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HYPERPRESSURE GAS CHROMATOGRAPHY

IV. GAS CHROMATOGRAPHY OF ETIOPORPHYRIN II METAL CHELATES*

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SUMMARY

The hyperpressure gas chromatography of fifteen etioporphyrin II metal chelates of fourteen different elements has been studied at 145° and 1000–1700 p.s.i., with dichlorodifluoromethane as the solvent gas. The chelates of Sn(IV) and Mn(III) are insoluble under the experimental conditions and they are, thus, sharply separated from the other elements studied. A column of 10% Epon 1001 resin on Chromosorb W effected the following separations: Mg(II), Cu(II) or Co(II), Ni(II) or Co(III), VO or TiO, Mn(II), Zn or Pt(II) and Pd(II); Ag(II) Pt(II) and Pd(II). A 10% Silicone Gum Rubber XE-60 on Chromosorb W column separated Mn(II) or Fe(III), Zn or TiO, Pt(II) and VO. All the chelates were eluted intact with the exception of that of Mg(II), which was demetallated in the column. A novel method for the insertion of metal ions in the porphyrin ring, involving the reaction of the corresponding metal acetylacetonate with the porphyrin in a suitable solvent, is also reported.

INTRODUCTION

The application of gas chromatography to the analysis of metallic compounds has been relatively limited in comparison to that of organic compounds or inorganic gases. This is due to the fact that those metallic compounds which are sufficiently volatile to be gas chromatographed are usually reactive and it is difficult to find appropriate column packing materials and detectors². Certain volatile metal halides, hydrides, alkyls^{2–5} and a considerable number of metal chelates³ have been successfully gas chromatographed, however. Many metal acetylacetonates are sufficiently volatile^{3,6} to permit elution without decomposition^{7–12}, but other acetylacetonates decompose^{13,14} at the temperatures necessary for volatilization³. Current research is devoted almost exclusively toward the discovery of more volatile metal compounds. Thus, the lanthanides and several second and third row transition metals were recently eluted without decomposition in the form of chelates with fluorinated β -diketones or

* Part III, *cf.* ref. 1.

dipivaloylmethane¹⁵⁻²¹. Progress to date indicates that eventually most of the metallic elements will be gas chromatographed by the extension of these methods.

Our recent separation of Cu or Mg, Ni, VO and Sn, in the form of etioporphyrin II chelates, by hyperpressure gas chromatography (HPGC)¹, indicates the possibility of a fresh approach to the gas chromatographic separation of metallic elements. Porphyrins and metalloporphyrins are unsuitable for conventional gas chromatography²², since they decompose at temperatures high enough for column operation. They have, nevertheless, been gas chromatographed under pressure and above the critical temperature of the solvent gas (CCl_2F_2) at much lower temperatures than those anticipated, if only volatility is taken into consideration^{1,22}. The substantial decrease of the temperature necessary for column operation with increase of pressure has been verified by other investigators²³. The present paper extends our recent HPGC studies of metal chelates^{1,24}, to include the etioporphyrin II chelates of Al(III), TiO, Mn(II) and (III), Fe(III), Co(II), Pd(II), Pt(II), Ag(II) and Zn(II). In general, the chelates of transition metals with porphyrins are very stable²⁵.

The HPGC of etioporphyrin II metal chelates can be considered as a prelude to the HPGC of tetraphenylporphin chelates. Tetraphenylporphin can be synthesized directly from benzaldehyde and pyrrole²⁶, while the synthesis of etioporphyrin II²⁷ leads to poorer overall yields. Tetraphenylporphin metal chelates need substantially higher pressures than etioporphyrin II metal chelates to be eluted in reasonably low retention times, as shown in a preliminary study with the Co(II) chelate. It is estimated that the less volatile metallotetraphenylporphins (*e.g.* VO, Pd, Pt) would require pressures in the region of 3000-4000 p.s.i., which are near to or beyond the upper limit of our present apparatus, in order to produce sufficiently sharp peaks in reasonably low retention times.

