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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF FREE-BASE PORPHYRINS

I. AN IMPROVED METHOD

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SUMMARY

A significantly improved method is described for the high resolution high-performance liquid chromatographic analysis of alkyl porphyrins on 3-μm silica. This improvement has been achieved by addition of varying amounts of both acetic acid and pyridine to the mobile phase. The technique is of special value in the analysis of complex mixtures of sedimentary alkyl metalloporphyrins after demetallation, and provides a complementary method to the gas chromatographic–mass spectrometric analysis of the bis-(*tert.*-butyldimethylsilyl)siloxyporphyrin derivatives.

INTRODUCTION

High-performance liquid chromatography (HPLC) provides a convenient means of analysis of synthetic and naturally occurring tetrapyrrole pigments. The methods commonly employed for carboxylated porphyrins[1], chlorophylls[2,3], and chlorins[4] usually utilise reversed-phase conditions. Despite the comprehensive literature on such compounds, the HPLC analysis of alkyl porphyrins is less well documented.

Interest in alkyl porphyrins arises through their widespread occurrence in sedimentary materials such as shales and coals, and in petroleums[5]. Such porphyrins

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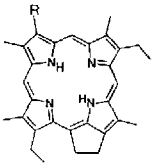
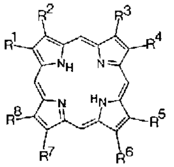


1 $R=CH_2CH_3$
4 $R=CH_3$



2 $R^1\text{-}R^8=CH_3$
3 $R^1,R^3,R^5,R^8=CH_3$ $R^2,R^4,R^6,R^7=CH_2CH_3$
5 $R^1,R^3,R^5,R^8=CH_3$ $R^2,R^4,R^7=CH_2CH_3$
$R^6=H$
6 $R^1\text{-}R^3,R^5,R^6,R^8=CH_3$ $R^4,R^7=CH_2CH_3$
OR $R^1\text{-}R^3,R^5\text{-}R^7=CH_3$ $R^4,R^8=CH_2CH_3$
7 $R^1\text{-}R^3,R^5\text{-}R^8=CH_3$ $R^4=CH_2CH_3$

(*e.g.* 1), which are thought to derive mainly from precursor chlorophylls[6–8], usually occur as metal complexes, most commonly of vanadyl and nickel[5], although copper[9], gallium[10], manganese and iron[11] have also been reported, as have free bases[12]. The application of these compounds in the assessment of the environment at the time of deposition of a sediment, and of its thermal history relies on a knowledge of their structures and of the analysis of their distributions. Hence, a number of the structures have been elucidated recently[6,7,13–21]. The complexity of the mixtures indicates, however, the need for a suitable high resolution chromatographic technique for separation. Computerised gas chromatography–mass spectrometry (C-GC–MS) of the bis-(*tert.*-butyldimethylsilyl)siloxyporphyrin derivatives has proved to be one such technique[22–25]. High-resolution HPLC provides a complementary procedure.

Initial investigations of the HPLC analysis of sedimentary alkyl porphyrins indicated that efficient separations could be obtained after demetallation, followed by normal-phase analysis on silica[26,27]. Separations were adequate for a preliminary study of the effects of thermal stress on the distributions of porphyrins from a series of shales from the Paris Basin[28]. More recently a significant improvement was achieved in the reversed-phase analysis of vanadyl porphyrins[29]. By comparison, however, available information on the reversed-phase analysis of nickel porphyrins shows at present relatively poor resolution with long retention times[30]. Conversely, reversed-phase HPLC of simple mixtures of free-base alkyl porphyrins has allowed separation of positional isomers[27,31].

The present study has concentrated on free-base alkyl porphyrins, and their HPLC behaviour on silica for several reasons:

(i) The range of basicities for the free-base porphyrins with known structure might be expected to be considerable, thus providing a basis for their separation, especially on a polar surface.

