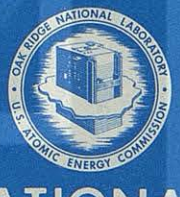
ORNL-TM-4434

GRAVITY ZERO (GØ) ANALYTICAL CLINICAL LABORATORY SYSTEM SEMIANNUAL PROGRESS REPORT FOR THE PERIOD JUNE 1, 1973, TO NOVEMBER 30, 1973



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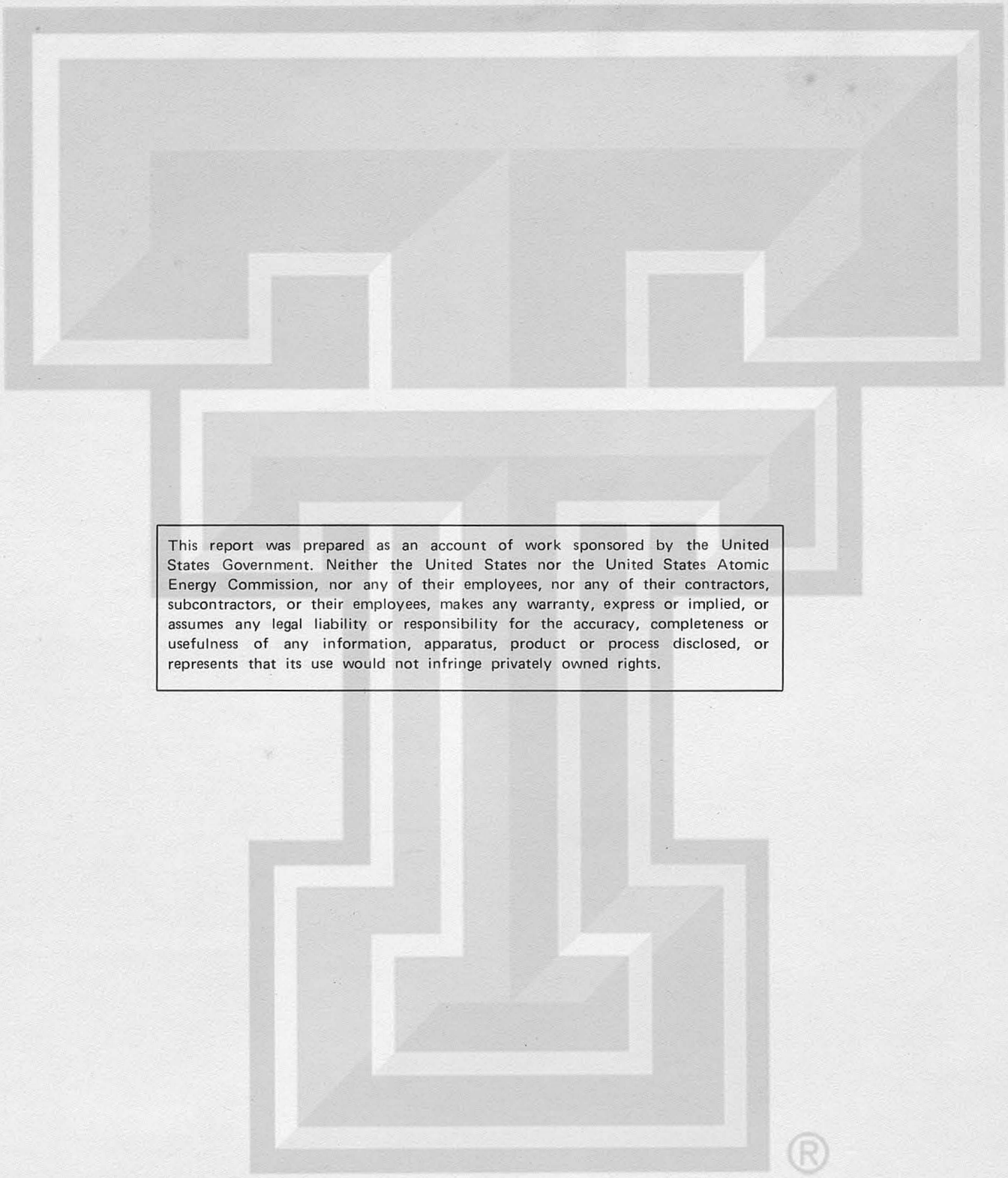

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GRAVITY ZERO (GØ) ANALYTICAL CLINICAL LABORATORY SYSTEM SEMIANNUAL PROGRESS REPORT FOR THE PERIOD JUNE 1, 1973, TO NOVEMBER 30, 1973*

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JANUARY 1974

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GRAVITY ZERO (GØ) ANALYTICAL CLINICAL LABORATORY SYSTEM SEMIANNUAL PROGRESS REPORT FOR THE PERIOD JUNE 1, 1973, TO NOVEMBER 30, 1973

SUMMARY

During this report period, a miniature analytical system was evaluated on-site at the Johnson Space Center, Houston, Texas. The system has operated and performed satisfactorily. Three minor equipment malfunctions were corrected by ORNL and NASA personnel. The technique of dynamic loading of liquids has been extensively studied, and factors affecting it have been identified and optimized. Equivalent results have been obtained using either discrete or dynamic loading of reagents. Work sponsored by other agencies which is of interest to NASA includes (1) development of fluorescence and light-scattering monitors, (2) development of a portable data processor, (3) development of a portable fast analyzer, (4) development of a uric acid procedure, and (5) evaluation of a miniature analytical system in a routine clinical laboratory.

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1. INTRODUCTION

The successful development of centrifugal Fast Analyzers for use in the clinical laboratory and other areas has led to consideration of the same principle for a miniature clinical analyzer that would be capable of operating in the zero gravity conditions of space flight as well as in ground-based operation. The unique centrifugal mode of sample and reagent handling, coupled with rapid automated processing of the data, makes this analytical approach particularly adaptable for space-flight applications. Further, the possibilities of miniaturizing the analyzer and using extremely small quantities of sample and prepackaged reagents are compatible with the weight and size limitations for space-flight application. In addition, these features are very attractive for ground-based operation, especially in the small clinical laboratory, the emergency laboratory, the pediatric laboratory, and medical research laboratories.

Previous studies have indicated that a miniature analyzer based on the GeMSAEC[1-4] concept is feasible, and that it is possible to design and fabricate operable, miniaturized systems that will be useful not only in space-flight applications but also in ground-based laboratories. A recent objective was to design and fabricate a miniature analytical system for evaluation as a ground-based clinical system. A complete system includes the miniaturized analyzer, several plastic rotors, a portable data printer, an automated sample and reagent loader, and a rotor washing station, as well as several assay protocols. Such a system has been fabricated and was delivered to the clinical laboratory of the NASA Johnson Space Center, Houston, Texas, on February 1, 1973.

In this report period the system was evaluated by NASA personnel with backup support supplied by the staff of the Oak Ridge National Laboratory. In addition, the technique of dynamic introduction of liquids into a spinning rotor was thoroughly studied, and the various factors affecting it were identified and optimized.

Parallel efforts, which are funded by agencies other than NASA, include the development of (1) a light-scattering and fluorescence detector for use with the miniature analyzer, (2) a digital data system, (3) a portable Fast Analyzer, and (4) a uric acid procedure. A comparison study of the performance of a 15-place GeMSAEC Fast Analyzer vs a miniature Fast Analyzer was also conducted in the Clinical Laboratory of the Health Division of the Oak Ridge National Laboratory.

2. SYSTEM EVALUATION AT THE JOHNSON SPACE CENTER

The miniature fast analytical system that was developed and fabricated for NASA evaluation was delivered and installed at the Clinical Laboratory of the NASA Johnson Space Center, Houston, Texas, on February 1, 1973. Prior to this delivery date Mr. Larry Wallace of Northrup spent a week at ORNL for training and familiarization.

As shown in Fig. 1, the NASA system consists of a miniature Fast Analyzer, several rotors, an automated sample-reagent loader, a data system, and a rotor cleaning station. The heart of the analytical system is an improved model of earlier miniature Fast Analyzer prototypes. As

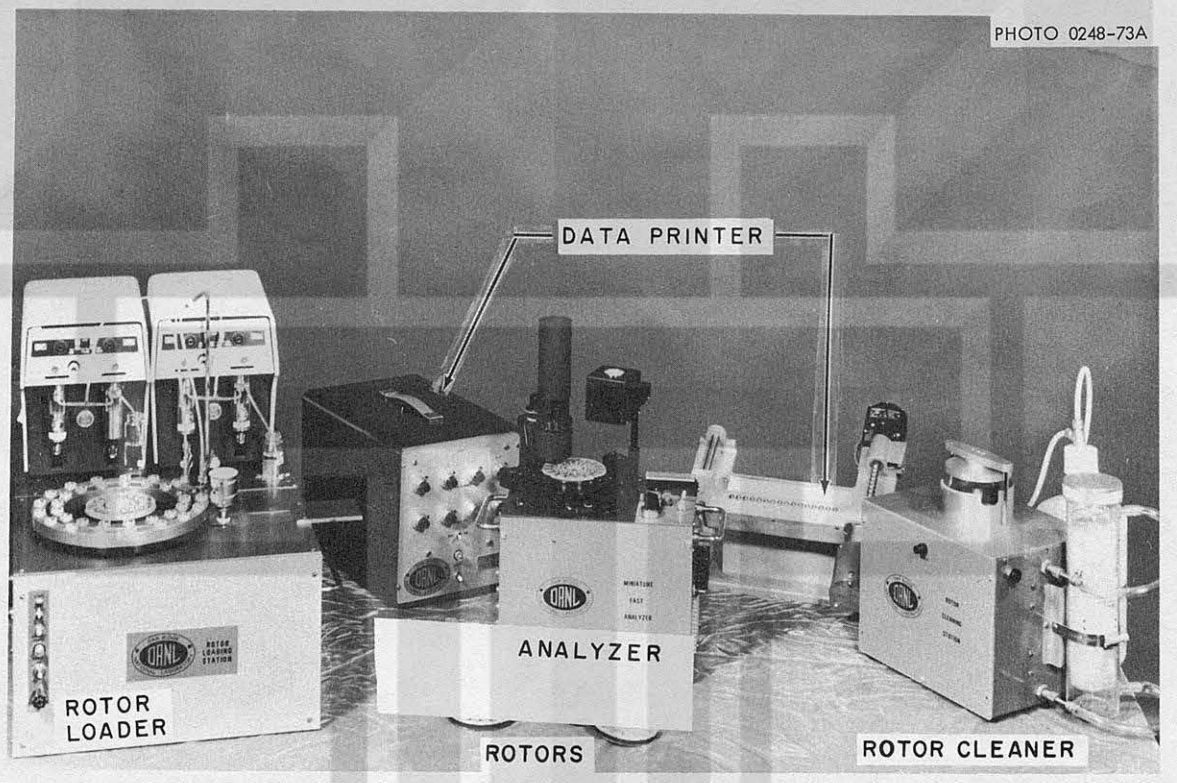

Fig. 1. The Miniature Fast Analytical System Currently Being Evaluated in the Clinical Laboratory of the NASA Johnson Space Center, Houston, Texas.

with the earlier prototypes, this model is compact (it occupies only a square foot of bench space) and weighs only 30 lb. Its primary function is to spin a 17-cuvet rotor at speeds up to 5000 rpm (normal operating speed is 1000 rpm) through a stationary photometer which consists of a quartz-iodine-tungsten light source located in a movable housing mounted above the rotor, interference filters, and a miniature photomultiplier (PM) tube. The six interference filters (340, 400, 415, 485, 550, and 620 nm, Ditric Company, Marlboro, Mass.) are contained in a movable filter wheel which is mounted just under the rotor housing and are positioned manually in the optical light path by means of the filter selector switch. As with all fast analyzers, the basic raw data generated by spinning cuvets through the photometer consist of a synchronized series of voltage signals which can be displayed by an oscilloscope and acquired and processed by means of either an on-line computer or other data systems.

2.1 Operation and Performance

The analytical system and several chemical procedures have been evaluated by Mr. Wallace in the Clinical Laboratory of the Johnson Space Center, including: (1) instrumental checkout of the system and its individual components, (2) determination of optical linearity at 340 nm and other wavelengths using standard solutions of NADH and Dextran Blue, and (3) systematic testing of all chemical procedures using commercial control sera. From this evaluation a report entitled, "The Evaluation of the GØ Miniature Analytical System," was prepared by Mr. Larry Wallace and Dr. Carter Alexander of NASA, and it should be available from NASA. In general the system performed as expected, and analytical results from it were comparable to those from reference methods.

2.2 Reliability

The system has been operating for 9 months, during which downtime has been minimal. A few problems have been encountered with the miniature system. The socket containing a photometer transistor cracked due to faulty fabrication of an associated heat-sink, which led to shorting of the transistor and a burn-out of the photometer lamp. In consultation

with the ORNL staff, NASA personnel replaced the heat-sink, transistor and socket, and optical lamp. This problem has not recurred. A second problem was a noisy drive motor which would not accelerate properly; it was replaced on-site by ORNL personnel, and no further problems were encountered. In the data printer, a zener diode burned out in the electronic module during one of the evaluation experiments, possibly due to the analyzer's being improperly connected to the data printer. The data printer was repaired at Oak Ridge and returned to the Johnson Space Center; it has since been operating satisfactorily. No problems have been encountered with the sample-reagent loader, the rotor cleaning station, or with the rotors.

3. DYNAMIC INTRODUCTION OF LIQUIDS

The primary objective during this report period has been the development and optimization of the technique of dynamic loading of liquids into the rotor of the miniature Fast Analyzer.

3.1 Introduction

Liquids may be introduced into the miniature Fast Analyzer in either a discrete or dynamic mode. In the discrete mode, aliquots of liquids are obtained by means of a pipetting device and transferred and dispensed into their respective cavities in a stationary rotor. A device (Fig. 1) to automatically load a rotor in the discrete mode has been developed and previously discussed.[5] The main disadvantages of discrete loading are the relatively high cost of instrumentation to automate it and the decreased portability of such a system. To circumvent these two disadvantages, the technique of dynamic loading of liquids into a spinning rotor is being developed. Basically the technique consists in dynamically introducing a volume of liquid (reagent or sample) into the centrifugal field generated by the spinning rotor of the Fast Analyzer (Fig. 2). The rotor can be designed to apportion the volume of liquid into discrete measured volumes that are automatically transferred into their respective cuvets for reaction initiation followed by photometric monitoring of the individual reactions.

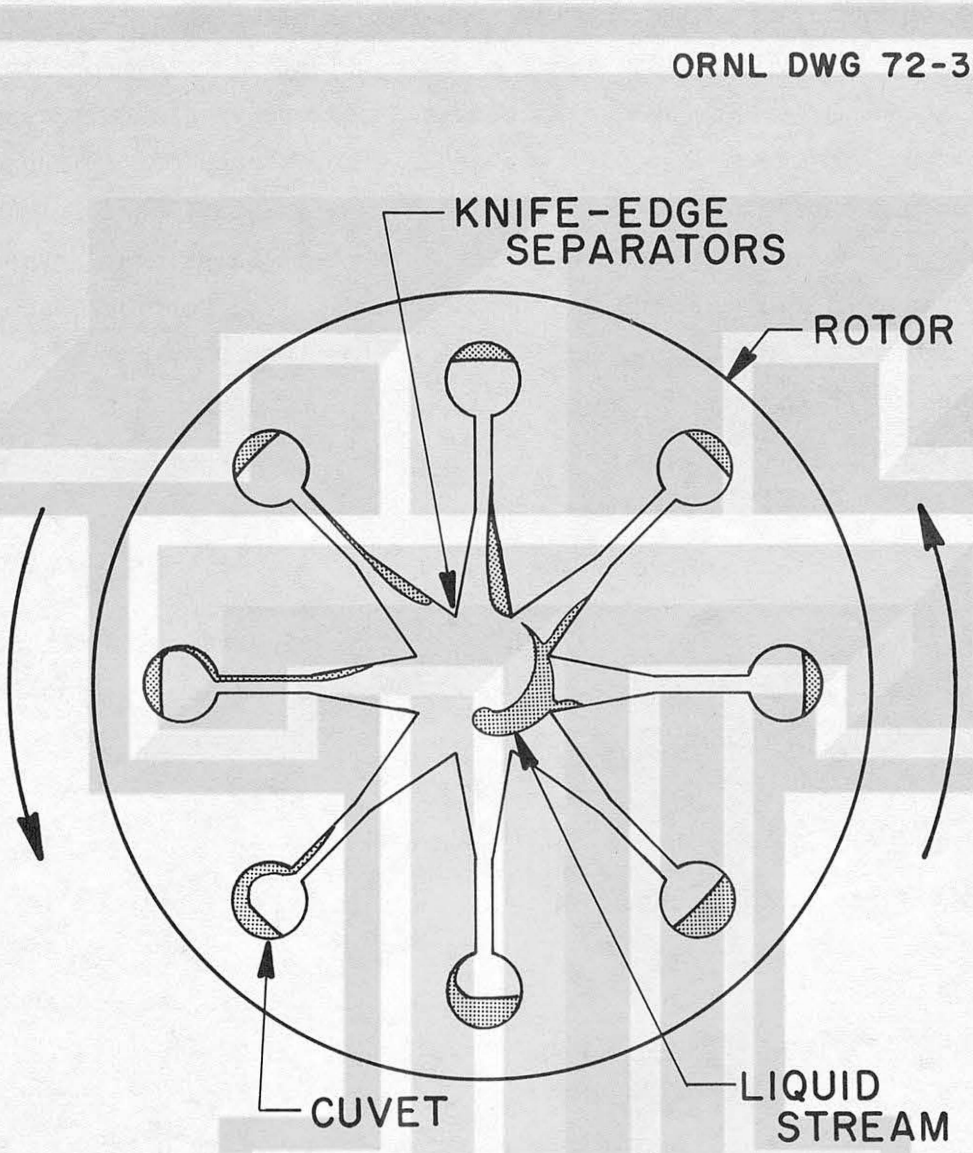

Fig. 2. Dynamic Apportionment of a Liquid Stream in a Centrifugal Field by the Rotor of a Fast Analyzer.

Initial studies[6] indicated the feasibility of using dynamic loading with a miniature Fast Analyzer. In the interim we have developed a dye dilution technique for evaluating the precision and accuracy of dynamic loading and have identified and investigated several parameters that influence its operation.

3.2 The Measurement of Dynamically Loaded Volumes

To measure the volume per cuvet obtained by dynamically loading a known volume of liquid into a spinning rotor, a dye dilution technique was developed in which precise and accurate aliquots of a Blue Dextran dye solution of known absorbance are discretely dispensed into cuvets 2-17 of a rotor. (Note: Cuvet 1 serves as a reference cuvet and is filled with water.) These aliquots are then transferred into their respective cuvets, a known volume of water is dynamically introduced into the spinning rotor, and, after mixing, the absorbances of the diluted dye solutions are measured. The volume dynamically introduced into each cuvet can be calculated using the following equation:

$$V_2 = \frac{V_1 (A_1 - A_2)}{A_2} , \qquad (1)$$

where

V_2 = volume of dynamically loaded liquid,
V_1 = volume of discretely loaded liquid,
A_2 = absorbance of diluted dye solution,
A_1 = absorbance of undiluted dye solution.

