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Analytical Techniques for Cell Fractions

XVII. The G-IIC Fast Analyzer System[1]

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The basic concept of the fast analyzer has been previously described using simple experimental systems suitable for orienting studies and for proof-of-principle (1–6). Methods for simultaneously moving measured sample and reagent volumes into a series of cuvets in a cuvet rotor, for achieving rapid mixing, and for displaying and recording the data have been devised and applied to simple analyses such as the biuret method for total protein. Further studies concerned with the application of fast analyzer techniques to a variety of clinical and biochemical analyses, including a number of enzyme assays, required the development of an experimental system for routine use which was also adapted for computer reduction of data. The present paper describes the G-IIC GeMSAEC fast analyzer designed to fill this requirement.[3]

THE G-IIC SYSTEM

The G-IIC system consists of a multiple cuvet rotor with associated drive electronics and air/vacuum and wash systems mounted on a movable table (Fig. 1). The operation of the system is similar to that previously described (2), but it has the advantage of being an integrated system adapted to direct computer interfacing.

[1] Research conducted under the joint NIH-AEC Molecular Anatomy (MAN) Program supported by the National Institute of General Medical Sciences, the National Cancer Institute, the National Institute of Allergy and Infectious Diseases, and the U. S. Atomic Energy Commission.

[2] Operated by Union Carbide Corporation Nuclear Division for the U. S. Atomic Energy Commission.

[3] Drawings of the G-IIC fast analyzer are available as CAPE package No. 1865 from the Clearinghouse for Federal Scientific and Technical Information, U. S. Department of Commerce, Springfield, Virginia 22151.

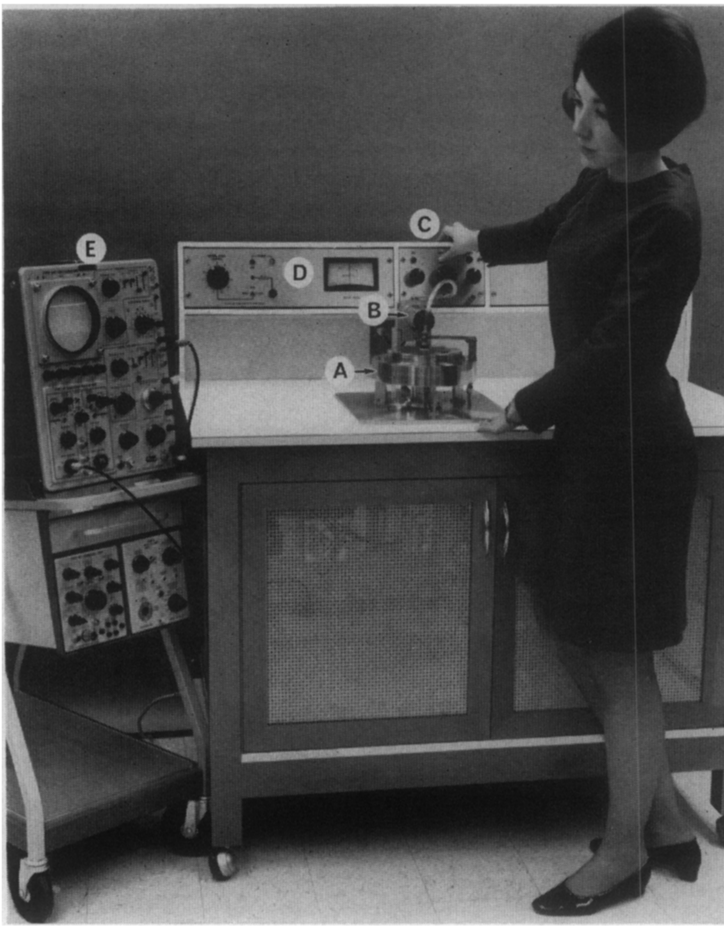

FIG. 1. Complete G-IIC fast analyzer system: (A) rotor with peripheral drainage ring, (B) photomultiplier housing, (C) air, vacuum, and water outlets, (D) control panel, (E) oscilloscope.

Rotor. The G-IIC rotor is similar to the G-IIB rotor previously described (2) with the following modifications.

The cuvets are formed by compressing a Teflon[4] annulus containing 15 slots between a glass disc and a glass annulus.[5] The modified Teflon annulus is constructed of black carbon filled Teflon,[6] as shown in Figure 2.

