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MOBILE PHASE SELECTIVITY MAPPING OF NICKEL AND VANADYL METALLOPORPHYRINS IN REVERSED-PHASE LIQUID CHROMATOGRAPHY

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SUMMARY

The retention behavior of nickel and vanadyl metalloporphyrins in reversed-phase high-performance liquid chromatography has been studied. Water or strong bases added to the otherwise non-aqueous mobile phase systems were found to enhance the separation of nickel metalloporphyrin homologues and the separation of vanadyl and nickel species. Differences in the retention shifts caused by the addition of water and strong bases to the mobile phase demonstrate two chromatographic variables: polarity and ligand coordination. A mixture of the octaethylporphyrins of zinc (II), nickel (II), copper (II), and cobalt (II) and the etioporphyrin-I of vanadium and nickel were separated with acetonitrile–ethanolamine (10:1) as the mobile phase.

INTRODUCTION

Since their discovery in oil and subsequent link to biological sources[1,2], the nickel(II) and vanadyl petroporphyrins, NiP and VOP, have been sought as geochemical markers in the study of the formation and maturation of oils and shales[3–7]. Prerequisite for the use of petroporphyrins as geochemical markers is their isolation from the crude oil matrix, After concentrating, their final separation by high-performance liquid chromatography (HPLC) is preferred because their lack of volatility has made gas chromatographic work difficult.

The use of HPLC to effect the isolation of the petroporphyrins has been studied with emphasis on the use of liquid–solid chromatography (LSC) on silica columns[8–12]. However, there is still a need for an improved understanding of how HPLC may be used to separate porphyrin isomers and homologues with the same metal center. Studies of demetallated petroporphyrins in reversed-phase liquid chromatography (RPLC) on octadecyl silane (C_{18}) columns have demonstrated its ability to separate structural isomers and members of a homologous series of porphyrins[13–15]. Unfortunately, the demetallation procedure for metalloporphyrins, which involves heating with methanesulfonic acid, is uncertain due to incompleteness of the reaction and due to possible structural degradation of the alkyl substituents on the porphyrin ring[15].

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Barwise and Whitehead[9] have reported the use of alkylsulfonic acid-functionalized silica to separate vanadyl metalloporphyrins. This depends, at least in part, on the protonation of the vanadyl oxygen to increase chromatographic selectivity. However, acid-catalyzed degradation is believed to accompany the separation. The ability to use the intrinsic acidic and basic properties of metalloporphyrins to chromatographic advantage in RPLC is, nonetheless, open to further investigation. This report describes the investigation of vanadyl and nickel metalloporphyrin separations through mobile-phase-based selectivity mapping in RPLC[16,17].

EXPERIMENTAL

Materials

All solvents were HPLC grade. The basic solvent additives were 99.9% pure liquids. Mobile phase solvent blends were prepared by volume-to-volume mixing before use in HPLC. Mobile phase systems containing these basic additives were handled so as to minimize water contamination which plays a detrimental effect, as will be described later. Metalloporphyrin standards used in the study were vanadyl(IV)-etioporphyrin-I (VOP) and nickel(II)-etioporphyrin-I (NiP-1) obtained through Mid-Century Chemicals (Pasen, IL, U.S.A.), octaethyl-21H,23H-porphine nickel(II) (NiP-2), octaethyl-21H,23H-porphine zinc(II) (ZnP), octaethyl-21H,23H-porphine copper(II) (CuP) and octaethyl-21H,23H-porphine cobalt(II) (CoP) obtained from Aldrich (Milwaukee, WI, U.S.A.). The NiP-1 and NiP-2 structures are C_{32} and C_{36} homologues that differ by four methylene groups. The purity of the metalloporphyrin standards is discussed at the end of this article. All metalloporphyrin standards were dissolved in dichloromethane. The preference for dichloromethane is based on their rapid solubilization as standards or as petroporphyrin fractions concentrated from petroleum and shales.

HPLC system

Metalloporphyrins were analyzed on a system consisting of a Waters Assoc. (Milford, MA, U.S.A.) Model 6000A pump, U6K injector, and a Model 440 UV absorbance detector with 405 nm and 546 nm filters. Additional UV–VIS detection was carried out with a Knauer digital spectral photometer Type 87 obtained through Sonntek (Woodcliff Lake, NJ, U.S.A.). Spectral monitoring was performed at 405 nm for both nickel and vanadyl metalloporphyrins with supplementary monitoring at 550 nm for nickel and 575 nm for vanadyl. Signal integration was performed by a Hewlett-Packard (Avondale, PA, U.S.A.) Model 3390A integrator. The start control on the HP keyboard was wired to a microswitch on the U6K injector to mark the injection time.

