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Portable Centrifugal Analyzer for the Determination of Rapid Reaction Kinetics

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A portable centrifugal analyzer prototype (*Clin. Chem.* 1977, *23*, 1416) is capable of rapidly initiating reactions and monitoring 17 optical channels as they rotate past a stationary photodetector. An advanced rotor drive permits transfer of discretely loaded sample and reagent into a cuvette within 60 ms. Various rotor designs have been employed to ensure efficient mixing concurrent with solution transfer, thus permitting absorbance or luminescence measurements to be made almost immediately after solution contact. Dye-dilution studies have been used to investigate transfer and mixing efficiencies. Rotor designs with parallel access for sample and reagent into the cuvette were found to promote efficient mixing during liquid transfer. The hypochlorite–luminol chemiluminescent reaction served to demonstrate the utility of the system for performing rapid kinetic analyses. Appropriate adjustment of reaction conditions allows first-order reaction half-lives as short as 0.04 s to be measured.

Approximately a decade ago, Anderson and co-workers introduced the concept of the centrifugal analyzer, in which a number of analyses are performed in parallel with a multicuvette rotor (*1–3*). Discrete aliquots of sample and reagent solution loaded into this rotor are transferred by centrifugal force into the optical cuvettes, establishing simultaneous starting times for the batch of reactions. Similar conditions (e.g., time, temperature) are maintained during the run. A stationary optical system monitors the cuvettes and samples the dark current during each revolution of the rotor. The analyzer is interfaced to a computer system which controls the timing and sequence of data acquisition and processes the data (*4*). The development of the early prototype instruments has been described in the literature (*1–3, 5–10*), and the instrumentation and its varied applications have been summarized in recent reviews (*11–14*) and a compendium (*15*). Centrifugal analyzers are well suited for precision kinetic photometry and are extensively used in the clinical laboratory; however, the versatile instrumentation also has general analytical utility.

Recently, we introduced an advanced prototype analyzer (*16*) which incorporates a microcomputer system to control several analyzer functions as well as to perform data acquisition and processing. The temperature of the rotor is controlled to within ±0.2 °C at one of three set points (25, 30, or 37 °C) by means of a thermoelectric heat pump located in the rotor holder. The use of an advanced rotor drive employing a clutch/brake enables discretely loaded sample and reagent to be transferred into the optical cuvette in times as short as 60 ms.

Various multicuvette–rotor designs have been evaluated with the objective of attaining efficient mixing of binary solutions concurrent with their transfer. The results for the most promising of these designs are described. The combination of precise time measurements (2-MHz internal clock with a resolution of 100 μs) made during each revolution, rapid acceleration via the clutch/brake, and the use of parallel-transfer channel rotor designs permit reactions to be monitored within 100 ms of their initiation. In this study, a number of relatively rapid reactions (i.e., those persisting for a least 200 ms) were monitored by photometry or chemiluminescence to determine the performance capabilities and limits of the analyzer for times of <1 s.

EXPERIMENTAL

Instrumentation. The portable centrifugal analyzer is described in detail in reference 16. An internal photomultiplier (PM) assembly (R 300, Hamamatsu Corp., Middlesex, NJ. 08846) was utilized for photometry and certain luminescence studies. For high-sensitivity luminescence studies, the system was modified to use an external PM assembly (8644, RCA Solid State Division, Lancaster, Pa. 17604). The external PM, with lens assembly, aperture, and preamplifier, gives improved signal-to-noise performance for low-level light emission by decreasing the cuvette-to-PM distance and employing more amplifying stages.

Some of the rapid reactions were also monitored by a stopped-flow spectrophotometer (Model D-110, Durrum Instrument Corp., Palo Alto, Calif. 94303). According to the manufacturer's specifications, this instrument provides efficient mixing of two components in 2 ms and permits observation of reactions with half-lives <5 ms. Data are recorded on a storage oscilloscope (Model 5111, Tekronix, Inc., Beaverton, Ore. 97005) and subsequently photographed for a permanent record.

Optical Studies of Concurrent Solution Transfer and Mixing. Equal volumes (65 μL) of NADH solution (reduced β-nicotinamide adenine dinucleotide, ~0.2 mg/mL in buffer) and buffer diluent [0.1 m tris(hydroxymethyl)methylamine, pH 9.0] were discretely placed within the loading cavities of a rotor using a hand-actuated micropipet (Pipetman Model P200, Rainin Instruments Co., Boston, Mass. 02215). The rotor was accelerated, and absorbance measurements (vs. buffer only in cuvette 1) were made at 340 nm.

In additional mixing and transfer studies, Blue Dextran 2000 (Pharmacia Fine Chemicals, Uppsala, Sweden) was used with buffer diluent. Both solutions contained 0–40% (w/v) sucrose. Absorbance measurements were made at 620 nm.

Studies of Rapid Chemical Reactions. *Photometry.* Reaction of phosphate and silicon with molybdate: 50 μL of sample and 80 μL of reagent (0.0193 M $Na_2MoO_4 \cdot 2H_2O$ in 0.144 N H_2SO_4) were discretely loaded into the sample and reagent chambers of a multicuvette rotor. The reaction was initiated and then monitored at 340 nm.