[4] Du Pont registered trademark. Mention of commercial products in this paper is meant to be informative rather than restrictive. Other products of similar characteristics may work equally well.

[5] Available from Esco Products, Oak Ridge, New Jersey.

[6] Available from John Dore Company, Houston, Texas, as Fluoroblack E-130, ½ in. thick, stress relieved.

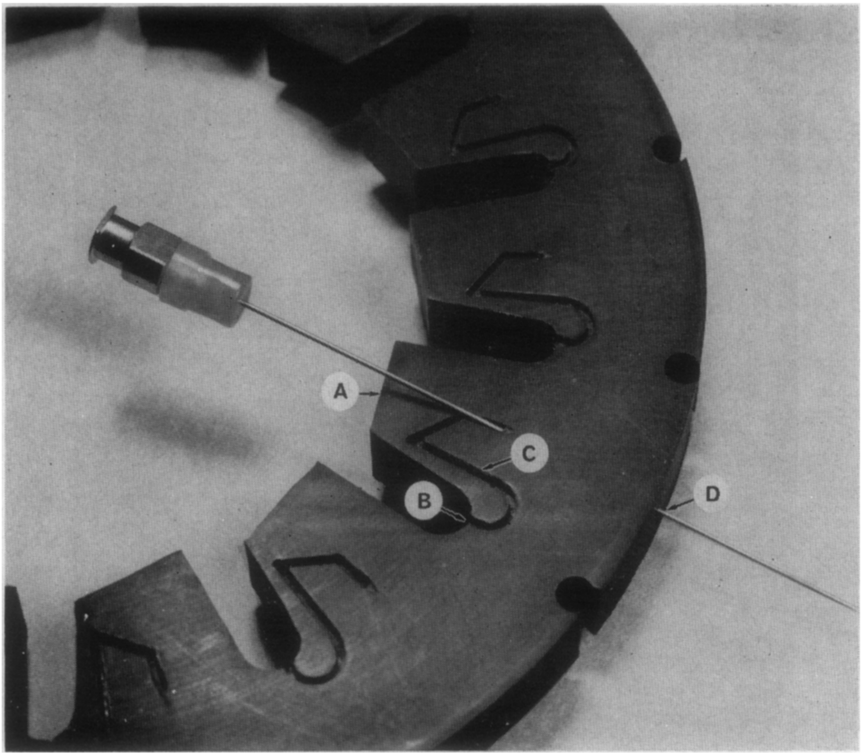

FIG. 2. Teflon annulus used to define the cuvets: (A) tapered inner edge to drain liquid into the cuvets, (B) sloping, rounded bottom of slots defining the cuvets—slope at outer edge drains all fluids into the syphon, (C) syphon groove, (D) syphon drainage hole.

The cross-section of the syphon groove has been increased to 3/64 in. × 3/64 in., while the drain line drilled through the Teflon is 3/64 in. in diameter.

Before machining, the Teflon was stabilized by heating to 525°F for 2 hr, followed by cooling to 300°F at the rate of 25°F/hr, after which the sheets were cooled to room temperature. When this pretreatment was omitted, subsequent shrinkage was noted.

An exploded view of the complete rotor is shown in Figure 3. The end windows are of 1/2 in. thick Pyrex, the end plates are of 316 stainless steel, while the remaining parts are of Teflon.

The rotor spins within a hollow stainless-steel drain ring which serves both as a rotor shield and as a collecting ring to collect liquid ejected from the rotor through the syphons. The liquid flows from the ring through a tube connecting to a waste bottle in the cabinet. The assembled rotor, light source, and photomultiplier are shown in Figure 4. A plastic cover

