Determination of Hematoporphyrin and Protoporphyrin by Ion-Pair Extraction with Chlorpromazine

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Abstract □ A sensitive method based on ion-pair extraction is described for the quantification of hematoporphyrin and protoporphyrin using chlorpromazine as an ion-pair-forming agent. Extraction of the ion-pair in chloroform is obtained quickly at an optimum pH of 6.5 for hematoporphyrin and of 6.5–6.8 for protoporphyrin, giving an excellent recovery of the porphyrin. A stoichiometric relationship of 1:2 betweeen porphyrin and chlorpromazine is proved. Cyanocobalamin and liver extract do not interfere with the assay.

Keyphrases □ Hematoporphyrin—determination by ion-pair extraction with chlorpromazine □ Protoporphyrin—determination by ion-pair extraction with chlorpromazine □ Ion-pair extraction—determination of hematoporphyrin and protoporphyrin using chlorpromazine

Hematoporphyrin[1] (I) and protoporphyrin[1] (II) have been found in human bile and in urine of patients with various porphyrias (1, 2). Compound II is present in cirrhosis, hepatic tumors, cholestasis (3), and in lead intoxication (4).

Recently, the pharmacological actions of I were reviewed (5). Compound I is used in anemia with cyanocobalamin and liver extract; I and some of its charge transfer complexes show antidepressant activity (6–8).

Analysis by direct spectrophotometry is not suitable for porphyrins in urine or when colored substances, such as liver extract and cyanocobalamin, are present in I. Ion-pair formation of I with dialkylaminoalkylphenothiazines was noted (9), and a red precipitate formed at pH 6.0–6.5. In the present study, a method based on ion-pair extraction (10–12) was applied for assaying I and II using chlorpromazine hydrochloride[2] (III) as a reagent.

EXPERIMENTAL

Materials—Hematoporphyrin dihydrochloride (I), protoporphyrin disodium salt (II), chlorpromazine hydrochloride (III), cyanocobalamin[1] (IV), liver extract (4 μg/ml of cyanocobalamin)[1] (V), and chloroform BP were used.

Reagents—The following solutions were used: 1×10^{-4} *M* I, 1×10^{-4} *M* II, and 1×10^{-1} *M* III in the required buffer. The ionic strength was not adjusted, but the solutions were prepared in 1×10^{-1} *M* phosphate buffer at various pH values.

Preparation of Solid Complexes between Porphyrins and Chlorpromazine—Compound I (60 mg) was dissolved in ~250 ml of pH 6.5 buffer and was precipitated by addition of 3 ml of III (1×10^{-1} *M*) with continuous stirring. The mixture was set aside for 30 min, filtered through a sintered-glass funnel (porosity 4), washed with 3-ml portions of ice-cold water, and dried under vacuum. The same procedure was followed for II but with a pH 6.8 buffer. A stoichiometric relationship was proved in both cases by NMR spectroscopy[3] and by spectrophotometry[4] according to the previously described methods (9).

Effect of Chlorpromazine on Porphyrins Recovery—Aliquots of I (6 ml, 1×10^{-4} *M*) and III (0.1–1.6-ml, 1×10^{-1} *M*) in pH 6.5 buffer were diluted in a 20-ml volumetric flask with the pH 6.5 buffer and then transferred to a separator. Extraction was accomplished by adding 20 ml of chloroform and then shaking thoroughly for 2 min. Absorbance was measured against chloroform from the blank experiment at 502 nm.

The same procedure was used for II with a pH 6.8 buffer and reading of the absorbance at 505 nm (Fig. 1).

Effect of pH on Recovery—Aliquots of I (6 ml, 1×10^{-4} *M*) and III (1 ml, 1×10^{-1} *M*) in buffered solutions of pH 5.8–7.0 were introduced into dry separators and diluted with 13-ml aliquots of the appropriate buffer. Each solution was shaken thoroughly for 2 min with 20 ml of chloroform. The absorbance of the separated organic phase was measured against chloroform from the blank experiment. The same procedure was used for II.