Reaction of Fe(III) with thiocyanate: Equal volumes (65 μL) of Fe(III) solution [0.04 M $Fe(NO_3)_3 \cdot 9H_2O$ in 0.8 N HNO_3] and thiocyanate solution (6.25×10^{-4} M KSCN in distilled water) were loaded into the rotor. The reaction was initiated and then monitored at 450 nm.

Chemiluminescence. *Cr(III)/Luminol System.* The procedure described by Bowling et al. (*17*) was adapted for the portable centrifugal analyzer. A 100-μL sample [0.26 mg/L Cr(III) as $Cr(NO_3)_3 \cdot 9H_2O$] and 30 μL of reagent [1.9×10^{-3} M luminol (5-amino-2,3-dihydro-1,4-phthalazinedione), 0.31 M H_2O_2, and 8.3×10^{-3} M EDTA in 0.10 M KOH/H_3BO_3 buffer, pH 10.3] were loaded into a multicuvette rotor. The light emission from the reaction (λ_{max} = 430 nm) was monitored without prior monochromation using the external PM assembly.

Hypochlorite/Luminol System. Equal volumes (65 μL) of sodium hypochlorite solution (prepared in 0.05 M NaOH) and luminol/peroxide reagent were used. Typical initial reaction conditions were: 1–25 mg/L NaOCl, 10^{-2} M H_2O_2, and 10^{-4} M luminol in borate buffer, pH 10–10.5.

RESULTS AND DISCUSSION

Transfer and Mixing Studies. In the original prototype centrifugal analyzers, the optical cuvettes are an integral part of the instrument and are cleansed in situ between analysis batches (*1*, *2*). In the miniature centrifugal analyzer, the cuvettes and transfer disk are combined into one removable unit (*7*, *9*). Special-purpose multicuvette rotors could be designed (*13*, *18*, *19*) and easily interchanged with the conventional rotor to provide a more rapid turnaround between sets of analyses. The conventional rotor (as used with later-generation prototype instruments) and the transfer of liquid under the influence of a centrifugal field are shown schematically in Figure 1. Sample and reagent are discretely loaded into their respective cavities (Figure 1A). The outermost loading cavity, located nearest the optical cuvette, is designated as the "sample" cavity; the innermost cavity is referred to as the "reagent" cavity. The loading of microliter volumes into this rotor design may be automatically performed, rapidly and precisely, by using an ancillary rotor loading station (*8*, *20*). Acceleration of the rotor causes the contents of the loading cavities to be transferred into their optical cuvettes (Figure 1B–D). With this sequential-transfer design, the "reagent" must flow through the "sample" cavity before entering the cuvette. Since the solutions enter the cuvette at different times, layering may occur (vide infra). In the conventional mixing cycle, the rotor is first accelerated to 4000 rpm to achieve liquid transfer, then rapidly decelerated (causing agitation, and thus mixing of the cuvette contents), and finally reaccelerated to a fixed speed (typically 1000 rpm) for data acquisition. The miniature centrifugal analyzer, with a direct-rotor drive assembly, requires 5–7 s for this sequence (*18*). The portable centrifugal analyzer, with an advanced clutch/brake rotor drive assembly, completes the same sequence in about 0.37 s (*16*).

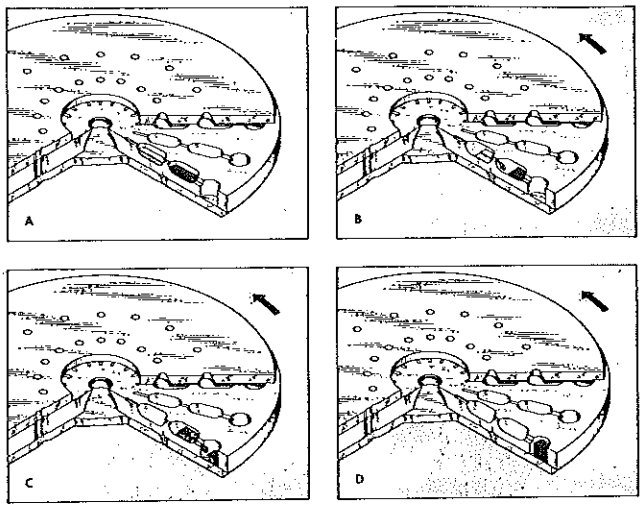

Figure 1. Schematic for conventional (sequential-transfer) multicuvette rotor. (A) Sample and reagent discretely loaded into their respective cavities. (B–D) radial transfer of liquid by centrifugal force into optical cuvette

It is desirable to achieve solution transfer and mixing concurrently since this would eliminate the brake-reaccelerate portion of the mixing cycle and permit more rapid initial optical measurements. In the studies described below, various rotor designs were evaluated for their ability to rapidly transfer and effectively mix two solutions using the force of acceleration only. Transfer time was estimated as the time required for the mixture to produce a constant or steady-state absorbance measurement. Mixing efficiency was judged by comparing this measurement with that for a homogeneous solution, premixed externally and pretransferred into certain of the cuvettes of the rotor. Since the automated rotor loading station was not compatible with all rotor designs, hand-actuated micropipets were used in the studies described here. The variation of absorbance measurements on the mixture attributable to the volumetric error of these pipets is estimated to be approximately 1%; therefore, the criterion for complete transfer and mixing of the two solutions is a steady-state absorbance measurement within ±1% of that of the premixed, pretransferred reference solution. A rotor speed of 2500 rpm was used in these studies; data were taken during each revolution of the rotor.

