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Evaluation of the Operating Characteristics of a Bichromatic Analyzer, the ABA-200

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We investigated certain operating characteristics of a new bichromatic analyzer, the ABA-200. The reliability of filter factor assignments was found to be within 3% for NADH and p-nitroaniline, 6% for p-nitrophenol. The accuracy and precision of the dilutor is a function of dilution ratio and sample size. Accuracy of dilution ratio was within 3% at all ratios tested except 1:201 at a sample volume of 1.25 μL. Coefficients of variation were <1.3% at all ratios. Photometric linearity was observed to 3.2 A₄ for all filter sets. Photometric precision measurements showed a CV of 0.27% at an absorbance difference of 1.0.

The introduction of the automated bichromatic analyzer by Abbott Laboratories (Diagnostics Division, Dallas, TX 75247) in 1972 (1) generated an intense interest in the technique, and the analyzers have been widely accepted. In the intervening years bichromatic analysis has been applied to nearly all of the commonly done clinical chemical tests. Although factors affecting each test have been exhaustively investigated—e.g., for the determination of cholesterol (2)—we find no critical evaluation of the factors relating to instrument performance.

Our laboratory was selected as one of 10 sites to evaluate and field test a prototype of the newest generation of bichromatic analyzers, the ABA-200. We elected to take this opportunity to evaluate certain of the operating variables, especially those over which the operator has little or no control. Therefore, we defined the following as our objectives: (a) to assess the reliability of the filter factor assignments by the manufacturer; (b) to assess the accuracy and precision of the dilutor; and (c) to assess the photometric linearity of the instrumental system at the wavelengths commonly used in clinical bichromatic analysis.

Materials and Methods

Apparatus

An ABA-200 Bichromatic Analyzer was used for these studies. Sample cups were filled uniformly in all experiments, with use of a 50-μL pipettor.

Reagents

Glucose (SRM 917) was obtained from the National Bureau of Standards.

p-Nitrophenol (pNP), from Matheson, Coleman and Bell (cat. no. NX645), was checked for purity by measuring its molar absorptivity at 402.5 nm. The solution was prepared in 2-amino-2-methyl-1-propanol (0.89 mol/L, pH 10.3) as the diluent. The measured molar absorptivity was 18 976 L mol⁻¹ cm⁻¹ (expected: 18 800 L mol⁻¹ cm⁻¹) (3).

p-Nitroaniline (pNA) (Matheson, Coleman and Bell; cat. no. NX450) was recrystallized from water, filtered, and dried under reduced pressure. Purified pNA was prepared by using Tris-HCl (0.1 mol/L, pH 8.1). Its molar absorptivity at 405 nm was 9710 L mol⁻¹ cm⁻¹ (expected: 9900 L mol⁻¹ cm⁻¹) (4).

Filter Factor Evaluation

340/380: Standards of certified glucose were prepared in a 1 g/L benzoic acid solution in concentrations ranging from 0 to 4.00 g/L. They were mixed with "A-gent Hexokinase" glucose reagent (Abbott Laboratories) in a 1:101 ratio and the A₄ at 340/380 was measured. We calculated the theoretical concentrations of NADH and analyzed the data for the standards, using least squares linear regression statistics.

415/450: p-Nitrophenol: Dilutions of pNP were prepared from a stock solution of 999.8 μmol/L pNP, with using the

1 Nonstandard abbreviations used: SRM, Standard Reference Material; pNP, p-nitrophenol; pNA, p-nitroaniline; and A₄, absorbance difference.
2-amino-2-methyl-1-propanol solution as diluent. The filter factor was estimated from linear regression analysis of the data.

**p-Nitroaniline**: Dilutions of pNA were prepared from a stock solution of 5.13 mmol/L pNA diluted in the Tris-HCl buffer. Linear regression analysis of the data was used to estimate the filter factor for pNA.

### Dilutor Performance

The dilutor section of the instrument was evaluated by comparison with manual dilutions made with use of Class A volumetric glassware. Stock solutions of pNP in 0.1 mol/L NaOH were prepared to yield dilutions having an $A_d$ of approximately 1.0. The same stock solutions were used for manual and instrument dilutions. Dilutions were made using 0.1 mol/L NaOH as the diluent.

