

크로마토그래피를 이용한 아스팔트로 부터 금속 포르피린의 분리 및 정제

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Chromatographic Techniques for the Isolation and Purification of Metalloporphyrins from Crude Asphalts

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요 약. 겔투과 크로마토그래피와 실리카겔크로마토그래피등을 이용하여 여러가지 천연아스팔트에서 금속포르피린을 얻었으며 크로마토그래피 용액을 알루미나로 처리한 후 피리딘으로 추출하여 보다 농축된 금속 포르피린을 얻을 수 있었다. 금속 포르피린에서 포르피린 고리를 파괴하지 않고 금속을 제거하여 여러가지 형태의 포르피린을 얻었다.

ABSTRACT. Porphyrin-rich materials were obtained from some crude asphalts by gel permeation chromatography and silica gel chromatography. After extraction of each chromatographic fractions through alumina with pyridine, more concentrated metalloporphyrins were obtained. Demetallation of metalloporphyrins was possible without destroying porphyrin ring to provide different type of metal free porphyrins.

INTRODUCTION

Since porphyrins occur in nature in only small amounts, one of the biggest problems has been that of separation and purification.

There has been considerable research effort aimed at getting fractions of petroleum much enriched in metalloporphyrins, which can be further treated to yield pure compounds.

A mixture of porphyrins is obtained in the acid phase as a result of the digestion of petroleum samples and oil shales with hydrogen bromide and acetic acid. Chromatography¹⁻⁴ and partitioning between ethyl ether and hydrochloric acid⁵ have been employed to bring about separation of such mixtures. A paper

chromatographic technique, using lutidine-water as the solvent phase to separate porphyrin-free acid, was introduced in 1949 by Nicholas and Rimington^{6,7}. Kehl and Stich⁸, Falk⁹, and Eriksen¹⁰ separated polycarboxylic porphyrins by this lutidine-water method.

Dunning² and Blumer¹¹ studied separation by paper chromatography of mesoporphyrin and etioporphyrin obtained from hemin, and two other porphyrins, deoxyphylloerythrin and deoxyphylloerythroetioporphyrin from Triassic oil shale. Adsorbents used in column chromatographic separations of porphyrin mixtures include aluminum oxide, magnesium oxide, magnesium carbonate, calcium carbonate, silica gel and celite.

Partitioning of porphyrins between ether and aqueous hydrochloric acids offers a means of separating porphyrins. This technique has been extensively utilized, although it does not allow isolation of single molecular species of porphyrins.

Since nonporphyrin compounds are less polar than porphyrins, the latter can be concentrated from asphaltene by various polar solvents. Sugihara *et al.*¹² demonstrated that by adding water to an asphaltene-pyridine mixture, a porphyrin-poor fraction can be precipitated. The filtrate, which contains dissolved porphyrins can be extracted into carbon tetrachloride to provide a porphyrin concentrate. Further treatment with alumina and various solvents provides relatively pure porphyrin mixtures.

As described above, many research works were performed to obtain porphyrin rich materials from crude asphalts, however, none of them were successful to obtain pure metalloporphyrins in large amounts.

During the last two decades, there were no research works about the separation of metalloporphyrins because of the difficulties of their utilization for the large amounts of porphyrins.

In this paper, a gel permeation chromatographic technique, which allows isolation of relatively large amounts of porphyrin rich materials from crude asphalts is introduced and discussed.

RESULTS AND DISCUSSION

Concentration of metalloporphyrins was possible by passing an asphalt-benzene solution through a gel permeation column. The metalloporphyrins were largely contained in the last emerging fraction (Fraction III, *Table 1*), containing the smallest molecules in the parent asphalts. In the fraction-III of gel permeation chromatography, the Soret band, which is

Table 1. Metals and porphyrins concentration of each GPC fractions

Fractions		Concentrations ($\mu\text{M/g}$)		
		Vanadium	Nickel	Porphyrin
Fraction I		79	7.0	20
Fraction II		54	4.0	
Fraction III		80	4.0	81
Fraction I 20 $\mu\text{M/g}$ Soret	DMSO-Soluble	83	6.2	41.2
	DMSO-Insoluble	84	8.5	11

characteristic of metalloporphyrins was observed. Thus the Fraction-III was subjected to silica gel chromatography and the chromatogram was developed with hexane, hexane-benzene and benzene-chloroform.