Sequential-Transfer Rotor. The performance of a conventional, sequential-transfer rotor for the transfer and mixing of NADH and diluent is illustrated in Figure 2. Note that although light-absorbing species (NADH) and diluent produce an approximate steady-state absorbance value by the fourth revolution of the rotor (0.16–0.19 s), mixing is incomplete as judged by comparison with the absorbance of a premixed, pretransferred solution. At long times, the reference absorbance value is approached, presumably by slow diffusional mixing of any initially layered liquid. The fact that layering occurs is suggested by the difference in absorbance within the cuvette, depending on whether NADH solution of diluent first enters the optical cuvet. The resulting absorbance measurements during the conventional sequence of accelerate–brake–accelerate is also illustrated. In the latter case, complete transfer and mixing occur independent of which solution first enters the cuvette; however, no optical measurements are possible until approximately 360 ms have elapsed.

Parallel-Transfer Rotor. A schematic diagram of a cuvette of the parallel-transfer rotor is given in Figure 3. Sample and reagent have separate access to the optical cuvette and may transfer simultaneously. This array of transfer channels has been used both in the original prototype centrifugal analyzer (*17*, *21*) and in the miniature centrifugal analyzer (*18*);

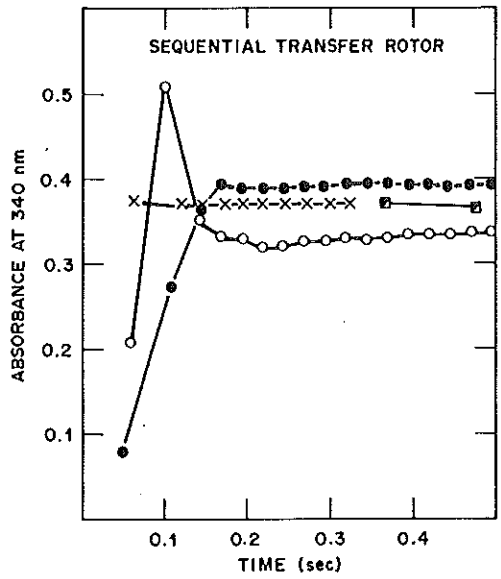

Figure 2. Solution transfer and mixing within a sequential-transfer rotor. x–x: premixed, pretransferred mixture (reference value); O–O: diluent initially loaded in innermost ("sample") cavity, NADH solution in outer ("reagent") cavity; •–•: NADH in "sample" cavity, and diluent in "reagent" cavity; ▨: NADH and diluent mixed by sequence of accelerate–brake–accelerate

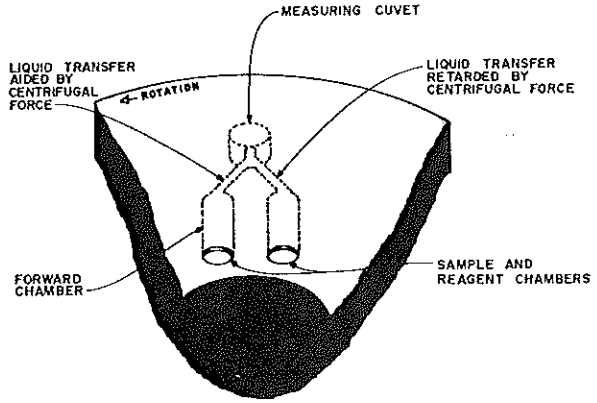

Figure 3. Schematic for one cuvette in parallel-transfer rotor (forward chamber designated as "sample" cavity)

however, the rapid rotor acceleration with the portable centrifugal analyzer especially takes advantage of this design (*16*). Figure 4 illustrates typical results for solution transfer and mixing within a multicuvette rotor of this design. Both transfer and mixing are typically complete by the second revolution of this rotor (0.10–0.13 s), irrespective of which loading channel was initially charged with light-absorbing species. The array of loading ports, however, prevents the use of the automated loading station as presently designed (*8*, *20*).

Rotated-Cavity Rotor. A schematic of a new rotor design, designated the rotated-cavity rotor, is shown in Figure 5. As with the sequential-transfer rotor (Figure 1), loading ports are arrayed radially with respect to an optical cuvette; thus the rotor is compatible with the automated loading station (a significant advantage). However, instead of a common transfer path into a radially aligned cuvette, each loading cavity empties its contents into an adjacent cuvette by means of a separate channel (as in the parallel-transfer design, Figure 3).

Figure 6 illustrates solution transfer and mixing within a rotated-cavity rotor. Solution transfer and mixing are somewhat slower (third or fourth revolution, ~0.15–0.18 s) than for the parallel-transfer rotor (cf. Figure 4), yet are more rapid and complete than in the sequential-transfer rotor when acceleration alone is used (cf. Figure 2).

