Interface Instrumentation between Computer and Spectrophotometer for Reaction Rate Measurements

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An instrument interface which generates an output voltage level proportional to rate of change of an input voltage is described. The interface permits direct connection of routine spectrophotometric rate measurements to a real-time laboratory computer system. The form of the voltage level output enables the computer to read rates in the same manner as peaks are read for AutoAnalyzers, permitting the clinical laboratory LABCOM system to compute and report results for rate methods with no software changes. Spectrophotometric rates from about 10 to 0.025 absorbance units per min can be measured with an accuracy and precision of less than 1% relative in measurement times of about 10 to 30 s, respectively. The interface is readily constructed from commercially available components. Test results are presented for simulated rates, lactic dehydrogenase, and alkaline phosphatase.

It is generally recognized that rates of enzyme reactions are best measured with a recording spectrophotometer and subsequent analysis of the recorded rate curve. However, the large amount of labor and skill required to do all enzyme assays routinely with a recording spectrophotometer encourages busy laboratories to select less satisfactory single-point manual or automated procedures. Placing the spectrophotometer on line with a real-time laboratory computer can eliminate tedious manual calculations and makes routine spectrophotometric rate measurements practical, while providing automatic computer entry of results and computer quality-control analysis.

An instrument is described for interfacing a recording spectrophotometer as well as other rate-producing instruments utilizing a retransmitting slidewire, directly to a LINC (Laboratory INstrument Computer) with LABCOM (Laboratory Aided By COMputer) system software. The interface is simple to operate. The operator places the reaction tube in the spectrophotometer and pushes a button. The ready light goes off, indicating that the measurement is in progress. The interface first pauses for a predetermined delay (set by the operator). After the delay, the rate signal from the strip chart recorder is sampled for 10 to 30 s and a voltage level proportional to the rate is transmitted to the computer. After the data is transmitted, the ready light turns on again, indicating that another reaction tube may be inserted. Up to 70 enzyme determinations per hour can be performed by one technologist using manual dilution techniques. No changes are necessary in the LABCOM computer programs to read reaction rates on-line with this interface.

The computer on-line enzyme reports include the usual LABCOM comments on quality control: standard drift, control samples that are out of acceptable limits, and excessive noise in the system.

A hardware interface to calculate rate was selected because we found that software techniques required the sampling of too many points on the rate curve to be practical in time-shared laboratory computing systems. Much interest in the automation of rate measurements has been exhibited, many aspects of which have been reviewed (1, 2). Various approaches have been proposed more recently. James and Pardue (3) described an instrument which essentially automated

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the variable time procedure of Blaedel and Hicks (4). Trayser and Seligson (5) designed a system using a dual beam spectrophotometer to measure the absorbance difference between two samples started after a fixed time interval. Weichselbaum et al. (6) described an entire instrument engineered for automatic determinations based on a spectrophotometric time-derivative measurement. Cordos et al. (7) described a signal integration-subtraction instrument that is generally directly adaptable to rate measurements from any type of instrument with linear concentration response. The latter approach was selected for the rate-measuring interface described in this report, since it is more versatile, more readily constructed, and more adaptable to equipment and procedures in most laboratories.

**System Description**

Figure 1 illustrates a typical configuration for which the interface was designed. The LABCOM system (8) is a special purpose time-sharing system for laboratories based on the use of a LINC (Digital Equipment Corp., Maynard, Mass., and Spear, Inc., Waltham, Mass.). Output voltages of up to 24 instruments can be sampled once each second on an analog channel (A/D input). LABCOM discerns the peak voltage of the signal and, with a linear interpolation program including scheduled calibration standards, it calculates and prints the results in the units of the standard.

In equilibrium or steady-state measurements, a voltage proportional to absorbance, for example, is sampled from a retransmitting slidewire on a recorder that indicates the final absorbance of a particular sample. The new interface allows rate methods to be automatically processed in the same manner by providing an output voltage proportional to the rate.

