

Short Communication

Modification of reversed-phase liquid chromatographic retention characteristics for metal-tetraphenylporphyrin complexes using octane as a mobile phase additive

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ABSTRACT

The effect of the addition of octane to a methanolic mobile phase on the reversed-phase liquid chromatographic separation of a series of porphyrin compounds is described. The capacity factors of tetraphenylporphyrin (TPP) and five metal-TPP complexes decreased with increase in the octane content of the mobile phase. Selective sorption of octane on the surface of ODS occurred. The addition of octane to the mobile phase caused an alteration in the sequence of **elution**, improved the resolution of some TPP complexes and reduced the time required for analysis.

INTRODUCTION

Reversed-phase liquid chromatography (RPLC) is today carried out mainly with octyl- or octadecyl-bonded silica gel (ODS) and one of three solvents, methanol, acetonitrile or tetrahydrofuran. Only a few studies have been carried out using other solvents [1,2] in place of or in addition to the above solvents. When the mobile phase additive is selectively sorbed on (or incorporated in) the stationary phase substance, the physical properties of the stationary phase are affected by the additive, and the retention characteristics of analyte compounds are influ-

enced by the mobile phase additive [3,4]. The so-called "non-aqueous" RPLC using methanol-dichloromethane or acetone-acetonitrile is often applied to some kinds of compounds.

This paper reports an investigation of the effect of an **alkane** as an additive to a methanolic mobile phase in RPLC using ODS, as alkanes are expected to have a strong affinity for the ODS phase. Octane was taken as a model **alkane** and selective sorption of octane on ODS was confirmed. The feasibility of improving separations by the addition of octane to a methanolic mobile phase was examined for the separation of metal complexes of tetraphenylporphyrin (TPP). Chromatography of metal **chelate** complexes, such as those of **β -diketones** [5], porphyrins [6] and chlorophylls [7], is one of our major interests.

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EXPERIMENTAL

Materials

Octane was distilled after stirring with 15% fuming sulphuric acid for 1 day. Methanol was distilled. Acetone and sodium nitrate were of analytical-reagent grade.

5,10,15,20-Tetraphenylporphine (H_2tpp), which is the free acid form of TPP (see Fig. 1), was synthesized by the method of Adler et al. [8] and purified by the method of Bamett et al. [9]. The metal complexes of TPP with oxovanadium(IV), nickel(II), copper(II), zinc(II) and palladium(II) [VO(tpp), Ni(tpp), Cu(tpp), Zn(tpp) and Pd(tpp), respectively] were prepared by the general method by Adler et al. [10].

HPLC

The HPLC system consisted of a Hitachi (Tokyo, Japan) Model 655-15 pump, a Rheodyne (Cotati, CA, USA) Model 7125 injection valve with a 10 mm³ sample loop, a Shimadzu (Kyoto, Japan) Model SPD-6AV UV-visible spectrophotometric detector and a Shimadzu Model C-R6A chromatographic data processor. An octadecyl-bonded silica gel column, TSK-gel ODS-120T (particle size 5 μm; 150 mm × 4.6 mm I.D.) (Tosoh, Tokyo, Japan), was used. A methanolic solution containing octane at a mole fraction (X_{oct}) smaller than 0.061 (corresponding to a 0.21 volume fraction) was used as the mobile phase.

The sample solution of each TPP compound was prepared at a concentration of 4×10^{-6} M in acetone. Typically, a 1-mm³ aliquot of the sample solution was injected into the column, and the elution was monitored at a detection wave-

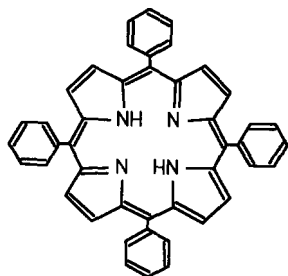


Fig. 1. Structural formula of H_2tpp .

length of 420 nm. All experiments were carried out in a thermostated room at $25.0 \pm 0.5^\circ\text{C}$.