### Photometer Linearity

Stock solutions of NADH, pNP, Ponceau S, and ferro- zine–iron(II) complex were prepared for use in the estimation of photometric linearity of the 340/380, 415/450, 500/600, and 550/650 nm wavelength pairs, respectively. The concentration of the stock solutions was empirically chosen to result in $A_d$'s greater than 3.5 at the primary wavelength of each filter pair. The stock solutions were diluted in 10% (by vol) steps and the $A_d$ was measured. Linearity of response was estimated by fitting the data to a polynomial of the form $y = b_0 + (b_1 + x) + (b_2 + x^2)$ and testing the value of $b_2$ to see if it differed significantly from zero (5, 6). The number of degrees of freedom used to calculate the $f$-value for the quadratic coefficient was one less than the number of dilutions included in the statistical analysis.

### Photometer/Cuvet Precision

A solution of pNP having an $A_d$ of approximately 1.0 was manually pipetted into 31 positions of a standard 32-position cuvet. The $A_d$ at 415/450 nm was measured with the ABA-200 photometer unit.

### Results

#### Filter Factor Evaluation

- **340/380**: The measured filter factor (millimolar absorptivity difference at the wavelength pair) was 5.00 (nominal: 5.08).
- **415/450**: The measured filter factor for pNP was 11.39 (nominal: 11.98).

For pNA, the measured filter factor was 5.53 (nominal: 5.68).

#### Dilutor Performance

The actual ABA-200 dilution ratio was calculated by dividing the mean $A_d$ for each instrument dilution ($n = 15$) by the mean $A_d$ for the manual dilution ($n = 7$) and multiplying the result by the actual manual dilution ratio. The results are tabulated in Table 1. The precision at each dilutor setting was obtained from the same data.

### Photometer Linearity

Table 2 shows the values of $A_d$ that define the measured limit of linearity at each wavelength.

### Discussion

The technique of bichromatic analysis involves modification of a familiar spectrophotometric concept, molar absorptivity. In conventional spectrophotometers, molar absorptivity may be well defined at a specific wavelength. However, the concept must be re-defined when one measures the absorbance difference ($A_d$) at a pair of wavelengths. $A_d$ is proportional to the difference in molar absorptivity of the compound at the two wavelengths. Abbott has defined a factor called the "filter factor," which is the millimolar absorptivity difference of a specific compound (e.g., NADH, p-nitrophenol, or p-nitroaniline) at two wavelengths. Interference filters are used to provide the wavelength isolation in Abbott instruments. Because of the variability in filter characteristics, filter factors must be measured for each filter set. Filter factors are assigned by the manufacturer and are stamped on each filter carriage. The filter factor value is used in all enzyme activity calculations and may also be used in "endpoint" assays that are coupled to NADH (e.g., glucose, urea, triglycerides, and lactate). Uncritical acceptance of the manufacturer's values may potentially lead to unrecognized error. We were concerned with the magnitude of this potential error. Our results showed that measured filter factors differed by 1.6% from the expected value for the 340/380 nm wavelength pair, 5.2% from the nominal value for p-nitrophenol, and 2.7% for p-nitroaniline at the 415/450 nm wavelength pair.

We do not know the technique used by the manufacturer to assign filter factors. Our method for measurement of filter

### Table 1. Dilutor Precision and Accuracy

<table>
<thead>
<tr>
<th>Nominal dilution</th>
<th>Sample vol, µL</th>
<th>Dilutor settings</th>
<th>$A_d$ ratio (manual/instrument)</th>
<th>Precision (CV, %)</th>
<th>ABA-200 dilution ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:11</td>
<td>25</td>
<td>06/07</td>
<td>0.989</td>
<td>0.86</td>
<td>1:10.9</td>
</tr>
<tr>
<td>1:25</td>
<td>12.5</td>
<td>07/04</td>
<td>1.003</td>
<td>0.79</td>
<td>1:25.1</td>
</tr>
<tr>
<td>1:26</td>
<td>10</td>
<td>06/03</td>
<td>0.986</td>
<td>0.68</td>
<td>1:25.6</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>08/05</td>
<td>0.979</td>
<td>0.37</td>
<td>1:25.5</td>
</tr>
<tr>
<td>1:51</td>
<td>5</td>
<td>06/02</td>
<td>0.993</td>
<td>0.37</td>
<td>1:25.8</td>
</tr>
<tr>
<td>1:101</td>
<td>2.5</td>
<td>06/01</td>
<td>0.993</td>
<td>1.03</td>
<td>1:100.3</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10/03</td>
<td>0.983</td>
<td>0.51</td>
<td>1:100.1</td>
</tr>
<tr>
<td>1:201</td>
<td>1.25</td>
<td>10/02</td>
<td>0.991</td>
<td>0.55</td>
<td>1:100.1</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>10/01</td>
<td>1.027</td>
<td>1.27</td>
<td>1:206.5</td>
</tr>
</tbody>
</table>