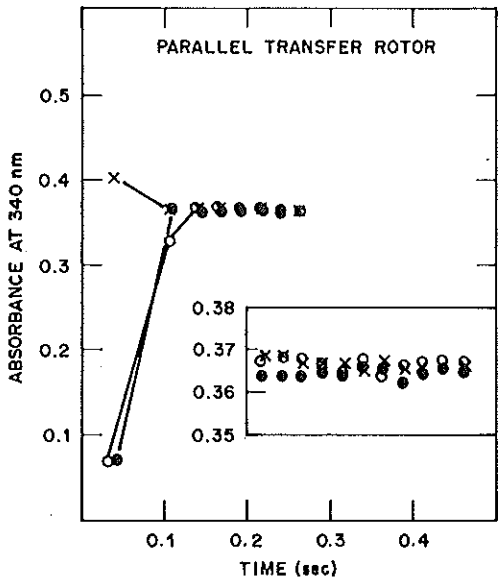

Figure 4. Solution transfer and mixing within a parallel-transfer rotor (cf. Figure 2)

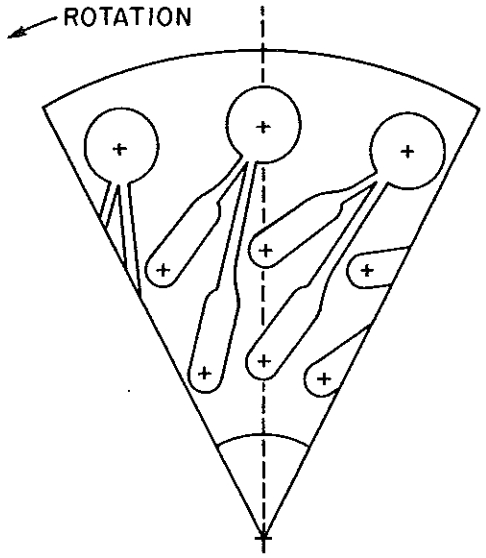

Figure 5. Rotor segment schematically detailing rotated-cavity design

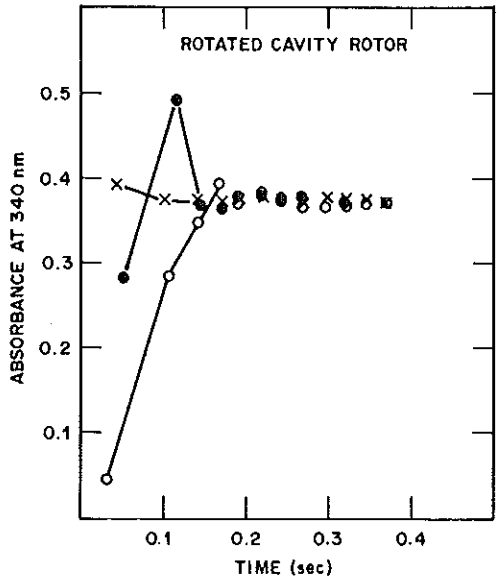

Figure 6. Solution transfer and mixing within a rotated-cavity rotor (cf. Figure 2)

Table I. Mixing of Disparate Volumes of Dye and Diluent[a]

volume of sample/total volume	time for transfer and mixing,[b] s	steady-state absorbance at 620 nm[c]
0.25	~0.16	0.2373
0.50	~0.11	0.4799
0.75	~0.15	0.7207
1.00	~0.11	0.9422

[a] Dye = 2 mg/mL Blue Dextran in buffer, diluent = buffer (0.10 M Tris, pH 9.0). A parallel-transfer rotor was used; rotor speed, 3000 rpm; temperature, 25 °C. [b] Time required to attain an absorbance value ≥99% of steady-state absorbance. [c] Average absorbance for five successive rotor revolutions after steady-state absorbance value had been obtained. Linear least-squares regression analysis of data (y = steady-state absorbance, x = volume fraction of sample): $y = (0.9422 \pm 0.0143)x + (0.0061 \pm 0.0138)$, $r = 0.9998$.

Table II. Sucrose in Water, 25 °C[a]

sucrose, %	density, g/mL	relative density	viscosity, cP	relative viscosity
0	0.9977	≡1.00	0.8913	≡1.00
10	1.0366	1.04	1.179	1.32
20	1.0790	1.08	1.696	1.90
30	1.1250	1.13	2.739	3.07
40	1.1745	1.17	5.160	5.79

[a] Data taken from H. A. Sober, Ed., "CRC Handbook of Biochemistry", The Chemical Rubber Co., Cleveland, Ohio, 1968, p J-250.

With all rotor designs, transfer and mixing times are dependent on rate of rotor acceleration. Transfer time and mixing efficiency for rotors with separate access of sample and reagent into the optical cuvette were found to vary somewhat, depending on transfer channel geometry and dimensions.

The studies described above involve the transfer and mixing of equal volumes of liquids with similar viscosities. The data presented in Table I were obtained by loading disparate volumes of dye and diluent into a parallel-transfer rotor. The larger volume was loaded into the transfer chamber where transfer is aided by the centrifugal force (see Figure 3). Mixing of these liquids via acceleration alone was very efficient.

