

# Formation of zinc complexes during chromatography of porphyrins on fluorescent thin-layer plates

Koichi Saitoh, Chikara Kiyohara and Nobuo Suzuki

Department of Chemistry, Faculty of Science, Tohoku University, Sendai, Miyagi 980 (Japan)

(First received August 6th, 1991; revised manuscript received March 5th, 1992)

## ABSTRACT

An undesirable chemical change of a porphyrin to its zinc complex occurred on a particular silica gel thin-layer chromatographic (TLC) coating that contained a fluorescent indicator. This effect was observed for different porphyrins, such as *meso*-tetraphenylporphine, *meso*-tetrakis(*p*-tolyl)porphine, octamethylporphyrin, etioporphyrin and octaethylporphyrin in development with *m*-xylene. The zinc complex formation was attributable to the reaction of porphyrin with the zinc compound contained as a fluorescent indicator in the TLC coating.

## INTRODUCTION

Thin-layer chromatography (TLC) [1,2] and also high-performance TLC (HPTLC) [3–5] are powerful techniques for the separation and identification of porphyrins and metalloporphyrins and also common organic compounds. A TLC plate coated with an adsorbent containing a fluorescent indicator is usually particularly convenient for the detection of colourless organic compounds in chromatograms. Such fluorescent TLC coatings are applicable, of course, to the separation of coloured compounds. It is required in any event that the fluorescent indicators do not affect the chemical stability of the compounds to be chromatographed.

This work was undertaken to examine whether the fluorescent indicator contained in the TLC coating would influence the TLC behaviour of porphyrins. Six synthetic porphyrins were tested on three commercial silica gel TLC plates precoated with or without fluorescent indicator.

Correspondence to: Dr. K. Saitoh, Department of Chemistry, Faculty of Science, Tohoku University, Sendai, Miyagi 980, Japan.

## EXPERIMENTAL

### *Porphyrins and their metal complexes*

Fig. 1(a) and (b) show the general formulae of porphyrins ( $H_2P$ ) and their metal complexes  $[M(P)]$ , respectively.

Porphine ( $H_2Por$ ) ( $R_1 = R_2 = R_3 = H$  in Fig. 1) [6], *meso*-tetraphenylporphine ( $H_2 tpp$ ) ( $R_1 = \text{phenyl}$ ;  $R_2 = R_3 = H$ ) [3], *meso*-tetrakis(*p*-tolyl)porphine ( $H_2 ttp$ ) ( $R_1 = 4\text{-methylphenyl}$ ;  $R_2 = R_3 = H$ ) [4] and etioporphyrin I ( $H_2 etio$ ) ( $R_1 = H$ ;  $R_2 = \text{methyl}$ ;  $R_3 = \text{ethyl}$ ) [5] were prepared previously. Octamethylporphyrin ( $H_2 omp$ ) ( $R_1 = H$ ;  $R_2 = R_3 = \text{methyl}$ ) was synthesized from 3,4-dimethylpyrrole and formaldehyde [7]. Octaethylporphyrin ( $H_2 oep$ ) ( $R_1 = H$ ;  $R_2 = R_3 = \text{ethyl}$ ) was obtained from Strem Chemicals (Newburyport, MA, USA).

The complexes of each porphyrin with Zn(II)  $[Zn(P)]$  and Cu(II)  $[Cu(P)]$  were prepared by reaction of  $H_2P$  (1 mM) with zinc acetate (30 mM) and copper acetylacetonate (10 mM), respectively, while refluxing in chloroform.  $Zn(P)$  was purified by recrystallization from chloroform–methanol and  $Cu(P)$  by alumina column chromatography. The complexes, such as  $Ni(P)$  and  $Pd(P)$  ( $P = tpp$  [3],  $ttp$  [4],  $etio$  [5]), were synthesized previously.

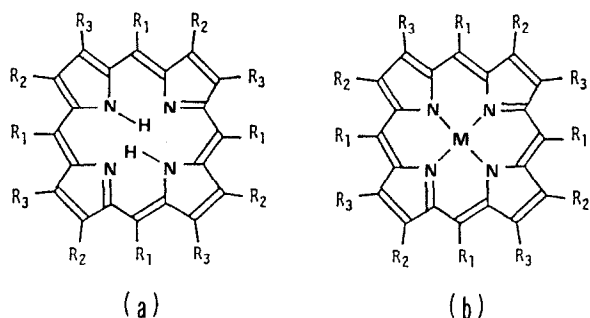


Fig. 1. General structural formulae of (a) porphyrin ( $H_2P$ ) and (b) its metal complex [(MP)].

