Thin-Layer Chromatographic Mobility of Aryl-Substituted Porphyrins and Their Metalloporphyrins

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Abstract

The migration behavior of aryl-substituted porphyrins and their metalloporphyrins with Zn(II), Ni(II), and Cu(II) were studied in high-performance thin-layer chromatography systems with silica gel and RP_{18} plates using different organic mobile phases having different polarities. The (bromohydroxy)phenyl, (phenoxy)phenyl, and oxy(acetic acid)phenyl porphyrins migrated at similar rate. Chloromethylphenylporphyrin was weakly adsorbed on silica gel using either polar or nonpolar mobile phases. The mobility of metal complexes generally tends to increase in the given order of central metal ions: Zn(II) < Ni(II) < Cu(II) on silica gel. The favorable mobile phase for the separation of ligands and the metalloporphyrins is a mixture of acetonitrile–benzene.

Introduction

Porphyrin is a group of chemical analogues possessing a macrocycle, which consists of four pyrrole rings linked by methines, giving rise to the characteristic bands (Soret band) with intense absorption in the visible region (400–500 nm) (1). The Soret band is regarded as the band of choice for the spectrophotometric determination of metalloporphyrins (2). Many porphyrins, including lipophilic and water-soluble types, have been synthesized and used for the spectrophotometric determination of metals (3–5). However, Soret bands of porphyrins and their metal chelates overlap so closely that the determination of a certain metal ion often suffers serious interference from other metals. By chromatographic techniques such as high-performance liquid chromatogrpahy (HPLC) and high-performance thin-layer chromatography (HPTLC), the quantitative separation and determination of metals and porphyrins has been solved.

Chromatography has been effectively used for the purification and separation of synthetic porphyrin compounds and their metal complexes. The migration of porphyrin derivatives in TLC depends on the nature of their substituents. Modification of the porphyrin system with small substituent groups leads to a substantial difference in compound polarity. Hence, the chromatographic behavior of large molecules is not predicted easily. Separation of many porphyrins and metalloporphyrins by TLC (6–10) and HPLC (11–14) has been described in the literature.

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Up to now, the mobility of porphyrins, such as the crown ethers, tetrahydroxyl and tetraalkyloxy derivatives of tetraphenylporphyrin and tetra-(p-tolyl)porphyrin have been investigated by TLC (6,15–18). Similarly, a few metalloporphyrins have been studied (9,10,16,18).

In our laboratory, the chromatographic behavior of previously uncharacterized or newly prepared tetrahydroxyphenylporphyrin and tetraphenylporphyrin derivatives and their complexes with Ni(II), Cu(II), and Zn(II) were investigated. The structural formulas of the studied porphyrin compounds are given in Figure 1. These compounds have bromohydroxy, chloromethyl (19), phenoxy, or oxy(acetic acid) (20,21) as substituents in the tetrahydroxyphenylporphyrin or tetraphenylporphyrin. Silica gel and reversed-phase plates were used as stationary phase; development was performed with the binary mobile phases having different compositions.

Experimental

Instrumentation

^{1}H NMR spectra were recorded on Bruker instruments (Billerica, MA) operating at 400 MHz using tetramethylsilane as an internal standard. FTIR spectra (4000–700 cm) in KBr were recorded with a PerkinElmer Spectrum (Ramsey, MN) BX Fourier Transform IR spectrometer. The elemental analysis was determined using a Fisons Carlo Erba EA-1108 elemental analyzer.

Chemicals

Unless specified otherwise, starting materials and solvents were reagent-grade (Aldrich, Seelze, Germany) and used as received.

Synthesis of porphyrin derivatives

5,10,15,20-tetra-4-oxy(acetic acid)phenylporphyrin (21)

An amount of 0.050 g tetrahydroxyphenylporphyrin was mixed with 0.042 g chloroacetic acid and 0.156 g sodium carbonate and 150 mL of ethanol in a round-bottom flask and refluxed for 12 h. The reaction mixture was left to cool, filtered off, and the ethanol evaporated. Then, 10 mL of distilled water was added to the final product, and the pH of this mixture was adjusted with 0.1 mol/L HCl. The mixture was filtered and finally dried under vacuum. The yield of TAPP was 80%. ^{1}H NMR