(ii) Despite a recent report of a method suitable for vanadyl porphyrins[29], this may not be generally applicable to other metals (*e.g.* nickel and copper) at present.

(iii) After demetallation, identical HPLC conditions can be used for the analysis of porphyrins chelated to different metals.

(iv) Initial studies have indicated the suitability of normal-phase HPLC for free-base alkyl porphyrins[26,27].

Modern HPLC materials and techniques provide the potential for a highly efficient separation procedure; these have been used in the problem of analysis of complex mixtures of sedimentary porphyrins.

EXPERIMENTAL

Sample preparation

In the present study free-base porphyrins were isolated from the bitumen (1 g) or oil (1 g) by treatment with excess methanesulphonic acid (MSA, 98%, 5–10 ml, 100°C, 4 h). The reaction was quenched by pouring the acid–oil mixture into water (double-distilled, 20 ml). After cooling, the coagulated organic material was removed by filtration. The organic residue was washed with aliquots of dilute MSA (3 × 5 ml; 50% v/v), until the filtrate was colourless. The combined aqueous extracts, containing the porphyrins as di-cations, were extracted with dichloromethane (3 × 5 ml), neutralised (sodium hydrogen carbonate), and dried (toluene azeotrope). The resulting extract was used for HPLC analysis without further purification. No significant discrimination or structural modification of alkyl porphyrins indigenous to geological materials has been observed in the acid extraction.

Instrumentation

Analyses were performed using a Spectra-Physics SP8700 ternary solvent delivery system, and a Rheodyne 7125 injector fitted with a 10-μl loop. Detection was carried out with a LDC 1202 Spectromonitor III variable-wavelength detector (400 nm), which was normally interfaced with a VG Minichrom data system. Unless stated otherwise analyses were performed using three columns (Spherisorb 3W; each 150 × 4.6 mm I.D.; Phase Separations) connected in series. Typical back-pressures were of the order of 2000 to 2500 p.s.i., at a flow-rate of 1.0 ml min^{-1}.

Solvents

All solvents were doubly distilled prior to use. Degassing was achieved by constant purging with helium.

The typical conditions for the analysis of the mixtures of sedimentary alkyl porphyrins utilise a stepped programme (Table I), and a ternary solvent system, which can be readily converted into a binary system. For the ternary system the solvent mixtures used were: (A) methylene chloride–acetone (4:1); (B) hexane–pyridine (99:1); (C) hexane–acetic acid (99:1).

Samples

An authentic sample of aetioporphyrin III (3) was kindly supplied by Dr. J. G. Erdman. All other standard porphyrins have been isolated from geological samples and characterised fully, except where stated otherwise[6,14,17–20].

RESULTS AND DISCUSSION

The basicity of free-base alkyl porphyrins is highly dependent on structure.

