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High-performance thin-layer chromatographic mobility of porphyrins and their copper complexes on different adsorbents

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ABSTRACT

The R_F values of twelve synthetic porphyrins including porphine and phenyl- and alkyl-substituted porphines, and also of their copper complexes, were obtained on thin-layer chromatographic plates coated with silica gel, cellulose, NH_2-bonded silica gel and octadecyl-bonded silica gel (ODS) with different developing solvents. On the first three plates, the mobilities (R_F values) of each porphyrin were always smaller than those of the corresponding copper complex, whereas the opposite occurred on the last plate. With an identical solvent, the mobilities of both a porphyrin and its copper complex increase in the following order of polar adsorbents: silica gel < NH_2-silica < cellulose. *meso*-Tetra(4-pyridyl)porphine was adsorbed so strongly on every polar adsorbent that it hardly migrated from the origin, whereas a large migration with the $R_F \approx 0.9$ occurred on an ODS plate specifically with N,N-dimethylformamide.

INTRODUCTION

The convenience of thin-layer chromatography (TLC) has been applied to the isolation or preliminary identification of porphyrins and porphyrin esters by many investigators [1,2]. High-performance TLC (HPTLC) has promised to give higher resolution and reproducibility than conventional TLC for the separation of porphyrins and related compounds. Only a limited numbers of paper have dealt with systematic studies of the HPTLC behaviour of porphytins and metalloporphyrins.

In our laboratory, a series of fundamental studies have been carried out on the HPTLC migration behaviour of metal complexes of various porphyrins, such as *meso*-tetraphenylporphine [3,4], *meso*-tetrakis(4-tolyl)porphine [5] and etioporphyrin [6], using both normal- and reversed-phase separation modes, and porphine [7], haematoporphyrin [8] and pheophorbide-a and -b (not of the porphyrin but the chlorin family) [9] only using the reversed-phase mode. As these studies were not always carried out under identical conditions, the mobilities of different porphyrins and metalloporphyrins could not be easily compared with each other.

This paper describes the comparison of the mobilities of different porphyrins in the forms of the free acids (H_2P) and copper(II) complexes [Cu(P)] under identical conditions of adsorbent and solvent. The HPTLC study covered twelve synthetic porphyrins, including porphine, which is the compound with the most basic structure of porphyrin, and its analogues substituted with alkyl, *para*-substituted-phenyl or pyridyl groups. The Cu(P)s were taken as model metalloporphyrins. Four kinds of adsorbents, *viz.*, silica gel, cellulose, aminopropyl-bonded silica gel (NH_2-silica) and ODS, were used.

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EXPERIMENTAL

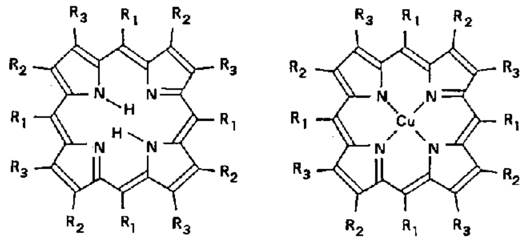
Porphyrins and their copper complexes

Fig. 1 shows the porphyrins studied. The preparations of H_2POR [7], H_2TPP [3], H_2TPP [5] and H_2ETIO [6] have been described in previous papers. H_2TMP [10] and H_2TPyP [11] were prepared by literature methods, respectively. Other porphyrins were synthesized by the general method of Adler *et al.* [12], in which pyrrole reacted, in refluxing propionic acid, with a suitable aromatic aldehyde, *e.g.*, fluorobenzaldehyde for H_2TFPP. These porphyrins were purified according to the procedures of Barnett *et al.* [13]. H_2OEP was obtained commercially (Strem Chemicals, Newburryport, MA, USA).

All Cu(P)s were prepared by the reaction of the porphyrin with ten times the equivalent amount of copper(II) acetylacetonate in refluxing chloroform [14]. They were then purified by alumina column chromatography followed by recrystallization.