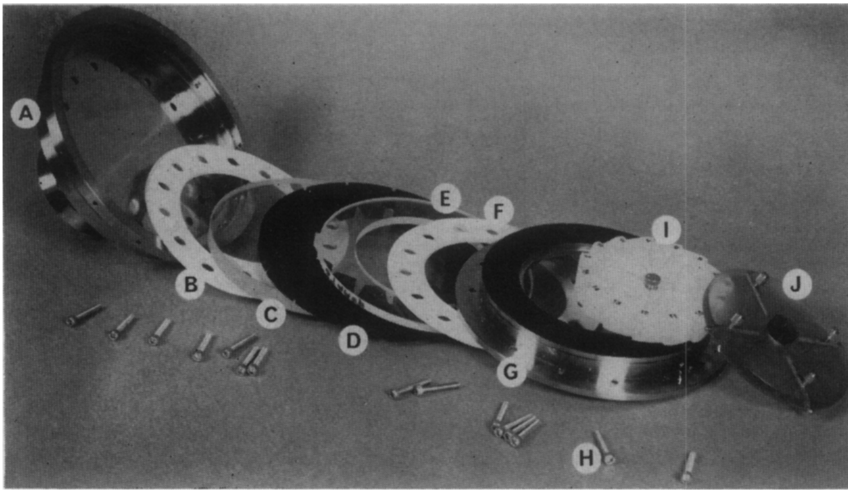

FIG. 3. Exploded view of complete G-IIC rotor: (A) lower supporting rotor disc, (B) lower Teflon gasket, (C) lower Pyrex window disc, (D) center cuvet annulus of black Teflon, (E) upper Pyrex window annulus, (F) upper Teflon gasket, (G) rotor cover, (H) rotor assembly screws, (I) transfer disc (Teflon), (J) cover with Rulon center seal. (Courtesy American Association for the advancement of Science; from N. G. Anderson, *Science* **166,** 317–324 (1969)).

attached to the rotor upper plate holds at its center a Rulon[7] rotating seal that matches a stainless-steel static seal attached to the air and vacuum systems.

A stainless-steel flange is attached to the lower rotor shaft to prevent drainage of fluid into the drive motor. The rotor is driven by a 1/4 hp, 1725 rpm motor[8] using a silicon autotransformer for controlling speed. A three-position switch, in addition to the autotransformer control, was provided with settings marked *stop, run,* and *accelerate.* The braking time from 1950 rpm to zero was 8 sec, while 500 rpm was reached from the same speed in 3 sec, and 1600 rpm in 0.8 sec. For fast acceleration, a special acceleration circuit was provided that accelerated the rotor from rest to 1000 rpm in 1.0 sec, and to 1600 rpm from rest in 2.5 sec. All measurements were made with a Teflon transfer disc in place.

There is danger of lateral splashing of liquid in the transfer disc if the initial acceleration is too rapid. In practice, therefore, the rotor is gradually accelerated to 300 rpm manually, and then rapidly accelerated past

[7] Rulon available from Dixon Corporation, Bristol, Rhode Island.

[8] Bodine ¼ hp, 1725 rpm, 115 V DC motor with SA25 silicon-controlled rectifier-autotransformer type of speed control. Stock No. B2422ZCXX; X1-flange mount, X2-dual shaft. Available from B & B Motor and Control Corp., New York, New York.

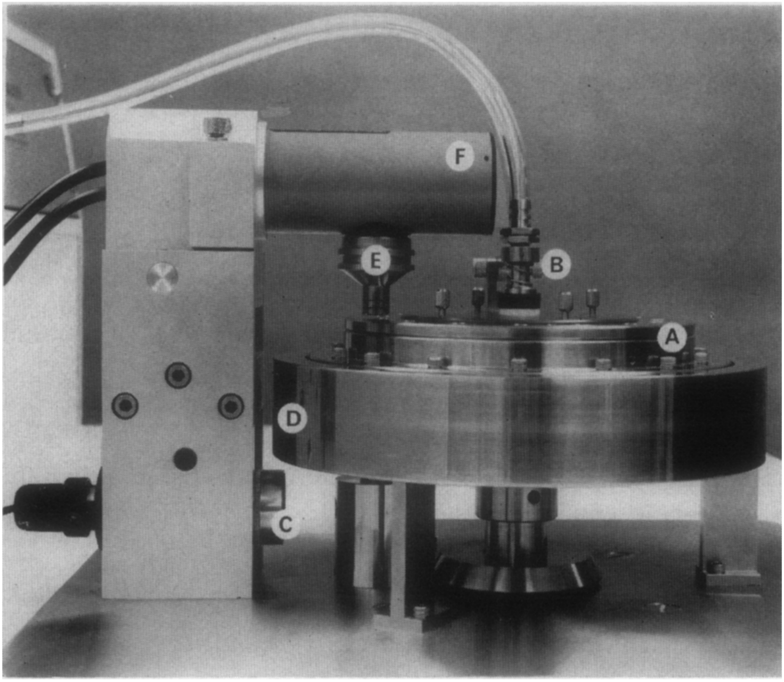

FIG. 4. G-IIC rotor and optical system completely assembled: (A) rotor, (B) static seal and air line, (C) light source, (D) collecting drain ring, (E) filter mount, (F) photomultiplier housing.

