

Control of the retention selectivity of rare earth octaethylporphyrins in reversed-phase high-performance liquid chromatography using amines as mobile phase additives

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ABSTRACT

The reversed-phase liquid chromatographic retention behaviour of the complexes of octaethylporphyrin (OEP) with eleven trivalent rare earth (REs), *viz.*, Y, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu, on an octadecyl-bonded silica gel column is described. All these complexes can be eluted without demetallation with a methanol–water mixture that contains a small amount (about 1%) of acetylacetone and an amine. The retention order of the RE–OEP complexes depends on the amine used. With a mono-*n*-alkylamine and a di-*n*-alkylamine, the retention of the RE–OEP complex decreases with increasing atomic number of the REs within the lanthanide series, whereas the reverse tendency occurs with a trialkylamine and a dialkylamine possessing branched alkyl side-chains or alcoholic side-chains. In all instances, the retention of the Y complex is close to that of the Dy complex. Successful separation of the OEP complexes of Lu, Yb, Tm, Er and Sm in 20 min is demonstrated with dihexylamine.

INTRODUCTION

There has been increased interest in the separation of metalloporphyrins from the biomedical [1], geological [2] and analytical [3] viewpoints. High-performance liquid chromatography (HPLC) is the most powerful tool for the separation of these compounds today [4]. HPLC separations of a limited number of metal complexes of synthetic porphyrins, have been investigated, e.g. porphine [5], haematoporphyrin [6], *meso*-tetraphenylporphine (TPP) [7], *meso*-tetrakis (*p*-tolyl)porphine [8] and pheophorbide-a (not a porphyrin, but a compound with a chlorin structure) [9].

In general, the separation of metalloporphyrins with different central metal ions is more difficult than the separation of different porphyrin complexes of a certain metal, because the metal ion is surrounded by a bulky macrocyclic porphyrin structure to which various organic substituents are at-

tached. The separation of metalloporphyrins becomes increasingly difficult as the metals become more similar chemically. The feasibility of the reversed-phase HPLC separation of the TPP complexes of trivalent rare earths (REs) was previously confirmed [10]. It was found that the separation selectivity for the RE–TPP complexes varied with the type of amine added to the mobile phase.

This work was undertaken to examine the effects of amines on the chromatographic retention characteristics of the RE complexes of octaethylporphyrin (OEP), which had been frequently used as a model porphyrin, similarly to TPP, in studies relating to porphyrin. OEP differs from TPP in its molecular structure. In the TPP molecule, four relatively bulky phenyl groups are bonded with four *meso*-carbons to the porphyrin nucleus, whereas in OEP eight ethyl groups are bonded to eight β -pyrrolic positions.

EXPERIMENTAL

Materials

The free acid form of OEP (H_2oep) was obtained from Strem Chemicals (Newburyport, MA, USA). The complexes of OEP with RE (III) (RE = Y, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu) were synthesized by reacting H_2oep (60 mg, 0.11 mmol) with the corresponding RE acetylacetonate $[RE(acac)_3]$ (110–130 mg, 0.25 mmol) while refluxing in 1,2,4-trichlorobenzene (12 ml) in a stream of nitrogen, a modification of the method used for the preparation of a mixed ligand complex of an RE with TPP and acetylacetonate, $RE(tpp)(acac)$ [11]. The reaction mixture diluted with 200 ml of toluene and then poured onto a neutral alumina column (70 mm \times 12.5 mm I.D.). After complete elution of the unreacted H_2oep with toluene–acetone (30:1, v/v), the RE complex was eluted with dimethyl sulphoxide–water (80:20, v/v). The eluate was mixed with water (1:1) and the RE–OEP complex was extracted with dichloromethane. After removal of the solvent, the complex was obtained as a crystalline material of reddish violet needles or as an amorphous solid. When examined by UV–VIS spectrophotometry, each material gave two sharp absorption peaks corresponding to the so-called α -band and β -band peaks in the 500–600-nm region, implying that the material was a metal complex of porphyrin [12]. A significant peak appeared in the mass spectrum (recorded with desorption electron impact ionization) at an m/z value consistent with the molecular ion $[RE(oep)(acac)]^+$; for example, the Lu(III) complex gave a peak at m/z 806. The complexes thus prepared were identified as mixed-ligand complexes with tetradentate oep and bidentate acetylacetonate (acac) anions, $RE(oep)(acac)$. The structural formula of this complex is illustrated in Fig. 1, assuming that it is analogous to that of $RE(tpp)(acac)$ [13]. The term RE–OEP complex is hereafter taken to mean $RE(oep)(acac)$, unless indicated otherwise.

