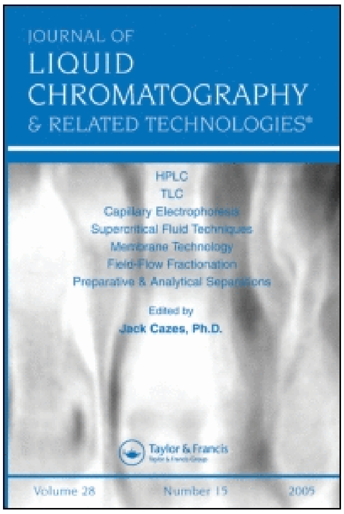

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Influence of Stationary Phase Selectivity on the HPLC Separation of Porphyrins

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Abstract: The chromatographic conditions for the resolution and determination of {5-[4-nitro-3-(toluene-4-sulphonylmethyl)phenyl]-10,15,20-triphenylporphyrinato} zinc(II) and *N,N*-dimethyl-*C*-{[2-nitro-5-(10,15,20-triphenylporphyrinato-5-yl) phenyl]methane-sulphonamide}zinc(II) by HPLC are presented. These compounds have potential use in the treatment of cancer in the form of photodynamic therapy. Several stationary phases were taken into consideration, such as chemically bonded octadecyl (RP SG-C18), octyl (RP SG-C8), and an aryl stationary phase (RP SG-NAF). The composition and type of mobile phase was also examined. The highest selectivity was observed when using the RP SG-C18 stationary phase [usually regarded as a standard (reference) phase] with methanol/water as the mobile phase. The highest separation factor was seen using the aryl stationary phase SG-NAF for an acetonitrile/water mobile phase.

Keywords: Stationary phases: aryl, octyl, and octadecyl, {5-[4-Nitro-3-(toluene-4-sulphonylmethyl)phenyl]-10,15,20-triphenylporphyrinato}zinc(II), *N,N*-Dimethyl-*C*-{[2-nitro-5-(10,15, 20-triphenylporphyrinato-5-yl)phenyl]methanesulphonamide} zinc(II), $\pi-\pi$ Interactions, Chromatography

INTRODUCTION

Continual progress has taken place in one of the most commonly used techniques in analytical chemistry, high performance liquid chromatography (HPLC). Recent advancements include the introduction of various methodologies in micro- and macroscale, in aqueous and anhydrous systems, as well as their modifications coupled with other techniques, e.g., with

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electromigration technique. However, the column and its packing is always a central component of the system, because the column is where the thermodynamic processes of resolution occur. Packing materials with chemically bonded phases are the most common. Popular stationary phases include hydrophobic adsorbents containing alkyl chains of different length (e.g., C_2, C_8, C_{18}), as well as phases of diverse polarity (alkyl chains terminated with various functional groups, e.g., $-OH$, $-CN$) are used. However, the use of these materials is not effective sometimes, especially when attempting to resolve some polar compounds. Band broadening and peak tailing can be observed in some cases.[1–4]

In this paper, we attempted to find the application of acetonitrile/water mobile phase with a new generation propyl-naphthalene stationary phase for the separation of two newly synthesized porphyrins. These moieties belong to a very important class of aromatic macrocycles, containing a total of 22 conjugated π-electrons (18 of which are incorporated into a delocalisation pathway). They also bear four nitrogen atoms in the core ring of the symmetrically arranged molecule.

The [18π]-electron porphyrin aromatic system is present in many well-known biological materials (e.g., chlorophyll, heme, vitamin B_{12}, etc.).[5] The first laboratory syntheses of naturally occurring porphyrins, including monitoring of the reactions, separation, and purification of the products, were reported by Woodward in the 1960's.[6] Many porphyrin derivatives are widely used as photosensitizers in photodynamic therapy (PDT)[7,8] for cancer treatment. These compounds are also used as molecular-based multi-bit memory storage,[9] electron-donor parts in artificial photosynthetic models,[10] and are known to serve as useful synthetic precursors to mono-oxygenase and allosteric enzyme model systems.[11,12] Some "synthetic" porphyrins were used in preparation of silica gel–porphyrin connected stationary phases in chromatography, e.g., Martin et al., applied such a system for separation of fullerenes.[13–15] The effect of π–π interactions between porphyrin and fullerene π-electrons are the key to effective separation herein.

The prevalence of porphyrins in nature, as well as their growing usefulness in scientific and medical applications, necessitated the development of analytical techniques for their identification and quantitation. Optimized and rapid chromatographic techniques were sought due to the typically low yields seen in their synthetic procedures, as well as the rapid photodegradation seen with these compounds.

