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Note

Determination of hemin by paper chromatography

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During a recent investigation of a photochemical reaction of hemin (which is the prosthetic group of hemoglobin), a simple, rapid and convenient technique was needed for both the qualitative and the quantitative determination of hemin. Reversed-phase paper chromatography[1] and TLC[2] are useful for studying the heterogeneity of hemin; consequently, these techniques would seem to be unsuitable for the separation and identification of hemin in general analysis. Paper chromatography[3] with methanol–acetic acid–water (90:3:7) as developing solvent is used as a simple and rapid technique for the separation and identification of hemin and blood stains. Hemin is known to react[4,5] with pyridine to form a red-orange complex (pyridine hemichrome) that is stable even in hydrochloric acid solution. It has also been found[6] that hemin reacts sensitively with pyridine in acetic acid to produce a stable red-orange color due to pyridine hemichrome, and that this color reaction can be applied to the identification and determination of hemin.

In this paper, a method based on paper chromatography and colorimetric analysis by using the pyridine hemichrome reaction is described for determining hemin.

EXPERIMENTAL AND RESULTS

Materials

Hemin (chlorohemin) was purchased from Wako Pure Chemical Industries (Osaka, Japan) and recrystallized by Fischer's method[7]. Toyo Roshi No. 51 paper in strips 40 cm long and 2 cm wide was used for the chromatography. Methanol, acetic acid and pyridine were of analytical grade and were distilled before use.

Spectrophotometers

The absorption spectrum of hemin was measured with a Hitachi EPS 3T recording spectrophotometer. Individual absorbance measurements were made with a Hitachi spectrophotometer 101 in 1-cm quartz cells.

Ultraviolet lamp

A PAN UV lamp (250–400 nm; Tokyo Optical Machine Co.) was used for the UV irradiation.

Procedure

Paper chromatography. A 0.01-ml portion of hemin solution in acetic acid–pyridine (17:3, by vol.) was applied as a circular spot to a line 6 cm from one end of a paper strip, and development was carried out by the ascending technique with methanol–acetic acid–water (40:3:7, by vol.) for 3 h; the strip was then dried in a stream of air at room temperature. Hemin gave a single pale-brown spot, which usually had an R_F value of 0.69 ± 0.03.

Colorimetric analysis. The area (2 cm × 3.5 cm) containing the hemin spot was cut into small pieces, which were mixed in a test tube with 5 ml of the acetic acid–pyridine (17:3); the tube was then stoppered with a glass stopper and allowed to stand for 1 h at room temperature, with occasional shaking. The liquid, which had a pale red-orange colour, was then filtered off, and its absorbance was measured at 405 or 410 nm; the color was stable for 90 min. The R_F value in paper chromatography and the absorption spectrum (see Fig. 1; $\lambda_{max.}$ 407 nm) of the filtrate served to identify hemin.

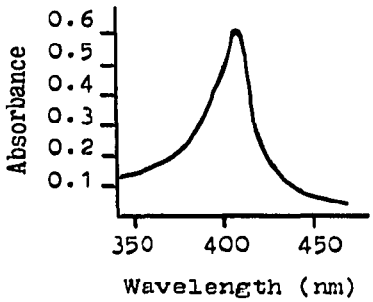

Fig. 1. Absorption spectrum of hemin (3.36 μg per ml) in acetic acid–pyridine mixture.

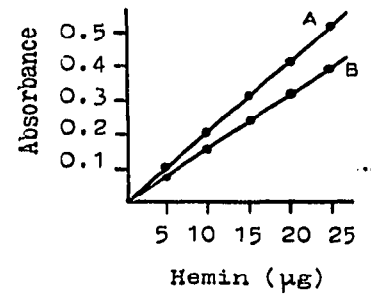

Fig. 2. Calibration curves for hemin, with absorbance measured at 405 nm (curve A) and 410 nm (curve B).

Calibration curve. To establish the calibration curve, a series of solutions containing from 500 to 2500 μg of hemin per ml was prepared in acetic acid–pyridine (17:3), and 0.01 ml of each solution was applied to a paper strip, developed, extracted and examined colorimetrically as described above. The absorbance values were plotted as a function of the weight of hemin applied to the strip; rectilinear calibration curves

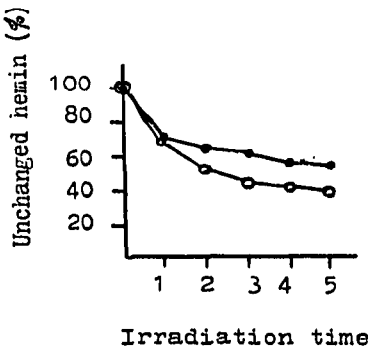

Fig. 3. Changes in hemin on exposure for 1–5 h to UV radiation (○) or summer sunlight (●).

were obtained, as shown in Fig. 2. Sample concentrations are determined by reference to such calibration curves.

Application of procedure

The effects of irradiation by sunlight and UV rays on hemin stains on paper strips were investigated by using the procedure described. The stains (each containing 25 μg of hemin) being directly exposed to sunlight or 250–400 nm radiation for 1–5 h; unchanged hemin was then determined by the recommended procedure. The results are shown in Fig. 3 and indicate that the amount of hemin decreases with increasing irradiation time.

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