Development of a Miniature Fast Analyzer

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Design features and operation of a prototype miniaturized Fast Analyzer are described, and some results obtained with it are presented. The Analyzer occupies only one cubic foot of space. It has a 17-cuvet plastic rotor that rotates through a stationary optical system at speeds up to 5000 rpm. The resulting centrifugal force is utilized to transfer and mix a series of sample(s) and reagent(s) into the cuvets. The ensuing reactions are monitored spectrophotometrically, and the data evaluated in real time by an on-line computer. Samples (1 to 10 μl) and reagents (70 to 110 μl) are loaded into the rotor either discretely or dynamically; various rotor configurations can be used to do this. Many of the standard clinical analyses, including most of the NADH-linked enzymatic analyses, have been adapted for use with this analyzer. Precision obtained ranges from 1 to 4%. This report considers, specifically, analyses of some serum enzymes. Results show that the small analyzer possesses the previously demonstrated advantages of Fast Analyzers and, in addition, has several beneficial features arising from miniaturization.

Additional Keyphrases *small sample volume* • *low reagent costs* • *rapid clinical analyses* • *disposable plastic rotors* • *spatial economy* • GEMSAEC • *enzyme activity measurements*

Fast Analyzers (*1–11*), also commonly known as GEMSAEC Fast Analyzers[3], are now being used in several clinical laboratories. In these machines centrifugal force is used to transfer and mix together samples and reagent(s) in a multicuvet rotor. A stationary photometer scans the cuvets during rotation. The signals thus generated are acquired and evaluated in real time by an on-line computer, allowing the reaction courses to be observed as they occur. Since all reactions are initiated simultaneously and are coupled with the continuous referencing of the spectrophotometric system of the analyzer, errors caused by electronic, mechanical, or chemical drift are minimized. In addition to the increased precision and accuracy obtainable with such instruments, significant advantages include: (*a*) low sample and reagent volume requirements (and hence low reagent cost); (*b*) high sample analysis rate and; (*c*) compatibility with true automation, i.e., feedback control.

A previous report (*12*) discussed the feasibility of miniaturizing a Fast Analyzer since a miniature system would be desirable for several reasons. For example, a miniature, compact unit would require a minimum of valuable laboratory space and be easily portable. In addition, its sample and reagent

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[3] Nonstandard abbreviations and nomenclature used: GEMSAEC, an acronym for *G*eneral *M*edical *S*ciences *A*tomic *E*nergy *C*ommission: ALP, alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1); CK, creatinine phosphokinase (ATP:creatinine phosphotransferase, EC 2.7.3.2); HBD, α-hydroxybutyrate dehydrogenase; LD-L, lactate dehydrogenase-lactate substrate (NAD oxidoreductase, EC 1.1.1.27); AST, serum glutamic oxaloacetic transaminase (L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1); ALT, serum glutamic-pyruvic transaminase (L-alanine:2-oxoglutarate aminotransferase, EC 2.6.1.2); NADH, nicotinamide-adenine dinucleotide, reduced.

volume requirements would be decreased to 1–10 μl and 70–110 μl, respectively. Also, the small rotors used in a miniature analyzer could be inexpensively manufactured and therefore could be considered disposable, thus eliminating the time-consuming tasks of washing and drying the rotor and the transfer disk between analytical runs now required in the operation of Fast Analyzers (*11*). It would be desirable to design the disposable rotors for dynamic loading, to eliminate problems arising from discrete dispensing of samples and reagents. Further, disposable rotors could be preloaded with reagents which could then be lyophilized and at the time of analysis reconstituted with water or buffer, thus minimizing and standardizing reagent preparation. Like the larger analyzers, the miniature one can be operated in either the single-chemistry:parallel-samples or the single-sample:parallel-chemistries mode of operation. In addition to the above special advantages, a miniaturized Fast Analyzer would have the same desirable features as the larger analyzer with regard to parallel analyses, precision, accuracy, and date reduction (*1, 7, 9, 11*).

With these considerations in mind, a miniature Fast Analyzer prototype has been designed and fabricated. The objectives of this report are to describe the design and operation of this prototype and to discuss its initial evaluation.