For extensive evaluation of the performance of the interface, a Heath Model EUA 19-2 Polarography Module was used as a rate simulator to generate voltage ramps to drive the recorder from 20 V/min to 0.001 V/min as shown in Fig. 1. A Bausch and Lomb Spectronic 20 was used for this work, modified to use a cell compartment (with thermostat) and a logarithmic amplifier (specially remodeled and built by Instrument Products Div., E. I. DuPont & Co., Wilmington, Del.) on the phototube output to drive a strip chart recorder to produce absorbance vs. time voltages. However, any good-quality recording spectrophotometer suitable for enzyme rate measurements could be used. Signals from the logarithmic amplifier or the rate simulator were recorded on a Moseley Model 680 strip chart recorder with a 5-kiloohm retransmitting slidewire.

Since the absolute rate in either volts per minute or absorbance units per minute depends on the sensitivity setting of the recorder, but not on the position, all rate values are expressed in units of fraction full-scale per minute. Absolute rates can be obtained from the recorder sensitivity and from the volt-absorbance unit change in the logarithmic amplifier output.

**Circuit Description**

The interface was constructed from modules and cards of the Heath EU-801 Analog Digital Designer, except for passive components and two FET input amplifiers (Burr Brown, Inc., Tucson, Ariz. Model 3115/12C). The schematic diagram of the circuit is shown in Fig. 2. The logic, relays, relay drivers, indicator lamps, three operational amplifiers, push-button trigger switch, and 1-Hz clock were interconnected in the EU-800-RC cabinet by a motherboard and 32-pin card connectors mounted directly over the cards and modular connections. The signal circuitry including most of the passive components and the FET amplifiers was wired on a Vectorbord panel, which was mounted above the motherboard by 40-mm standoffs. An additional 50-mm panel was attached to the top of the cabinet to mount the input-output connectors, gain control, range switch, output bias control, and battery. This construction provides a semipermanent instrument unit, which can be quickly disassembled if necessary for modifications or other functions.

Functional operations and signal waveforms of the interface are illustrated in Fig. 3. The circuit
is more readily understood if Figs. 2 and 3 are considered together. The input circuit can be adapted to any recorder to which a retransmitting slidewire can be attached to obtain a signal. A high input impedance follower amplifier was used to avoid any error caused by loading the 5-kilohm potentiometer. The supply voltage across the potentiometer was maintained at 9.1 V to avoid damage to the input follower and succeeding circuits. The 1-μF capacitors in parallel with the slidewire tend to minimize noise generated by the windings of the potentiometer.

The signal modification circuit is used to reduce the voltage offset of the input signal to nearly zero at the beginning of a measurement cycle to prevent driving the integrator amplifier, OAm, to its 10-V output limit by possible offset voltages of up to 9.1 V. The inverting track-hold long-term memory circuit has been described (10) and is used to null the output of amplifier 3 when RY1 is closed during the initial delay period. At all other times RY1 is open to add a constant voltage to the input of amplifier 3 so that its output inverts the input slope only during the measurement interval. The potentiometer in the feedback loop is used to attenuate the signal by a factor of up to 4 to provide a

![Schematic diagram of circuit](image)

**Fig. 2.** Schematic diagram of circuit

All resistance values indicated are in kilohms. Resistances marked * are closely matched values. All capacitance values are in μF. C1 and C2 are polycarbonate capacitors, 200 V, 1 μF and 5 μF, respectively. Amplifiers 1, 3, and 4 are Heath EU-900-NA cards. Amplifiers # and $ are Burr Brown, 3115/12C. RY1-δ are relay contacts and drivers on a Heath EU-800-JD card. Flip-flops F1-δ are three Heath EU-800-CB cards. The NAND gates are on a Heath EU-800-JC card. Lamps Q to T and drivers and P0 switch are on a Heath EU-801-12 Binary Information Module. M1 and M2 are Heath EU-800-LA, Dual Monostable Card. The 1-Hz clock is a Heath EU-801-13 Digital Timing Module. Power is obtained from a Heath EU-801-11 Digital Power Module.

![Block diagram of interface](image)

**Fig. 3.** Block diagram of interface, illustrating the signal waveforms, timing, and functions of the subcircuits of Fig. 2.
full-scale control that overlaps the basic fourfold sensitivity settings.

Because any drift in the output of amplifier 2 will appear as a signal to the slope-measuring circuit, it is essential to use a high quality, 1- to 10-µF capacitor of polycarbonate or polystyrene dielectric and an amplifier with low input current (10). The same considerations apply to amplifier 5 and its integrating capacitor.