EXPERIMENTAL

Materials and Methods

Newly synthesized porphyrin derivatives: {5-[4-nitro-3-(toluene-4-sulphonylmethyl)phenyl]-10,15,20-triphenylporphyrinato}zinc(II) (**1**) and

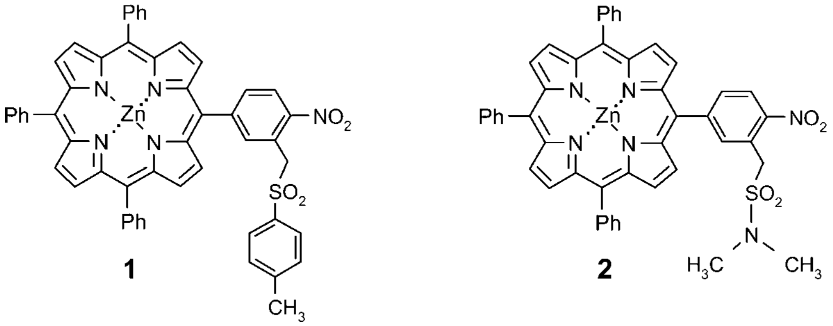

Figure 1. Structures of porphyrin derivatives: {5-[4-nitro-3-(toluene-4-sulphonylmethyl)phenyl]-10,15,20-triphenylporphyrinato}zinc(II) (**1**) and *N,N*-dimethyl-*C*-{[2-nitro-5-(10,15,20-triphenylporphyrinato-5-yl)phenyl]methanesulphonamide}zinc(II) (**2**).

N,N-dimethyl-*C*-{[2-nitro-5-(10,15, 20-triphenylporphyrinato-5-yl)phenyl] methanesulphonamide}zinc(II) (**2**) (Fig. 1) were dissolved in dichloromethane (HPLC purity, Fluka AG, Buchs, Switzerland), obtaining a concentration of *ca* 10 μg/mL in each sample. Because of their instability and spontaneous decomposition in solvents, the samples were analyzed by HPLC immediately after preparation (at wavelength 420 nm and a flow rate of 1 mL/min; temperature –20°C). Three different stationary phases were used in the investigation: octadecyl (S. Witko–J.T. Baker, Łódź, Poland), octyl (S. Witko–J.T. Baker, Łódź, Poland), and aryl (RP SG-NAF, Fig. 2a, home made,[3] Table 1). Stainless steel columns were used in the following dimensions: RP SG-C_{18}—250 mm × 4.6 mm, RP SG-C_8—125 mm × 4.6 mm, and RP SG-NAF—125 mm × 4.6 mm, respectively. Several mobile phase systems were also used: acetonitrile/water (65/35, 55/45, 40/60) and methanol/water (95/5, 85/15).

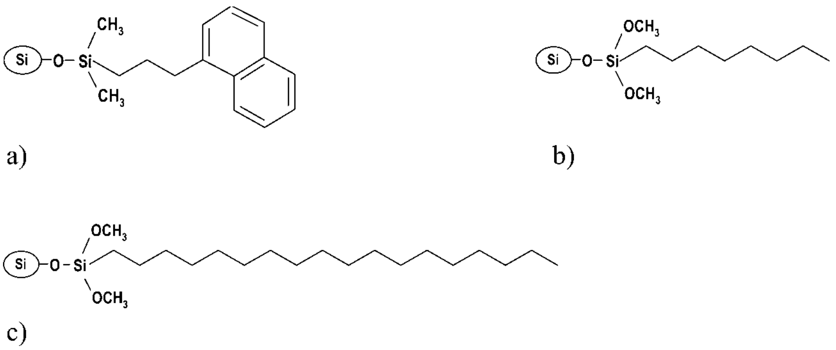

Figure 2. Scheme of chemically bonded stationary phases: a) aryl (SG-NAF) b) octyl (SG-C8), and c) octadecyl (SG-C_{18}).