(DMSO, 400 MHz) δ (ppm): 10.25 ppm (s, 4H), 7.23 ppm (s, 2H), 7.01–6.38 ppm (m, 24H). FTIR (KBr): 3466 cm^{-1} (C-OH), 1755 cm^{-1} (C=O). Elemental analysis, expected (calculated): C-63.55 (63.75), H-4.10 (4.29), N-5.66 (5.72)

5,10,15,20-tetra-(3-bromo-4-hydroxyphenyl) porphyrin

An amount of 0.170 g tetrahydroxyphenylporphyrin was mixed with 1.2 mL concentrated H_2SO_4 and 150 mL of tetrahydrofuran (THF) in a round-bottomed flask and refluxed for 3 h at 70°C. The reaction mixture was left to cool, and 5 mL of 0.6 M NaOH solution and 250 µL of bromine were added drop by drop. The mixture was refluxed for 1 h at 70°C and after left to cool; 5 mL of 1.0 M H_2SO_4 was also added and mixed for 30 min. The reaction mixture was extracted with diethyl ether, and the THF in the upper phase was evaporated by rotary evaporator. After 10 mL of distilled water was added, the formed residue was filtered off and finally dried under vacuum. The yield of TBHPP was 75%. ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 9.88 ppm (s, 4H), 7.55 ppm (s, 2H), 7.05–6.70 ppm (m, 20H). FTIR (KBr): 3125 cm^{-1} (C-OH), 897 cm^{-1} (C-Br). Elemental analysis, expected (calculated): C-49.55 (49.67), H-2.76 (2.82), N-5.01 (5.26).

5,10,15,20-tetra-(4-phenoxyphenyl) porphyrin

An amount of 0.2 g tetrahydroxyphenylporphyrin was stirred in 15 mL dimethylformamide with 0.120 g of crushed sodium hydroxide. 0.4 g of chlorobenzene was added over a period of an hour from a dropping funnel. After 30 h, 10 mL ethanol was added to the solution, followed by 80 mL of distilled water. The product was filtered off and washed with absolute ethanol and then dried. It was chromatographed on alumina with chloroform. The TPPP moves with the solvent front and separates easily from any unreacted starting material and from two bands near the top of the column. The yield of TPPP was 82%. ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.30 ppm (s, 2H), 8.28–7.77 ppm (m, 44H). FTIR (KBr): 2100 cm^{-1} (Ar-O-Ar). Elemental analysis, expected (calculated): C-77.49 (77.86), H-4.65 (4.28), N-5.16 (5.82)

5,10,15,20-tetra-p-chloromethylphenyl porphyrin

An amount of 0.123 g tetraphenylporphyrin was mixed with 0.002 g *p*-formaldehyde, 0.027 g anhydrous $ZnCl_2$ powder, and 150 mL of THF in a round-bottomed flask and refluxed for 30 min at 75°C. Ten milliliters of 1.0 M HCl was added to the reaction mixture drop by drop and refluxed for more than 15 min. Then the reaction mixture were transferred to a separatory funnel and washed with 2.0–3.0 mL of cold distilled water, 2.0–3.0 mL of saturated Na_2CO_3 solution, and 2.0–3.0 mL of distilled water. The mixture was filtered off, the solvent was evaporated, and finally the product was dried under vacuum. The yield of CMPP was 71%. ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.28 ppm (s, 2H), 8.05–7.54 ppm (m, 34H). FTIR (KBr): 2920 cm^{-1} (Ar-CH_2-Cl), 895 cm^{-1} (C-Cl). Elemental analysis, expected (calculated): C-65.52 (65.97), H-4.20 (4.54), N-5.66 (5.26).

Preparation of metal complexes

One millimole of the porphyrin was dissolved in 50 mL of tetrahydrofuran. The metal nitrate salt as 10 mmol was added to porphyrin solution, and then the last mixture was refluxed at 70–80°C for 2 h. After evaporation of the solvent, the solid was washed with distilled water. The resulting colored solid was filtered by use of filter paper. For further purification, the mixture was chromatographed on alumina column with THF–C_2H_5OH (9:1, v/v), and the yield was 62–71%.