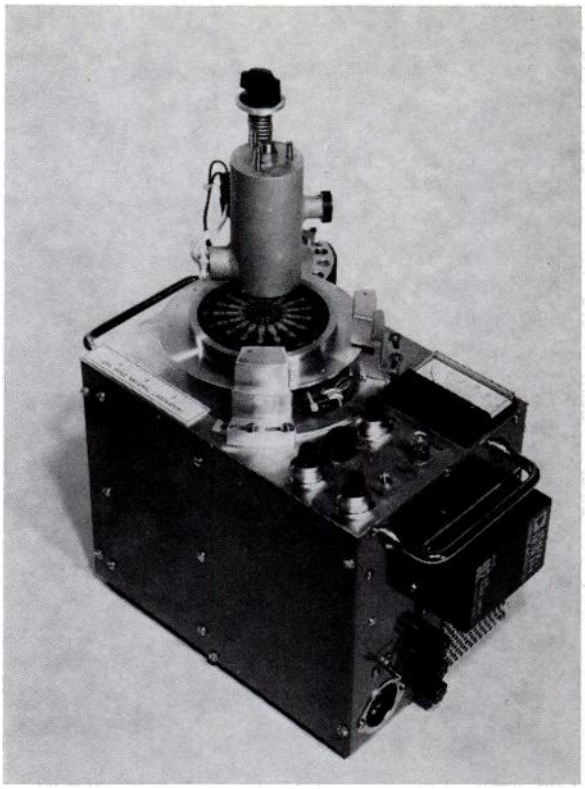

Fig. 1. Miniature Fast Analyzer: Side view (scale in inches)

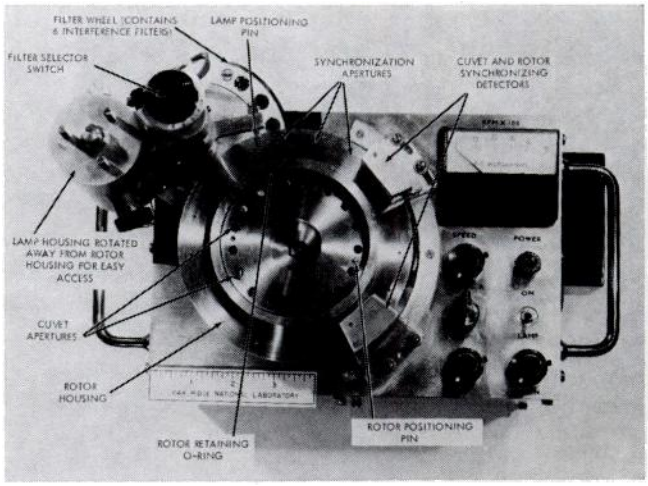

Fig. 2. Miniature Fast Analyzer: Top view

Materials and Methods

Instrumentation

Miniature Fast Analyzer. The miniature Fast Analyzer is shown in Figures 1 and 2. The analyzer is compact, requiring one cubic foot of space and weighing only 25 lb. It is contained in (and on) a small, sheet-metal cabinet (10 × 7 × 8 in). The heart of the system is a 17-cuvet rotor, which, when placed in the rotor support housing, is rotated through a stationary optical system at the normal operating speed of 500 rpm. Provisions are available for accelerating the rotor to speeds as high as 5000 rpm. Once placed and indexed in its housing, the rotor is retained in position by means of an O-ring. The rotor turns with the rotor housing, which is directly driven by a dc servomotor. This, in conjunction with a tachometer generator, is situated in the instrument cabinet (Figure 3).

Synchronization signals for the computer and analog display scope are provided by the rotor and cuvet synchronization detectors shown in Figure 2. Signals are generated when the synchronization apertures, located in the rim of the rotor housing, pass through the photometric detectors and allow light from a small tungsten lamp to reach and activate a photodiode. One detector provides a rotor signal on every revolution of the rotor and the other a cuvet signal just after each cuvet has passed through the optical system. The use of these signals to synchronize the analyzer output with the computer has been previously discussed (*8*). Proper synchronization is accomplished by moving either photometric detector along the slide bar until the desired adjustment is achieved. At this point, both detectors are physically secured to the top of the instrument cabinet by means of locking screws.

Optical system. The optical system (see Figure 4) consists of a light source located in a movable housing mounted above the rotor and a miniature photomultiplier located below the rotor. A quartz-

