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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY FOR OPTICAL RESOLUTION ON A COLUMN OF AN ION-EXCHANGE ADDUCT OF SPHERICALLY SHAPED SYNTHETIC HECTORITE AND OPTICALLY ACTIVE METAL COMPLEXES

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SUMMARY

Optical resolution was accomplished by liquid chromatography on a column packed with an ion-exchange adduct of synthetic hectorite and optically active metal complexes. The metal complexes used were optically active tris(1,10-phenanthroline)-nickel(II) and -ruthenium(II), and were ion exchanged into synthetic hectorite that had been spray-dried to a spherically shaped particle with an average diameter of 5.5 μm . When methanol or ethanol was used as the eluting solvent, the following compounds were resolved completely into enantiomers: 1,1'-binaphthol, 2,2'-diamino-1,1'-binaphthyl, 2,2'-diselecyano-1,1'-binaphthyl, [5]-helicene, $\text{Co}(\text{acac})_3$ (acac = acetylacetonato), $\text{Cr}(\text{acac})_3$, $\text{Rh}(\text{acac})_3$ and $[\text{Co}(\text{acac})_2(\text{en})]^+$ (en = ethylenediamine). The effect of the solvent on selectivity was studied in the resolution of 1,1'-binaphthol with acetonitrile-methanol as the eluent.

INTRODUCTION

In recent years, we have developed a liquid column chromatographic method for resolving organic and inorganic enantiomers on a column of a clay-metal complex adduct¹. For example, $[\Delta\text{-Ni}(\text{phen})_3]^{2+}$ -montmorillonite (phen = 1,10-phenanthroline) was used as a column material for resolving $\text{M}(\text{acac})_3$ (M = metal; acac = acetylacetonato)². In comparison with other known chiral adsorbents³, these materials are characterized by ease of preparation and a high efficiency in resolving wide varieties of compounds with aromatic functional groups.

One difficulty in the present method is the preparation of a clay of uniform particle size. A clay is composed of microcrystalline particles of diameter 0.1-1 μm . These particles are too small to be used as a packing material. In addition, they may collapse to smaller particles under high pressure. In order to overcome this difficulty,

we have tried to coat a silica gel particle with a film of a clay. Such materials have been found to improve the resolution efficiency greatly⁴.

In this study, synthetic hectorite (Laponite XLG, Laporte) was used as the clay material. Tohkubo *et al.*⁵ have recently made spherically shaped particles of this material by the spray-dry method. We expected that an adduct of such hectorite particle and an optically active chelate would be ideal as a packing material in high-performance liquid chromatography (HPLC). As a result, high resolutions were attained for a number of inorganic and organic compounds when methanol or ethanol was used as the eluting solvent.

EXPERIMENTAL

[Ru(phen)₃]Cl₂ was prepared according to the literature⁶. The complex was resolved by use of potassium antimony *d*-tartrate as a resolving agent. [*Δ*-Ru(phen)₃] [antimony *d*-tartrate]₂ was converted into the chloride salt by mixing with an anion-exchange resin in the chloride form. [*Δ*-Ni(phen)₃]Cl₂ was synthesized as described in the literature⁷. 2,2'-Diamino-1,1'-binaphthyl was also synthesized according to the literature⁸. 2,2'-Diselecyano-1,1'-binaphthyl was donated by Prof. S. Tomoda (University of Tokyo). Co(acac)₃, Cr(acac)₃, Rh(acac)₃ and [Co(acac)₂(en)]⁺ (en = ethylenediamine) were synthesized according to the literature⁹. Other compounds were used as purchased.

