Molecular Devices

MAXline
Application Bulletin
6

Eliminate Temperature Errors in Pathlength Measurements Using PathCheck Sensor

Accurately and precisely measure optical pathlength in microplate wells with the PathCheck® Sensor.

- **Only sensor of its kind not affected by temperature**
- **Self-contained in the instrument– no accessories needed**
- **Calculations automated in the software**
- **Patent allowed in the U.S.**
- **Featured in two peer-reviewed publications[1,2]**
- **Winner of R&D 100 award**
- **Invented by Molecular Devices scientists**

Materials

- SpectraMax® Microplate Spectrophotometer with PathCheck Sensor
- Microplates (regular or half-area plates)

Methods

Principles: Water is essentially transparent from 200 nm to 900 nm, but has a distinctive absorbance peak in the near infrared (NIR) region at approximately 977 nm (Figure 1).

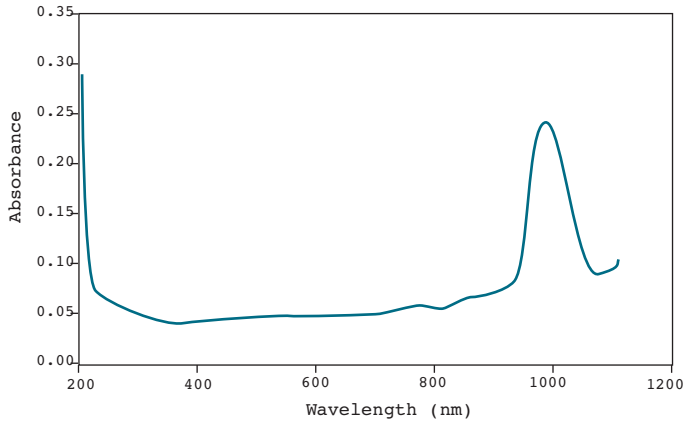

Figure 1: Absorbance spectrum of water.

This characteristic absorbance can be used to measure the pathlength of an aqueous sample. As predicted by Lambert's Law concerning light absorption, absorbance is proportional to the distance that light travels through the sample (water). The pathlength through an aqueous sample in a microplate well is calculated by comparing the height of the water peak in the well with the height of the water peak in a 1 cm cuvette.

The baseline absorbance measurement is made at a wavelength distant from the water absorbance peak, e.g., 900 nm.

$$\frac{(A_{977}-A_{900})_{Reagent\ in\ well}}{(A_{977}-A_{900})_{Reagent\ in\ 1\text{-}cm\ cuvette}} = \text{Pathlength in well (in cm)}$$

SoftMax® Pro Software will do the calculations automatically using a stored value for the 1 cm pathlength. If desired, the Cuvette Reference Method may be used (not described here) to accommodate samples containing small amounts of organics or highly-concentrated buffers. Comparatively few substances absorb in the NIR, so most aqueous solutions used in typical biochemistry laboratories do not contain interfering species. Pathlength measurement can be used to verify performance of multichannel pipettors and automated liquid dispensers.[1-3] It can also be used to normalize absorbance values to a 1 cm pathlength equivalent. For example, to normalize nucleic acids to a 1 cm pathlength, the A_{260} value for each well is divided by its pathlength:

$$\frac{(A_{260})_{Sample\ in\ well}}{\text{Pathlength (cm)}} = A_{260} \text{ normalized to 1 cm pathlength}$$

Wavelength selection to avoid temperature effects: The near infrared absorption of water is quite dependent upon temperature. As the temperature increases, the absorbance increases and the location of the peak maximum shifts to a lower wavelength (Figure 2). A temperature isosbestic point, where absorbance does not change with temperature, is located near the peak at 998 nm. PathCheck Sensor measures the water peak at the temperature isosbestic point in order to avoid temperature-related errors.