Columns

Anticipating the possible destructive nature of some of the mobile phase additives, columns of two types were used. For feasibility studies and clean-up, 5 cm × 0.4 cm I.D. Upchurch (Oak Harbor, WN, U.S.A.) stainless-steel columns were used. These were packed with Whatman (Clifton, NJ, U.S.A.(LRP-1 (13–24 μm) octadecyl silane (C_{18}) stationary phase. Packing was done by means of a liquid chromatograph in 100% ethanol. Further studies were conducted using Perkin-Elmer

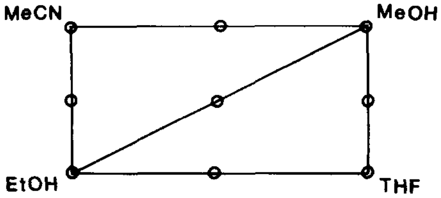
(Norwalk, CT, U.S.A.) Pecosphere 3 cm × 3 mm C_{18} cartridge-type (PE 3 × 3 C_{18}) short columns.

RESULTS AND DISCUSSION

Retention and selectivity mapping of NiP and VOP

An RPLC study was conducted on VOP and NiP standards. Sorptive behavior was investigated using two interrelated solvent triangles, shown in Fig. 1, to map mobile phase effects upon non-aqueous reversed-phase (NARP) and RPLC behavior[16,17]. The retention behavior and selectivity of the metalloporphyrins are summarized in Fig. 2 and Table I, respectively. Retention behavior is described using the capacity factor, k', as follows:

$$k' = (V_r - V_0)/V_0 \tag{1}$$



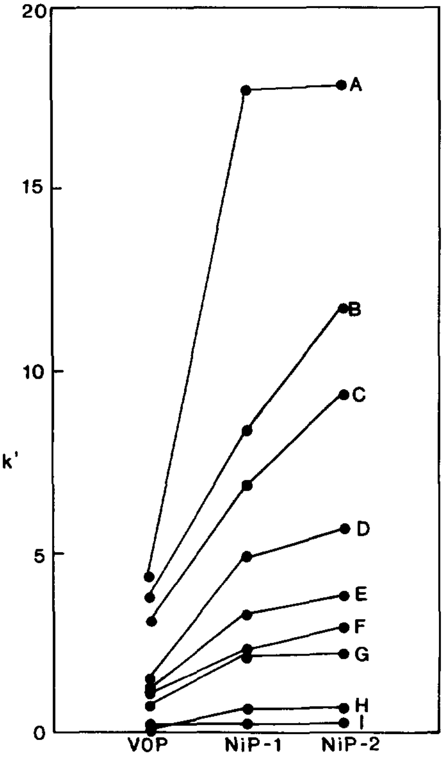


Fig. 1. The two solvent selectivity triangles mapped in this study involve the four solvents ethanol (EtOH), acetonitrile (MeCN), methanol (MeOH) and tetrahydrofuran (THF). Metalloporphyrin sorptivity was studied using individual solvents as well with their mixtures, as non-aqueous media and with water, and with nucleophilic modifiers added.

Fig. 2. Plots of k' values for NiP and VOP species obtained on a PE 3 × 3 C_{18} column. Flow-rate, 1.00 ml/min with detection at 405 nm; 1.0 a.u.f.s. Mobile phases are pure solvents or equal volume 1:1 or 1:1:1 blends: (A) methanol, (B) acetonitrile, (C) acetonitrile–methanol, (D) methanol–ethanol, (E) methanol–acetonitrile–ethanol, (F) ethanol, (G) acetonitrile–ethanol, (H) acetonitrile–methanol–tetrahydrofuran, and (I) acetonitrile–ethanol–tetrahydrofuran.

