Note

Electronic system for chart recorder absorbance output of the multicuvette system for liquid chromatography

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We have previously described a computer-interfaced multicuvette system for liquid chromatography in which quantitation is performed in specially built 40-section PTFE and glass multicuvettes that are transported by a motor drive across a monochromatic light beam[1]. A photomultiplier converts the transmitted light to a time varying signal which is then processed by the computer to extract the maximum transmission from each section, calculate absorbance and subtract a base value. After further data reduction a graph is formatted for an electrostatic printer/plotter. Such a system gives the convenience of computer reduction of chromatographic data at the cost of a minicomputer, a printer/plotter and interfacing.

The present note describes an electronic system that makes it possible to use the multicuvette system without these costly components providing a usable recording of absorbance values on an ordinary potentiometric recorder without sacrificing operating speed. The system is not only a substitute for the computer, but provides back-up capability when the computer or other components are not operating, since it can operate at about the same speed, about one chromatogram per min, preventing the pile-up of chromatograms in a routine operation.

APPARATUS

The heart of the system shown in Fig. 1 is the peak detector (Model 4084/25; Burr-Brown, Tucson, Ariz., U.S.A.) whose output follows the rise of the signal as the analyzing light beam is gradually uncovered by the cuvette window of the moving multicuvette. A diode within this module prevents the output from falling as the back edge of the window begins to eclipse the light beam. This maximum value is then transferred to a sample/hold module (Burr-Brown Model 4032/15) whose output then represents the transmittance of the cuvette. A logarithmic amplifier (Burr-Brown Model 4116) converts transmittance to absorbance. These circuits are gated by the position of the window being used so that the peak detector is reset as the leading edge is detected and the peak signal is transferred to the sample/hold when the trailing edge is detected. The window detector consists of an infrared (IR) light source

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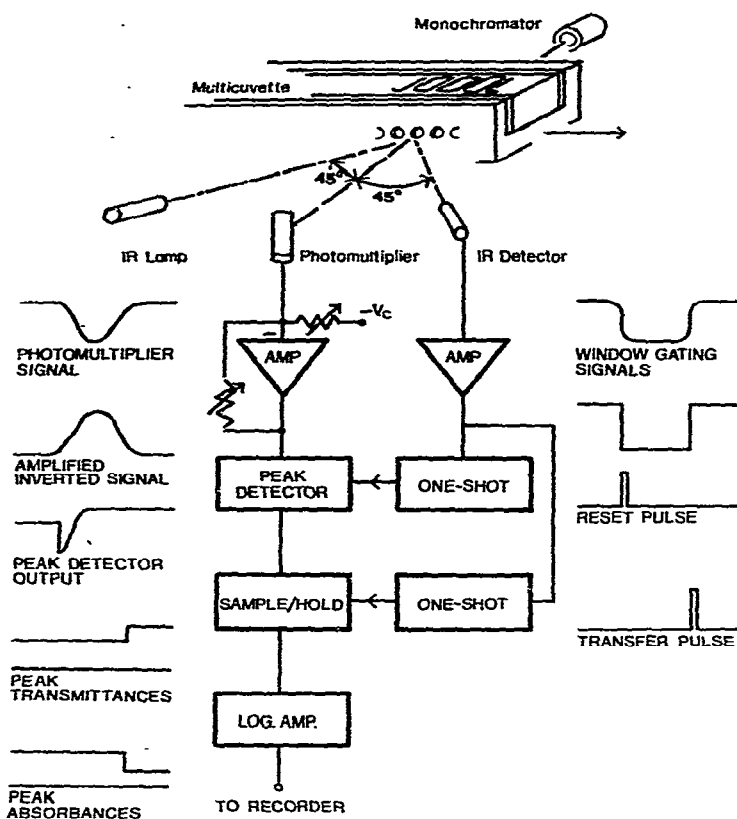
Fig. 1. Composite diagram showing at top the geometry of the window position detector and light absorption measuring system, at left the main signal units of amplifier, peak detector, sample/hold and logarithmic amplifier with typical waveforms, and at the right the window position gating circuit and its waveforms.

(Model L150; Skan-a-matic, Elbridge, N.Y., U.S.A.) mounted at 45° to the analyzing light beam and an IR detector (Skan-a-matic Model P171 IRC) at 45° on the other side of the monochromatic light beam, as shown at the top of Fig. 1. The IR light is at a wavelength of 900 nm so that it is clearly above the visible region of the spectrum used in spectrophotometry. The IR beam is reflected from the aluminium sides of the multicuvette between the windows so that almost no reflection occurs when a window is in the analyzing position. The detector output is shaped by an amplifier (Skan-a-matic Model T330D) and pulses are generated at both leading and trailing edges by two one-shots (Motorola MC74121P).