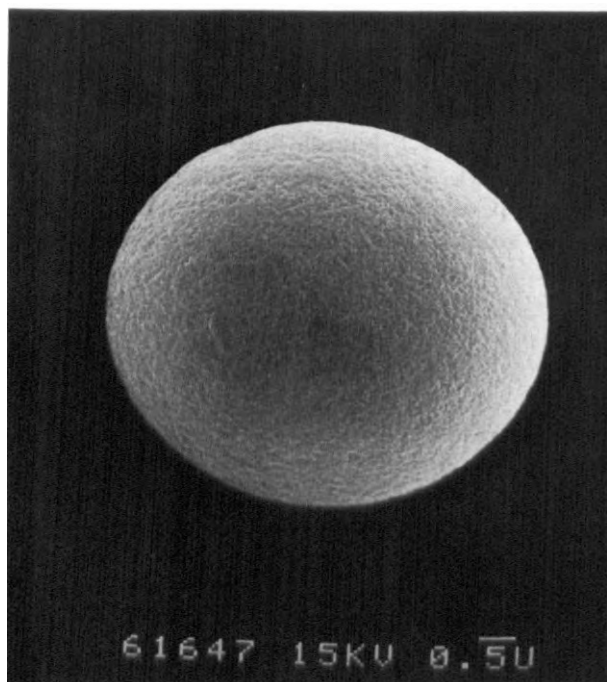


Fig. 1. Electron microphotograph of a particle of spray-dried synthetic hectorite. The diameter of the particle is about 6.5 μm .

The column material was prepared as follows. About 3 g of spray-dried synthetic hectorite (Laponite XLG) (Fig. 1) was dispersed in 100 ml of methanol, to which $1 \cdot 10^{-3}$ mol of optically active $[M(\text{phen})_3]\text{Cl}_2$ ($M = \text{Ru}$ or Ni) was added as an methanol solution. The mixture was centrifuged and the supernatant solution was separated. Based on a value of the cation-exchange capacity (CEC) of 79 mequiv. per 100 g, about 50% of the CEC was exchanged with the metal complexes. The above material was packed as a slurry in a stainless-steel tube (25 cm \times 0.4 cm I.D.). The columns in which larger or smaller amounts of the Ru chelates were exchanged into a clay were prepared in a similar way.

HPLC was performed with a BIP-1 chromatograph (JEOL) equipped with a UVIDEC-100-VI UV spectrophotometer (JEOL). About 10^{-6} mol of a compound was injected and eluted with methanol or a methanol-organic solvent mixture at a flow-rate of 0.1–1.0 ml/min. The temperature of the column was about 20°C unless indicated otherwise. The UV spectrum of the eluent was measured at 250 nm. The absolute configuration of an enantiomer was determined from the circular dichroism (CD) spectrum. The CD spectra were recorded on a J-20 polarimeter (JEOL).

RESULTS

Fig. 2 shows the chromatogram obtained when 1,1'-binaphthol was eluted with ethanol on a $[\Lambda\text{-Ru}(\text{phen})_3]^{2+}$ -hectorite column. Two peaks are observed at elution volumes of 6.8 and 10.6 ml. From the CD spectra, the first and second peaks correspond to the *R* and *S* enantiomers, respectively. The small peak at 3.0 ml is the peak due to chloroform, which was injected to determine the dead volume of the column. The separation factor, α , is defined by

$$\alpha = (t_2 - t_0)/(t_1 - t_0)$$

where t_0 , t_1 and t_2 are the elution time for the dead volume, the first peak and the second peak, respectively; α is calculated to be 2.0.

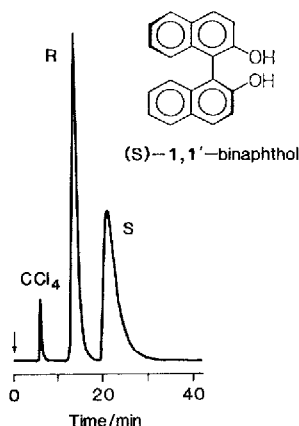


Fig. 2. Chromatogram obtained on elution of 1,1'-binaphthol on a $[\Lambda\text{-Ru}(\text{phen})_3]^{2+}$ -hectorite column with ethanol at a flow-rate of 0.2 ml/min.

TABLE I

EFFECT OF FLOW-RATE ON THE ELUTION OF 1,1'-BINAPHTHOL ON A COLUMN OF $[\Delta\text{-Ru}(\text{phen})_3]^{2+}$ -HECTORITE WITH ETHANOL AS ELUENT

Flow-rate (ml/min)	Retention time (min) ^a	α	R_s
0.2	$t_R = 20.58$ (622), $t_s = 30.18$ (500)	2.02	2.21
0.4	$t_R = 11.64$ (588), $t_s = 17.10$ (446)	2.00	2.12
1.0	$t_R = 5.82$ (465), $t_s = 8.28$ (339)	1.98	1.71

^a Theoretical plate numbers are shown in parentheses.