Acetylacetonate (Hacac), octylamine (OA), diethylamine (DEA), dipropylamine (DPA), diisopropylamine (DIPA), dihexylamine (DHA), diethanolamine (DEOLA), triethylamine (TEA), tripropylamine (TPA), dichloromethane and sodium hydroxide (NaOH) were of analytical-reagent grade (Wako, Osaka, Japan). Methanol and water were distilled in glass.

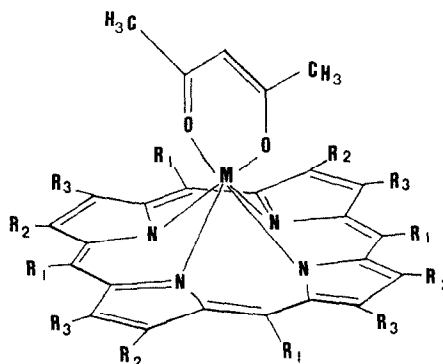


Fig. 1. Structural formula of the RE–OEP ($R_1 = H$; $R_2 = R_3 =$ ethyl) and the RE–TPP ($R_1 =$ phenyl; $R_2 = R_3 = H$) complexes. M indicates the RE(III) ion.

HPLC

A Twinkle solvent delivery pump, a VL-611 sample injection valve (Jasco, Tokyo, Japan) and a Model SPD-M6A photodiode-array UV–VIS spectrophotometric detector (Shimadzu, Kyoto, Japan) were used with a Model TSK ODS-80TM column (150 mm \times 4.6 mm I.D.) packed with octadecyl-bonded silica gel (5 μ m) (Tosoh, Tokyo, Japan).

The mobile phase was prepared, unless indicated otherwise, by adding an amine to the mixture of methanol–water–Hacac (95:5:1, v/v/v) so that the amine was of equimolar concentration with respect to Hacac in the final solution (about 0.1 M). In order to avoid damaging the column, when NaOH was used in place of an amine, it was added to the methanol–water–Hacac mixture so as to be approximately half the equimolar concentration with respect to Hacac in the final composition of the mixture. The flow-rate of the mobile phase was 1 ml/min.

The sample solution of an RE–OEP complex was prepared at a concentration of about 0.1 mM in dichloromethane containing DEA (2%, v/v) and a 10- μ l aliquot of each solution was injected into the column. All experiments were carried out at $25 \pm 1^\circ C$.

RESULTS AND DISCUSSION

Capacity factors

The OEP complexes of light lanthanides, particularly of Sm(III) and Eu(III), showed low stability

in common solvents such as methanol, acetonitrile, acetone, dichloromethane and benzene: demetallation of the complexes occurred within 1 h after the preparation of the solutions at about the 0.1-mM level. These complexes could be stabilized by addition of a small amount of an amine, such as DEA, to the solution, as for RE complexes of TPP [4]. The solution of an RE-OEP complex to be applied in HPLC was prepared in dichloromethane-DEA (50:1, v/v).

The capacity factor (k') of an RE-OEP complex was calculated from its retention volume and the column void volume, which was assumed to be equal to the retention volume of sodium nitrate (a nearly saturated solution was injected). The k' value was determined from triplicate measurements, with a relative standard deviation of less than 1%.