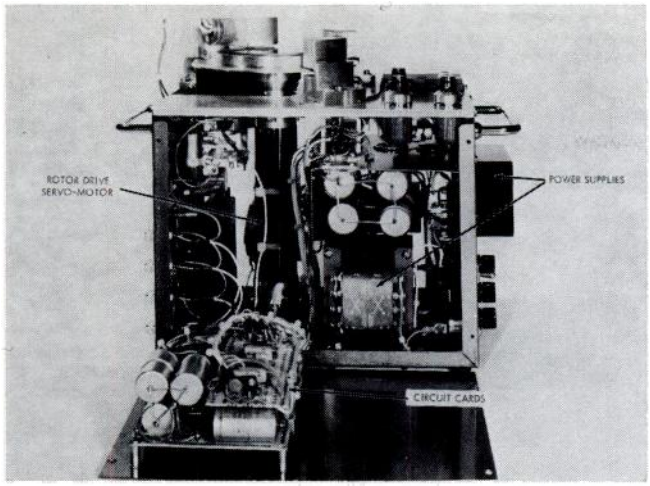

Fig. 3. Miniature Fast Analyzer: Inside cabinet view

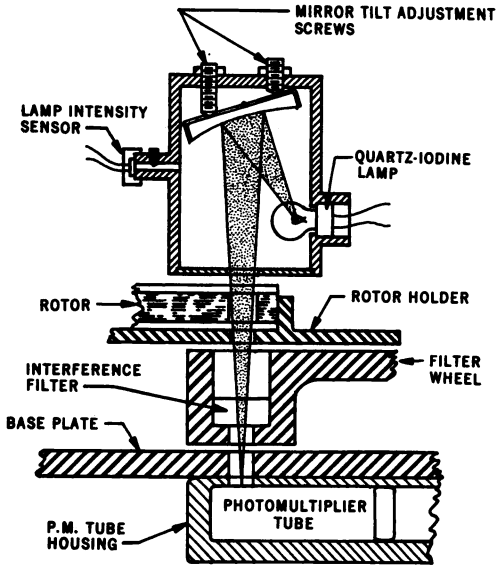

Fig. 4. Optical system of the miniature Fast Analyzer

iodine lamp (GE 1974, 20 W, 6 V; General Electric Co., Charlotte, N. C.) and a concave mirror are contained in the movable aluminum housing, which can be rotated away from the rotor housing area. As shown in Figure 5, this facilitates the insertion and removal of the rotor from its housing. Provisions have also been made for radial, azimuth, and height adjustments of the lamp housing. The concave mirror can be adjusted so that the image of the filament of the lamp is focused on the photomultiplier tube. The miniature photomultiplier tube (Type HTV R-300; Hamamatsu Corp., Lake Success, N. Y. 11040) is mounted in a shock-protected holder located just under the baseplate, which supports the motor and filter wheel. Light of the proper wavelength is obtained by placing an interference filter in the optical light path. The six interference filters (340, 405, 415, 485, 560, and 620 nm; Spectro-Film Inc., Winchester, Mass. 01890) are contained in an adjustable filter wheel, which is mounted just under the rotor housing.

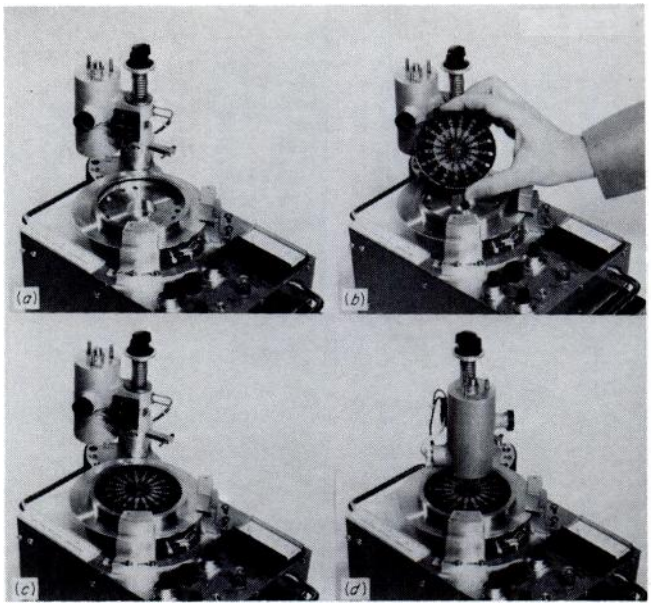

Fig. 5. Placing of rotor into the housing of the miniature Fast Analyzer

(*a*) lamp housing rotated away from rotor for easy access, (*b*) rotor aligned by matching pin in rotor housing with hole in rotor, (*c*) rotor snapped into place and retained by O-ring, (*d*) lamp housing moved into monitoring position

The appropriate filter is placed in the light path by means of the filter selector switch. Each of the filters has a band width of 10 nm at one-half transmission and a minimum peak transmission ranging from 20% for the 340-nm filter to 50% for the 620-nm unit. For each filter, the photomultiplier voltage is adjusted to a signal level of 9 to 10 V by means of the H.V.-labeled potentiometer located on the top of the instrument (Figure 2).

An oscilloscope, which is used to display the signals generated by the photomultiplier tube of the optical system, results in 17 signals representing the light transmittance of the individual cuvets (Figure 6). In this type of display, the upper baseline represents 0% transmittance (infinite absorbance), while the full-scale signal obtained with cuvet 1 represents 100% transmittance (zero absorbance). The signals from the other cuvets can then ae compared with the 100% transmittance signal of cuvet 1 to obtain their relative transmittance.