HPLC

Commercially available HPTLC plates (10 × 10 cm) (Merck, Darmstadt, Germany) precoated with silica gel (silica gel 60), cellulose, NH_2-silica (NHY$_2$ F254S), and ODS (RP-18 F254S), all from Merck, were used. The silica gel plate was cleaned by development with methanol and then activated prior to use by heating at 110°C for 30 min followed by cooling in a silica gel desiccator for 2 h. The other plates were used without preliminary treatment. A portion of the thin-layer coating was preliminarily scraped off each plate so that the development would stop automatically when the solvent front had migrated 75 mm from the origin.



H_2P Cu(P)

Porphyrin (H_2P)	Abbr. P	R_1	R_2	R_3
porphine	POR	H	H	H
meso-tetramethylporphine	TMP	CH_3	H	H
meso-tetraphenylporphine	TPP	ph–H	H	H
meso-tetrakis(4-tolyl)porphine	TTP	ph–CH_3	H	H
meso-tetrakis(4-methoxyphenyl)porphine	TMPP	ph–OCH_3	H	H
meso-tetrakis(4-fluorophenyl)porphine	TFPP	ph–F	H	H
meso-tetrakis(4-chlorophenyl)porphine	TCPP	ph–Cl	H	H
meso-tetrakis(4-nitrophenyl)porphine	TNPP	ph–NO_2	H	H
meso-tetrakis(4-biphenyl)porphine	TBPP	ph–ph–H	H	H
meso-tetrakis(4-pyridyl)porphine	TPyP	4-pyridyl	H	H
etioporphyrin I	ETIO	H	C_2H_5	CH_3
octaethylporphyrin	OEP	H	C_2H_5	C_2H_5

ph = para-substituted phenyl; $-(C_6H_4)-$.

Fig. 1. The H_2P and Cu(P) species studied.

A sample solution of an H_2P or Cu(P) was prepared at a concentration of 0.1 m*M* or less in chloroform. A 0.5-μl portion of the solution was charged with the aid of a glass capillary micropipette (Drummond Scientific, Broomall, PA, USA) on to the sample origin located 5 mm from the edge of the TLC plate. All porphyrins or their copper complexes were spotted on identical plates so that their mobilities could be compared with each other.

The chromatogram was developed horizontally in a Camag (Muttenz, Switzerland) Model 28510 sandwich-type chamber in a room thermostated at 25°C. After the migration of the solvent front had automatically stopped at the preset distance (75 mm), the development was allowed to continue for an additional 5 min so that the irregularity of the amount of solvent particularly in the vicinity of the solvent front was reduced. The chromatogram was recorded spectrophotometrically at the absorption maximum wavelength of the Soret band of each compound (about 500 nm) with a Shimadzu (Kyoto, Japan) Model CS-920 densitometer.

RESULTS AND DISCUSSION

One sharp spot was detected for each compound in the chromatogram on all the chromatographic plates used. The R_F values obtained for H_2P and Cu(P) on different HPTLC plates are given in Tables I and II, respectively. For discussing the mobility of each compound, the migration factor R_M calculated from the following equation is used hereafter:

$$R_M = \log\left(\frac{1}{R_F} - 1\right) \qquad (1)$$

For convenience, R_M values above 1.7 or below −1.7 will be entered on the graphs as 1.7 or −1.7, respectively.