1000 rpm using the acceleration switch setting. From 300 rpm, 1000 rpm is reached in 0.9 sec. Liquid began to flow from the transfer disc at 350 rpm, and was essentially quantitatively transferred by 1000 rpm (2).

Light Sources. A commercial microscope light source[9] with a diaphragm to adjust light intensity was found suitable for work in the near-ultraviolet and visible range. A DC power supply was used to ensure stable operation.[10] One instrument was constructed with a high-intensity monochromator suitable for use down to 340 nm.[11]

Photomultiplier. A type 1P28 or R136 photomultiplier[12] mounted in a suitable housing[13] was used with a filter holder mounted in front of the

[9] Microscope illuminator without transformer power supply, Supply Cat. No. 653 A. O., American Optical Company.

[10] 0–7 V DC modular power supply capable of 4 A output at 40°C, model LM225, available from Lambda Electronics Corp., Melville, Long Island, New York.

[11] Monochromator No. 33-86-02 obtained from Bausch & Lomb, Rochester, New York.

[12] Available from Radio Corporation of America, Camden, New Jersey.

[13] Photomultiplier housing D-500 for 1P28 tube, equipped with dynode resistor chain and cables, from Schoeffel Instrument Company, 15 Douglas Street, Westwood, New Jersey.

sensitive surface. Two power supplies have been used for the photomultiplier.[14] The light intensity was adjusted so that the voltage output from the amplifier, with the cuvets filled with water, was approximately 3 V.

Synchronization Signals. For control of the oscilloscope display and for computer input, a number of different signals are required. It is necessary to provide a horizontal sweep-ramp signal which returns to zero between cuvets 15 and 1, and reaches the same voltage regardless of speed. This ensures that the cuvet signals will always be displayed in the proper order and that the observed pattern will have the same width regardless of rotor speed. The circuit used requires two input signals from the rotor. The first is a voltage proportional to rotor speed and is provided by a DC tachometer generator[15] coupled to the lower end of the motor shaft. The second is a pulse occurring between cuvets 15 and 1 that controls the return sweep of the oscilloscope. Both signals are fed into the circuit shown in Figure 5. The resulting oscilloscope display is shown in Figure 6, together with plots of the synchronizing signals.

The tachometer produces a voltage proportional to shaft speed. This voltage is converted into a charge-independent current to the capacitor by the action of the resistor and operational amplifier. The voltage across the capacitor is equal to the time integral of the charging current. Since the photodiode signal to the silicon-controlled rectifier discharges (resets to zero) the capacitor once per revolution, the voltage across the capacitor is proportional to the angular position of the rotor.

The synchronizing pulses are generated by a radially slotted disc attached to the lower end of the motor shaft which rotates between three stationary photodiodes[16] and three miniature lamps.[17] Signals are generated when the slots allow light to reach the photodiodes.

Fifteen slots on the periphery of the disc expose the outer photodiode to correspond in time with the passage of the 15 cuvets through the analytical light beam. One slot is elongated and exposes two inner photodiodes once per revolution between cuvets 15 and 1. The light sources and photodiodes are adjustably mounted so that the sync signals may be precisely set. The signals from the outer and one inner photodiodes are supplied

[14] Model K-15 photomultiplier power supply, output 1500 V max at 1 mA with input of 15 V, available from Venus Scientific, Inc., 25 Bloomingdale Rd., Hicksville, New York, and model 651A, output 1600 V max at 5 mA, available from Hewlett-Packard, 1101 Embarcadero Road, Palo Alto, California 94303.

[15] Tachometer generator, 7 V/1000 rpm, SA-757A-2, available from Servo-Tek Products Company, 1086 Goffe Road, Hawthorne, New Jersey.

[16] No. 2N2175, available from Texas Instrument Company, Box 66027, Houston, Texas, 77006.

[17] 5 V lamp. TL1, CM-8-1415, Chicago Miniature Lamp Works, 4433 Ravenswood Ave., Chicago Ill., 60640.