The slope-measuring circuit on which the design of the interface is built has been described in detail by Cordos et al. (7). Basically, the input to the integrator during the first cycle, Δt, is obtained from the output of the unity inverter, amplifier 4. The integrator holds during the next cycle and finally subtraction is obtained by integrating the noninverted signal during the last Δt cycle. The output voltage, e1, is proportional to the rate, r, unless the input voltage is too large, so as to limit the integrator. Although the principle of circuit operation has been described (7), an explicit expression for the output voltage in terms of circuit parameters and input rate has not been given.

Referring to Fig. 3, the input voltage to the integrator, e1, may be expressed as

\[ e_1 = V_o + rg t \text{ for the first } \Delta t \]
\[ e_1 = 0 \text{ for the second } \Delta t \]
\[ e_1 = V_o - r g(2 \Delta t) - r g t \text{ for the third } \Delta t \]  

(1)

where G is the total gain of the circuit before the input to the integrator, r is the rate to be measured from the retransmitting potentiometer, and V0 is the initial offset voltage at the integrator input. The final output voltage, e0, is obtained by integrating Equation 1 term by term between the limits 0 and Δt and taking the sum of terms

\[ e_0 = \sum \frac{-1}{RC} \int_0^{\Delta t} e_1 dt = \frac{2rG(\Delta t)^2}{RC} \]  

(2)

where RC is the integrator time constant. The gain, G, may be varied by the 150-kiloohm potentiometer in the signal modifer and Δt may be varied in precise steps of 1, 2, 4, and 8 s by SW4 in the logic circuit which, because of squared dependence on Δt, provides for relative sensitivity settings of 1, 4, 16, and 64. These settings correspond to nominal 0.1, 0.4, 1, 6, and 6.4 full-scale-per-min rates for a 2-V output to the Limc, to provide maximum precision with the 2-V full-scale, 9 bit Α/Δ converter.

An analysis of Equations 1 and 2 clearly demonstrates the necessity for the track-and-hold circuit—e.g., an offset V0 of about ±0.6 V to the 0.5-s integrator will cause amplifier 5 to limit during an 8-s Δt.

By attenuating the signal with the gain control it is possible to switch to the next most sensitive scale to improve the signal-to-noise ratio, because doubling Δt increases the sensitivity four times, which in turn allows a fourfold increase in RC. Long-term drifts, however, the largest source of error in the measurements, are integrated twice as long, for a doubled net improvement of signal-to-noise ratio. Since the readout is obtained from an integrator that inherently tends to reject noise, especially short-term noise, all scale settings are relatively immune to such noise. Most short-term noise is derived from 60-Hz hum, the windings of the input potentiometer, and from oscillations due to underdamping of the recorder. It is a fortunate part of the design that at higher sensitivities, more noise rejection is inherently built in. This is similar to the approach of the variable-time method (4), in which the slower rates are expressed to a greater precision. Serious errors is caused by recorder underdamping and dead-zone because long-term noise does not tend to be averaged. It is therefore recommended that the recorder be used in a slightly underdamped mode. Results have shown no difference between readings from an oscillating pen and a critically damped recorder.

The output to the computer consists of RY5 and a voltage divider to apply a bias of about +0.2 V (Fig. 2). RY5 is held open for 5 s by the monostable multivibrator, M2. A 470-kiloohm resistor between pins 7 and 8 and a jumper between pins 1 and 16 on the EU-500-IA card are used to obtain the 5-s period. The +0.2 V bias is necessarily added as a threshold, so the computer can distinguish between a zero signal and no signal present. Addition of the bias causes no calculation problems because a linear interpolation program is used for the calculations. The 5-s delay ensures that the peak will be read at least three times by the computer.