The analyzing light signal ($+1$ to -1 V) is inverted and scaled (typically 0 to $+5$ V) by an operational amplifier with adjustable gain and offset. Power supplies of $+5$, $+15$ and -15 V are provided. A switch allows selection of push-button control during the process of gain and offset setting. Almost any available chart recorder may be used. The operating speed of the transport is limited by the response of the recorder. With a variety of recorders we have operated at speeds of 1–5 min for a 120 fraction

readout, typically operating at 2 min with a Honeywell Electronik 194 recorder at a chart speed of 10 sec/in. At this speed the recorder has 1 sec to respond to the absorbance signal, for which the photomultiplier has been exposed for perhaps 0.1 sec.

The cost of the system is about US$ 500 for electronic components and modules, US$ 120 for the IR window detecting system, and in addition building a mount for the IR detector and lamp is required.

RESULTS AND DISCUSSION

The performance of the electronic system is illustrated by the simultaneous recording of the chromatograms of four common urinary corticosteroids (Fig. 2) using both the computer output on an electrostatic printer/plotter (top of Fig. 2) and the electronic system described here and recording on a chart recorder (bottom part). The corticosteroids run are *allo*-tetrahydrocortisone (a-THE), tetrahydrocortisone (THE), *allo*-tetrahydrocortisol (a-THF) and tetrahydrocortisol (THF). Gradient elution chromatography with a gradient of acetone in chloroform was used with quantitation by the blue tetrazolium reaction as described elsewhere[2]. Numerical
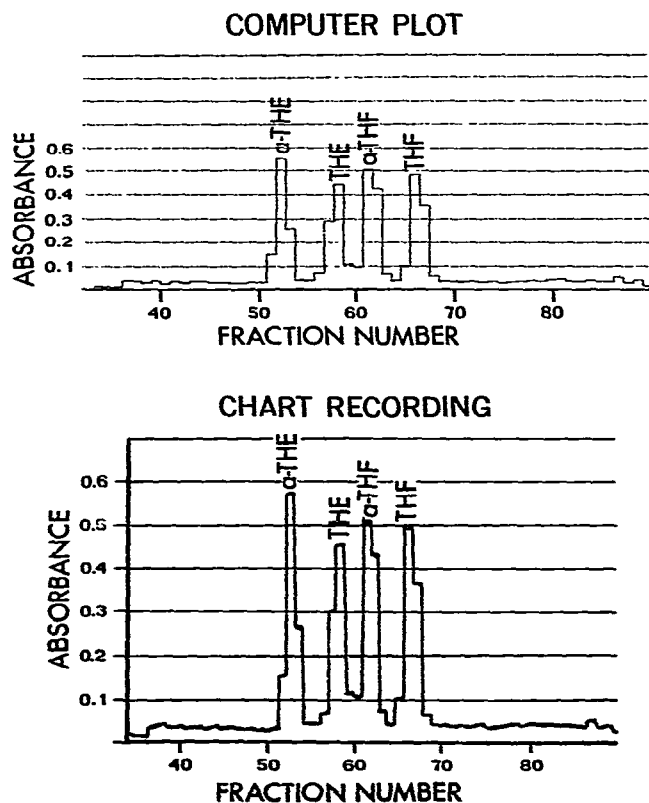
Fig. 2. Top: section of a printer/plotter output of chromatogram of corticosteroids (see text) from the computer based system. Bottom: chart recorder output from the electronic system for the identical cuvettes.

values taken from the chart outputs have agreed within 2 % with the computer output.

This system may also be applied to a dual-beam spectrophotometer by placing the logarithmic amplifier at the input end together with a second logarithmic amplifier for the reference beam and an operational amplifier for subtracting the two absorbances to provide an inverted and offset signal for the peak detector.

The window detector circuitry is also useful during computer operation since threshold detection logic will sometimes fail to detect a fraction whose absorbance is greater than 2.

Additional details may be obtained from the authors.

ACKNOWLEDGEMENTS

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2 P. Vestergaard and J. F. Sayegh, in *Advances in Automated Analyses, Technicon International Congress, 1969*, Vol. I, Mediad, White Plains, N.Y. 1970, p. 327.