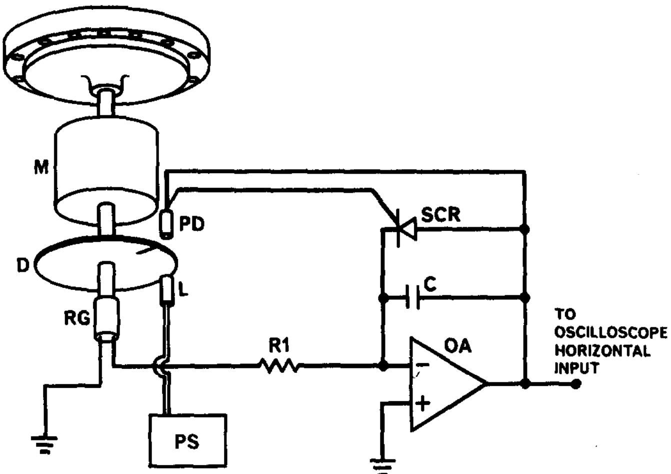

FIG. 5. Horizontal sweep generator for oscilloscope monitor. A slotted disc (D) is spun with the GeMSAEC rotor (R) by a motor (M). The photodiode (PD) is illuminated by a small lamp (L) as the slot passes and provides one pulse per revolution to a silicon-control rectifier (SCR). A rate generator (RG) tachometer attached to the rotor shaft provides a voltage proportional to rotor speed to an operational amplifier (OA) which charges a capacitor (C) to provide a linear ramp voltage. The capacitor is discharged each time the photodiode provides a current to the silicon-control rectifier.

to the computer interface; the remaining signal is provided to the sweep-signal generator.

Air, Vacuum, and Water System. The cuvets may be emptied with air pressure applied to the center of the rotor during rotation, while rapid mixing is effected by applying a vacuum to the same line (2). Air and vacuum lines are provided with regulators and solenoid valves controlled from the console. Water for washing the rotors is supplied at 0.5 psi (adjustable) from a Pyrex reservoir in the cabinet, with the air pressure set at 13.5 psi to the control valve on the console.

Transfer Disc. The 15-place transfer disc employed with the G-IIC rotor is shown in Figure 7. The modifications include matching the peripheral surfaces to the cuvet annulus and provision for washing during rotation. Channels are provided to the rotor so that water introduced at the center flows out to all cavities in the disc and on to the rotor cuvets. The discs have been fabricated from Teflon, polyethylene, and polypropylene with equally satisfactory performance in the tests run thus far, except for the usual differences in machinability of these plastics.

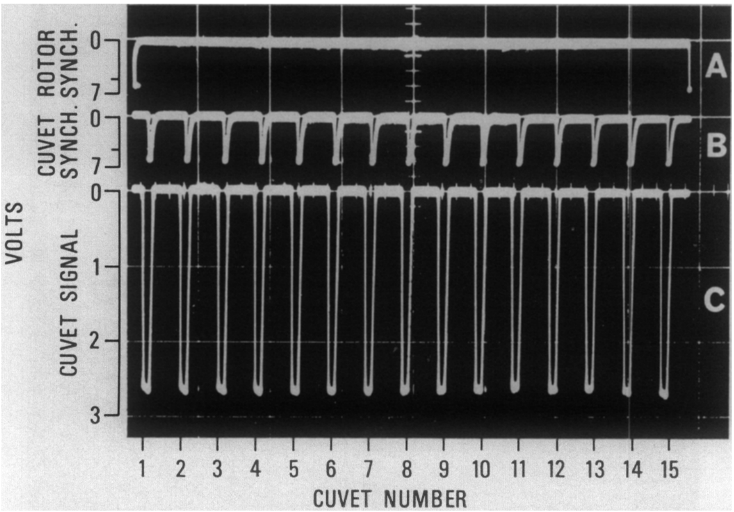

FIG. 6. Oscilloscope display of signals provided from GeMSAEC G-IIC system. Rotor synchronizer pulse (upper trace) is provided for computer interfacing. A similarly timed pulse (not shown) is also provided to reset the sweep generator. The cuvet synchronizer pulses (middle trace) are set to provide a signal starting at the beginning of the last third of the plateau of the cuvet signal, and is used for computer interfacing. The cuvet signal (lower trace) is obtained from the photomultiplier and contains in each trace information on the dark current, the blank readings, standards, and experimentals, as previously described. In the trace shown, all cuvets were empty.