Rotor design. A 17-place rotor (shown in Figure 7) was designed and fabricated for use with the miniature Fast Analyzer. This rotor is an integrated multicuvet rotor and transfer disk, and is a multifunctional device in that sample(s) or reagent(s) can be loaded either discretely or dynamically. With the discrete loading side of the rotor up, sample(s) and reagent(s) are loaded discretely into either of two concentric series of cavities, which are then transferred into their respective cuvets when rotation begins. With the dynamic loading side up, solutions can be loaded-

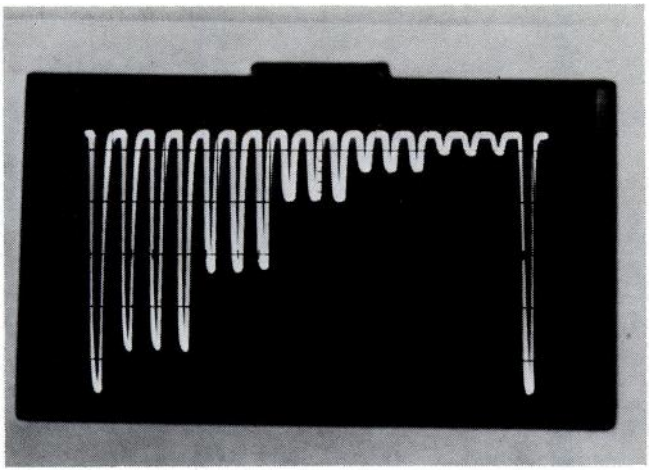

Fig. 6. Photomultiplier signals obtained at 405 nm from the miniature Fast Analyzer as displayed on an oscilloscope

(The first cuvet is filled with water. The remaining cuvets are filled with various solutions of *p*-nitrophenol in 0.1 *M* Tris buffer)

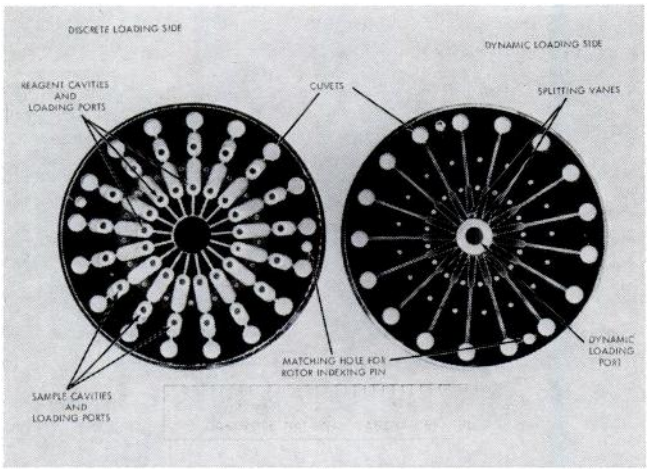

Fig. 7. Seventeen-place rotor used in the miniature Fast Analyzer (scale in inches)

dynamically into the spinning rotor and the resulting apportioned aliquots simultaneously transferred into their respective cuvets.

Three modes of operation are possible with this rotor: (*a*) samples and reagents can be discretely dispensed into their individual cavities on the discrete loading side of the rotor and then transferred to the cuvets; (*b*) with the dynamic loading side of the rotor facing up, a reagent can be dynamically loaded, apportioned and aliquots of it transferred into the cuvets, the rotor can then be turned over and samples dispensed and transferred into the cuvets (in this mode, multiple samples are analyzed for a single component); and (*c*) a sample can be dynamically loaded and transferred, and a series of reagents can be discretely dispensed and transferred (in this mode, a single sample is analyzed for several components). Thus, this rotor is very versatile and has proved to be very useful in evaluating the analyzer and for developing chemical procedures for use with it.

Rotor fabrication. The rotor bodies are machined from a 0.5-cm-thick sheet of black acrylic plastic, and the windows are machined from a 3.2-mm-thick sheet of ultraviolet-transmitting acrylic plastic (Rohm and Haas, Philadelphia, Pa. 19105). This window material allows the rotor to be usable down to wavelengths as low as 300 nm (Figure 8). The black acrylic body also serves as an internal light mask for the cuvets.

An unassembled rotor consists of three separate parts (i.e., the body and two windows), which are joined and sealed together to produce the finished rotor. In screening various methods for accomplishing this task, gluing the components with a low-viscosity epoxy glue (CIBA Araldite 502 resin and 951 hardener, thinned with xylene to 20–30% xylene content) was found to be the most effective method.

