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A MINIATURIZED ULTRAVIOLET FLOW PHOTOMETER FOR USE IN LIQUID CHROMATOGRAPHIC SYSTEMS*

LOUIS H. THACKER, CHARLES D. SCOTT AND W. WILSON PITT, JR.
*Oak Ridge National Laboratory**, *Oak Ridge, Tenn. 37830 (U.S.A.)*

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SUMMARY

A miniaturized ultraviolet photometer for continuously monitoring chromatographic column effluents at 254 nm and 280 nm has been built and tested at the Oak Ridge National Laboratory. The instrument is of double-beam design and employs dual solid-state electronic circuits for operation without wavelength switching and multipoint recorder synchronization. Performance tests have shown that the ultraviolet photometer can provide greater sensitivity and less band spreading of chromatographic peaks than the modified spectrophotometer that was used previously.

INTRODUCTION

Several analytical instruments now in use or under development use high-resolution liquid chromatography for the separation step. One instrument of this type, called the UV analyzer, is being developed in the Body Fluids Analyses Program at the Oak Ridge National Laboratory (ORNL). It utilizes high-pressure ion-exchange column chromatography for separation and UV photometry for detection of the UV-absorbing constituents in body fluid samples[1,2].

Prototype systems of the UV analyzer were built at ORNL and are now being tested at several clinical laboratories[3]. Examination of the design of the UV analyzer and of the service records for the prototypes showed that the UV detector (a commercial spectrophotometer modified for automatic wavelength shifting) was a major contributor to the cost, size, and maintenance requirements of the systems. Therefore, it was desirable to replace the large, general-purpose spectrophotometer with a small UV photometer designed specifically for the application at hand.

The criteria established for an acceptable UV photometer include the following:

(1) The detector head must be of the smallest size practical to permit mounting as close to the column discharge as possible.

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(2) The flow cells for the photometer must have a minimal volume, and their bubble-clearing characteristics should be optimized.

(3) Two wavelengths, 280 nm and either 254 or 260 nm, should be monitored continuously.

(4) The photometer must operate as a double-beam instrument at both of the designated wavelengths so that it will be useful with chromatographic systems in which gradient elution is used.

(5) The sensitivity of the photometer must be as high as that of a moderately-priced spectrophotometer equipped with flow cells having a path length of 1 cm.

(6) The instrument should be simple in design, require little maintenance, and have a low fabrication cost.

A number of commercially available UV photometers were examined or tested; however, while each had some acceptable feature, no single model contained all of the required characteristics. Thus the two-wavelength, dual-beam photometer (designated as Mark I) described below was developed to meet the established criteria.

DESCRIPTION

The Mark I photometer system (Fig. 1) consists of a photometer head, a small electronic chassis, and an interconnecting cable.

The photometer head (Figs. 1 and 2) is 1½ in. in diameter and 5 in. long. Reference and sample streams flow upward through 3-mm-I.D. flow cells made of drawn quartz. A low-pressure mercury pencil lamp supplies the 254 nm radiation. Excess 254 nm radiation excites a pair of phosphor rods, which emit 280 nm radiation normally absent from the mercury spectrum. The 254 nm channel consists of reference and measuring beams that cross the respective flow cells near their lower end, a com-