### HPTLC

HPTLC plates (10 cm  $\times$  10 cm) were obtained from Merck (Darmstadt, Germany), including silica gel 60 (plate I), silica gel 60 F<sub>254</sub> (plate II) and silica gel 60 F<sub>254S</sub> (plate III). The first plate was free from the fluorescent indicator and the other two were of the fluorescent indicator-added type. Each HPTLC plate, after having been developed with methanol for cleaning, was activated by heating at 110°C for 30 min and then cooled in a silica gel desiccator for 2 h.

A 0.5- $\mu$ l aliquot of a chloroform solution of a porphyrin (at 0.1 mM or lower concentration) was placed on the TLC plate used. The chromatogram was developed 45 mm from the sample origin with *m*-xylene in the so-called solvent vapour saturation mode or the non-saturation mode in a horizontal sandwich chamber (Model 28510, Camag, Muttenz, Switzerland). In the former mode, a TLC plate coated with cellulose, which had been wetted with *m*-xylene, was used as a counter plate in the chamber. The temperature was 25°C. Each porphyrin was detected spectrophotometrically at about 400 nm with a Shimadzu (Kyoto, Japan) Model CS-920 densitometer.

### HPLC

A Shimadzu Model LC-6A HPLC system including an SPD-M6A photodiode-array spectrophotometric detector was used with an Inertsil ODS-2 (5  $\mu$ m) column (150 mm  $\times$  4.6 mm I.D.) (GL Sciences, Tokyo, Japan).

## RESULTS AND DISCUSSION

### HPTLC of porphyrins

*m*-Xylene was a convenient solvent for comparing the TLC behaviours of different porphyrins. Every porphyrin gave single spots with almost identical mobilities on plates I and III. On plate II, however, two poorly resolved spots appeared for each porphyrin whether it was developed in the solvent vapour saturation mode or the non-saturation mode (Fig. 2). One of the spots appeared with a migration comparable to those of the same porphyrin on plates I and III, and the other spot appeared with a larger migration. This spot was assigned to an unknown compound. When exposed to UV radiation of wavelength 365 nm, both spots found on plate II fluoresced with intense reddish colours.

A comparative study of the TLC behaviour was carried out for several examples of metalloporphyrins [M(P)], such as the Ni, Cu, Pd and Zn complexes of tpp, ttp and etio, on plate II. All the M(P)s of the first three metals moved near to the solvent front, which meant larger mobilities than the unknown compounds found with corresponding porphyrins ( $H_2P$ s) on plate II. In addition, the M(P)s of these three metals did not fluoresce under 365-nm UV radiation. However, each Zn(P) showed almost the same extent of migration as the unknown compound observed with the corresponding  $H_2P$ . Fig. 2 shows the comparative chromatograms of six  $H_2P$ s and corresponding Zn(P)s on plates I and II. In addition, it was confirmed that every Zn(P) fluoresced with a reddish colour under UV radiation.

It was considered at this stage that the unknown spot found in the chromatogram of an  $H_2P$  on plate II could be assigned to Zn(P) that was formed presumably from  $H_2P$  on the TLC coating of plate II.

### Effect of fluorescent indicator

In order to verify the hypothesis that an  $H_2P$  could be converted into its Zn complex on a particular TLC coating, the reactivities of  $H_2P$  etio, which was taken as an example of  $H_2P$ , with the TLC coatings of plate I and II were compared.

About 250-mg portions of the TLC coatings (I and II) were scraped from plates I and II. Each portion of the coating, after having been activated by heating at 110°C for 30 min, was wetted with toluene and then packed in a short glass column tube of 4