The logic and relay circuit is essentially that described by Cordos et al. (7). The fourth flip-flop, F4, determines the integrator timing, Δt. By varying the frequency to the toggle input of F4 with SW4 and F1, F2, and F3, it is possible to control the circuit sensitivity. NAND gate 1 prevents the flip-flop string from being toggled except during the measurement period; NAND gate 2 prevents retriggering during a measurement. The cross-coupled NAND gates which input to gate 2 serve to eliminate contact bounce from the spring-load push-button switch pair PB1 on the Binary Information Module. Monostable circuit M1 provides a variable (3 to 30 s) delay time before sampling. This permits ample time to set up the circuit by allowing the track-and-hold circuit to develop an accurate track, and to discharge the integrating capacitor C9. Long delay times can be used reproducibly to cancel effects of initiation times, stirring delays, etc., in the chemical measurement. The output of M2 also sets F1, F2, and F3 so that no additional delay is made. F4 is
toggled on the first positive clock pulse after the delay time has passed.

The lamps Q, R, S, and T on the Binary Information Module are used to indicate the status of the measurement—i.e., ready to initiate, premeasurement delay, measurement being taken, and computer reading peak, respectively. The polarity of the relays and relay drivers was chosen so that the relay coils are normally not energized except during the measurement cycle.

**Evaluation**

The interface was first evaluated for precision and accuracy by measuring output voltage rates of the Heath Polarograph Module. Resulting recorder slopes were carefully measured by manual estimation and by a sophisticated software technique which forms a part of the ELLA (Experimental Link Laboratory Analytic) system (11), currently under development in these laboratories. Slopes were also measured for enzymatic determinations and compared to the output voltage of the interface. Finally, dilution studies and comparison studies against the SMA-12/60 (Technicon Corp., Tarrytown, N. Y.) were made for lactic dehydrogenase (LDH) and alkaline phosphatase (1-lactate: NAD oxidoreductase, EC 1.1.1.27, and orthophosphoric monoester phosphohydrolase, EC 3.1.3.1., respectively).

All data were analyzed by calculations and plotting packages of the ELLA system. The graphs prepared for this paper were photographed directly from the LINC driven COMPLT plotter (Houston Instruments, Inc., Houston, Tex.). The graphs represent least-squares fits of the data. The slope, intercepts, and standard error of estimation in y (approximately the standard deviation in y) were also calculated by the ELLA software system.

**Simulated Rates**

Figure 4 shows a typical computer on-line schedule and report used in the study. The first two results (cup no.) are standards having relative values of 0 and 1000. The next seven results are for unknown samples. The computer calculates the sample values by linear interpolation. For nonlinear working curves, up to 12 standards can be accepted by the system. The name of the particular schedule in Fig. 4 is FAKETEST, especially prepared for seven settings on the polarographic rate simulator in values of 66 to 500 on the 1000-unit scale. The numbers next to the standards are the voltages obtained by the A/D converter on a 512-unit (9 bit) scale for 0- to 2-V input. The expected value is shown in parentheses in Fig. 4 next to the computer-calculated value. In all cases the voltage output was monitored by

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**ON-LINE SCHEDULE FOR FAKETEST**

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<th>CUP NO.</th>
<th>IDENTIFICATION</th>
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<td>0 STD</td>
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<tr>
<td>2</td>
<td>1000 STD</td>
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<tr>
<td>3</td>
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<td>4</td>
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<td>(100)</td>
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<td>7</td>
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<td>9</td>
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[COMPANY UPDATE REPORT]

**FAKETEST**

06-26-69

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<td>9</td>
<td>SAMPLE 63</td>
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</table>

Fig. 4. Schedule (upper) and computer report (lower) for the simulated reaction rates (FAKETEST)

Numbers in parentheses indicate expected values

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**Fig. 5. Output voltage vs. absolute-recorder rate (full-scale-per-min pen travel)**

For lines A and B, use upper abcissa. Sensitivity is decreasing from A to D. The slopes are in the order 16.90, 4.183, 1.042, and 0.2560. Relative standard error of estimation for the voltage output is on the order 1.1%, 0.7%, 0.5%, and 0.2%
Table 1. Summary of System Tests

<table>
<thead>
<tr>
<th>Line in Fig. 5</th>
<th>Nominal scale setting (full-scale/min)</th>
<th>Integration time, s</th>
<th>Slope of regression</th>
<th>Rel. std. error of estimation, %</th>
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<tr>
<td>A</td>
<td>0.1</td>
<td>8</td>
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<tr>
<td>B</td>
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<td>4</td>
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<tr>
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<td>2</td>
<td>1.021</td>
<td>1.1</td>
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<tr>
<td>D</td>
<td>6.4</td>
<td>1</td>
<td>0.988</td>
<td>1.7</td>
</tr>
<tr>
<td>...</td>
<td>All scales</td>
<td>...</td>
<td>1.002</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* 45 points are included for each scale setting.
* Standard error of estimation × 100/full-scale value (1000).
* Normalized results for all 180 data points considered individually.