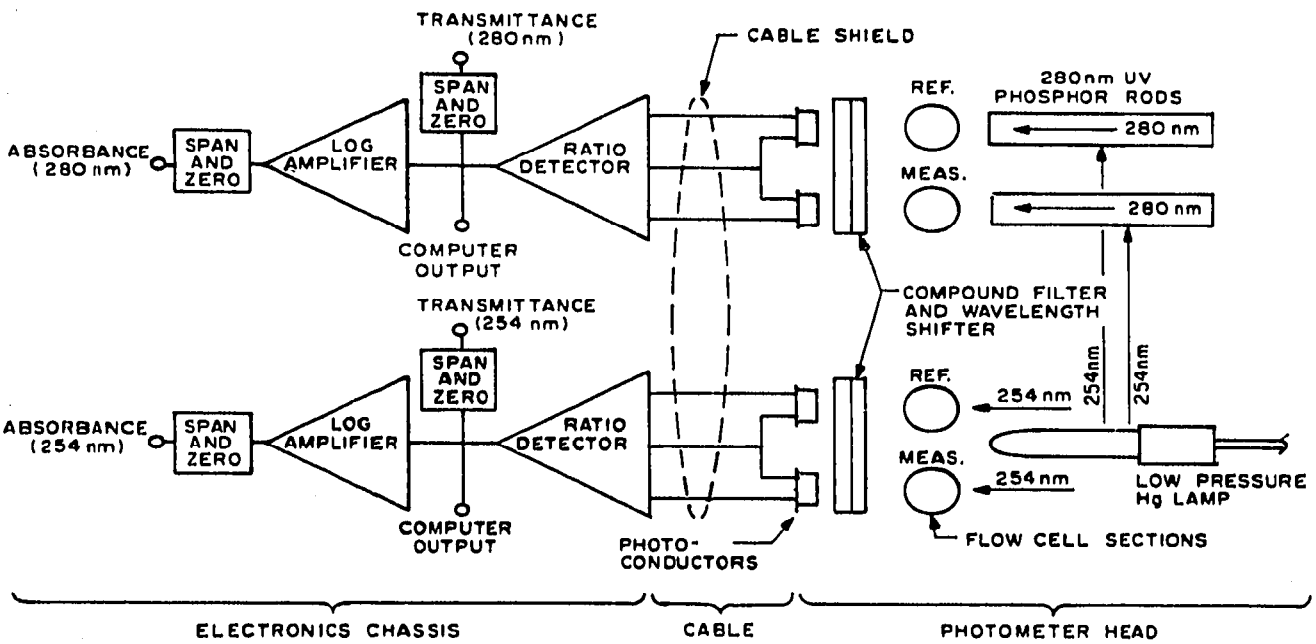

Fig. 1. Schematic diagram of the photometer system.

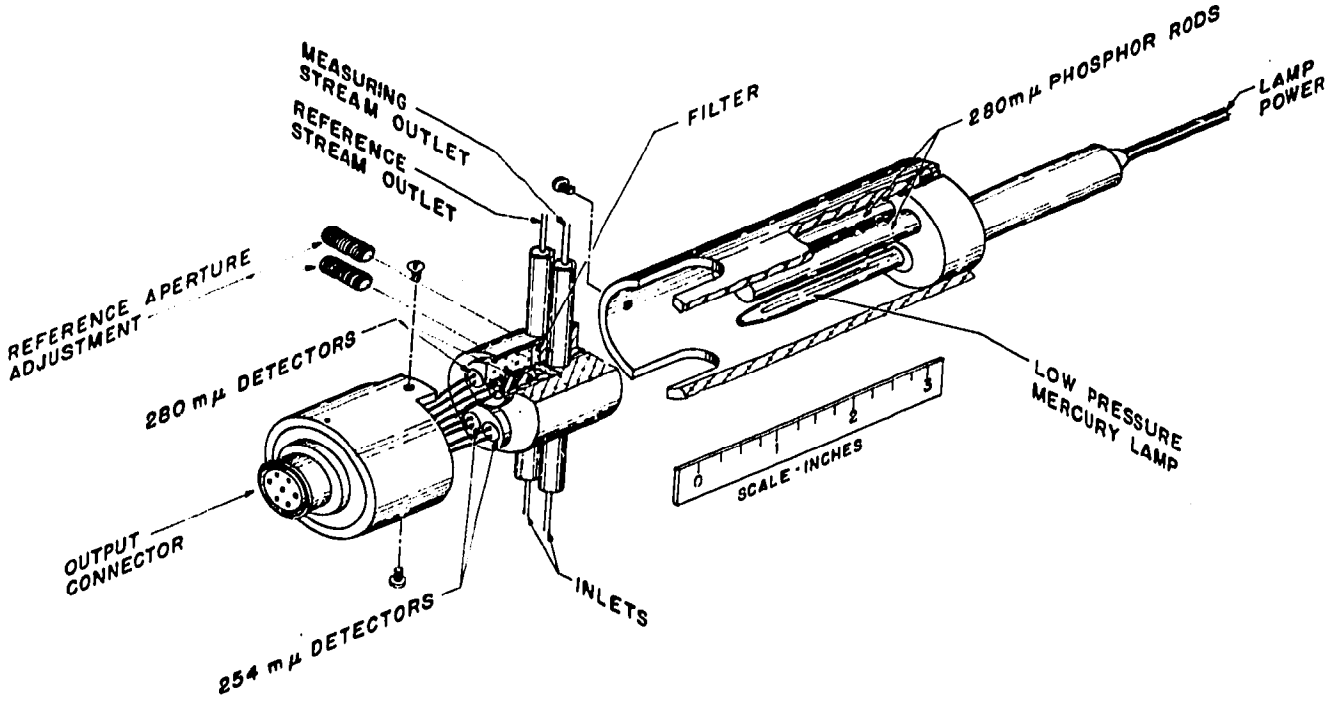

Fig. 2. Two-wavelength UV photometer detector head.