Table 1. Characteristics of bonded phase

Type of packing	Column parameters (mm)	Manufacturer of column	Carbon content vol.%
SG-C18	250 × 4.6	S. Witko– J.T. Baker	18.09
SG-C8	125 × 4.6	Home made	13.49
SG-NAF	125 × 4.6	Home made	16.10

Porphyrin **2** was obtained according to the method described in earlier literature for analogous compounds[16]: M.p. $> 300°C$. ^1H NMR ($CDCl_3$): $\delta = 9.02$ (d, $J = 4.7\,Hz$, 2H, H^{β}-pyrrole), 8.97 (s, 4H, H^{β}-pyrrole), 8.91 (d, $J = 4.7\,Hz$, 2H, H^{β}-pyrrole), 8.50 (d, $J = 1.6\,Hz$, 1H, H-2 of $Ar(NO_2)$), 8.44 (part of AB, $J = 8.3\,Hz$, 1 H, H-5 of $Ar(NO_2)$), 8.39 (part of AB coupled with another proton, $J = 8.3, 1.6\,Hz$, 1H, H-6 of $Ar(NO_2)$), 8.27–8.18 (m, 6H, H-Ph), 7.82–7.71 (m, 9H, H-Ph), 5.00 (s, 2 H, CH_2), 2.93 (s, 6 H, $N(CH_3)_2$). ^{13}C NMR ($CDCl_3$): 37.5 ($N(CH_3)_2$), 51.7 (CH_2), 121.6, 123.8, 126.6, 127.6, 130.2, 130.9, 132.2, 132.4, 132.9, 134.4, 135.3, 139.9, 142.5, 148.7, 149.1, 150.1, 150.2, 150.3, 150.5, 154.5, 156.5. UV/Vis ($CHCl_3$): λ_{max} ($\lg\varepsilon$) = 588.0 (3.72), 548.0 (4.40), 511.5 (3.63), 419.5 nm (5.61, Soret). MS (ESI): m/z (% rel. int.) = 849 (14), 848 (27), 847 (60), 846 (67), 845 (83), 844 (80), 843 (100), 842 (34) [isotopic M^+ and $M + H$]. HR-MS (ESI) calcd. for $C_{47}H_{35}N_6O_4SZn$ ($M + H$) −843.1732, found −843.1756.

Data for porphyrin **1**—see literature [16b].

Equipment

Chromatographic measurements were performed on a liquid chromatograph (model SPD-6A, Shimadzu, Kyoto, Japan), equipped with a gradient pump (Shimadzu, model LC-6A, Kyoto, Japan), a UV-Vis detector, a sampling valve (Rheodyne, model 7125; Berkeley, CA, USA), with a 20 μL sample loop, and a Shimadzu model C-R6A data recorder.

RESULTS AND DISCUSSION

The selective derivatization of *meso*-tetraarylporphyrins, leading to highly substituted derivatives, is one of the most important problems in the chemistry of synthetic porphyrins. From this process, the hydrophobic moieties can be transformed into hydrophilic compounds (sought in PDT). However, attempts to prepare porphyrins possessing a high degree of complexity lead, in some cases, to complicated mixtures of several products. Thus, the thorough analytical investigations including chromatographic

Table 2. Effect of the water content of the mobile phase on the separation of porphyrins (with the use of stationary phases SG-NAF, SG-C8, and SG-C18; mobile phase: aqueous methanol; flow rate: 1 mL × min^{-1}; detection—420 nm)

Methanol content vol.%	ln k′		
	SG-NAF	SG-C8	SG-C18
100	0	0	0
85	1.25	1.68	1.53
75	2.21	2.51	2.31
70	2.91	2.76	2.52
60	3.51	3.13	3.01
0	9.40	8.74	8.83

resolution and structure elucidation are necessary and complementary parts of the research program.

The effect of the water content of the mobile phase on the separation of porphyrins, with the use stationary phases SG-NAF, SG-C_8, and SG-C_{18}, is shown in Table 2. In these cases, comparison of the ln k′ values clearly shows that π-electron aryl phases have considerable participation in $\pi-\pi$ interactions during the separation of π-electron moieties.

In order to determine and compare the selectivity of the packings prepared (RP SG-C_8, RP SG-NAF), a mixture of benzene and its alkyl

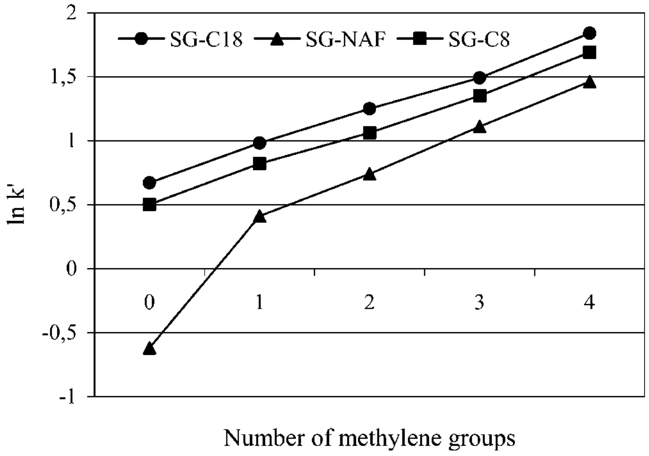

Figure 3. Dependence ln k′ on the number of carbon atoms in alkyl chain of alkylbenzenes for octyl and aryl packings. Chromatographic conditions: mobile phase 75/25 vol.% methanol/water, flow—1 mL × min^{-1}, wavelength–254 nm, temperature—20°C.