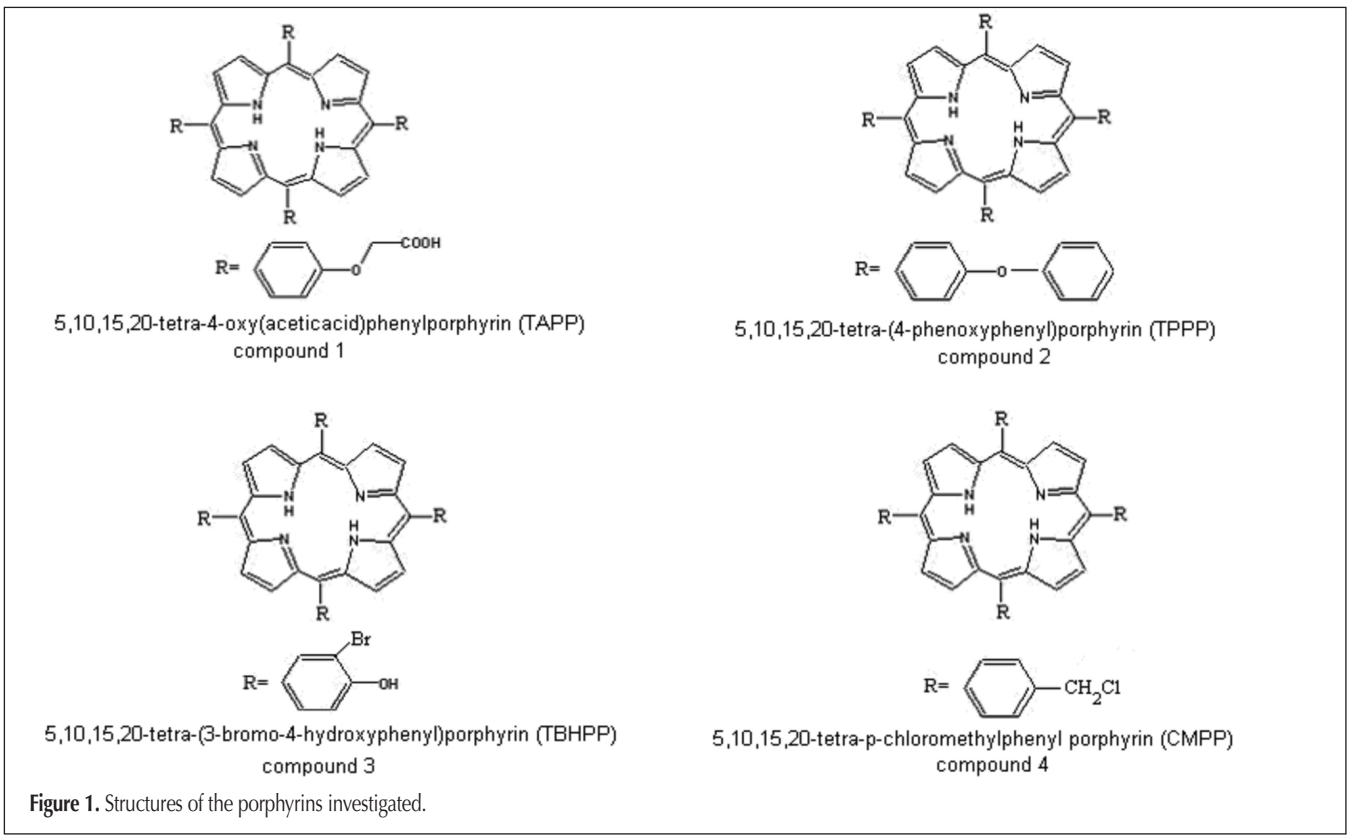

Figure 1. Structures of the porphyrins investigated.

HPTLC

HPTLC was performed on commercially available precoated silica gel $60F_{254}$ and $RP_{18}F_{254}$ glass plates from Merck (Darmstadt, Germany). RP_{18} plates were used without preliminary treatment. Before use, silica gel plates were activated at 110°C for 30 min and then cooled in a desiccator. Standard solutions of the TAPP, TPPP, CMPP, and their metal complexes in chloroform, and TBHPP and its metal complexes in ethanol, were prepared at 1 mg/mL and 1 µL volumes. Using a Linomat V sample applicator they were spotted on the plates. Chromatograms were developed to a distance of 5 cm with binary mobile phases at room temperature (22–25°C) in a 10 × 10 cm horizontal Camag TLC tank. After development the plates were dried at room temperature. Each experiment was repeated three times. The migration of the test compounds was measured with a Camag TLC Scanner 3 at 420–424 nm on the silica gel plate.

