Programmed Multiparameter Monitoring of Effluents from Chromatographic Columns

S. M. Mozersky and J. A. Connelly

Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Philadelphia, Pa. 19118

A low-cost system is described which is capable of programmed monitoring of as many as eight parameters of the effluent of a chromatographic column. With no change in fundamental design, the number of parameters monitored can easily be increased by a factor of two or more. The parameters may include those of reaction streams resulting from reaction of the column effluent with one or more confluent reagent streams. The present system utilizes a spectrophotometer whose wavelength wheel can be programmed to stop at any number of preselected wavelength settings while measurements are made. A system in which this spectrophotometer will be eliminated is discussed.

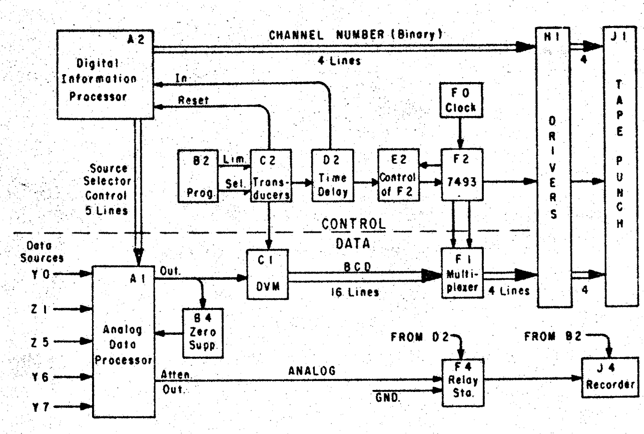

Figure 1. Block diagram of the monitoring system

In our work on the fractionation of enzyme (myosin) preparations for preparative and analytical purposes, it is necessary to monitor the effluent of a chromatographic column at intervals of 5–10 minutes over a period of about 5 days for the concentrations of protein and enzyme. Most of the column effluent is, in preparative runs, collected fractionally, in which case it is also necessary to relate the chromatograms of protein and enzyme content to the various fractions.

The system to be described here provides eight monitoring channels, and, therefore, has the capability of monitoring eight parameters. We use four of the channels (No. 1–4) to monitor protein concentration and one (No. 5) for enzyme concentration. A sixth channel (No. 6) is used to keep track of the fraction number. This leaves two channels (No. 0 and 7) which can be used for other parameters, such as pH and conductivity. We generally use these to monitor 0 and +5.000 volt signals, as a check on the integrity of the whole system.

Protein concentration is determined from measurements of the transmittance of the effluent, made with a Beckman DB-G spectrophotometer, at 279, 330, and 259 nm, the latter two wavelengths serving to correct for light scattering (*1*) and nucleic acid content.

The effluent is also monitored at 296 nm, at which wavelength the absorptivity of protein is low and the range of recorded transmittance values correspondingly reduced, to provide a somewhat crude but quick guide to the variations in protein content. A portion of the column effluent is continuously drawn off with a stream splitter, and the concentration of enzyme (ATPase) is determined by a continuous assay procedure to be described elsewhere. The necessary observations are made, at 340 nm, with a second spectrophotometer and a Gilford Model 2000 Absorbànce Recorder.

The analog data are collected on recorder chart paper and, after conversion to digital form, on paper tape. The perforated tape is processed with an IBM 1130 computer. A computer-controlled plotter then provides the desired chromatograms of protein concentration, enzyme concentration, and specific enzymatic activity, as well as plots of the raw data and, when desired, partially processed data.

GENERAL FEATURES OF OPERATION

To provide a general understanding of the operation of the system, its principal parts and their functional roles will be described first. A discussion of the details of operation, in terms of the sequence of events involved, will be given in the section titled Control Logic.

The Programmer. The programmer unit of the system (B2 of Figure 1) consists of the wavelength wheel of a Beckman DB-G spectrophotometer and its fast (200 nm/min) drive motor, the clips which attach to the wheel, the clip-sensing photocells, and an automatic wavelength "programmer" module (Beckman Catalog No. 135970). The "programmer" module is set in the "point program" mode and on the "medium" readout period. (Some familarity with the spectrophotometer and "programmer" module will be assumed.)

