Quantitative Photochemical Decomposition of Drugs I

Design and Calibration of an Instrument

By JAMES C. PRICE and GEORGE E. OSBORNE

An instrument for facilitating photochemical decomposition studies has been designed, constructed, and calibrated. The instrument supplies light of narrow bandwidth, which can be varied with respect to wavelength and to some extent with respect to intensity; the instrument provides for monitoring, recording, and integration of the incident and exit beams to and from the reaction mixture; it provides for keeping the reaction mixture at a constant and predetermined temperature and for stirring the mixture. Stoppered reaction cells exclude air when desirable. Short and long term stability of the instrument appear to be good, and response to light of any one wavelength with respect to intensity is practically linear.

THE PROBLEM of the decomposition of drugs as a result of the absorption of radiant energy in the form of light has become more acute as the number and complexity of pharmaceuticals have increased. The quantitative study of the influence of light upon drug stability is unfortunately difficult. Not only are many of the drug entities very complex, but also the presence of adjuncts in a finished, marketable, and acceptable preparation may tend to confound the study of such systems. Add to these problems the complicated task of measuring and controlling the light energy absorbed by the system and of separating the effects produced by the light from other unknown and uncontrollable factors, and it becomes little wonder that investigations in this area in the discipline of pharmacy have, until recently, been more or less qualitative. It is to the problem of the measurement and control of the quality and quantity of light that the efforts of this work have been primarily directed.

INSTRUMENT DESIGN

The design of an instrument for photochemical studies must include the following considerations.

The instrument should supply radiant energy of proper wavelength range, of suitably narrow bandwidth, and of sufficient intensity to make decomposition studies practicable.

The instrument should measure the quantity of energy absorbed by the drug sample by accounting for (*a*) the intensity and quantity of the light energy entering the drug system and (*b*) for the intensity and quantity of energy leaving the system.

Ideally, the instrument should provide for concurrent and constant analysis of the effects of the radiant energy on the drug system.

The components of such an instrument then should include: (*a*) a light source, (*b*) a monochromator or other means of isolating a narrow portion of spectrum, (*c*) radiant energy detection devices and associated equipment for the measurement of light entering and leaving the sample, and (*d*) a means of keeping the reaction system in a constant and controlled environment.

When the components of this system are to be selected, it is helpful in some instances to postulate properties of ideal components, considering both the behavior of radiant energy and the requirements for the study of photochemical decomposition reactions. Available components of design which most closely approximate those of the ideal can then be selected within budgetary limits.

Design and Selection of Components

Light Source.—The ideal light source for photochemical studies should produce line spectra, the intensity of which could be varied at will by changing the input energy. The source should be stable, have a long life, be economical of space and energy requirements, be readily obtainable and inexpensive. The lamps which most nearly approach these requirements are the mercury vapor medium pressure arcs.

The lamp selected for the subject instrument was 250-watt mercury vapor Uviarc, manufactured by the General Electric Co. The lamp was rated for 1000 hours useful life, and it supplied useful mercury spectral lines at 254, 265, 281, 297, 303, 313, 365, 405, 436, 546, and 578 mμ wavelengths. The lamp was readily obtainable and had a low first cost. In

Received June 3, 1963, from the College of Pharmacy, University of Rhode Island, Kingston.
Accepted for publication September 3, 1963.
Abstracted from a thesis submitted by James C. Price to the Graduate School, University of Rhode Island, in partial fulfillment of Doctor of Philosophy degree requirements.
This investigation was supported in part by a fellowship from the American Foundation for Pharmaceutical Education.
Presented to the Scientific Section, A.PH.A., Miami Beach meeting, May 1963.

addition, it could be operated from the regular alternating current lines through a current-limiting transformer. The addition of a variable transformer ahead of the current-limiting transformer made it possible to vary the output intensity of the lamp through a limited range.

The Monochromator.—Quantitative photochemical studies almost always require that the light used to irradiate a chemical substance or a drug be of narrow bandwidth, *i.e.*, as nearly monochromatic as possible. Ideally, one should be able to select any bandwidth for a given experiment, and the device used for limiting the bandwidth should not cause any light loss in the process.

Although filters can be used to isolate a narrow portion of spectrum, they do not offer the versatility and ease of preselection of wavelength that is possible with a prism or diffraction grating monochromator. For these reasons, a diffraction grating monochromator was designed as a part of the instrument. A diffraction grating was chosen as the dispersing element in the monochromator, rather than a prism, primarily because its linear spectral dispersion facilitated wavelength calibration; in addition, good quality replica gratings were available at a reasonable cost.