RESULTS AND DISCUSSION

Selective incorporation of octane into the ODS phase

In order to elucidate the equilibration of the column with a methanol-octane mixture as the mobile phase, the retention time (t_R) of Zn(tpp) was measured during conditioning of the column. As a result, 50 cm³ of the mobile phase were found to be sufficient to make the t_R constant in every case. After conditioning of the column, the liquid retained in the column was eluted with acetone and the eluate was collected in a 25-cm³ volumetric flask. Octane and methanol in the eluate were determined by gas chromatography.

The amount of octane incorporated in the stationary phase ($Q_{oct,s}$) is related to the total amounts of octane contained in the column ($Q_{oct,t}$) and just in the mobile phase ($Q_{oct,m}$):

$$Q_{oct,s} = Q_{oct,t} - Q_{oct,m} = Q_{oct,t} - C_{oct,m}V_m$$

where $C_{oct,m}$ is the molarity of octane in the mobile phase and V_m is the volume of the mobile phase, that is, the column void volume. In a similar manner, the amount of methanol contained in the stationary phase ($Q_{MeOH,s}$) was calculated. In this work, V_m was determined by injection of a sodium nitrate solution (nearly

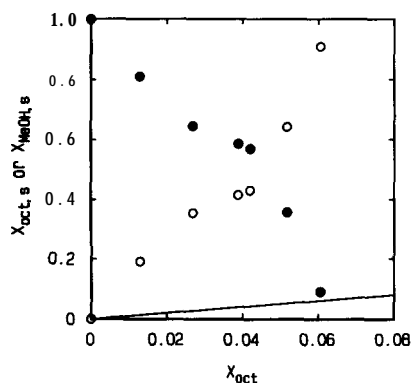


Fig. 2. Plots of (○) $X_{oct,s}$ and (●) $X_{MeOH,s}$ as a function of X_{oct} . The line represents the relationship $X_{oct,s} = X_{oct}$.

saturated solution in the methanol-octane used as the mobile phase). The V_m value was independent of the injection volume in the range 10–25 mm³.

The mole fractions of octane ($X_{\text{oct},s}$) and methanol ($X_{\text{MeOH},s}$) in the sorbed portion of the mobile phase components on the stationary phase are given by

$$X_{\text{oct},s} = Q_{\text{oct},s} / (Q_{\text{oct},s} + Q_{\text{MeOH},s})$$

and

$$X_{\text{MeOH},s} = Q_{\text{MeOH},s} / (Q_{\text{oct},s} + Q_{\text{MeOH},s})$$

Fig. 2 shows the plots of $X_{\text{oct},s}$ and $X_{\text{MeOH},s}$ as a function of the mole fraction of octane in the mobile phase (X_{oct}). It is obvious that all the $X_{\text{oct},s}$ points show a positive deviation from the line indicating equality of the compositions of the methanol-octane in the mobile and stationary phases. This implies that the incorporation of octane into the surface layer of ODS occurs preferentially to methanol, and in this manner the stationary phase was modified with a larger proportion of octane and a smaller proportion of methanol than those in the mobile phase.

Capacity factors of TPP compounds

The capacity factor (k') of a TPP compound was calculated from its retention volume (V_r) and V_r . The V_r value was obtained with a relative standard deviation of less than 1% in each instance ($n = 3$). The effect of the octane content of the mobile phase on the retention of each TPP compound was shown by plotting $\log k'$ versus X_{oct} (Fig. 3).