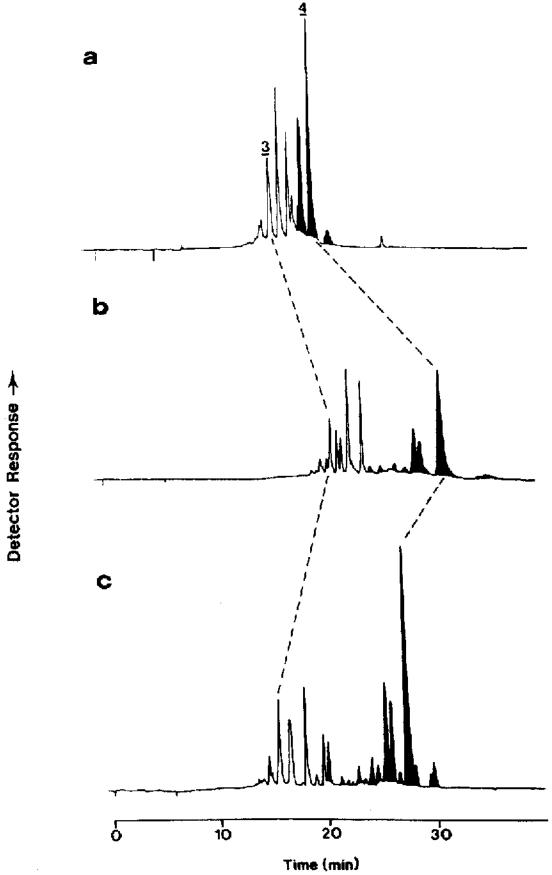


Fig. 1. HPLC chromatograms of demetallated nickel porphyrins of Gilsonite bitumen (Eocene; Utah, U.S.A.): (a) without addition of acetic acid to solvent C (*cf.* Table I); (b) with acetic acid (1%) added to solvent C (*cf.* Table I); (c) with acetic acid (1%) added to solvent C, and pyridine (1%) added to solvent B (Table I). Analysed on 5-μm silica (250 × 4.6 mm I.D. column). Shaded peaks indicate the main elution range of components with an exocyclic alkano ring. Dotted lines indicate common peaks.

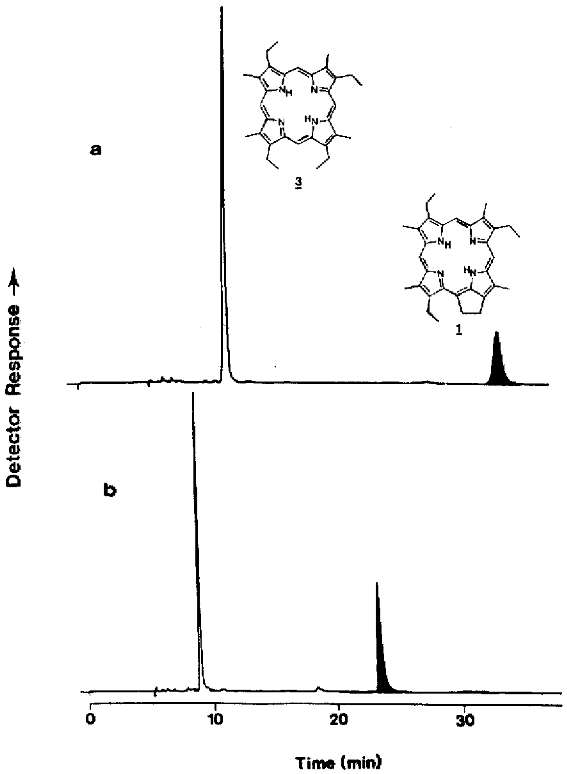


Fig. 2. HPLC chromatograms of 1 and 3 using isocratic conditions described in Table II: (a) 5-μm silica; (b) 3-μm silica.

TABLE I

PROGRAMME FOR TERNARY SOLVENT SYSTEM USED TYPICALLY IN ANALYSES OF DEMETALLATED SEDIMENTARY PORPHYRINS

Solvents: A = methylene chloride–acetone (4:1); B = hexane–pyridine (99:1); C = hexane–acetic acid (99:1).

Time (min)	*Solvent*		
	A (%)	*B (%)*	*C (%)*
0	5	5	90
5	5	5	90
20	15	7.5	77.5
35	30	30	40
45	30	30	40
{50*	5	5	90
{70	5	5	90

* Re-equilibration.

For example, introduction of an N-methyl group in octamethylporphyrin (2) causes a concomitant increase in basicity, due to the puckering of the aromatic ring system[32]. Structural investigations have shown that a large number of sedimentary porphyrins possess features which may give rise to significant deformation of the planar ring system. The most notable is the presence of an exocyclic alkano ring (*e.g.* in 1)[8,33]. Previous studies of HPLC behaviour have indeed shown that such components are readily separated on silica from those having no exocyclic ring (*e.g.* aetioporphyrin III, 3)[26,27]. This result is presumably in part due to the acidic nature of the silica used. Furthermore, a previous investigation[34] indicated that, on silica, addition of a small percentage of acetic acid to the mobile phase significantly improved separation of uroporphyrin III octamethyl ester from its uroporphyrin I counterpart. In that case, the improved resolution was thought to derive from a reduction of association, via protonation of the porphyrins in their microenvironment.