This equation has been incorporated into a newly developed program for use with an on-line computer which automatically calculates the dynamically loaded volume for each cuvet. To evaluate the splitting performance of a rotor, a statistical analysis is performed on the calculated volumes and absorbances of the diluted dye solution in cuvets 2-17. A correction for cuvet-to-cuvet variability inherent in the rotor is also made. To evaluate run-to-run variability within a cuvet, the data from several runs can be stored for subsequent recall and statistical processing. A typical computer output from this program is shown in Fig. 3.

```
DAY 310  TIME 10 : 31 : 35
PROCEDURE:DYNAMIC LOADING OF 340UL H2O AT 3000 RPM.
OPERATOR:JBO

ROTOR NUMBER  L-:8

GEMSAEC UNIT:17.2
FILTER:620NM
DISCRETE LOADED VOLUME (UL)=:100

ABS. OF DYE STANDARD=:.404893
SIGMA OF ABS. STANDARD=:.000870

DELAY INTERVAL--SEC:5
OBS. INTERVAL--SEC:5
NUM OF SETS OF OBS.:10
READINGS PER CUVET:20
RUN NUMBER:7
READY!
```

ROTOR L- 8

CUVET	MEAN ABS.	SIGMA	C.V.	LOADED VOLUME (UL)
2	0.3351	0.0002	0.0621	20.9
3	0.3388	0.0002	0.0435	19.5
4	0.3363	0.0002	0.0592	20.4
5	0.3329	0.0002	0.0502	21.6
6	0.3341	0.0003	0.0817	21.2
7	0.3304	0.0002	0.0469	22.6
8	0.3353	0.0002	0.0496	20.8
9	0.3315	0.0002	0.0568	22.1
10	0.3357	0.0002	0.0522	20.6
11	0.3336	0.0002	0.0652	21.4
12	0.3379	0.0001	0.0387	19.8
13	0.3358	0.0002	0.0533	20.6
14	0.3388	0.0002	0.0419	19.5
15	0.3377	0.0001	0.0402	19.9
16	0.3416	0.0002	0.0564	18.5
17	0.3361	0.0002	0.0541	20.5

```
AVERAGE CUVET VOLUME (UL)=     20.6
SIGMA                  =      1.0
C.V.                   =     5.00


AVERAGE CUVET ABS.     =   0.3357
SIGMA                  =   0.0029
C.V.                   =   0.8541


AVERAGE CUVET VOLUME (UL)=    20.6
CORRECTED SIGMA          =     1.0
CORRECTED C.V.           =     4.7


AVERAGE CUVET ABS.       =   0.3357
CORRECTED SIGMA          =   0.0027
CORRECTED C.V.           =   0.8117
```

Fig. 3. Output of Computer Program Used to Assess the Precision and Accuracy of the Technique of Dynamic Loading of Liquids into the Rotor of a Miniature Fast Analyzer.

3.3 Parameters Influencing Dynamic Loading

Using the technique described in Sect. 3.2, several of the parameters important in dynamic loading were identified and investigated, including rotor design and geometry, rotor speed, injection rate, and probe diameter.

3.3.1 Rotor Design and Geometry

A multifunctional 17-place rotor has been previously designed and fabricated[5] for use with the miniature Fast Analyzer which can load sample(s) or reagent(s) either discretely or dynamically. In the dynamic mode, solutions can be injected into the spinning rotor and diverted into the rotary path of splitting vanes; the resulting apportioned aliquots are simultaneously transferred into their respective cuvets. In testing the original design several modifications were found to be necessary (Fig. 4). The computer-controlled machining method used to fabricate the rotor components tended to produce small, thin curls of plastic on the face of the splitting vanes with a concurrent loss in the splitting performance. However, these curls were easily removed by a polishing procedure which resulted in sharp, smooth surfaces on the face and walls of the splitting vanes.

A second modification concerned the volume capacity of the receiving chambers of the rotor. In the original design, each chamber had a capacity of approximately 20 µl. Testing of this design showed reasonable splitting performance when the injected volume per cuvet did not exceed 30 µl per cuvet. However, when the volume per cuvet was increased to either 40 or 50 µl, splitting performance decreased. This was attributed to an overfilling of the individual receiving chambers during the dynamic loading operation. When the chamber capacity was increased to 50 µl, equivalent performance was obtained independent of the volume loaded per cuvet (Fig. 5). At a volume of 60 µl per cuvet, equivalent performance was obtained irrespective of the volume capacity of the receiving chamber. A possible explanation for this anomaly is that the total injected volume required to load 60 µl per cuvet is 1.020 ml. This volume is relatively large considering the internal geometry of the rotor, and has been observed to flood the center portion of the rotor upon injection. Thus, under the centrifugal field of the analyzer, this flooding results in the formation

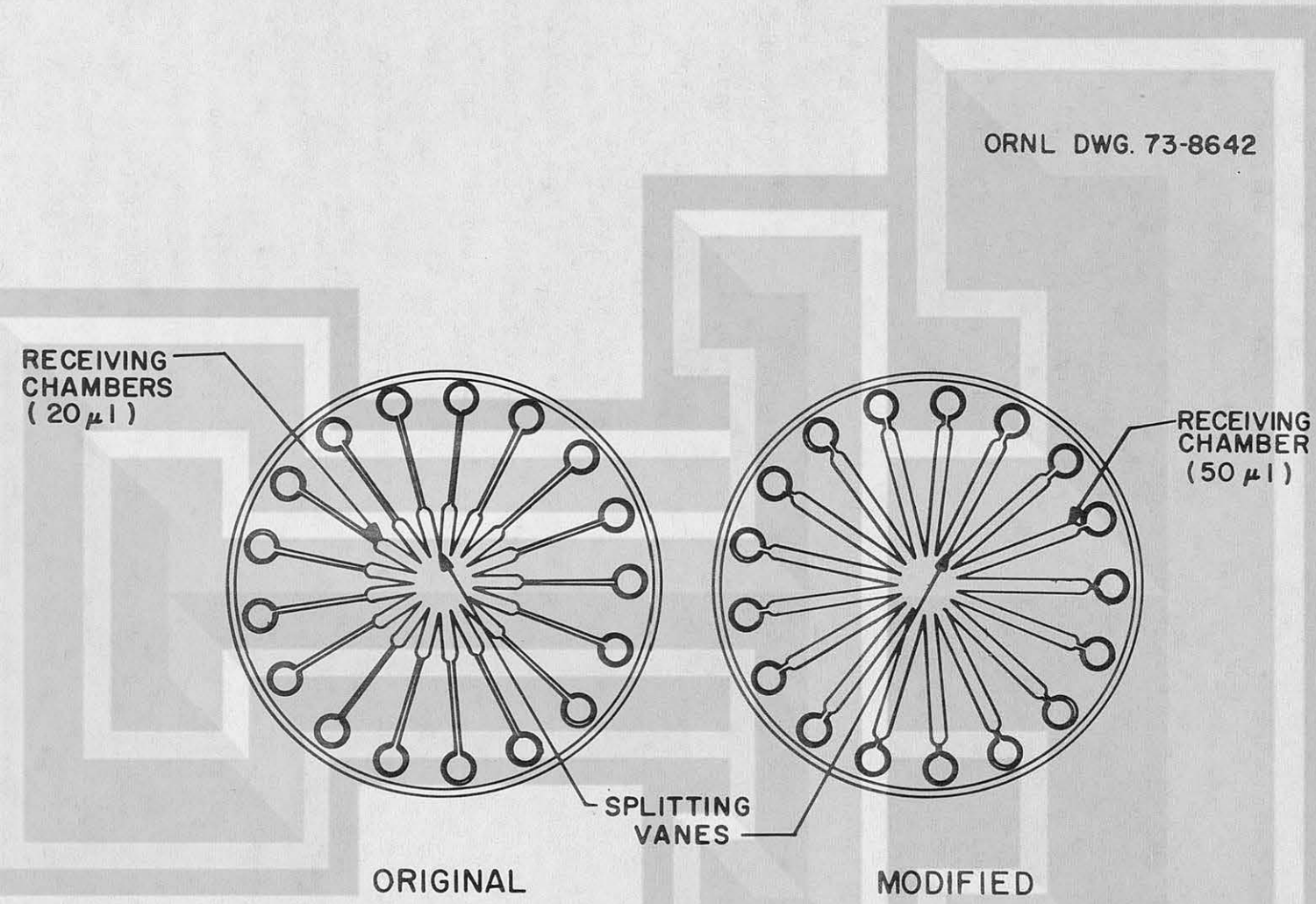

Fig. 4. Modification of the Dynamic Loading Side of a 17-Cuvet Rotor Used in a Miniature Fast Analyzer.

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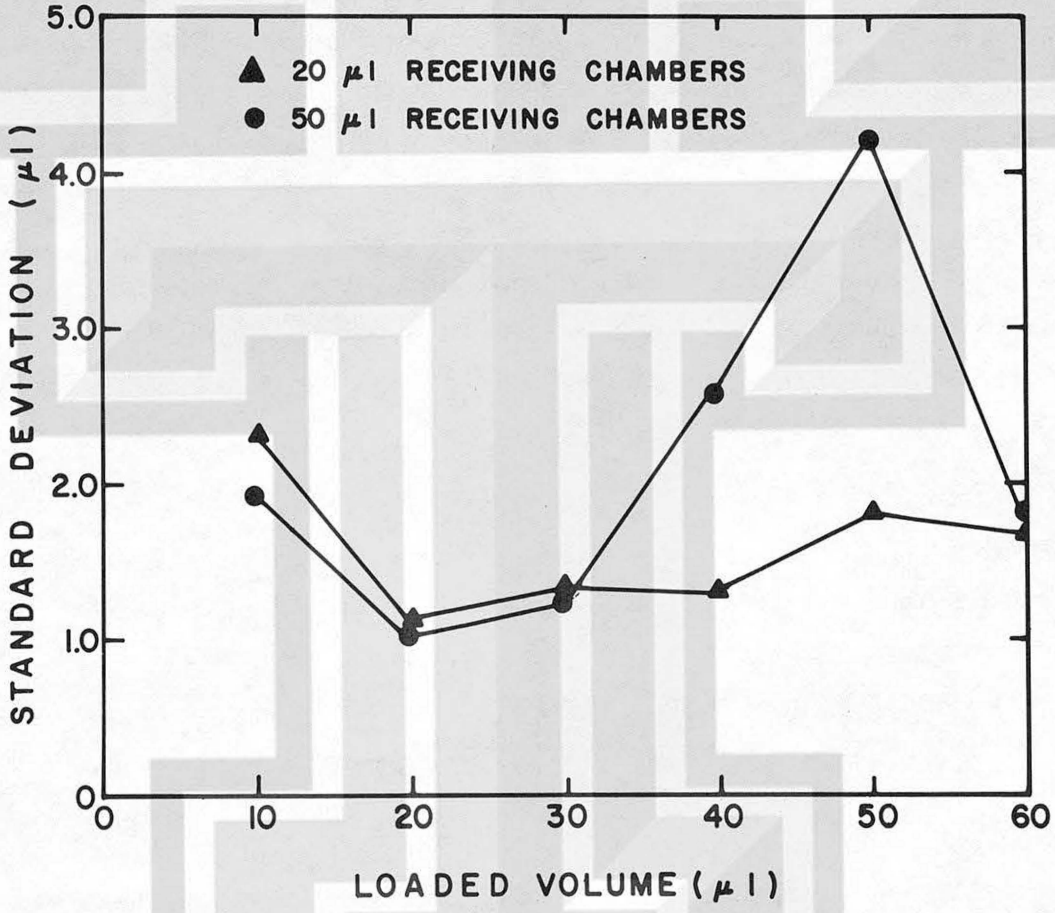

Fig. 5. Precision of Dynamic Loading as a Function of Volume Capacity of Rotor Receiving Chambers.

of a symmetrical, volumetric annulus similar to a donut near the center of the rotor. Due to the centrifugal field, this volumetric donut is forced towards the rotor periphery and drains at an equal rate through the receiving chambers into the cuvets of the rotor. Evidently, at lower volumes this flooding phenomenon does not occur, and unequal apportionment results.

A third modification was a change in the internal geometry of the rotor immediately adjacent to the splitting vanes. When the injected liquid was directed onto the walls of the splitting vanes, an aerosol was often formed, resulting in poor splitting performance. This problem was eliminated by machining a hemispherical cavity into the center of the rotor. In the modified rotor the injected liquid stream strikes the smooth face of the hemispherical cavity, which improves the rotor's splitting performance.

In addition to rotor modifications, a syringe and probe guide were added to the analyzer. The accurate and reproducible positioning of the syringe and probe for each analysis has greatly improved the splitting performance of the system.

3.3.2 Rotor Speed

Before the above modifications were made to the rotor, it was observed that the optimum rotational speed of the rotor was dependent on the volume injected per cuvet. For example, when the volume injected was 10 µl per cuvet, optimum performance was obtained at 4000 rpm; when the volume loaded per cuvet was increased to 50 µl, optimum performance was achieved at 2000 rpm. However, this inverse relationship was not observed when the modified rotor was tested. As shown in Table 1, essentially equivalent performance was obtained for each of the four rotational speeds tested. Consequently, a rotational speed of either 2000 or 3000 rpm is now routinely used to dynamically load the rotors.

3.3.3 Probe Diameter

An earlier report[6] indicated that if a liquid is dynamically introduced into a rotor in the form of large drops rather than as a continuous stream, unequal apportionment could result, since the individual drops might be directed to only a few cuvets. This effect

Table 1. Effect of Rotor Speed on Splitting Performance During Dynamic Loading[a]

Volume Injected (µl)		Rotation Speed (rpm)	Measured Volume (µl)	
Total	Per Cuvet		Mean	Standard Deviation
170	10	1000	9.7	1.4
		2000	9.8	0.4
		3000	10.0	0.6
		4000	9.0	0.1
340	20	1000	18.7	1.3
		2000	20.0	0.4
		3000	18.7	0.4
		4000	19.2	1.1
510	30	1000	29.5	1.7
		2000	29.7	0.3
		3000	30.6	0.7
		4000	29.7	0.2
850	50	1000	50.1	2.1
		2000	50.4	1.7
		3000	50.2	1.0
		4000	50.3	1.0

[a]Conditions: Total volume injected in 1.5 sec using a Fast Micromedic Automatic Pipette; injection probe, 0.01 in. ID; wavelength = 620 nm.

can be minimized by introducing the liquid through a small-diameter delivery tip as indicated by tests in which various volumes of liquids were introduced into the spinning rotor through delivery probes having different tip diameters. A Fast Micromedic Automatic Pipette was used to inject the liquid through probes having an internal tip diameter of either 0.010, 0.020, or 0.030 in. For each of the three probes, the standard deviations obtained from the four injected volumes (i.e., 10, 20, 30, and 50 µl per cuvet) were averaged, and this value was plotted against the internal tip area of the delivery probe.

The data in Fig. 6 clearly indicate the advantages of using a delivery probe having a tip of very small internal diameter. There is a practical problem to be considered in selecting a delivery probe. For operational ease, mechanical stability, cost, and availability, it would be desirable to use a hypodermic needle as a delivery probe. However, a needle having an internal diameter of 0.010 in. (equivalent to a 33-gauge needle) is often difficult to obtain and is easily obstructed during operation. The problem can be circumvented by using Teflon delivery probes which are tapered down to an internal tip diameter of 0.010 in. The probe tips can be cut 1 in. from their furthermost end and slipped over the end of a 26-gauge needle. The result is an easily obtainable delivery probe having a small-diameter, flexible tip. Since only the tip and a short section of the probe is of the small diameter, possibility of plugging is reduced. In addition, the flexible Teflon tip will not scratch the rotor when the delivery probe is inadvertently inserted too far into a spinning rotor.

3.3.4 Injection Rate

To investigate the effect of injection rate on splitting performance, a Fast Micromedic Automatic Pipette was used as a dispensing device. With this pipette, the volume dispensed can be easily varied, but it is always delivered in 1.5 sec. In addition, the delivery probe of the pipette can be easily changed, which allows one to vary the linear velocity of the dispensed liquid. The experimental procedure included placing the pipette above and behind the miniature analyzer. A 1-ml pump was placed into the pipette and an attached delivery probe was placed into the probe guide of

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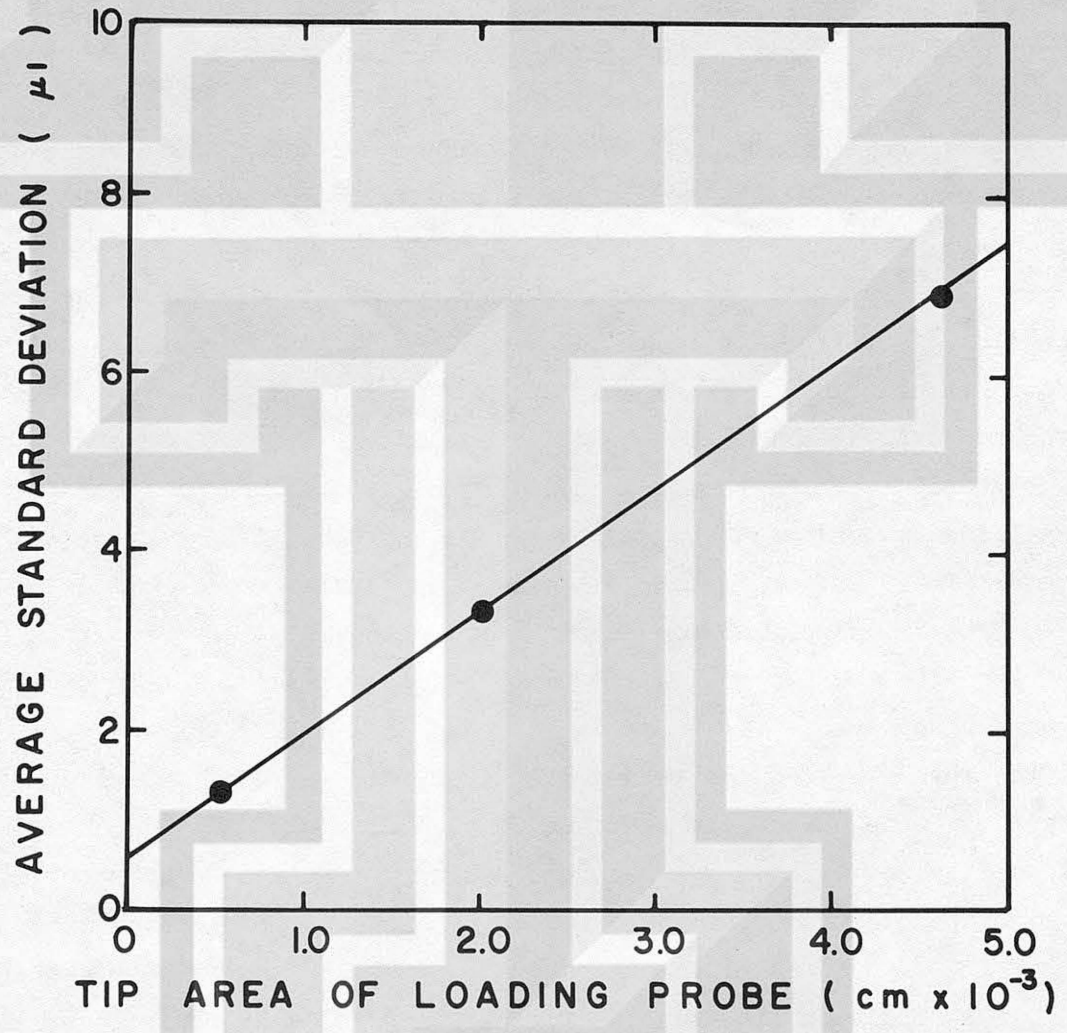

Fig. 6. Precision of Dynamic Loading as a Function of Tip Area of Loading Probe.

the analyzer at a 60° angle relative to the horizontal plane of the rotor. The probe tip was inserted into and positioned within the hemispherical cavity of the rotor such that its injected stream would be delivered onto the smooth wall of the cavity. Experiments were conducted in which the injected volume and tip diameter were varied and their effect on splitting performance determined. In general, for a given probe diameter, equivalent splitting performance was obtained when the volumetric flow rate was varied from 0.113 to 0.567 ml/sec. However, splitting performance was found to improve with an increase in linear velocity (Fig. 7). This improvement was quite marked when the linear velocity was increased from 25 to 200 cm/sec; above 200 cm/sec, the performance curve flattened and equivalent performance was obtained in the linear velocity range of 200 to 1000 cm/sec.