To program the wavelength wheel to stop at the four wavelength settings used for monitoring protein concentration, point clips were carefully placed at these positions on the wheel, corresponding to channels 1–4 (Table I). For channels 0 and 5–7, for which the wavelength settings are meaningless, one of the point clips was placed at a wavelength higher than that for channel 1, and three at wavelengths lower than that for channel 4. These are dummy clips in the sense that no transmittance readings are taken by the DB spectrophotometer at these wavelengths. When the wavelength wheel stops at one of these settings, the control system to be described directs that a reading be taken of the voltage output of the corresponding data source. Thus, when the wheel stops at 232 nm, which corresponds to channel 5, the system reads the output of the Gilford spectrophotometer, data source Z5. (All designations consisting of a capital letter followed by a number refer to Figure 1).

No low-wavelength (reverse) clip is used; the wheel, therefore, moves only in the forward direction, which is the direction of decreasing wavelength. A high-wavelength (limit) clip was placed at approximately the 375 nm posi-

Table I. Selection of Data Sources and Wavelengths

Data source (Fig. 1)	Channel No.	Type of clip	Wavelength, nm	Function
...	...	High (limit)	375	Start cycle
Y0	0	Point (select)	355	0 V
Z1	1	Point	330	Protein concentration
Z1	2	Point	296	Protein concentration
Z1	3	Point	279	Protein concentration
Z1	4	Point	259	Protein concentration
Z5	5	Point	232	Enzyme concentration
Y6	6	Point	206	Fraction count
Y7	7	Point	180	+5.000 V

tion on the wavelength wheel. After the last channel (No. 7) has been monitored, the wheel moves in the forward direction, and a new cycle begins when the wheel passes through the 375-nm (limit) position, at which time the digital processor A2 is reset to channel 0. This, in turn, sets the analog processor A1 to process data from source Y0.

The wheel does not stop at the high-wavelength clip; the first stop is at the first point clip (at 355 nm), at which time the output of data source Y0 is read. When the wheel moves away from any given point clip, the information processor A2 is set to the next higher channel number, in anticipation of the reading to be taken on reaching the next wavelength clip.

On leaving channel 7, the digital information processor is set to channel 8. However, the next clip seen (by the clip-sensing photocells) is the high-wavelength (limit) clip, which resets the processor to channel 0. Hence, no data are collected for the nonexistent channel 8.

One revolution of the wheel (a period) takes about 6½ minutes.

Generation and Recording of Channel Number. Information for the control circuitry (A2–F2) is generated by the limit and select lamps on the face of the "programmer" module. The limit lamp turns on when the high-wavelength (limit) clip passes its detector, a photocell, and the select lamp turns on when any of the point (select) clips activates the point clip detector, another photocell. The light signals from these two lamps are observed by photoconductive cells, and, after transduction to electrical pulse signals and suitable time delay (C2 and D2), they reset to zero or advance by one, respectively, the binary (7493) counter of the digital information processor A2. Since the output of the counter never exceeds eight (in effect, it never exceeds seven), only one decimal digit is required to record it in BCD, the code used on the paper tape.

Assignment of the Paper Tape Coding. Of the eight columns across the width of the paper tape (Figure 2) the first four, 1–4, are used to record the channel number, and the last four, 5–8, to record the voltage outputs of the monitoring instruments, after conversion from analog to digital form. These voltages are read to the nearest millivolt. Four rows of the paper tape are used for each reading, corresponding to four decimal digits, δ, γ, β, and α, thus providing a range of 0 to 9999 mV. The channel number, which requires only one decimal digit, and therefore, one row, is punched four times, thus identifying each of the four successive decimal digits of the data as belonging to a given channel. This simplifies reading the paper tape visually, which is occasionally helpful.