Table I shows the effect of the flow-rate on the chromatogram for the resolution of 1,1'-binaphthol. When the flow-rate was varied from 0.3 to 1.0 ml/min, the compound was resolved completely with little change in the separation factor. The resolution factor, R_s , is defined by

$$R_s = 2(t_2 - t_1)/(t_{w1} + t_{w2})$$

where t_{w1} and t_{w2} are the peak widths of the first and second peaks, respectively, R_s was found to decrease with increase in the flow-rate.

The effect of temperature on the separation factor was studied by varying the column temperature from 30 to 50°C. As shown in Fig. 3, the separation factor decreased from 2.9 to 2.5 with this increase in temperature. The peaks of the separated

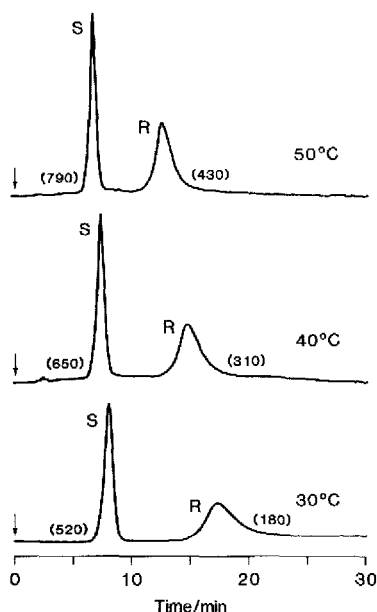


Fig. 3. Effect of the temperature on the elution curve of 1,1'-binaphthol on a column of $[\Delta\text{-Ru}(\text{phen})_3]^{2+}$ -hectorite. The figures in parentheses are the theoretical plate numbers of the peaks.

enantiomers were sharper at higher temperature, leading to an increase in the theoretical plate number as indicated in Fig. 3.

The effect of the amount of $[\Delta\text{-Ru(phen)}_3]^{2+}$ adsorbed in a clay was examined when $[\text{Co}(\text{acac})_3]$, $[\text{Cr}(\text{acac})_3]$ or 1,1'-binaphthol was eluted with ethanol. As shown in Table II, the retention volumes of the $[\text{Cr}(\text{acac})_3]$ and 1,1'-binaphthol enantiomers increase with increase in the amount of preadsorbed Ru complex, whereas the retention volumes of the $[\text{Co}(\text{acac})_3]$ enantiomers decrease with increase in the amount of preadsorbed Ru complex. The separation factor was lowered with increase in the amount of Ru complex in all the three instances.

Other kinds of binaphthyl derivatives, such as 2,2'-diamino-1,1'-binaphthyl and 2,2'-diselecyano-1,1'-binaphthyl, were completely resolved in similar way. 2,2'-Dibromo-1,1'-binaphthyl was partially resolved, since the two peaks overlap with each other. A racemic mixture of [5]-helicene was eluted with ethanol. M(-)-[5]-helicene was eluted at a retention volume of 19.2 ml as determined from the CD spectrum¹⁰. The antipode, P(+)-[5]-helicene, was not eluted with this solvent, but it was recovered from the column when acetonitrile was used as the eluent. P(+) and M(-) indicate the right- and left-handed helices, respectively.

The neutral metal complexes $[\text{Co}(\text{acac})_3]$, $[\text{Cr}(\text{acac})_3]$ and $[\text{Rh}(\text{acac})_3]$ were eluted with ethanol and were completely resolved. A positively charged complex, $[\text{Co}(\text{acac})_2(\text{en})]\text{ClO}_4$, was eluted with ethanol and completely resolved. The absolute configurations of the eluted enantiomers were determined from their CD spectra⁹. It was found that all these acetylacetonato complexes were eluted in the order Δ - followed by A -. Hence the charges of the complexes do not affect the selectivity of resolution on the present column. The results for these complexes are summarized in Table III.

The effect of the addition of acetonitrile to the methanol eluent was studied with the resolution of 1,1'-binaphthol. Table IV gives the chromatographic results when the compound was eluted with acetonitrile-methanol mixtures containing 0, 5, 10, 20 and 100% (v/v) of acetonitrile. The elution curves for the *R* and *S* enantiomers were obtained when each enantiomer was eluted independently with the same solvents.