Calibration Curve—Stock solutions of I (1×10^{-4} *M*) and III (1×10^{-1} *M*) were used to prepare mixed standard solutions. The mixed standards were diluted to 20 ml with the buffer in a volumetric flask to yield a series of mixed working solutions containing 1–2.5 $\times 10^{-5}$ *M* I and 5×10^{-3} *M* III. The working solutions in the separators were gently shaken for 2 min with 20 ml of chloroform. The absorbance of the chloroform extract was measured against the chloroform extract from the blank experiment.

The mean value of three experiments was taken in to obtain the calibration curve. The molar absorptivity (ϵ) was 10,000 ± 100 at 502 nm. This value was confirmed by direct measurement of the absorbance of the solid complex in chloroform.

The same procedure was used for II; the molar absorptivity (ϵ) was 11,000 ± 120 at 505 nm and was also confirmed by direct measurement of the absorbance of the solid complex in chloroform.

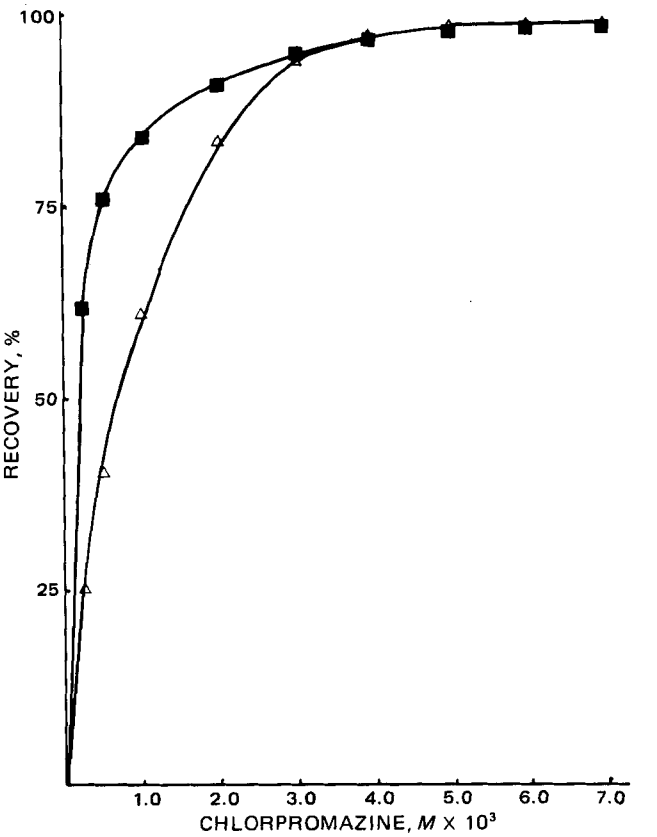

Figure 1—*Recovery of the chloroform extract as a function of chlorpromazine with concentrations of* 3×10^{-5} M *hematoporphyrin* (△) *and* 3×10^{-5} M *protoporphyrin* (●) *in the aqueous layer.*

[1] Fluka.
[2] Rhône-Poulenc.
[3] ^{1}H-NMR (Varian A-60).
[4] Perkin-Elmer EPS-3T spectrophotometer.

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Table I—Recovery of 200 μg of Hematoporphyrin from 20-ml Solutions

Buffer	In Presence of 20 μg of Cyanocobalamin	In presence of 25% Liver Extract
197.5	199.0	201.0
197.0	197.3	196.5
198.2	200.0	197.5
199.8	197.5	197.2
200.0	196.5	199.2
199.0	197.5	198.0
198.5 ± 1.2[a]	198.0 ± 1.4[a]	198.2 ± 1.6[a]

[a] Mean ± *SD*.

Analysis of Porphyrins in Presence of Cyanocobalamin and Liver Extract—A preliminary experiment performed using a chlorpromazine ion-pair method with a 5-ml aliquot of 4 μg/ml of cyanocobalamin or of liver extract showed no formation of colored complex in chloroform. Consequently, on assaying porphyrin (200 μg) in the presence of either of these solutions, no interference occurred and hematoporphyrin or protoporphyrin was easily and quantitatively extracted. Table I lists the replicate recovery results of 200 μg of I from 20-ml buffer solutions alone, in the presence of IV, and with 25% of V. The porphyrins were also completely extracted from urine according to the described ion-pair method with chlorpromazine.