Figure 2 illustrates a section of tape containing one reading. The channel number is 7; hence, $\delta = \gamma = \beta = \alpha = 7$. Therefore, D = 0 and C, B, and A are 1 (columns 1–4). A

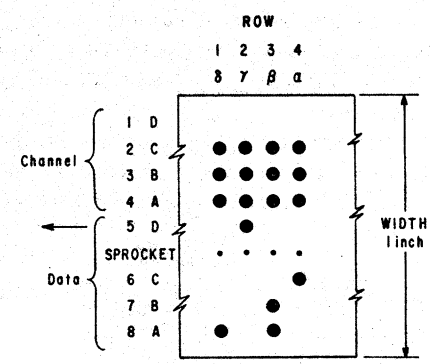

Figure 2. A section of perforated tape

The channel number is 7, the data value 1834 (millivolts). The Greek letters represent decimal digits; the English letters, binary digits. As it is punched, the tape moves, over the palls, in the direction shown (arrow). The rows are, therefore, perforated in the order 1 to 4

"1" appears on the tape as a hole. The data value, recorded in columns 5–8, is 1834, *i.e.*, $\delta = 1$, $\gamma = 8$, $\beta = 3$, and $\alpha = 4$. Converting to binary, $\delta = 1$, $\gamma = 1000$, $\beta = 11$, and $\alpha = 100$.

The Analog Data Processor (A1). It will be noted that while there are eight channels, 0–7, there are only five data sources (Figure 1). This is because four wavelength settings, corresponding to channels 1–4, are used for monitoring protein concentration, and the data source for these four channels is the same, *viz.*, Z1, the DB spectrophotometer. To achieve this, the output of the counter in A2 is fed to a circuit, also located in A2, which is, in essence a binary to 1/5 line decoder. Counts of 1, 2, 3, and 4 from the counter, all result in the same choice of output line. The five lines of the decoder output connect to the coils of five relays in A1 (Figure 1), thereby connecting the output of the analog data processor to the proper one of the five data sources Y0 to Y7.

The DVM (C1) and Multiplexer (F1). To convert the selected analog signal to the BCD form required by the tape punch J1, a digital voltmeter, C1, is used. The DVM consists of a Hewlett-Packard No. 3470 measuring system, which converts dc voltage into parallel BCD information. The voltmeter is triggered, by C2, to take a reading one second after the wavelength wheel has been stopped by a point (select) clip, and this reading is held until the voltmeter is triggered again. Sixteen of the output lines (C1–F1) of the digital voltmeter provide the four decimal digits needed (δ, γ, β, and α in columns 5–8 of Figure 2). The sixteen lines are connected, by the multiplexer F1, four at a time, to four drivers (H1), which, when in the "on" state, activate the palls of the punch for columns 5–8 (D, C, B, and A) of the paper tape (Figure 2). The first four of the

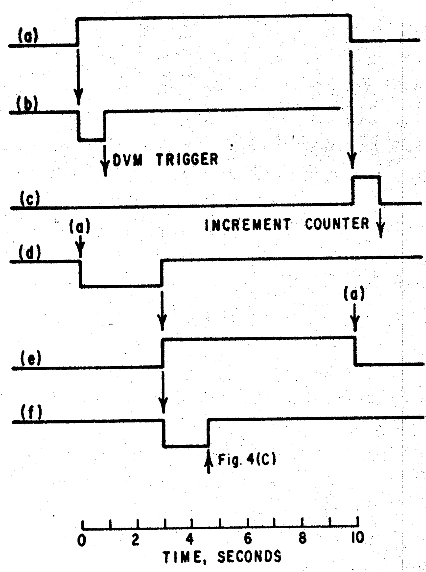

Figure 3. Triggering the digital voltmeter C1(*b*), incrementing the channel counter in the Digital Information Processor A2(*c*), and controlling the activity of counter F2(*f*)

Referring the pulse diagrams (*a–f*) to the block diagram (Figure 1), the flow of information is as follows: (*a*) B2 to C2 to D2, (*b*) C2 to C1, (*c*) D2 to A2, (*d*) D2, internal, (*e*) D2 to E2 and F4, (*f*) E2 to F2. The pulse widths, in seconds, are as follows: (*a*) 10, (*b*) 1, (*c*) 1, (*d*) 3, (*e*) 7, (*f*) 1.6