With increase in the acetonitrile concentration, the separation factor decreased until the column no longer resolved the enantiomers at all at 18% (v/v) of acetonitrile.

TABLE II

EFFECT OF THE AMOUNT OF $[\Delta\text{-Ru(phen)}_3]^{2+}$ EXCHANGED IN THE HECTORITE COLUMN ON THE RETENTION VOLUMES AND ON THE SEPARATION FACTORS (α) OF THE ENANTIOMERS

V_d = Dead volume.

Amount adsorbed (mequiv.)	<i>Co(acac)</i> ₃			<i>Cr(acac)</i> ₃			1,1'-Binaphthol		
	<i>V</i> (Δ) (ml)	<i>V</i> (Δ) (ml)	α	<i>V</i> (Δ) (ml)	<i>V</i> (Δ) (ml)	α	<i>V</i> (<i>S</i>) (ml)	<i>V</i> (<i>R</i>) (ml)	α
30	9.3	17.8	2.3	9.6	23.5	3.2	4.6	10.0	4.3
40	7.7	13.8	2.2	10.5	24.3	2.9	8.9	22.1	3.2
50	6.4	10.5	2.1	11.4	24.6	2.6	14.3	37.1	3.0
	$V_d = 2.5$ ml			$V_d = 3.2$ ml			$V_d = 3.0$ ml		

TABLE III

SEPARATION FACTORS FOR THE RESOLUTION OF VARIOUS RACEMIC MIXTURES ON A $[\Delta\text{-Ru(phen)}_3]^{2+}$ -HECTORITE COLUMN USING ETHANOL AS ELUENT

Compound	Order of elution	Separation factor (α)
1,1'-Binaphthol	$R \rightarrow S$	2.0
2,2'-Diselecyano-1,1'-binaphthol	$R \rightarrow S$	1.5
$[\text{Co}(\text{acac})_3]$	$A \rightarrow A$	2.4
$[\text{Cr}(\text{acac})_3]$	$A \rightarrow A$	2.9
$[\text{Rh}(\text{acac})_3]$	$A \rightarrow A$	3.3
$[\text{Co}(\text{acac})_2(\text{en})]^+$	$A \rightarrow A$	2.6
[5]-Helicene	$M \rightarrow P$	Very large

When the concentration of acetonitrile exceeded 18% (v/v), the order of elution of the enantiomers reversed from R - S to S - R . Fig. 4 shows the effect of solvent composition on the separation factor. A similar reversal of the elution order was also confirmed on a column packed with $[\Delta\text{-Ni(phen)}_3]^{2+}$ -hectorite. On this column, the reversal of selectivity from S - R to R - S took place at 14% (v/v) acetonitrile.

Fig. 5 shows the CD spectra of an adduct of $[\Lambda\text{-Ru(phen)}_3]^{2+}$ -hectorite dispersed in either methanol or acetonitrile. The CD spectrum due to adsorbed $[\Lambda\text{-Ru(phen)}_3]^{2+}$ was displaced downwards when methanol was replaced with acetonitrile. When the amplitude of the negative peak at 425 nm, A_{425} , was plotted against the acetonitrile content, it varied as shown in Fig. 6. A_{425} decreased rapidly until it became constant value at an acetonitrile concentration of 5% (v/v).

Other kinds of nitriles were added to the eluent to study the effect of the structure of the nitrile on the efficiency of resolution of 1,1'-binaphthol. Table V shows the separation factors when the eluent contained 5% (v/v) of nitrile. It is concluded that nitriles of simpler structure such as acetonitrile and propionitrile give the lowest separation factors.

TABLE IV

EFFECT OF MOBILE PHASE COMPOSITION ON THE OPTICAL RESOLUTION OF 1,1'-BINAPHTHOL ON A COLUMN OF $[\Delta\text{-Ru(phen)}_3]^{2+}$ -HECTORITE

Mobile phase: acetonitrile-methanol.