In our preliminary studies, therefore, acetic acid (less than 1%) was added to the eluent. This provided a significant improvement in the resolution of the aetioporphyrins from those having an exocyclic alkano ring (Fig. 1a and b; Gilsonite bitumen, Eocene, UT, U.S.A.; demetallated nickel porphyrins). It was apparent, however, that further improvement was necessary since, whilst reasonable peak shape was evident for the early eluting compounds, the peaks arising from the later eluting compounds showed bad tailing (Fig. 1b). This problem was overcome by introducing increasing amounts of pyridine into the mobile phase as the run progressed, greatly improved peak shapes and reduced retention times for these later eluting components being obtained (Fig. 1c). The improvement is presumably a result of the competition of pyridine and the more basic of the porphyrins for the acidic active sites on the surface of the silica.

Further improvements were achieved by using 3-μm silica. Fig. 2 shows a comparison of the results for a mixture of 1 and 3 analysed under isocratic conditions, using three columns coupled in series, to provide the resolution and number of the-

TABLE II

COMPARISON OF HPLC DATA FOR 1 AND 3 ANALYSED ON 3-μm AND 5-μm SILICA UNDER ISOCRATIC CONDITIONS

Solvent composition was 12% dichloromethane, 3% acetone, 0.2% pyridine and 1.5% acetic acid in hexane.

Data	*Silica particle size (μm)*	
	5	*3*
Dead time (min)	5.7	5.3
t for 3*	12.0	9.2
t for 1*	34.0	23.8
No. theoretical plates (*N*)	24 620	46 890
Plate height (*H*; μm)	31	10
Plates m^{-1}	32 830	104 200
Resolution (3 *vs.* 1)	29	37
Column length (cm)	3 × 25	3 × 15
Eluent flow-rate (ml min^{-1})	2	1

* *t* = Total retention time (min).

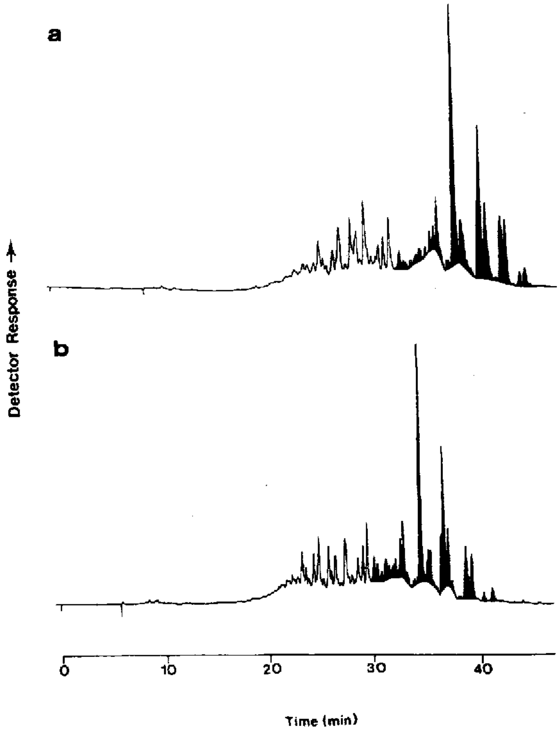


Fig. 3. HPLC chromatograms of demetallated vanadyl porphyrins of Boscan crude oil (Venezuela) using solvent conditions in Table I: (a) 5-μm silica (three 250 × 4.6 mm I.D. columns, flow-rate 2.0 ml min^{-1}); (b) 3-μm silica (three 150 × 4.6 mm I.D. columns, flow-rate 1.0 ml min^{-1}).

oretical plates listed in Table II. The improvement in resolution can also be seen in Fig. 3, which shows a comparison on 5-μm and 3-μm silica of the distributions of the vanadyl porphyrins obtained from a crude oil (Boscan, Venezuela) after demetallation, using the solvent programme in Table I. The improvement in using 3-μm silica and three columns in series is emphasised by comparison of the distribution of the demetallated nickel porphyrins in Gilsonite shown in Fig. 4 with that in Fig. 1c. The identities of the components shown in Fig. 4 were confirmed by coinjection of the components after isolation and structure assignment by NMR.