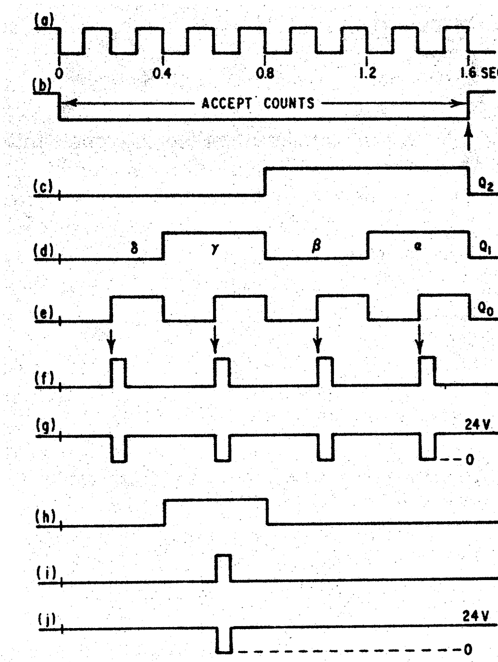

Figure 4. Control of the multiplexer (*c, d*), the tape punch clut[illegible] and its palls (*j*)

Pulse *b* is the same as that in Figure 3*f*, the time scale being expanded. The flow of information is as follows: (*a*) F0 to F2, (*b*) E2 to F2, (*c*) F2 to E2 and F1, (*d*) F2 to F1, (*e*) F2 to H1, (*f*) H1, internal, (*g*) H1 to J1, (*h*) F1 to H1, (*i*) H1, internal, (*j*) H1 to J1

sixteen lines multiplexed provide the most significant decimal digit δ (row 1), which is in thousands of millivolts.

The Strip Chart Record. In addition to the digital (BCD) record on paper tape, an analog record is also kept of the outputs of the monitoring instruments Y0–Y7. The output of the analog data processor A1 is first fed to a precision, autoranging zero suppressor B4 (*2*), which reduces the output by an integral number of volts to a value between 0 and 1. The latter value is then fed to the voltage divider section of the analog data processor, which attenuates the signal by a factor of 10. The attenuated output of A1 is thus in the range 0–100 mV, as required by the potentiometric recorder (J4) used (Beckman No. 100502).

When a reading is to be taken, the input to the recorder J4 is connected by the relay station F4 to the attenuated analog signal from A1, and the recorder drive motor is turned on for a few seconds by the programmer B2. When a reading is not being taken, the input to the recorder is connected to ground.

CONTROL LOGIC (TTL)

The events occurring at the beginning of a cycle are as follows. When the high-wavelength (limit) lamp of B2 is turned on, the output of the limit transducer in C2 goes high, resetting the channel counter in the digital information processor A2 to zero. The reset control voltage remains high for one second, keeping the counter immune during this time. Return of the voltage to the low state makes the counter receptive to pulses arriving at its clock input from D2. The first such pulse occurs when the wavelength wheel starts moving from the channel 0 to the channel 1 setting.

The sequence of events in the subsequent phases of operation is shown in Figures 3 and 4. When the wavelength wheel arrives at a point (select) clip it stops, and the select lamp goes on (Figure 3*a*). This generates a one-second negative pulse (3*b*) at one of the outputs of the select transducer in C2. The positive-going transition at the terminal edge of this pulse triggers the digital voltmeter C1 to take a reading of the analog input from A1, which reading is held until the next such pulse.

When the wavelength wheel moves away from a given wavelength setting, the select lamp (3*a*) goes off. This produces a one-second positive pulse (3*c*) to the input of the channel counter in A2. The negative-going trailing edge of this pulse advances the counter output one count.

As the wavelength wheel approaches a given setting, the input to the DVM comes from the data source to be observed next. There is, therefore, no large change at the input of the voltmeter when the wheel stops. (A large change could occur if the channel selection, *i.e.*, an increment in the channel count, were made when the wheel stopped.) In addition, the one-second delay gives more than adequate time for the input to settle down to its proper value.