Data acquisition. Fast Analyzers are analytical instruments that yield output data in a form and at a rate suitable for direct input into a computer (*3*, *10*, *11*). Consequently, both 15- and 42-cuvet Fast Analyzers have been successfully interfaced with the GEMSAEC computer module, which is built around a PDP-8/I computer (Digital Equipment Corp., Maynard, Mass. 01754) having 8K of fast memory and 64K words of disk memory (*11*). The miniature Fast Analyzer has been found to be completely compatible with the hardware and the software of the GEMSAEC computer module. Routine operating programs that have been found to be useful with the miniature analyzer include calibration, end-point, and enzyme rate analysis routines. These programs are written in FOCAL (a conversational computer language developed by Digital Equipment Corp.) and have been described elsewhere (*9*, *11*).

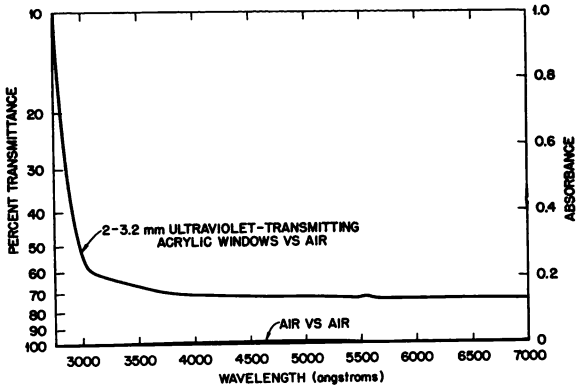

Fig. 8. Absorption spectrum of ultraviolet-transmitting acrylic windows used in the fabrication of the 17-place rotor. (Spectrum was obtained with a Cary 14 recording spectrophotometer)

Clinical Chemical Determinations

Assays studied. Standard clinical methods for serum albumin, bilirubin, blood urea nitrogen, calcium, glucose, protein, and uric acid and for the serum enzymes ALP, CK, HBD, LD-L, AST, and ALT have been adapted for use with the miniature Fast Analyzer. However, only the data obtained from the serum enzyme analyses are reported here.

Reagent kits, marketed under the trade name "Stat-Packs," were purchased from Calbiochem, Los Angeles, Calif. 90036. The two vials contained in each individual kit were reconstituted with distilled water and then mixed and diluted to a combined volume of 10 ml. Depending on the analysis, 70-μl aliquots of the appropriate reagent were metered into the reagent cavities of the 17-place rotor by manual actuation of a "Precision Liquid Dispenser" (Hamilton Co., Whittier, Calif. 90608). The 50-μl aliquots of serum samples that had been diluted five-fold with distilled water (equivalent to 10 μl of the original serum) were dispensed into the individual sample cavities by means of a "Sampler Pipette" (Oxford Laboratories, San Mateo, Calif. 94402).

The enzyme rate analyses computer program (*11*) was used to determine the various serum enzyme activities. By means of a linear regression curve-fitting routine, this program determines, within a preset time interval, the change of absorbance per minute, which is then multiplied by a preset enzyme factor to convert the rate of change of absorbance to enzyme units. Since the enzyme factor includes the molar absorptivity of the absorbing species, the sample volume, total reaction volume, and dilution factors, the enzyme activities are computed in terms of U per liter per minute. Immediately after each analytical run, the rotor temperature was manually determined and the computed enzyme activities were converted to U per liter per minute at 30.0 °C by application of the appropriate temperature factor. Computer parameters required by this program are listed in Table 1.

Sample preparation. To obtain serum samples having a wide range of enzyme activities, we obtained "Multi-Enzyme Reference Sera" from Hyland Laboratories, Costa Mesa, Calif. 92626. The contents of the individual vials were dissolved in 5.0 ml of distilled water and then diluted five-fold with distilled water. Sample volumes were 50 μl of the diluted serum (10 μl of the original serum) in each assay; reagent volume was 70 μl. The methods for ALP (*13*), CK (*14*), HBD (*15*), LD-L (*16*), AST (*17, 18*) and ALT (*18, 19*) were unmodified.

Operating procedure. Reagents and samples are manually loaded into their respective cavities in the rotor, as described earlier. The operator uses the teletype to call the required computer program from the memory disks; the program is then automatically loaded into the fast memory core of the computer. The required program constants and identifying information are added to the program through the teletypewriter. The computer subsequently gives a "ready-for-analysis" signal. The loaded rotor is placed in its rotor housing and is properly indexed by aligning the matching hole of the rotor with the location pin of the rotor housing and then pressing the rotor into the retaining O-ring. To prevent lateral splashing of liquid in the transfer disk, the rotor is gradually accelerated to 500 rpm with the rotor speed control. Next, the rotor is accelerated to 4000 rpm to ensure the transfer of small droplets of fluids adhering to the walls of the sample and reagent cavities of the rotor. Finally, the rotor is stopped by reversing the polarity of the current to the drive motor and allowing the motor to act as a brake. This reversal of current polarity is obtained when the rotor switch (Figure 2) is manually turned to the stop position. By alternately accelerating and braking the rotor, the contents of the individual cuvets are thoroughly mixed. After mixing is complete, the rotor is accelerated to its normal operating speed of 500 rpm and the computer program is started by manual closure of a relay contact. After the analytical run has been concluded (3 to 12 min), the rotor is stopped, removed from its housing, emptied by application of vacuum to the dynamic loading part on the bottom side of the rotor, and thoroughly washed by flowing distilled water through the rotor. The rotor is then rinsed with methanol and air dried. This emptying, washing, rinsing, and drying of the rotor requires about 2 min, after which the rotor is ready for reuse.