pound optical filter and wavelength shifter, and a pair of photoconductive detectors to sense the intensity of the two transmitted beams. The parallel 280 nm channel crosses the two flow cells near their upper end. The optical apertures of the reference channels can be adjusted to provide reference cell illumination at the same level as that of the measuring cell. The outer cylindrical case with its two end caps supports the photometer capsule, the UV lamp, the phosphor rods, and the signal connector; it also aligns the optical components. The photometer head is connected to the electronics chassis by a six-conductor shielded cable.

The reference and measuring photoconductors* in each channel have the relationship:

$$R = CI^{-k},$$

where R = resistance of photoconductor,

I = illumination level

C = resistance at unit illumination,

k = slope constant for photoconductor (approximately 1 at low light levels).

The signals from the two photoconductors provide the input to a ratio detector (Fig. 1), which, in turn, provides an output voltage E_o:

$$E_o = E_i \left(\frac{R_{REF}}{R_{MEAS}}\right) = E_i \left(\frac{I_{MEAS}}{I_{REF}}\right)^k = E_i T^k,$$

where E_i = 10 V, T = transmittance, and $k \approx 1$. Accomodations are available for setting the span and the zero point of the ratio detector output so that the trans-

* Clairex type 905 HLL-T, manufactured by Clairex Corp., N.Y., U.S.A.

mittance of the test liquids can be displayed on a 10-mV recorder at any sensitivity from 0–100% to 90–100%.

The output of the ratio detector can also be fed to a logarithmic amplifier, which provides an output E_L:

$$E_L = g \log E_i T^k + gz = g(\log E_i + z) + gk \log T,$$

where g is an adjustable gain and z is an adjustable voltage that can be set as $z = -\log E_i$.
Thus

$$E_L = gk \log T = GA_z,$$

where A_z is the absorbance for an optical path length, z (in cm). However, since $A_{1.0\ cm} = A_z(1/z)$, the scale factor G can be set to display $A_{1.0\ cm}$.

The transmittance and absorbance output signals are displayed on a 10-mV multipoint recorder. In addition, transmittance output of 0 to −10 V is available for interfacing with a digital computer.