TABLE I

R_F × 100 VALUES OF H_2P

Plate	Solvent	R_F × 100											
		POR	TMP	TPP	TTP	TMPP	TFPP	TCPP	TNPP	TBPP	TPyP	ETIO	OEP
Silica gel	Carbon tetrachloride	2	0	5	2	0	3	10	0	1	0	1	0
	m-Xylene	42	8	85	87	6	97	SF[a]	8	90	0	17	39
	Benzene	54	14	97	SF	21	SF	SF	24	SF	0	21	48
	Trichloroethylene	15	2	25	21	0	33	59	0	17	0	4	8
	1,2-Dichloroethane	97	4	SF	SF	66	SF	SF	78	SF	0	40	64
Cellulose	Hexane	25	9	21	14	0	5	7	0	0	0	16	24
	Cyclohexane	39	11	43	34	0	8	18	0	0	0	14	32
	Methylcyclohexane	–	14	52	39	–	–	38	–	–	0	23	37
	Carbon tetrachloride	SF	64	SF	SF	75	87	96	7	84	0	92	SF
NH_2-silica	Methylcyclohexane	–	0	3	3	–	–	3	–	–	0	2	4
	Carbon tetrachloride	15	13	53	57	6	51	67	0	24	0	32	48
	m-Xylene	55	52	91	97	41	97	SF	23	86	0	79	91
	Trichloroethylene	43	57	93	98	43	95	SF	17	88	0	82	94
	Carbon disulphide	12	5	27	24	0	22	43	0	9	0	8	11
	Benzene	74	72	SF	SF	82	SF	SF	59	SF	0	92	SF
ODS	Methanol	42	11	10	7	13	20	5	47	3	0	10	12
	Ethanol	57	19	44	35	36	60	39	80	26	0	26	33
	1-Propanol	55	23	63	63	66	72	60	92	49	0	69	76
	1-Butanol	64	48	86	98	76	91	82	SF	81	0	95	SF
	Acetone	94	78	81	75	91	90	78	97	80	0	76	77
	Acetonitrile	42	7	10	10	19	17	4	42	2	0	2	2
	Ethyl acetate	SF	SF	SF	SF	SF	SF	SF	SF	SF	0	91	95
	DMF	90	82	75	61	83	80	72	89	76	89	63	44

[a] SF = Moved with the solvent front.

Silica gel plate

With an identical solvent, H_2P generally has a larger R_M value than corresponding Cu(P); an example of the comparison of the R_M values is shown in Fig. 2. This implies that the chelation of the nitrogen atoms in the porphyrin nucleus with a copper ion weakens the interaction between the porphyrin nucleus and the active sites (presumably silanol groups) on the surface of the silica gel.

In both series of H_2P and Cu(P) with *meso*-substituted porphyrin structures, the mobility tends to decrease in the order of porphyrin ligands TPP > POR > TMP > TPyP, which means that the adsorption increases in the reverse of this order of the porphyrins.

In the H_2TPP molecule, four phenyl groups are bonded with four *meso*-carbons in the porphyrin nucleus, with rotation out of the porphyrin plane by more than 60° [15]. Such a steric configuration of

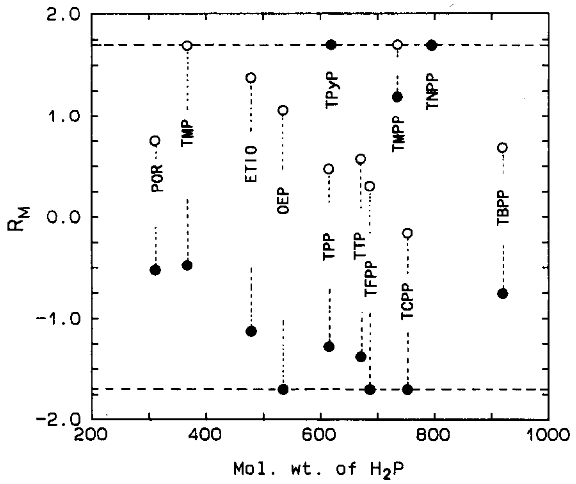


Fig. 2. R_M values of (○) H_2P and (●) Cu(P) on a silica gel plate with trichloroethylene. The broken line indicates R_M = −1.7 or 1.7.