This flattening of the performance curve at the higher linear velocities is of practical significance since it indicates that equivalent performance can be obtained within a range of injection times. Since linear velocity is a function of the volumetric flow rate and area of the flow channel, the time required to inject a given volume of liquid at a given linear velocity can be easily calculated when given a probe of known diameter (Table 2). For example, equivalent results can be obtained when a volume of 170 µl (10 µl per cuvet) is injected into a rotor within a time range of from 0.3 to 1.7 sec. At 850 µl this range increases from 1.7 to 8.3 sec.

The practical advantage achieved from this range of injection times is that a simple device can be manually used to dynamically load liquids. A manual device, such as a hypodermic syringe, requires an operator; thus the time of injection would be expected to vary due to operator individuality (Fig. 8). However, almost equivalent results can be obtained even though the injection time varies slightly. Essentially equivalent results were obtained in an experiment in which performance of an automatic dispensing device was compared with that of manual operation (Table 3).

3.4 Precision of Technique

Utilizing the previously mentioned improvements, successive experiments were performed to determine the run-to-run precision of dynamic loading. As

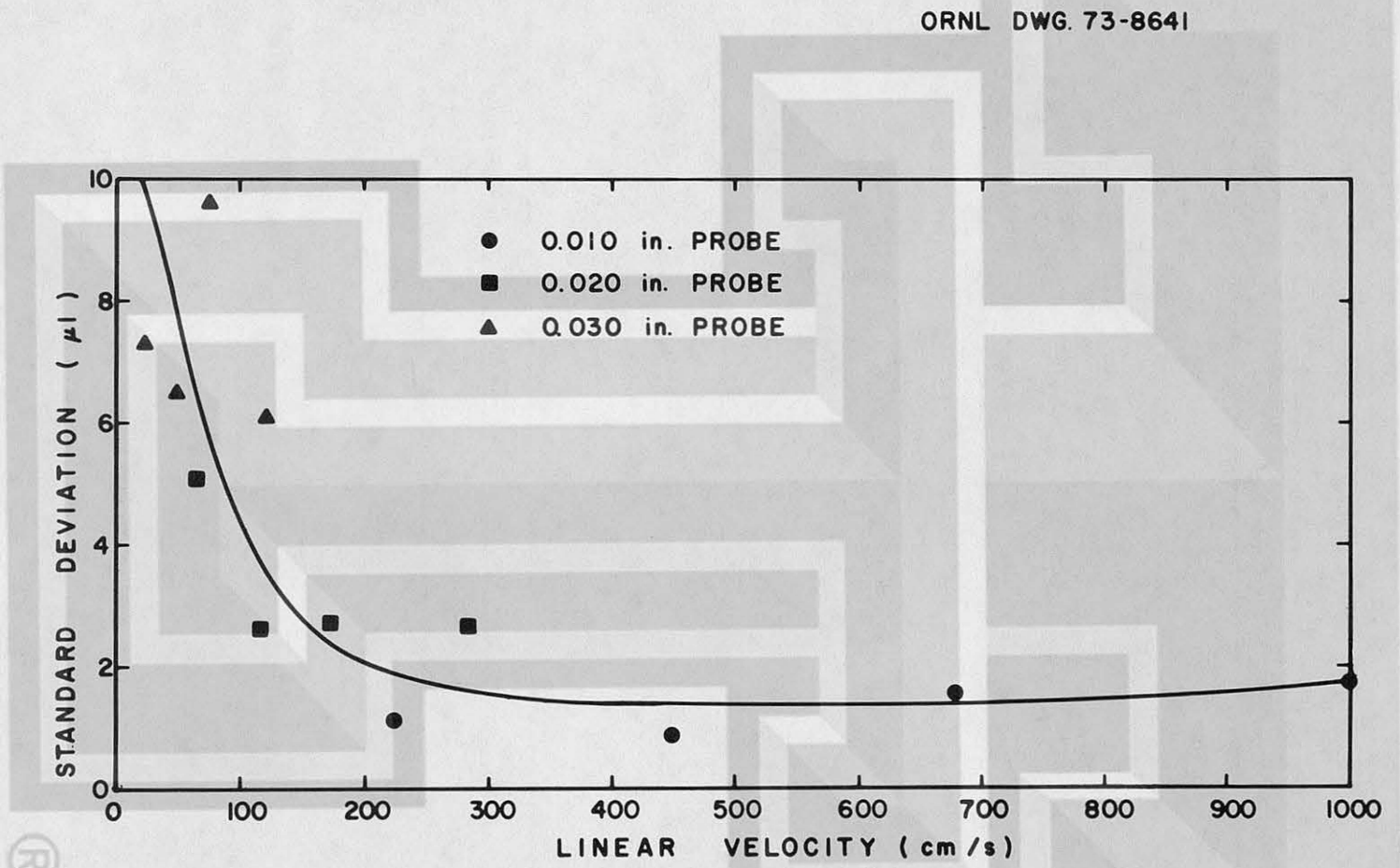

Fig. 7. Precision of Dynamic Loading as a Function of the Linear Flow Velocity of the Injected Liquid.

Table 2. Time Required to Deliver a Fixed Volume at a Given Linear Velocity[a]

Linear (cm/sec)	Volumetric[b] (ml/sec)	Injection Time[c] (sec) for Listed Delivered Volumes			
		170 µl	340 µl	510 µl	850 µl
200	0.102	1.7	3.3	5.0	8.3
300	0.153	1.1	2.2	3.3	5.6
500	0.255	0.7	1.3	2.0	3.3
700	0.357	0.5	1.0	1.4	2.4
1000	0.510	0.3	0.7	1.0	1.7

[a] Calculations made given a tip diameter of 0.010 in. (area = 5.1×10^{-4} cm^2).

[b] Volumetric rate = linear velocity x tip area.

[c] Injection time = volume delivered/volumetric flow rate.

Table 3. Comparison of the Splitting Performance of a Manual vs an Automatic Dynamic Loading Device[a]

	Volume (µl)		Standard Deviation (µl)	
	Injected	Calculated	Within Cuvet	Cuvet-to-Cuvet
Manual[b]	50	50.5	0.8	1.5
Automatic[c]	50	50.4	1.3	1.8

[a] Conditions: Rotor speed at time of injection = 3000 rpm, wavelength = 620 nm.

[b] 1-ml Hamilton syringe, 26-gauge needle having a flexible 0.010-in.-ID tip.

[c] Fast Micromedic Automatic Pipette, 1-ml pump.

PHOTO 0820-73

Fig. 8. The Use of a Hypodermic Syringe and Needle to Dynamically Introduce a Liquid into the Spinning Rotor of a Miniature Fast Analyzer.

shown in Table 4, excellent run-to-run precision was obtained; the average cuvet volume over a six-run test (n = 96) was 50.4 ± 1.0 µl. Table 5 summarized data from a series of experiments in which precision vs volume injected was determined.

3.5 Analytical Comparison of Discrete vs Dynamic Loading of Reagents

A series of samples was analyzed for various enzyme activities using the miniature Fast Analyzer. In one set of experiments both the samples and reagents were loaded discretely using the automated sample-reagent loader. In a second set of experiments the samples were loaded discretely with the automated sample-reagent loader, then the reagents were dynamically introduced. As seen in Table 6, essentially equivalent results were obtained using either discrete or dynamic loading of the reagents. In a subsequent, similar experiment 48 patient serum samples were analyzed in parallel for five blood constituents using both discrete and dynamic loading of reagents (see Table 7). The data in Tables 6 and 7 indicate that reagents may be dynamically loaded into a rotor with no loss in analytical performance.

4. PERTINENT DEVELOPMENTS FOR OTHER AGENCIES

Other aspects of the miniature Fast Analyzer program which are of interest to the NASA program are being supported by other government agencies. These include (1) development of a fluorescence and light-scattering monitor, (2) development of a portable data printer, (3) development of a portable analyzer, (4) development of a chemical protocol for the measurement of uric acid, and (5) evaluation of a miniature analytical system in a routine clinical laboratory.

4.1 Fluorescence and Light-Scattering Monitors

A fluorescence monitor for fast analyzers has been previously developed for use with a 15-place GeMSAEC Fast Analyzer.[7,8] Due to mechanical and physical constraints of the analyzer rotor assembly, this optical system utilizes a top frontal excitation and emission optical configuration instead

Table 4. Run-to-Run Precision of Dynamic Loading[a]

Run No.	Volume (µl)		Standard Deviation (µl)
	Injected	Mean[b]	
1	50	49.2	1.6
2	50	51.1	1.2
3	50	50.8	1.3
4	50	49.7	0.4
5	50	51.0	0.7
6	50	50.5	1.0
1-6, avg.	50	50.4	1.0

[a]Rotor speed of 3000 rpm; introduction by 1-ml Hamilton syringe with 26-gauge needle having a flexible 0.010-in.-ID tip.

[b]Sixteen values per run.

Table 5. Precision of Dynamic Loading vs Volume Injected[a]

Volume Injected (µl)	Measured Volume Mean (µl)	Standard Deviation (µl)
10	10.4	0.6
20	20.0	0.5
30	33.2	1.0
50	50.5	1.1

[a]Conditions: Miniature Fast Analyzer, 620 nm; rotor speed, 3000 rpm; injection device, 1-ml Hamilton syringe with a 26-gauge needle having a flexible 0.010-in.-ID tip; for each volume mean, N = 16.

Table 6. Comparison of Analytical Results Obtained with Either Discrete[a] or Dynamic[b] Loading of Reagents

Sample Code Number	Enzyme Activity (I.U. liter^{-1} min^{-1}, 30°C)					
	ALP[c]		LDH-L[d]		SGOT[e]	
	Discrete	Dynamic	Discrete	Dynamic	Discrete	Dynamic
1	30.6	31.9	62.8	59.9	11.4	10.8
2	210.2	201.5	217.6	213.2	43.4	41.9
3	37.6	38.9	70.8	67.8	5.7	5.5
4	38.9	39.8	67.9	63.3	6.8	5.2
5	32.5	33.1	62.2	58.9	10.1	8.0
6	58.7	57.7	70.1	76.5	12.9	12.0
7	27.7	27.0	100.0	86.8	11.0	9.6
8	43.9	43.7	97.2	95.9	10.2	8.3
9	42.3	41.6	87.8	87.3	25.0	23.5
10	52.3	52.0	84.4	83.0	14.2	12.6
11	58.7	58.5	93.4	96.8	12.8	8.3
12	56.0	56.9	56.8	53.9	6.7	5.6
13	38.7	38.4	66.6	66.1	6.7	4.0
14	37.2	38.3	68.0	67.5	4.6	3.8
15	30.0	30.5	61.3	57.7	10.3	8.9
16	210.1	202.9	216.2	220.7	42.2	42.3
Average	62.8	62.1	92.7	91.0	14.6	13.1

[a]Volumes loaded: 10 µl sample + 50 µl diluent (discrete); 20 µl reagent + 50 µl diluent (discrete).

[b]Volumes loaded: 10 µl sample + 50 µl diluent (discrete); 60 µl reagent (diluted 1:3) (dynamic).

[c]Conditions: 400 nm; 30°C; dynamic loading speed, 3000 rpm; delay interval = 10 sec; observation interval = 10 sec; N = 20.

[d]Conditions: 340 nm; 30°C; other conditions identical to those in footnote c.

[e]Conditions: 340 nm; 30°C; other conditions identical to those in footnote c except observation interval = 15 sec.

Table 7. Statistical Comparison of Analytical Results Obtained with Either Discrete or Dynamic Loading of Reagents

Assay	N	Slope	Intercept	Average Y (Dynamic)	Average X (Discrete)	Coefficient Correlation	Standard Error of Estimate	Relative Standard Error of Estimate
Glucose[a]	48	1.023	-0.40	108.7	106.6	0.9990	2.08	1.92
Triglycerides[a]	45	0.980	4.22	196.1	195.9	0.9962	7.57	3.86
Alkaline phosphatase[b]	48	1.051	-0.53	59.3	56.9	0.9995	1.44	2.42
LDH-L	48	1.017	0.13	86.4	84.9	0.9914	6.28	7.26
SGOT[b]	48	1.027	-0.040	13.1	13.1	0.9958	1.06	8.08

[a]Results in mg/dl.

[b]Results in I.U. liter^{-1} min^{-1} at 30°C.

of the more widely used right-angle excitation/emission configuration. This has proved to be successful in its initial evaluations with respect to both sensitivity and versatility in methodology development.

When transferring the fluorescence monitoring technology to the miniature Fast Analyzer, some mechanical and physical constraints of the 15-place GeMSAEC Fast Analyzer can be circumvented. Since the rotor of the miniature Fast Analyzer is small, portable, and self-contained, at least two approaches can be made: (1) adaptation of right-angle excitation/emission using specially designed rotors with end-windows in each cuvet normal to the standard top rotor, and (2) frontal excitation/emission as previously developed for the 15-place GeMSAEC Fast Analyzer. The former can also be used for monitoring light scattering. Two such systems have been built for the miniature Fast Analyzer. Each is currently undergoing evaluation for sensitivity, versatility, and practicality with regard to operation of the system in both fluorometric and photometric modes and maintenance of the compactness and mobility of the miniature analyzer.

4.1.1 Right-Angle Fluorescence and Light-Scattering Monitor

A right-angle rotor and detection system developed for the miniature analyzer which can be used for both fluorescence and light-scattering measurements is shown in Fig. 9. The rotor was designed specifically for low-level fluorescence measurements for which a right-angle rotor would have the advantage of reduced reflection and light scatter over the frontal system. Also, the top window and the emission detector can be positioned to minimize problems associated with the inner filter effect. The emission volume element (as seen by the detector) was placed at the outer (excitation window side) edge of the cuvet, in contrast to centering it in the middle of the cuvet window. The rotor, as seen in Fig. 10, has solid cylinders of ultraviolet transmitting (UVT) acrylic plastic windows glued into the end of each cuvet, allowing excitation to occur directly through the end window and normal to the overhead detection system. The emission signal (90° to the excitation source) is monitored by a photomultiplier tube and filter system positioned above the rotor. This arrangement was developed to use an existing 1P28 photomultiplier tube which would also allow evaluation of the performance with respect to the frontal system on the 15-place analyzer.

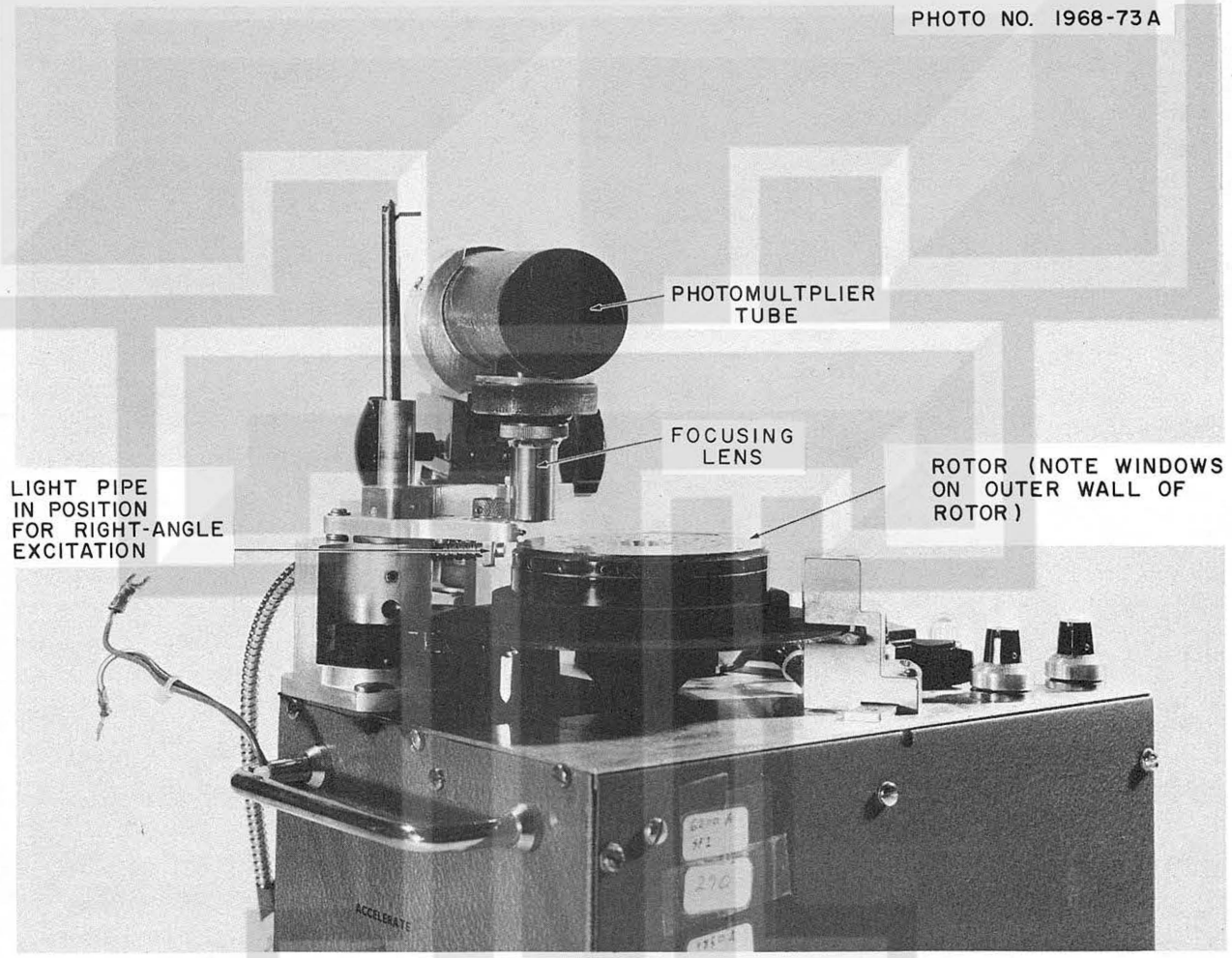

Fig. 9. Optical Configuration of a Right-Angle Monitoring System to Measure Both Fluorescence and Light-Scattering with a Miniature Fast Analyzer.