Table 1. Computer Operating Parameters Required for the Analysis of Various Serum Enzymes by Means of a Miniature Fast Analyzer[a]

Enzyme	Delay intervals	No. sets of observations	Total analysis time, min
ALP	60	3–10	2–5
CK	120	8–40	4–12
HBD	60	6–40	2–11
LD-L	60	8–30	3–9
AST	60	13–40	5–11
ALT	60	10–40	4–11

[a] 10 readings per interval; observation interval was 15 s.

Results

Optical Performance

Because many of the clinical chemistry determinations are performed by measuring the absorbance of NADH at 340 nm, the optical system of the miniature Fast Analyzer must provide a linear, accurate, and precise absorbance measurement at

this wavelength. Consequently, reference solutions of NADH in Tris buffer (0.1 mol/liter, pH 7.4) were prepared to have calculated absorbances ranging from 0.1 to 2.0 at 340 nm for a 0.5-cm cell. These solutions were loaded into a rotor and transferred; then their absorbances were measured. The resultant absorbances of the individual solutions are plotted against their NADH concentrations in Figure 9, demonstrating that the optical system of the miniature analyzer is linear up to an absorbance of 2.0 at 340 nm. By use of various dye solutions, the optical linearity of the analyzer was also confirmed at other wavelengths.

To evaluate the spectrophotometric accuracy of the analyzer, the data displayed in Figure 9 were utilized to calculate the molar absorptivity of NADH. This resulted in a calculated molar absorptivity for NADH of 6150, which agrees to within 1% of the accepted value of 6200 (*20*). As an external check, the absorbances of the NADH solutions were determined by using a Model 300 N spectrophotometer (Gilford Instrument Laboratories Inc.. Oberlin, Ohio 44074) and were found to be in agreement, to within 1%, with those from the miniature Fast Analyzer data.

In regard to spectrophotometric precision, the absorbances of the NADH solutions were repetitively determined by use of a tabular absorbance-versus-time computer program. With this program, the absorbances of the individual solutions were determined every 10 s for a total of 100 s. At each 10-s interval, the absorbances were determined by averaging the data obtained from ten successive revolutions of the rotor (i.e., 10 data points averaged per cuvet). The results thus obtained were statistically processed and yielded the data listed in Table 2. As may be seen, the spectrophotometric precision of the analyzer is quite satisfactory; as the absorbances of the individual solutions were determined with a variation of only ±0.02 to 0.12%, which corresponds to actual absorbance values within 0.2 to 0.8 of a milliabsorbance unit.

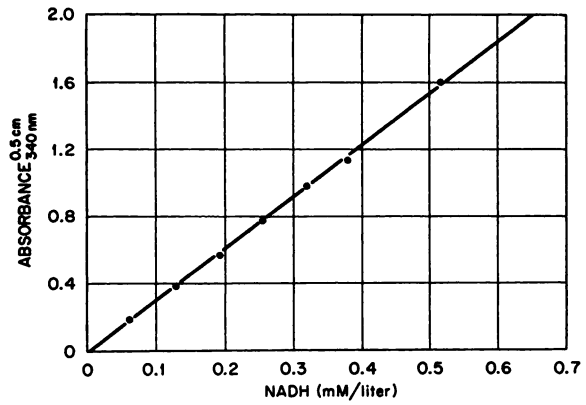

Fig. 9. Spectrophotometric linearity of the miniature Fast Analyzer at 340 nm

Table 2. Spectrophotometric Precision Obtained with the Miniature Fast Analyzer

Mean[a] absorbance 0.5 cm, 340 nm	Standard deviation	RSD, %[b]
0.1925	0.0003	0.12
0.3819	0.0002	0.06
0.5755	0.0003	0.04
0.7716	0.0003	0.04
0.9828	0.0004	0.03
1.1891	0.0004	0.02
1.6437	0.0008	0.04

[a] n = 10 observations (10 data points averaged per observation).
[b] Relative standard deviation (coefficient of variation).

These results indicate that the miniature Fast Analyzer provides the optical linearity, accuracy, and precision required for the chemical procedures to be performed with the system.