derivatives (containing one to four carbon atoms in the side chain) were used (Fig. 3). According to Jandera's classification,[17,18] we view these phases as different. The slopes of alkyl packings are significantly different as compared to that observed for the aryl phase, and the values of the retention parameters (ln k′) are twice higher for benzene, toluene, and ethyl benzene. The above results show the specific character of aryl phases which may be used for chromatographic analysis of hydrophobic analytes.

In this paper, we present the effect on the separation of two new synthesized porphyrins [{5-[4-nitro-3-(toluene-4-sulphonylmethyl)phenyl]-10,15,20-triphenylporphyrinato}zinc(II) (**1**) and *N,N*-dimethyl-*C*-{[2-nitro-5-(10,15,20-triphenylporphyrinato-5-yl)phenyl]methanesulphonamide}zinc(II) (**2**)], and the optimal conditions found for chromatographic resolution and determination, with the use of stationary phases SG-NAF, SG-C_8 and SG-C_{18} (Fig. 4). All the results obtained are listed in Table 3 and in Figure 5.

Most of the synthetic porphyrins usually exhibit moderate polarity. During optimization, various mobile phase systems and three stationary phases (octadecyl, octyl, and aryl) were taken into consideration. The octadecyl stationary phase has recently become almost an industry standard

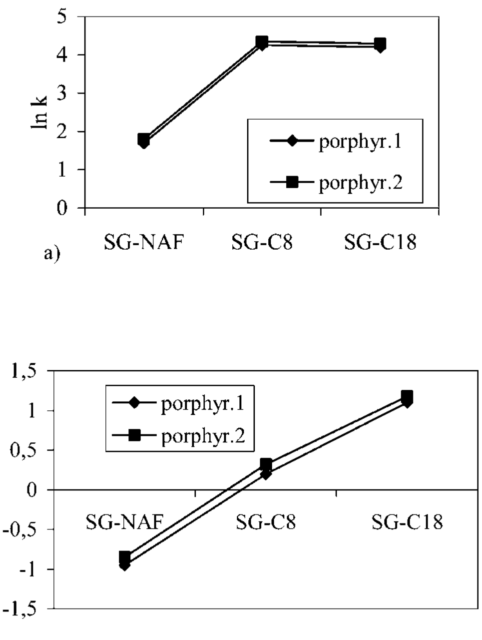

Figure 4. Effect of the separation of porphyrins with the use of stationary phases SG-NAF, SG-C8, and SG-C_{18}. Mobile phase: (a) 85/15 vol.% methanol/water and (b) 65/35 vol.% acetonitrile/water, flow rate: 1 mL × $\min^{-1}$; detection—420 nm (see Table 3).

Table 3. Chosen dependence ln k′ for porphyrins 1 and 2 on type of stationary and mobile phase. Chromatographic conditions: flow—1 mL × min^{-1}, wavelength—420 nm, temperature—20 °C

Type of stationary phase	Mobile phase	k_1'	k_2'	$\alpha = k_2'/k_1'$
RP SG-C18	Methanol/water (95/5)	11.83	13.43	1.14
	Methanol/water (85/15)	72.39	73.99	1.02
	Acetonitrile/water (55/45)	3.94	3.98	1.01
	Acetonitrile/water (40/60)	6.53	6.61	1.01
RP SG-C8	Methanol/water (95/5)	19.43	23.58	1.21
	Methanol/water (85/15)	74.34	75.47	1.02
	Acetonitrile/water (55/45)	1.39	1.41	1.01
	Acetonitrile/water (40/60)	3.40	3.43	1.01
RP SG-NAF	Methanol/water (95/5)	0.32	0.33	1.03
	Methanol/water (85/15)	5.92	6.05	1.02
	Acetonitrile/water (55/45)	0.42	0.45	1.07
	Acetonitrile/water (40/60)	1.44	1.51	1.05

in many HPLC methodologies; as well, octyl phase has been used, however not so often. So-called dedicated stationary phases, often utilized for only one specific group of compounds, are also used. One example is the aryl-bonded stationary phase (SG-NAF), used primarily for the separation of π-electron containing compounds.[3] Interactions of $\pi-\pi$ type between this stationary phase and analytes are predominant during the chromatographic process. Due to these interactions, retention time is often shortened, and the resolution of compounds (e.g., isomers) can be improved. This effect is seen