EXPERIMENTAL

Materials and methods

The hyperpressure gas chromatograph and its initial modifications have been described elsewhere²⁴. The apparatus was modified by replacement of the back-pressure regulator with a completely sealed all-metallic diaphragm-type valve, designed to eliminate leaks at "O" rings. The monochromator was used as the detector, instead of the filter photometer^{1,24}. The sample size varied between 0.5-10 μg . The eluted samples were examined by comparison of their visible and UV absorption spectra with those of the original sample, in a Beckman DK-2 recording spectrophotometer.

Columns. Titanium or stainless steel columns were utilized throughout this work. The solid supports were Chromosorb W, 60-80 mesh (Johns Manville Co.) and Chromosorb W, acid-washed (AW), hexamethyldisilazane (HMDS)-treated, 80-100 mesh (Nester and Faust). The stationary phases were Epon 1001 resin (Shell Chemical Co.) and Silicone nitrile Gum Rubber XE-60 (Nester and Faust). The trapping materials were acid (anionotropic) Woelm Alumina, activity grade I, and magnesol. Finally pyrex wool plugs were used instead of aluminum wire plugs²⁴ in the first two components of the chromatographic column. The samples were introduced at the column inlet in the form of crystals. For calibration, a measured volume of a standard

ethylene dichloride solution of the sample was applied to a pyrex wool plug and the solvent was evaporated by heating at 110° before introducing the plug at the column inlet.

Chemicals. The purest commercially available reagents and solvents were utilized. Etioporphyrin II was prepared by the method of FISCHER AND NEUMANN²⁸, the solvent gas was Genetron 12 (Allied Chemical).

Etioporphyrin II metal chelates. The Mg²⁹, Cu(II)³⁰, Ni(II), Co(II), Zn(II), Fe(III) monoacetate³¹, Ag(II)²⁸, Sn³² and VO³³ etioporphyrin II chelates were prepared according to methods described in the literature. The Al(III) monochloride etioporphyrin II was prepared according to the method of HILL³⁴ (spectra in ethylene dichloride: 398, 530, 570 m μ ; shoulder at 380 m μ ; $\alpha > \beta$). The Pd(II) and Pt(II) etioporphyrins II were prepared by the methods used for the tetraphenylporphin chelates³⁵ (spectra in ethylene dichloride: Pd—395.5, 511, 549.5 m μ ; $\alpha \gg \beta$; Pt—380, 500, 533 m μ ; $\alpha \gg \beta$). The method of TAYLOR³⁶ was applied for the preparation of Mn(III) etioporphyrin II (spectra in ethylene dichloride: 359, 461, 552, 582 m μ ; $\beta \gg \alpha$).

Mn(II) porphyrins have been obtained in dilute alkaline solution by reduction of the Mn(III) chelate³⁶⁻³⁸. We prepared Mn(II) etioporphyrin II as follows: Etioporphyrin II was added to a solution of an excess of Mn(II) acetylacetonate in pyridine and the mixture refluxed for 15 min. The solvent was evaporated under reduced pressure and water added. After filtration the precipitate was dissolved in benzene and the chelate precipitated with cold methanol and filtered. Microgram quantities of the pure chelate in crystalline form were obtained by HPGC of the crude, during which the impurities remained at the column inlet. Spectra: ethylene dichloride—400, 529, 566 m μ ; $\alpha > \beta$; benzene—402, 530, 568 m μ ; $\alpha > \beta$; ethylene dichloride + 10% pyridine—411, 539, 574 m μ ; $\beta > \alpha$; 0.1 N NaOH + 10% pyridine—415.5, 541, 579 m μ ; $\beta > \alpha$. The alkali-pyridine solution spectrum of the pure product is in agreement with those of Mn(II) mesoporphyrin³⁶, hematoporphyrin³⁷ and methyl phaeophorbide³⁸. The same method can be used for the preparation of Zn(II) etioporphyrin II. The Ni chelate required a 3:1 mixture of pyridine-glacial acetic acid as solvent.

Only one titanium porphyrin has been reported in the literature³⁹. Titanyl acetylacetonate and etioporphyrin II were refluxed in nitrobenzene and the solvent was subsequently removed by evaporation under reduced pressure. The unreacted free base was separated from its TiO chelate by HPGC from a 10% Epon 1001 resin on Chromosorb W column. Spectrum in ethylene dichloride: 401, 529, 567 m μ ; $\alpha > \beta$. Vanadyl etioporphyrin II was also prepared by exactly the same procedure and identified by comparison of its spectra with those of the authentic chelate.