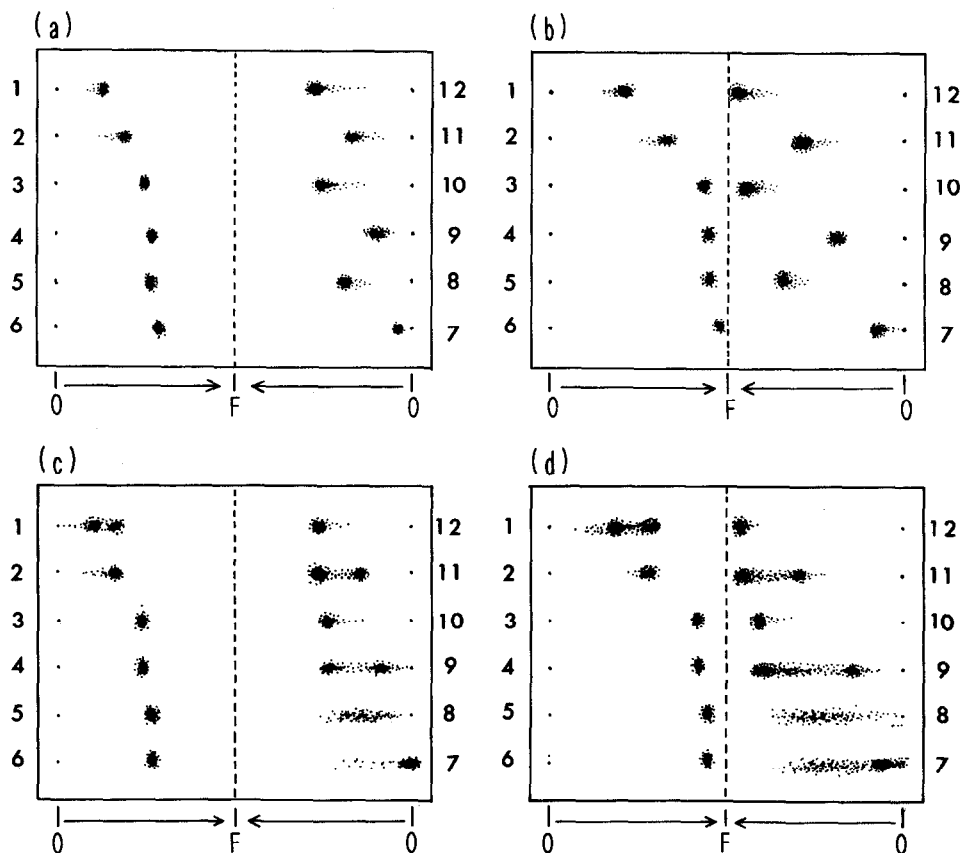


Fig. 2. HPTLC of porphyrins and their zinc(II) complexes on (a,b) plate I and (c,d) plate II. Development: 45 mm with *m*-xylene, (a,c) in the solvent vapour saturation mode with a 30-min preliminary exposure to the vapour and (b,d) the non-saturation mode. O, sample origin; F, solvent front. Compounds: 1 = H<sub>2</sub>Por; 2 = Zn(por); 3 = H<sub>2</sub>tpp; 4 = Zn(tpp); 5 = H<sub>2</sub>ttp; 6 = Zn(ttp); 7 = H<sub>2</sub>omp; 8 = Zn(omp); 9 = H<sub>2</sub>etio; 10 = Zn(etio); 11 = H<sub>2</sub>oep; 12 = Zn(oep).

mm I.D. A 50- $\mu$ l aliquot of a toluene solution of H<sub>2</sub>etio (0.1 mM) was placed on the column, which was then allowed to stand for 1 h at room temperature. Some coloured substance was eluted from the column with 0.8 ml of toluene, and the eluate was analysed by reversed-phase high-performance liquid chromatography (HPLC). The same procedures were also applied to the experiment using the TLC coating of plate II which was washed with 0.1 M hydrochloric acid in preference to the activation (hereafter denoted TLC coating II-aw).

The HPLC traces recorded for the eluate from the columns packed with different TLC coatings are compared with reference chromatograms of H<sub>2</sub>etio and Zn(etio) in Fig. 3. The eluates from coatings I and II-aw gave peaks at the same retention time as

H<sub>2</sub>etio, and that from coating II gave a peak at the retention time corresponding to Zn(etio). The visible absorption spectra recorded with the photodiode-array detector for the peaks in chromatograms (a) [and also (c)] and (b) matched perfectly those of H<sub>2</sub>etio and Zn(etio), respectively. It was reasonable to conclude that no chemical change had occurred for H<sub>2</sub>etio on TLC coating I, whereas a conversion from H<sub>2</sub>etio to Zn(etio) occurred on TLC coating II. Such an undesirable chemical change did not occur on TLC coating II-aw.

