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THE SEPARATION BY THIN-LAYER CHROMATOGRAPHY OF TRACE METALS AS THEIR TETRAPHENYLPORPHYRIN CHELATES

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SUMMARY

The tetraphenylporphyrin chelates of iron, cobalt, manganese, nickel, zinc, copper, rhodium, lead, cadmium and mercury were prepared and their chromatographic behaviour investigated using four different adsorbents and eleven different solvent systems. Mixtures of lead, cadmium and mercury chelates exhibited isographic behaviour in all conditions; mixtures of the other metal chelates were adequately resolved on silica (MN Polygram Sil SHR) by the solvent system light petroleum (b.p. 80–100°)–toluene–acetic acid–water (66:33:85:15, v/v, upper phase). The intense colours of the chelates permit their visual detection at levels of about 10^{-10} moles; using the integrated ion-current mass spectrometric procedure, as little as 10^{-14} moles of metal could be detected and assessed.

INTRODUCTION

In an attempt to develop a procedure for the analysis of those trace metals present in biological tissues by a mass spectrometric (MS) technique, various metal chelates were investigated. As the metals we wished to investigate possessed similar mass and several isotopes, separation of the metals prior to a qualitative and quantitative analysis by MS was required. A further requirement for MS is that the metals exist in a form, such as a chelate, which is volatile at 330° or less. After an examination of several chelating agents, which included β-diketones, fluorinated β-diketones, oximes and EDTA, tetraphenylporphyrin (see Fig. 1) was selected as being the most suitable from the chromatographic and MS points of view. Thin-layer chromatography (TLC) of metals as their chelates has been undertaken in the past[1], but only vanadium, copper and nickel appear to have been studied in the form of their tetraphenylporphyrin (TPP) derivatives[2]. The quantitative analysis of a number of chromatographically separated unchelated tetraphenylporphyrins has also been reported[3]. In this paper we describe the TLC properties of the tetraphenylporphyrin chelates of manganese, iron, cobalt, nickel, copper, zinc, rhodium, cadmium, mercury and lead.

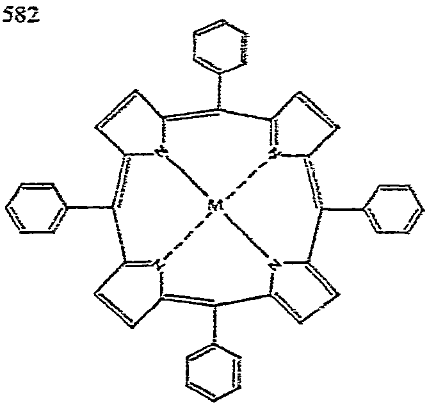

Fig. 1. Structure of metal tetraphenylporphyrin.

EXPERIMENTAL

Preparation of tetraphenylporphyrin and the metal chelates

α, β, γ, δ-TPP was prepared by the method of Adler *et al.*[4], in which freshly distilled benzaldehyde and pyrrole are added to refluxing propionic acid.

The metal chelates were prepared by heating TPP and a five-fold excess of metal chloride in refluxing dimethylformamide (180°) for 30 min[5]. The dimethylformamide was removed by evaporation in a stream of nitrogen, the residue triturated in benzene which was then washed several times with distilled water to remove unreacted metal salt. The benzene was evaporated, the residue dissolved in chloroform and then adsorbed on a column of neutral alumina. The metal chelate was eluted and its identity checked by MS. TPP or a contaminating metal chelate was never detected in a purified metal TPP.

Materials

Reagent-grade solvents as obtained commercially were used without further purification.

The solvent systems were prepared as indicated in Table I; in biphasic systems the upper organic phase was used.

The following TLC plates were used: MN Polygram Sil SHR (Macherey, Nagel & Co., Düren, G.F.R.), silica gel (Brinkmann, Westbury, N.Y., U.S.A.), Kieselgel (Camag, Muttenz, Switzerland), alumina (Analtech, Newark, Del., U.S.A.), polyamide (Schleicher & Schuell, Keene, N.H., U.S.A.) and Eastman 6064 cellulos (Eastman-Kodak, Rochester, N.Y., U.S.A.). All plates were 20 × 20 cm in size wit layers 250 μm thick, except for the MN Polygram and polyamide plates which we 100 μm thick. They were used without prior activation. The plates were developed glass tanks (Desaga, Heidelberg, G.F.R.) containing freshly mixed solvent (Table) added to a depth of 1 cm 1 h before introduction of the plate.

Methods

Samples (10 μl) of synthetic metal TPPs containing 50 μg of chelate per were applied to the origin line on each type of layer and placed in the appropriate sc- vent system. Development was allowed to proceed at room temperature (20°) un the solvent front was 15 cm from the origin (usually 30–60 min). After removal fro the tanks, the chromatograms were air dried and examined in daylight and und

TABLE I

COMPOSITION OF SOLVENT SYSTEMS USED TO SEPARATE TETRAPHENYLPORPHYRIN CHELATES OF SOME METALS

The boiling range of the light petroleum used is 80–100°.