The benzene-chloroform eluate showed strong peaks at 408nm (Soret band), 529nm (β -band) and 567nm (α -band) which indicate vanadyl porphyrins (*Fig. 1*). The hexane and the initial fraction of benzene-hexane eluate showed a 390 nm (Soret band) and a 546nm peak, which indicate that most of the porphyrins in the hexane eluate are nickel porphyrins (*Fig. 2*). Because of the $V=0$ group, vanadyl porphyrins are more polar than nickel porphyrins, elution with a nonpolar solvent such as hexane gives nickel porphyrins and a polar solvent gives vanadyl porphyrins. Fraction-I of gel permeation chromatography contains most of the nonporphyrin metals (*Table 1*) which exhibits no Soret absorbance. Dimethyl sulfoxide (DM. SO) treatment was carried out to remove all the Soret absorbing materials from the crude asphalts, fraction-I and fraction-II of gel permeation chromatography.

Since nonporphyrin compounds are less polar than porphyrins, the latter can be concentrated from asphalts by various polar solvents such as water¹² and DMSO. The DMSO-soluble frac-

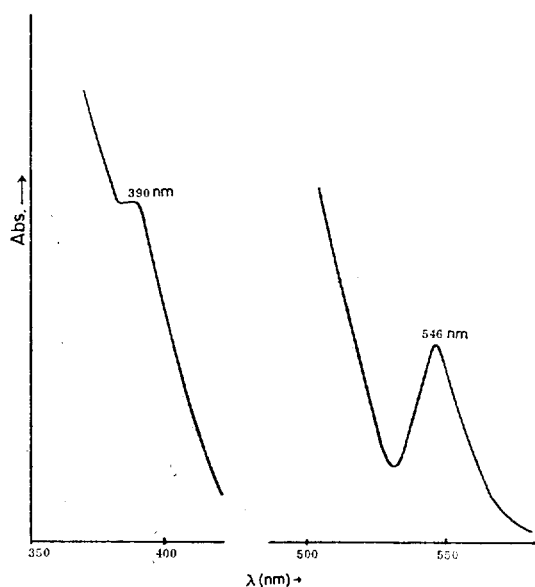


Fig. 1. Visible spectrum of benzene-chloroform eluate from silica gel chromatography.

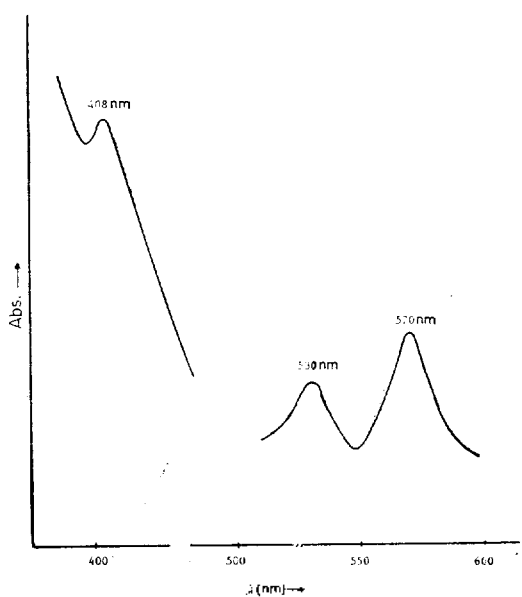


Fig. 2. Visible spectrum of hexane eluate from silica gel chromatography.

tions were treated with alumina and extracted with the mixture of benzene and pyridine.

Table 2. The ratio of vanadium to porphyrin concentration after alumina treatment

DMSO-Soluble fractions	Concentration ($\mu\text{M/g}$)		
	Vanadium	Porphyrin	Vanadium to porphyrin ratio
15 ml-pyridine-135 ml-benzene Extracts	458	456	1.0
75ml-pyridine-75 ml-benzene Extracts	319	290	1.1
DMSO-F-I 15ml-pyridine-135 ml-benzene Extracts	80	75	1.07
DMSO-F-I 75ml-pyridine-75 ml-benzene Extracts	80	66	1.21
DMSO-F-II 2.1ml pyridine-150 ml-benzene Extracts	157	148	1.06

Table 3. The ratio of vanadium to porphyrin concentration before alumina treatment

DMSO-Soluble Fractions	Concentration ($\mu\text{M/g}$)		
	Vanadium	Porphyrin	Vanadium to porphyrin ratio
DMSO-Soluble	82	41	2.00
DMSO-Soluble F-I	78	38	2.04
DMSO-Soluble F-II	85	69	1.23

The vanadium to porphyrin ratio found in the pyridine-benzene extracts of alumina treatment was approximately in 1 to 1 ratio, which indicates that the pyridine molecules associate mainly with vanadium in porphyrins and not with nonporphyrin vanadium (Table 2).