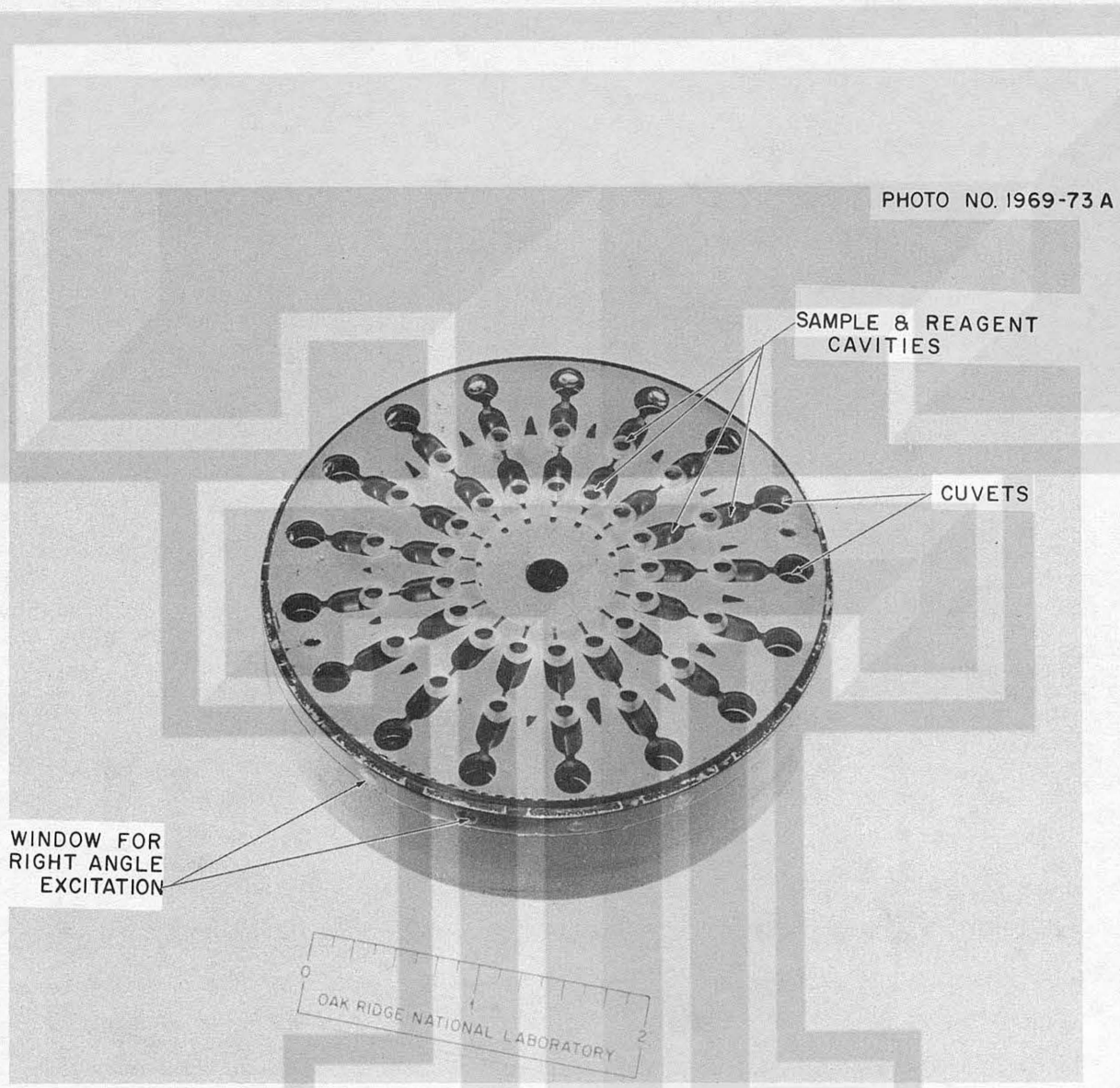

Fig. 10. Rotor Used to Measure Light Scattering with a Miniature Fast Analyzer. (Note that individual plastic windows have been sealed into the peripheral wall of each cuvet.)

A semiquantitative evaluation of the rotor and detection sensitivity was made using solutions of sodium fluorescein in 0.01 N NaOH. Sodium fluorescein solutions with concentrations of 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 4, 6, 8, and 10 ng/ml (5.0×10^{-10} mole/liter to 2.5×10^{-8} mole/liter) were prepared and placed in the rotor, the first five solutions being pipetted in duplicate. The excitation source was a 150-W xenon arc lamp coupled to a Bausch and Lomb high-intensity gradient monochromator. The analog emission signals of these samples are shown in Fig. 11. The blank emission for 0.01 N NaOH can be seen (cuvets 16 and 17) to be very small as compared with cuvets 1 and 2, which contain 0.2 ng/ml (5.0×10^{-10} mole per liter) sodium fluorescein. This is indicative of a detection limit in the range of 1.0×10^{-10} mole/liter. At this level, adsorption of the fluorescence material onto walls of the dilution vials becomes a limiting factor (along with blank emission) due to many factors, including Raman emission and filter leakage of scattered light.

4.1.2 Surface Fluorescence Monitor

A second approach to fluorescence measurements has been to adapt a surface fluorescence optical system to the analyzer. The advantage of such an approach is twofold: First, no redesign of rotors is required, and the same rotors can be used for fluorescence as are currently used for photometric analyses. Second, the system would use the existing photomultiplier tube of the analyzer and, with the successful adaptation of a miniature high-intensity lamp source, the compactness and mobility of the present analyzer would be maintained.

A surface fluorescence optical system has been developed for the analyzer. The primary component of the system is a mirror assembly that fits in a slot cut into the end of the filter wedge (Fig. 12). The mirror is set at an angle of 30° to the filter holder plane, which directs excitation light at a 60° angle of incidence into the bottom window of the rotating rotor. The mirror extends into the first cylindrical filter holder to permit direction of the exciting light to the maximum possible depth in the cuvet as the cuvet is centered above the aperture of the photomultiplier tube. An objective lens is used to focus the emission signal, and 0.5-in.-diam barrier filters of the desired wavelength are used

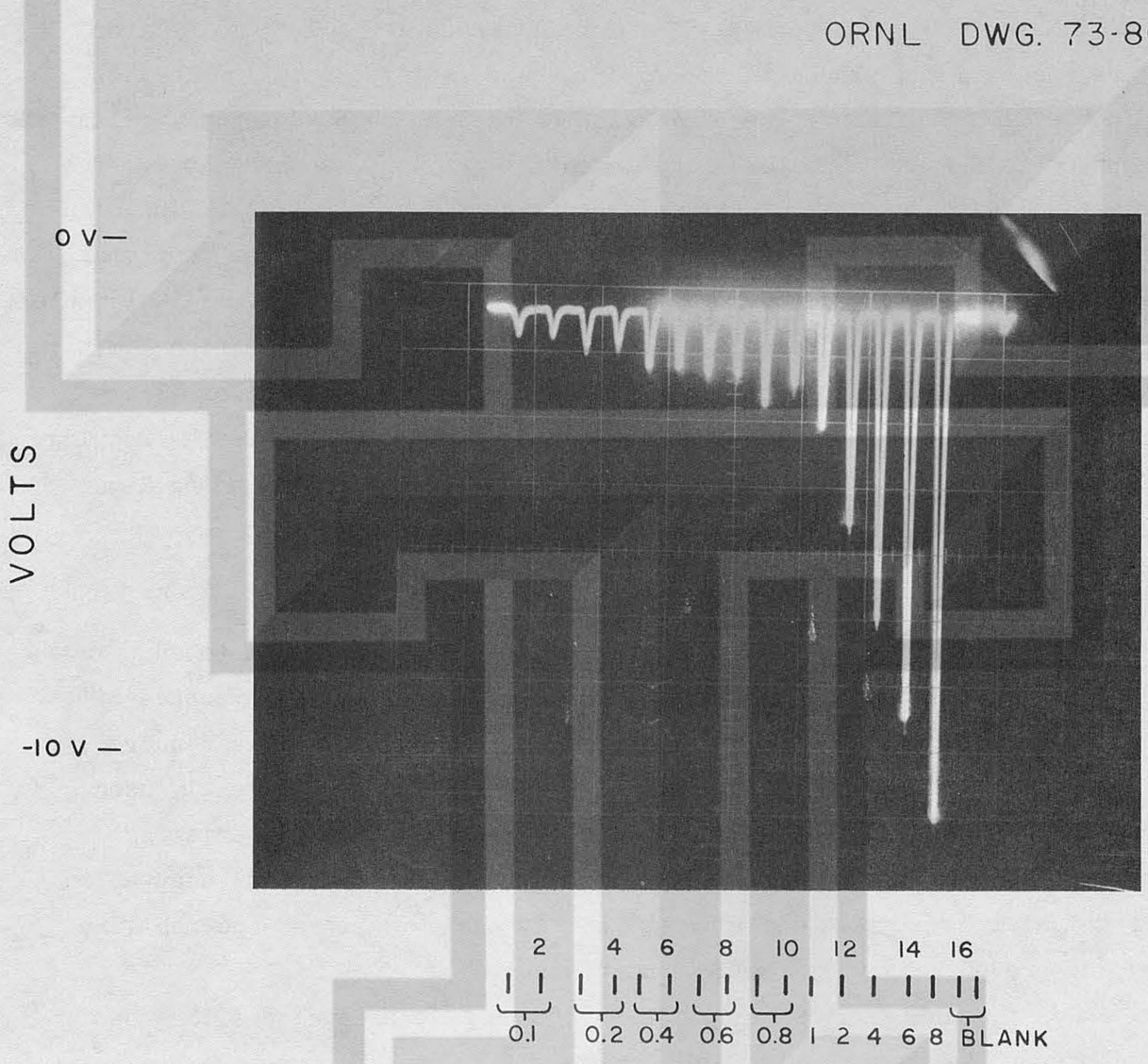

Fig. 11. Fluorescence Signals Obtained Using a Right-Angle Fluorescence Monitor with a Miniature Fast Analyzer.

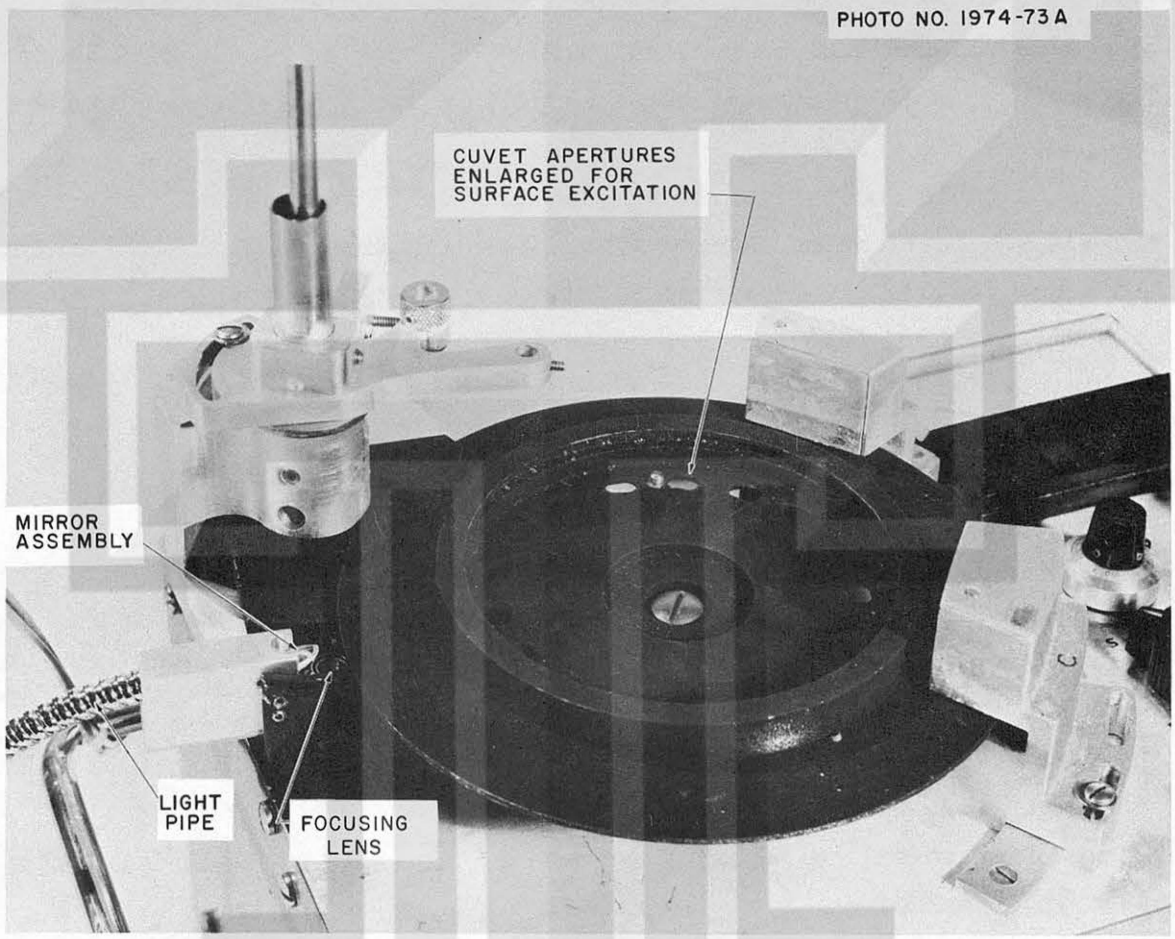

Fig. 12. Surface Fluorescence Optical System for Use with the Miniature Fast Analyzer.

to isolate the emission signal. A quartz fiber optical bundle has been employed for direction of excitation light to the spinning rotor from a xenon lamp source. The sensitivity of the system is about 10 ng/ml or 2.5×10^{-8} mole/liter of sodium fluorescein in 0.01 N NaOH. The sensitivity can be improved by replacing the current 1/4-in. aperture in the photomultiplier holder with a variable aperture, which would allow most of the emission signal to fall on the photomultiplier tube window in the fluorescence mode of operation, but would provide a 1/8-in. aperture for the photometric mode.

A suitable small light source that has sufficient intensity in the uv and visible portions of the energy spectrum to permit fluorescence measurements and photometric measurements is under consideration. The mirror assembly has made it possible to eliminate the fiber optical bundle completely and mount the lamp source close to the mirror system.

4.1.3 Application of the Right-Angle Light-Scattering Monitor

Although the right-angle monitor and rotor system shown in Fig. 9 was developed specifically for low-level fluorescence, particularly as such measurements relate to competitive binding fluoroimmunoassays, it can also be used as a monitor for light scattering. Consequently, one of the important and practical uses of the rotor will be for specific protein analyses. The monitoring of the intensity of light scattering as a function of antigen concentration after aggregate equilibrium is of increasing interest for so-called specific protein analyses. Serum IgG, IgA, IgM, β-1-trypsin inhibitor, transferrin, and C'3 complement factor are among some 40 specific serum proteins that can be assayed. These analyses are based on the aggregation of immunoglobulins with their respective antibodies, which can be measured by determining the change in light scattering either during the aggregate formation (kinetic) or after aggregate formation (equilibrium).

Because of the small volume requirements and light-scattering monitoring capabilities, the miniature Fast Analyzer with the right-angle rotor is being developed to perform specific protein analyses. In an initial study, 100 µl of antihuman IgG diluted 1:25 with 0.9% NaCl solution was pipetted in reagent cavities 1 through 17 of the right-angle rotor. Fifty microliters

of diluent was added to sample cavities 1 and 2; 50 µl of human IgG (2.73 mg/ml) diluted 1:1000 was added to sample cavities 3 through 6; 50 µl of human IgG (6.1 mg/ml) diluted 1:1000 was added to sample cavities 7 through 10; 50 µl of human IgG (11.5 mg/ml) diluted 1:1000 was added to sample cavities 11 through 14; and samples containing 23.0 mg/ml IgG diluted 1:1000 were added to sample cavities 15 through 17. Excitation was set at 410 nm, and the rotor containing the IgG samples and antibody was accelerated to 500 rpm. A photograph of the analog signal was taken immediately (T_o), and a similar photograph was taken at 16 min. The results, shown in Fig. 13, indicate that increasing intensity was obtained with increasing concentrations of IgG at 16 min. The system is apparently very sensitive in that it can detect microgram-per-milliliter quantities of IgG and it requires the use of only 4 µl of antibody per test (100 µl of a 1:25 dilution). This volume of antibody can be reduced further by both decreasing the reaction volume and using more specific antibody.

The quantitation of the IgG by light scattering is a somewhat unique approach in which the rate of IgG--anti-IgG aggregation is monitored (Fig. 14). The change in light-scattering intensity ($I_{90°}$) as a function of IgG concentration is shown in Fig. 15. These data indicate that an assay based on a nonlinear fixed-time approach could provide direct kinetic determination of IgG concentration in less than 3 min from the initiation of the reaction. The time required for equilibrium light-scatter procedures is approximately 20 min for IgG and longer for other assays, while a time of 18 hr is generally used for double-immunodiffusion procedures which are commonly employed. There is some literature evidence which suggests that the initial rate of formation of antigen/antibody complex as monitored by light scattering tends to reach a constant value at higher antigen concentration, and the rate does not decrease at antigen excess. Therefore, this type of data could be used to establish a cut-off value for the acceptable rate, and any samples that had values appearing at this limiting rate would be diluted and analyzed again.

