Automated Turbidimetric Bioassay Readout Instrument Using a Multiple Flow-Cell System

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Abstract

An instrument has been developed which permits the automatic quantitation of the turbidities of microbiological assay samples. Assay tubes were fed to the instrument at the end of the incubation period. The turbidity readings were automatically converted to digital data which were printed on International Business Machines (IBM) cards and from which potencies were calculated by an IBM computer. The instrument operated at a speed of over 240 tube readings per hour and was totally automatic in sample-mixing, readout, and data recording. The instrument is being used routinely at The Upjohn Co. for the turbidimetric bioassay of vitamins, with a coefficient of variation among repeated turbidity readings of 0.12 to 0.23%.

Because of the demanding and repetitive nature of microbiological assays of antibiotics and vitamins, there is a strong trend among industries toward automation. Most attempts at automation have been based on a continuous-flow system (3, 4). However, when the incubation period of a microbioassay is longer than 3 or 4 hr, it is less troublesome to adopt a batch incubation system. McMahan (2) designed an elaborate instrument which would dispense samples and medium, incubate, and record turbidity in digital form, all automatically.

This paper deals with an instrument which is less cumbersome and less expensive to construct, yet is totally automatic in sample-mixing, readout, and digital recording on International Business Machines (IBM) cards, and which is capable of handling more than 240 tubes per hour.

Materials and Methods

Instrumentation. The automated turbidimetric readout instrument has four major components: a sampler, a pump, a spectrophotometer, and a digital converter with an IBM 526 summary punch unit (Fig. 1).

The GME linear fractionator model LB1 (Gilson Medical Electronics, Middleton, Wis.) was modified and used as a sampler. The modification was the installation of a sampling arm, which consists of a 7-inch (17.8-cm) air cylinder and a stainless-steel horizontal plate. Three stainless-steel tubes (outer diameter, 0.3 cm) were attached to the plate for the sample intake together with 12 air sample mixing prongs and 6 magnetic sample vibrators. Magnetic vibrators are made of 26-gauge, stainless-steel plates (0.3 cm wide) which are twisted one turn every 4.4 cm to insure good sample-mixing (see Fig. 2). The racks used with the linear sampler were designed to hold nine test tubes (20 by 150 mm) and were made of stainless steel. The linear sampler takes sample from three tubes in a rack, and sample change-over is made by moving racks to a new position automatically.

The GME automatic transferator model AT6 (Gilson Medical Electronics) was modified to operate two syringe pumps instead of one to increase the pumping capacity required to aspirate turbid samples to three flow cells and to increase the overflow about 3 ml to minimize the carry-over effect.

The Hitachi Perkin-Elmer spectrophotometer model 139-PM was used to measure turbidity. Flow cells (model 127-OS, 5-mm light path) were obtained from Precision Cells, Inc., New York, N.Y. Three matched flow cells were used. The automatic cell positioner and flow-through cover (no. 9097-H10, both from A. H. Thomas, Philadelphia, Pa.) were attached to the spectrophotometer to move three flow cells sequentially at 6-sec intervals.

The digital converter unit consisted of a Datex Encoder (model C-104B-0; Datex Corp., Skokie, Ill.) which was installed on an indicator shaft of the Speedomax H model R indicator (Leed & Northrop Co., Philadelphia, Pa.) to digitize the millivolt output from the Hitachi spectrophotometer. The Encoder was connected to the Datex Control Chassis (model K-154) and, via the Datex junction box (model J-113), to an IBM 526 Summary Punch Unit.

The automated turbidimetric readout instrument was electronically integrated and controlled by use of a multiple relay and a step switch. The instrumental sequence of movements for the readout was as follows.
Turbid samples were first mixed with air, followed by a magnetic vibrator, for 45 sec each. The pump moved to aspirate samples into the flow cells. A portion of each sample, approximately 3 ml, was forced through a flow cell into an overflow check valve. Samples were held in each flow cell for 10 sec to stabilize turbidity readings. Sequential readout of the three samples followed at 6-sec intervals. Turbidity readings were converted to digital form and were punched on an IBM card. Then the pump moved to discharge the samples. The overflow check valve opened to discharge excess samples, and the sampler moved to a new position to take new samples.

The assay data thus obtained were processed with an IBM computer 1620 to compute product assay potencies.

**Assay procedure.** The niacin microbiological assay (1) was used to evaluate the instrument.

**RESULTS AND DISCUSSION**

Since the computer program used successfully to calculate vitamin potency is based on three dose levels of a standard and three dose levels of a sample (K. Tsuji et al., in preparation), three flow cells are arranged so that one flow cell takes only one given turbidity level. Sample-to-sample turbidity carry-over is minimized, thus eliminating the need of a rinse. Also, the multiple flow-cell

![Fig. 1. Automated turbidimetric bioassay readout instrument.](image1)

![Fig. 2. Magnetic and air sample mixing prongs.](image2)

**FIG. 1.** Automated turbidimetric bioassay readout instrument.

**FIG. 2.** Magnetic and air sample mixing prongs.

**FIG. 3.** Relation between the Hitachi Perkin-Elmer spectrophotometer reading and the Encoder unit (linear regression: $y = 1.009x - 1.302$).

**TABLE 1.** Effect of delay readout intervals on the stability of readings

<table>
<thead>
<tr>
<th>Sampling</th>
<th>Delay readout time (sec)</th>
<th>± 4 sec and after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Sample read-out</td>
<td>13.8*</td>
<td>14.4*</td>
</tr>
<tr>
<td>Medium read-out</td>
<td>84.1*</td>
<td>89.1*</td>
</tr>
</tbody>
</table>

* Significant difference at 95%.
system helped to obtain a high speed in read-out. The racks used with the linear sampler were constructed to stop three tube positions at a time to facilitate sampling of three levels of turbid sample to three flow cells. Since the rack was made of stainless steel, it withstood repeated autoclaving and incubation process, thereby minimizing the manual handling of test tubes.

**Encoder unit versus percentage of transmittance.** A relation between percentage of transmittance with the Hitachi spectrophotometer and Encoder units was determined by use of the niacin bioassay samples, and was found to be linear between 10 and 100% (Fig. 3). Therefore, in a practical sense, Encoder units can be treated as percentage of transmittance.

**Readout delay time interval.** When the turbid sample was introduced into the flow cell, it took a little while to stabilize the spectrophotometer reading. Therefore, it was important to determine a suitable delay time interval between the time the sample was introduced into the flow cell and the time the reading was taken. The instrument was set to record at 1-sec intervals from the time of sample introduction into the flow cell. The results are shown in Table 1. Statistically, there was no significant difference between readings taken after 4 sec. Therefore, 4 sec was considered the minimal delay time required to obtain a stable reading of a turbidimetric sample.

**Turbidimetric sample readout.** When the automated instrument was used to read plain medium repeatedly, the coefficient of variation among 12 readings was approximately 0.13% (Table 2). Also, Table 2 indicates that the three flow cells used were closely matched. The coefficient of variation among 12 readings was slightly higher (0.23%) when turbid suspensions were used (Table 2). However, this increase can easily be tolerated since the instrument is intended for use with bioassays.

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**Literature Cited**