TABLE II

R_F × 100 VALUES OF Cu(P)

Plate	Solvent	R_F × 100											
		POR	TMP	TPP	TTP	TMPP	TFPP	TCPP	TNPP	TBPP	TPyP	ETIO	OEP
Silica gel	Cyclohexane	4	0	0	0	0	0	0	0	0	0	1	2
	Carbon tetrachloride	37	18	44	42	0	54	77	0	18	0	25	50
	m-Xylene	93	88	SF[a]	SF	32	SF	SF	24	SF	0	SF	SF
	Toluene	–	87	SF	SF	–	–	SF	–	–	0	SF	SF
	Trichloroethylene	77	75	95	96	6	SF	SF	2	85	0	93	SF
Cellulose	Hexane	35	13	24	22	0	6	4	0	0	0	18	32
	Cyclohexane	47	19	44	43	3	9	12	2	0	0	21	41
	Methylcyclohexane	–	27	60	60	–	–	24	–	–	0	26	54
	Carbon tetrachloride	SF	95	SF	SF	80	82	94	11	87	0	97	SF
NH_2-silica	Cyclohexane	4	0	4	4	0	3	5	0	0	0	4	7
	Carbon tetrachloride	43	53	76	82	16	77	88	0	49	0	76	88
	m-Xylene	82	84	98	SF	64	SF	SF	32	95	0	SF	SF
	Trichloroethylene	80	88	SF	SF	64	SF	SF	26	SF	0	SF	SF
	Carbon disulphide	52	54	80	84	8	69	91	0	48	0	70	79
ODS	Methanol	17	5	5	4	7	10	4	34	0	0	2	3
	Ethanol	32	10	28	21	31	43	20	70	15	0	12	20
	1-Propanol	36	16	47	44	54	63	51	85	36	0	26	41
	1-Butanol	45	25	67	64	75	81	70	SF	58	0	42	60
	Acetone	79	59	72	64	75	81	70	SF	58	0	42	60
	Acetonitrile	19	7	3	0	5	6	0	25	0	0	4	4
	Ethyl acetate	89	74	SF	98	SF	SF	SF	SF	SF	0	85	91
	DMF	91	77	73	55	84	77	68	88	53	87	50	40

[a] SF = Moved with the solvent front.

phenyl groups presumably increases the distance separating the porphyrin plane from the silica gel surface. The bulky phenyl moieties weaken the adsorption of H_2TPP and Cu(TPP) compared with H_2POR and Cu(POR), respectively. With TMP, the methyl groups bonded to the porphyrin nucleus are relatively compact so that the groups interfere little with the interaction between the porphyrin nucleus and the silica gel surface. The enhancement of charge density localization in the porphyrin nucleus due to the bonding of electron-donating methyl groups with the porphyrin nucleus is regarded as one of the factors contributing to the stronger adsorption of H_2TMP than H_2POR and also Cu (TMP) than Cu(POR). H_2TPyP and Cu(TPyP) were adsorbed so strongly that they hardly migrated from the origin with a variety of solvents. Strong interaction between pyridyl group(s) [basic nitrogen atom(s) in particular] and acidic silanol group(s) on the surface of silica gel is suggested as the reason.

In the series of *meso*-tetrakis(*para*-substituted-phenyl)porphines and also of their copper complexes, the mobility tends to decrease in the order of porphyrin ligands TCPP > TFPP > TTP $\geqslant$ TTP > TBPP > TMPP > TNPP, although some irregularities are found with different solvents. A few examples of the plots of R_M *versus* the Hammett constant σ_p of the substitutent group [16] bonded to the *para*-position of the phenyl moiety of such a porphyrin are shown in Fig. 3.

Fig. 3 shows that the adsorption of both H_2P and Cu(P) tends to be weakened, approximately in proportion to the increase in the electron-withdrawing ability of the substituent group, except for NO_2, bonded with the phenyl moieties of the TPP structure. It is concluded that the substituent group affects indirectly the electron density and its localization on the porphyrin nucleus interacting with the active site on the surface of silica gel. The nitro group is the most polar of the substituents of pres-

