Experiment 16: Erythrocite Protoporphyrin

SYNOPSIS Fluorescence of zinc protoporphyrin in a blood sample is used to indirectly determine lead in the blood.

READINGS Pages 311-313 in Critical Reviews. Attached is an article relating the protoporphyrin measurement to the actual blood lead measurement.

MATERIALS

Bleach
10 mM phosphate buffer, pH 7.4.
Blood sample, obtained from biochemistry lab.
Zn proto-porphyrin (P.O. Box 31, Logan Utah, Porphyrin Products, Utah)
10 mg/100 mL
pyridine (EtOH is a possible alternative)
0.05 µg ZnPP/mL pyridine

PROCEDURE

A. Calibrating the fudge factor or internal standard

1. Place 0.05 µg ZnPP/ml pyridine secondary standard in the sample compartment.
2. Set instrumental parameters
3. Scan the $ë_{emission}$ from 450 to 700 nm.
4. Measure $I_{emission}$ at 400 nm.

B. Measurement of whole blood fluorescence

1. Take undiluted whole blood and obtain a spectrum.
2. Dilute 20 µL whole blood with 10 mL phosphate buffer.
3. Obtain a spectrum.
4. Spike with 10 mL of ZPP solution. Obtain a spectrum.
5. Dispose of blood by adding bleach to sample, then pour down drain with more bleach, followed by 15 minutes of water rinse.
6. Take 10 ml of phosphate buffer and spike with 10 mL of ZPP solution. Obtain a spectrum.
7. For your whole blood, spiked whole blood, and spiked phosphate buffer determine the µg Pb/100 mL blood:

$I = K'P_o 2.303åbC$ (Skoog and West p. 182)

where K' is a quantum yield related constant.

$$\frac{I_{emission}}{I_{ZPP\ pyridine\ std}} = \frac{K'P_o 2.303 \text{åb} C_{sample}}{K'P_o 2.303 \text{åb} C_{std}} = \frac{C_{sample}}{C_{std}}$$

$$C_{sample}\ (\mu g\ ZPP/100\ mL\ blood) = \frac{I_{em}}{I_{std}}(5\ \mu g\ ZPP_{pyridine}\ std/100\ mL) \times 500 \times (fudge = 1.34)$$

$$\frac{8}{\text{dilution factor for 20 µL blood/10 mL buffer}}$$

REPORT

1. How reliable a measure of blood lead do you think this measurement will be?
2. What is the chemistry of Zn protoporphyrin that makes it a good candidate for a fluorescence assay.
3. Why does whole blood contribute to the measured signal? Why does it have to be diluted?