The sequence of events involved in control of the tape punch is as follows. In addition to the one-second pulse output (3*b*) to the DVM, the select transducer has an output to the time delay D2. This output is the simple electrical analog of the state of the select lamp. Figure 3*a* therefore serves to indicate the state of this output as well as that of the lamp. When the select lamp goes on, the positive-going transition in the output of the transducer triggers a three-second negative pulse (3*d*) in the delay circuit D2. The positive-going transition at the terminal edge of this pulse then triggers a one-shot with a long time constant, the output (3*e*) of which is sent to E2 and to relay station F4. This pulse never lives out its full life; when the lamp goes off, the negative-going transition at the terminal edge of pulse (3*a*) resets the output (3*e*) to the low state. The leading edge of pulse (3*e*) triggers the counter control E2 to produce a very long (20-second) negative pulse (Figures 3*f* and 4*b*) which likewise never lives a full life, being subjected to a clear signal after 1.6 seconds (4*c*). When the reset control (3*f*) is high, the counter F2 is inactive and its output is zero. F2 counts only during the 1.6-second period when the reset is low.

The clock input to the counter F2 (Figure 4*a*) has a period of 200 msec and a pulse width of 100 msec. The outputs Q1 and Q2 (4*d* and 4*c*) provide a count of 0 to 3 to the multiplexer, thereby selecting, successively, the four decimal digits δ, γ, β, and α.

Output Q0 (4*e*) of counter F2 is fed to a one-shot with a time constant of 30 msec; the latter is contained in the first stage of the driver circuitry (H1). The leading edge of each input pulse (4*e*) produces a 30-msec positive pulse (4*f*) at the Q output of the one-shot. Each such pulse gives rise to a 24-volt pulse (4*g*) at the clutch driver output, thereby activating the clutch solenoid of the tape punch J1 (Friden Model Sp-2).

The output of the one-shot (4*f*) is also fed to each of eight AND gates in H1. Each AND gate has, as a second input, one of the four lines (D, C, B, A) from the channel counter in A2 or one of the four lines (D, C, B, A) from the multiplexer F1 (4*h*). The output (4*i*) of a given AND gate is high only when both inputs, that from A2 or F1 (4*h*) and that from the oneshot (4*f*) are high. Consequently, in any one of the four periods δ, γ, β, or α (4*d*), a 30-msec pulse is produced at the output (4*i*) of the AND gate if, and only if, its input from A2 or F1 is high. The output of the AND gate produces a 24-volt pulse (4*j*) as the output of the driver, which, in turn, activates the corresponding pall in the tape punch (J1). Activation of the palls is simultaneous with activation of the clutch.

The inputs from the multiplexer (F1) are constant for a duration of 400 msec (4*d* and 4*c*). (Those from A2 are constant for a much longer time.) The positive-going transitions of Q0 (4*e*) occur in the middle of these periods. The leading edge of any given driver pulse, therefore, occurs in the middle of the period during which the input to the AND gate from the multiplexer is constant, and the driver pulse terminates 170 msec before the period ends.

As an illustration of the logic involved in controlling a pall, consider column 5 (binary digit D) of the section of tape shown in Figure 2. The holes in this column are produced by pall No. 5. Here $D(\delta) = D(\beta) = D(\alpha) = 0$, and $D(\gamma) = 1$. Hence, the output of the multiplexer at line D is given by Figure 4*h*, being high only during the γ-period (4*d*). The inputs to AND gate No. 5 are 4*h* and 4*f*. The output of this gate (4*i*) is, therefore, high only during the first 30 msec of the second half of the γ-period. Pulse 4*i* produces a 24-volt pulse at the output of driver No. 5 (4*j*), which activates the solenoid of pall No. 5 (row 2 of the tape).

CONSTRUCTION

Of the blocks in Figure 1, B2, C1, J1, and J4 are instruments or accessories available commercially; B4 was made in this laboratory (*2*). The circuitry of the remaining blocks was constructed on printed circuit boards measuring 3¾ × 3¾ inches. Each block occupies one board, except for F1 and F2, which together occupy one board, and H1, for which seven boards were used. In most cases, sockets were soldered into the boards, so that components could be easily replaced. The circuit boards were manufactured according to our design and specifications by a small company specializing in custom work.