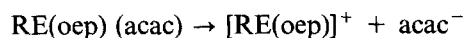
Effects of amines on the retention of the RE-OEP complexes

No RE-OEP complex could be eluted from the HPLC column with a mixture of methanol and water (e.g., 95:5, v/v) or methanol alone; in every instance, H_2O_{ep} was not detected in the eluate. [The k' value of H_2O_{ep} was the smallest ($k' = 4.24$) when using methanol as the mobile phase, and increased with increasing water concentration in the methanol-water mixture.] This implied that the dissociation of the OEP ligand from the RE complex did not occur to any detectable extent, and that the complex adsorbed strongly on the column packing material.

The OEP complexes of Lu(III), Yb(III), Tm(III) and Er(III) could be eluted with a mobile phase containing Hacac, e.g., with methanol-water-Hacac (95:5:1, v/v/v). All other RE-OEP complexes were eluted successfully by addition of both Hacac and an amine, such as DEA, to the mobile phase.

It was considered that the dissociation of the anionic bidentate ligand, $acac^-$, from the RE-OEP complex occurred in the Hacac-free mobile phase, and resulted in a cationic species, $[RE(oep)]^+$, that was adsorbed by a cation-exchange site (presumably dissociated silanol group) on the surface of the alkyl-bonded silica gel. Similar phenomena have been reported for the TPP complexes of trivalent metals, such as Mn(tpp)Cl and Co(tpp)Cl, in reversed-phase HPLC, where strong adsorption of the complexes was suppressed by addition of am-

monium chloride to the mobile used [15]. Higher $acac^-$ concentrations in the mobile phase were considered to eliminate the anomalous retention behaviour of the RE-OEP complex by suppression of the following dissociation:



One of the functions of the amine added to the mobile phase is to act as a base and promote the dissociation of the weak acid Hacac, thereby increasing the concentration of the $acac^-$ anion. In this respect, all amines (and also simple inorganic bases, such as NaOH) are expected to be useful mobile phase additives for the successful elution of RE-OEP complexes.

It was confirmed, by means of real-time monitoring of the UV-VIS spectra of the eluate with a photodiode-array spectrophotometric detector, that undesirable chemical reactions did not occur to any detectable extent between the injected complex and the metal components of the HPLC system.

Effects of the molecular structure of the amine additive

When a methanol-water-Hacac-amine mixture was used as the mobile phase, the k' of the RE-OEP complexes and also their retention sequence varied with the type of amine used, as shown in Fig. 2. With mobile phases containing monoalkylamines, such as OA, and dialkylamines, such as DEA, DPA and DHA, the k' of RE-OEP increased with decreasing atomic number (Z) of the RE within the lanthanide series. On the other hand, with trialkylamines, such as TEA and TPA, the opposite trend was observed for these metal complexes. It is shown in Fig. 3 that the retention trends observed with DIPA and DEOLA are different from those obtained with other dialkylamines, but similar to the trends observed with trialkylamines (see Fig. 2). If amines functioned only as bases promoting the dissociation of the weak acid, Hacac, the retention order should depend little on the molecular structure of the amine, and should be similar to the trends observed with NaOH. In fact, when NaOH was used in the place of an amine, the k' values decreased with decreasing Z of the lanthanides, as shown in Fig. 3. This retention trend is similar to those observed with DIPA and trialkylamines, such as TEA and TPA, and DIPA.

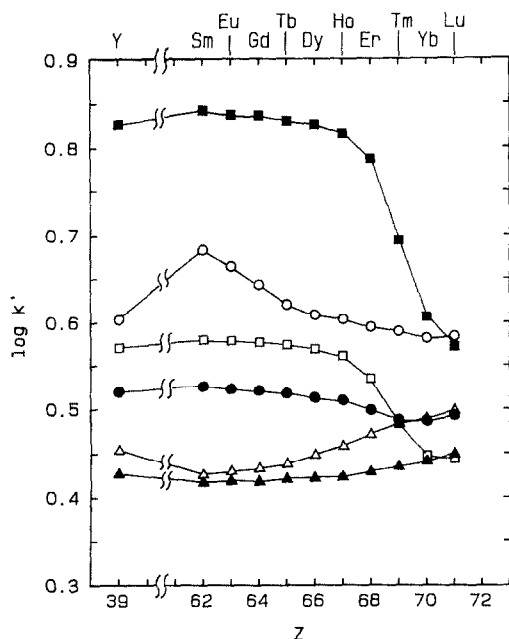
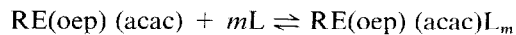


