High-Performance Colorimeter with an Electronic Bubble Gate for Use in Miniaturized Continuous-Flow Analyzers


We describe a high-performance colorimeter with an electronic bubble gate for use with miniaturized continuous-flow analyzers. The colorimeter has a flow-through cuvette with optically flat quartz windows that allow a bubbled stream to pass freely without any breakup or retention of bubbles. The fluid volume in the light path is only 1.8 µl. The electronic bubble gate selectively removes that portion of the photodetector signal produced by the air bubbles passing through the flow cell and allows that portion of the signal attributable to the fluid segment to pass to the recorder. The colorimeter is easy to use, rugged, inexpensive, and requires minimal adjustments.

Numerous colorimeters and spectrophotometers are commercially available for use with continuous-flow analyzers; most of them are expensive, fragile, bulky, require excessive quantities of fluid, and do not have flow-through cuvettes adequate for the passage of a bubbled stream.

Wishing to construct an efficient, low-cost miniaturized high-speed continuous-flow system (1), we found it essential to design and develop a high-performance colorimeter. Our prime considerations in the colorimeter design were:

1. It must be capable of accurate and precise transmittance measurements.
2. The photodetectors and associated circuitry must be highly stable, sensitive, and respond linearly to low light intensities.

3. The flow-through cuvette must employ optically flat quartz windows that allow a bubbled stream to pass without any breakup or retention of the bubbles.
4. An electronic bubble gating system must selectively remove that portion of the photodetector signal caused by the air bubbles passing through the flow cell and allow that portion of the signal from the fluid segment to pass to the recorder.
5. It must be of low cost, easy to use, rugged, require minimal quantities of raw materials, and have minimal adjustments.

Here we describe the design and construction of such a colorimeter.

Colorimeter Construction

General description. Figure 1, a simplified block diagram of the colorimeter, illustrates the main components. The light source is a high-intensity quartz halogen lamp. The light passes from the lamp through an interference filter, a biconvex lens, a flow-through cuvette, and then reaches the photodetector. The photodetector signal is amplified and enters the electronic bubble gate. The electronic bubble gate eliminates that portion of the signal produced by the bubble artifact and allows the signal from the transmittance of the fluid segments to pass to the strip-chart recorder.

The optical feedback (optical servo) system, which continually monitors and controls the lamp intensity, increases stability. The light passes through an aperture and an interference filter to a photodetector. The photodetector signal is amplified and enters the light-regulation circuit that controls the current to

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1. Divisions of Biochemistry, Instrumentation, and Neuropsychiatry, Walter Reed Army Institute of Research, Washington, D. C. 20012; and the Department of Laboratory Medicine, Yale-New Haven Hospital, New Haven, Conn. 06510.
2. Reference to products by manufacturer does not constitute endorsement by any government agency and is included only for purpose of description.
3. Received Oct. 1, 1975; accepted Nov. 21, 1975.
4. Xerographic copies of the colorimeter components, detailed parts listing, and printed circuit board layouts may be obtained on specific request to either the authors or the Editorial Office.
the lamp. A quartz halogen lamp is used because of its high intensity and relatively low power consumption. As noted by Hewitt and Pardue (2), the high-intensity lamp provides a high signal/noise ratio and minimizes the independent and proportional errors in absorbance measurements.

*Interference filters.* The interference filters (1.3 × 1.3 cm, approximately 0.5 cm thick) are inexpensive (about $4.05 in lots of 20) and have excellent blocking properties in the near-infrared range as required when silicon P.I.N. photodiode detectors are used (Ditric Optics, Inc., Valencia, Calif. 91355).