It was found for every TPP compound that the use of an octane-containing mobile phase causes a lower retention than that in pure methanol. This effect is attributable to the decrease in the polarity of the mobile phase by the addition of such a non-polar component as octane. The retention, however, does not always decrease monotonously with increasing octane content of the mobile phase. When the X_{oct} value has been increased to 0.061, a significant enhancement of retention is observed for the TPP compounds having relatively large k' values, *viz.*, H_2tpp ,

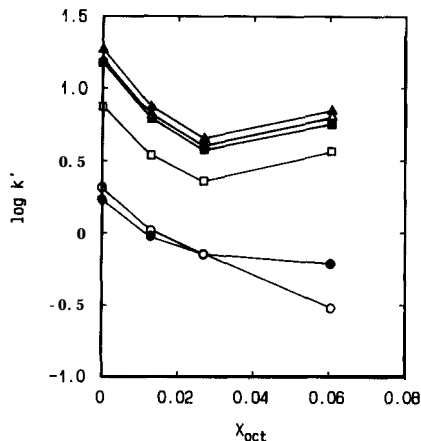


Fig. 3. Retention ($\log k'$) of TPP compounds plotted as a function of the mole fraction of octane (X_{oct}) in the mobile phase. $\circ = \text{Zn}(\text{tpp})$; $\bullet = \text{VO}(\text{tpp})$; $\square = \text{H}_2\text{tpp}$; $\blacksquare = \text{Pd}(\text{tpp})$; $\triangle = \text{Ni}(\text{tpp})$; $\blacktriangle = \text{Cu}(\text{tpp})$.

$\text{Pd}(\text{tpp})$, $\text{Ni}(\text{tpp})$ and $\text{Cu}(\text{tpp})$. This implies that the distribution of a TPP compound in the column is influenced by the alterations both in the composition of the stationary phase and in the composition of the mobile phase. It is shown in Fig. 2 that the larger the X_{oct} value, the more the stationary phase is modified with octane in preference to methanol.

It is shown in Fig. 3 that the retention order for $\text{Zn}(\text{tpp})$ and $\text{VO}(\text{tpp})$ in particular depends on the octane content in the methanolic mobile phase. This is a useful characteristic of octane as a mobile phase additive for the separation of metal-TPP complexes.

Separation of TPP compounds

Fig. 4a shows the chromatogram for the separation of a series of TPP compounds with methanol as the mobile phase. The retention sequence for the TPP compounds is consistent with those observed with different solvents in both column liquid chromatography and thin-layer chromatography [11,121].

When octane was added to the mobile phase, the separation characteristics depend on the octane content in the mobile phase, as shown in Fig. 4b–d. The retention time of each TPP compound decreased with increasing octane content in the mobile phase. In addition, the elution