Acetonitrile concentration (% v/v)	Retention time (min) ^a	α	R_S
0	$t_R = 19.12$ (1125), $t_S = 29.04$ (672)	2.09	2.39
5	$t_R = 18.40$ (1006), $t_S = 21.20$ (917)	1.35	1.09
10	$t_R = 16.28$ (1656), $t_S = 16.97$ (1422)	1.11	0.41
15	$t_R = 14.93$ (1744), $t_S = 15.15$ (1509)	1.05	0.15
20	$t_S = 13.38$ (1525), $t_R = 13.48$ (1548)	0.97	0.07
50	$t_S = 10.00$ (1299), $t_R = 10.22$ (1194)	0.68	0.20
80	$t_S = 9.66$ (1701), $t_R = 9.80$ (1789)	0.43	0.15
100	$t_S = 9.74$ (1848), $t_R = 9.91$ (1870)	0.32	0.18

^a Theoretical plate numbers are shown in parentheses.

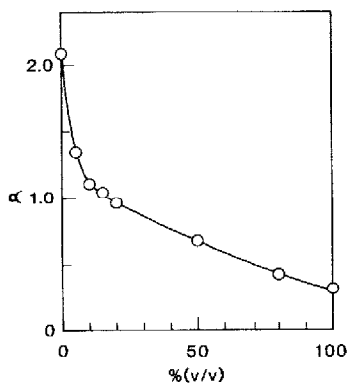


Fig. 4. Plot of the separation factor (α) against the acetonitrile concentration in the eluent. The data were obtained from the results in Table IV.

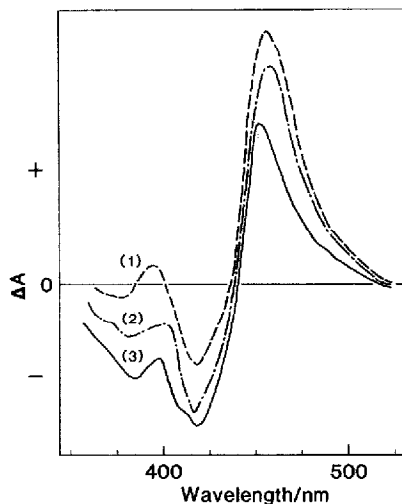


Fig. 5. Circular dichroism spectra of a free $[A\text{-Ru(phen)}_3]^{2+}$ ion or $[A\text{-Ru(phen)}_3]^{2+}$ bound to a colloiddally dispersed hectorite. (1) Free $[A\text{-Ru(phen)}_3]^{2+}$ ion in methanol; (2) colloiddally dispersed $[A\text{-Ru(phen)}_3]^{2+}$ hectorite in methanol; (3) colloiddally dispersed $[A\text{-Ru(phen)}_3]^{2+}$ hectorite in acetonitrile.

DISCUSSION

$[A\text{-Ru(phen)}_3]^{2+}$ and $[A\text{-Ni(phen)}_3]^{2+}$ hectorite resolve a number of organic and inorganic racemic mixtures when they are eluted with methanol or ethanol. The present results were compared with those observed on a column of silica gel coated with $[A\text{-Ru(phen)}_3]^{2+}$ montmorillonite³. On the latter column, 1,1'-binaphthol was resolved with $\alpha = 2.3$ when the compound was eluted with methanol-water (1:2, v/v) and α decreased with increase in the methanol content in the eluent until no resolution was attained on elution with pure methanol. On the other hand, the present hectorite

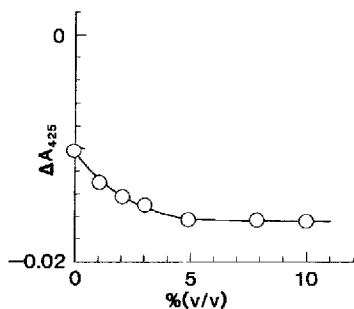


Fig. 6. Plot of the amplitude at 425 nm in the circular dichroism spectra in Fig. 5 against the concentration of acetonitrile in an acetonitrile-methanol mixture.