RESULTS AND DISCUSSION

In previous work^{1,24} packed columns were preconditioned under a stream of CCl₂F₂ at 3100 p.s.i. and 180°. The use of lower pressures (*ca.* 2000 p.s.i.) and temperatures (150-155°) during preconditioning has a favorable effect on the separating ability of the column. Thus, better separations of Cu and Ni etioporphyrins II were effected by columns treated in this manner (Fig. 1) than by those treated at higher pressures and temperatures¹.

Only the chelates of Sn and Mn(III) proved to be insoluble in CCl₂F₂ under the

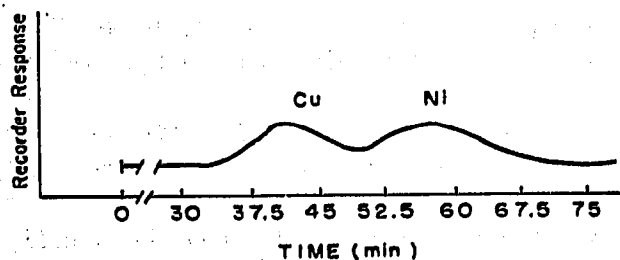


Fig. 1. Separation of Cu(II) and Ni(II) etioporphyrins II. Column: 46 in., $\frac{1}{8}$ in. I.D., of 10% Epon 1001 on Chromosorb W, 60–80 mesh. Pressure: 1275 p.s.i. Column temperature: 145°. Flow (gas): 172 ml/min. Monochromator setting: 394 $m\mu$.

experimental conditions. Both could be recovered unchanged at the column inlet and are, therefore, sharply separated from all the other chelates. The Al(III) chelate is completely volatilized but cannot be eluted from either of the two columns. The Fe(III) chelate is also volatilized and can be eluted intact from a Silicone XE-60 column but it is retained in an Epon 1001 column. Finally, the Mg(II), TiO, VO, Mn(II), Co(II), Ni(II), Pd(II), Pt(II), Ag(II) and Zn(II) etioporphyrins II can be volatilized and eluted from both chromatographic columns. Table I shows the relative retention values of the above metalloporphyrins from these columns.

TABLE I

RELATIVE RETENTION VALUES OF ETIOPORPHYRIN II METAL CHELATES

Etioporphyrin II chelate	Monochromator setting during detection ($m\mu$)	Relative retention time	
		Epon 1001 ^a	XE-60 ^b
Mg(II)	397	0.90	1.05
TiO	401	2.26	1.16
VO	405	2.39	2.36
Mn(II)	400	2.13	1.08
Mn(III)	359	insoluble	
Fe(III)	392	retained	1.07
Co(II) 1st peak (Co(II))	391	1.08	0.86
2nd peak (Co(III))		1.41	1.06
Ni(II)	390	1.27	1.14
Pd(II)	395	2.90	2.19
Pt(II)	380	2.31	1.68
Cu(II)	398	1.00	1.00
Ag(II)	405	1.19	1.12
Zn(II)	400	2.07	1.03
Al(III)	398	retained	retained
Sn	406	insoluble	
Etioporphyrin II	397	0.95	1.15
Retention time of Cu(II) etioporphyrin II (min)		29.250	18.5625

^a Column: 64 in., $\frac{1}{8}$ in. I.D., column of 10% Epon 1001 resin on Chromosorb W, 60–80 mesh. Pressure: 1500 p.s.i. Column temperature: 145°. Flow rate: 162 ml/min.

^b Column: 46 in., $\frac{1}{8}$ in. I.D., column of 10% Silicone XE-60 on AW-HMDS-treated Chromosorb W, 80–100 mesh. Pressure: 1350 p.s.i. Column temperature: 145°. Flow rate 136 ml/min.