The results for the simulated rates obtained by repeating the FAKE TEST schedule while simultaneously recording the interface output voltages five times at each basic sensitivity scale of 0.1, 0.4, 1.6, and 6.4 full-scale per minute are summarized in Fig. 5. Each data point shown in Fig. 5 is the average of the five replicate schedules run for each scale setting. The voltage intercepts of about 0.20 V in each case represents the added threshold voltage required by LABCOM (8). The most and least sensitive scales, lines A and D, exhibit the poorest reproducibility owing to internal noise and drift in the most sensitive scale and probably to recorder response speed on the least sensitive scale. The sensitivity of each scale, the slope of the four lines, differs by an average factor of 4.04 compared to the theoretical slope of 4 predicted by Equation 2.

Table 1 summarizes the data and represents the calculated values for the entire system of Fig. 1. The data are essentially equivalent to the final results of the working curves of Fig. 5, but include all of the inherent errors of interpolation of seven readings between the two points, i.e., 0 and 1000. Again, the two inner scales are seen to produce better results for the same reasons given above. The standard errors of estimation indicate the excellent linearity and reproducibility of the system. The last line of Table 1 summarizes all 180 data points for the simulated runs as reported on the FAKE TEST computer reports. The "1000 point," for example, represents the standard or maximum value for the particular scale: 0.1, 0.4, 1.6, and 6.4 full-scale-per-minute rates as input to the recorder and its retransmitting potentiometer. The regression slope of 1.002 indicates the accuracy of the reported values for the system. This value represents a relative accuracy of 0.2%. The 3.010 standard error of estimation approximates a relative standard deviation of 0.3% for the highest values. For values of about one quarter scale, the relative accuracy and precision is about 1%. Precision of these results are of the order of experimental error associated with the 9-bit A/D converter or 1/512, or about 0.25%.

Manually Obtained Rates and Noise Rejection

A calibration curve was prepared correlating output voltage from the interface with slopes obtained manually and spectrophotometrically recorded rates, in this case for LDH determination (Fig. 6). The slopes' change in absorbance per minute (ΔA/min) was manually estimated from the recorder chart. The standard error of estimation for this least-squares fit corresponds to 0.0017 ΔA/min, indicating that the interface obtains values that agree closely with the standard manual techniques and are of the order of the drift (0.001 ΔA/min) of the particular spectrophotometer.

The noise-rejection features of the interface were tested by comparing the output voltages for inputs with and without an oscillating recorder pen. For oscillations amounting to about 5% of full scale, no measurable difference was noted in the output voltage. This corresponds to about 500 mV peak-to-peak noise in the input circuit.

Enzymic Dilution Studies

To evaluate performance of the entire system in a laboratory situation, enzymic dilution studies
Comparison with SMA-12/60

To illustrate that reliable values for enzymic rate measurements can be obtained from the system, a study was made to compare the results of sera analyzed by the system to those analyzed by the routine clinical laboratory. The “token” results were obtained with the SMA-12/60 by the method described in the instrument manual (14). Since the methods for SMA-12/60 are not true rate methods, large discrepancies may be expected.

Figure 7 compares alkaline phosphatase determinations. The results of this experiment indicate excellent agreement of the two methods and the precision of the system. The regression line of Fig. 7 was obtained from the results of 12 different sera and had a slope of 1.003 with a standard error of estimation of 5.0. The intercept value of 2 units is within the precision of the method. This study indicates a relative correlation of 0.3% and a relative precision of 0.25% for high sera. More results would certainly be required for clinical evaluation. The alkaline phosphatase reagents were the same for both methods.

A similar experiment for LDH determinations was performed. A regression line slope of 1.08, standard error of estimation of 8 Wacker units, and an intercept of 8 Wacker units were obtained for five samples run in quadruplicate. The agreement for LDH was not so good, probably because of the difference in methods.

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References