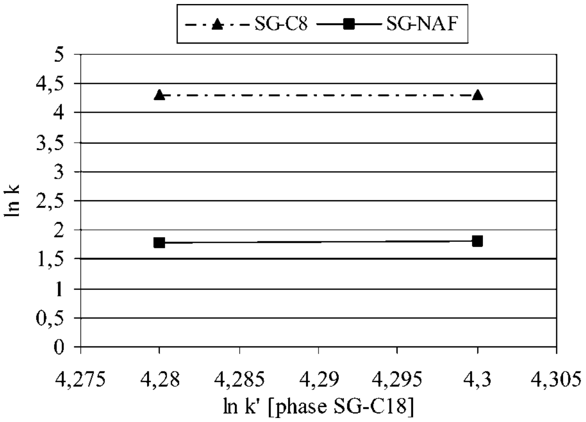

Figure 5. Dependence of ln k′ of the SG-C8 and SG-NAF phases on ln k′ obtained for the octadecyl phase for porphyrins **1** and **2**.

very clearly in Table 2 and in the diagram presented on Figure 3. During the determination of {5-[4-nitro-3-(toluene-4-sulphonylmethyl)phenyl]-10,15,20-triphenylporphyrinato}zinc(II) (**1**), when using methanol/water (85/15), and a RP SG-C_{18} column, a retention time of 49.69 min was obtained (for *N,N*-dimethyl-*C*-{[2-nitro-5-(10,15,20-triphenylporphyrinato-5-yl)phenyl] methanesulphonamide}zinc(II) (2)—50.77 min), while for the same solvent system and a SG-NAF column, the retention times were 8.63 min and 9.77 min, respectively (Fig. 6). A similar relationship was observed when other solvent systems were applied. A comparison of the results obtained for the octyl and octadecyl phases (Table 3) shows significantly increasing retention times, and as a consequence, capacity factors (k′). Results of retention time data obtained for methanol/water systems: 70/30 and 50/50 were very high (over 100 min) and then they were omitted in Table 3.

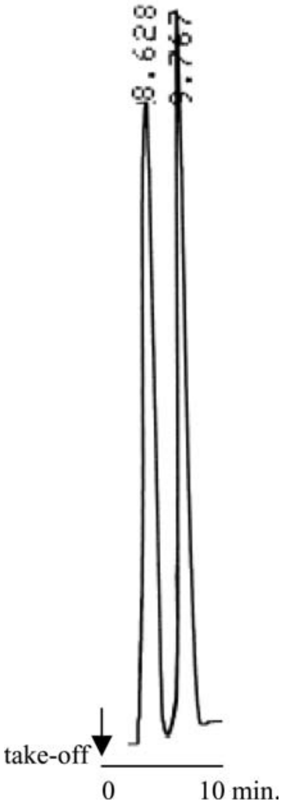

Figure 6. A chromatogram of separation of the porphyrins **1** (8.63 min) and **2** (9.77 min.) on the stationary SG-NAF phase. Mobile phase: acetonitrile/water (25/75 vol.%); flow—1 mL × min^{-1}, wavelength—420 nm, temperature—20°C.

Retention times and capacity factors (k′) for all stationary phases were lower when acetonitrile/water systems were used (65/35, 55/45, and 40/60).

Conditions for the resolution of the two porphyrin derivatives **1** and **2** were optimized according to the data listed in Table 3, and it was found that for the methanol/water solvent system (95/5), the most selective phase is RP SG-C_{18} (separation factor $\alpha = 1.14$), although the retention time was very long. The RP SG-C_8 phase exhibited for the same solvent system a relatively lower separation factor ($\alpha = 1.21$). Conversely, the aryl-bonded stationary phase SG-NAF showed the highest selectivity ($\alpha = 1.15$) for the acetonitrile/water system (25/75) (see Fig. 6).

CONCLUSIONS

The analytical chemistry of porphyrin derivatives is of great importance due to the biological activity of these compounds and their occurrence in nature. In this paper we presented the optimal conditions for the chromatographic resolution and determination of two *meso*-tetraaryl-porphyrins. The investigations have shown that the above-mentioned compounds can be separated with the use of octadecyl stationary phase, as well as octyl and aryl ones. The application of an aryl-bonded stationary phase allowed a decrease in run time (1 : 6) compared to the octadecyl phase (used in many analytical laboratories as reference phase).

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