Fig. 2. Relationship between the retention of RE-OEP complexes and the atomic number (Z) of the RE. Mobile phase: methanol-water-Hacac-amine (95:5:1: x , v/v). Amines: \circ = OA ($x = 1.6$); \bullet = DEA ($x = 1.0$); \square = DPA ($x = 1.3$); \blacksquare = DHA ($x = 2.3$); \triangle = TEA ($x = 1.3$); \blacktriangle = TPA ($x = 1.8$).

Function of the amine additive

Trivalent REs can have coordination numbers (CN) larger than 6 [16]. Accordingly, The coordination sphere of the RE ion in an RE-OEP is not necessarily saturated by the tetradentate ligand OEP and bidentate acac, rather coordination with an additional ligand (L) in the mobile phase, such as water, methanol or an amine, may be possible:



In the case of RE-TPP complexes in dimethyl sulphoxide, a CN of 8 was determined for the light lanthanides from Ce to Tb and a CN of 7 for the heavy lanthanides from Dy to Lu [17].

It is reasonable to predict that the stronger the coordinative interaction that occurs between the RE ion and the hydrophilic ligand(s) such as water and/or methanol, the more the capacity factor of the RE-oep is reduced in the reversed-phase system. The retention trend for the RE-OEP complexes observed without an amine (see Fig. 3 with NaOH instead of an amine) implies that the coord-

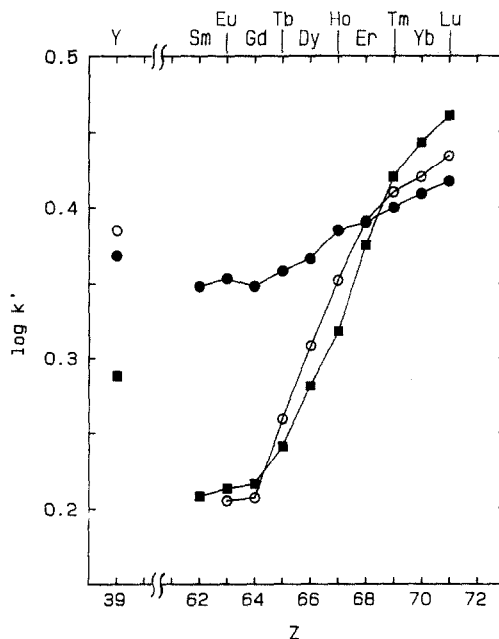


Fig. 3. Relationship between the retention of RE-OEP complexes and the atomic number (Z) of the RE. Mobile phase: \bullet = methanol-water-Hacac-DIPA (95:5:1:1.4, v/v); \circ = methanol-water-Hacac-DEOLA (95:5:1:0.93, v/v); \blacksquare = 0.045 M NaOH in methanol-water-Hacac (95:5:1, v/v).

ination strength of such hydrophilic ligand(s) increases with decreasing atomic number of the lanthanide.

Amines are stronger electron donors than water and methanol. Most amines possess hydrophobic alkyl moieties in their molecules. When an amine coordinates to the RE ion in a OEP complex more strongly than water and methanol, the k' of the RE-OEP complex may be larger than those observed without the amine in the reversed-phase system. The results shown in Figs. 2 and 3 imply that the stability of the additional coordination of the mono- and dialkylamines to the RE ion in the OEP complexes tends to increase with decreasing atomic number of the lanthanide. Also, trialkylamines (TEA and TPA) and the dialkylamines with branched alkyl side-chains (DIPA) cannot coordinate to the RE ion as strongly as the mono- and di- n -alkylamines. This suggests that the structure of the alkyl moiety of the amine affects the strength of the additional coordination of the amine to the RE ions in the RE-OEP complexes.

