UV-Vis spectrophotometry: monochromators vs photodiode arrays

Comparing absorbance measurements between the Quad4 Monochromators™-based Infinite® M200 PRO and a multimode reader using photodiode array technology

Introduction

The most widely established technology for UV-Vis absorbance measurement is a monochromator-based microplate reader. Spectrophotometers have undergone a great deal of development since their introduction in the early 1950s (1) and, in recent years, multimode readers using linear photodiode array (PDA) technology for absorbance measurements have become available. PDA-based readers incorporate an optical grating and a solid state array detector, enabling measurement of light intensity throughout the UV and visible regions of the spectrum. Similar to a monochromator, but much faster, they allow the entire UV-Vis spectrum of a sample to be captured within a few seconds per well.

However, this technology suffers from a number of drawbacks, mainly due to high levels of stray light. This results in a dramatically limited dynamic measurement range (2). This technical note compares the results of basic absorbance measurements performed on an Infinite M200 PRO multimode reader equipped with Quad4 Monochromators with those from a multimode reader equipped with a PDA.

Materials and methods

- Infinite M200 PRO multimode microplate reader
- Multimode microplate reader with a PDA
- Herring sperm DNA standard
- Tris-EDTA
- Orange G (OG)
- ddH2O
- 96-well, transparent UV-Star® plates

Experiment 1

Linearity in the visible spectrum using Orange G

In the first experiment, the OD (optical density) linearity of the PDA spectrophotometer in the visible wavelength range was compared with the OD linearity of the monochromator-based Infinite M200 PRO. An OG dilution series was prepared in ddH2O (200, 150, 112.5, 84.4, 63.3, 47.5 and 35.6 mg/ml) and 200 µl of each concentration was pipetted into a 96-well UV-Star plate in triplicate. The plate was then measured on the Infinite M200 PRO (492 nm, 25 flashes, 100 ms settle time) and on the PDA-based reader (492 nm, 25 flashes,
100 ms settle time). The results were plotted in Microsoft Excel®, and the R² values were calculated to estimate the signal linearity (dynamic measurement range).

Experiment 2
DNA absorbance spectrum
A sample containing 3.125 ng/µl of herring sperm DNA standard was prepared in Tris-EDTA and a 200 µl aliquot was pipetted into one well of a 96-well UV-Star plate. For the blank, 200 µl of Tris-EDTA buffer was pipetted into a second well of the same plate. The absorbance spectrum of both wells was recorded on the Infinite M200 PRO (230 to 350 nm, 1 nm steps, 25 flashes) and the PDA reader (220 to 350 nm, 1 nm steps, 25 flashes). Blank values were subtracted from the sample values, and the spectrum was plotted in Microsoft Excel.

Experiment 3
DNA A260/A280 reproducibility and UV linearity
The absorbance of a low concentration DNA sample (200 µl of 0.8 µg/ml solution) was measured ten times on the Infinite M200 PRO (260/280 nm, 25 flashes, 100 ms settle time) and the PDA reader (260/280 nm, 25 flashes, 100 ms settle time). This demonstrates the OD reproducibility (% CV) at the instrument’s detection limit, and is a good indicator of the overall performance in absorbance mode for both instruments.

To estimate the UV linearity, a DNA dilution curve (0.8, 1.56, 3.125, 6.25, 12.5, 25, 50, 62.5, 125, 250 and 500 µg/ml; 200 µl/well) was measured in a 96-well UV-Star plate on the Infinite M200 PRO (260/280 nm, 25 flashes, 100 ms settle time) and the PDA reader (260/280 nm, 25 flashes, 100 ms settle time).

Results and discussion

Experiment 1
Linearity in the visible spectrum using Orange G
Table 1 shows the OD values for the Orange G dilution curve measured on the Infinite M200 PRO and the PDA reader. A critical issue with the PDA reader is that there is no indication that values with an OD above 3.5 are outside the instrument’s measurement range. Consequently, incorrect values are displayed that could easily be misinterpreted by the user.