Results and Discussion

Solvent dependence of the mobility of porphyrin derivatives

Structures of the investigated porphyrin derivatives having different organic functional groups, such as (bromohydroxy)phenyl, phenoxy, oxy(acetic acid)phenyl, and (chloromethyl)phenyl are given in Figure 1. They were chromatographed on silica gel and RP_{18} stationary phases and several binary mobile phases. On the high-performance thin-layer plates, every porphyrin derivative gave a compact spot without tailing. The rate of migration of investigated compounds fundamentally depends on the substituent groups attached to phenyls in tetrahydroxy–phenylporphyrin.

Polar interactions like hydrogen-bonding take place between the hydroxyl groups of the silica and the oxygen or halogen in the substituent groups and also between the oxygen atoms of the stationary phase and the "inner" hydrogen atoms of the porphyrin ring. However, the second interaction should be weakened by the "projecting" phenyl groups, which separate the porphyrin plane from the sorbent surface (15).

Analysis of Table I–II reveals that compound 4, containing chloromethylphenyl, is less adsorbed on silica gel plate and show higher mobility in both polar and non polar mobile phases. The greater hydrophobicity of this compound leads to less hydrogen-bonding interaction between the compound and the adsorbent and also to its greater solubility in the mobile phases. The migration rates of compounds 1–3 are similar and have moderate mobilities in polar mobile phases. These compounds are strongly adsorbed on silica gel for non-polar mobile phases because they have one or more oxygen atoms in their substituent groups. However, the interaction is weakened by using polar mobile phases. Conversely, compound 4 is retained longer on RP_{18} stationary phase as shown in Table III. The mobilities of the others, phenoxyphenyl, bromohydroxyphenyl, and oxy(acetic acid) phenyl derivatives, are higher and similar because of their higher solubilities in the polar mobile phases.

Table I. The hR_F Values of Tetrahydroxyphenylprophyrin Derivatives on Silica Gel on Binary Solvents*

Developer	TAPP*	TPPP†	TBHPP‡	CMPP§
Acetonitrile–chloroform (2:8)	24 ± 2	28 ± 3	28 ± 2	96 ± 1
Acetonitrile–chloroform (3:7)	45 ± 2	50 ± 1	46 ± 2	91 ± 2
Acetonitrile–chloroform (4:6)	76 ± 1	81 ± 2	78 ± 3	96 ± 1
Methanol–chloroform (1:9)	37 ± 2	39 ± 1	42 ± 3	98 ± 2
Methanol–chloroform (2:8)	62 ± 2	64 ± 3	68 ± 2	86 ± 3
Methanol–chloroform (3:7)	84 ± 2	85 ± 2	90 ± 3	91 ± 2
Methanol–tetrahydrofuran (1:9)	84 ± 3	84 ± 2	88 ± 3	79 ± 2
Acetonitrile–benzene (3:7)	55 ± 2	52 ± 1	58 ± 3	90 ± 1
Carbon tetrachloride-chloroform (9:1)	0	0	0	56 ± 3

* TAPP = 5,10,15,20-tetra-4-oxy(acetic acid)phenylporphyrin
† TBHPP = 5,10,15,20-tetra-(3-bromo-4-hydroxyphenyl) porphyrin
‡ TPPP = 5,10,15,20-tetra-(4-phenoxyphenyl) porphyrin
§ CMPP = 5,10,15,20-tetra-p-chloromethylphenyl porphyrin

Table II. The hR_F Values of Tetrahydroxyphenylprophyrin Derivatives on Silica Gel as a Function of the Composition of the Acetone–Chloroform Binary Mobile Phases

Developer	TAPP	TPPP	TBHPP	CMPP
Acetone–chloroform (0:10)	9 ± 1	9 ± 1	9 ± 1	97 ± 1
Acetone–chloroform (1:9)	10 ± 3	9 ± 4	18 ± 2	91 ± 1
Acetone–chloroform (2:8)	27 ± 1	26 ± 4	25 ± 3	89 ± 5
Acetone–chloroform (3:7)	44 ± 3	45 ± 2	52 ± 3	93 ± 1
Acetone–chloroform (4:6)	55 ± 4	57 ± 1	53 ± 1	94 ± 1
Acetone–chloroform (1:1)	61 ± 2	63 ± 1	62 ± 3	82 ± 2
Acetone–chloroform (6:4)	74 ± 2	75 ± 1	75 ± 3	90 ± 1
Acetone–chloroform (7:3)	82 ± 1	81 ± 3	85 ± 4	93 ± 2
Acetone–chloroform (8:2)	87 ± 2	88 ± 1	85 ± 2	95 ± 1
Acetone–chloroform (9:1)	96 ± 1	95 ± 3	94 ± 2	95 ± 2
Acetone–chloroform (10:0)	97 ± 1	98 ± 1	98 ± 1	97 ± 1