The RE(III) ionic radii are larger than the best fit (64 pm) for the cavity in the N_4 -moiety of porphyrin [18]. In a mixed complex of RE with porphyrin and acetylacetonone, the RE ion is displaced from the porphyrin plane towards the additional ligand, acac (see Fig. 1), and the estimated out-of-plane distance increases with increasing ionic radius of the RE [13,19]. It is assumed that the longer the out-of-plane distance, the stronger is the interaction between the RE ion and the additional ligand. Also, the bulkier the alkyl moiety in the vicinity of the nitrogen atom in an amine molecule, the greater is the extent of its steric hindrance to the coordination of the amine with the RE ion.

It was previously found that the retention sequence of RE-TPP complexes depended on the type of amine used as the mobile phase additive [10]. Such peculiar retention characteristics were explained in terms of the steric effects of the alkyl moiety of the amine on the coordination to the RE ion complexed with both porphyrin and acetylacetonone.

The retention trends of RE-OEP complexes observed with and without amines are represented as a

function of the ionic radius (r_i) of the RE in Fig. 4, where the r_i values for a CN of 8 [20] are applied. It is noted that the k' value of the complex of Y(III) lies, in every instance, near to that of the complex of Dy(III), whose ionic radius is close to that of Y(III). With mono- and di-*n*-alkylamines, the k' of RE-OEP tends to increase monotonously with increasing r_i of the RE. The opposite retention trends observed with trialkylamines is regarded as being due to the bulky alkyl moieties of these amines. The steric effect of branched alkyl groups appears clearly in the comparison of DIPA and DPA, which possess the same numbers of carbon atoms in their alkyl moieties. The retention trend observed with DEOLA, which is similar to that with NaOH, is attributed to the polar alcoholic structure of the side-chains in the molecule; the formation of the hydrophilic DEOLA adduct of an RE-OEP complex resulted in a reduction of the k' of the RE complex.

Control of the elution sequence

Methanol and water were used as the main components of the mobile phase to which Hacac and

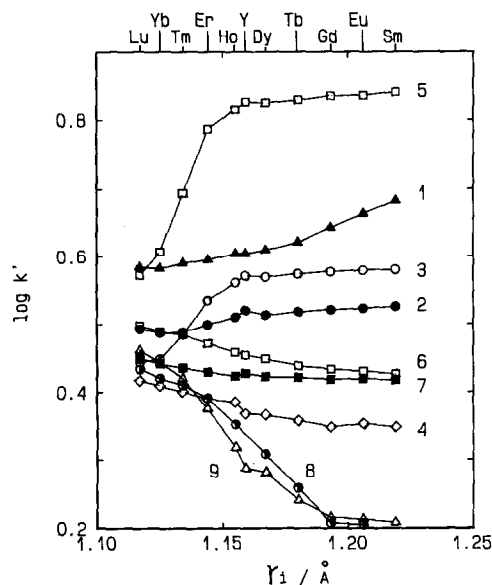


Fig. 4. Retention of RE-OEP complexes as a function of the ionic radius (r_i) of the RE(III) (r_i values for CN = 8). Basic mobile phase additives: 1 = OA; 2 = DEA; 3 = DPA; 4 = DIPA; 5 = DHA; 6 = TEA; 7 = TPA; 8 = DEOLA; 9 = NaOH. For the mobile phase compositions, see Figs. 2 and 3.