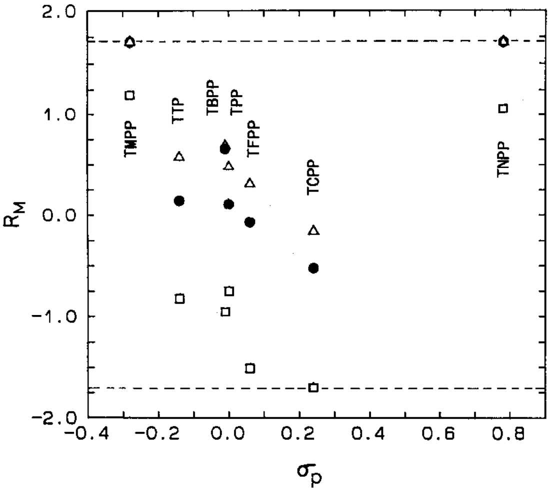


Fig. 3. Plots of the R_M of (■ and △) *para*-substituted tetraphenylporphines and (●) the copper complex *versus* the Hammett constant σ_p of the substituent. HPTLC plate: silica gel. Solvents: □ = *m*-xylene; △ = trichloroethylene; ● = carbon tetrachloride. The broken line indicates R_M = −1.7 or 1.7.

ent interest, and electron resonance occurs between the nitro and phenyl groups. The strong adsorption of H_2TNPP and Cu(TNPP) on silica gel is presumably attributable to the direct interaction of the nitro group with the active sites on the surface of silica gel in addition to the interaction between the porphyrin nucleus and silica gel.

In the series of alkylated porphyrins, such as H_2TMP, H_2ETIO and H_2OEP, and also of their copper complexes, the mobility tends to increase with increase in the bulk of the alkyl moieties of the porphyrin molecule.

Cellulose plate

The mobilities of both H_2P and Cu(P) on a cellulose plate were larger, in most instances, than those on both silica gel and NH_2-silica plates with an identical solvent. H_2TPyP and Cu(TPyP) were so strongly adsorbed on cellulose that they did not leave the origin with any of the solvents tested. The mobility of a H_2P was smaller than that of Cu(P) with an identical solvent.

NH_2-silica plate

The chromatographic migration characteristics of H_2P and Cu(P) on an NH_2-silica plate are similar to those found on both silica gel and cellulose in the following respects: (1) the mobility of H_2P is smaller than that of Cu(P) with an identical solvent; (2) the mobilities of H_2TNPP and H_2TMPP are smaller than those of other *para*-substituted tetraphenylporphines, and analogous mobility trends are found with their copper complexes; (3) both H_2TPyP and Cu(TPyP) are adsorbed so strongly that they hardly move with any of the solvents tested; (4) the mobility of alkylated porphyrins increases with increasing the bulk of the alkyl substituent group, *i.e.*, TMP < ETIO < OEP. Fig. 4 shows typical mobility trends for H_2P and Cu(P) on an NH_2-silica plate.

The mobility of H_2P on an NH_2-silica plate was between those on silica gel and cellulose plates with an identical solvent, and similar mobility trends were found also for Cu(P).

ODS plate

The mobility of H_2P was larger than that of the corresponding Cu(P) with an identical solvent. TPP analogues substituted with polar groups, such as methoxy [H_2TMPP and Cn(TMPP)] and nitro [H_2TNPP and Cu(TNPP)], showed larger mobilities than the parent compounds [H_2TPP and Cu (TPP)]. These migration trends are the reverse of those found on polar adsorbents, such as silica gel, cellulose and NH_2-silica.

The migration sequences of the different H_2Ps

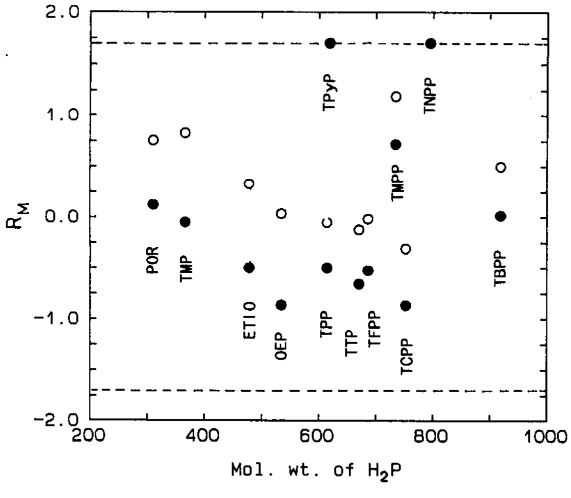


Fig. 4. R_M values of (○) H_2P and (●) Cu(P) on an NH_2-silica plate with carbon tetrachloride. The broken line indicates R_M = −1.7 or 1.7.