Solvent system	*Composition*	*Proportions (v/v)*	*Comments*
1	Acetic acid–chloroform	1:99	
2	Acetic acid–chloroform	2:98	
3	Acetic acid–chloroform	4:96	
4	Light petroleum–toluene	67:33	
5	Acetic acid–water–toluene	70:30:100	Upper phase used
6	Acetic acid–water–toluene	85:15:100	Upper phase used
7	Acetic acid–water–light petroleum	85:15:100	Upper phase used
8	Acetic acid–water–light petroleum	90:10:100	Upper phase used
9	Acetic acid–water–light petroleum–toluene	85:15:50:50	Upper phase used
10	Acetic acid–water–light petroleum–toluene	85:15:67:33	Upper phase used
11	Acetic acid–water–light petroleum–toluene–methanol	85:15:67:33:1	Upper phase used; methanol added to upper phase after separation of phases.

ultraviolet light (365 nm). In some cases the metal TPP zones were scraped from the plate, eluted from the adsorbent with methanol and an aliquot (5 μl) introduced on a direct insertion probe into the AEI MS-902S mass spectrometer. Spectra and quantitative analysis by the integrated ion-current procedure were obtained as previously described[6–9].

RESULTS AND DISCUSSION

Adequate resolution and zone definition was obtained in the case of silica gel

TABLE II

R_F VALUES OF TETRAPHENYLPORPHYRIN CHELATES OF SOME METALS SEPARATED ON SILICA GEL

Plates: MN Polygram Sil SHR, pre-coated.

Solvent system	*Mn*	*Fe*	*Co*	*Ni*	*Cu*	*Zn*	*Rh*	*Cd*	*Hg*	*Pb*	*TPP*
1	0.19	0.43	0.00	0.97	1.00	0.98	0.00	0.96	0.97	0.96	0.76
2	0.30	0.45	0.00	0.96	0.98	0.97	0.00	0.88	0.95	0.97	0.88
3	0.78	0.94	0.00	0.96	1.00	1.00	0.00	0.96	0.97	0.98	0.87
4	0.00	0.00	0.00	—	0.44	0.08	—	—	—	—	0.08
5	0.27	0.86	0.94	0.94	0.98	0.97	0.00	0.83	0.82	0.82	0.75
6	0.57	0.99	0.97	0.97	0.97	0.98	0.07	0.92	0.92	0.92	0.87
7	0.00	0.22	0.00	0.65	0.60	0.32	0.00	0.15	0.16	0.16	0.07
8	0.00	0.38	0.00	0.73	0.81	0.50	0.00	0.17	0.15	0.15	0.19
9	0.18	0.65	0.00	0.96	0.95	0.91	0.00	0.61	0.64	0.58	0.56
10	0.13	0.66	0.00	0.97	0.90	0.80	0.00	0.48	0.45	0.47	0.41
11	0.14	0.73	0.00	0.96	0.98	0.94	0.00	0.60	0.52	0.55	0.74

TABLE III

R_F VALUES OF TETRAPHENYLPORPHYRIN CHELATES OF SOME METALS SEPARATED ON SILICA GEL

Plates: Brinkmann, pre-coated.

Solvent system	*Mn*	*Fe*	*Co*	*Ni*	*Cu*	*Zn*	*Rh*	*Cd*	*Hg*	*Pb*	*TPP*
1	0.05	0.13	0.98	0.94	0.98	0.89	0.00	0.93	0.92	0.92	0.90
2	0.11	0.26	0.95	0.95	0.96	0.88	0.00	0.92	0.93	0.94	0.83
3	0.28	0.43	0.98	0.96	0.98	0.92	0.00	0.92	0.92	0.91	0.74
4	0.00	0.00	0.50	0.49	0.49	0.12	0.00	0.09	0.10	0.10	0.09
5	0.09	0.42	0.98	0.97	0.97	0.95	0.00	0.84	0.83	0.85	0.46
6	0.33	0.60	0.98	0.98	0.97	0.89	0.08	0.93	0.89	0.88	0.60
7	0.00	0.11	0.27	0.35	0.29	0.21	0.00	0.18	0.18	0.18	0.11
8	0.00	0.12	0.28	0.38	0.28	0.22	0.00	0.22	0.20	0.21	0.09
9	0.17	0.51	0.83	0.85	0.81	0.62	0.00	0.49	0.50	0.50	0.50
10	0.04	0.40	0.60	0.67	0.73	0.48	0.00	0.44	0.42	0.43	0.40
11	0.05	0.38	0.41	0.79	0.79	0.59	0.00	0.48	0.47	0.47	0.55

and alumina. Poor quality chromatograms were produced on cellulose and polyamide layers. Tables II–V list the R_F values obtained for ten metal chelates on alumina and on three different types of silica gel.

