A custom method for hemoglobin measurements

Using the NanoDrop One Spectrophotometer

Abstract

Thermo Scientific™ NanoDrop™ Microvolume UV-Vis Spectrophotometers have been used for many years to reliably measure nucleic acids and proteins using as little as 1–2 µL of sample. However, NanoDrop spectrophotometers may also be used for many other applications. This application note discusses how the NanoDrop One/One^{C} Spectrophotometer can be used to measure hemoglobin using a custom method.

Introduction

Hemoglobin is a tetrameric protein found in erythrocytes that transports oxygen to tissues throughout the body and carries CO_2 away from tissues [1]. Hemoglobin can reversibly bind oxygen and typically contains iron in the reduced ferrous state (Fe^{2+}). When the iron is oxidized to the ferric state (Fe^{3+}), hemoglobin is converted to methemoglobin and can no longer bind oxygen [2].

A spectrophotometer may be used to quantitate various forms of hemoglobin using the appropriate extinction coefficient and peak absorbance value. Oxyhemoglobin contains bound oxygen and exhibits absorbance peaks at 414 nm, 541 nm, and 576 nm [3]. Deoxyhemoglobin does not contain bound oxygen and exhibits an absorbance peak at 431 nm. Methemoglobin cannot bind oxygen due to containing iron in the ferric state, and exhibits an absorbance peak at 406 nm [4].

A spectrophotometer may also be used to assess a plasma or serum sample for hemolysis. Hemolysis occurs when the red blood cell membrane breaks down and releases hemoglobin and other intracellular components into the surrounding serum or plasma [5]. The estimation

of hemolysis is important as it can affect the accuracy of many laboratory assays. A spectrophotometer may be used to evaluate hemolysis using the absorbance of a plasma or serum sample at 414 nm. An elevated absorbance at 414 nm is associated with increased free oxyhemoglobin [3, 6].

It is important to keep in mind that spectrophotometers measure the total absorbance of a sample, and cannot distinguish the analyte of interest from any other component in the sample that absorbs at the same wavelength. Spectrophotometric hemoglobin measurements in plasma may be subject to interference from increased bilirubin levels, plasma proteins, albumin, lipids, and other absorbing components [5].

If you would like to quantitate hemoglobin in whole blood, a colorimetric assay may be performed at 540 nm using Drabkin's reagent. A colorimetric assay is beyond the scope of this application note, but users can set up a custom method for the assay on the NanoDrop One/One^{C} Spectrophotometer.

Experimental Procedures

A custom method was created for the NanoDrop One/One^{C} instrument to measure the absorbance of hemoglobin at 406 nm, 414 nm, 431 nm, 541 nm, and 576 nm. The method includes an analysis wavelength at 414 nm to calculate the concentration of oxyhemoglobin using an extinction coefficient of 524,280 M^{-1} cm^{-1} and molecular weight of 64.5 kDa [7]. The method also includes an additional calculation to determine the concentration of deoxyhemoglobin using the absorbance at 431 nm, an extinction coefficient of 552,160 M^{-1} cm^{-1}, and molecular weight of 64.5 kDa [7]. The method includes wavelengths 541 nm and 576 nm to monitor, as oxyhemoglobin exhibits absorbance peaks at these wavelengths [5]. The method includes wavelength 406 nm to monitor for methemoglobin. A baseline correction at 750 nm was included for the entire spectrum, as well as an analysis wavelength correction based on the sloping baseline correction from 360–500 nm.

Custom Method Download

1. Navigate to www.thermofisher.com/nanodrop
2. On the left, select "NanoDrop Software Download"
3. Choose the "NanoDrop One/One^{C}" tab
4. Select "Local Control Software Download Instructions"
5. Scroll to "How to add a NanoDrop One/One^{C} Custom Method file" and click on "Oxy-hemoglobin Method"
6. Unzip the custom method file and copy the .method file to a USB device and then follow the online "Instructions for uploading a Custom Method to the instrument from a USB device".