Transfer and Mixing of Fluids

Considering the micro-scale volumes involved, the quantitative transfer of the individual volumes of reagent and sample into their respective cuvets and the subsequent thorough mixing of these solutions in their cuvets before spectrophotometric monitoring of the ensuing reactions are critical steps in the analytical operation of the miniature analyzer. To determine the efficiency of the transfer and mixing in the miniature analyzer, 50-µl aliquots of distilled water and 50-µl aliquots of a dextran blue dye: serum solution that had been diluted fivefold with distilled water were loaded into the reagent and sample cavities of the rotor, respectively. These solutions were then transferred to their cuvets by slowly accelerating the rotor to 500 rpm, followed by rapid acceleration to 4000 rpm and then rapid braking to achieve an abrupt stop. The rotor was then accelerated to 500 rpm, and the absorbances of the solutions were subsequently determined. By assuming that a thoroughly mixed solution would have a constant absorbance value when measured repetitively, it was found that two rapid acceleration and braking operations, requiring about 10 s, were needed for thorough mixing. Comparison of the constant absorbances of the mixed solutions with the absorbance obtained for a solution that had been prepared by manually and thoroughly mixing equal quantitative volumes of the dyed serum and water showed that these conditions resulted in over 99% of the solutions being transferred. Therefore, the operational procedure of slowly accelerating the rotor to 500 rpm, followed by two rapid accelerations to 4000 rpm and abrupt braking operations and, finally acceleration to the normal operating

speed of 500 rpm for monitoring, ensures that quantitative transfer and homogeneous mixing of the sample and reagent solutions are attained.

It should be mentioned that an effect of the total reaction volume on homogeneous mixing was observed. Since the total volume containable in each cuvet was 130 μl, it was found that the combined sample and reagent volumes should not exceed 120 μl. This ensures that each cuvet is not overfilled and that each will contain a small air bubble observed to be necessary to facilitate mixing. Thus, for routine analysis, diluted sample and reagent volumes of 50 and 70 μl, respectively, are used.

Precision of Chemical Analyses

To determine the analytical precision obtainable with the miniature Fast Analyzer, we analyzed five replicate aliquots of three Hyland Control Sera containing enzymes at various levels of activity. The results from these analyses are listed in Table 3.

The relative standard deviation for these analyses ranged from 0.9 to 4.8% for enzyme activities in the normal range (Serum I) to 0.4 to 1.5% for elevated enzyme activities (Serum III). This degree of analytical precision compares quite favorably with that previously reported for the larger analyzers (*11*, *21*).

The data in Tables 1 and 3 also confirm the inverse relationship between analytical precision and analysis time. Previous papers (*21*, *22*) have demonstrated that the determination of enzymatic rates frequently requires that a small absorbance change with time be measured at moderately high absorbances. Under these conditions, instrumental noise can contribute substantially to analytical variation. This effect of instrumental noise can be minimized by increasing analysis time. However, from a practical viewpoint, it is advantageous to perform the analysis as rapidly as possible since the analytical throughput of the instrument is directly proportional to the analysis time. Thus, a compromise between length of analysis time and analytical precision confronts the analyst. For example, in a normal population (as represented by serum sample I), activities of the enzymes CK, AST, and ALT are relatively low. Therefore, to obtain the precision listed for these analyses in Table 3 requires an analysis time of from 10 to 12 min per rotor. However, since the unique parallel mode of operation of Fast Analyzers (*7*) allows several samples to be analyzed simultaneously (in the miniature Fast Analyzer this number would be 16, because cuvet 1 is used for referencing purposes), a 10- to 12-min analysis time per rotor is not unreasonable and, in fact, extrapolates to a sample analysis rate of 80 to 96 samples per hour. In addition, for samples having greater enzyme activities (serum samples II and III) or enzymes having normally greater activities (i.e., ALP and LD-L), the analysis time can be reduced to as little as 2 to 4 min per rotor.

Table 3. Analytical Results Obtained from Multiple Enzyme Analyses of Three Hyland Control Sera by Using the Miniature Fast Analyzer

	Enzyme activity, U liter^{-1} min^{-1} at 30°C					
	Serum I		Serum II		Serum III	
Enzyme	Mean	RSD	Mean	RSD	Mean	RSD
ALP	50.3	0.9	81.2	1.2	131.9	1.3
CK	21.5	3.5	241.1	1.3	379.4	0.4
HBD	16.0	4.3	122.3	2.8	291.5	1.5
LD-L	62.3	1.4	185.5	2.7	279.2	1.0
AST	13.8	4.8	59.3	2.4	91.6	1.2
ALT	11.0	3.9	74.4	1.5	150.5	1.4

Discussion

We have demonstrated that a miniature Fast Analyzer can be fabricated from mechanical and electronic components that are smaller than those currently used in the larger analyzers. A recent publication (*23*) has given an indication of the high total cost required to produce one linear foot of usable bench-top space. Since the miniature Fast Analyzer requires only one cubic foot of bench-top space, an important advantage of the smaller unit over the larger analyzers would be spatial economy. In addition, the components of a miniaturized system are generally integrated into a few replaceable components or modules; therefore, the miniature unit has the potential of being easily maintained and repaired because it allows a relatively inexperienced operator to test and check the individual components or modules and replace them if necessary. Also, owing to the small size of the system, the entire unit can be returned to repair specialists instead of the specialists being required to service it on-site. Consequently, the miniature system would ultimately be less expensive because of its ease and low cost of maintenance.