According to the manufacturer's literature (Merck), the coating on plate II contains zinc silicate as the fluorescent indicator and that on plate III some acid-resistant fluorescent indicator (more detailed information about this indicator is not

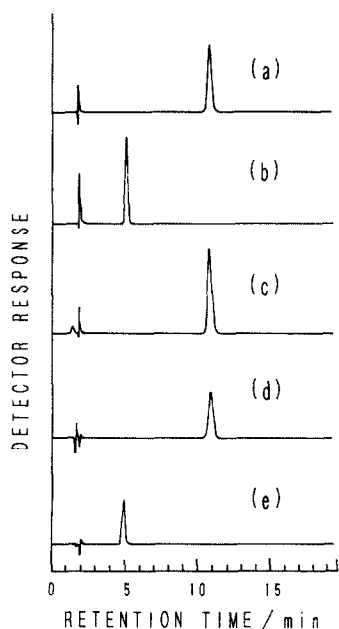


Fig. 3 HPLC of the eluate from  $H_2$ etio-charged small columns packed with adsorbents (a) I, (b) II and (c) II-aw and references chromatograms of (d)  $H_2$ etio and (e) Zn(etio). Mobile phase: methanol at 1 ml/min. Detection at 400 nm.

available). It is noted that TLC coating II, after having been washed with the acid, became non-fluorescent even on exposure to UV radiation, and the zinc washed from the coating was determined to be about 1% dry weight of the coating by atomic absorption spectrometry at 213.8 nm (Model 180-80 spectrometer, Hitachi, Tokyo, Japan).

It is considered that the foregoing peculiar HPTLC behaviour observed for porphyrins on plate II is attributable to the reactions of the porphyrins with the zinc compound (presumably zinc silicate) contained as the fluorescent indicator. Accordingly, plate II is not recommended for use in HPTLC separations of porphyrins. It should be noted that no metal complex formation was observed on plate III even though its coating contains a fluorescent indicator. HPTLC plates coated with both the non-fluorescent and the  $F_{254}S$  type of silica gel 60 (plates I and III, respectively) are recommended for the chromatography of porphyrins.

When copper complexes [Cu(P)] were tested in place of  $H_2P$ , no transmetallation from Cu(P) to Zn(P) occurred on plate II. This was attributable to the higher stability of Cu(P) than Zn(P) (the stability classes of Cu(P) and Zn(P) are II and III, respectively [8,9]).

In conclusion, a preliminary check is required for the effect of the fluorescent indicator contained in the thin layer of adsorbent on the chemical stability of porphyrins in preference to application of the HPTLC plate to the separation of porphyrins. The formation of metal porphyrin complexes usually does not occur easily. Heating of a solution containing a high concentration of porphyrin and metal salt is applied many instances [10,11]. The formation of zinc-porphyrin complexes on an HPTLC plate is undesirable with respect to chromatography, whereas it is presumably useful for the preparation of the complex under such mild conditions in a solvent of low polarity at room temperature.

#### ACKNOWLEDGEMENT

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture.

#### REFERENCES

- 1 J.-H. Fuhrhop and K. M. Smith, in K. M. Smith (Editor), *Porphyrins and Metalloporphyrins*, Elsevier, Amsterdam, 1975, pp. 858–859.
- 2 W. I. White, R. C. Bachmann and B. F. Burnham, in D. Dolphin (Editor), *The Porphyrins*, Vol. I, Academic Press, New York, 1978, pp. 553–576.
- 3 K. Saitoh, M. Kobayashi and N. Suzuki, *Anal. Chem.*, 53 (1981) 2309.
- 4 M. Kobayashi, K. Saitoh and N. Suzuki, *Chromatographia*, 5 (1985) 417.
- 5 S. Miyake, K. Saitoh and N. Suzuki, *Chromatographia*, 22 (1986) 160.
- 6 Y. Wakui, K. Saitoh and N. Suzuki, *Chromatographia*, 17 (1976) 1157.
- 7 J. N. Phillips, *Rev. Pure Appl. Chem.*, 10 (1960) 35.
- 8 J. W. Buchler, in K. M. Smith (Editor), *Porphyrins and Metalloporphyrins*, Elsevier, Amsterdam, 1975, p. 197.
- 9 A. D. Adler, F. R. Longi, F. Kampas and J. J. Kim., *J. Inorg. Nucl. Chem.*, 32 (1976) 2443.
- 10 J. W. Buchler, in K. M. Smith (Editor), *Porphyrins and Metalloporphyrins*, Elsevier, Amsterdam, 1975, pp. 177–187.