PHOTOGRAPHIC DATA REDUCTION

The cathode ray tube (CRT) display[18] is photographed using Royal Pan film and an exposure of 1/5 sec at *f*8, and developed for 5 min in D-11 at 70°F. The beam intensity is reduced and carefully focused to give a fine trace which, when photographed and developed to high contrast, gave patterns with very sharp edges. The negative is projected on a Benson-Lehner OSCAR[19] and digitalized, and the data are recorded automatically on punched cards. The complete system is shown in Figure 8. The lower edge of the tracing is therefore used for data reduction, avoiding the problem of determining the location of the center of the trace lines. For each data point, values for both the X and Y coordinates are recorded.

The data required for absorbance determination include a value for the dark current (zero per cent transmission or infinite absorbance), a value

[18] Tektronic oscilloscope, model 505, equipped with camera Model C-27, available from Tektronix, Inc., Portland, Oregon.

[19] Model F OSCAR, oscillogram, and strip-chart record recorder with decimal converter made by Benson-Lehner Corp., 1860 Franklin St., Santa Monica, California.

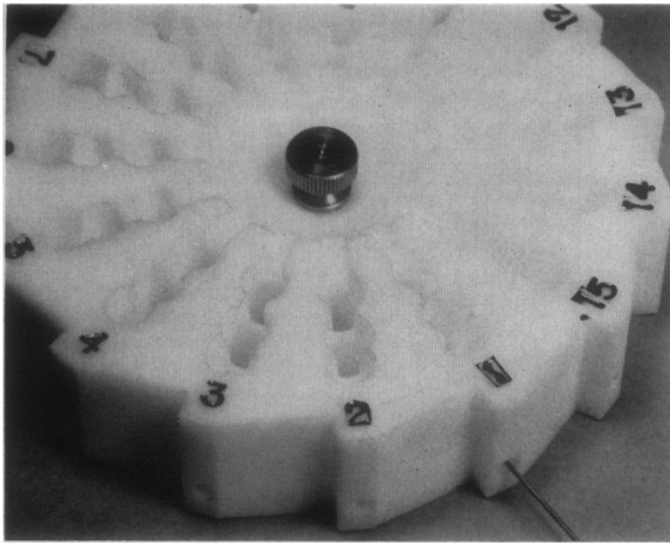

FIG. 7. 15-place transfer disc employed in G-IIC rotor. Inner and middle row of holes slope outward 15° from vertical and drain during rotation into the peripheral row of holes which slant inward 15°. Each peripheral hole collects fluid from two inner holes and then drains outwardly through small holes which slant upward 20° from horizontal in a radial direction, thus transferring all fluid simultaneously to the cuvets by centrifugal force.

for the water blank, and all experimental values. These may all be obtained from each negative as follows:

The negative cannot be inserted in the projector with high precision; consequently, the dark current tracing (the apparent baseline along the top of the tracing) may slope very slightly or may conceivably curve. The position of the tracing to the right of each cuvet is therefore recorded to give a series of 15 values for the dark current.

The coordinates of the peaks are then recorded. All values in both coordinates range from −999 to +999. The computer program used to reduce this data accordingly adds 1000 to all values so that all are positive. A subroutine is included to determine that the spacings of the dark current values along the X coordinate are approximately equal and to print an error signal if values are too close, indicating that one position has been read twice. An equation for the zero per cent transmittance line is then generated and used to provide a series of dark current values having X coordinate positions equal to those of the peaks. Peak heights are then determined by subtraction. If the water blank peak heights are less than some of the heights observed for experimental values, negative absorbances will be recorded. To avoid this, the water blank in the first cuvet is assumed to transmit actually only 95% of the light (i.e., to have an

FIG. 8. Data reduction system for use with photographic negatives. Negatives projector (OSCAR) shown in center. Positions of manually positioned cross hairs are digitalized by analog-to-digital converter (left) and automatically recorded on punched cards (right).

absorbance of 0.0223) and is therefore set equal to this value by multiplying it by C where

$$C = \frac{1}{0.95} = 1.0526$$

The absorbance of each remaining peak is then determined by

$$A = \log \frac{H_1 \times C}{H_n}$$

where H_1 is the height of the water blank (distance from the zero per cent transmission upper baseline), and H_n is the height of any other peak similarly measured. The results are then suitably tabulated.

Direct Computer Data Reduction. Output connectors for the photomultiplier signal, start, and cuvet pulses are provided on the side of the console for direct input into a small computer, as is described in a subsequent paper (8).