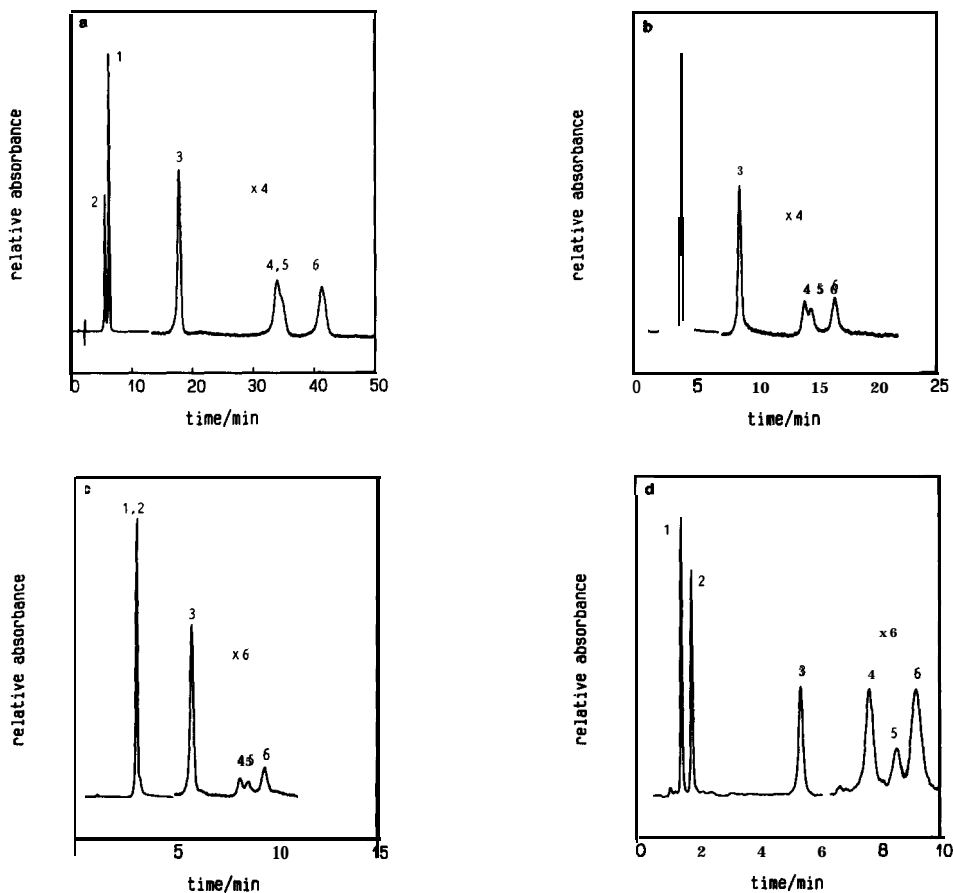


Fig. 4. Chromatographic separation of TPP compounds. Column: TSK-gel ODS-120T (150 mm \times 4.6 mm I.D.). Mobile phase: methanol-octane, X_{oct} = (a) 0, (b) 0.013, (c) 0.027 and (d) 0.061. Flow rate: 0.80 cm^3/min . Detection wavelength: 420 nm. 1 = Zn(tpp); 2 = VO(tpp); 3 = H_2tpp ; 4 = Pd(tpp); 5 = Ni(tpp); 6 = Cu(tpp).

sequence for the Zn(tpp) and VO(tpp) was the reverse of that given with pure methanol.

Ni(tpp) could not be resolved from Pd(tpp) with methanol (see Fig. 4a), but their resolution was improved with increasing octane content in the methanolic mobile phase. When the octane content was increased to 0.061 mole fraction (methanol:octane = 79:21, v/v), almost a baseline separation was achieved for six TPP compounds, as shown in Fig. 4d.

In conclusion, octane is an effective mobile phase additive for varying the elution sequence and the time for the separation of TPP and its metal complexes in RPLC using an ODS stationary phase and a methanolic mobile phase.

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REFERENCES

- 1 G.J.-L. Lee, R.M.K. Carlson and S. Kushinsky, *J. Chromatogr.*, **212** (1981) 108.
- 2 J.W. Huber, III, and C.L. Seaver, *J. Liq. Chromatogr.*, **10** (1987) 2337.
- 3 J.H. Knox and A. Pryde, *J. Chromatogr.*, **112** (1975) 171.
- 4 R.M. McCormick and B.L. Karger, *J. Chromatogr.*, **199** (1980) 259.

- 5 S. Tsukahara, K. Saitoh and N. Suzuki, *J. Chromatogr.*, 547 (1991) 225.
- 6 N. Suzuki, K. Saitoh and Y. Sugiyama, *Chromatographia*, **22 (1986) 132**.
- 7 K. Adachi, K. Saitoh and N. Suzuki, *J. Chromatogr.*, **457 (1988) 99**.
- 8 A.D. Adler, F.R. Longo, J.D. Finavelli, J. Goldmacher, J. Assour and L. Korsakoff, *J. Org. Chem.*, **32 (1967) 478**.
- 9 G.H. Barnett, M.F. Hudson and K.M. Smith, *J. Chem. Soc., Perkin Trans. 1*, **(1975) 1401**.
- 10 A.D. Adler, F.R. Longo, F. Kampas and J. Kim, *J. Inorg. Nucl. Chem.*, 32 (1970) 2443.
- 11 K. Saitoh, M. Kobayashi and N. Suzuki, *Anal. Chem.*, **53 (1981) 2309**.
- 12 K. Saitoh, M. Kobayashi and N. Suzuki, *J. Chromatogr.*, **243 (1982) 291**.