*Lens.* In place of an expensive and complicated lens system, excellent results are obtained by the use of a simple biconvex lens ($1.50 each; A. Jaegers, Inc., Lynbrook, N.Y. 11563). The lens holder is a very simple design, yet precise adjustments are easy. The lens is mounted in a holder and a small spring applies upward pressure. Two small adjustment screws are used to position the lens precisely. By adjusting each of the two screws with an adjustment tool, the lens can be easily moved over a small circular area to provide optimal alignment. To align the lens, the flow-through cuvette is filled with water and the adjustment screws are rotated until the voltage output of the photodetector amplifier (Figure 3, IC2, pin 6) is maximum. At this point a maximum amount of light is passing through the flow-through cuvette. If at a particular wavelength the light intensity is too great the lens may be slightly defocused to decrease it.

*Flow-through cuvette.* The flow-through cuvette is constructed from a polyvinyl chloride or Kel-F block. Polyvinyl chloride is satisfactory for most reagents used in the clinical chemistry laboratory and is used in most cases because it is easily machined and inexpensive. A jig was first designed and the flow-through cuvette was constructed in clear acrylic to assure correct internal design. The jig was then used to manufacture the actual flow-through cuvettes in opaque polyvinyl chloride blocks. A cross section of the flow-through cuvette is diagrammatically illustrated in Figure 2. The light path (B) is 0.8 cm long and 0.5 mm in diameter (E). The volume in the light path is only 1.8 μl. The inlet ports are 0.79 mm in internal diameter (G) and approach the horizontal path from an angle of 66° 17' (A). Quartz rods with flat, polished ends (Honde Glass Co., Newark, N.J. 07104) measuring 0.635 cm in length (C) and 0.10 cm in diameter (D), are press-fitted in the block to provide optically clear windows. Adhesive is not applied to the rods, because it can easily coat the inner face and thus partially block the transmitted light. The great advantage of this flow-through cuvette is that a large amount of light passes through the flow cell because of the quartz windows. Unlike flow cells constructed from bent glass tubing, there is no light reaching the detector through the glass tubing walls. In earlier designs, a major problem arose: small bubble fragments would break off from the larger bubbles and would attach to the interface between the glass ends and the plastic body. In the present design this does not occur. By allowing the quartz rod on each end to protrude slightly into the flow path, a slight turbulence is created as the solution enters the light path over the edge of the quartz rod. This greatly aids in "cleansing" the quartz rod face and prevents bubble entrapment. At the distal end of the light path, the increased fluid velocity and the tendency for bubbles to float upwards aids in preventing bubble entrapment. Alternatively, one can substitute a commercially available flow-cell that is used on the SMAC analyzer (cat. No. 178-B724-02; Technicon Instruments Corp., Tarrytown, N.Y. 10591).

*Photodetectors.* A silicon P.I.N. photodiode is the photodetector. Used for measurements between 430 and 1000 nm, its advantages are small size, low noise, low cost, long-term stability, linearity over wide ranges of incident light, ruggedness, and low voltage operation (5–15 V d.c.). The particular photodetector we used ($6.25 each) is guaranteed for 10 years by the manufacturer (Monsanto Commercial Products, Cupertino, Calif. 95014). In contrast, photomultipliers are expensive, temperature-sensitive, require high-voltage supplies (1100 V d.c.), extensive and expensive shielding, and complicated electronic circuits, and are easily damaged by excess light.
The photodiode (PD1) is connected to an operational amplifier (IC1) with a field-effect transistor input to provide high input impedance (Figure 3). The operational amplifier serves as a current-to-voltage converter. As contrasted with most other photodetectors, the P.I.N. photodiode is operated without bias. This is the correct method of operation in this application, as it ensures zero dark current. Used in this fashion, the diodes are linear over a range of almost twelve decades. The value of the feedback resistor (R1) ranges from 200 to 1000 MΩ (Victoreen Instrument Division, Cleveland, Ohio 44104). At low wavelengths (430–540 nm) a 1000-MΩ resistor is used; for higher wavelengths, a 200-MΩ resistor. Capacitor C1, used to reduce high-frequency noise, should be carefully selected, because too small a value will result in excessive noise and too large a value will result in a low slew rate, which precludes proper operation of the electronic bubble gate. PD1, IC1, R1, and C1 must be electronically shielded. Power-supply bypass disc capacitors are used (not shown) in close proximity to each operational amplifier. The second operational amplifier (IC2) inverts and amplifies the analog signal to provide the necessary 0–8 V input range required by the electronic bubble gate. P1 is used to adjust the photodetector signal to approximately 8 V for 100% T.