In addition to the special advantages of physical miniaturization of the analyzer, reagent and sample volume requirements are further decreased in the miniature system. For example, sample and reagent volumes for the miniature analyzer are only 1 to 10 μl and 70 to 110 μl, respectively. Thus the analyzer would have application in areas where obtaining a sufficient volume of sample for analyses is difficult. For example, with the miniature analyzer in a pediatric laboratory, several analyses could be performed on the small volume of blood obtained from a finger- or toe-prick of a newborn infant. Accompanying the sample economy ad-

vantage of the analyzer is the reagent economy, which results in a lower reagent cost per analysis. This advantage is significant in assays in which the reagents are expensive, for example the reagents necessary for the CK analysis. With the small analyzer the reagent cost per CK analysis would only be 2¢.

It should be emphasized, however, that a need for hardware to accurately and precisely measure small volumes accompanies the decreased volume requirements. Currently available technology for manually or automatically (*24*) sampling and dispensing volumes in the 20- to 500-μl range can be used to load reagent into the miniature rotors; it may even be used to load samples provided they are prediluted as was the case in the present study. This method of sample and reagent loading is useful for evaluating various features of the instrument, and it could be automated as is currently done with the larger Fast Analyzers (*24*). However, the sample must be prediluted, which is tedious, time consuming, and contributes to imprecision and inaccuracy in the analytical method. Therefore, in the future, emphasis must be placed on developing technology that will result in instrumentation capable of accurately and precisely measuring and dispensing small fluid volumes in the 1- to 20-μl range.

Obviously, because of its central role, the rotor is a primary component of the analyzer; consequently, rotor development appears to be the most fruitful area for further investigation. For example, rotors now being developed (*25*) are planned to contain preloaded reagents and be capable of accepting a sample of whole blood, separating the plasma from the cellular components, and then accurately and precisely transferring measured volumes of plasma to their respective cuvets, where they are mixed with the preloaded reagent. Accompanying the design and development of these specialized rotors is the development of fabrication techniques that we hope will result in the rotors being made inexpensive and disposable. One of the most promising techniques appears to be injection molding of the individual rotor components, which are later assembled and sealed by ultrasonic welding.

In summary, these studies indicate that a Fast Analyzer can be significantly miniaturized and still retain the high degrees of optical performance and analytical accuracy and precision that have been previously demonstrated to be obtainable with these analyzers. In addition, miniaturization results in a four- to fivefold decrease in the sample and reagent volume requirements of the analyzer; and small (potentially disposable) plastic rotors have been designed and fabricated to process these small volumes of sample (1 to 10 μl) and reagent (70 to 110 μl). As with the larger analyzers, the miniature system can be conveniently interfaced with a computer and used to perform precise and accurate clinical analyses; this is demonstrated by the encouraging results obtained with the serum enzyme analyses. Although some technical problems such as techniques for measuring and transferring small volumes (1 to 10 μl) of undiluted serum and precision fabrication of rotors by injection molding remain to be solved, we conclude that many advantages can be obtained by miniaturizing a Fast Analyzer. With its unique advantages, a miniature Fast Analyzer should prove useful in many areas of application. As mentioned earlier it would be quite useful in instances where obtaining a sufficient sample volume is difficult, for example, in a pediatric laboratory. In addition, it would be useful in diverse areas such as emergency wards, small clinics, under emergency conditions, and even as an on-board analyzer for use in an orbiting space laboratory.

The development of a sophisticated device such as the miniature Fast Analyzer requires the cooperation, advice, and assistance of several individuals representing a broad spectrum of technical specialties. At ORNL we are fortunate to have available to us such a staff of highly competent and trained personnel, and we gratefully acknowledge the assistance of J. B. Overton, L. H. Thacker, J. E. Attrill, C. A. Hahs, E. L. Collins, R. A. Mathis, W. Walker, M. T. Kelley, J. M. Jansen, and D. D. Willis. The technical advice and constructive criticism provided by Dr. R. S. Melville of the National Institute of General Medical Sciences, Bethesda, Md., and by Drs. E. Harris and S. R. Pool of the National Aeronautical and Space Administration, Manned Space Center, Houston, Texas, are also acknowledged.

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