TABLE I

RELATIVE RETENTION MEASUREMENTS OF NiP-2 AND NiP-1 HOMOLOGUES (C_{36} AND C_{32}), AND NiP AND VOP METALLOPORPHYRINS (C_{32})

$k' = k'_{NiP-2}$; $\alpha_1 = k'_{NiP-2}/k'_{NiP-1}$; $\alpha_2 = k'_{NiP-1}/k'_{VOP}$

Liquid	k'	α_1	α_2
Tetrahydrofuran	0.0	1.0	1.0
Ethanol	2.9	1.3	2.2
Acetonitrile	11.7	1.4	2.3
Methanol	17.8	1.0	4.3
Acetonitrile–ethanol (1:1)	2.2	1.0	3.0
Ethanol–methanol (1:1)	5.6	1.1	2.3
Acetonitrile–methanol (1:1)	9.3	1.4	2.3
Acetonitrile–ethanol–methanol (1:1:1)	3.8	1.1	3.1
Ethanol–water (100:1)	7.0	1.1	3.1
Acetonitrile–water (100:1)	29	1.6	2.4
Methanol–water (100:1)	41	1.1	4.7
Acetonitrile–*n*-hexylamine (10:1)	3.9	1.3	2.0
Acetonitrile–morpholine (10:1)	4.5	1.3	2.5
Acetonitrile–ethanol (10:1)	7.5	1.4	2.2
Acetonitrile–ethylamine (10:1)	9.0	1.4	2.2
Acetonitrile–ethanolamine (10:1)	9.7	1.4	2.8

where V_r is the retention volume. The void volume, V_0, was referenced to pure tetrahydrofuran on the basis of the observed reproducibly minimal and constant retention behavior (to within experimental error) of both NiP and VOP standards. On this basis it was concluded that these standards showed negligible affinity and negligible permeation differences with the porous C_{18} sorbent in that solvent.

Selectivity was measured by the retention ratio, α, which was calculated as

$$\alpha = k'_2/k'_1 \tag{2}$$

All of the experiments were carried out at fixed carrier composition and a flow-rate of 1.00 ml/min.

The wide range of solvent-based selectivity shown in Table I has a maximum α value for the NiP-1 and NiP-2 homologues of 1.41 in 100% acetonitrile with k' values of 8.19 and 11.58. The observed effectiveness of 100% acetonitrile as a mobile phase contrasts with the opposite conclusion in a previous study[18]. Further, the use of tetrahydrofuran in any mobile phase system did not permit practical retention of the metalloporphyrins; thus it is too strong a solvent for NARP chromatography of these species. Separations based on these mapping studies can be used for various purposes, *i.e.* metalloporphyrin separations in terms of different metals, or as homologues of a given metal, as shown in Figs. 3. These chromatograms show the selectivity variations obtained by varying the mobile phase.

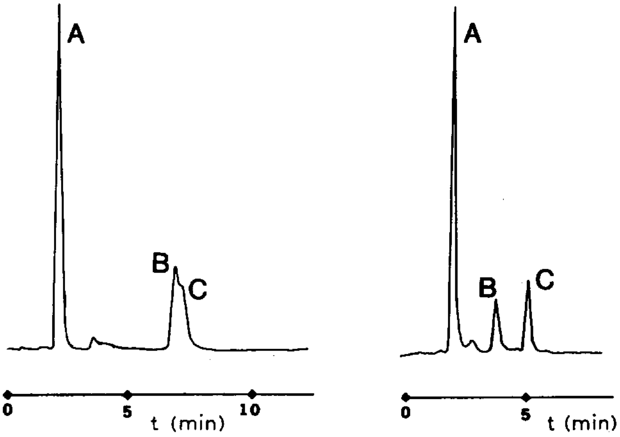


Fig. 3. Separation of VOP (A) from the NiP-1 (B) and NiP-2 (C) homologues on a C_{18} column in methanol (left) and in acetonitrile (right). Same chromatographic conditions as Fig. 2.

Additives to mobile phase systems

Water as an additive. Small amounts of water were added to several points in the mapping triangles. A solvent/water ratio of 100:1 was chosen due to the limited solubility of metalloporphyrins in water systems. (With the more polar standards, *i.e.* VOP, significantly higher water concentrations are also useful.) The retention behavior of the VOP and NiP standards is summarized in Fig. 4 and Table I. The tetrahydrofuran system, again, gave no retention while the methanol–water system gave high retention of NiP and a large selectivity between NiP and VOP species. The acetonitrile–water mobile phase system showed the most useful range of k' values