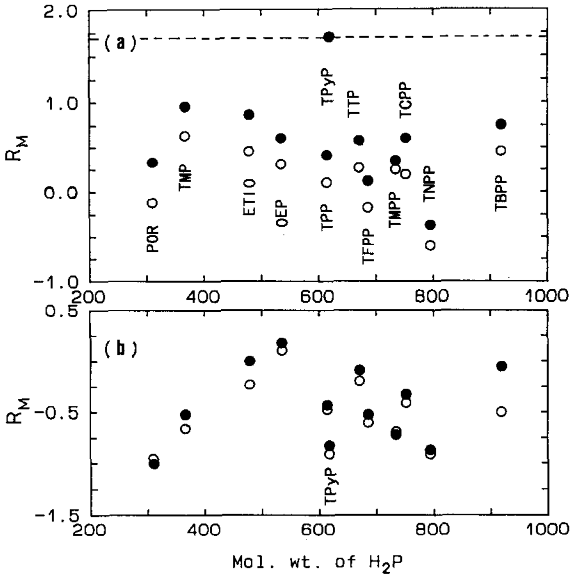


Fig. 5. R_M values of (○) H_2P and (●) Cu(P) on an ODS plate with (a) ethanol and (b) DMF. The broken line indicates R_M = 1.7.

and also of the Cu(P)s depended on the solvent used. The mobility trends for porphyrins observed with ethanol and dimethylformamide (DMF) are compared in Fig. 5. It is noted that both H_2TPyP and Cu(TPyP) do not leave the origin with various polar solvents, whereas they can migrate close to R_F = 0.9 with DMF.

The dependence of the mobility trend of alkylated porphyrins on the solvent is complicated. The increasing orders of mobility are as follows: $H_2ETIO \approx H_2TMP \approx H_2OEP$ in methanol; $H_2TMP < H_2ETIO < H_2OEP$ in alcohols, such as ethanol, 1-propanol and 1-butanol; $H_2ETIO \approx H_2OEP < H_2TMP$ in aprotic solvents such as acetone, ethyl acetate and acetonitrile; and $H_2OEP < H_2ETIO < H_2TMP$ in DMF. Further, the mobility order Cu(TMP) < Cu(ETIO) < Cu(OEP) is found with acetone, ethyl acetate and alcohols other than methanol, whereas the reverse order occurs with DMF. Solvent effects on the dimerization or aggregation of alkylated porphyrins, the solvation of the alkyl moiety of ODS and the interaction with unalkylated sites on the surface of ODS are possible reasons, but not clear evidence sufficient for an explanation has yet been obtained.

CONCLUSIONS

It has been confirmed that the mobilities of H_2Ps and Cu(P)s depend considerably on the thin-layer adsorbent and on the developing solvent used. Each porphyrin always has a smaller mobility than its copper complex on the HPTLC plates coated with polar adsorbents, such as silica gel, cellulose and NH_2-silica, whereas the reverse tendency occurs on an ODS plate. Both H_2TPyP and Cu(TPyP) show small mobilities on all the HPTLC plates tested with different solvents, whereas they show specifically large mobilities with the combination of an ODS plate and DMF.

The difference in mobilities between H_2Ps and Cu(P)s on a silica gel plate is greater than those observed on other plates. Accordingly, silica gel is recommended as an effective adsorbent for the HPTLC separation of a Cu(P) from the corresponding H_2P. For the separation of porphyrins or their copper complexes, the most effective combination of the adsorbent and solvent should be chosen according to the compounds to be separated. The migration behaviour of alkylated porphyrins on an ODS plate in particular depends considerably on the solvent used.

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