Table III. The hR_F Values of Tetraphenylprophyrin Derivatives on RP18 on Single and Binary Solvents

Developer	TAPP	TPPP	TBHPP	CMPP
Methanol–THF (5:95)	93 ± 4	97 ± 3	92 ± 1	85 ± 1
Acetone	95 ± 3	94 ± 2	86 ± 3	72 ± 2
Methanol–acetone (20:80)	92 ± 3	91 ± 1	84 ± 1	63 ± 1

Table IV. The hR_F Values of Zn(II), Ni(II), and Cu(II)-porphyrins (TPPP, TBHPP, TAPP, CMPP) on Silica Gel with Binary Solvents

	hR_F		
	Ni(II)	Zn(II)	Cu(II)
Mobile phase: Acetone–chloroform (2:8)			
TPPP	27 ± 1	27 ± 0	26 ± 0
TBHPP	25 ± 3	16 ± 3	83 ± 2
TAPP	24 ± 3	23 ± 2	26 ± 3
Mobile phase: Acetonitrile–benzene (3:7)			
TPPP	76 ± 1	65 ± 2	81 ± 1
TBHPP	78 ± 2	50 ± 2	72 ± 3
TAPP	65 ± 1	73 ± 3	74 ± 1
Mobile phase: Carbon tetrachloride–chloroform (9:1)			
CMPP	54 ± 3	57 ± 3	86 ± 1

Solvent dependence on mobility of metal-porphyrin complex

The chromatographic behaviors of metal complexes of compounds 1–4 were also investigated and the hR_F ($R_F \times 100$) values of these metal complexes were summarized in Table IV for the best binary mobile phases on silica gel. In adsorption chromatography on silica gel, the optimum separations of TPPP, TAPP, and TBHPP and their Zn(II), Ni(II), and Cu(II) complexes were obtained with acetonitrile–benzene, 3 + 7 (v/v) and acetone–chloroform, 2 + 8 (v/v) as mobile phases. Carbon tetrachloride–chloroform, 9 + 1 (v/v) was found to be the best mobile phase for the separation of CMPP and its metal complexes. The metal complexes showed generally higher hR_F values than their ligands, indicating that the polarity of these metal complexes is lower. The reason for the higher mobilities of the metalloporphyrins could be a decrease in dipole moment and also disappearance of polar NH bond. They were separated from the ligand and partially from each other in acetonitrile–benzene (3 + 7). In carbon tetrachloride–chlorofirm (9:1), Ni(II) complexes of the studied porphyrins were not separated from their ligands. The complexes of Cu(II) has the highest hR_F value. However, the Zn(II)-TBHPP had lower mobility than its porphyrin in both mobile phases. This can be explained by its lower electrostatic field strength than the other studied ions as mentioned by Kobayashi and coworkers before (18). When the positive charge of metal cation is more dispersed over the π-electron system of the porphyrin derivatives and then the metal-ligand bond is more delocalized, the dipole-dipole interaction between the metal-porphyrin complex and polar stationary phase is reduced, and so increased mobility may be observed (18). Unfortunately, ın partition chromatography, the prepared compounds and their metal complexes did not migrated separately on either RP plates at different combination of chloroform–methanol, acetone–methanol, and tetrahydrofuran–methanol.

Conclusion

The synthesized aryl-substituted porphyrin derivatives could be used in quantitative analysis of zinc, nickel, and copper in various analytical matrices using adsorption TLC.