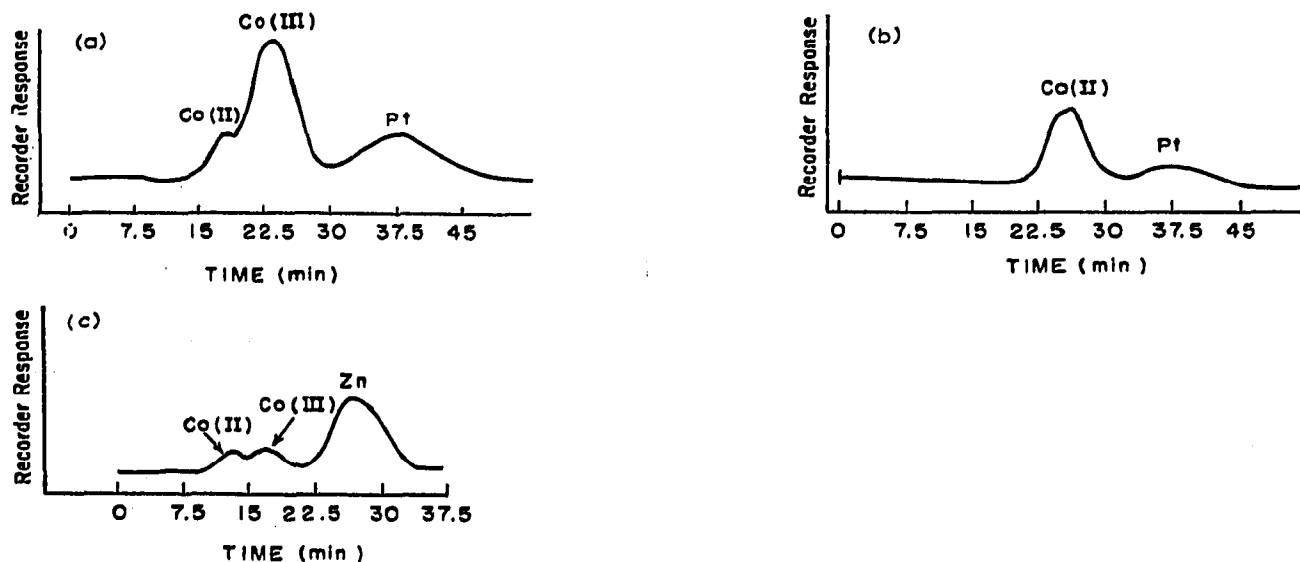


Fig. 2. (a) Separation of Co(II) and Pt(II) etioporphyrins II, in the presence of air. Column: 46 in., $\frac{1}{8}$ in. I.D., of 10% Epon 1001 on Chromosorb W, 60–80 mesh. Pressure: 1750 p.s.i. Column temperature: 145°. Flow (gas): 202 ml/min. Monochromator setting: 385 $m\mu$. (b) Separation of Co(II) and Pt(II) etioporphyrins II, in the absence of air. Column: 46 in., $\frac{1}{8}$ in. I.D., of 10% XE-60 on AW-HMDS-treated Chromosorb W, 80–100 mesh. Pressure: 1100 p.s.i. Column temperature: 145°. Flow (gas): 123 ml/min. Monochromator setting: 385 $m\mu$. (c) Separation of Co(II) and Zn(II) etioporphyrins II, in the absence of air. Column: the same as in Fig. 2a. Pressure: 1650 p.s.i. Column temperature: 145°. Flow (gas): 277 ml/min. Monochromator setting: 397 $m\mu$.

The TiO, VO, Ni(II), Pd(II), Pt(II), Cu(II) and Ag(II) chelates were recovered intact from both columns on an anionotropic alumina trap. The eluates of Mn(II) and Zn(II) etioporphyrins II, when trapped on acid alumina, showed the presence of *ca.* 20% etioporphyrin II but were recovered unchanged from a magnesol trap. The Mg(II) chelate is completely demetallated during its migration through the chromatographic column¹. The etioporphyrin peak which appears in the chromatogram is, however, reproducible and the method can be utilized for the identification and determination of Mg.