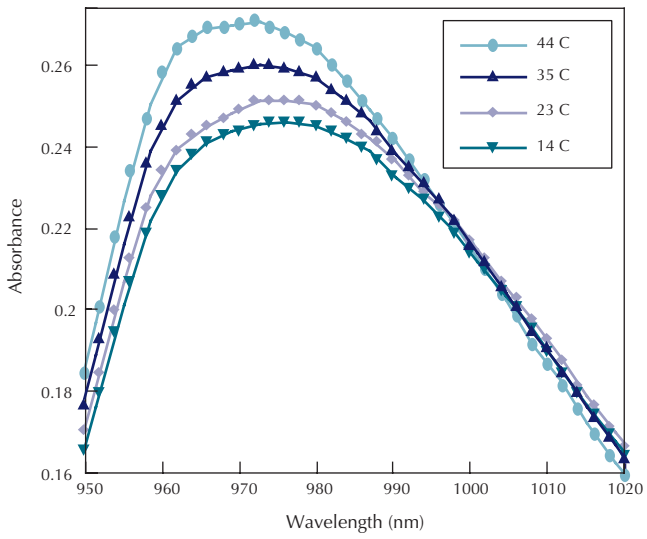

Figure 2: Effect of temperature on NIR absorbance of water.

If 977 nm measurements were used for pathlength calculations, it would be extremely important that the samples and the 1 cm reference be measured at exactly the same temperature. If they were not, the pathlength-corrected absorbances would be in error by approximately 0.5% per °C difference. This can be a common problem when the temperature in the lab varies during morning *vs.* afternoon or summer *vs.* winter.

The implication of measurement error due to temperature fluctuation is illustrated with a 100 ng/mL DNA solution (Figure 3). In the example, the 1 cm reference measurements were made at 27 °C, whereas the temperature of the DNA solution ranged from 5 °C to 44 °C. Pathlength-corrected absorbance (and therefore apparent concentration) varied significantly with temperature when the absorbance measurements were made at 977 nm. In contrast, when the measurements were made at 998 nm, the apparent concentration was essentially unaffected by temperature.

The PathCheck Sensor avoids temperature dependency by making the absorbance measurements at the isosbestic point of water (998 nm).

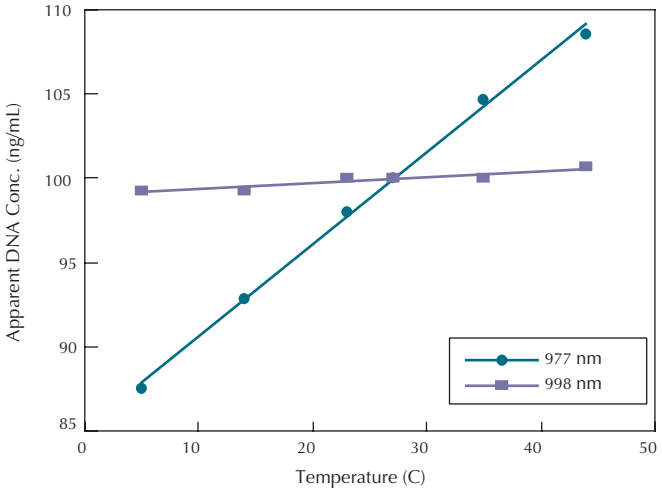

Figure 3: Effect of temperature on pathlength-corrected DNA concentrations when pathlength measurements are made at 977 nm or 998 nm (temperature isosbestic point of water).

PathCheck Sensor

- Ensures consistent results, regardless of temperature
- Offers one-step measurement of pathlength without any daily adjustments by the user
- Is proprietary technology with claims allowed by the U.S. Patent Office
- Represents a significant contribution, recognized by scientific leaders[4]

[1]McGown, E.L. and D.G. Hafeman. 1998. *Anal. Biochemistry* 258: 155-157.

[2]McGown, E.L. et al. 1998. *Clinical Chemistry* 44(10): 2206-2208.

[3]Anders, A. et al. 1998. *LaborPraxis* 10: 78-82.

[4]Winner of the R&D Magazine Top 100 Technology Products of 1998.

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