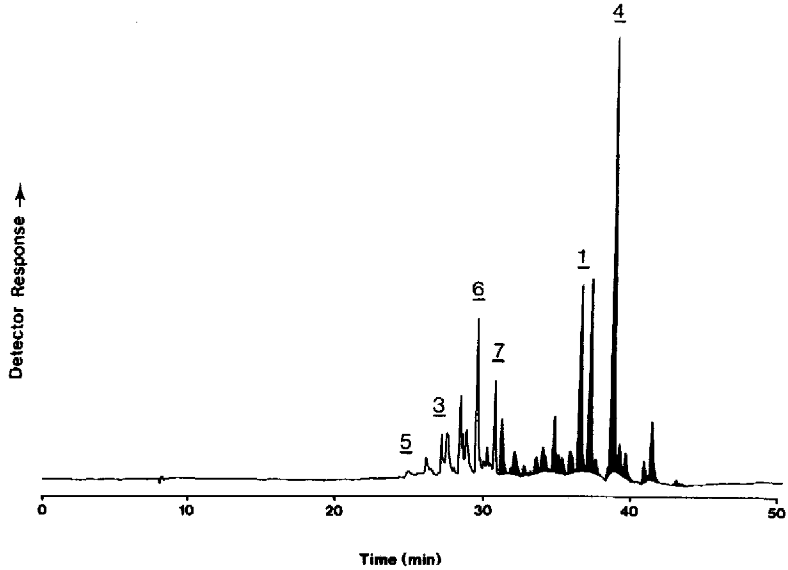


Fig. 4. HPLC chromatogram of Gilsonite demetallated nickel porphyrins on 3-μm silica using conditions in Experimental and in Table I. Peaks labelled show coinjected components.

Back-pressures using three columns in series were well within the specification limits for the HPLC equipment (see Experimental). The nature of the silica was found to be important; Spherisorb W was the most favourable of a number of silica phases evaluated. Our laboratories have employed the solvent system in Table I and the 3-μm silica column combination for several years in petroporphyrin analysis, and we have found the results to be reproducible. Some reduction of column performance is observed during prolonged periods of analysis of crude porphyrin extracts. This becomes evident by a shortening of retention times. Operating efficiency can be restored by purging the columns with dry tetrahydrofuran (100 ml) at low flow-rate (0.5 ml min^{-1}). With such treatment the lifetime of a set of columns used essentially on a daily basis, was up to two years.

CONCLUSIONS

A method is described for the separation of alkyl porphyrins. There are several advantages of the method:

(i) It is generally applicable to complex mixtures of free-base porphyrins and, after demetallation, of metalloporphyrins isolated from geological sources. The range of sedimentary metalloporphyrins includes all those which can be readily demetallated, *i.e.* nickel, vanadyl, copper, iron and manganese.

(ii) The procedure is rapid and reproducible, and the columns can be easily regenerated on loss of performance.

(iii) The basic method is suitable for semi-preparative HPLC studies as indicated in the following paper. Indeed, this technique has allowed the isolation and characterisation of a large number of the porphyrins that commonly occur in geological samples. Furthermore, it has already been applied to several geochemical studies[35–38].

(iv) Initial investigations indicate that the method is applicable to, and gives good separation of, porphyrin carboxylic acids as their methyl esters. Application to the separation of geologically-occurring carboxylated porphyrins[39,40], as well as to biologically-occurring tetrapyrroles of this type shows considerable promise[41].

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