When the air enclosed in the column during the introduction of the sample was not removed prior to heating, two peaks appeared in the chromatogram of the Co(II) chelate (Fig. 2a). The first peak is the unchanged Co(II) chelate, and the second peak, which is larger, is Co(III) etioporphyrin II with, probably, a hydroxyl as the appended anion. The trapped eluate shows two Soret bands in ethylene dichloride: 391 $m\mu$ (Co(II)) and 414 $m\mu$ (Co(III))⁴⁰. When the air was displaced by CCl_2F_2 under a pressure of 200–250 p.s.i. from the column inlet before heating, the Co(II) etioporphyrin II was eluted intact from the Silicone XE-60 column (Fig. 2b). The Epon 1001 column, however, produced an approximately 1:1 mixture of the Co(II) and Co(III) chelates (Fig. 2c), indicating an oxidative attack of the metalloporphyrin by the stationary phase. Co(II) tetraphenylporphyrin exhibited a similar behavior to that of the etioporphyrin II chelate.

The fact that the Mn(II) chelate produces only one peak from either column does not indicate that it is unchanged. If partial oxidation occurred, the Mn(III) chelate would be deposited on the column packing at the spot of its formation.

Data available on the volatilities of porphyrin⁴¹ and phthalocyanine⁴² metal chelates do not seem to correlate to the gas chromatographic behavior of etioporphyrin II chelates. Thus, the order of increasing retention times (Table I) from the Epon 1001 is $\text{Cu} < \text{Co} < \text{Ni} \ll \text{Zn} < \text{Mn} < \text{VO}$ and from the XE-60 column $\text{Co} < \text{Cu} < \text{Zn} < \text{Fe(III)} < \text{Mn} < \text{Ni} \ll \text{VO}$. Volatility data would suggest the following order of increasing retention: $\text{Ni, Fe(III)} < \text{Cu, VO}^{41}$ and $\text{Ni, Cu} < \text{Mn, Co, Zn}^{42}$. No definitive conclusions as to mechanisms can be reached on this until retention data from non-polar columns are available.

The results of analysis of the Mn(III) hematoporphyrin monochloride monohydrate, showing less than one chloride per molecule, led LOACH AND CALVIN³⁷ to the postulation that the chelate is aggregated. In the case of the etioporphyrin II chelate, although no carboxylic side-chains are present, aggregation may be caused by intermolecular forces between the large flat porphyrin rings⁴³. Since both ferric and cobaltic etioporphyrins II can be gas chromatographed, a combination of low vapor pressure and partial aggregation might account for the resistance of the manganic chelate to volatilization.

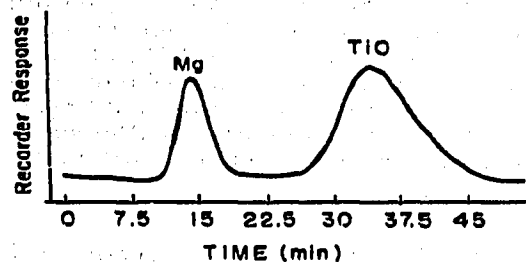


Fig. 3. Separation of Mg(II) and TiO etioporphyrins II. Column: 46 in., $\frac{1}{8}$ in. I.D., of 10% Epon 1001 on Chromosorb W, 60–80 mesh. Pressure: 1350 p.s.i. Column temperature: 145°. Flow (gas): 336 ml/min. Monochromator setting: 400 $m\mu$.

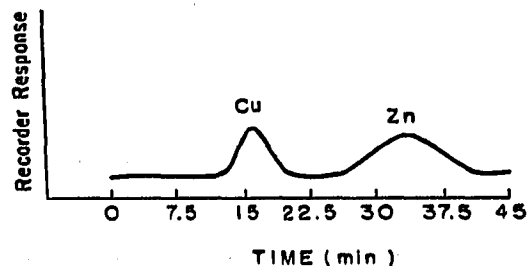


Fig. 4. Separation of Cu(II) and Zn(II) etioporphyrins II. Column: the same as in Fig. 3. Pressure: 1750 p.s.i. Column temperature: 146°. Flow (gas): 202 ml/min. Monochromator setting: 399 $m\mu$.

In the case of the tin etioporphyrin II, its resistance to volatilization can be satisfactorily explained by the fact that it is very insoluble in most solvents³². The chloride ion is, presumably, responsible for the retention of the Al chelate in both columns utilized. Finally, the Pd(II) chelate should be less volatile, less soluble or partially aggregated to explain its great retardation in relation to the Pt(II) analog.