EXPERIMENTAL RESULTS

The Mark I UV photometer was tested for linearity, sensitivity, and general

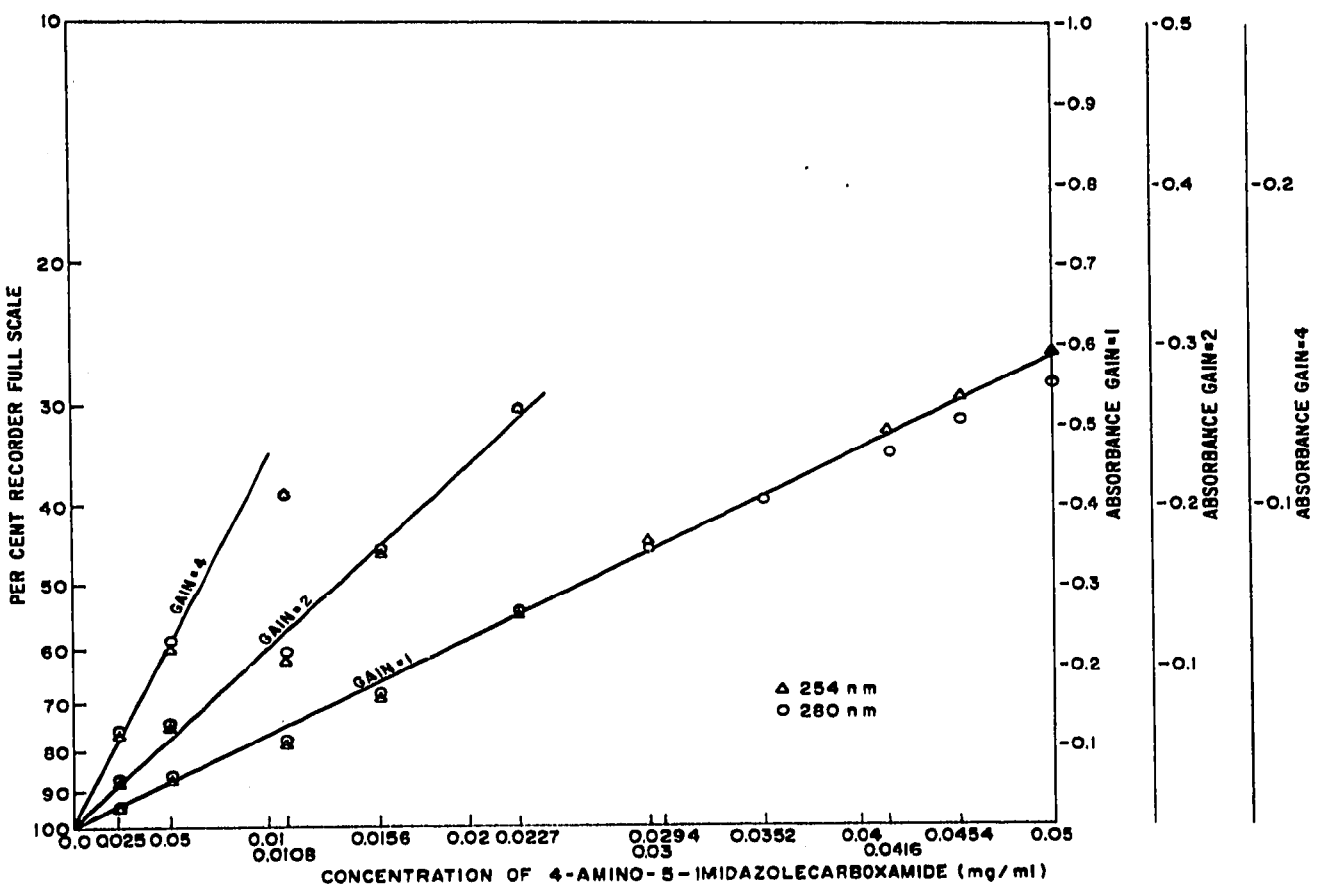

Fig. 3. Calibration curves for the Model Mark I UV photometer.

utility and was found to be suitable for use as a detector in liquid chromatographic systems.

Calibration

The response of the Mark I photometer to aqueous solutions of known compounds was determined and compared with that of a modified Beckman DB spectrophotometer. The photometer was calibrated at absolute electronic gains of 1X, 2X, and 4X, using solutions of 4-amino-5-imidazolecarboxamide. Calibration curves obtained from these tests are shown in Fig. 3. The data show that the dual-beam photometer has essentially a linear response to changes in concentration. Results of similar calibration tests made using the modified DB spectrophotometer indicate that the linearity of the Mark I UV photometer is about the same as that of the DB spectrophotometer.

The effective path length of the photometer, using 3-mm-I.D. quartz tubes as flow cells, was determined by comparing the absorbance values of the reference solution in the photometer with those obtained from the spectrophotometer with cells of 10-mm path length (see Fig. 4). From the slope of the straight line of this plot, the effective path length of the photometer flow cells was found to be 2.6 mm.

Peak broadening

The dispersion number of the Mark I photometer was measured by the pulse injection technique in order to determine the extent to which the photometer contributes to extra-column peak broadening[4]. In this technique, a UV-absorbing substance is injected, as a pulse, into a stream flowing through the flow cells, and the absorbance of the stream is measured as a function of time and recorded on a strip-