The hemoglobin custom method was validated using hemoglobin obtained from MP Biomedicals, LLC (catalog number 100714, lot number 7541J). The hemoglobin preparations available through MP Biomedicals may be primarily methemoglobin, which cannot bind oxygen, as the iron in hemoglobin is easily oxidized by air.

A hemoglobin stock was prepared at 8 mg/mL using reagent grade water. Serial dilutions were then performed to create standards at 4 mg/mL, 2 mg/mL, 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, and 0.125 mg/mL. The instrument was blanked with water, and five replicates of each standard were measured using the Oxy-hemoglobin Custom Method on the NanoDrop One/One^{C} Spectrophotometer.

Results

Each hemoglobin standard was measured in replicates of five on the NanoDrop One instrument using the hemoglobin custom method. The standard deviation based on five replicates of each solution ranged from 0.015–0.552A. The coefficient of variation for each solution ranged from 0.61–2.79%.

	Average A406 n=5	Standard Deviation n=5	%CV
Solution 1	44.493	0.487	1.09
Solution 2	22.846	0.552	2.41
Solution 3	11.127	0.068	0.61
Solution 4	5.549	0.058	1.05
Solution 5	2.660	0.019	0.71
Solution 6	1.245	0.035	2.79
Solution 7	0.579	0.015	2.66

Table 1. Results for hemoglobin standards measured using the hemoglobin custom method on the NanoDrop One/One^{C}.

Conclusion

The hemoglobin custom method described in this application note can be used to determine oxyhemoglobin and deoxyhemoglobin concentrations on the NanoDrop One/One^{C} Spectrophotometer. Depending on your needs, the method may be used to monitor the

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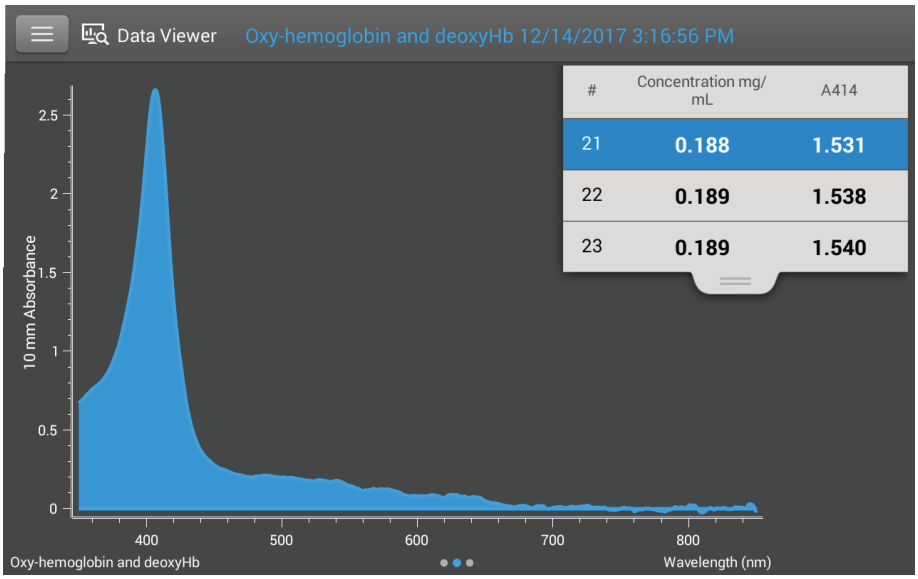

Figure 1. Typical absorbance spectrum for methemoglobin measured on the NanoDrop One with a peak at 406 nm. The spectra also lacks peaks at 541 nm and 576 nm which are present in oxyhemoglobin.

absorbance at 414 nm to determine if the sample may be hemolyzed. If measuring oxyhemoglobin, the method may be used to monitor the absorbance at 541 nm and 576 nm. If measuring methemoglobin, the method also may be used to monitor the absorbance at 406 nm.

References

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Further Assistance and Technical Support

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