Disparate solution viscosities present a much greater obstacle to rapid transfer and efficient mixing, as illustrated in Figure 7. The addition of sucrose to the diluent solution increases its relative viscosity (see Table II), with the result that transfer of the less-viscous dye component is favored. However, if the viscosities of the component solutions are matched, transfer and enhanced mixing occur simultaneously (see Figure 8).

Photometric Kinetic Measurements. *Reaction of Phosphate and Silicon with Molybdate.* Bostick et al. (22) used a miniature centrifugal analyzer to investigate the reaction of soluble silica and orthophosphate with molybdate reagent. With this instrument, which incorporates a direct coupling between the motor and rotor holder, the initial optical measurement could not be obtained prior to ~7 s after the reactions had been initiated. Under the conditions used, the reaction of phosphate with the reagent was essentially complete before the first possible observation. However, the reaction with silicon was sufficiently slow to permit initial rate kinetic analysis of this constituent, with no significant interference by phosphate ion. The estimated absorbance at zero time, computed by extrapolation of the absorbance–time data for $t > 10$ s, reflected the phosphate content of the sample, whereas the slope for these data was proportional to

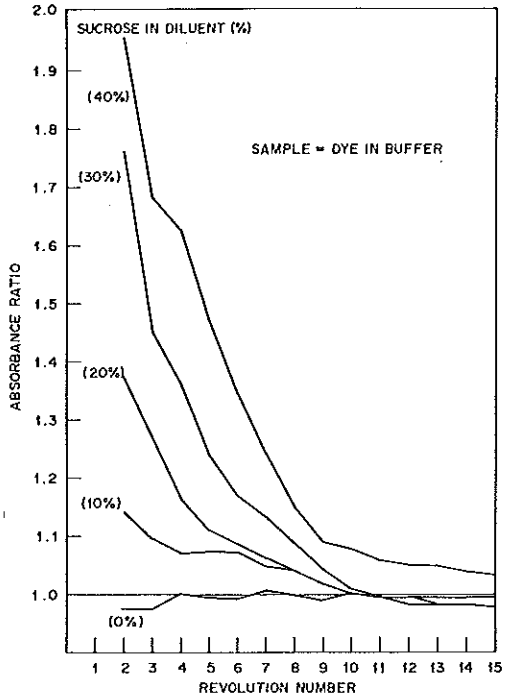

Figure 7. Transfer and mixing for solutions of disparate relative viscosities (cf. Table II). Dye solution, 2 mg/mL Blue Dextran in buffer (0.10 M Tris pH 9.0); diluent solution, buffer with added sucrose. A parallel-transfer rotor is used; rotor speed, 3000 rpm; T, 25 °C. The absorbance ratio is the observed absorbance at 620 nm referenced to a premixed, pretransferred solution

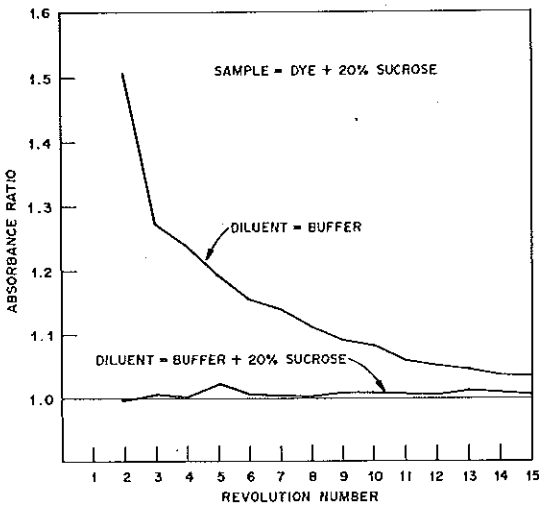

Figure 8. Transfer and mixing for dye and diluent. Dye, 2 mg/mL Blue Dextran in buffer + 20% sucrose; diluent, buffer alone or buffer + 20% sucrose (cf. Figure 7)

the silicon concentration. Mrochek et al. (16) using a modified molybdate reagent and monitoring the reaction with the portable centrifugal analyzer, found the rate of the phosphate–molybdate reaction for the time interval 0.1–1.2 s to be proportional to the phosphate ion concentration.

We have reinvestigated the reactions with silicon and phosphate; our results are summarized in Figure 9. A sequential transfer rotor was used, and an accelerate–brake–accelerate cycle was employed to ensure mixing. Observations were obtained at 0.5-s intervals (each observation is the average of five successive revolutions of the rotor at 3000 rpm). The phosphate reaction has a $t_{1/2}$ of ~1.6 s for the time interval 0.37–3.85 s. The silicon reaction has a $t_{1/2}$ of ~32 for the time interval 15–120 s.

