II.8.6 Methemoglobin

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Introduction

Determination of methemoglobin (Met-Hb) in blood is important for the diagnosis of poisoning by oxidants, such as nitrite, nitrate, chlorate, chlorite, alkyl nitrites, nitroglycerin, aniline and other compounds. In 1938, Evelyn and Malloy [1] had devised a photoelectric method for determination of Met-Hb in blood. Minor modifications of this method were made by several researchers to increase sensitivity [2–4]. These methods are based on a phenomenon that the absorbance maximum of weakly acidic Met-Hb at 630 nm disappears by addition of cyanide. Met-Hb concentration can be easily determined as the ratios of the absorbance changes induced by cyanide before and after addition of potassium ferricyanide.

In forensic science practice, there are many cases, in which Met-Hb has to be measured for blood specimens containing high concentrations of carboxyhemoglobin (CO-Hb); *e.g.*, especially in the cases of fire and exhaust gas poisoning [5]. However, the above methods [1–4] are not suitable for specimens containing high levels of CO-Hb. In 1981, the author et al. [6] developed a modification of the methods, which enabled accurate determination of blood Met-Hb even in the presence of high concentrations of CO-Hb by using about a 100-fold excess of potassium ferricynade to be added to the Hb iron. In this chapter, a simple procedure of the modified spectrophotometric method for Met-Hb is briefly described.

Reagents and their preparation

- Potassium ferricyanide, 4 % (w/v) in distilled water. It should be stored in a dark bottle, and be prepared monthly.
- Phosphate buffer solution, 0.1 M, pH 6.8.
- Phosphate buffer solution, 40 mM, pH 6.9. It can be prepared by dilution of two parts of the above reagent with three parts of distilled water.
- Potassium cyanide, 5 % (w/v) dissolved in the above 40 mM phosphate buffer solution. It should be prepared just before use.

Analytical instrument

A Hitachi 557 dual-wavelength spectrophotometer[a] (Hitachi, Ltd., Tokyo, Japan).

Procedure

i. A 6-mL volume of distilled water is placed in a test tube. A 0.2-mL volume of whole blood is added to the tube and mixed well.

ii. After allowing the tube to stand for 5 min, 4-mL of 0.1 M phosphate buffer solution is added to the mixture and mixed well.
iii. The hemolysate is centrifuged at 3,000 rpm for 10 min, and the clear supernatant is transferred to another test tube. The pH of the supernatant should be around 6.9.
iv. Four 4-mL volume cuvettes of the same type are cleaned well by washing with distilled water. The four cuvettes are designated as A, B, C and D.
v. To cuvettes A and B, a 0.5-mL volume each of distilled water is added. To cuvettes C and D, a 0.5-mL volume each of 4 % potassium ferricyanide solution is added.
vi. To cuvettes A and C, a 3-mL volume each of 40 mM phosphate buffer solution is added. To cuvettes B and D, a 3-mL volume each of the supernatant of the above hemolysate is added. Each cuvette is mixed well.
vii. The absorbance of cuvette B at 630 nm is read using cuvette A as reference, and this reading is A_1. After allowing cuvette D to stand for 10 min, the absorbance at 630 nm is read using cuvette C as reference, and this reading is A_3.
viii. To all cuvettes, a 30-μL volume each of 5 % potassium cyanide solution is added and mixed well.
ix. After allowing the cuvettes to stand for 2 min, the absorbances of cuvettes B and D at 630 nm are read using cuvettes A and C as references, respectively. These readings are A_2 and A_4, respectively.
x. The percentage of Met-Hb is calculated by the following equation: Met-Hb % = 100 $(A_1–A_2)/(A_3–A_4)$.

Assessment and some comments on the method

The present method for analysis of Met-Hb in blood is simple and rapid, and is not interfered with by the coexistence of high concentrations of CO-Hb [6]. As low as about 0.2 % of Met-Hb can be measured accurately.

However, when high concentrations of sulfhemoglobin (SHb) may interfere with the present assay [7]; but SHb concentrations in putrefied blood not older than 7 days are usually not high, and do not probably influence the present assay. Caution should be, therefore, made for blood specimens obtained from living subjects or cadavers of sulfide or polysulfide poisoning (see the chapter "Hydrogen sulfide and its metabolite" of this book).

Met-Hb in blood is not stable at room temperature and even at –30 °C.

When analysis of Met-Hb has to be made later, the blood specimens should be stored at –80 °C or even lower temperature [8], or at –30 °C in the presence of a cryoprotective solution[b] [9, 10].

Toxic and fatal concentrations

Clinical symptoms according to the concentrations of Met-Hb are presented in *Table 5.1* of the previous chapter (Chapter II.8.5, "Nitrate and nitrite compounds").

Notes

a) Any type of spectrophotometers can be used, regardless of manufacturers.
b) The cryoprotectant is aqueous solution containing 28 % glycerol, 3 % mannitol and 0.65 % NaCl. It should be added to blood samples at the ratio of 1:1.

References

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