

EXERCISE 1

PREPARATION OF HAEMIN CRYSTALS

Structure

1.1	Introduction	1.5	Observations
	Objectives	1.6	Discussion
1.2	Materials Required	1.7	Precautions
1.3	Principle	1.8	Terminal Questions
1.4	Procedure		

1.1 INTRODUCTION

Haemin or haemin (Ferric chloride heme) is an iron-containing porphyrin with chlorine that can be formed from a haem group, such as haem group in haemoglobin of human blood. The purpose of this exercise is to enable you to understand how can we prepare crystals from the haem part of the haemoglobin.

Objectives

After completing this exercise, you will be able to:

- ❖ identify the haemin crystal, and
- ❖ appreciate importance of haemin crystal test in medico-legal cases.

1.2 MATERIALS REQUIRED

Glass slides, Cover slips, Pricking needles, Dropper, Spirit lamp, Cotton, Compound microscope

90% Alcohol, Nippe's reagent

Preparation of Nippe's Reagent: Dissolve 100 mg KCl, 100 mg KBr and 100 mg KI in 100 mL of glacial acetic acid.

1.3 PRINCLIPLE

Haemoglobin is present in RBCs which imparts red colour to the blood and helps in the transport of gases. Haemoglobin is a conjugated **chromoprotein** which is composed of two parts- non-protein haem and protein globin. Haem is made up of four pyrrole rings and a central iron ion in ferrous state.

The haemin crystals are prepared by heating of the blood with Nippe's reagent. This ruptures the RBCs and the haemoglobin is released. Also, in this process the ferrous form of iron is converted to ferric form. The globin protein gets denatured by heating with acetic acid; while, heme is converted to oxidized heme called **haematin**. The haematin combines with halogens such as chloride ions to form **insoluble haemin** which appear as rhombic crystal of chocolate brown colour. The haemin crystals or hydrochloride of heme (Fig. 1.1) are characteristic of blood and are thus, used for identification of blood stains.

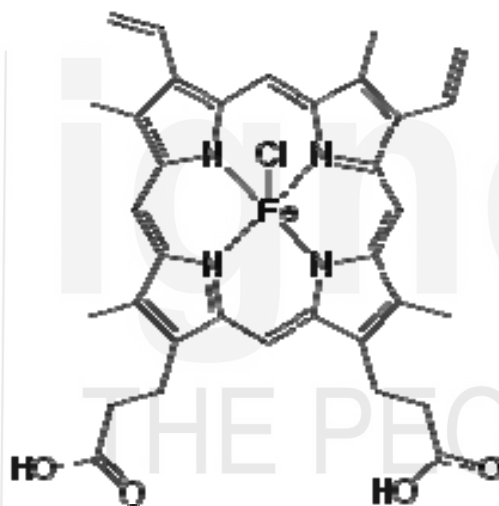


Fig. 1.1: Haemin.

Haemin was first crystallized by **Ludwik Karol Teichmann** in 1853. Therefore, they are also referred to as '**Teichmann Crystals**'.

1.4 PROCEDURE

1. Sterilize the tip of finger with cotton swab soaked in 90% alcohol.
2. Prick the finger with the sterilized pricking needle.
3. Place a drop of blood on a clean slide and spread it to form a thin film.
4. Add 2-3 drops of the Nippe's reagent on the blood and place a cover slip.
5. Gently heat the slide over low flame until the reagent starts boiling.
6. Remove the slide from the flame and add few drops of Nippe's reagent from the side of the coverslip. Heat it again for few more seconds.
7. Cool the slide and observe under the microscope, first under low magnification (10x) and then under high magnification (40x).

1.5 OBSERVATIONS

Rhomboid, chocolate brown crystals as shown in Figure 1. 2. are observed. Crystals are homogeneous solids, bounded by plane faces and having a geometric shape.

A lyophilized form of haemin is used as a pharmacological agent in certain cases for the treatment of porphyria attacks.

Porphyria is a group of diseases in which substances called porphyrins build up, negatively affecting the nervous system.

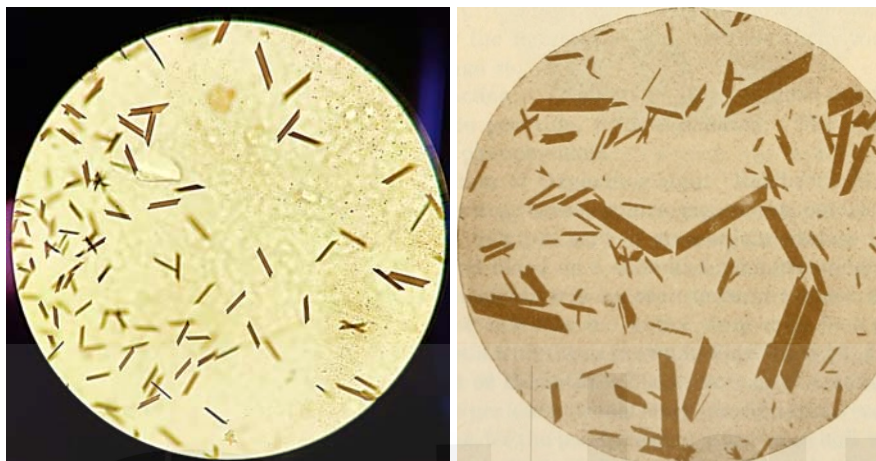


Fig. 1.2: Haemin Crystals of Human Blood.

1.6 DISCUSSION

Haemin crystals are used in medico-legal practices to distinguish fresh or dried blood stains from other red-coloured stains. The shape of haemin crystals varies in different species and thus, blood stains of human can be confirmed. Another advantage of the haemin test is that it can be performed with the dried blood stains.

1.7 PRECAUTIONS

- Clean the finger with alcohol before pricking.
- Discard the first drop of blood.
- Avoid overheating of the slide.
- Keep the slide undisturbed at the time of cooling.

1.8 TERMINAL QUESTIONS

1. Write the composition of Nippe's reagent.
2. Write the chemistry behind formation of haemin crystals.
3. What is the importance of haemin crystals test in medico-legal cases?
4. Name the blood pigment involved in haemin crystal test.
5. Differentiate between haematin and haem.

FURTHER READING

1. Tortora, G.J. and Derrickson, B.H. (2009). *Principles of Anatomy and Physiology*, XII Edition, John Wiley & Sons, Inc.
2. Victor P. Eroschenko. (2008). *diFiore's Atlas of Histology with Functional correlations*. XII Edition. Lippincott W. & Wilkins.

Acknowledgement of Figures

Fig.1. 2: (a) Self-Clicked

(b) <https://www.flickr.com/photos/internetarchivebookimages/14780801025>