In an earlier study using a miniature centrifugal analyzer (22), the occurrence of the phosphate–molybdate reaction

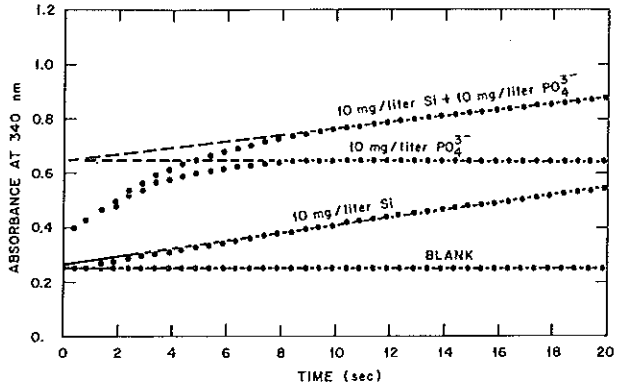

Figure 9. Reaction of phosphate ion and silicon with molybdate at 25 °C

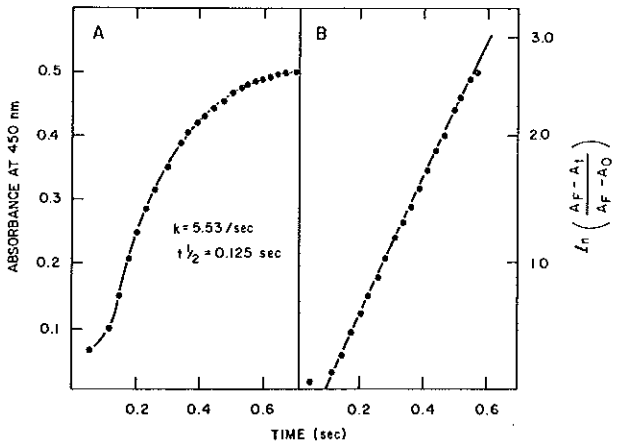

Figure 10. Reaction of Fe(III) with SCN^-. (A) Reaction progress curve; (B) data plotted as first-order kinetics reaction

could only be inferred from the extrapolated absorbance-vs.-time intercept. With the portable centrifugal analyzer, much of the relatively rapid phosphate reaction can be followed directly. However, extrapolation of the observable absorbance–time data for the phosphate reaction still yields a value greater than the observed reagent blank (see Figure 9). When this reaction is monitored by a stopped-flow spectrophotometer, an initial, very rapid ($t_{1/2}$ of ~5 ms) reaction phase is observed. This phase is thus essentially complete prior to the first permissible observation with the portable centrifugal analyzer.

Reaction of Fe(III) with Thiocyanate. Mieling and Pardue (*23*) describe conditions under which the reaction of Fe(III) with SCN^- obeys pseudo-first-order kinetics. The absorbance-vs.-time relationship for such a reaction as monitored may be described by (*23*):

$$A_t = A_F - (A_F - A_0)e^{-kt} \quad (1)$$

were A_0, A_t, and A_F are the initial, instantaneous, and final absorbances, respectively, and k is the first-order rate constant. The left-hand side of Figure 10 illustrates the absorbance-vs.-time progress curve for the Fe(III)/SCN^- reaction as monitored with the portable centrifugal analyzer. A parallel-transfer rotor was used to facilitate rapid mixing by acceleration alone and absorbance data were taken for successive rotor revolutions at 2500 rpm. The data shown on the right of Figure 10 are plotted in a normalized linear transformation of Equation 1 above. The data obey the expected pseudo-first-order kinetic relationship for times greater than that required for transfer and mixing (~0.1 s).

Chemiluminescence Kinetic Measurements. Measurement of absorbance with the centrifugal analyzer requires the comparison of light transmission between reference and

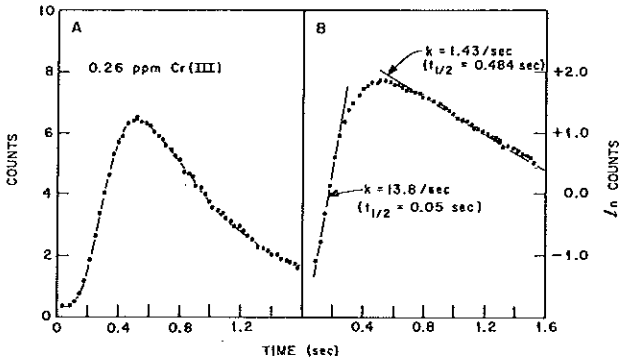

Figure 11. Cr(III)-catalyzed luminol–peroxide chemiluminescence. (A) Emission intensity (analog counts) vs. time (B) semilogarithmic plot of counts vs. time

analysis cuvettes. When this difference is small, as is the case when the reaction is first initiated, the error in measurement is more significant. Chemiluminescence, in which light emission is generated by a chemical reaction, should provide a more sensitive means of assessing the performance of the analyzer for early optical measurements.

Cr(III)–H_2O_2–Luminol Chemiluminescence. Bowling et al. (*17*) used a modification of the original prototype centrifugal analyzer to determine chromium, based on the chemiluminescence produced by the catalytic oxidation of luminol by hydrogen peroxide in basic solution. We have reexamined this reaction in order to contrast the performance capabilities of the two centrifugal analyzer systems.