4.2 Portable Data Printer

A portable digital Data Printer is currently being developed for use with the miniature and portable Centrifugal Fast Analyzers. It will print

ORNL DWG. 73-8636

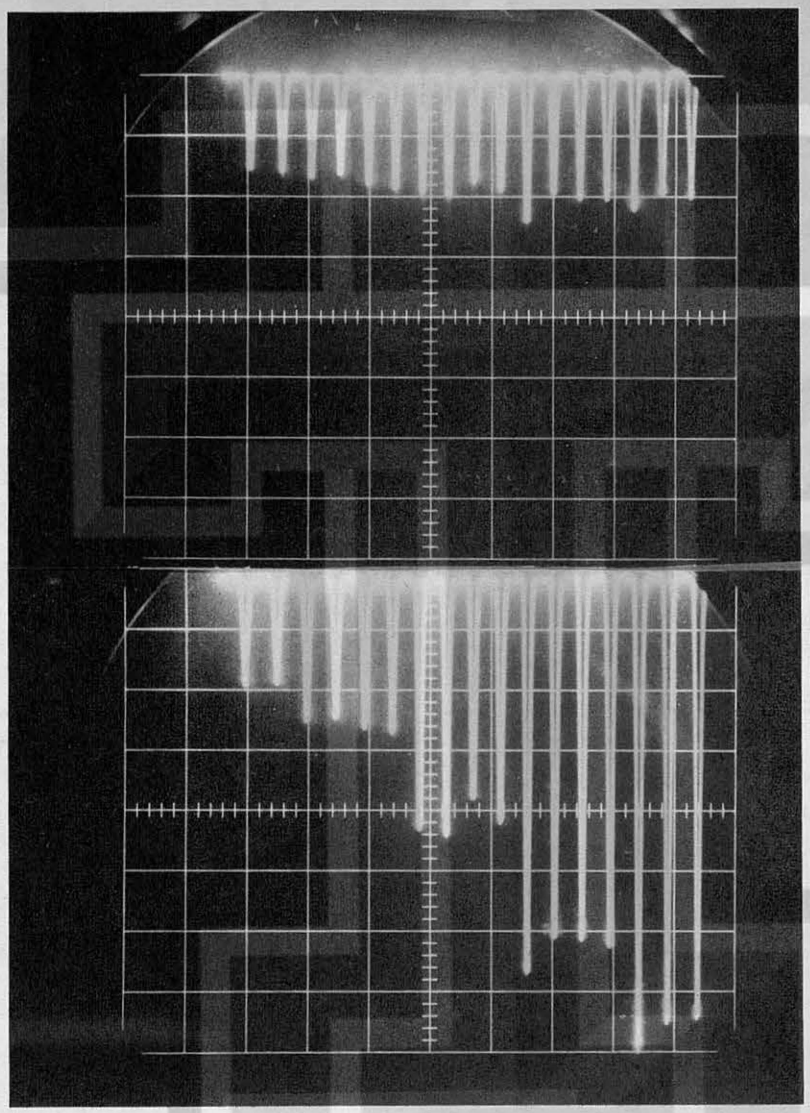

Fig. 13. Light Scattering from IgG-Anti-IgG as Determined on the Miniature Fast Analyzer.

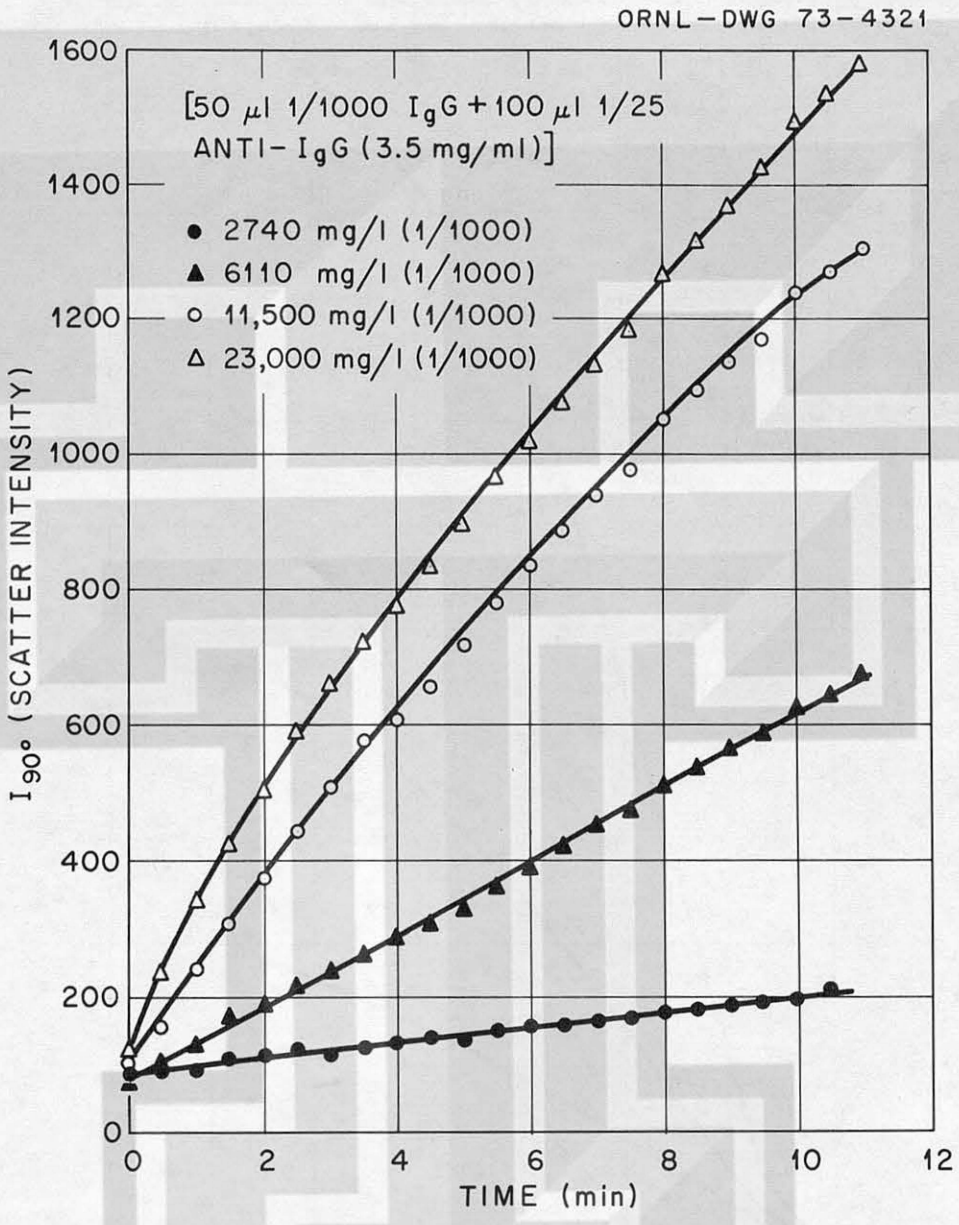

Fig. 14. The Interaction of Human IgG-Anti-IgG vs Time as a Function of IgG Concentration.

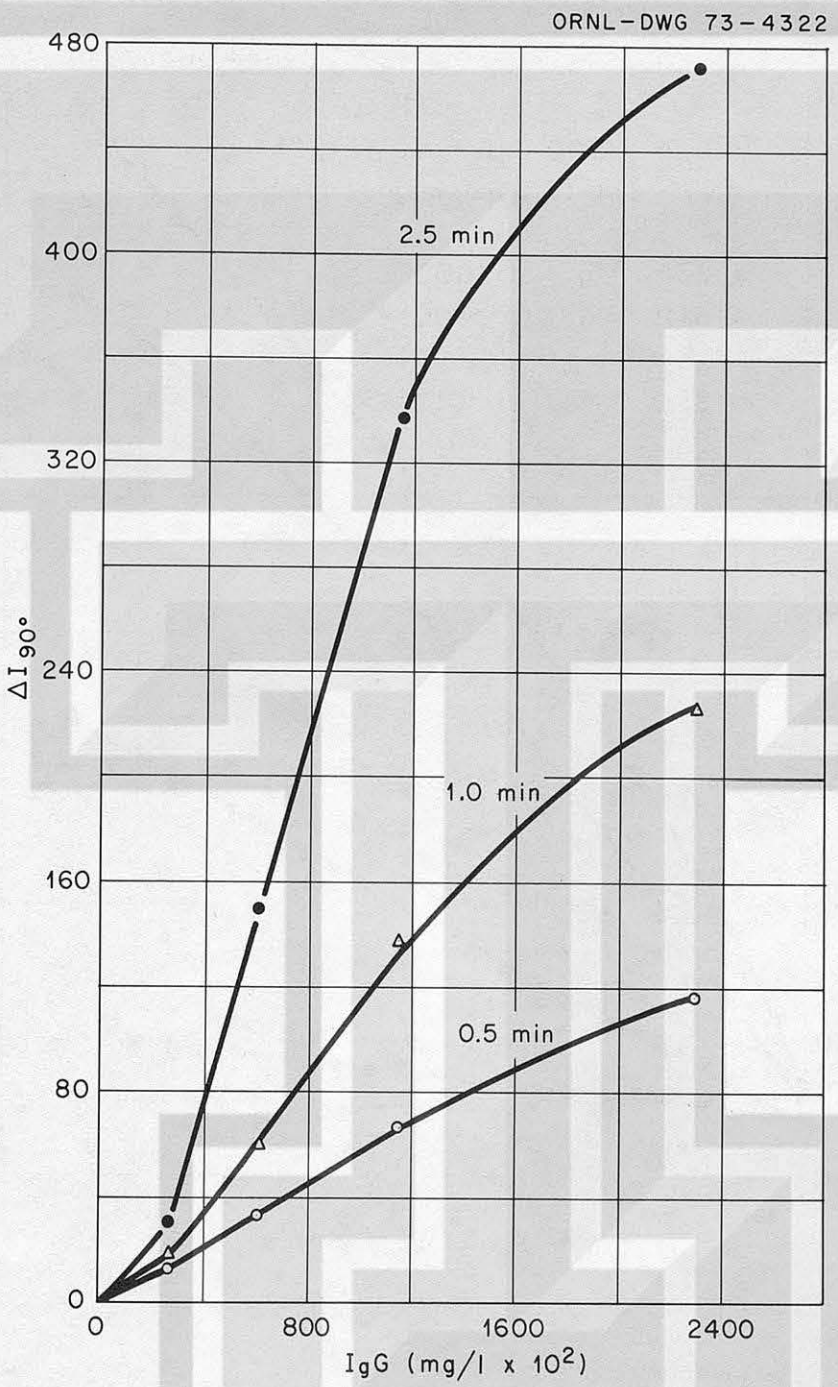

Fig. 15. Fixed Time Rate of Change of IgG-Anti-IgG Interaction as a Function of Time.

instantaneous absorbance values of each of 17 cuvets on thermal-sensitive paper in response to an external print command signal. The electronic package, which has been fabricated and is currently being tested, utilizes a logarithmic amplifier to convert the transmission pulses to absorbance data pulses; these are then digitized by a 12-bit A/D converter, and the resultant digital data are stored in a 384-bit random-access memory arranged as 32 words of 12 bits each. After the data are accumulated in memory, printing begins. The recording device is an 8-digit printer with a 7-segment thermal print head and thermal-sensitive paper. It prints the 2-digit cuvet number, followed by a blank space (or an 8 if the reference cuvet 1 is dirty), a decimal point, then a 4-digit absorbance value at the rate of 2 lines per second.

This first model has been designed to record the absorbance in the range of 0 to 0.8192 utilizing the total count capacity of 4096 counts of the 12-bit A/D converter. Although this is somewhat less capacity than is desirable, it will allow the techniques for storage and data retrieval to be tested. A more sophisticated digital calculator integrated circuit will be employed as the central data processor in the next model.

4.3 Portable Fast Analyzer

A portable fast analyzer system has been developed, and a working prototype has been fabricated and tested. A 4-in. cubic case contains the system power supply (a Ni-Cd battery pack), rotor drive motor, a miniature photomultiplier (PM) tube, a converter to develop the required high voltage for the PM tube, and optics to direct the light from transmission samples to the PM tube. Controls provide rotor speed and PM voltage adjustments and allow the PM tube to be rotated to positions for sensing either transmitted light or emitted fluorescence. The 12-W quartz-halogen source lamp is powered by the Ni-Cd battery pack, but is externally mounted for improved heat dissipation.

On top of the 4-in. cubic case are the 2-1/4-in.-diam, 8-cuvet rotor and a mount for the distal end of a bifurcated, half-quartz, half-glass fiber optic illuminator and fluorescence detection system. The proximal end of the quartz-fiber arm is inserted in the source lamp holder, which also contains an interference filter for selecting the incident wavelength

for each type of analysis. The proximal end of the glass fiber arm is returned to an adapter, on top of the instrument case, which contains an interference filter for isolating emitted fluorescent wavelengths and provides an aperture to the photomultiplier tube. Output coaxial connectors transmit light measurement signals and rotor and cuvet pulses.

A series of tests was performed to evaluate the capabilities of this prototype to make both absorbance and fluorescence measurements. Absorbance tests were made with solutions of Blue Dextran in water (1/2% formaldehyde as preservative) with known absorbances at 620 nm. The corresponding range of absorbances for the solutions in the rotor cuvets provided a comparison (Fig. 16). Results indicate reasonable linearity up to an absorbance of about 0.6.

Fluorescence measurements were made using solutions of fluorescein in 0.01 N NaOH. The excitation light was defined by a 480-nm cutoff interference filter, and a 520-nm cuton barrier filter was used to pass emitted light to the PM tube. Because of the limited PM tube voltage available in the battery-operated prototype, concentrations below 10 µg/ml were not detectable; however, the results indicate essentially linear response in the concentration range of 10 to 100 µg/ml (Fig. 17).

4.4 Development of a Chemical Procedure for Measuring Uric Acid

The typical assay for uric acid in the clinical laboratory involves the chemical oxidation of uric acid with phosphotungstic acid. The procedure, although effective for this purpose, is not specific, and the oxidation of a variety of serum metabolites also occurs. Uric acid can be enzymatically oxidized to allantoin and carbon dioxide by means of the enzyme uricase.

Based on the uricase method, a uric acid procedure is being developed for use with the miniature Fast Analyzer. The adaptation presents a twofold problem involving both instrumental changes and chemistry development. First, a suitable light source must be obtained for operation at 292 nm, and a rotor capable of uv transmission must be available. Second, the chemistry must be suitably adapted to the existing automated sampling and diluting system.

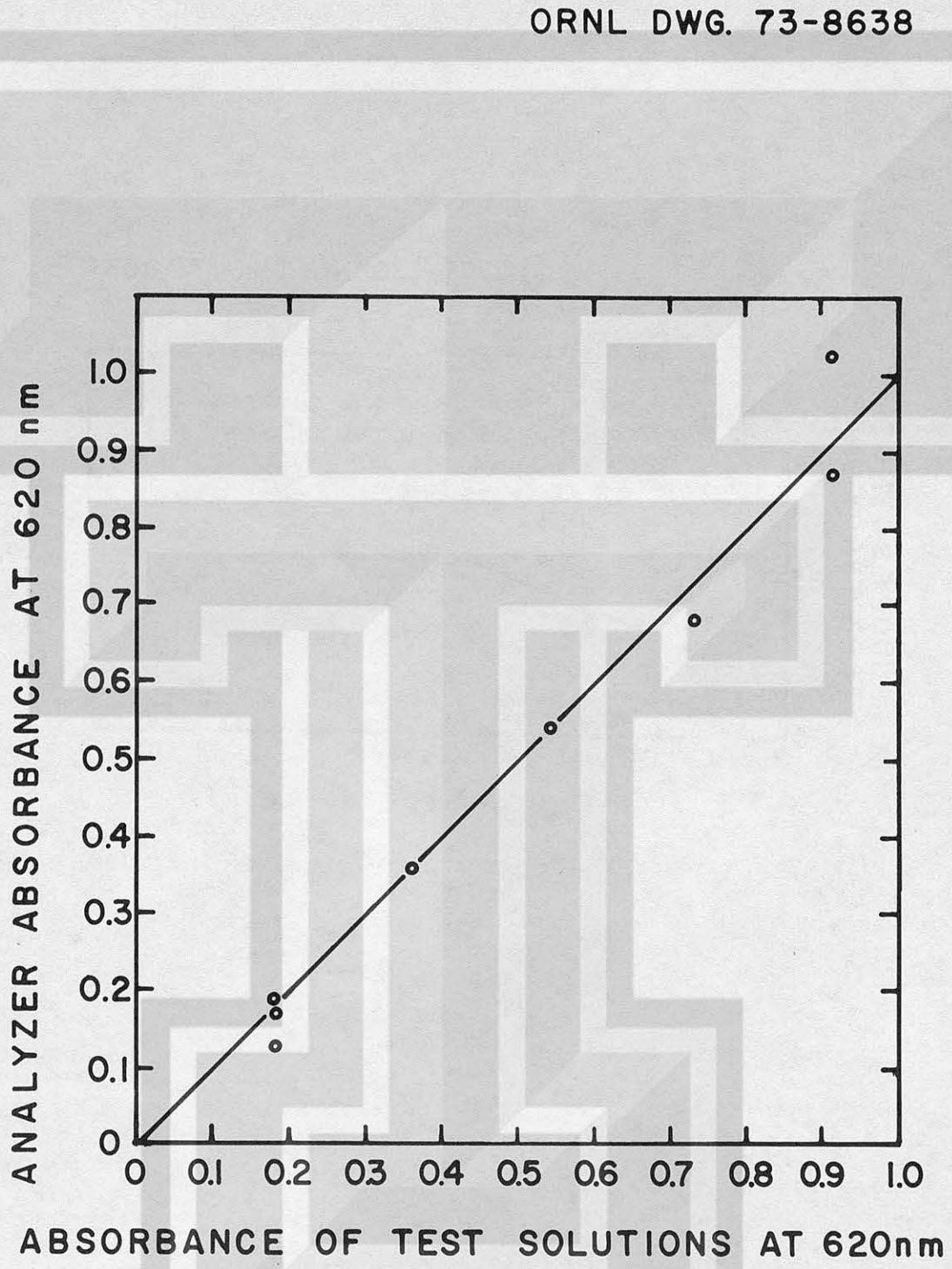

Fig. 16. Absorbance Measurements Using the Portable Fast Analyzer.

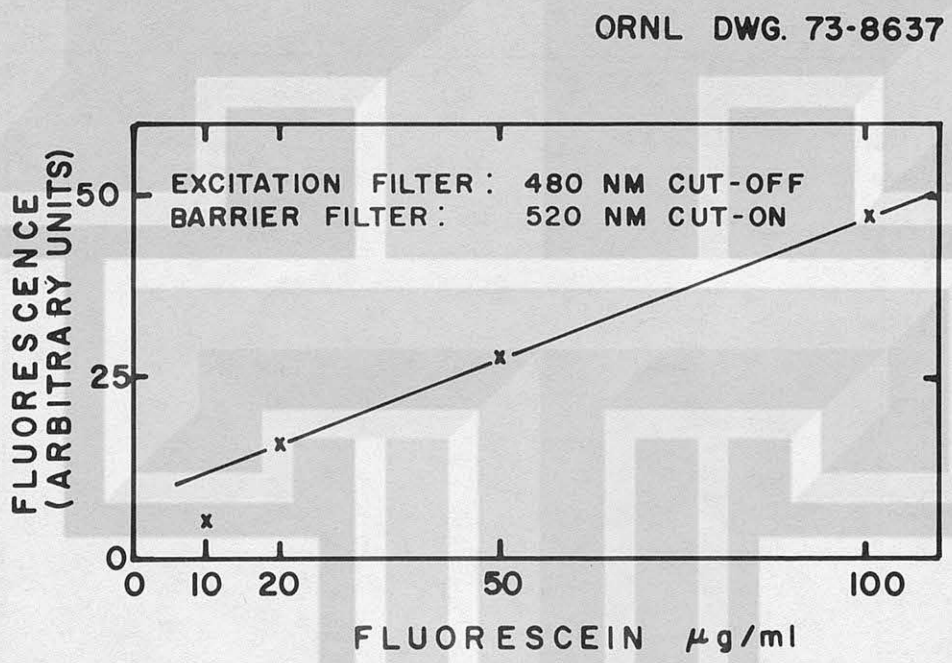

Fig. 17. Response of the Portable Fast Analyzer to Solutions of Fluorescein.