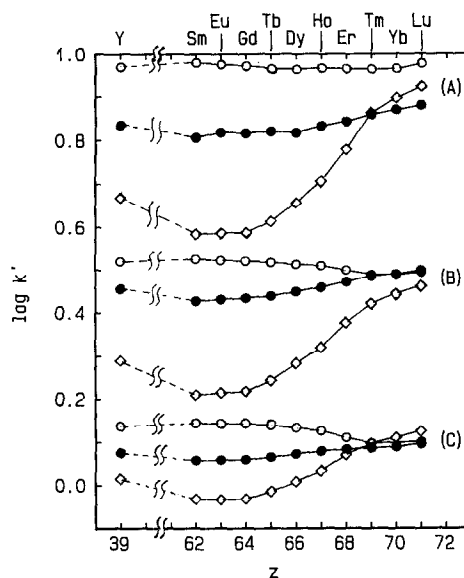


Fig. 5. Effect of the mobile phase composition on the retention of RE-OEP complexes. Mobile phase: (A) methanol-water-Hacac-base (90:10:1: x , v/v); (B) methanol-water-Hacac-base (95:5:1: x , v/v); (C) methanol-Hacac-base (100:1: x , v/v). Bases: \circ = DEA ($x = 1$); \bullet = TEA ($x = 1.3$); \diamond = NaOH (0.045 M).

amine were added. When the methanol-to-water ratio in the mobile phase was increased, the k' value decreased considerably for each RE-OEP complex, whereas their retention order changed little, as illustrated in Fig. 5 as an example. This means that the elution sequence for these complexes varies little with either the methanol or the water content of the mobile phase. According to the results shown in Figs. 2-4, the selectivities for the RE-OEP complexes can be varied with basic mobile phase additives such as amines and NaOH. It can be predicted that the elution order of the OEP complexes follows the increasing atomic number of the lanthanides when using trialkylamines, DIPA, DEOLA and NaOH as the mobile phase additives, whereas the reverse elution order is possible with a di- n -alkylamine or OA.

Separations of the RE-OEP complexes with different elution orders are demonstrated in Fig. 6.

Comparison with RE complexes of TPP

The effects of amines on the retention behaviour of RE-OEP complexes are compared here with those of RE-TPP complexes [10]. When the mobile

phase contains a trialkylamine, the retention trends for RE-OEP complexes are similar to those for RE-TPP; the capacity factor increases with increasing atomic numbers of lanthanides, as shown in Fig. 7. However, with respect to the effects of dialkylamines, such as DPA, the retention trends for the complexes of OEP are different from those for the complexes of TPP. With the TPP complexes, the capacity factor tends to decrease with increasing atomic number of the RE for relatively light lanthanides from Sm to Tb, whereas the reverse trend, which is similar to the results obtained with trialkylamines, is obtained for the complexes of heavy lanthanides from Dy to Lu. The capacity factors of the OEP complexes decrease monotonically with increasing atomic number of the lanthanide. This implies that the additional coordination of the dialkylamine with heavy lanthanides in the OEP complexes is stronger than in the TPP complexes.

According to the results shown in Fig. 7, the retention sequences for the complexes of heavy lanthanides in particular differ between the TPP complexes and OEP complexes, even though a dialkylamine is used. In an actual HPLC separation as

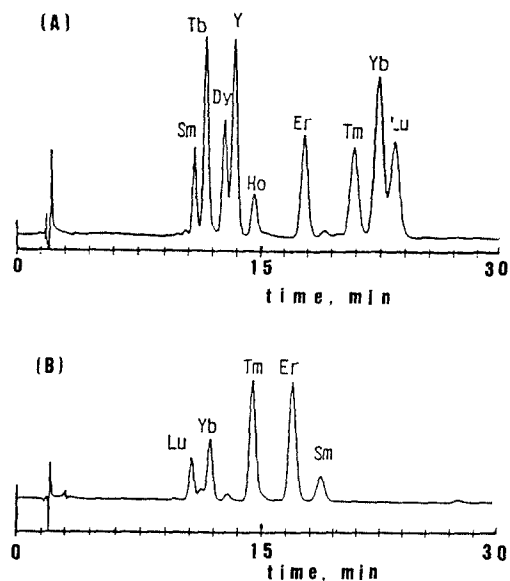


Fig. 6. HPLC separation of the RE-OEP complexes. Column: 50- μ m TSK Gel ODS-80TM (150 mm \times 4.6 mm I.D.). Mobile phase: (A) 0.045 M NaOH in methanol-water-Hacac (87:13:1, v/v/v); (B) methanol-water-Hacac-DHA (93:7:1:2.3, v/v); flow-rate, 1.0 ml/min. Detection at 565 nm.