Figure 11 illustrates the chemiluminescent emission from the reaction under conditions similar to those reported by Bowling et al. (*17*). Because of limitations of both instrumentation and software, Bowling and co-workers could acquire a maximum of 21 sets of observations in a minimum time of ~2.5 s. In contrast, the portable centrifugal analyzer can acquire and process up to 99 successive observations (*16*); the time required (including that for rotor acceleration) is <2.8 s at 2000 rpm and <1.8 s at 3500 rpm.

An external photomultiplier assembly (see Experimental section) was used to measure the low-level light emission for the reaction of 0.26 ppm Cr(III) as shown in Figure 11A. If the chemiluminescent emission obeys exponential decay, it may be described by:

$$I_t = I_0 e^{-kt} \quad (2)$$

where I_t and I_0 are the instantaneous and maximum observed emission intensity, respectively. The semilogarithmic plot of the data (Figure 11B) indicates a relatively rapid ($t_{1/2}$ = 0.05 s) rise in emission followed by a much slower ($t_{1/2}$ = 0.48 s) decay.

Hypochlorite–H_2O_2–Luminol Chemiluminescence. Aqueous chlorine and hypochlorite function equivalently as co-oxidants of luminol, and peroxide enhances chemiluminescent response (*24*). This reaction is considered to be an appropriate model with which to evaluate the portable centrifugal analyzer since the decay of luminescence is known to obey pseudo-first-order kinetics over a range of reaction conditions, and with appropriate selection of conditions the light emission is initially intense and of short duration (*24*, *25*).

The chemiluminescent response for this reaction, as monitored with the portable centrifugal analzyer, is illustrated in Figure 12A; the semilogarithmic transformation of the data is given in Figure 12B. For $t > 0.07$ s, the emission decay appears to obey pseudo-first-order kinetics according to Equation 2. For comparison purposes, this reaction was also examined with a stopped-flow device (Figure 13). Logarithmic amplification of the PM signal (Figure 13, curve b) produces a linear response (cf. Figure 12) and the estimated half-life

Table III. Hypochlorite–Luminol Chemiluminescence in Test Rotor[a]

parameter	cuvette configuration[b] rotated cavity	parallel transfer	sequential transfer
I_{max}	7.826 ± 0.190 (CV = 2.43%)	5.982 ± 0.117 (CV = 1.95%)	4.195 ± 0.130 (CV = 3.09%)
ΣI[c]	29.45 ± 0.53 (CV = 1.81%)	29.74 ± 1.32 (CV = 3.81%)	27.53 ± 0.98 (CV = 3.55%)
semilogarithmic least-squares regression			
interval selected:[d]			
revolutions	2–11	3–12	5–14
avg. time, s	0.11–0.33	0.16–0.35	0.21–0.40
slope, k, s^{-1}	11.94 ± 0.31 (CV = 2.57%)	13.06 ± 0.04 (CV = 0.30%)	11.42 ± 0.27 (CV = 2.36%)
correlation coefficient	0.9999	0.9998	0.9997
$t_{1/2}$ s	0.0581	0.0531	0.0607

[a] Initial reaction conditions: NaOCl, 12 mg/L; luminol, 1.1×10^{-4} M; H_2O_2, 0.011 M; pH 10.0; temperature, 25 °C; rotor speed, 3000 rpm. [b] Four replicates of each configuration were used. All data under this subheading are average values. [c] Sum of counts, first 20 revolutions of rotor (dark current, $\Sigma I = 1.70$). [d] By use of linear-search computer subroutine (see text).

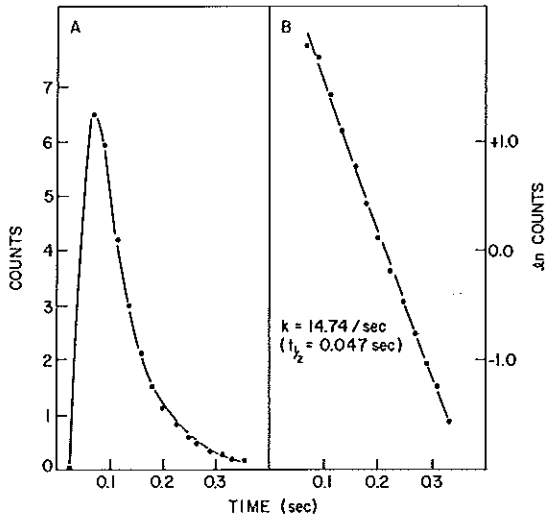

Figure 12. Hypochlorite–peroxide–luminol chemiluminescence. (A) emission intensity vs. time; (B) semilogarithmic plot of data. Initial reaction conditions: NaOCl, 25 mg/L; luminol, 1.1×10^{-4} M; H_2O_2, 0.04 M; pH, 10.5 (0.25 M KOH–H_3BO_3 buffer); temperature, 30 °C. A parallel-transfer rotor and internal photomultiplier are used; rotor speed is 3000 rpm