EXPERIMENTAL EVALUATION

The G-IIC fast analyzer was designed for photometric analysis in the range from approximately 340 to 700 nm. The glass windows used limit the lower range, as is shown in Figure 9, where per cent transmission of a 1/2 in. thick sample of Pyrex is plotted against wavelength. From these data the absorbance of two such windows would be 0.228 at 340 nm—an acceptable value. The transmission of the 340 nm filter[20] used is also shown in Figure 9. Sufficient light is available to yield acceptable oscilloscope patterns at this wavelength. Filters through the visible range have also been employed with no difficulties. Care must be taken, however, to adjust either the light source intensity or the photomultiplier voltage to ensure that the photomultiplier is not saturated.

Evaluation of the System. The evaluation of the system is done in stages to determine the magnitude and sources of error. A potassium chromate stock solution having twice the concentration of the chromate and KOH used as an absorbance standard (7) was used. Substandards containing 25, 50, 75, and 100% of the stock concentration were introduced into the rotor and the oscilloscope pattern was photographed, as previously described. Six exposures made from the same pattern were read and the results, together with the standard errors, are givn in Table 1. The average of all the standard errors was 0.0039 absorbance unit. As expected, the

[20] First order 340 ± 3 nm peak wavelength, 5% half-peak bandwidth, blocked to x-ray on low side and to far-infrared on high side. Peak transmission at least 25%, 1 in. in diameter, supplied by Baird Atomic, Inc., 33 University Road, Cambridge, Massachusetts 02138.

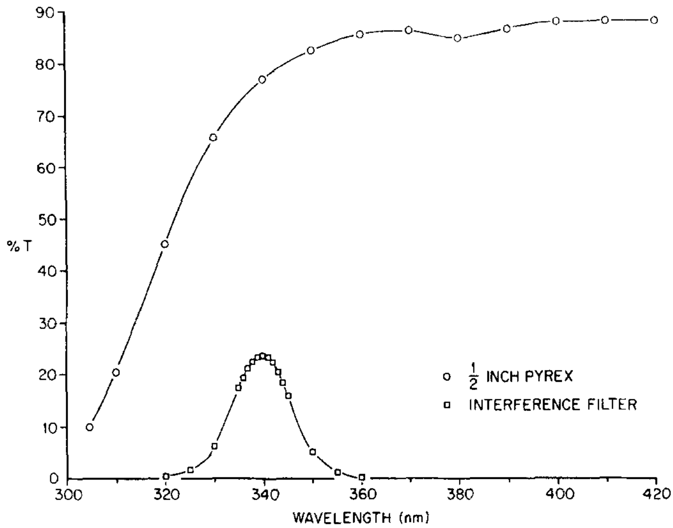

FIG. 9. Optical characteristics of ½ in. Pyrex window used for GeMSAEC rotor (upper curve) and of interference filter used for NADH measurements (lower curve).

TABLE 1
Error in Reading a Series of Negatives of One Experiment Compared with Errors Arising from Repeated Reading of One Negative

Cuvet No.	Av. 6 different negatives of same expt.	σ_1 ±	Av. 7 readings of same negative	σ_2 ±	$(\sigma_2/\sigma_1) \times 100$
2	0.2371	0.0013	0.2473	0.0008	62
3	0.4575	0.0027	0.4664	0.0023	85
4	0.6674	0.0122	0.6728	0.0055	45
5	0.8330	0.0070	0.8925	0.0069	99
6	0.0036	0.0019	0.0064	0.0009	47
7	0.2279	0.0021	0.2431	0.0016	76
8	0.4502	0.0028	0.4689	0.0087	311
9	0.6535	0.0020	0.6686	0.0035	175
10	0.8325	0.0067	0.8627	0.0046	69
11	0.0141	0.0015	0.0086	0.0005	33
12	0.2419	0.0029	0.2379	0.0009	31
13	0.4540	0.0034	0.4569	0.0018	53
14	0.6624	0.0040	0.6645	0.0010	25
15	0.8343	0.0045	0.8530	0.0039	87
		Av. 0.0039		*Av.* 0.0031	*Av.* 85%

errors increased as absorbance increased and averaged ±0.0061 at the highest absorbance readings with the concentrated stock solution. The errors observed are the sum of all occurring in the system. To determine the fraction of error due to reading of the negatives (as distinguished from variations in the patterns displayed on the oscilloscope), one negative was read five times, with the negative being removed from the projector and reinserted between readings. The results are shown in Table 1. Comparison of the two sets of results suggests that approximately 85% of the variation occurred in the reading and digitalization process.