Before alumina treatment, the sample always contains vanadium at a level beyond that accountable as porphyrins (Table 3). After evaporation of benzene from the benzene-pyridine extracts of alumina treatment, the spectrum of the pyridine-extracts showed two different Soret bands (Fig. 3). The one Soret band exhibits a maximum at 408nm and the second Soret at 430nm. Also the relative intensities of the peaks at 570nm (α -band) and

530nm (β -band) were changed from those of uncomplexed vanadyl porphyrins (Fig. 3, 1)

The hexane eluate of the silica gel chromatography which contains nickel porphyrins was also treated with alumina for further purification. After alumina treatment, the spectrum showed two Soret bands. A second species (Soret band-II) appeared whose spectrum has a maximum peak at about 20nm longer wavelength (Fig. 4). Basic solvent molecules such as pyridine are serving as ligands at the nickel or vanadium atom giving rise to species II. The proportion of species II increase with increasing solvent basicity. A difference in spin

state with species I diamagnetic, as it is in the solid, and species II paramagnetic might be expected on theoretical ground.

Whitten and co-workers¹³ attribute the Soret band at longer wavelengths to a new species in which nickel exists in an octahedral configuration such that the ligand is directed along the "Z" axis. The ligand (pyridine molecule) then perturbs the porphyrin ring system by disturbing the π -electron system¹⁴. Evidently the electron system of the ligand disturbs the π -electron clouds of porphyrin rings changing the position of the Soret band. In addition, the distortion of the π -electron clouds would be greater in the ground state than in excited states, giving rise to the observed shift to the red.

DMSO-soluble fraction was not demetallated with hydrogen bromide in acetic acid and in formic-acid. Wu¹⁵ tried to demetallated some metalloporphyrins with methane sulfonic acid at different temperatures. In all case, the spectra of the metalloporphyrins were not

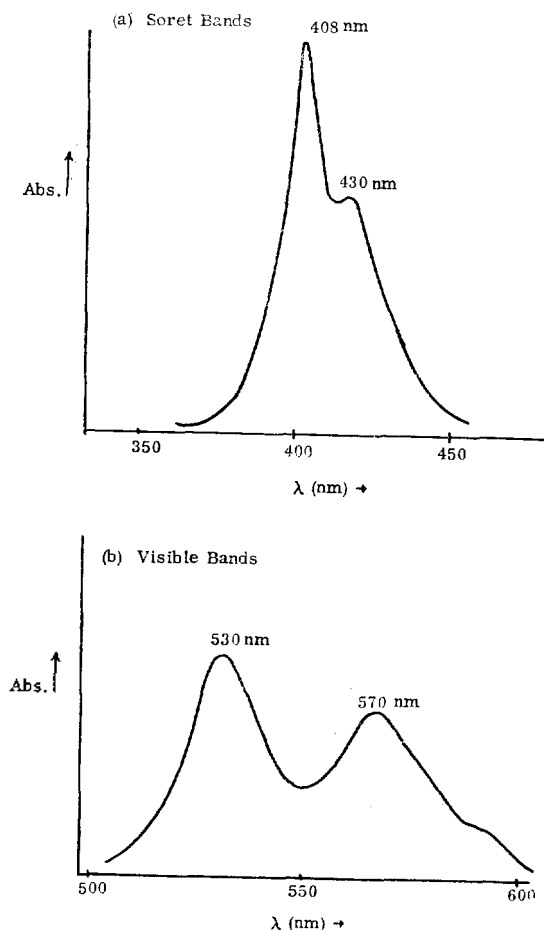


Fig. 3. Visible and soret bands of vanadyl porphyrin complex with pyridine.

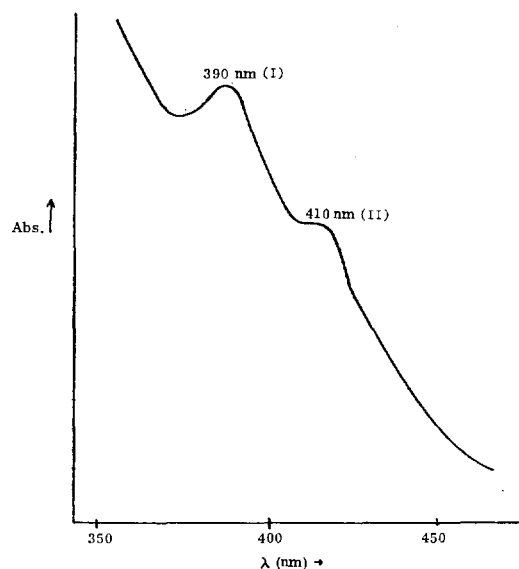


Fig. 4. Soret bands of nickel porphyrin in pyridine-methylene chloride solution.

altered. Hydrofluoric acid demetallated metalloporphyrins in DMSO-soluble fraction at -78°C for 3 hours, affording etio- and phyllo-type porphyrins. These porphyrins were purified by silica gel chromatography by elution with benzene, and the ethyl acetate mixture, and each metal free porphyrins were identified by the spectrum for those of authentic samples.

