

# Spectrophotometry of hemoglobin derivatives

Hemoglobin and its derivatives have characteristic absorption spectra in the visible light region, which are used for their analysis and rapid identification. Significant absorption maxima in the 400–430 nm region, the so-called Soret band, are typical for all hemoproteins. Other absorption peaks are considerably lower. Oxyhemoglobin is characterized by two incompletely separated maxima in the region of 540 and 578 nm. Deoxyhemoglobin has a single absorption maximum at 555 nm. The main absorption maximum of methemoglobin is at 630 nm and a second faint peak at 500 nm is pH dependent. When methemoglobin reacts with potassium cyanide, the maximum at 630 nm disappears, as cyanmethemoglobin is formed. The decrease in absorbance at 630 nm is proportional to the methemoglobin concentration. **Cyanmethemoglobin** shows a broad absorption maximum at 540 nm, which is used in determining the concentration of hemoglobin in the blood. The spectrum of **carbonylhemoglobin** resembles that of oxyhemoglobin, but the position of the peaks is shifted towards lower wavelengths.

Absorption maxima of hemoglobin and its derivatives

Hemoglobin derivative	Absorption maxima [nm]
Hemoglobin reduced	431, 555
Oxyhemoglobin	414, 540, 578
Methemoglobin	404, 500, 630
Carbonylhemoglobin	420, 538–540, 568–569
Cyanmethemoglobin	421, 540

## Determination of carbonyl hemoglobin:

The determination of carbonylhemoglobin in the blood is one of the basic toxicological tests. It is an objective criterion in the assessment of acute and chronic carbon monoxide poisoning.

- *Spectrophotometric evaluation.* Carbonylhemoglobin can be determined rapidly spectrophotometrically by subtracting the shift of the absorption maximum of diluted blood from 586 nm<sup>[1]</sup>. The shift of the maximum in the spectrum is dependent on the ratio of COHb and O<sub>2</sub> Hb in the sample.
- *Reaction with tannin.* As a guide, carbonylhemoglobin can be determined by reaction with tannin or Ajatin (from about 10% COHb). Tannin forms a strawberry-red precipitate in the presence of carbonylhemoglobin. In the absence of carbonyl hemoglobin, the color of the precipitate is brownish gray.
- *Acid base balance analyzers.* The analysis of the toxicologically most important derivatives of hemoglobin COHb and metHb is also made possible by modern acid-base balance analyzers that have a built-in photometric system for their measurement.

## Links

### Related articles

- Spectrophotometry
- Acid-base balance

### Reference

1. LEDVINA, M. Rapid spectrophotometric determination of carbonylhemoglobin in blood. *Biochem Clin Bohemoslov.* 1987, vol. 16, pp. 493-495, ISSN 0139-9608.