4.4.1 Light Source and Rotor

Three light sources were available for testing with the miniature Fast Analyzer: (1) a 22-W quartz-iodine lamp, (2) a low-pressure mercury lamp coupled to a 300-nm emitting phosphor, and (3) a deuterium lamp source coupled to a monochromator and quartz optical fiber bundle. Each source was evaluated with respect to linearity of absorbance at 290 to 293 nm by using calibrated uric acid solutions whose absorbances ranged from 0 to 2.0 absorbance units. A rotor with quartz windows (Fig. 18) was fabricated by sandwiching a black acrylic body between a bottom quartz disk and top quartz annular ring. A plastic insert, which was cut to fit in the top center portion of the rotor, contains the sample reagent and dynamic loading ports. The procedure for assembling the rotor was identical to that used for assembling the all-plastic rotors.[5]

Figure 19 shows the linearity of absorbance of the uric acid solutions at 292 nm as a function of window material and lamp used. The linearity of absorbance at 292 nm for the uv/phosphor source, and the deuterium source with the quartz rotor, is shown in Fig. 20. Both figures demonstrate that the most nearly linear response was obtained with the quartz rotor. In addition, the uv/phosphor source had reasonable linearity and potential application. The main disadvantage of this lamp source is its extremely low intensity, which results in a higher noise level (e.g., $\pm 1.5 \times 10^{-3}$ absorbance unit uncertainty as contrasted to $\pm 0.7 \times 10^{-3}$ absorbance unit with the deuterium source). The performance of the quartz-iodine lamp was not acceptable. Because the deuterium source was immediately available, the uric acid procedure was developed using the quartz rotor in conjunction with it and a 200- to 400-nm monochromator as the optical system (Fig. 21).

4.4.2 Chemistry Development

Due to its high specific activity, the procedure was developed using bacterial uricase (obtained from Nova Industry, New York), which has been found to be stable for a year in 0.1 *M* borate buffer, pH 9.1. The development of the method was straightforward and primarily involved developing the procedure within the operating conditions of the automated sample-reagent loader. This device aspirates small aliquots of samples and reagents into their probes, then dispenses and follows them with a preset

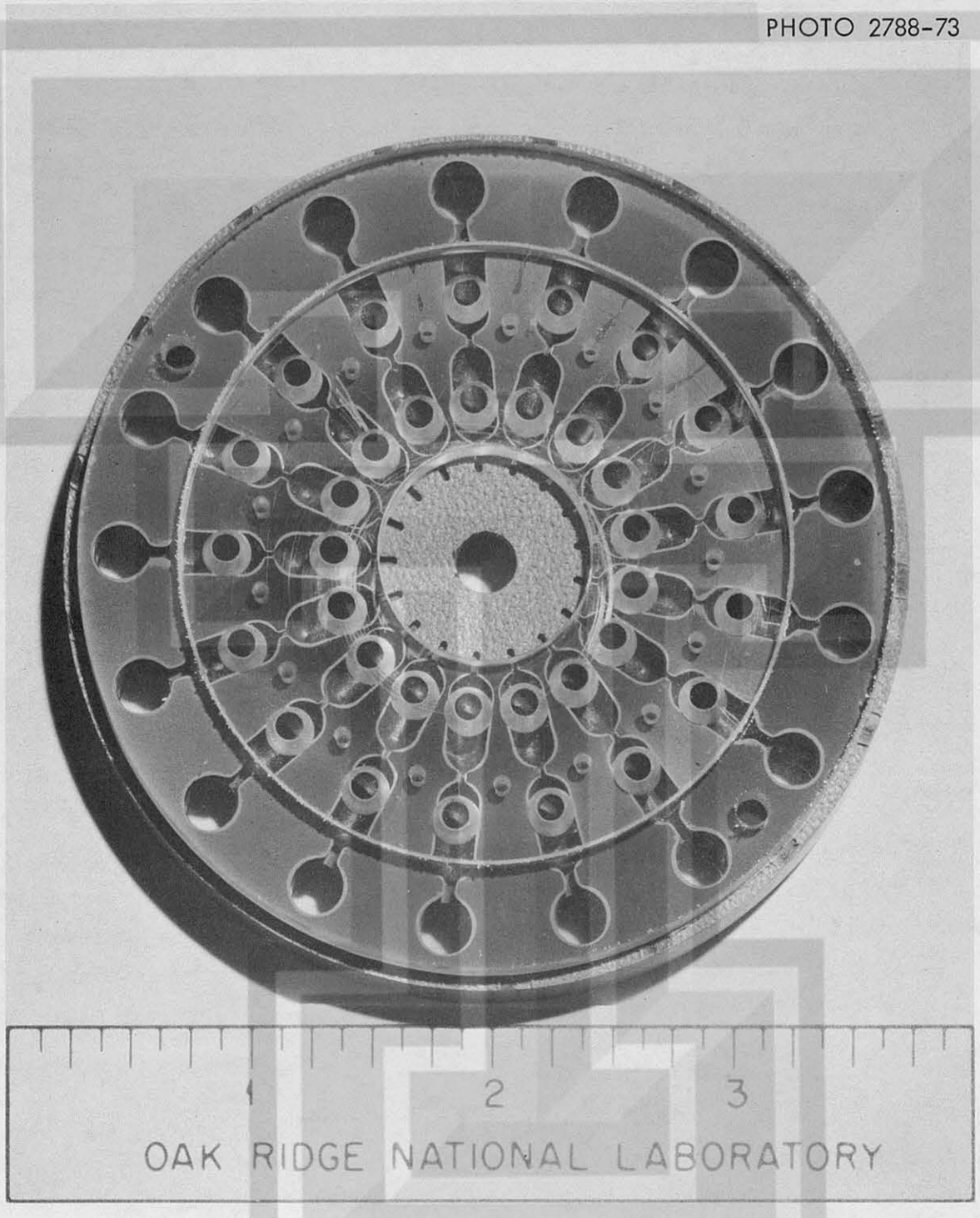

Fig. 18. A Miniature Fast Analyzer Rotor with Quartz Windows.

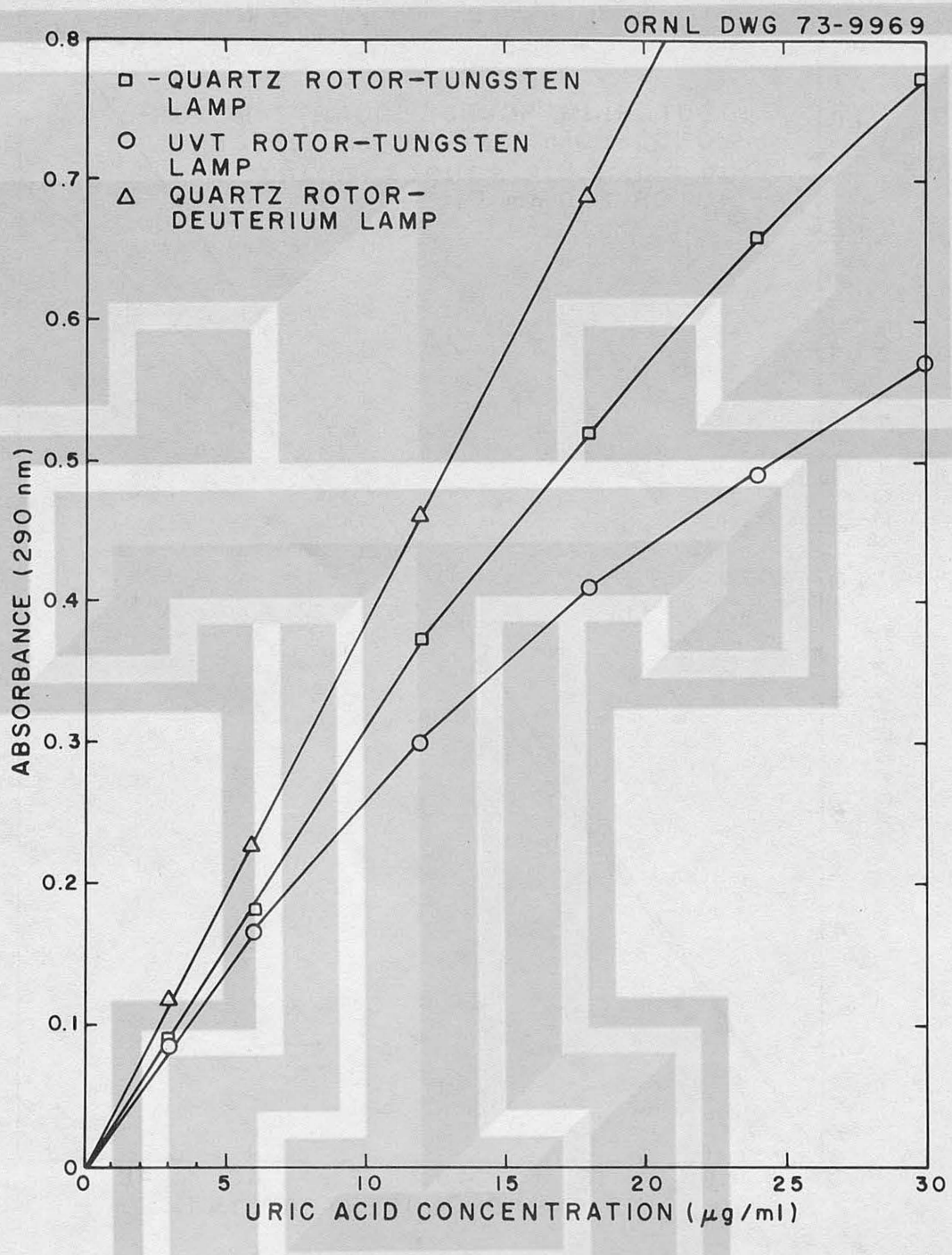

Fig. 19. Linearity of Absorbance of Uric Acid Solutions at 292 nm Measured with the Miniature Fast Analyzer as a Function of Quartz Iodine Lamp, Deuterium Lamp, Plastic UVT, and a Quartz Rotor.

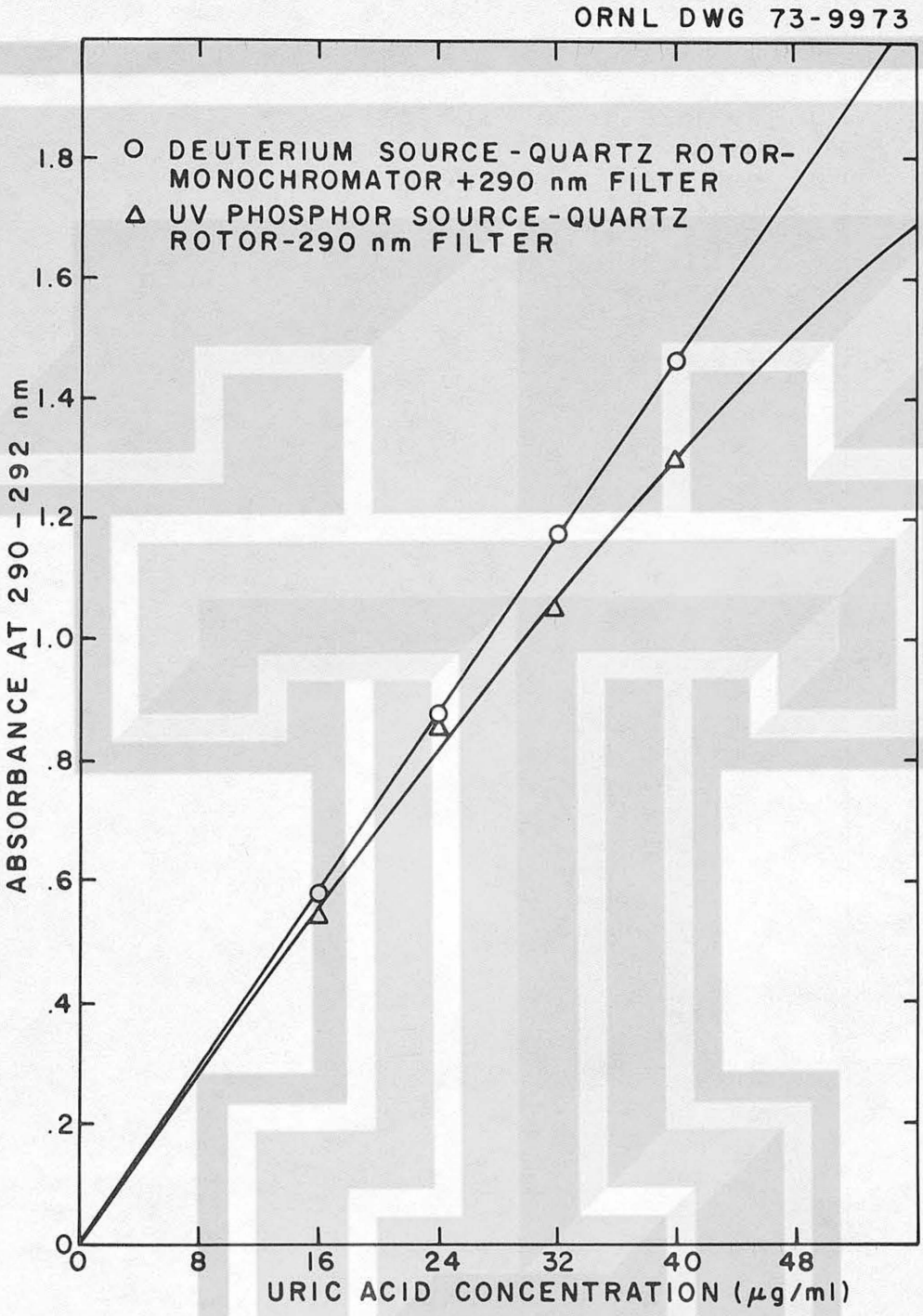

Fig. 20. Linearity of Absorbance of Uric Acid Solutions at 292 nm as Measured by the Miniature Fast Analyzer with a Deuterium Lamp Source and a UV Phosphor Source.

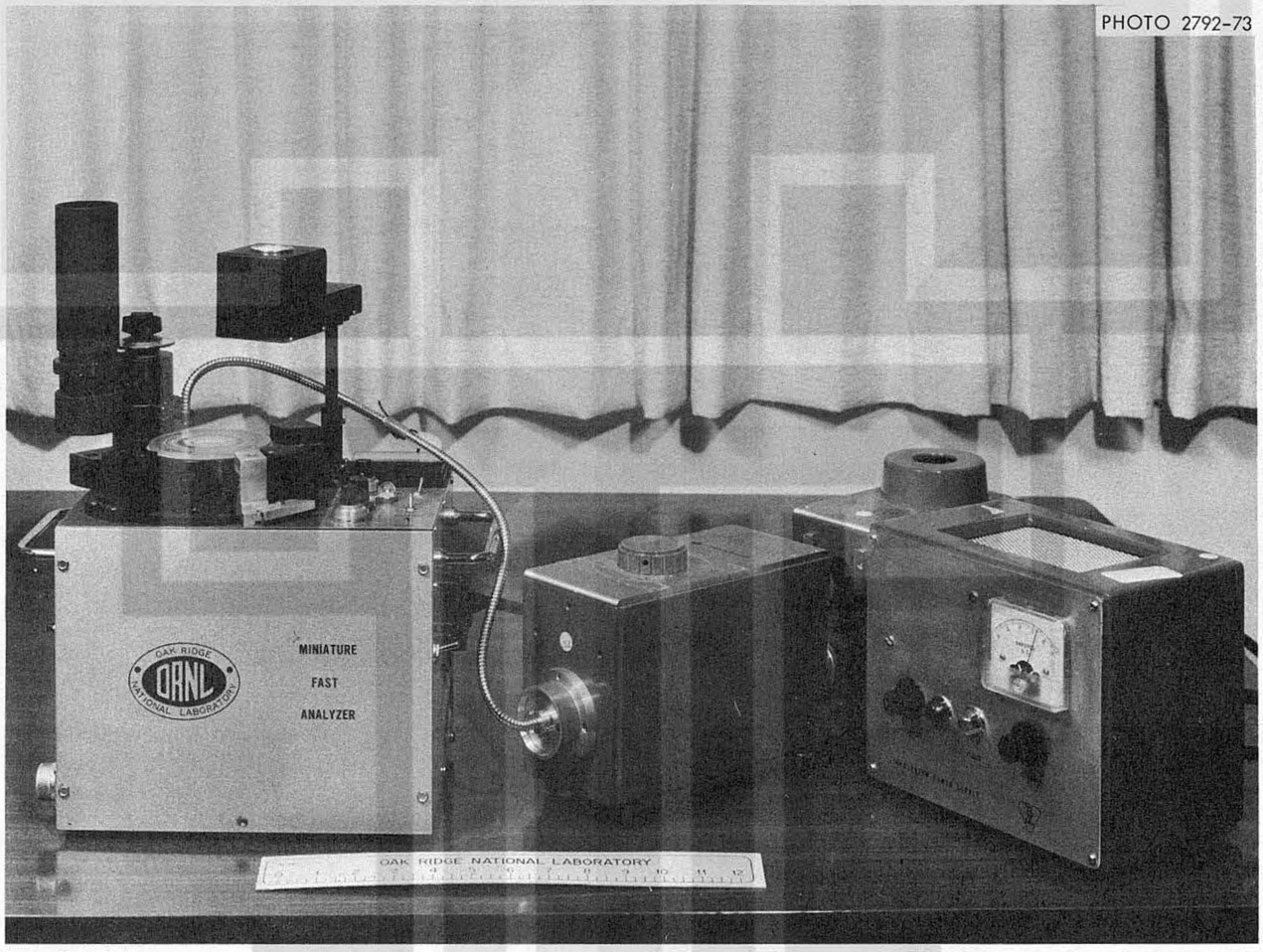

Fig. 21. Interfacing the Miniature Fast Analyzer with a Deuterium Light Source.

quantity of diluent into their respective rotor cavities. Thus, a concentrated preparation of the reagent must be used. To meet this condition, the uricase reagent was prepared in a 1.0 *M* borate buffer (pH 9.1). During the loading procedure, 15 µl of reagent was aspirated and diluted approximately 1:10 in the final reaction volume. The analysis was thus performed at a buffer concentration of 0.1 *M* and a pH of 9.1, which are the optimal conditions for this assay. The preparation of the enzyme in the concentrated form did not affect its activity. The detailed analysis procedure is presented in the Appendix.