The most useful solvent system was number 10, although it will not completely resolve a mixture of the ten listed metals. Two groups of metal chelates in particular are difficult to resolve; these are cadmium, mercury and lead, and nickel and copper. Although there are small differences with respect to the R_F values of the metal chelates in these two groups, they should be considered essentially as isographic.

For reasons that are not clear, solutions of cobalt TPP, unless freshly prepared, produce poorly defined zones in several different solvent systems, R_F values that are lower than expected and extensive tailing. This may be because most, or all, of the cobalt in freshly prepared CoTPP exists in the divalent state, but that after standing

TABLE IV

R_F VALUES OF TETRAPHENYLPORPHYRIN CHELATES OF SOME METALS SEPARATED ON SILICA GEL

Plates: Camag Kieselgel, pre-coated.

Solvent system	*Mn*	*Fe*	*Co*	*Ni*	*Cu*	*Zn*	*Rh*	*Cd*	*Hg*	*Pb*	*TPP*
1	0.12	0.09	0.32	0.84	0.97	0.91	0.00	0.88	0.87	0.93	0.91
2	0.21	0.21	0.40	0.89	0.96	0.90	0.00	0.86	0.87	0.86	0.81
3	0.35	0.39	0.61	0.94	0.94	0.93	0.00	0.90	0.85	0.83	0.72
4	0.00	0.00	0.00	0.50	0.44	0.08	0.00	0.09	0.08	0.08	0.07
5	0.32	0.49	0.71	0.93	0.97	0.46	0.00	0.84	0.77	0.77	0.60
6	0.49	0.60	0.91	0.98	0.95	0.58	0.09	0.90	0.92	0.92	0.59
7	0.00	0.05	0.00	0.30	0.11	0.07	0.00	0.14	0.13	0.13	0.03
8	0.02	0.20	0.00	0.34	0.27	0.24	0.00	0.17	0.20	0.20	0.15
9	0.15	0.43	0.42	0.83	0.79	0.23	0.00	0.49	0.44	0.43	0.32
10	0.13	0.43	0.28	0.64	0.69	0.19	0.00	0.38	0.38	0.36	0.37
11	0.09	0.33	0.04	0.76	0.73	0.60	0.00	0.52	0.43	0.41	0.55

TABLE V

R_F VALUES OF TETRAPHENYLPORPHYRIN CHELATES OF SOME METALS SEPARATED ON ALUMINA

Plates: Analtech alumina pre-coated.

Solvent system	*Mn*	*Fe*	*Co*	*Ni*	*Cu*	*Zn*	*Rh*	*Cd*	*Hg*	*Pb*	*TPP*
	0.16	0.85	0.62	0.96	0.98	0.68	0.12	0.94	0.88	0.96	0.93
	0.00	0.75	0.84	0.96	0.98	0.65	0.35	0.95	0.96	0.97	0.95
	0.55	1.00	0.85	0.98	1.00	1.00	0.50	0.94	0.93	0.96	1.00
	0.00	0.00	0.00	0.79	0.37	0.00	0.00	0.15	0.11	0.15	0.06
	0.60	0.57	1.00	1.00	1.00	0.70	0.41	0.92	0.70	0.71	0.87
	0.74	0.89	1.00	1.00	1.00	0.96	0.64	0.93	0.94	0.93	0.92
	0.10	0.34	0.37	0.68	0.62	0.32	0.00	0.60	0.42	0.45	0.45
8	0.08	0.43	0.44	0.75	0.67	0.39	0.00	0.59	0.56	0.63	0.51
9	0.57	0.58	0.90	0.94	0.95	0.72	0.08	0.73	0.74	0.81	0.76
10	0.45	0.50	0.76	0.93	0.92	0.67	0.02	0.74	0.67	0.68	0.69
11	0.52	0.86	0.61	0.92	0.94	0.90	0.33	0.68	0.65	0.64	0.80

in solution it is oxidized to the trivalent state which results in a different behaviour on TLC. MS analysis, however, revealed that CoTPP was present in the same form in both fresh and stored solutions after extraction from the plate. It has been found[10] that iron and manganese TPP are formed in the trivalent state (even when the starting material is a divalent salt), with the third valence being taken up by a chlorine atom (if the salt was a chloride). In the mass spectrometer, however, we have found that this third ligand is so readily lost that no molecular ion is detectable, the apparent molecular ion is the same as that of the divalent metal TPP. This even applies to tin TPP which is tetravalent and loses two ligands (*e.g.*, two chlorine atoms) to give an apparent molecular ion equivalent in mass to divalent SnTPP.