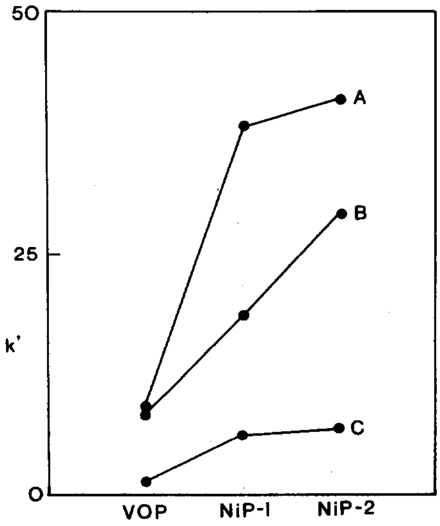


Fig. 4. The k' values for VOP, NiP-1 and NiP-2 (approximately doubled) when 1% water is added to the individual solvents (A–C) shown in Fig. 2.

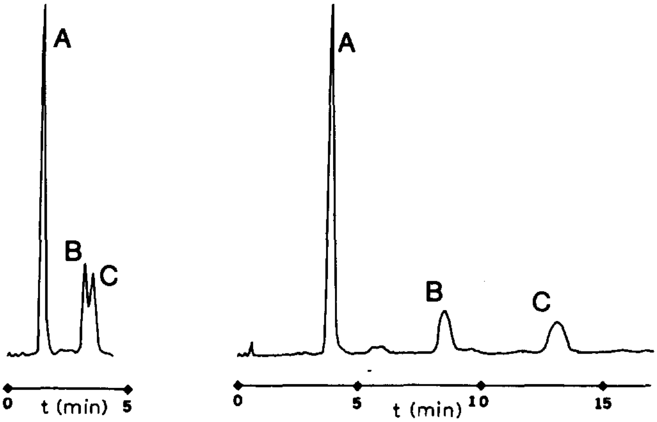
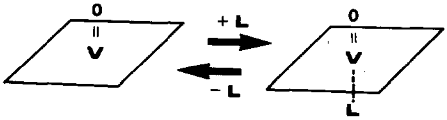


Fig. 5. Separation of the VOP (A), NiP-1 (B) and NiP-2 (C) on C_{18} column. The presence of 1% water in ethanol–water (left) and acetonitrile–water (right) increases selectivity in comparison to Fig. 3. Same chromatographic conditions as Fig. 2.

and the greatest selectivity between the two nickel porphyrins. In all cases, the addition of water to the mobile phase blend increased retention. Surprisingly, the separation of NiP improved with the addition of water to acetonitrile and methanol, but decreased with the same addition to ethanol (see Fig. 5).

NARP chromatography with nucleophilic (basic) additives. The possibility of using the ligand coordination properties of the metalloporphyrins to chromatographic advantage was studied using several nucleophilic compounds added to the mobile phase solvent system. Unlike water, the basic amine additives were found to promote the solubility of the highly lipophilic metalloporphyrins. Studies of the chemistry of



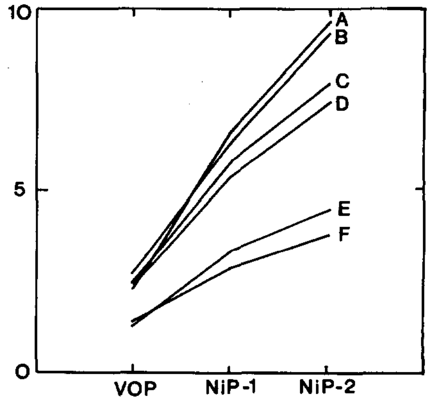


Fig. 6. Model illustrating the nucleophilic ligand which coordinates the central vanadium along the metal-oxygen axis. Bronsted acids would be expected to interact by protonation of the oxygen[19].

Fig. 7. Plot of the k' values for NiP and VOP with different mobile phase mixtures of the mobile phase consisted of 10% acetonitrile mixed with 90% of the following nucleophilic additives: (A) ethanolamine, (B) dimethyl sulfoxide, (C) ethylamine, (D) ethanol, (E) morpholine, (F) *n*-hexylamine. Same chromatographic conditions as in Fig. 2.

VOP structures have shown that certain nucleophiles coordinate as axial ligands attached to the metal side of the VO bond (see Fig. 6). This causes an apparent splitting of the Soret band absorption (380–410 nm) in the UV–VIS spectrum, the result of a new band due to presence of both the metalloporphyrin and the corresponding metalloporphyrin–ligand complex[19–21]. NiP structures showed similar but smaller spectral shifts of the Soret band in basic media. This has been attributed to symmetric di-coordination at vacant sites above and below the metal center[22,23]. The extent of coordination and Soret band shifts are related to concentration of the nucleophile in the solvent[24]. Hence, a solvent-based mechanism for retention control is available.