Light-regulation circuit. The light-regulation circuit is based on the earlier work of Pardue et al. (3, 4), in which optical feedback was used to stabilize their photometers. The photodetector circuit constantly monitors the lamp output, and if any change in light intensity is detected, the current supply to the lamp is proportionally decreased or increased, resulting in a very nearly constant lamp intensity. Light striking the photodiode PD1 (Figure 4) produces a small current, which is converted to a voltage by the high-impedance operational amplifier (IC1). IC2 further amplifies the signal and provides an adjustment for the gain. A third operational amplifier (IC3) serves as a voltage comparator and controls the base current of transistor Q1, which in turn controls the power transistor (Q2). A large capacitor (C4) prevents oscillation. Q1 and Q2 form a Darlington circuit with a large current gain. For example, if the lamp intensity should increase slightly, PD1 senses this increase. With the elevated voltage signal at the inverting input of the comparator (IC3) as compared to the fixed voltage at its noninverting input, the output of the comparator will decrease the base current to transistors Q1 and Q2. This then decreases the current to the lamp. If the lamp intensity should decrease slightly below its set level the circuit will respond to increase the lamp current. With a 7-V lamp power supply, the maximum voltage available to the lamp is about 6.3 V. For the light regulation circuit to operate properly, the lamp voltage is adjusted with P1 to about 5.8 V.

Electronic bubble gate (Figure 5). The first bubble gate (5) allowed a bubbled stream to pass through a colorimeter and, by a specific method not mentioned, would interrupt the photodetector signal to the recorder whenever a bubble would pass through the
light path. Later, Habig et al. (6) eliminated the need for a debubbler by their design and construction of a device called a “bubble-gated flow cell,” which monitored flow cell conductance and deactivated the recorder servo motor whenever bubbles passed through the colorimeter flow cell. They concluded that as a result of the bubble gate system, efficiency was significantly increased but that their particular design, although useful for testing the method, would not withstand daily technician use and abuse. Neeley et al. (7) recently described a new, rugged, electronic bubble gate (signal comparator) that would eliminate the bubble artifact from the photodetector signal while allowing the signal produced from the fluid segment to remain intact. This instrument is reliable, easy to use, presently in routine use in a hospital laboratory, and does not require any adjustments by the technician.

Our new electronic bubble gate is based on the principles and logic described in our earlier article (7). This electronic bubble gate has been improved by the addition of several newly designed electronic components that provide greater flexibility and a significant reduction in overall cost.

The circuit design, printed circuit board layout, photoetching, and assembly is done entirely within our laboratories. The commercial window comparator has been replaced by a newly designed window comparator constructed from two inexpensive operational amplifier chips, a transistor, and a few passive components. The window width (in millivolts) of the window comparator is fully adjustable upward from a minimum of 1 mV. In addition, the window can be symmetric or adjusted over a wide asymmetric range. The three light-emitting diodes incorporated into the circuit provide a quick visual check to verify proper operation of the window comparator at any time. The recorder output can be set to any voltage range from 10 mV to 8 V. An optional bias voltage is provided to allow the use of Bristol (AutoAnalyzer) recorders. A separate analog signal of 0–8 V is provided to facilitate interfacing with any on-line data-processing equipment.

Performance

Linearity. Rather than measure the linearity of individual components, a truer estimate of overall performance is to use the colorimeter in several high-speed continuous-flow systems that are performing actual chemical determinations. Several chemical systems were chosen to represent various portions of the operating spectrum of the instrument. A commercial instrument (see Table 1) was used. A Hewlett-Packard 9810A programmable calculator was used on-line with the continuous-flow system to convert voltage to transmittance and then to absorbance (8). Several standards were run and a least-squares linear regression was performed on-line and the correlation coefficient obtained (Table 1).