This procedure was evaluated for several weeks to determine its precision and to correlate the results obtained from it with parallel data obtained from an existing procedure using a continuous flow analyzer. In the correlation, both analytical methods used the same standards. The main difference between the two systems was the reducing procedure that was used with the continuous flow analyzer. A phosphotungstic acid procedure has been designed to minimize nonuric acid interference. More than 200 samples have been analyzed with each system, and the results have been correlated by linear regression methods. Typical correlation coefficients of 0.91 to 0.96 have been obtained, with mean uric acid values of 5.85 mg/100 ml and 5.95 mg/100 ml for the continuous flow analyzer and the enzymic uric acid procedure respectively. The coefficient of variation (C.V.) of the analysis is 3 to 4% in the normal range (e.g., 4 to 6 mg/100 ml), and 2% in the abnormal range of 8 to 10 mg/100 ml. Because of these encouraging results, the enzymatic uric acid procedure on the miniature Fast Analyzer will replace the continuous flow analyzer method and will be routinely used by the ORNL Health Division.

4.5 Evaluation of a Miniature Fast Analytical System in a Routine Clinical Laboratory

To evaluate the miniature Fast Analyzer under routine conditions, an analytical system based around a miniature Fast Analyzer has been placed in the Clinical Laboratory of the Health Division of the Oak Ridge National Laboratory.

4.5.1 System Description

The analytical system consists of a miniature Fast Analyzer, several rotors, an automated sample-reagent loader, a rotor cleaning station, and a computerized data system. This system is similar to one being evaluated at the NASA Johnson Space Center, Houston, Texas (Fig. 1), except that it is interfaced to an on-line computer for data processing (Fig. 22). The data system is composed of a PDP-8/E computer (Digital Equipment Corp., Maynard, Mass.) with 8192 words of core memory, a 1200-Hz line-frequency-based clock, an ARS 33 Teletype, a Sykes cassette-tape unit (Sykes Datatronics Inc., Rochester, N. Y.), and the necessary analog and digital interfaces for coupling the analyzer to the computer hardware.

The high-level language, FOCAL*, was modified and extended with appropriate software to accept data under real-time control of the line-frequency clock. Modified subprograms have been added to permit program and data storage on and retrieval from the cassette tape unit. Direct access features of the tape transport permit storage of data from many analyses for later correlation and study, as well as reprocessing of the original data with new data processing techniques.

Using this computer system, an operating program has been developed for routine operation of the system. The program includes an executive file which initiates the daily operation and, by a series of operator question:response routines, will automatically load the required program into the computer core. A typical output from this executive program is shown in Fig. 23. After the operator initiates the program, a summary of the stored quality control data is printed, after which the operator is queried as to rotor calibration. If so desired, the rotor calibration program is automatically loaded and initiated. If the answer is no, the operator is then asked to enter the code of one of the listed chemistries. In this example, the code for glucose analysis has been entered and followed by automatic loading of the glucose program. The operator enters identification information in a question:answer format, and the system is ready for analysis. The operator responses in this particular example have been underscored in Fig. 23.

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*FOCAL (FOrmula CALculation) is a conversational language and is a registered trade name of the Digital Equipment Corporation, Maynard, Mass.

Fig. 22. Computerized Data System Used with the Miniature Fast Analyzer.

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ORNL HEALTH DIVISION MINIATURE FAST ANALYZER RESULTS

DAY 317  TIME  8 : 23 :  7

QUALITY CONTROL DATA

DAY 317

ASSAY          CONTROL   N     MEAN     SIGMA     C.V.     95% RANGE

GLUCOSE          M-1     67     78.1     3.28     4.20     71.5 -  84.6
                 M-2     67    211.6     7.26     3.43    197.1 - 226.1
                 PS      67     84.4     3.33     3.95     77.7 -  91.0

TRIGLYCERIDE   STD#1     36    296.1     7.36     2.49    281.4 - 310.9
               STD#2     36    305.1    13.24     4.34    278.7 - 331.6

URIC ACID        M-1      3     10.0     0.10     0.97      9.8 -  10.2
                 M-2      3      9.4     0.64     6.84      8.1 -  10.7
                 PS       3      5.5     0.41     7.46      4.7 -   6.3

ALK. P'HASE      M-1     34     33.1     2.72     8.22     27.7 -  38.6
                 M-2     34    213.3     7.54     3.53    198.2 - 228.4
                 PS      34     35.8     4.48    12.51     26.8 -  44.7

LDH-L            M-1     33     49.2     5.06    10.27     39.1 -  59.3
                 M-2     33    203.2    13.31     6.55    176.6 - 229.8
                 PS      33     50.1     7.36    14.67     35.4 -  64.9

SGOT             M-1     37     18.8     1.98    10.53     14.8 -  22.8
                 M-2     37     51.1     2.41     4.72     46.3 -  55.9
                 PS      37      7.3     1.39    18.94      4.6 -  10.1

DO YOU WISH TO CALIBRATE A ROTOR (YES OR NO):NO

TO SELECT A CHEMICAL PROCEDURE ENTER ONE OF THE FOLLOWING CODES

  CODE    CHEMICAL PROCEDURE
-------  --------------------
  401-   GLUCOSE (FASTING)
  402-   GLUCOSE (1 HOUR)
  403-   GLUCOSE (2 HOUR)
  404-   GLUCOSE (TOLERANCE)
  411-   GLUCOSE (RANDOM)
  405-   URIC ACID
  408-   ALKALINE PHOSPHATASE
  409-   SGOT
  410-   LDH-L
  412-   TRIGLYCERIDES
  700-   GLYCEROL CALIBRATION
  800-   TERMINATE RUN
  900-   RESTART

ENTER DESIRED CODE :401

                GLUCOSE ASSAY

DAY 317  TIME  8 : 28 :  4
OPERATOR:CB
GEMSAEC UNIT:17.1
RUN NUMBER:1
ROTOR NUMBER:1
READY!
```

Fig. 23. Typical Computer Output Obtained from the Executive Program Used to Routinely Operate a Miniature Fast Analytical System in a Routine Clinical Laboratory.

4.5.2 Chemical Procedures

In the routine mode of operation, ORNL personnel undergoing periodic 18-month health examinations were asked to present themselves at the time of the examination in a fasting state (12 to 16 hr without food). The Fast Analyzer was then used to perform the following tests on the blood sample drawn at that time: glucose (fasting and 2-hr post-100-g glucose load), triglycerides (fasting), uric acid, SGOT, LDH, and alkaline phosphatase. The protocols for these procedures are listed in the Appendix. In addition, the ORNL Clinical Laboratory has also been participating in two outside quality control problems, one set up by the Center for Disease Control (CDC), and the other directed by the College of American Pathologists (CAP).

4.5.3 Performance Comparison with a 15-Cuvet GeMSAEC Fast Analyzer

Before the miniature fast analytical system was placed into routine operation in the ORNL Clinical Laboratory, its performance was compared with that of a 15-cuvet GeMSAEC Fast Analyzer which had been routinely operated in that laboratory for the past 2-1/2 years. In this comparison study both analyzers were used to daily analyze routine serum samples for four blood constituents. The resultant data were then statistically correlated using linear regression analysis. As shown in Table 8, essentially equivalent results were obtained from either analyzer; the daily coefficient of correlation averaged 0.9962, 0.9977, and 0.9942 for the glucose, alkaline phosphatase, and LDH-L assays respectively. The average daily coefficient of correlation for SGOT was lower, at 0.9375, but considering the low enzyme activities that were statistically processed, this degree of correlation was acceptable.

Training of four medical technologists to operate the miniature system went quite smoothly, possibly because they had been previously trained to routinely operate the GeMSAEC Analyzer. In general the technologists have preferred to operate the new miniature system due to its simplicity and improved ease-of-operation.

Because of the high correlation between the data obtained from either system, the older, 15-cuvet GeMSAEC Fast Analyzer was replaced with the miniature analytical system on July 1, 1973. For the past 5 months the

Table 8. Correlation of the Data Obtained from a 15-Place GeMSAEC and a Miniature Fast Analyzer (MA)

Date	Glucose			Alkaline Phosphatase[c]			LDH-L			SGOT		
	GeMSAEC[a]	MA[a]	CC[b]	GeMSAEC[c]	MA[c]	CC[b]	GeMSAEC[c]	MA[c]	CC[b]	GeMSAEC[c]	MA[c]	CC[b]
6-15-73	97	100	0.9980	60	67	0.9985	81	82	0.9941	14.0	15.0	0.9560
6-20-73	98	114	0.9810	60	63	0.9984	91	92	0.9979	14.0	12.3	0.8940
6-21-73	104	108	0.9985	57	60	0.9995	91	82	0.9985	14.0	14.4	0.8950
6-22-73	88	83	0.9973	50	53	0.9977	88	83	0.9973	24.5	23.2	0.9995
6-25-73	114	116	0.9977	50	51	0.9995	88	82	0.9752	17.9	13.6	0.8520
6-26-73	94	102	0.9982	54	61	0.9993	90	82	0.9987	22.7	20.5	0.9333
6-27-73	106	116	0.9984	53	62	0.9993	85	77	0.9979	20.1	18.2	0.9111
7-3-73	95	111	0.9988	59	62	0.9899	91	89	0.9774	17.0	15.0	0.9415
7-5-73	100	103	0.9970	60	63	0.9920	87	85	0.9930	14.7	12.9	0.8895
7-9-73	111	121	0.9970	61	67	0.9990	83	78	0.9970	15.2	15.6	0.9147
7-10-73	119	122	0.9980	57	62	0.9990	81	72	0.9970	12.8	12.0	0.9891
7-11-73	107	119	0.9960	66	62	0.9997	79	67	0.9980	14.4	14.2	0.9774
7-12-73	101	109	0.9940	60	61	0.9980	77	74	0.9991	13.1	13.8	0.9733
7-13-73	97	104	0.9970	68	66	0.9980	88	83	0.9980	13.5	13.2	0.9742
7-17-73	99	104	0.9967	72	72	0.9981	87	85	0.9950	14.4	14.6	0.9624
Average	102	108	0.9962	59	62	0.9977	86	81	0.9942	16.1	15.2	0.9375

[a] Average daily mean result (mg/dl).

[b] Coefficient of correlation.

[c] Average daily mean result (I.U./liter, 30°C).

miniature system has been routinely operated in the ORNL Clinical Laboratory, and the downtime has been minimal (<1 day). The only serious problem encountered has been incomplete cleaning of the rotors between analytical runs; this was eliminated by increasing the efficiency and duration of the cleaning and drying cycles of the rotor cleaning station. A minor problem was encountered with leaking seals in the pumps used in the dispensers used in the sample-reagent loader; this problem has been eliminated by periodic replacement of the pump seals.

4.5.4 Development of an On-Line Quality Control Program

For every rotor routinely analyzed in the miniature analytical system, cuvets 2, 3, and 4 are dedicated to quality control (QC) samples (the only exception is the triglyceride assay in which cuvet 2 is a reagent blank channel with cuvets 3 and 4 being QC cuvets). A computer program was developed to automatically store this QC data on the cassette tape after each analytical run. These data may be recalled, statistically processed, and utilized in the overall QC program. Four program files are used in the QC program: The first file (the FOCAL text of which is listed in Fig. 24) is used to initialize a tape and reserve specified storage locations on the tape for a particular chemistry method. In general, storage location for 6 months of QC data is reserved for each chemistry. The second file (Fig. 25) is used to store the QC data after each run. To recall the data, one program (Fig. 26) will statistically process all of the stored data and print the data in the format previously shown in Fig. 23. An additional program (Fig. 27) will print all of the stored data in a chemistry-date-run number-result format. These QC programs were incorporated into the general operating program on October 1, 1973.

5. ACKNOWLEDGMENTS

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```
C-GEMSAEC 2A 730643W

01.01 C-INITIALIZATION OF ALL QC FILES (CAUTION WHEN USING)
01.11 S TB=2000
01.21 S DU=FSTR(TB,-1);S DU=FSTR(TB+64,-1);S SU=TB+128
01.50 A !!!"ENTER DESIRED CODE "CO
01.54 IF (CO-401)1.56,2.1,1.56
01.56 IF (CO-402)1.58,2.1,1.58
01.58 IF (CO-403)1.6,2.1,1.6
01.60 IF (CO-404)1.7,2.1,1.7
01.70 IF (411-CO)1.8,2.1,1.8
01.80 IF (405-CO)1.82,2.2,1.82
01.82 IF (408-CO)1.84,2.3,1.84
01.84 IF (409-CO)1.86,2.4,1.86
01.86 IF (410-CO)1.87,2.5,1.87
01.87 IF (800-CO)1.88,1.88,1.88
01.88 IF (900-CO)1.89,1.89,1.89
01.89 IF (412-CO)1.90,2.6,1.90
01.90 IF (700-CO)1.99,2.6,1.99
01.99 T !!"ILLEGAL CODE ";G 1.5

02.10 S E=1;D 3;G 1.5
02.20 S E=2;D 3;G 1.5
02.30 S E=3;D 3;G 1.5
02.40 S E=4;D 3;G 1.5
02.50 S E=5;D 3;G 1.5
02.60 S E=6;D 3;G 1.5

03.01 S C=-1
03.02 S C=C+1; IF (FSTR(TB+64+2*C))3.06,3.11,3.11
0
 3.06 D 4;D 5;S SU=SU+17+5*NU;G 3.51
03.11 IF (FSTR(TB+64+2*C)-E)3.02,3.16,3.02
03.16 D 7;R
03.51 S DU=FSTR(TB+64+2*C,E)
03.56 S DU=FSTR(TB+64+2*C+1,CO)
03.61 S C=C+1;S DU=FSTR(TB+64+2*C,-1);R

04.01 A !"HOW MANY DATA SETS IN TABLE",NU;IF (NU)4.01,4.01;D 4.15
04.02 IF (SU+17+NU*5-32768)4.06;G 4.01
04.06 S DU=FSTR(SU,0);S DU=FSTR(SU+1,NU)
04.11 F I=1,3;S TI=SU+(I-1)*5+2;D 6;R
04.15 IF (NU-FITR(NU))4.01,4.02,4.01
04.16 R

05.01 S A=-1
05.06 S A=A+1;IF (FSTR(TB+2*A))5.11,5.06,5.06
05.11 S DU=FSTR(TB+2*A,CO);S DU=FSTR(TB+2*A+1,SU)
0
 5.16 S DU=FSTR(TB+2*(A+1),-1);R

06.01 S DU=FSTR(TI,1E600);S DU=FSTR(TI+1,-1E600)
06.06 S DU=FSTR(TI+2,0);S DU=FSTR(TI+3,0)
06.07 T !"APPROXIMATE VALUE OF CONTROL#(",I,")=";A VL
06.16 S DU=FSTR(TI+4,VL);R

07.01 S TC=FSTR(TB+2*C+65)
07.06 S B=-1
07.11 S B=B+1;IF (FSTR(TB+2*B)-TC)7.11,7.16,7.11
07.16 S T2=SU;S SU=FSTR(TB+2*B+1)
07.21 D 5;S SU=T2;R
*
```

Fig. 24. FOCAL Text of Computer Program Used to Initialize a Cassette Tape for Storage of Quality Control Data.

```
C-GEMSAEC 2A 730643W

01.01 C-FIND STORAGE FILE FOR QC DATA;UPDATE WITH NEW DATA
01.03 A !!"IS QC DATA ACCEPTABLE (YES OR NO)"AN
01.04 IF (AN-0YES)1.05,1.08,1.05
01.05 IF (AN-0NO)1.03,1.54,1.03)
01.08 S TB=2000
01.09 S C=TB
01.10 IF (FSTR(C))1.11,1.16,1.16
01.11 T !!"THERE IS NO SUCH CODE AVAILABLE ";G 1.54
01.16 IF (FSTR(C)-CODE)1.21,1.26,1.21
01.21 S C=C+2;G 1.10
01.26 S SU=FSTR(C+1)
01.30 G 2.01
01.54 IF (CO-401)1.56,3.1,1.56
01.56 IF (CO-402)1.58,3.1,1.58
01.58 IF (CO-403)1.6,3.1,1.6
01.60 IF (CO-404)1.7,3.1,1.7
01.70 IF (411-CO)1.8,3.1,1.8
01.80 IF (405-CO)1.82,3.2,1.82
01.82 IF (408-CO)1.84,3.3,1.84
01.84 IF (409-CO)1.86,3.4,1.86
01.86 IF (410-CO)1.87,3.5,1.87
01.87 IF (800-CO)1.88,3.80,1.88
01.88 IF (900-CO)1.89,3.70,1.89
01.89 IF (412-CO)1.90,3.6,1.90
01.90 IF (700-CO)1.99,3.6,1.99
01.99 T !!"ILLEGAL CODE ";D 3.7

02.01 F I=1,3;S II=SU+2+(I-1)*5;D 5
02.06 S TU=FSTR(SU,FSTR(SU)+1)-1
02.11 S TS=FSTR(SU+1)
02.16 S PS=TU-FITR(TU/TS)*TS;D 6
02.21 G 1.54

03.10 L G 5,G 4.41
03.20 L G 18,G 4.41
03.30 L G 2,G 6.40
03.40 L G 3,G 6.40
03.50 L G 4,G 6.40
03.60 L G 8,G 4.41
03.70 L G 0,G 1.5
03.80 L R;Q

0
 5.01 IF (CN(I+1)-FSTR(II))5.06,5.11,5.11
05.06 S DU=FSTR(II,CN(I+1))
05.11 IF (FSTR(II+1)-CN(I+1))5.16,5.21,5.21
05.16 S DU=FSTR(II+1,CN(I+1))
05.21 S DU=FSTR(II+2,FSTR(II+2)+CN(I+1))
05.26 S DU=FSTR(II+3,FSTR(II+3)+CN(I+1)*CN(I+1))
05.31 R

06.01 S DU=FSTR(SU+17+PS*5,DY)
06.02 S DU=FSTR(SU+17+PS*5+1,RN)
06.03 F I=1,3;S DU=FSTR(SU+17+PS*5+I+1,CN(I+1))
06.04 R
*
```