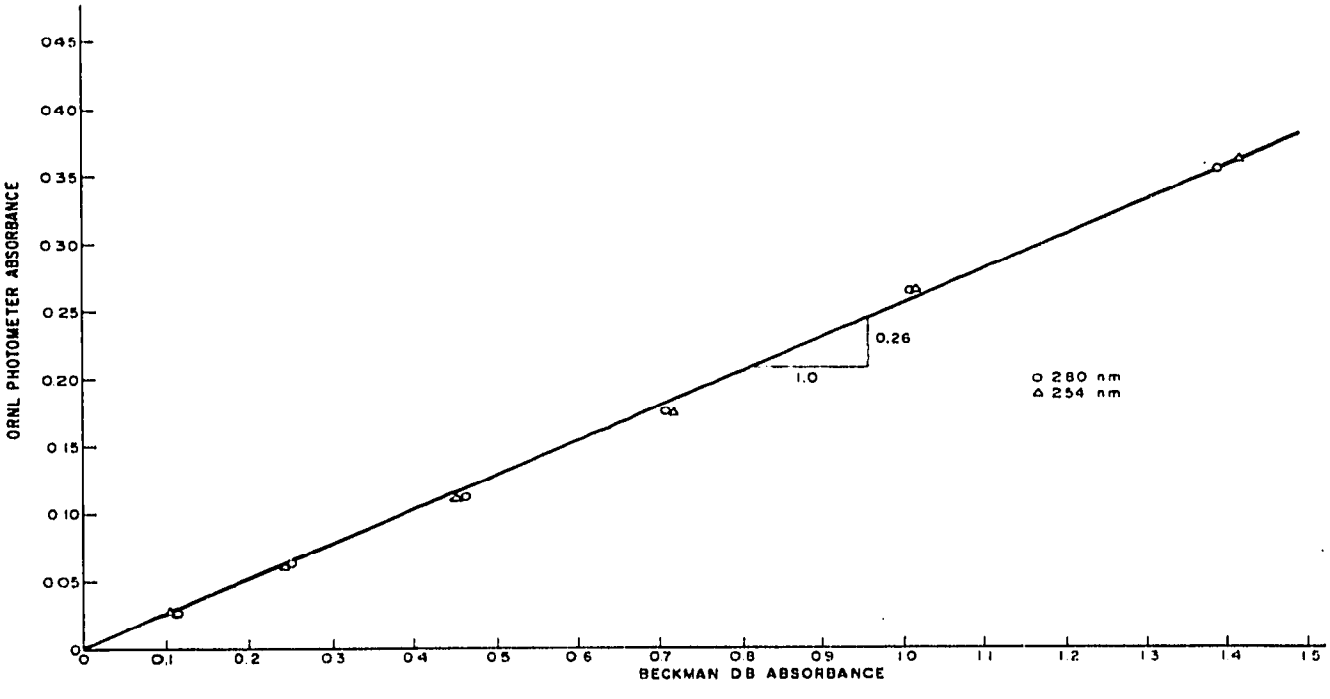

Fig. 4. Comparison of ORNL photometer with Beckman DB.

chart. The resulting increase in peak width is a result of flow through the flow cell and is thus the measure of the peak broadening due to the cell.

The dispersion number is a dimensionless group that uniquely characterizes the degree of longitudinal mixing that occurs during flow. Depending on the experimental setup, this parameter can be determined from experimental concentration *vs.* time curves or by measuring the standard deviation of a chromatographic peak. For the experimental setup used, the dispersion number was determined by:

$$8\left(\frac{D}{\mu L}\right)^2 + 2\left(\frac{D}{\mu L}\right) = \frac{J^2}{E^2}$$

where $D/\mu L$ is the dispersion number; J is the standard deviation of the peak in time units; and E is the mean resident time. A small value for the dispersion number