Stability. Stability of the filter photometer depends highly on the wavelength used, because voltage output of the photodetector amplifier is directly related to the emission of the lamp at the particular wavelength and the particular wavelength response of the photodetector. At the longer wavelengths, the high photodetector output combined with the high emission of the tungsten lamp yields a large output signal, whereas at 430 nm the output signal is relatively low because of the combination of low lamp emission and relatively low photodetector sensitivity. Thus, at longer wavelengths there is maximum stability, owing to the large signal, and at low wavelengths there is minimum stability, owing to the small signal. To describe the stability of the photometer, transmittance measurements were made at 660 nm and at 430 nm by allowing a bubbled stream of fluid to pass through the flow-through cuvette. After a 10-min warmup, drift during 1 h was 0.3% T at 660 nm and 0.7% T at 430 nm. This is a negligible drift when one considers that in this time period, 150 samples could have been analyzed on each channel.

Table 1. Results for Glucose Analyses

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Method</th>
<th>Wavelength, nm</th>
<th>Conc. of standards</th>
<th>Correl. coeff., r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon dioxide</td>
<td>Cresol reda</td>
<td>430</td>
<td>10, 20, 30, &amp; 40 mmol/liter</td>
<td>0.9999</td>
</tr>
<tr>
<td>Glucose</td>
<td>Glucose oxidaseb</td>
<td>510</td>
<td>200, 2500, 3000 mg/liter</td>
<td>1.0000</td>
</tr>
<tr>
<td>Glucose</td>
<td>Glucose oxidasec</td>
<td>660</td>
<td>200, 2500, 3000 mg/liter</td>
<td>0.9999</td>
</tr>
</tbody>
</table>

a Carbon dioxide procedure for SMA systems, Technicon Instrument Corp., Tarrytown, N.Y. 10591
b "GOD/PAP (Trinder)", Boehringer Mannheim Corp., New York, N.Y. 10017
c "GOD/PERID", Boehringer Mannheim.

![Fig. 6. Glucose analyses at 120 and 150 samples per hour by the Trinder glucose oxidase method](image)

Steady state (SS) for 300 mg/100 ml standard. Standards range from 50–300 mg/100 ml in 50 mg/100 ml increments. Repeated analysis of a single serum sample is shown (S)
Evaluation of serum analysis. Figure 6 shows a strip-chart tracing of glucose analyses by the Trinder glucose oxidase method (9). At 120 samples per hour the results were: \( \bar{x} = 1340, \) SD = ±11 mg/liter, CV = 0.8% (n = 25) and at 150 samples per hour is \( \bar{x} = 1340, \) SD = ±1.7 mg/liter, CV = 1.3% (n = 25).

Discussion

We have now expanded the colorimeter from a single channel to four channels by the simple addition of three sets of flow-through cuvettes, interference filters, lens, and photodetectors. These colorimeters are an integral part of our four-channel high-speed continuous-flow systems that are currently performing our routine clinical chemistry determinations. The overall result has been to more than double analysis rates and to decrease reagent consumption by eight to 10-fold as compared to AutoAnalyzer II or SMA (Technicon) systems. We included on-line programmable calculators to provide automatic calculation and printing of results. The current overall cost is less than $600 for a single-channel colorimeter. In addition to the design and construction of the instruments we are also responsible for their repair. As a result, each colorimeter is totally modular, with great emphasis being placed on ruggedness and simplicity in design. Each four-channel instrument is equipped with its own digital panel meter and a multiposition switch to allow the operator to monitor voltages at strategically located positions throughout the system. Should electronic difficulty develop, it can be accurately diagnosed without extensive test equipment or removal of the surrounding case. Furthermore, all of the circuits are on plug-in printed circuit boards that can be easily and quickly replaced.

References


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