The grating selected was a Bausch & Lomb Certified-Precision replica, plane reflection type, with 52 × 52-mm. ruled area, and 1200 lines per millimeter. The grating was blazed for 3000 Å. wavelength.

The optical layout for the monochromator was based on the Czerny-Turner modification of the system described by Ebert, as reported by Fastie (1, 2). The Czerny-Turner modification differs from the Ebert system only in that two small, off-axis, spherical mirrors are used instead of one large one.

The optical arrangement of the parts of the monochromator are shown in Fig. 1, which shows also the reaction cell, the optical blank, the detectors, and the light path taken in the instrument. Light from the source is collimated by a large, first surface, spherical, concave mirror and is focused at the entrance slit with the aid of a small plane mirror. Light from the entrance slit is deflected by a second plane mirror to a spherical concave mirror which renders the beam parallel and sends it to the diffraction grating. The diffracted light is then focused by a second spherical concave mirror on the reaction cell exit slit. However, a portion of the light is focused on the optical blank exit slit with the quartz plate beam splitter. Light from the exit slits goes through the reaction cell and the optical blank, respectively; the photocell detectors monitor the light exiting from the reaction cell and the optical blank cell. The rectangular objects on either side of the silica cells are water jackets and holders for the

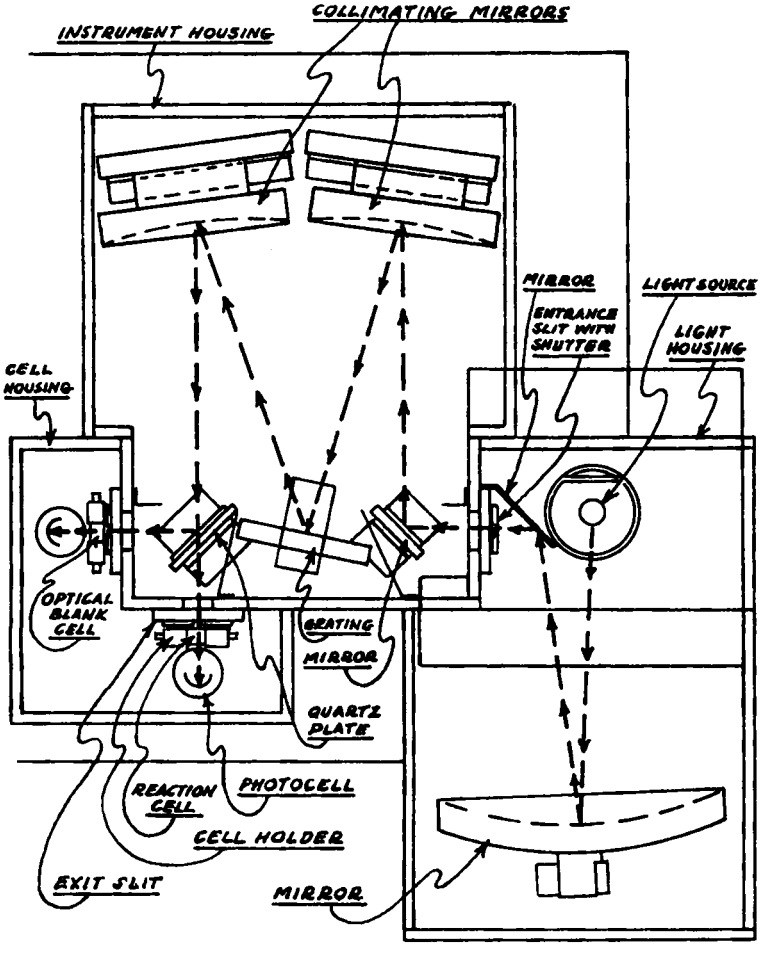


Fig. 1.—Light path diagram of the instrument showing the light source, the monochromator, the cells and cell holders, and the photocell light detectors.