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References

1. J.I. Itoh, J. Liu, and M. Komata. Novel analytical applications of porphyrin to HPLC post-column flow injection system for determination of the lanthanides. *Talanta* **69:** 61–67 (2006).
2. Z. Shi, and C. Fu. Porphyrins as ligands for trace metal analysis by high-performance liquid chromatography. *Talanta* **44:** 593–604 (1997).
3. M. Tabata, J. Nishimoto, and T. Kusano. Spectrophotometric determination of lithium ion using a water-soluble octabromoporphyrin in aqueous solution. *Talanta* **46:** 703–708 (1998).
4. K.Kilian and K. Pyrzynska. Spectrophotometric study of Cd(II), Pb(II), Hg(II) and Zn(II) complexes with 5,10,15,20-tetrakis(4-carboxylphenyl)porphyrin. *Talanta* **60:** 669–678 (2003).
5. R. Giovannetti, V. Bartocci, F. Pucciarelli, and M. Ricciutelli. Reactions of anionic porphyrin with group 11 elements: a spectrophotometric and electrospray ionization mass spectrometry study. *Talanta* **63:** 857–864 (2004).
6. M. Podgorna and P. Kus. Separation of tetraphenylporphyrin derivatives by adsorption and partition thin-layer chromatography. *J. Planar Chromatogr.* **13:** 166–170 (2000).
7. C.K. Lai, C.W. Lam, and Y.W. Chan. High-Performance Thin-Layer Chromatography Of Free Porphyrins For Diagnosis Of Porphyria. *Clin. Chem.* **40:** 2026–2029 (1994).
8. K. Saitoh, R. Kashiwa, R. Tada, C. Kiyohara, and N. Suzuki. High-Performance Thin-Layer Chromatographic Mobility Of Porphyrins And Their Copper-Complexes On Different Adsorbents. *J. Chromatogr.* **609:** 341–347 (1992).
9. M. Podgorna, J. Dziegielewski. Effect of the structure of selected metalloporphyrins on their chromatographic properties. *J. Planar Chromatogr.* **19:** 48–51 (2006).
10. M. Stefaniak. Lipophilic and physicochemical properties of metalloporphyrins separated by RP-TLC. *J. Planar Chromatogr.* **20:** 361–364 (2007).
11. Q. Hu, G. Yang, J. Yin, and Y. Yao. Determination of trace lead, cadmium and mercury by on-line column enrichment followed by RP-HPLC as metal-tetra-(4-bromophenyl)-porphyrin chelates. *Talanta* **57:** 751–756 (2002).
12. S. Igarashi, N. Ide, and Y. Takagai. High-performance liquid chromatographic-spectrophotometric determination of copper(II) and palladium(II) with 5,10,15,20-tetrakis(4N-pyridyl)porphine following homogeneous liquid-liquid extraction in the water-acetic acid-chloroform ternary solvent system. *Anal. Chim. Acta* **424:** 263–269 (2000).
13. J.F. Zhang, H. Wang, A.X. Hou, C.F. Wang, and H.S. Zhang. Behavior of new complexes of tetrakis(4-methoxylphenyl)porphyrin with heavy rare earth elements in reversed-phase high performance liquid chromatography. *J. Sep. Sci.* **27:** 1037–1041 (2004).
14. J.I. Itoh, J. Liu, and M. Komata. Novel analytical applications of porphyrin to HPLC post-column flow injection system for determination of the lanthanides. *Talanta* **69:** 61–67 (2006).
15. P. Kus. Thin-layer chromatographic mobility of crown ether derivatives of meso-tetraphenylporphyrin. *J. Planar Chromatogr.* **9:** 435–438 (1996).
16. M. Podgorna. Separation of porphyrins and metalloporphyrins by TLC. *Acta Chromatogr.* **12:** 226–233 (2002).
17. P. Kus. Thin-layer chromatographic mobility of some tetraphenylporphyrin derivatives. *J. Planar Chromatogr.* **15:** 383–386 (2002).
18. M. Kobayashi, K. Saitoh, and N. Suzuki. High-Performance Thin-Layer Chromatography Of Metal-Complexes Of Meso-Tetrakis(Para-Tolyl)Porphyrin On Cellulose And Silica-Gel. *Chromatographia* **18:** 441–444 (1984).
19. H.G.Liu, D.J. Qian, X.S. Feng, Q.B. Xue, and K.Z. Yang. Monolayer studies on an acetic acid substituted porphyrin and its complexes with aliphatic amines or quaternary ammonium salts at the air-liquid interface. *Langmuir* **16:** 5079–5085 (2000).
20. R.H. Jin, K. Motoyoshi, and J. Porphyr. Porphyrin-centered water-soluble star-shaped polymers: Poly(N-acetylethylenimine) and poly(ethylenimine) arms. *Phthalocya.* **3:** 60–64 (1999).
21. S. Seyhan, M. Merdivan, N. Demirel, and H. Hosgoren. Polymer supported 5,10,15,20-tetrakis(phenoxy acetic acid)porphyrin derivative for separation and preconcentration of d- and f-electron metals. *Microchim. Acta* **161:** 87–93 (2008).

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