TABLE V

EFFECT OF NITRILES ON THE EFFICIENCY OF RESOLUTION OF 1,1'-BINAPHTHOL USING NITRILE-ETHANOL (5:95, v/v) MIXTURES AS ELUENT

<i>Nitrile</i>	<i>Separation factor (α)</i>
Acetonitrile (CH ₃ CN)	1.3
Propionitrile (CH ₃ CH ₂ CN)	1.3
<i>n</i> -Butyronitrile (CH ₃ CH ₂ CH ₂ CN)	1.5
<i>n</i> -Valeronitrile [CH ₃ (CH ₂) ₃ CN]	1.7
Isobutyronitrile [(CH ₃) ₂ CHCN]	1.7
Trimethylacetone [(CH ₃) ₃ CCN]	1.9

column resolved the same racemic compound completely when pure methanol or ethanol was used as the eluent. Therefore, it is concluded that the present column improves the efficiency of resolution greatly in comparison with the previous column.

There are two possible reasons for the enhancement of resolution efficiency attained with the present column. The first is that the present column contains about 50 times more optically active chelates than the previous column. The number of binding sites may increase by this amount if all of the chelates in a hectorite particle can interact with the analyte molecules. This is confirmed for 1,1'-binaphthol and [Cr(acac)₃] from the results in Table II. The retention volumes for these compounds increase with increase in the amount of adsorbed chelates. For [Co(acac)₃], however, the retention volume decreases with increase in the amount of adsorbed chelate, and it is not clear why this compound exhibits this reverse tendency. The increased affinity towards an analyte molecule enables one to use organic solvents as eluents. The second reason is that the uniform size distribution and the spherical shape of a particle reduce the disturbances to the flow of the eluent. As a result, the width of a resolved peak is expected to be narrowed. In fact, the widths of the first and second peaks in the chromatogram were about 40 and 100 ml, respectively, when 1,1'-binaphthol was eluted with methanol-water (1:1, v/v) on the previous column of silica gel coated with [A-Ru(phen)₃]²⁺-montmorillonite. In contrast, the corresponding first and second peaks with the present column had widths of only about 2 and 4 ml, respectively. The observed narrow width is partly due to the use of pure methanol instead of methanol-water, as the clay layers swell to a lesser extent in methanol than in water. This leads to less penetration of an analyte molecule into the interlayer space. The non-equilibrium nature of such a penetration process is considered to be one of the main factors in increasing the widths of the separated peaks. The effect of temperature on the chromatogram (Fig. 3) supports this conclusion. The equilibration rate of the penetration increases with increase in temperature, resulting in a narrower peak width at higher temperatures.

The reversal of selectivity with the addition of acetonitrile (Table IV) implies that solvent molecules affect the binding states of the enantiomers in different ways, otherwise a change in solvent composition would never lead to reversal of selectivity. One possibility for the occurrence of this effect is that the enantiomers are adsorbed at different binding sites on the column and that acetonitrile molecules interact specifically with either one of the sites.

The spectral results in Fig. 5 suggest that acetonitrile molecules solvate the Ru chelate more strongly than methanol so that the solvation is complete at an acetonitrile concentration of 5% (v/v). Based on this, the observed effect of acetonitrile in resolving 1,1'-binaphthol might be ascribed to the solvation of $[A\text{-Ru(phen)}_3]^{2+}$ by acetonitrile molecules, which results in blockage of the binding site for the *S* enantiomer, but has little effect on the binding site for the *R* enantiomer. As a result, the *S* enantiomer is eluted faster than the *R* enantiomer at acetonitrile concentrations higher than 14–18% (v/v). The results in Table V that the smaller nitriles decrease the separation factor more efficiently are consistent with this view, because it is natural to expect that the smaller nitriles will solvate the Ru chelates more strongly.

Several examples have been reported in which a change in solvent composition causes a reversal in chiral selectivity. For example, Lappin *et al.*¹¹ observed that the electron transfer between $[\text{Co(en)}_3]^{2+}$ and $[\text{Co(edta)}]^-$ exhibits different stereoselectivity in water and dimethyl sulphoxide. This result was ascribed to the hydrogen-bonding solvation structures of the reacting complexes in water collapsing in dimethyl sulphoxide. In the present instance also the alcohol molecules are liable to solvate a resolved molecule by hydrogen bonding whereas such bondings collapse in acetonitrile-containing solvents. Therefore, the difference in the abilities of the investigated solvents to form hydrogen bonds is considered to be one of the reasons for the present phenomena.

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