RESULTS AND DISCUSSION

The red complexes of hematoporphyrin and protoporphyrin assayed by the described method showed adherence to Beer's law. Figure 1 shows that a 5×10^{-3} *M* concentration of chlorpromazine was necessary for a total recovery of the porphyrin at a concentration of 3×10^{-5} *M*(ratio 3:500). Figure 2 shows the recovery profile of hematoporphyrin using a fixed ratio of 3:500 between porphyrin and chlorpromazine in chloroform from buffer solution as a function of pH. At pH 6.5, hematoporphyrin was completely extracted; protoporphyrin was completely extracted in a wider pH range.

The extracted species are well defined and the reported method can be considered sensitive and accurate. Table I shows that the mean recovery for hematoporphyrin was 99.0–99.2%.

The method can be applied for determining porphyrins in pathological urine.

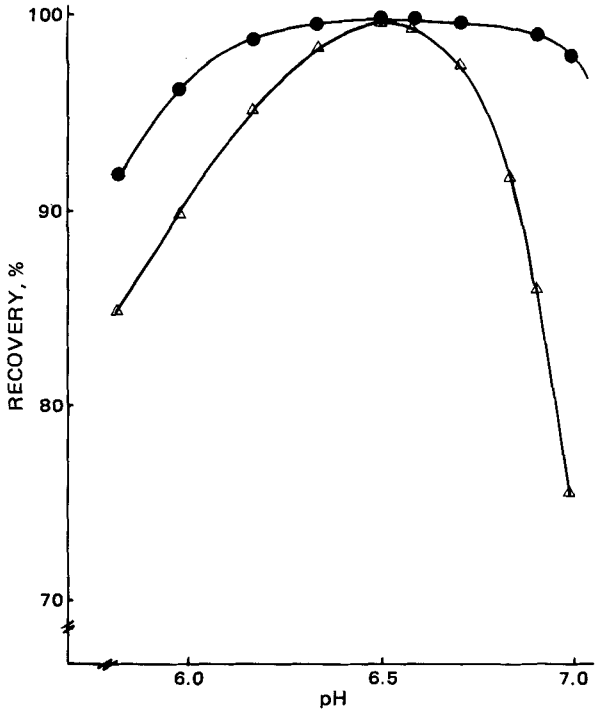

Figure 2—*Hematoporphyrin and protoporphyrin recovery–pH profile after 20-ml chloroform extraction of 20 ml of aqueous solution containing* 5×10^{-3} M *hematoporphyrin (△) and* 3×10^{-5} M *protoporphyrin (■).*

REFERENCES

(1) A. A. Schotorst, J. Stevenick, L. N. Went, and D. Suurmond, *Clin. Chim. Acta*, **33**, 207 (1971).

(2) S. G. Smith, R. V. Belcher, R. Mahler, and J. Yudkin, *ibid.*, **23**, 241 (1969).

(3) E. De Salamanca, J. A. Herruzo, F. Arnalich, J. Pardo Rueda, *Rev. Esp. Enferm. Apar. Dig.*, **47**, 805 (1976); through *Chem. Abstr.*, **85**, 157559s (1976).

(4) A. Alvares, A. Fischbain, S. Sassa, K. Anderson, and A. Kappas *Clin. Pharmacol. Ther.*, **19**, 183 (1976).

(5) V. N. Luzgina, E. I. Filoppovich, and R. P. Eustigneeva, *Khim. Farm. Zh.*, **11**, 26 (1977); through *Chem. Abstr.*, **87** 47834m (1977).

(6) D. DeMaio and F. Belloni, *Clin. Ther.*, **47**, 591 (1968).

(7) G. Di Modica, French Demande 2,299,864 (Sept. 3 1976); through *Chem. Abstr.*, **86**, 165525p (1977).

(8) A. Brucato, G. Di Modica, F. Marletti, S. Mazzarese, D. Murgolo, and P. M. Provenzano, *Arzneim.-Forsch.*, **27**, 1015 (1977).

(9) M. R. Gasco, M. Trotta, and M. E. Carlotti, *Pharmazie*, **36**, 276 (1981).

(10) E. M. Girgis, Z. Gad, and M. Maram, *J. Pharm. Sci.*, **63**, 1764 (1974).

(11) F. S. Hom and W. R. Ebert, *ibid.*, **66**, 710 (1977).

(12) B. A. Persson and S. Eksborg, *Acta Pharm. Suec.*, **7**, 353 (1970).