The retention behavior and selectivity of VOP, NiP-1, and NiP-2 standards in strongly basic mobile phase systems is summarized in Fig. 7 and Table I. All basic additives were combined with fresh acetonitrile and chromatographed in the NARP mode.

It is emphasized that the strongly basic reagents were added to reagent or HPLC grade acetonitrile. Water contamination was minimized during short term experiments. Contact with air humidity was lessened with a metal foil wrap at the neck of a glass mobile-phase reservoir. The pump was also flushed after use with pure solvent to minimize exposure of the seals to the additives. The need for such precautions was indicated by experiment. For example, acetonitrile–ethanolamine (10:1) with 3% water added showed prompt and marked loss of sorptive retention after return to pure acetonitrile mobile phase.

Column performance was checked after base additive runs were completed. This showed that the column was restabilized with only a slight overall loss of retention of the metalloporphyrins. This provided improved peak sharpness. The small loss of retention and increased peak sharpness may be due in part to the base interaction with residual silanol groups on the column. These experiments show that a highly basic carrier composition does not interfere with, and may even improve, stable column performance in the NARP mode.

Fig. 8 shows the peak sharpness, rapid elution and high selectivity of two of the basic mobile phase systems on a mixture of VOP, NiP-1 and NiP-2. When Figs.

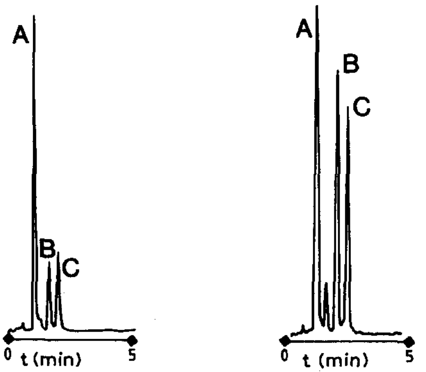


Fig. 8. Separation of VOP (A), NiP-1 (B) and NiP-2 (C) on C_{18} column in containing a nucleophilic additive: (left) acetonitrile–*n*-hexylamine (10:1), and (right) acetonitrile–morpholine (10:1). Same chromatographic conditions as in Fig. 2.

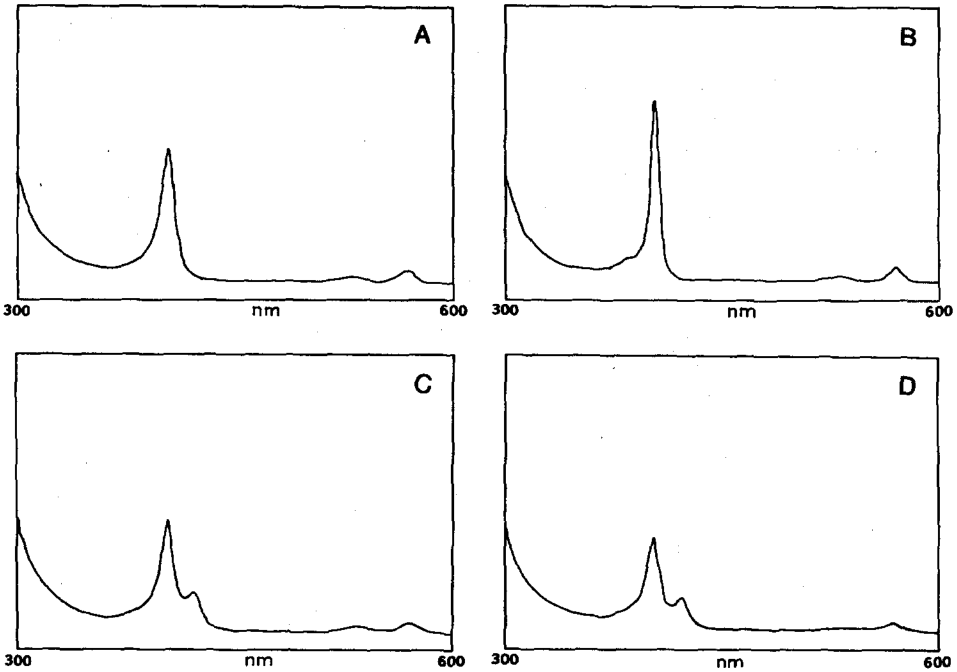
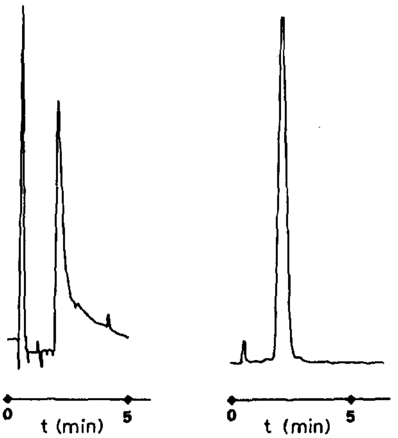


