

HPLC SEPARATION OF PORPHINE, SELECTED PORPHYRINS, AND THEIR METAL DERIVATIVES

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SUMMARY

Porphine, *meso*-mono-*tert*-butylporphyrin, *meso*-tetra-*tert*-butylporphyrin, and metalloporphyrins containing Zn(II), Cu(II), and Ni(II) have been separated by adsorption HPLC on a Hypersil column with *n*-hexane–chloroform mixtures (7:3, 8:2, 9:1, and 10:0, *v/v*) as mobile phases. The separations obtained were compared.

INTRODUCTION

Because porphyrins and metalloporphyrins are compounds of both biological [1–5] and chemical [6–12] importance, their chromatographic characteristics have long been investigated in our laboratories [13–21]. The aim of this study was to compare HPLC separations of porphine (P),

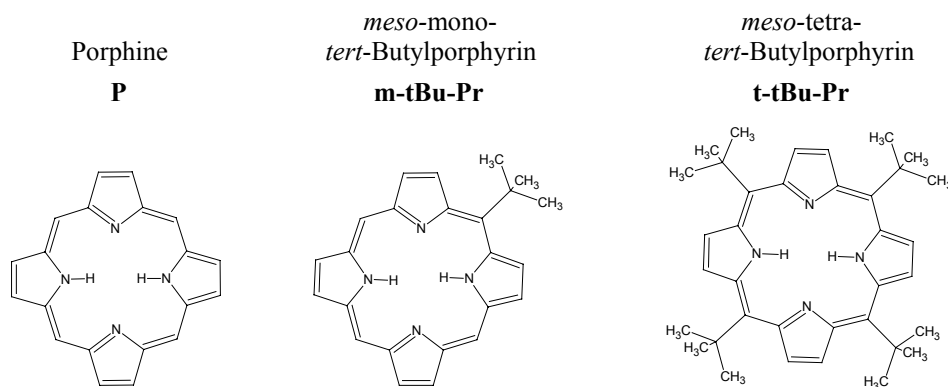


Fig. 1

Chemical formulae of the porphyrins investigated

meso-mono-*tert*-butylporphyrin (m-tBu-Pr), and *meso*-tetra-*tert*-butylporphyrin (t-tBu-Pr) with those of their Ni(II), Cu(II), and Zn(II) derivatives. The structures of the compounds investigated are given in Fig. 1.

EXPERIMENTAL

Materials

The porphyrins investigated and their metal derivatives were synthesized as reported elsewhere [22,23].

Normal-Phase HPLC

HPLC was performed with a Laboratorni Pristroje (Praha, Czech Republic) chromatograph equipped with a UV-visible LCD detector. Separations were performed at room temperature on a 250 mm × 4.6 mm i.d., 5 μm particle, Hypersil silica column. *n*-Hexane-chloroform mixtures (7:3, 8:2, 9:1, and 10:0, *v/v*) were used as mobile phases. The mobile phase flow rate was 0.8 mL min⁻¹. Solutions (50 μg mL⁻¹) of the porphyrins were prepared in chloroform and 5-μL volumes were injected on to the column. Chromatograms were recorded at two wavelengths, 405 nm for porphine, *meso*-mono-*tert*-butylporphyrin, and their metal derivatives and 436 nm for *meso*-tetra-*tert*-butylporphyrin and its metal derivatives.

RESULTS AND DISCUSSION

The results obtained from this investigation are shown in Tables I and II and Figs 2 and 3. Tables I and II show retention times, t_R (min), and Δt_R (min) values obtained after separation by adsorption HPLC. Figure 2 shows the relationship between the t_R values of the porphyrins and those of their derivatives with different cations. Figure 3 shows the relationship between the t_R values of derivatives of P, m-tBu-Pr, and t-tBu-Pr with the same cations.