With the Infinite M200 PRO, all values that are outside the measurement range are displayed as ‘OVER’ values, avoiding any misinterpretation.

<table>
<thead>
<tr>
<th>O.G. concentration (mg/ml)</th>
<th>Infinite M200 PRO (OD @ 492 nm)</th>
<th>MMR with PDA spectrophotometer (OD @ 492 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0.030</td>
<td>0.025</td>
</tr>
<tr>
<td>35.6</td>
<td>0.939</td>
<td>0.924</td>
</tr>
<tr>
<td>47.5</td>
<td>1.187</td>
<td>1.170</td>
</tr>
<tr>
<td>63.3</td>
<td>1.601</td>
<td>1.585</td>
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<td>84.4</td>
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<td>112.5</td>
<td>2.753</td>
<td>2.77</td>
</tr>
<tr>
<td>150</td>
<td>3.706</td>
<td>3.34</td>
</tr>
<tr>
<td>200</td>
<td>OVER</td>
<td>OVER</td>
</tr>
</tbody>
</table>

Table 1: OD values at 492 nm (average of triplicate values).

Figure 1 compares the OD linearity in the visible range for the Infinite M200 PRO and the PDA reader. It is clear that the linearity of the PDA reader is limited above an OD of 2.5. Even when the highest value – which should be indicated as OVER – is removed, the R² of the PDA reader is 0.9914 (data not shown), a value that is still not acceptable for a well performing absorbance spectrophotometer (R² ≥0.999 is a widely-accepted performance value).
**Technical Note**

**Experiment 2**

**DNA absorbance spectrum**

Figure 2 shows the DNA spectrum recorded with both the Infinite M200 PRO and with the PDA reader. For the PDA reader, even after subtraction of the blank value, the values between 220 and 240 nm are clearly incorrect, with some negative OD values. This demonstrates that the results for the PDA reader are not reliable below 240 nm, and a correct A\textsubscript{230}/A\textsubscript{260} ratio cannot be determined.

**Experiment 3**

**DNA A\textsubscript{260}/A\textsubscript{280} reproducibility and UV linearity**

Table 2: A\textsubscript{260}/A\textsubscript{280} reproducibility using a low concentration DNA sample (0.8 µg/ml herring sperm DNA).

Table 2 shows the reproducibility of measurements at 260 and 280 nm, as well as the A\textsubscript{260}/A\textsubscript{280} ratios. The Infinite M200 PRO reader shows exceptional reproducibility at both 260 and 280 nm, indicating very good performance for DNA quantification and purity checks. The PDA reader is less reproducible, with all CVs greater than 10%, bringing into question whether this reader should be used for DNA quantification.
out of the measurement range for the PDA-based instrument (not even ‘3.5’ is displayed), whereas the two highest concentrations are clearly displayed as ‘OVER’ on the Infinite M200 PRO. This critically influences the outcome of the experiment for UV linearity, as the user would include these erroneous values in the dilution curve, leading to poor linearity. Excluding these two values results in an acceptable $R^2$ value of 0.9991 (data not shown).

**Conclusion**

PDA-based spectrophotometry is a well-established technology for absorbance measurements, offering ultra-fast spectral scanning capabilities. However, it has significant drawbacks, particularly due to increased stray light, resulting in a limited dynamic measurement range. Although fast scanning capabilities are important for some applications, such as full spectral scans of low volume DNA samples, this feature is not required for most absorbance-based measurements.

In this study, we show that the monochromator-based Infinite M200 PRO multimode reader offers significant advantages over a PDA-based reader for many applications. These benefits include an extended dynamic measurement range, better OD linearity in the visible and the UV range, and greater measurement reproducibility at 260 and 280 nm.

For the majority of applications, these characteristics are of greater importance than high speed spectral scanning, making a monochromator-based multimode reader a better choice for most users.

**Literature**


**List of abbreviations**

- CV: Coefficient of variation
- MMR: Multimode microplate reader
- OD: Optical density
- OG: Orange G
- PDA: Photodiode array