The 10% Epon 1001 resin on Chromosorb W, 60–80 mesh column (column E) is, in general, more effective for the separation of metalloetioporphyrins and elution of these chelates is considerably retarded in relation to the 10% Silicone XE-60 on AW-HMDS-treated Chromosorb W, 80–100 mesh column (column X) under the same conditions. Column E retards selectively the Zn(II), Mn(II) and TiO chelates, allowing the complete separation of any of these elements from Mg(II), Cu(II), Co(II) and Co(III), Ag(II) and Ni(II) (Figs. 2c, 3–5) and their partial separation from Pd(II). Cu(II), Mg(II) or Co(II) can be separated from Ni(II) or Co(III) (Figs. 1, 2c, 6). The Ag(II) peak, which is relatively broad, overlaps completely with the peaks of the latter elements. Ag(II), Pt(II) and Pd(II) can be separated in column E (Fig. 7). Pd and Pt can also be separated from Mg, Cu, Co or Ni. Finally, vanadyl is easily

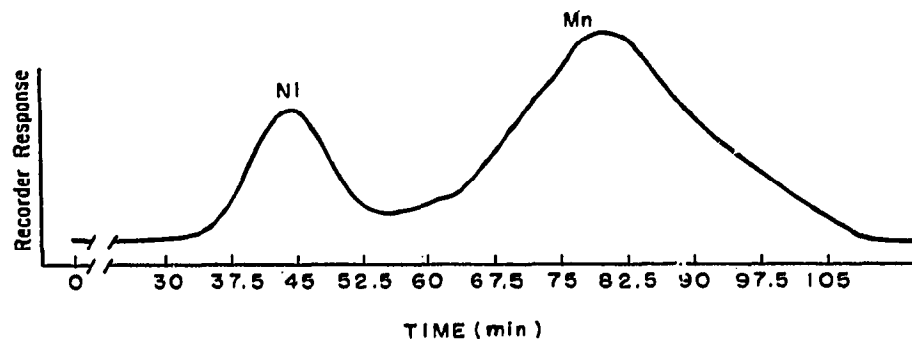


Fig. 5. Separation of Ni(II) and Mn(II) etioporphyrins II. Column: 46 in., $\frac{1}{8}$ in. I.D., of 10% Epon 1001 on Chromosorb W, 60–80 mesh. Pressure: 450 p.s.i. Column temperature: 145° . Flow (gas): 105 ml/min. Monochromator setting: 397 $m\mu$.

separated from Mg, Cu, Co, Ni or Ag but overlaps considerably with Pd and is not separated from any of the other elements. Column E retains the Fe(III) chelate and promotes the partial oxidation of the Co(II) porphyrin.

Column X, in contrast, allows the elution of the Fe(III) etioporphyrin II and that of the unchanged Co(II) chelate. The retention times of the Zn(II), Mn(II) and TiO chelates are quite near to those of Co(II) and Co(III), Cu(II), Mg(II), Fe(III), Ag(II) and Ni(II) and only relatively poor separations between some of these

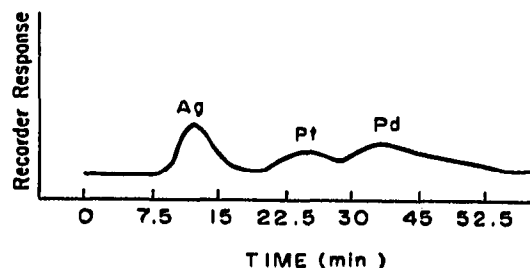
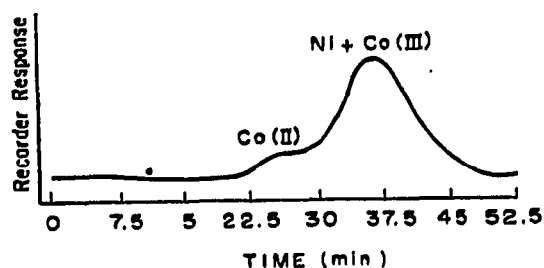


Fig. 6. Separation of Co(II) and Ni(II) etioporphyrins II, in the absence of air. Column: the same as in Fig. 5. Pressure: 1525 p.s.i. Column temperature: 145° . Flow (gas): 163 ml/min. Monochromator setting: 391 $m\mu$.