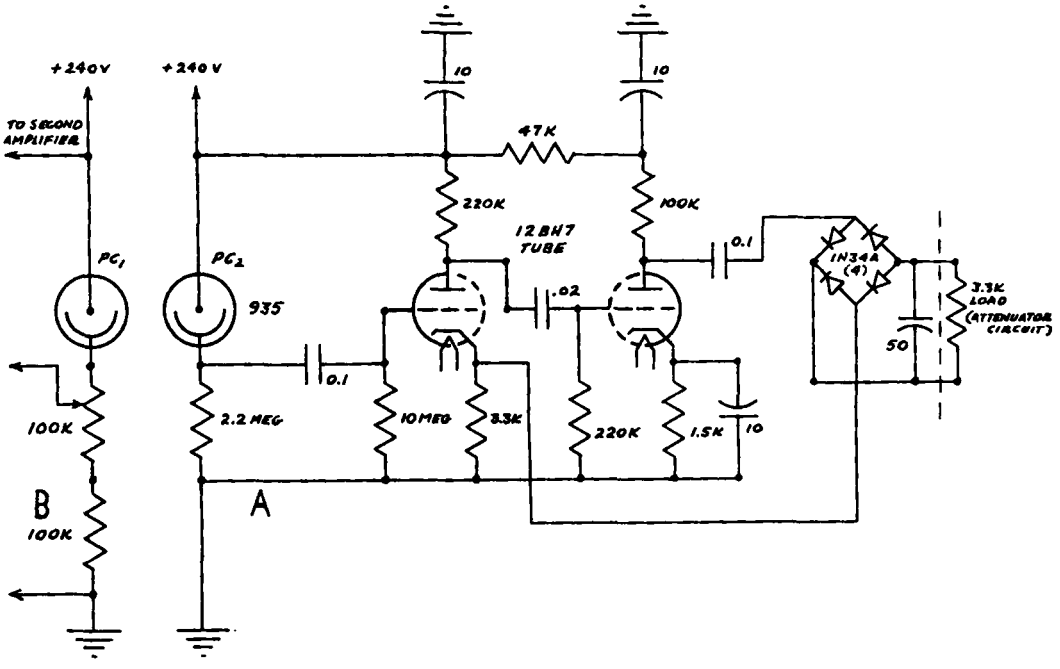


Fig. 2.—*A*, Photocell and photocell amplifier circuit for the optical blank channel. *B*, Photocell circuit for the reaction cell channel (amplifier circuit is identical to *A*). All resistance values are in ohms: k = 1000, meg = 1,000,000. Capacitance values are in microfarads.

cells. Not shown on the diagram is a small electric motor with a magnet attached to its shaft, which serves to stir the reaction mixture (*via* a small magnetic stirrer).

The collimating mirror for the light source is a concave mirror, 6.5 in. in diameter, 3.8-in. focal length; the two concave mirrors in the monochromator proper are identical 4.25-in. diameter, 9-in. focal length, aluminized mirrors. The quartz plate beam splitter is a 1-in. by 2-in. silica microscope slide. The slits are fixed at 2.1-mm. width and 18-mm. length. The diffraction grating, described previously, is rotated with a gearbox from a surplus type A-10 sextant. The degree indicator on the sextant gearbox serves as a means of selecting wavelength.

The reaction cells are ordinary, 1-cm. light path, silica spectrophotometer cells with Teflon stoppers. They are held in place against the slits by the water jacket assembly.

Electronic Components

Light Detectors.—The ideal detector should give a linear response with respect to intensity, should respond only to the spectral range of interest, and should show the same response for the same intensity for any wavelength within the range of interest. Thermopiles are classically the detectors of choice for photochemical studies (chiefly because of their linear response which does not change with wavelength); they have the disadvantages of low output, slow response, and sensitivity to temperature changes and heat radiation. In addition, a display of their output requires either very sensitive galvanometers or elaborate amplifiers. Vacuum type photocells, though having a varying response with wavelength, are insensitive to heat radiation and temperature changes; when a modulated signal is used, their output is easily amplified. Their response time is rapid, and for a given wavelength, their output is linear with respect to intensity. For these reasons, photocells were selected as detectors in this instrument. They were type 935 vacuum phototubes having an S-5 spectral response.

The output signals from the photocells were approximately electrically balanced by using different values of cathode resistors in each phototube. A voltage divider potentiometer in the cathode resistance of the photocell monitoring the light from the reaction cell permitted balancing the two signals exactly (Fig. 2).

Photocell Amplifiers.—The primary requirement of the amplifier is to give stable, linear amplification. Alternating current amplifiers with negative feedback to compensate for nonlinearity fulfill these requirements. Since the signal from the photocells was modulated at a 120 cycle rate (the light output from the mercury vapor lamp was modulated at this rate because of the alternating current operation), an alternating current amplifier could be used, and the difficulties of direct current amplification were avoided. The amplifier constructed for the photocells consisted of a dual-triode tube and a full-wave bridge rectifier to provide a rectified output signal. Negative feedback through the rectifier to the cathode of the first stage helped to correct for nonlinearity in the amplifier and the rectifiers. In the circuit shown in Fig. 2, a type 12BH7 tube gave very nearly linear response over the full range of output from the phototubes. A type 12AX7 tube gave slightly more linear response over a shorter range.