Some modification of the Beckman "programmer" module was necessary. The three 10-kohm resistors at Q21, Q22, and Q26 were pulled so as to separate them from the +12-V supply, and the +12-V supply to relay K6 was cut. These modifications inactivated the recorder input and print command relays, which produce interference. On terminal strip TB2, mounted on the rear of the "programmer" module, terminals 1 and 2 were grounded to a nearby point on the chassis, and terminals 6 and 7 were shorted together.

The driver circuitry (H1), as initially constructed, gave rise to irregular operation of the tape punch. This resulted from false pulses appearing at the output of the one-shot, these presumably occurring during the intervals when the Q0 output of counter F2 (4*e*) was low. To prevent these false pulses from triggering the pall drivers, three input AND gates are used in the eight driver circuits described above, rather than two-input gates. In addition to the input from the one-shot and that from the digital information processor A2 or the multiplexer F1, output Q0 (4*e*) of the counter (F2) is fed, as a third input, to each of the AND gates. This provides a coincidence circuit for pulses 4*e* and 4*f*, so that pulses put out by the one-shot are effective only if and when the output Q0 (4*e*) of the counter (F2) is high. False triggering of the clutch driver is likewise prevented by a ninth three-input AND gate. Two of its inputs are the output (4*f*) of the one-shot; the third input is the Q0 output (4*e*) of the counter F2.

Two other precautions were found useful for the prevention of interference by noise. First, the outputs of the spectrophotometers, Y1 and Y5 (not shown), are fed to the corresponding inputs of the analog data processor A1 through instrumentation amplifiers, Z1 and Z5, the inputs of which float. Second, the ground of the 24-volt power supply used to power the drivers is not connected to the ground of the TTL system. Signals from the TTL logic are transferred to the drivers with optical couplers.

RESULTS AND DISCUSSION

Major advantages of the monitoring system described, besides achieving the purpose of programmed monitoring of several parameters, are its excellent reliability, the ease of repairing it, and its low cost. The system has been trouble-free during approximately two years of operation. As an additional benefit, we found design and assembly of the circuits to be an invaluable experience in laboratory instrumentation. The type of circuitry involved has very wide application in the laboratory, and first-hand familiarity with it impresses upon the investigator the ease and precision with which formerly difficult tasks may be performed.

Because of the impact which the system described has had on our work, we plan to build a second such system. However, we anticipate certain changes in design. Spectrophotometers (Y1 and Y5, not shown) will not be used. Light of the desired wavelengths will be selected by light filters instead of monochromators, and pin photodiodes will be used as detectors in place of photomultiplier tubes. Variations in the intensity of the light source will be compensated for by taking a ratio of the intensity of the light beam observed to that of the source. Hard-wired electronic circuitry will be used to perform the programming function now performed by the wavelength wheel, the clips attached to it, the clip sensors, the wheel drive motor, and the commercial "programmer" module. The modifications will make it unnecessary to purchase some of the costly commercial instruments and accessories which are part of the present system. These instruments and accessories were included in the present system because they were available, and using them reduced the time required to make the system operational.

Since the fast response of TTL is not required, MOS circuits will be used in the new system. The latter are preferable for several reasons: 1) MOS circuitry is virtually immune to ground noise, thereby eliminating the need for construction of a complicated ground plane. 2) MOS facilitates construction of circuits with long time constants. 3) It does not need the sharp triggering pulse required by TTL. 4) Power supplies for MOS circuits need less regulation than those for TTL. 5) The power consumption will be decreased by a factor of at least 1000. And, finally, 6) MOS eliminates almost all technical problems associated with handling TTL.

CONCLUSION

A highly reliable, relatively inexpensive system is described for programmed monitoring of the effluent of a chromatographic column for as many as eight parameters. These may include the transmittance of the effluent, and of products of reactions used for assay of the effluent, at each of several wavelengths. The data are collected on paper tape, for processing by computer, and on recorder chart paper.

LITERATURE CITED

(1) S. M. Mozersky, J. D. Pettinati, and S. D. Kolman, *J. Chromatogr.*, **65**, 387 (1972).
(2) J. A. Connelly, S. M. Mozersky, and H. Pessen, *Rev. Sci. Instrum.*, **42**, 773 (1971).

RECEIVED for review July 29, 1974. Accepted November 15, 1974. Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.