Fig. 7. Separation of Ag(II), Pt(II) and Pd(II) etioporphyrins II. Column: the same as in Fig. 5. Pressure: 1475 p.s.i. Column temperature: 145° . Flow (gas): 351 ml/min. Monochromator setting: 390 $m\mu$.

elements can be achieved (*cf.* Table II). Any of these elements can, however, be separated from Pt(II), Pd(II) or VO (Figs. 2b, 8, 9). Column X, thus, is advantageous over column E, in the case of separation of Zn, Mn(II) or TiO from Pt, Pd or VO. The order of elution of VO and Pd is reversed in this column. The separation of Pt and VO is satisfactory in column X but both these chelates partially overlap with that of Pd.

Table II summarizes the separations that can be effected from these columns. HPGC at lower pressures and flow rates or use of longer columns lead to better separations, but the peaks obtained under such conditions are usually too broad and cannot be measured. Thus, Pt and Pd, for example, can be completely separated at 1100 p.s.i. and a flow of 150 ml/min, but their peak widths cover periods of over 20 min.

TABLE II

SEPARATIONS OF ETIOPORPHYRIN II METAL CHELATES

Column E = 46 in., $\frac{1}{8}$ in. I.D., column of Epon 1001 on Chromosorb W, 60-80 mesh. Column X = 46 in., $\frac{1}{8}$ in. I.D., column of XE-60 on AW-HMDS-treated Chromosorb W, 80-100 mesh. c = Complete separation. s = Satisfactory partial separation (small overlapping of the peaks). p = Poor separation (considerable overlapping of the peaks). n = No separation (complete overlapping of the peaks).

Chelate	Mg (II)	TiO	VO	Mn (II)	Fe (III)	Co (II)	Co (III)	Ni (II)	Pd (II)	Pt (II)	Cu (II)	Ag (II)	Zn (II)	
	EX	EX	EX	EX	EX	EX	EX	EX	EX	EX	EX	EX	EX	
Mg(II)	-	-	c p	c c	c n	- n	n n	s n	s p	c c	c c	n n	n n	c n
TiO	c p	-	-	n c	n n	- n	c p	c n	c n	s c	n c	c p	c n	n n
VO	c c	n c	-	-	n c	- c	c c	c c	c c	p p	n s	c c	c c	n c
Mn(II)	c n	n n	n c	-	-	- n	c n	c n	c n	s c	n c	c n	c n	n n
Fe(III)	- n	- n	- c	- n	-	-	- n	- n	- c	- c	- n	- n	- n	- n
Co(II)	n n	c p	c c	c n	- n	-	s p	s p	c c	c c	n n	n n	c n	c n
Co(III)	s n	c n	c c	c n	- n	s p	-	n n	c c	c c	s n	n n	c n	c n
Ni(II)	s p	c n	c c	c n	- n	s p	n n	-	c c	c c	s p	n n	c n	c n
Pd(II)	c c	s c	p p	s c	- c	c c	c c	c c	-	s p	c c	c c	s c	s c
Pt(II)	c c	n c	n s	n c	- c	c c	c c	c c	s p	-	c c	c c	n c	n c
Cu(II)	n n	c p	c c	c n	- n	n n	s p	s p	c c	c c	-	n n	c n	c n
Ag(II)	n n	c n	c c	c n	- n	n n	n n	n n	c c	c c	n n	-	c n	c n
Zn(II)	c n	n n	n c	n n	- n	c n	c n	c n	s c	n c	c n	c n	-	-

Calibration curves for the Zn(II) and Ag(II) etioporphyrins II (Fig. 10), show that a linear relationship exists between the amount of sample and the peak height or peak area, for sample sizes up to 2-8 μ g. These results, together with the calibration curves for the Cu(II) chelate, reported elsewhere¹, indicate that satisfactory quantitative determinations can be carried out.

The reproducibility of the chromatograms of all the chelates studied during the present work is quite satisfactory.