### Table 2. Photometric Linearity

<table>
<thead>
<tr>
<th>Filter set</th>
<th>Limit of linearity, $A_d$</th>
<th>$f$</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>340/380</td>
<td>3.260</td>
<td>0.49</td>
<td>5</td>
</tr>
<tr>
<td>415/450</td>
<td>3.450</td>
<td>0.42</td>
<td>8</td>
</tr>
<tr>
<td>500/600</td>
<td>3.623</td>
<td>1.65</td>
<td>4</td>
</tr>
<tr>
<td>550/650</td>
<td>3.521</td>
<td>1.11</td>
<td>8</td>
</tr>
</tbody>
</table>

*At higher $A_d$'s, the $f$ value for the quadratic coefficient, $b_2$, was significant at the 5% level.
factors was recommended by the manufacturer. The values we obtained were the results of several experiments and were calculated from the slopes of the linear regression equations obtained from the data. We have confidence in our results and believe that they and the nominal values corroborate each other.

The original pipettor/diluter system on bichromatic analyzers was composed of interchangeable pairs of syringes with a fixed volume ratio. In the absence of leaking valves, fittings, or syringe barrels, the dilution ratio for a pair of syringes was held constant for all volume combinations. In the new dilutor system fixed syringes are driven by microprocessor-controlled stepping motors. Thumbwheel switches are used to select sample and reagent volumes.

The dilution ratio is an integral part of the calculation for all enzymatic assays as well as with some "endpoint" assays when filter factors are also used. Any deviation from the nominal value will add to the error of the test.

We evaluated dilutor accuracy and precision by using solutions of pNP at concentrations selected to yield high final absorbances (approximately 1.0 $A_d$), to minimize the contribution of photometric error to our estimate of actual dilutor ratios. The dilutor performed better than we had expected, both in accuracy and precision. The worst performance was seen with a 1:201 ratio when a sample volume of 1.25 $\mu$L was chosen. At any dilutor ratio, precision was better for larger sample volumes. With decreasing sample volumes, factors such as the position of the pick-up probe and the depth to which the sample cup is filled assume increasing importance. Small absolute errors in sample aspiration or carryover are magnified as proportional error. The accuracy for all dilution ratios and sample volumes other than 1:201 (1.25 $\mu$L sample volume) was within 2.7% of the nominal value. Precision (CV) was 1% or better for all ratios except 1:201. We believe that even the 1.27% CV we observed at 1:201 dilution (2.5 $\mu$L sample volume) is acceptable.

The quality of photometric response of the optical components of the system was estimated as a function of the photometric linearity at the commonly used wavelength pairs (i.e., 340/380, 415/450, 500/600, and 550/650) and the photometric precision at 415/450 nm. The optical/electronic design of the photometer/signal conversion system permits reproducible measurement of $A_d$ over a remarkably wide range. We measured solutions having $A_d$'s in excess of 3.2 at all wavelength pairs and found that the photometric response did not differ significantly from linearity. These $A_d$'s were well in excess of those claimed for linearity by the manufacturer (<2% deviation from linearity at 2.5 $A_d$).

The photometric precision measurement included contributions from the integral optical/electronic system as well as the contribution due to inter-cuvet variability. Abbott claims an inter-cuvet variability of 0.6% or less. Our observation of a CV of 0.27% at 1.0 $A_d$ supports this claim.

The combination of high $A_d$ linearity and good precision at high $A_d$ values leads us to the opinion that the system may be used to measure optically dense or turbid samples without sacrificing precision.

In summary, we believe that the instrument performs reliably.

References