the evaluation board and/or the imperfect circuit built in our laboratory. Consequently, it may be possible to reduce the noise level below $6 \cdot 10^{-5}$ AU if a better circuit and a higher speed computer are employed.

Fig. 7 shows the chromatogram for 1 ng of biphenyl measured at 230 nm by using the RL 512 S as detector. Besides the high-frequency noise, there is a drift in the baseline. More precise stabilization of the light source is presumably necessary. Attempts to reduce the thermodynamic noise of the array would seem to be unwarranted until these factors are improved. However, it should be possible in this system to attain noise levels of the order of $1 \cdot 10^{-5}$ AU similar to that of recent highly sensitive UV spectrophotometers.

Chromatogram at a single wavelength

Fig. 8 shows the chromatogram of a mixture of acenaphthene, acenaphthylene and fluoranthene at 230 nm obtained by using system I equipped with the RL 1024 C/17 detector. Although the results are comparable to those obtainable by a conventional LC detector, in system I the wavelength can freely be selected and also the simultaneous monitoring of absorbances at two or more wavelengths is possible if a multi-pen recorder is used. Fig. 9 shows the linear response of system I equipped with the RL 512 S for $5 \cdot 10^{-1}$–$1 \cdot 10^{3}$ ng biphenyl measured at 230 nm. Each point was obtained by averaging three measurements. The dynamic range is about four decades for the RL 512 S and three decades for the RL 1024 C/17.

Fig. 10 shows a pseudo three-dimensional chromatogram obtained by using system II equipped with the RL 1024 C/17. Fig. 11 shows a plot of total integrated absorbance at all wavelengths *vs.* elution time (total absorbance chromatogram)

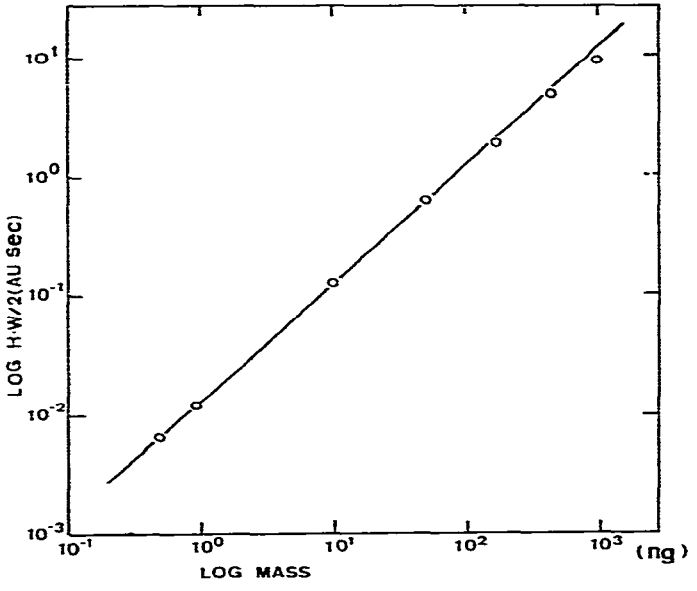

Fig. 9. Plot of peak area *vs.* mass of biphenyl injected.

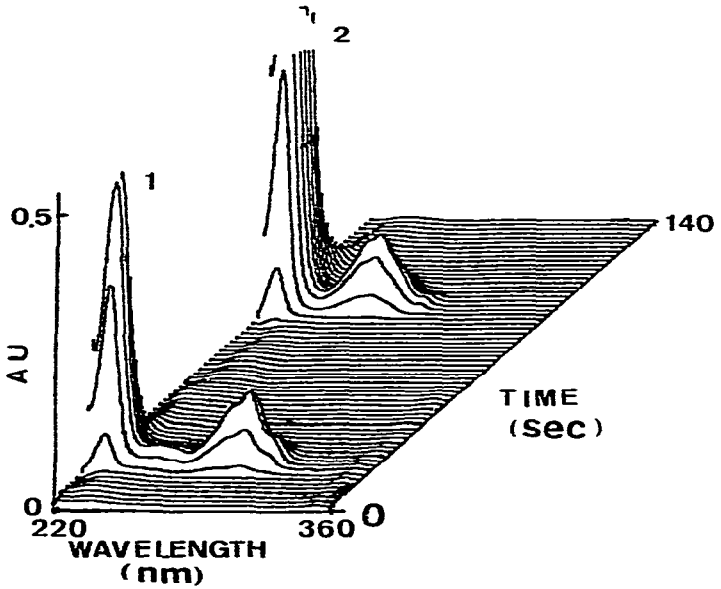

Fig. 10. Three-dimensional chromatogram for separation of acenaphthene (peak 1, 200 ng) and acenaphthylene (peak 2, 100 ng). LC conditions, as in Fig. 8.

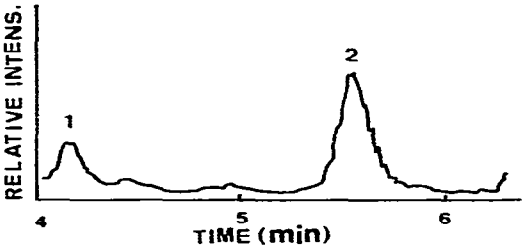

Fig. 11. Total absorbance chromatogram of acenaphthene (peak 1, 10 ng) and acenaphthylene (peak 2, 5 ng). LC conditions as in Fig. 8.

using the same system. In system II, all transmittance data obtained during an LC run are stored in the computer memory. Then, at the completion of the run, calculation of absorbance is undertaken, which takes about 5 min. Such post-run data processing is too time consuming for routine analysis and requires a large amount of computer memory. Work is therefore continuing in order to develop a real time display for three-dimensional chromatograms by using data compression techniques and real time retrieval by using an analogue–analogue correlator (charge coupled device).

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