In conclusion, the first major attempt to elute and separate metallic elements

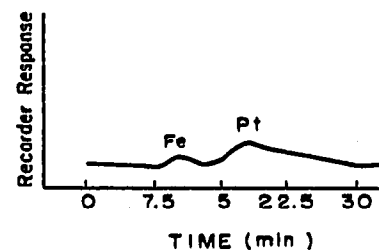
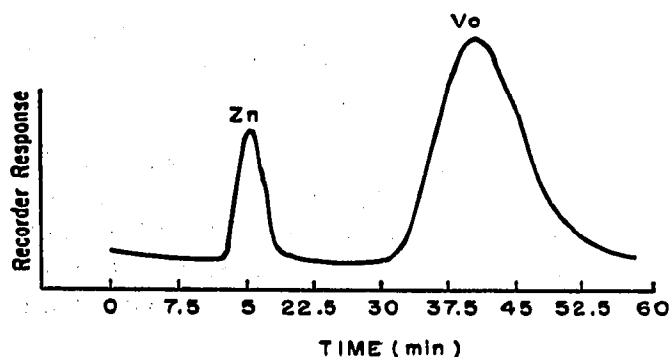


Fig. 8. Separation of Zn(II) and VO etioporphyrins II. Column: 60 in., $\frac{1}{8}$ in. I.D., of 10% XE-60 on AW-HMDS-treated Chromosorb W, 80-100 mesh. Pressure: 1450 p.s.i. Column temperature: 145°. Flow (gas): 306 ml/min. Monochromator setting: 402 $m\mu$.

Fig. 9. Separation of Fe(III) and Pt(II) etioporphyrins II. Column: the same as in Fig. 8. Pressure: 1750 p.s.i. Column temperature: 145°. Flow (gas): 367 ml/min. Monochromator setting: 385 $m\mu$.

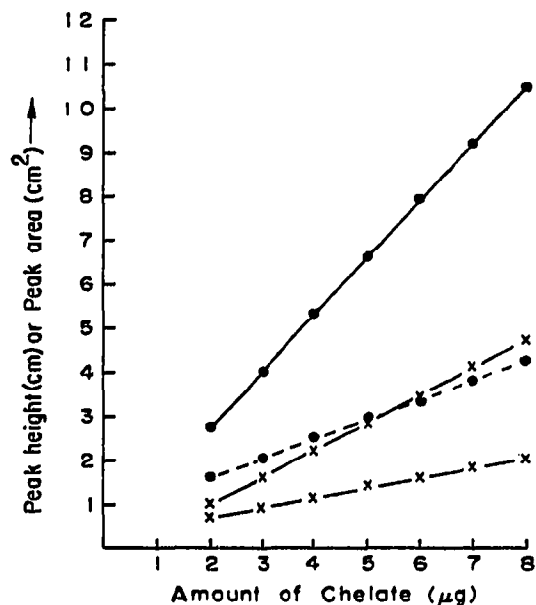


Fig. 10. Calibration curves for Zn(II) (—●—) and Ag(II) (—x—) etioporphyrins II. Pressure: 1600 p.s.i. Column temperature: 145°. Flow (gas): 336 ml/min. Column: 60 in., 1/8 in. I.D., column of 10% Silicone XE-60 on AW-HMDS-treated Chromosorb W, 80-100 mesh. ● = Peak height (cm); x = peak area (cm²). Monochromator setting: 400 mμ.

in the form of their etioporphyrin II chelates by HPGC has been successful. The outstanding feature of this work is the volatilization and separation of Ag(II) and Pt(II) chelates. The method has the advantage of the possibility of operation at substantially lower temperatures than those required by the conventional method suggesting that it become the indicated gas chromatographic method for this type of compound. Many interesting separations have been effected by using two different polar columns which permit reversal of the order of elution of some pairs of elements. The possibility of HPGC of the chelates of metals with some of the most common and relatively inexpensive chelating agents is easily foreseeable. The conventional gas chromatography of metal chelates is, in contrast, mainly based on polyfluorinated β -diketonates⁴⁴. An extension of the present HPGC study to all the known stable etioporphyrin II chelates⁴⁵⁻⁴⁷, combined with attempts to prepare new chelates with other elements would undoubtedly result in the elution and separations of many other elements.

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