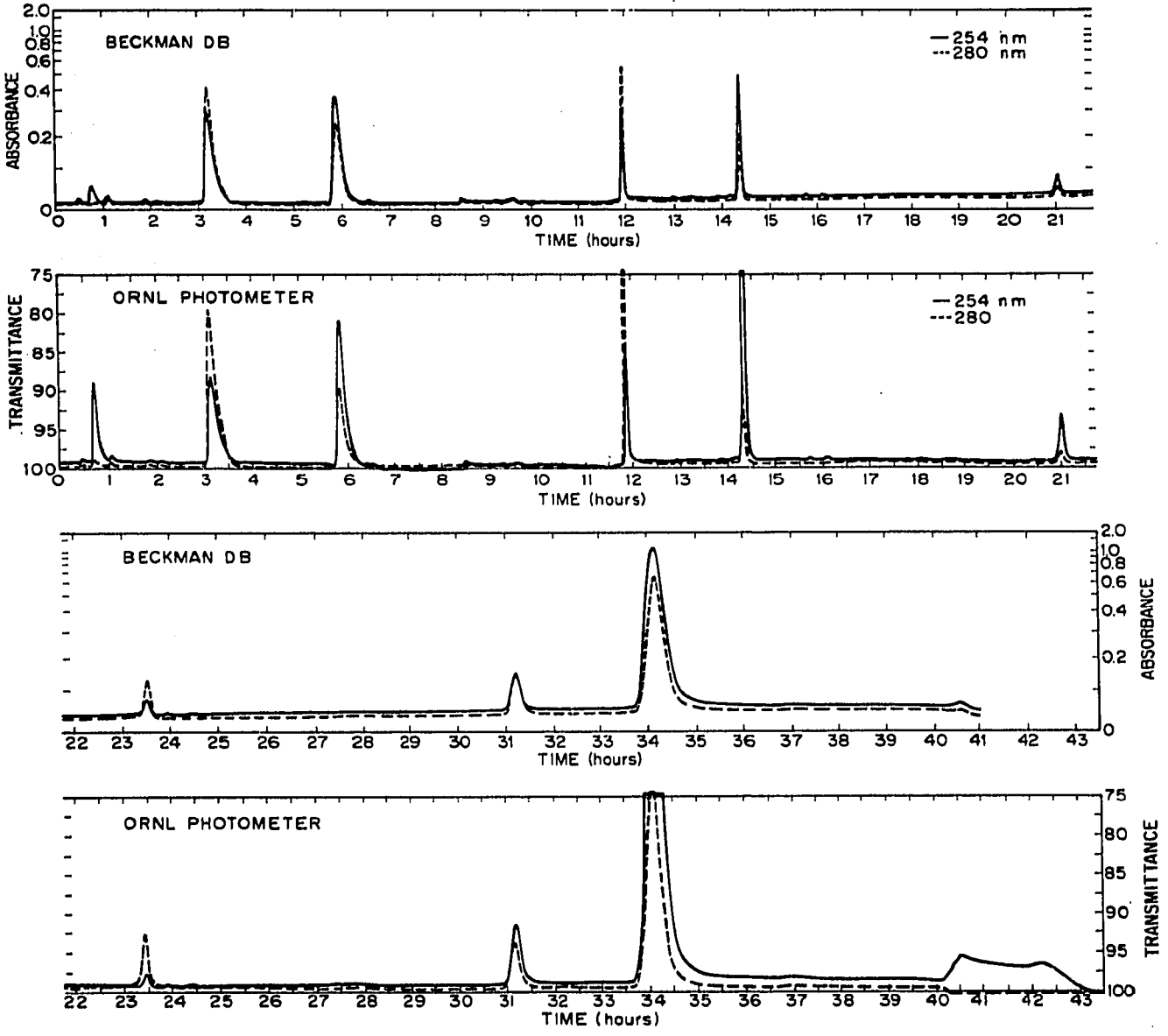

Fig. 5. Comparison of the UV photometer with the Beckman DB spectrophotometer as column monitors.

indicates that there is only a small amount of backmixing or peak broadening and suggests that the cell is well designed.

The dispersion numbers determined for a flow rate of 5.0 ml/h (*i.e.*, the flow rate used with a 0.22-cm-diam. column in the UV analyzer) were 0.032 and 0.045 for the 254-nm portion and the 280-nm portion, respectively, of the photometer flow cell. For comparison, a typical dispersion number for the DB spectrophotometer with a Pyro-Cell* was 0.065. This indicates that the contribution to peak broadening of the Mark I photometer flow cell is significantly less than that of the flow cell used in the DB spectrophotometer.

Chromatographic detector

In order to determine the comparative utility of the Mark I UV photometer and the modified Beckman DB spectrophotometer as chromatographic column monitors, the two instruments were placed in series on a standard Mark II UV analyzer prototype**. Fig. 5 shows the detector signals, as recorded on strip-chart recorders, of the 280 and 254 nm wavelength channels of each instrument. The UV photometer was operated at an electronic gain of 4 × (transmittance range of 75–100%) during these tests, and its response to the separated urinary constituents was somewhat greater than that of the DB spectrophotometer. Also, the chromatographic peaks showed less band spreading. The gain of the photometer can be increased additionally by about a factor of 2 without significantly increasing the noise level.

After this initial test, the Mark I UV photometer was used successfully as the primary detection system in more than 50 UV analyzer runs.

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* Flow cell No. 5011, with a 1-cm path length, from Pyro-Cell Manufacturing Co., Westwood, N.J., U.S.A.

** Available as CAPE-1753 from the Clearing House for Federal Scientific and Technical Information, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, Va. 22151, U.S.A.