Fig. 9. UV–VIS spectra of vanadyl etioporphyrin-I in (A) acetonitrile, (B) cyclohexane, (C) acetonitrile–morpholine (10:1) and (D) acetonitrile–ethanolamine (10:1). The splitting of the Soret band arises from the red-shifted Soret band absorption of the equilibrium metalloporphyrin adduct formed (see Fig. 6). Wavelength span is from 300–600 nm.



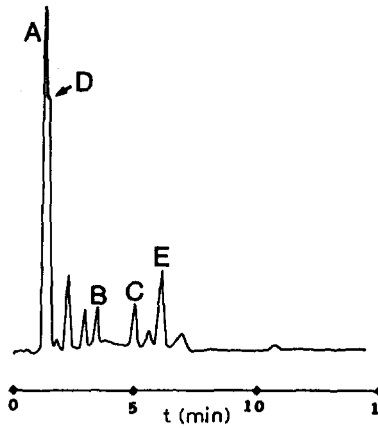


Fig. 10. (left) NARP chromatography of VOP standard in acetonitrile after a series of chromatographic runs. (right) Result of the same experiment, but following injection of 4 ml of acetonitrile–ethanolamine (10:1). The C_{18} column is stable in the presence of the strongly basic nucleophile, providing water is absent. Same chromatographic conditions as in Fig. 2.

Fig. 11. Separation of a mixture of VOP (A), NiP-1 (B), NiP-2 (C), ZnP (D), CuP (E) and CoP (F). CoP was not eluted. Same conditions as in Fig. 2 with o.5 a.u.f.s. The mobile phase was acetonitrile–methanol (1:1).

2 and 7 are compared, it can be seen that the basic additives to acetonitrile span the retention behavior between acetonitrile–methanol–ethanol (1:1:1) and 100% acetonitrile with good selectivity between the NiP-1 and NiP-2 homologues.

Evidence of the coordination of the metal centers was found in the splitting or shifting of the Soret band (*ca.* 400 nm) in the UV–VIS spectra of VOP and NIP standards in the basic mobile phases (see Fig. 9). The NiP standards showed a smaller effect with less than 2 nm red shift of the Soret band as expected for symmetric dicoordination. These coordination effects were found to be reversible; the splitting of the VOP Soret and red shift in NiP both disappeared after evaporation of the basic additive and redissolving the metalloporphyrin in a non-coordinating solvent such as cyclohexane.

The strong affinity of the metalloporphyrins for coordinating media was also put to use in the C_{18} column clean-up. After using the PE 3 × 3 C_{18} column for studies with the various solvents in the solvent triangles, the VOP peak exhibited increased tailing. This was especially noticeable after runs with methanol. After injection of a 4-ml "slug" of acetonitrile–ethanolamine (10:1), VOP was again chromatographed. The result was a decrease in the tailing as shown in Fig. 10. The column showed no evidence of sorptive degradation.

MULTIMETAL METALLOPORPHYRIN SEPARATION

Given the goal of isolating metalloporphyrine in crude oil and shale matrices, the RPLC behavior of several different metalloporphyrins was also investigated. A mixture of VOP, NiP-1, NiP-2, ZnP, CuP and CoP was resolved as a first test of mobile-phase optimization strategy. Chromatograms in Figs. 11 and 12 show the ability of the basic additives in the mobile phase to separate ZnP from VOP species and to elute the very non-polar CoP species, which was not eluted by any other mobile phase system. Saitoh *et al.*[18], used an acetone–acetonitrile (60:40) mobile phase to separate *meso*-tetraphenylporphyrins of zinc(II), vanadium(IV), copper(II) and nickel(II). This mobile phase system gave α values between ZnP and VOP complexes of approximately 1.35 with k' values below unity. Our basic mobile phase system gave a better result: α of 1.68, with k' values of 1.37 and 2.30 for VOP and ZnP, respectively. The relative order of polarity of the various metalloporphyrins was the same as in ref. 18.