The problem of linearity in the over-all system is important, especially since the photomultiplier is easily saturated at many wavelengths. Fortunately, the pattern observed on the oscilloscope is on a calibrated scale, and the signal voltage may be easily kept near 3 V for the water blanks. Linearity was checked by comparing the curves obtained with the potassium chromate stock and three dilutions of it in the G-IIC cuvet rotor and in a standard spectrophotometer. The results are shown in Figure 10. At higher concentrations, the solution does not obey Beer's law exactly, yet the same deviations were noted with the G-IIC system as with conventional spectrophotometers. However, at the same wavelength a higher absorbance was always observed with the cuvet rotor. The path length of the rotor cuvets was carefully measured and found to be 0.9953 cm, $\sigma = 0.0037$. This error, which is barely significant, is in the wrong direc-

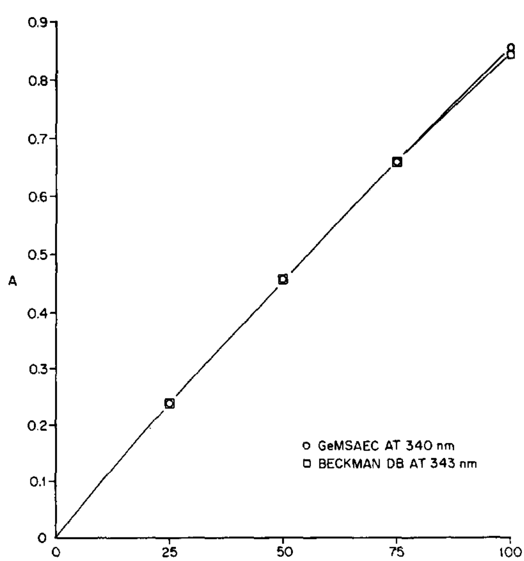

FIG. 10. Comparison of results obtained with potassium chromate standards using GeMSAEC at 340 nm and Beckman DB spectrophotometer at 343 nm.

tion and does not account for the difference. The explanation appears to be as follows:

The amount of light incident on the filter as a function of wavelength decreases very rapidly with decreasing wavelength because the tungsten lamp emission intensity declines and because the absorbance of the thick cuvet window increases. As a result, the wavelength of the actual intensity maximum passing the filter may be shifted toward a longer wavelength as observed. With potassium chromate solutions, 340 nm is in a steep portion of the curve. A slight shift in the absorbance maximum toward a higher wavelength therefore produces a higher absorbance, as observed.

The 340 nm filter was used in these studies since it represents the lower limit of usefulness with the present system. The chromate standards used in this region, while very useful for developing data reduction systems, are inferior to nicotine-adenine dinucleotide, which would actually be used. As a final test of the system, a series of NADH solutions were prepared and the absorbance values measured in the GeMSAEC system and in a Beckman DB spectrophotometer. The results agreed within 1.1%.

At higher wavelengths (up to 700 nm), much less noise was observed in the tracings and no problems were encountered in obtaining sufficient sensitivity.

DISCUSSION

The GeMSAEC G-IIC multiple-cuvet rotor system allows the absorbance of the contents of 15 cuvets to be observed in real time, the display pattern photographed, and the data transcribed from the negative to punched cards and reduced by a computer. The over-all precision with standard solutions is bettter than 1%, but would be larger with multisolution analyses because of the pipetting error. The system is suitable for a variety of colorimetric analyses but is most readily adapted to those in which the rate of change of absorbance is measured.

The principle errors observed in the present system arise during reading of the photographs; hence, further improvements appear to depend upon the development of purely electronic methods of data reduction, as are described in a subsequent paper.

The rapid acceleration rate achieved with the G-IIC system allows all reactions to be initiated during a much shorter time interval and makes possible determination of reaction rates early in the course of the reaction. Additional studies are required to determine the minimum time intervals for complete transfer, mixing, and determination of reaction rates, and will necessitate construction of additional special equipment.

It is evident that the system described may be fabricated as a more compact, portable system using direct reading of Polaroid prints for use in small clinical laboratories.

The problems associated with real time data reduction with this system are discussed in a subsequent paper.

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