The rectified output from each photocell amplifier goes to an attenuator circuit, which allows the selection of one-half or full output to go to the recorder.

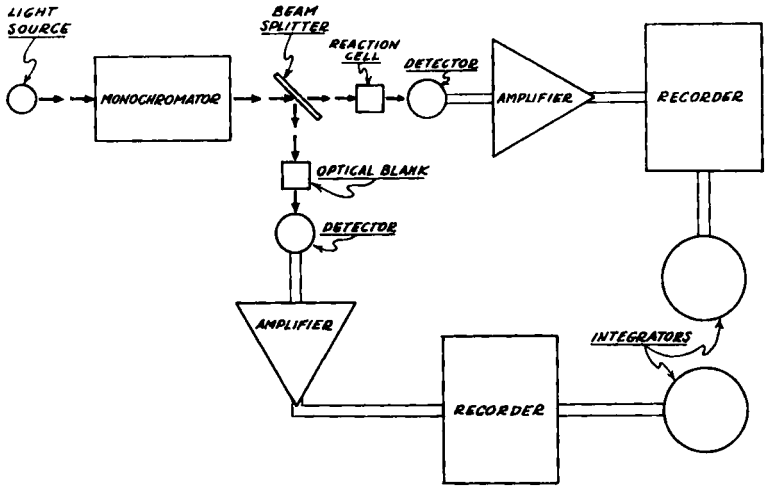


Fig. 3.—Block diagram showing the relationship of the components of the instrument.

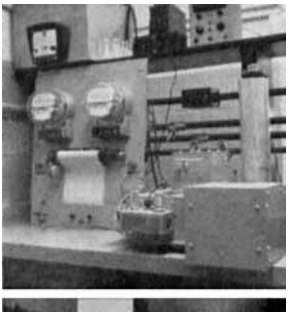
Recorder and Integrator Systems.—The recorder and integrator systems were based on a light integrator described by Miller (3). Two servo-motor-amplifier systems were used to record and integrate the signal from each photocell amplifier. A complete description of the recorder and integrator systems is included in the thesis (4) from which this report is derived.

Summary of Instrument Design

The relationship of the individual components in the instrument is shown in Fig. 3. Light from the Uviarc source is rendered monochromatic by the monochromator; the monochromatic beam is split; the main portion of the beam is directed to the reaction cell, and the other portion of the beam is directed to the optical blank. Output from each photocell detector is amplified, and the signal intensity is recorded. The signal is then integrated or summed over the time period of exposure of the reaction cell to light. Figure 4 (*A* and *B*) shows the instrument.

CALIBRATION OF THE INSTRUMENT

Monochromator Calibration.—The monochromator was calibrated with the mercury arc source, using known lines of the mercury spectrum as references. A plot of wavelength *versus* dial indication was linear.



A

B

Fig. 4.—*A*, Overall view. The recorder and integrators and their associated controls are on the left. The housing on the right contains the light source, monochromator, reaction and optical blank cells, and the photocell detectors. *B*, Top view of light source, monochromator, and cell housing assembly. Cell compartment is shown open.

Recorder Calibration.—The recorder was calibrated to determine its linearity by using known fractions of the voltage required to drive the recorder to full scale indication. The response was linear within the readability of the chart indication. This result was expected since the recorder linearity is primarily dependent upon the linearity of the balancing potentiometer, and this unit is specified by the manufacturer to be linear within 0.5%.

Integrator Linearity Calibration.—The integrator assemblies were calibrated for linearity in terms of recorder chart indication *versus* revolutions per minute of the integrator rotors. The results confirmed those of Miller (3) that the linearity and accuracy of the integrators were better than 1% of the full registration rate.

Calibration for Linearity of Over-All Response.—The over-all response linearity (excluding the integrators) was determined by utilizing Beer's relationship. Dilutions of dyes were made up, and the transmittance of several dilutions were determined for each channel of the instrument. This procedure was repeated for the most useful spectral lines of the mercury lamp from 254 mμ wavelength to 436 mμ wavelength. The results showed that the instrument was practically linear down to about 15% transmittance for all wavelengths tested. The responses of both channels were very closely parallel.

Calibration of Integrators in Terms of Quanta Per Revolution of Integrator Rotor.—This calibration was performed using the uranyl oxalate actinometer system first studied in detail by Leighton and Forbes (5). The quantum yields for this reaction have been carefully worked out by these and other workers (6, 7) for several wavelengths of the mercury spectrum.