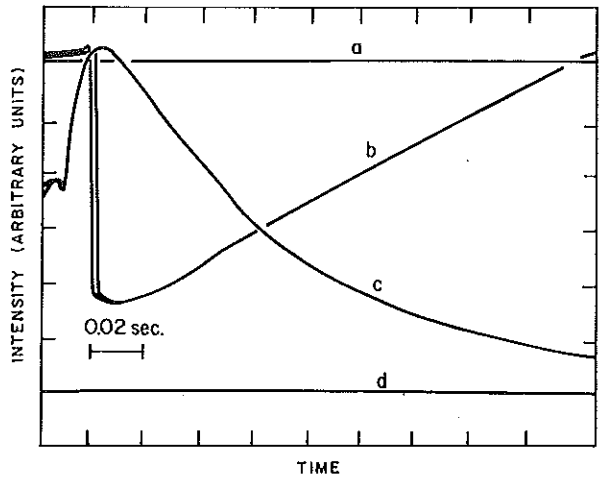

Figure 13. Hypochlorite–peroxide–chemiluminescence, as studied by a stopped-flow device. (A) Base line, semilog mode (absorbance mode); (B) chemiluminescent response, semilog mode; (C) chemiluminescent response, linear mode (transmission mode); (D) base line, linear mode. External source and monochrometer are not used. Initial reaction conditions are given in Figure 12

of the reaction is similar to that observed with the portable centrifugal analyzer (approximately 50 ms). Peak emission occurs at approximately 0.02 s, an interval that is again too rapid for accurate measurement with the portable centrifugal analyzer. However, the slower emission decay is accurately monitored.

A special test rotor was fabricated which incorporates replicate rotated-cavity, parallel-transfer, and sequential-transfer configurations within a single unit. Thus, the relative performance of these configurations for monitoring rapid reactions may be evaluated in a single run in which reagent composition, temperature, detector sensitivity, etc., are similar.

Table III summarizes the results for hypochlorite–luminol chemiluminescence produced in this test rotor. In the table, I_{max} (the maximum observed intensity) and ΣI (sum of counts) are estimates of the peak and integrated intensities, respectively. The closeness with which I_{max} approximates the true peak intensity is dependent on the kinetics of the reaction and the observation interval of the analyzer. For quantitation of light emission, one may use either the sum of counts, based upon a number of discrete observations, or the single maximum value observed. The instantaneous intensity is arbitrarily scaled to 0–10, corresponding to the output (0–16, 384 counts) of the 14-bit analog-to-digital converter.

Pseudo-first-order kinetic parameters were estimated by semilogarithmic linear regression analysis, based on the expression given in Equation 2. Time and emission data from the portable centrifugal analyzer were reentered into a PDP-8 laboratory computer, and regression analyses of the emission decay were performed with use of a "linear-search" subroutine (*26*). This subroutine automatically selects the segment of the linearized data with the greatest slope (rate constant) compatible with a minimum linearity criterion (correlation coefficient) selected by the operator. For the data summarized in Table III, in which the observation frequency (~0.02 s) is less than the half-life of the reaction (~0.06 s), assessment of the rate constant is precise (CV 1–3%). Both the rotated-cavity and parallel-transfer configurations of this test rotor appear to achieve transfer and mixing more rapidly than the sequential transfer configuration, as confirmed by dye-dilution studies.

CONCLUSIONS

The portable centrifugal analyzer is capable of precise measurement of time and light intensity. Although the instrument does not permit initial optical measurements as rapidly as does the stopped-flow technique, it may be used

for many rapid kinetic applications. The use of special rotors having separate transfer paths to the optical cuvettes allows solution transfer and mixing to be completed in approximately 100 ms, providing solution viscosities are similar. Pseudo-first-order rate constants for reactions with half-lives of 50 ms or greater may be determined with a precision of 2–3%. This complements and fills a void between the sequential, very rapid (milliseconds to tens of milliseconds) kinetic measurement capability of stopped-flow devices and the capabilities of conventional automated spectrophotometer systems, including earlier prototype parallel analyzers. The centrifugal analyzer compares very favorably with various flow systems designed to provide reproducible measurement of relatively rapid chemiluminescent reactions (*25*, *27–29*).

The centrifugal analyzer has many advantages for kinetic analysis, including high sample throughput; the multicuvette rotor can process, in parallel, up to 16 samples (plus blank) for absorbance measurements or 17 samples (or various permutations of replicates) for luminescence measurements. The data obtained are in a form suitable for on-line computer analysis.

A minicomputer can be interfaced to the microprocessor in the analyzer to expand data analysis and program storage capabilities. We recently fabricated such systems for the National Institute of Environmental Health Sciences and the University of Michigan. A portable centrifugal analyzer was coupled to a Digital Equipment Corporation (DEC) Model PDP-11VO3 minicomputer with dual discette drives. A Tektronix graphics terminal was included with the latter system. The minicomputer system was configured to use the DEC RT-11 operating system and the FOCAL (FOrmula CALculator) interpretive language. This allows the operator to write his own programs when sophisticated, special-purpose data processing is required. Examples of such data processing may include complex enzyme kinetics (*30*), simplex optimization (*31*), multifactoral experimental designs (*32*), and differential kinetic analysis (*33*). The capability of the portable centrifugal analyzer to obtain up to 99 discrete observations under two subsets of observation conditions ("Dual condition" data acquisition, see reference 16) will be quite useful for the latter application.

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