The observable colours of the metal TPP chelates dissolved in chloroform and after development in four different solvent systems are listed in Table VI. In ultraviolet light (365 nm) the separated zones exhibited either a red fluorescence (ZnTPP, CdTPP,

TABLE VI

COLOUR OF SOME METAL TPP CHELATES IN CHLOROFORM AND AFTER SEPARATION IN VARIOUS SOLVENT SYSTEMS

Condition	*Mn*	*Fe*	*Co*	*Ni*	*Cu*	*Zn*	*Rh*	*Cd*	*Hg*	*Pb*	*TPP*
Chloroform*	gray-green	brown-yellow	brick red	orange	orange-red	cherry red	brown-orange	beige	gray-violet	magenta	magenta
Chloroform**	gray-green	brown-yellow	orange-red	brick red	brick red	purple	orange-red	beige	purple	purple	purple
Solvent system 3	gray-green	brown-yellow	orange-red	orange-red	brick red	purple	orange-red	green	green	green	green
Solvent system 4	gray-green	brown-yellow	orange-red	orange-red	red	pale green	orange-red	green	purple	purple	purple
Solvent system 10	gray-green	brown-yellow	orange-red	orange-red	red	purple	orange-red	green	green	green	green

* Metal TPP complex dissolved in chloroform.

** Colour on TLC following development in chloroform.

HgTPP, PbTPP and TPP itself) or else a dark one against the lighter background o the rest of the plate. This latter behaviour was not affected by the nature of the medium. The differences in colour between the zones separated in different solvent systems appears to depend upon the presence or absence of acetic acid. The colours o some metal TPP complexes developed in solvent systems containing acetic acid exhibit a change in colour following air drying; these changes may be prevented b exposing the plate after solvent development to an ammonia-saturated atmosphere

Although the metal TPP chelates exhibit clean mass spectra and significan molecular ions (usually the base peak) suitable for analysis by the mass spectrometri IIC technique, where as little as 1 pg of metal (1.5×10^{-14} moles) may be detecte and quantitated[11], it is interesting to note that because of their intense colours the can also be detected and therefore presumably quantitated by spectrophotometric procedures in nanogram quantities following TLC. In the case of Mn, Co and Cd, as little as 0.5 ng of metal (5 ng of chelate) may be seen by the naked eye; some 1–5 ng of the other metals) are required.

In preliminary experiments some metals have already been identified in rat brain. Briefly the brain (and this procedure applies to any tissue or body fluid) was excised, dried overnight in a platinum crucible at 100° and then heated (ashed) at a temperature that was gradually increased to 700° and then maintained at this value for 4 h. The ash, containing metals as their oxides, was heated with 0.5 ml concentrated hydrochloric acid (*i.e.*, to convert oxides to chlorides), and after removal of the acid by evaporation, the residue was triturated in 10 ml water. An aliquot (0.1–0.5 ml), after evaporation, was refluxed in dimethylformamide containing an excess of TPP. After removal of the dimethylformamide under a stream of filtered nitrogen, the residue was dissolved in chloroform and subjected to chromatographic separation followed by MS analysis. The metals Cu, Co, Zn, Fe and Mn were identified. Further details with respect to the identification and quantitative analysis of these (and other) metals in mammalian tissues will be published elsewhere[11].

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REFERENCES

1 K. Saitoh and N. Suzuki, *J. Chromatogr.*, 92 (1974) 371.
2 M. Sato and T. Kwan, *Chem. Pharm. Bull.*, 20 (1972) 840.
3 R. W. Balek and A. Szutka, *J. Chromatogr.*, 17 (1965) 127.
4 A. D. Adler, E. R. Longo, J. D. Finarelli, J. Goldmacher, J. Assour and L. Korsakoff, *J. Or. Chem.*, 32 (1967) 476.
5 A. D. Adler, F. R. Longo, F. Kampas and J. Kim, *J. Inorg. Nucl. Chem.*, 32 (1970) 2443.
6 D. A. Durden, S. R. Philips and A. A. Boulton, *Can. J. Biochem.*, 51 (1973) 995.
7 S. R. Philips, D. A. Durden and A. A. Boulton, *Can. J. Biochem.*, 52 (1974) 366.
8 S. R. Philips, D. A. Durden and A. A. Boulton, *Can. J. Biochem.*, 52 (1974) 447.
9 S. R. Philips, B. A. Davis, D. A. Durden and A. A. Boulton, *Can. J. Biochem.*, 53 (1975) 65.
10 E. B. Fleischer, J. M. Palmer, T. S. Srivastava and A. Chatterjee, *J. Amer. Chem. Soc.*, 93 (1971) 316.
11 B. A. Davis, K. S. Hui and A. A. Boulton, in A. Frigerio and D. M. Desiderio (Editors *Advances in Mass Spectrometry in Biochemistry and Medicine*, Vol. 2, Raven Press, New Yor in press.