The actinometer solution was exposed to a given wavelength of light for a sufficient length of time to give a titratable difference between the exposed and unexposed solutions; the number of molecules of oxalate decomposed was calculated; then from the known quantum yield of the reaction, the number of quanta absorbed by the solution was calculated. The number of integrator rotor revolutions of the channel monitoring the reaction cell exit was sub-

tracted from the number of rotations of the optical blank integrator, and the result was divided by the number of quanta absorbed by the solution to obtain the number of quanta per revolution of the integrator rotor.

Because of the difference in phototube response at different wavelengths, the calibration procedure was carried out for the most useful wavelengths of the mercury spectrum, *i.e.*, 254, 265, 281, 303, 313, 365, and 405 mμ.

STABILITY OF THE INSTRUMENT

Short term stability of the instrument has been observed to be excellent with no noticeable drift after a 30-minute warm-up period. Long term stability has been checked at one wavelength (254 mμ) using the potassium ferrioxalate chemical actinometer system described by Parker (8) and later tested by Baxendale (9); the calculated values for quanta per revolution of the integrator rotor were within 1% of the original calculated values.

SUMMARY AND CONCLUSIONS

An instrument for facilitating photochemical decomposition studies has been designed, constructed, and calibrated. The instrument supplies light of narrow bandwidth, which can be varied with respect to wavelength and to some extent with respect to intensity; the instrument provides for monitoring, recording, and integration of the incident and exit beams to and from the reaction mixture; it provides for keeping the reaction mixture at a constant and predetermined temperature and for stirring the mixture. Stoppered reaction cells exclude air when desirable. Short and long term stability of the instrument appear to be good, and response to light of any one wavelength with respect to intensity is practically linear.

Considering that the cost of the parts and materials to build the instrument was minimal, this instrument or a modification of it would be a useful tool for pharmacy laboratories doing photochemical decomposition studies.

REFERENCES

(1) Fastie, W. G., *J. Opt. Soc. Am.*, **42**, 641(1952).
(2) *Ibid.*, p. 647.
(3) Miller, O. E., *ibid.*, p. 661.
(4) Price, J. C., unpublished thesis, University of Rhode Island, Kingston, 1963.
(5) Leighton, W. G., and Forbes, G. S., *J. Am. Chem. Soc.*, **52**, 3139(1930).
(6) Brackett, F. P., Jr., and Forbes, G. S., *ibid.*, **55**, 4459 (1933).
(7) Forbes, G. S., and Heidt, L. F., *ibid.*, **56**, 2363(1934).
(8) Parker, C. A., *Proc. Roy. Soc. (London)*, **A220**, 104 (1953).
(9) Baxendale, J. H., and Bridge, N. K., *J. Phys. Chem.*, **59**, 783(1955).

Use of Models in Determining Chemical Pharmaceutical Stability

By LLOYD KENNON

The area of chemical kinetics as it has been applied to the prediction of the stability of drugs in pharmaceutical formulations has been quite extensively discussed and reviewed during the past few years. In spite of this, a method has been developed which will, it is hoped, be especially useful in pharmaceutical product development work as distinguished from academic research studies in which the goals are to determine reaction rates or orders or to elucidate reaction mechanisms. The present method is based upon the construction of reference reaction paths which result logically when one considers the usual values of pertinent heats of reaction and considers normal shelf-life goals. Two results which have emerged from consideration of these paths are rules of thumb having a sound theoretical basis and a naturally arising logical assay schedule. The schedule arises because of the temperatures chosen for sample storage (RT, 37°, 45°, 60°, and 85°) and would change if another set of storage conditions was employed.

MORE FREQUENT USE of chemical kinetics is now being made in the course of product development work in the pharmaceutical industry. The intrinsic value of such approaches is now considered to be significant—as the many academic and industrial publications in the scientific literature bear witness.

The pharmaceutical literature is replete with examples of stability data which are presented much in the same manner as product development laboratory reports. Many papers present tables which show stability assay results obtained after formulations were stored under the usual accelerated conditions or tables which indicate composition changes induced or prevented when modifications are made in the formulation under study. Although useful, such information must be considered qualitative or semiquantitative when compared to the results of more rigorous studies made since 1950 wherein parameters such as reaction rate constants and heats of activation were determined. It may be noted, however, that concepts such as kinetic studies and

Received May 27, 1963, from Bristol-Myers Products, Hillside, N. J.
Accepted for publication December 2, 1963.
Presented before the A.PH.A. Industrial Section Stability Symposium, A.PH.A., Miami Beach meeting, May 1963.