The principal goal of this study was to compare adsorption HPLC separations of P, m-tBu-Pr, and t-tBu-Pr with those of their metal derivatives (zinc, copper, and nickel). Four different mobile phases were used: *n*-hexane-chloroform (7:3, 8:2, and 9:1 *v/v*) and hexane (one-component phase). It is apparent from Figs 2 and 3 that addition of chloroform to the hexane results in general reduction of retention times. Of the substances examined the longest retention times and, therefore, the strongest adsorp-

Table I

Effect of changing the cation on t_R (min) and Δt_R (min) values of the substances examined

Substance	<i>n</i> -Hexane–chloroform							
	7:3 (v/v)		8:2 (v/v)		9:1 (v/v)		10:0 (v/v)	
	t_R	Δt_R	t_R	Δt_R	t_R	Δt_R	t_R	Δt_R
ZnP	4.67	0.74	6.00	1.67	8.50	3.33	14.80	9.13
P	3.93		4.33		5.17		5.67	
NiP	3.80		4.17		4.67		4.83	
CuP	3.70		4.17		4.50		4.50	
Zn m-tBuPr	3.80	0.00	5.83	1.66	7.83	2.83	14.34	8.84
m-tBuPr	3.80		4.17		5.00		5.50	
Ni m-tBuPr	3.73		4.00		4.58		4.33	
Cu m-tBuPr	3.67		4.00		4.33		4.17	
Zn t-tBuPr	3.80	0.20	5.00	0.84	5.17	0.84	7.17	1.84
t-tBuPr	3.60		4.16		4.33		5.33	
Ni t-tBuPr	3.53		3.83		3.83		4.33	
Cu t-tBuPr	3.47		3.83		3.67		4.17	

Table II

Comparison of t_R (min) and Δt_R (min) for derivatives with the same cation

Substance	<i>n</i> -Hexane–chloroform							
	7:3 (v/v)		8:2 (v/v)		9:1 (v/v)		10:0 (v/v)	
	t_R	Δt_R	t_R	Δt_R	t_R	Δt_R	t_R	Δt_R
ZnP	3.93	0.13	4.33	0.16	5.17	0.17	5.67	0.17
Zn m-tBuPr	3.80		4.17		5.00		5.50	
Zn t-tBuPr	3.60		4.16		4.33		5.33	
P	4.67	0.87	6.00	0.17	8.50	0.67	14.84	0.50
m-tBuPr	3.80		5.83		7.83		14.34	
t-tBuPr	3.80		5.00		5.17		7.17	
NiP	3.80	0.07	4.17	0.17	4.67	0.09	4.83	0.50
Ni m-tBuPr	3.73		4.00		4.58		4.33	
Ni t-tBuPr	3.53		3.83		3.83		4.33	
CuP	3.70	0.03	4.17	0.17	4.50	0.17	4.50	0.33
Cu m-tBuPr	3.67		4.00		4.33		4.17	
Cu t-tBuPr	3.47		3.83		3.67		4.17	

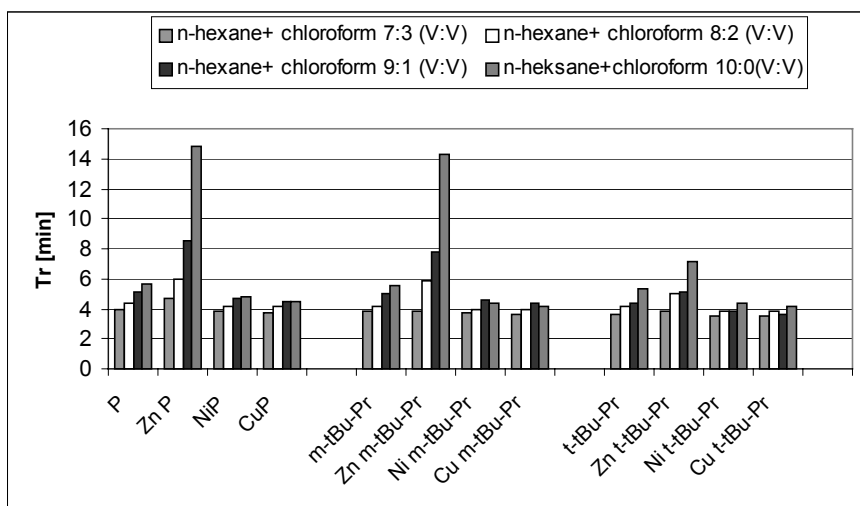


Fig. 2

Comparison of the t_R values of porphyrins and metalloporphyrins with different cations separated by adsorption HPLC