Fig. 25. FOCAL Text of Computer Program Used to Store Quality Control Data After Every Analytical Run.

```
C-GEMSAEC 2A 730643W

01.01 C-RETRIEVE AND PRINT OUT OF QC DATA
01.02 T !!!"QUALITY CONTROL DATA"
01.04 S DY=FDAY(0)
01.06 T !!!"DAY"%3,DY
01.28 D 10
01.30 S CO=401;G 1.54
01.31 S CO=700;G 1.54
01.32 S CO=405;G 1.54
01.34 S CO=408;G 1.54
01.36 S CO=410;G 1.54
01.38 S CO=409;G 1.54
01.54 IF (CO-401)1.56,3.1,1.56
01.56 IF (CO-402)1.58,3.1,1.58
01.58 IF (CO-403)1.6,3.1,1.6
01.60 IF (CO-404)1.7,3.1,1.7
01.70 IF (411-CO)1.8,3.1,1.8
01.80 IF (405-CO)1.82,3.2,1.82
01.82 IF (408-CO)1.84,3.3,1.84
01.84 IF (409-CO)1.86,3.4,1.86
01.86 IF (410-CO)1.87,3.5,1.87
01.87 IF (800-CO)1.88,3.80,1.88
01.88 IF (900-CO)1.89,3.70,1.89
01.89 IF (412-CO)1.90,3.6,1.90
01.90 IF (700-CO)1.99,3.6,1.99
01.92 L G 0,G 1.15
01.99 T !!"ILLEGAL CODE "%Q

02.01 C-LOCATION OF TEST STORAGE FILE AND STATISTICAL CALCULATION"
02.10 S TB=2000
02.12 S C=TB
02.14 IF (FSTR(C))2.16,2.18,2.18
02.16 T !!"NO FILE AVAILABLE";R
02.18 IF (FSTR(C)-CODE)2.20,2.22,2.20
02.20 S C=C+2;G 2.14
02.22 S SU=FSTR(C+1)
02.40 F I=1,3;S II=SU+2+(I-1)*5;S NS(I)=FSTR(SU);D 5
02.50 R

03.10 D 2;D 8.1;D 9.6;G 1.31
03.20 D 2;D 8.2;D 9.6;G 1.34
03.30 D 2;D 8.3;D 9.6;G 1.36
03.40 D 2;D 8.4;D 9.6;G 1.92
03.50 D 2;D 8.5;D 9.6;G 1.38
03.60 D 2;D 8.6;D 9.7;G 1.32

05.01 S XB(I)=FSTR(II+4)-(FSTR(II+4)-FSTR(II+2)/NS(I))
05.06 S SG(I)=FSQT[(NS(I)*FSTR(II+3)-FSTR(II+2)^2)/NS(I)*(NS(I)-1)]
05.11 S CV(I)=(SG(I)/XB(I))*100
05.16 S LL(I)=FSTR(II);S UL(II)=FSTR(II+1)
05.20 R

08.10 T !!"GLUCOSE      "
08.20 T !!"URIC ACID    "
08.30 T !!"ALK. P'HASE  "
08.40 T !!"SGOT         "
08.50 T !!"LDH-L        "
08.60 T !!"TRIGLYCERIDE"

09.10 T " M-1   "%3,NS(1)," "%6.01,XB(1)," "%5.02,SG(1),"    ",CV(1);D 92
09.12 T %6.01, XB(1)-2*SG(1)," -"%4.01,XB(1)+2*SG(1),
09.20 T !"              M-2   "%3,NS(2)," "%6.01,XB(2)," "%5.02,SG(2);D 9
09.22 T "   ",CV(2),""%6.01,XB(2)-2*SG(2)," -"%4.01,XB(2)+2*SG(2)
09.30 T !"              PS    "%3,NS(3)," "%6.01,XB(3)," "%5.02,SG(3);D 9
09.32 T "   ",CV(3),""%6.01,XB(3)-2*SG(3)," -"%4.01,XB(3)+2*SG(3)
09.40 T " STD#1 "%3,NS(2)," "%6.01,XB(2)," "%5.02,SG(2),"    ",CV(2);D 92
09.42 T %6.01, XB(2)-2*SG(2)," -"%4.01,XB(2)+2*SG(2)
09.50 T !"              STD#2 "%3,NS(3)," "%6.01,XB(3)," "%5.02,SG(3);D 9
09.52 T "   ",CV(3),""%6.01,XB(3)-2*SG(3)," -"%4.01,XB(3)+2*SG(3)
09.60 D 9.1;D 9.2;D 9.3;R
09.70 D 9.4;D 9.5;R

10.10 T !!!"ASSAY       CONTROL   N     MEAN    SIGMA     C.V.      "
10.20 T "95% RANGE"
*
```

Fig. 26. FOCAL Text of Computer Program Used to Recall and Statistically Process and Print Stored Quality Control Data.

```
C-GEMSAEC 2A 730643W

01.01 C-RETRIEVE AND PRINT OUT OF ALL STORED QC DATA
01.02 T !!!"QUALITY CONTROL DATA"
01.04 S DY=FDAY(0)
01.06 T !!!"DAY"%3,DY
01.28 D 10
01.30 S CO=401;G 1.54
01.31 S CO=700;G 1.54
01.32 S CO=405;G 1.54
01.34 S CO=408;G 1.54
01.36 S CO=410;G 1.54
01.38 S CO=409;G 1.54
01.40 S CO=800
01.54 IF (CO-401)1.56,4.1,1.56
01.56 IF (CO-402)1.58,4.1,1.58
01.58 IF (CO-403)1.6,4.1,1.6
01.60 IF (CO-404)1.7,4.1,1.7
01.70 IF (411-CO)1.8,4.1,1.8
01.80 IF (405-CO)1.82,4.2,1.82
01.82 IF (408-CO)1.84,4.3,1.84
01.84 IF (409-CO)1.86,4.4,1.86
01.86 IF (410-CO)1.87,4.5,1.87
01.87 IF (800-CO)1.88,4.80,1.88
01.88 IF (900-CO)1.89,4.70,1.89
01.89 IF (412-CO)1.90,4.6,1.90
01.90 IF (700-CO)1.99,4.6,1.99
01.92 T !!;D 1.93
01.93 L R;Q
01.99 T !!"ILLEGAL CODE ";Q

03.01 C-SPECIAL ROUTINE TO RECALL AND PRINT DAILY QC DATA
03.02 S TB=2000;S C=TB
03.03 IF (FSTR(C))3.04,3.05,3.05
03.04 T !!"NO FILE AVAILABLE";R
03.05 IF (FSTR(C)-CO)3.06,3.07,3.06
03.06 S C=C+2;G 3.03
03.07 S SU=FSTR(C+1)
03.08 S JJ=FSTR(SU);S JK=FSTR(SU+1)
03.09 IF (JJ)3.61,3.61,3.11
03.11 S LP=JJ-FITR(JJ/JK)*JK-1
03.16 IF (-LP)3.21,3.21;S LP=JK-1
03.21 IF (JJ-JK)3.26,3.31,3.31
03.26 S P1=0;G 3.36
03.31 S P1=JK-1-LP
03.36 IF (LP-P1)3.41,3.56,3.56
03.41 F II=P1,JK-1;S PS=SU+17+II*5;D 5
03.46 F II=0,LP;S PS=SU+17+II*5;D 5
03.51 G 3.61
03.56 F II=P1,LP;S PS=SU+17+II*5;D 5
03.61 R

04.10 D 8.1;D 9.1;D 3;G 1.31
04.20 D 8.2;D 9.3;D 3;G 1.34
04.30 D 8.3;D 9.1;D 3;G 1.36
04.40 D 8.4;D 9.1;D 3;G 1.92
04.50 D 8.5;D 9.1;D 3;G 1.38
04.60 D 8.6;D 9.2;D 3;G 1.32
04.80 T !!!; L R,Q

05.10 IF (700-CO)5.12,5.30,5.12
05.12 IF (412-CO)5.14,5.30,5.14
05.14 IF (405-CO)5.16,5.16,5.16
05.16 T !%3,"                ",FSTR(PS),"      ",%2,FSTR(PS+1)
05.17 T "      ",%6.01,FSTR(PS+2),"    ",FSTR(PS+3),"  "FSTR(PS+4);R
05.20 R
05.30 T !%3"                 ",FSTR(PS),"      ",%2,FSTR(PS+1)
05.32 T "      ",%6.01,FSTR(PS+3),"  "FSTR(PS+4)
05.40 R

08.10 T !!"GLUCOSE      "
08.20 T !!"URIC ACID    "
08.30 T !!"ALK. P'HASE  "
08.40 T !!"SGOT         "
08.50 T !!"LDH-L        "
08.60 T !!"TRIGLYCERIDE"

09.10 T "                            M-1       M-2";D 9.12
09.12 T "        PS";R
09.20 T "                            STD#1     STD#2";R
09.30 T "                            STD#1     STD#2     STD#3";R

10.10 T !!"ASSAY          DATE   RUN #          STORED  QC  DATA"
*
```

Fig. 27. FOCAL Text of Computer Program Used to Recall and Print All of the Stored Quality Control Data.

6. REFERENCES

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7. APPENDIX

CHEMICAL PROCEDURES USED WITH THE ORNL MINIATURE FAST ANALYTICAL SYSTEM

A. Glucose
B. Triglyceride
C. Uric Acid
D. Alkaline Phosphatase
E. LDH-L
F. SGOT

A. Serum Glucose

1. Reagent: Calbiochem glucose reagent kit: reconstitute by adding 2 ml of H_2O to Vial B (NADP), swirl to dissolve, and add contents to a vial of A (glucose reagent). Cap and dissolve contents by gentle inversion.
2. Sample: blood serum
3. Wavelength: 340 nm
4. Temperature: 30°C
5. Loading Volumes
   a. Reagent Pipette
      Reagent Pump 50 µl at 40% = 20 µl
      Diluent Pump 50 µl at 100% = 50 µl
   b. Sample Pipette
      Sample Pump 20 µl at 10% = 2 µl
      Diluent Pump 50 µl at 100% = 50 µl
6. Procedure
   a. Set sample-reagent loader in single-chemistry:multi-sample mode
   b. Place rotor on turntable
   c. Move sample and reagent probes in position and depress start button
   d. Place loaded rotor into analyzer and proceed as indicated in the Analyzer manual
7. SF = 353

B. Serum Triglyceride

1. Reagent:

   a. Reagent 1: Calbiochem 3-vial triglyceride reagent kit: reconstitute by adding 4 ml of H_2O to the contents of Vial A (ATP, PEP, NADH, PK, LDH, buffer), swirl to dissolve, and add contents to Vial C (lipase)

   b. Reagent 2: Vial B containing the glycerol kinase is made up to a volume of 2.0 ml and is subsequently refrigerated at 4°C until use

2. Sample: blood serum

3. Wavelength: 340 nm

4. Temperature: 30°C

5. Loading Volumes

   a. Reagent Pipette (H_2O diluent)
   Reagent Pump 50 µl at 60% = 30 µl
   Diluent Pump 50 µl at 80% = 40 µl

   b. Sample Pipette
   Sample Pump 20 µl at 20% = 4 µl
   Diluent Pump 50 µl at 60% = 30 µl

6. Procedure

   a. Set sample-reagent loader in single-chemistry:multi-sample mode

      1) Sequentially place water samples into cups 1 and 2 of the sample carousel

      2) Sequentially place samples into cups 3-17 of the sample carousel

      3) Place reagent 1 into reagent cup

   b. Place rotor on turntable

   c. Move sample and reagent probes into position and depress start button

   d. Place rotor into analyzer and transfer and mix the aliquots of samples and reagents into their respective cuvets

   e. Remove rotor, cover with Lucite cover, and allow to stand 10 min

   f. Using an Oxford pipette, add 20 µl of H_2O to sample cavity 1 and 20 µl aliquots of reagent 2 to sample cavities 2-17

   g. After hydrolysis, place the rotor in the analyzer and proceed as indicated in the Analyzer manual

7. All samples that exceed 400 mg/dl should be diluted with saline azide solution and rerun

8. SF = 880.0

C. Uric Acid

1. Reagent:

   a. 1.0 *M* borate buffer, pH 9.1

   b. Nova Industry bacterial uricase: reconstitute by dissolving contents of vial in 200 ml of 1 *M* borate buffer, pH 9.1. Refrigerate at 4°C. (Does not need to be made up fresh every day, should be used until gone.)

2. Sample: blood serum

3. Filter: 292 nm (deuterium source, quartz rotor)

4. Temperature: 30°C

5. Loading Volumes (Sample-Reagent Loader)

   a. Reagent Pipette
   Reagent Pump 50 µl at 30% = 15 µl
   Diluent Pump 50 µl at 100% = 50 µl

   b. Sample Pipette
   Sample Pump 20 µl at 25% = 5 µl
   Diluent Pump 50 µl at 100% = 50 µl

6. Procedure

   a. Set sample-reagent loader on single-chemistry:multi-sample mode

      1) Sequentially place samples into the cups in the sample carousel

      2) Place reagent into reagent cup

   b. Place rotor on turntable

   c. Move sample and reagent probes into position and depress start button

   d. Place loaded rotor into analyzer and proceed as indicated in the Analyzer manual

7. SF = 66

D. Alkaline Phosphatase

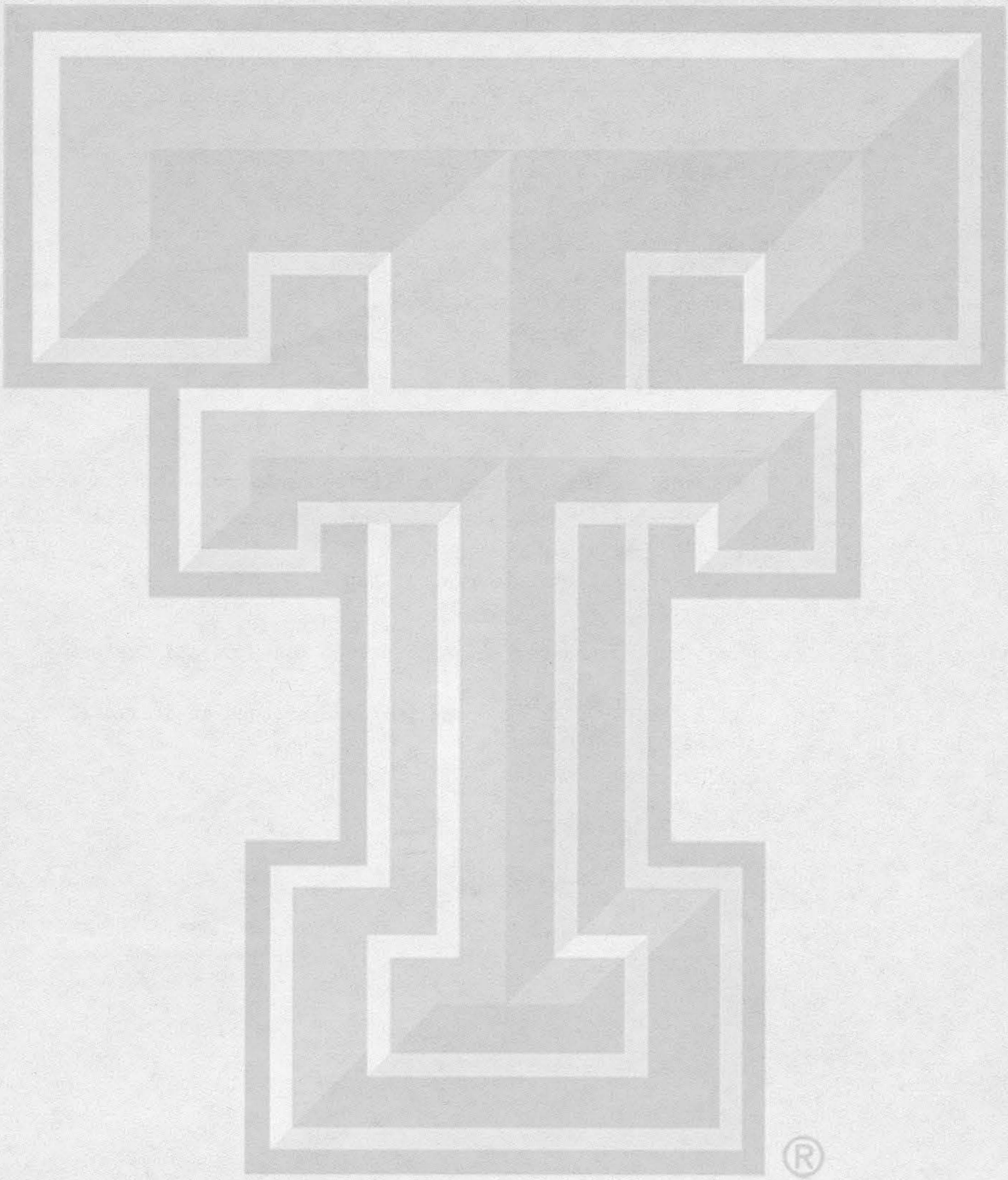
1. Reagent: Calbiochem alkaline phosphatase kit: reconstitute by adding 2 ml of Vial A (buffer) to Vial B (substrate). Cap, and dissolve contents by gentle inversion.
2. Sample: blood serum
3. Filter: 400 nm
4. Temperature: 30°C
5. Loading Volumes (Sample-Reagent Loader)
   a. Reagent Pipette
      Reagent Pump 50 µl at 40% = 20 µl
      Diluent Pump 50 µl at 100% = 50 µl
   b. Sample Pipette
      Sample Pump 20 µl at 50% = 10 µl
      Diluent Pump 50 µl at 100% = 50 µl
6. Procedure
   a. Set sample-reagent loader on single-chemistry:multi-sample mode
      1) Sequentially place samples into the cups in the sample carousel
      2) Place reagent into reagent cup
   b. Place rotor on turntable
   c. Move sample and reagent probes into position and depress start button
   d. Place loaded rotor into analyzer and proceed as indicated in the Analyzer manual
7. KE = 1400

E. Lactic Dehydrogenase-Lactate Substrate (LDH-L)

1. Reagent: Calbiochem LDH-L reagent kit: reconstitute by adding 2 ml of H_2O to Vial B (NAD), swirl to dissolve, and add to Vial A (LDH-L reagent). Cap, and dissolve contents by gentle inversion.
2. Sample: blood serum
3. Wavelength: 340 nm
4. Temperature: 30°C
5. Loading Volumes (Sample-Reagent Loader)
   a. Reagent Pipette
      Reagent Pump 50 µl at 40% = 20 µl
      Diluent Pump 50 µl at 100% = 50 µl
   b. Sample Pipette
      Sample Pump 20 µl at 50% = 10 µl
      Diluent Pump 50 µl at 100% = 50 µl
6. Procedure
   a. Set sample-reagent loader in single-chemistry:multi-sample mode
      1) Sequentially place samples into the cups in the sample carousel
      2) Place reagent into reagent cup
   b. Place rotor on turntable
   c. Move sample and reagent probes into position and depress start button
   d. Place loaded rotor into analyzer and proceed as indicated in Analyzer manual
7. KE = 4141

F. Glutamate Oxaloacetate Transaminase (SGOT)

1. Reagent: Calbiochem GOT reagent kit: reconstitute by adding 2 ml of H_2O to Vial B (NADH), swirl to dissolve, and add to Vial A (GOT reagent). Cap, and dissolve contents by gentle inversion.
2. Sample: blood serum
3. Filter: 340 nm
4. Temperature: 30°C
5. Loading Volumes (Sample-Reagent Loader)
   a. Reagent Pipette
      Reagent Pump 50 µl at 40% = 20 µl
      Diluent Pump 50 µl at 100% = 50 µl
   b. Sample Pipette
      Sample Pump 20 µl at 100% = 20 µl
      Diluent Pump 50 µl at 80% = 40 µl
6. Procedure
   a. Set sample-reagent loader in single-chemistry:multi-sample mode
      1) Sequencially place samples into the cups in the sample carousel
      2) Place reagent into reagent cup
   b. Place rotor on turntable
   c. Move samples and reagent probes into position and depress start button
   d. Place loaded rotor into analyzer and proceed as indicated in the Analyzer manual
7. KE = 2070



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