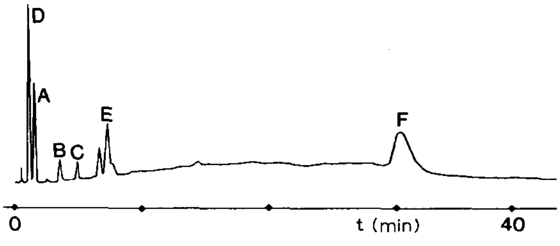


Fig. 12. Same experiment as in Fig. 11 except that the mobile phase has been modified by the presence of a nucleophilic additive, acetonitrile–ethanolamine (10:1). The resulting metalloporphyrin retentions are lessened without serious loss of selectivity and CoP (peak F) is eluted.

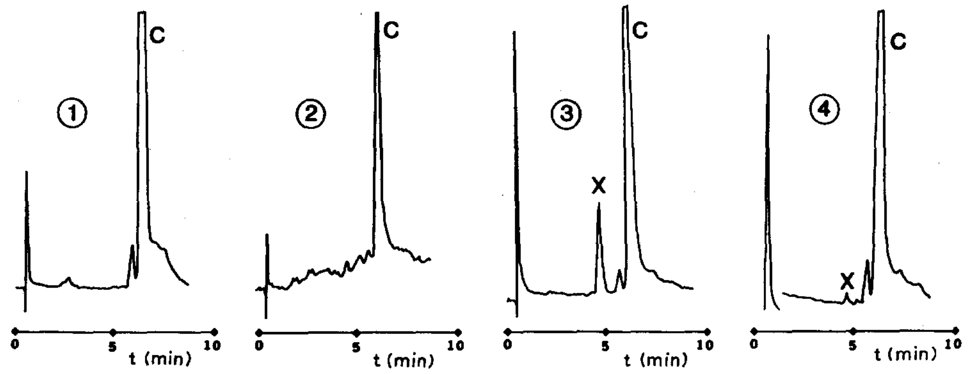


Fig. 13. Chromatograms of NiP-2 (C) standard in methylene chloride in acetonitrile mobile phase. Chromatographic conditions are similar to those in Fig. 2 except for the detector wavelength: 1, freshly prepared standard monitored at 405 nm; 2, same but monitored at 550 nm; 3, three month old standard monitored at 405 nm; 4, same but monitored at 550 nm. X denotes an impurity.

SUPPLEMENTAL STUDY OF STANDARDS

All metalloporphyrin standards showed chromatographic impurities in freshly prepared solutions. VOP, NiP-1 and NiP-2 standards were found to show an increase in the amount of impurity during approximately three months storage at room temperature, mainly in the dark. The NiP standards showed a marked increase, monitoring at 405 nm, of an impurity peak as shown in Fig. 13.

CONCLUSIONS

The chromatographic behavior of metalloporphyrins with water or basic additives in the mobile phase system enables fine-tuned mobile phase selectivity and retention. Two chromatographic variables are believed responsible, polarity and ligand coordination. As is consistent with the usual experience in RPLC, the addition of water increased the relative polarity of the mobile phase and increased k', while the addition of strong nucleophiles to the carrier increased interaction with the mobile phase and decreased k' values. Either of these two variables in the mobile phase can be adjusted to optimize NiP and VOP metalloporphyrin separations in RPLC.

The changes in the chromatographic behavior of metalloporphyrins in basic media point toward possible differences in the chromatography of standards and actual petroporphyrins. The petroporphyrins may be subject to coordination with known nucleophilic compounds whose analogues occur naturally in the oil matrix. Thus, the chromatography of the petroporphyrins will be influenced by the type, availibility and concentration of these analogous nucleophiles. Petroporphyrin isolation from petroleum and related source materials is therefore not confined to a class of metalloporphyrins, but more likely to a class that includes axial ligand complexes that vary with sample composition. Related studies are being conducted on the isolation of petroporphyrins and other indicator compounds from petroleum and related energy source materials[26].

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