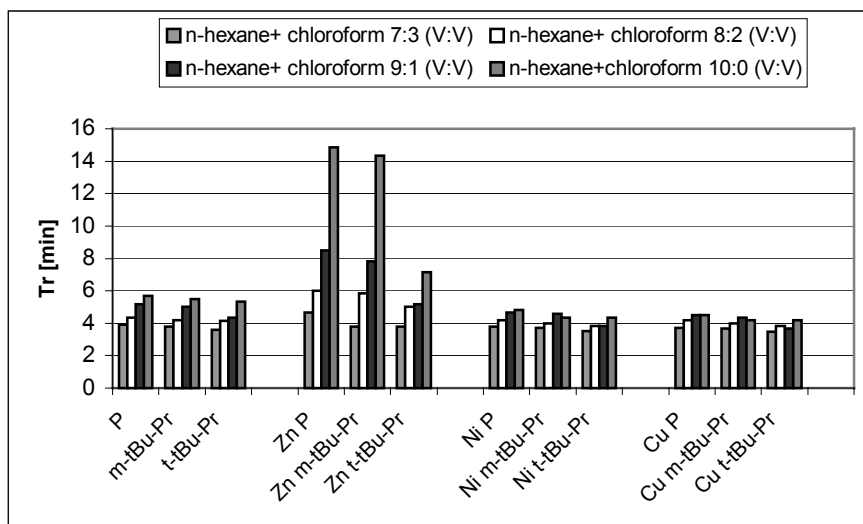


Fig. 3

Comparison of the t_R values of porphyrins and metalloporphyrins with the same cation separated by adsorption HPLC

tion was observed for ZnP and porphine. Similar short retention times were observed for NiP and CuP. Zn m-tBu-Pr was distinctly more strongly adsorbed than m-tBu-Pr, Ni m-tBu-Pr and Cu m-tBu-Pr. Similar behaviour was observed for the t-tBu-Pr derivatives. Among all the substances examined the zinc derivatives were the most strongly adsorbed; this enabled separation of the Zn(II) metalloporphyrins from the other metalloporphyrins.

Similar retention times and similar weak adsorption were observed for the Cu(II) and Ni(II) derivatives of the porphyrins. The retention data show there was no separation of these metalloporphyrins. Comparison of the data presented in Fig. 3 reveals the absence of separation of the nickel and copper derivatives, especially when the mobile phases were hexane–chloroform 7:3 or 8:2 (v/v). Good chromatographic separation of the zinc derivatives and of the free porphyrins was achieved irrespective of the mobile phase used. Increasing the volume of hexane in the mobile phase resulted in a general increase in the retention times of the zinc derivatives whereas for the nickel and copper metalloporphyrins changing the composition of the mobile phase did not cause distinct changes in the retention times of the substances. Addition of hexane caused a distinct increase in the retention time of t-tBu-Pr. For the other porphyrins no distinct increases of retention times were observed.

The retention times of the investigated substances are principally influenced by the presence of a metal ion in a porphyrin co-ordination centre. Retention times were little influenced by the presence of aliphatic chains on the porphyrin rings except for the zinc derivatives – for these retention times increased distinctly as the number of aliphatic chains attached to the porphyrin ring was reduced.

This adsorption HPLC investigation demonstrates that the zinc derivatives of the porphyrins have structures different from those containing copper or nickel. The presence of the zinc(II) cation results in formation of a planar porphyrin structure [24–26] whereas metalloporphyrins containing copper(II) and nickel(II) have non-planar structures [27,28]. The retention data corroborate the similar spatial structures of the nickel and copper derivatives of the porphyrins examined.

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