EXPERIMENTAL

Separation of Asphaltenes from the Petrolenes. Two hundred eighty-two grams of a crude oil was placed in a 6l Florence flask and 4.6l of *n*-pentane was added in 11 increments, swirling the contents of the flask for several minutes between each addition. After all the solvent had been added, the mixture was swirled for a while and allowed to stand for one week with occasional swirling. The supernatant was then filtered off, and the remaining solids washed with *n*-pentane until the color of the wash was very lightly colored. The recovered black solids placed in a vacuum desiccator and evacuation was continued until removal of the solvent was accomplished.

Gel Permeation Chromatography (GPC). Three grams of asphaltenes was dissolved in 30 ml of benzene and the resulting solution poured on the gel column 4.3 by 112cm. The column had been packed with a polystyrene gel of about 2,000 molecular weight exclusion limits.

Development of the chromatogram was accomplished with benzene and a flow rate of 6ml/min was maintained. The first 120ml of effluent provided the first fraction, the next 180ml the second fraction and the remaining 500ml the third fraction. Each fraction was evaporated under reduced pressure. A third fraction afforded 0.5~0.8g of metalloporphyrins.

Dimethyl Sulfoxide Treatment (DMSO). Seventy-two grams of GPC fraction I of Boscan

asphaltenes was dissolved in 1.4l of pyridine at room temperature. Two hundred ten milliliters of dimethylsulfoxide (DMSO) was added to the pyridine solution and the mixture filtered. The filtrates, DMSO-soluble materials, were cooled in a refrigerator and filtered in the same manner seven times more. About 300ml of the final filtrates, DMSO-soluble materials, were evaporated at a reduced pressure. When the volume was reduced to about 100ml, 500ml of water was added to the solution and the resulting mixture filtered. The precipitates, DMSO-soluble dark materials, were kept at room temperature overnight under reduced pressure to remove solvent.

Alumina Treatment. About 0.5g of sample was dissolved in 400ml of benzene. Three hundred grams of alumina was shaken with the solution, and after an hour the light-brownish benzene solution was decanted. This step was followed by two or three washings of the alumina with benzene, discarding each of the washings. Metalloporphyrin material was removed from the alumina by treatment with a solvent mixture of 150ml benzene-2.1ml pyridine until the extracts showed no intensive brownish-purple color. The benzene-pyridine solution was then evaporated by a rotatory evaporator to remove benzene and the remaining pyridine solution was dissolved in methylene chloride to run the visible spectrum. The visible spectra were run on a Beckman DK2 spectrophotometer before evaporation of pyridine. After extraction of metalloporphyrins with 2.1ml pyridine-150 ml benzene mixture, the same procedures were followed using 15ml-pyridine-135ml benzene, 75ml pyridine-75ml benzene and 75ml dimethyl formamide-75ml benzene mixtures to extract more metalloporphyrin materials.

Silica Gel Column Chromatography. A

sample of 0.1~0.5g of metalloporphyrin material was dissolved in a minimum amount of benzene. The solution was carefully added to a column 3.8 by 25cm. The column had been packed with a slurry of 15g silica gel and 15g celite in benzene. The following solvents were used to elute metalloporphyrin material, hexane, benzene-hexane (1:1), benzene-chloroform (1:1) and chloroform. Each of the benzene-hexane and benzene-chloroform eluates afforded 60~300mg of metalloporphyrin and the visible spectra of each eluates were run.

Hydrofluoric Acid Treatment. An amount of 0.37g of porphyrin-rich material was dissolved in 10ml toluene. This solution was added to 20ml of anhydrous HF while stirring with a magnetic stirrer at -78°C . After 3~10 hours the solution was washed with water and dissolved in a minimum amount of benzene. This benzene solution was subjected to silica gel chromatography. The chromatogram was eluted with benzene and benzene-ethyl acetate-methylalcohol (85:13:2) mixture. Each eluate was extracted with dilute HCl and after neutralization, visible spectra were run on a Beckman DK2 spectrophotometer.

Analysis for Metals and Porphyrins. The concentration of metal and porphyrins was determined according to the procedure of R. Bean (16).

CONCLUSION

Gel permeation chromatographic techniques allowed separation of nickel and vanadyl porphyrins from crude asphalts in relatively large amounts and these metalloporphyrins were further purified by silica gel chromatography and alumina treatment with pyridine, which react as ligand of complex. Some metallopor-

phyrins were demetallated by hydrogen fluoride to produce some metal free porphyrins such as etio-and phyllo-type porphyrins.

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