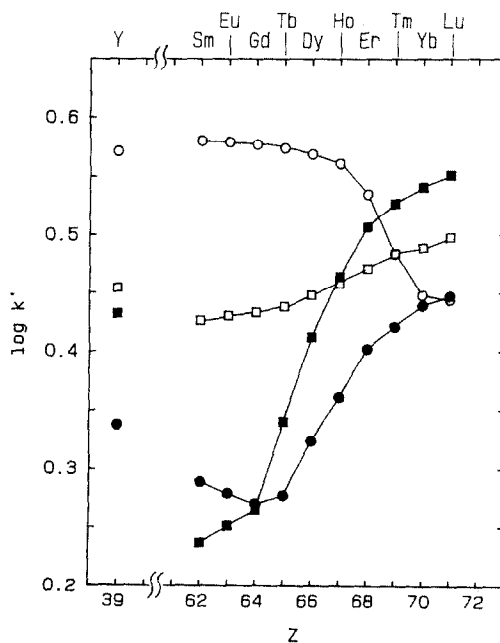


Fig. 7. Comparison of the retention trends for RE-OEP complexes (\circ , \square) and RE-TPP complexes (\bullet , \blacksquare). Mobile phase: \circ , \bullet = methanol-water-Hacac-DPA (95:5:1:1.3, v/v/v); \square , \blacksquare = methanol-water-Hacac-TEA (95:5:1:1.3, v/v/v).

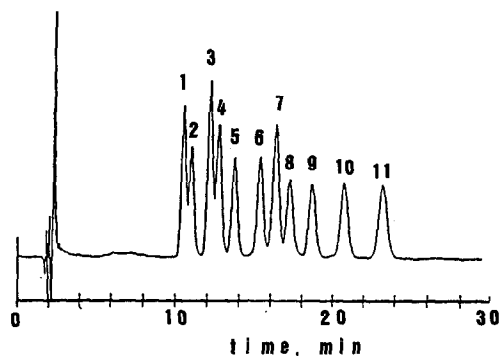


Fig. 8. HPLC separation of RE porphyrins. Mobile phase: methanol-water-Hacac-DPA (89:11:1:1.3, v/v); flow-rate, 1 ml/min. Detection at 555 nm. Column: as in Fig. 6. Peaks: 1 = Gd-TPP; 2 = Tb-TPP; 3 = Dy-TPP; 4 = Y-TPP; 5 = Ho-TPP; 6 = Er-TPP; 7 = Tm-TPP; 8 = Yb-OEP; 9 = Tm-OEP; 10 = Er-OEP; 11 = Sm-OEP.

demonstrated in Fig. 8, seven TPP complexes (peak 1-7) are eluted in order to the atomic numbers of the lanthanides, and then four OEP complexes (peaks 8-11) are separated in the reverse order of the atomic numbers of the RE.

CONCLUSIONS

An amine added to the mobile phase with a small amount of Hacac functions as a base which enhances the stability of the RE-OEP complexes to be separated and, accordingly, suppresses their undesirable adsorption on the stationary phase material. The second useful function of the amine is with regard to the selectivity and the elution sequence for the RE-OEP complexes. The retention selectivity depends on the amine used. The separation of RE-OEP complexes in increasing order of the atomic number of the REs is achieved with a trialkylamine as well as NaOH. The separation sequence can be reversed by changing the amine to either a mono-*n*-alkylamine, such as OA, or a di-*n*-alkylamine, such as DHA. The effect of dialkylamines in particular on the retention sequence of RE-OEP complexes is different from that for RE-TPP complexes.

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