CHROM. 5365

STUDIES ON LIGAND-EXCHANGE CHROMATOGRAPHY

III. SEPARATION OF NITROSONAPHTHOL ISOMERS

KAZUMI FUJIMURA, MASAHARU MATSUBARA AND WATARU FUNASAKA
Department of Industrial Chemistry, Faculty of Engineering, Kyoto University, Sakyo-ku, Kyoto (Japan)

(Received March 22nd, 1971)

SUMMARY

The separation of α-nitroso-β- and β-nitroso-α-naphthols by ligand-exchange chromatography was studied. The best results were obtained by use of a strong acid type resin in the Fe^{3+} form as a stationary phase and a 50 % v/v ethanolic ammonia solution (pH 9.5 and 12.0) as the mobile phase for stepwise elution. In view of the slow adsorption rate of both isomers to the resin, the Fe(III)–nitrosonaphthol complex formed in the resin phase seems to be a low-spin 1:1 complex for which the oxygen of the hydroxyl group and the nitrogen of the nitroso group are both responsible.

INTRODUCTION

The separation of isomers, homologues or analogues of organic substances from each other is generally difficult because of the similarity of their physico-chemical properties. If the substances to be separated are capable of coordinating with a metal ion, however, ligand-exchange chromatography using a cation exchanger or chelate resin in a metallic form, as a stationary phase, is very promising. In recent years, many studies on ligand exchange have been reported by several groups of workers[1–5], but the separation of isomers has rarely been tried.

In the previous work of this series[6,7], it was found that phenylenediamine isomers[6] could be completely separated on a column of Amberlite CG-120 in the Fe^{3+} form by eluting with very dilute aqueous ammonia (5×10^{-3} *M*) at room temperature. Similarly, aminobenzoic acid isomers[7] were separated on a column of the same resin in the Cu^{2+} form on eluting with either aqueous ammonia at pH 8.4 or distilled water alone. It was also found that dilute sodium hydroxide solution could be used as a developer with no metal leakage.

In order to apply the ligand-exchange reaction to elution chromatography, no metal leakage from the support and a fast rate of adsorption must be assured in order to achieve a successful separation. The former requirement may be satisfied to some extent by employing a resin which has a functional group that can form a complex with metal ions, *e.g.* a carboxylic acid type or iminodiacetic acid type resin, or by employing a developer which is as dilute as possible. On the other hand, to satisfy the second requirement, a metal ion which forms a labile complex, not neces-

sarily a complex of larger formation constant, with the substances to be separated must be selected as the counter ion of the resin.

The purpose of the present work is to find out the conditions appropriate for separating nitrosonaphthol isomers, to study the effect of alcohol on ligand-exchange adsorption, to determine the relationship between the rate of adsorption and the composition of the complex, the effect of physical adsorption on ligand-exchange adsorption, and the coordination site of the nitroso group.

EXPERIMENTAL

Reagents

α-Nitroso-β-naphthol and β-nitroso-α-naphthol: commercially available reagents (reagent grade; Tokyo Kasei Kogyo Co. Ltd.) were purified by recrystallization from 50% v/v ethanol until the melting points agreed with the valves in the literature. Stock solutions were prepared by dissolving 0.1732 g of these substances in 230 ml of 50% v/v ethanol and diluting to 250 ml with water; they were then stored in the dark. The concentration of this solution corresponds to 4×10^{-3} M (0.6928 mg/ml). Working standard solutions, whose concentrations ranged from 5×10^{-4} to 2×10^{-3} M, were prepared from the stock solution.

All the other reagents used were of analytical grade purity.

Ion-exchange resin: A strong acid cation-exchange resin, Amberlite CG-120, was used. After conditioning in the usual way, the resin was converted to the Fe^{3+} form with ferric chloride solution and was air-dried at room temperature by spreading on filter paper. The resin of a 100–200 mesh grade was used for batch operation and that of a 200–400 mesh grade for column operation.

Apparatus

A Shimadzu QV-50 spectrophotometer and a Toa Denpa HM-5A pH meter were used for spectrophotometric determination of the nitrosonaphthols and for the pH measurements, respectively. A RadiRac 3410B fraction collector (siphon type) was used for collecting the column effluents.

Distribution coefficients

The distribution coefficients for α-nitroso-β-naphthol and β-nitroso-α-naphthol on the Fe^{3+} form of the resin were measured by batch operation at room temperature as a function of the concentration of aqueous ammonia. To 1 g samples of the resin which had been weighed into 50-ml conical flasks with a glass stopper, 25 ml of aqueous ammonia of varying concentration was added. The flasks were then shaken gently for 1 h in order to swell the resin after which 1 ml of a nitrosonaphthol solution, corresponding to 0.1732 mg (1 μmole) of nitrosonaphthol, was added to the flasks. After being shaken vigorously for 1 h in a mechanical shaker, the flasks were allowed to stand for 24 h for equilibration to take place and then the resin was separated from the aqueous phase by filtration using a sintered-glass filter without suction. The amount of nitrosonaphthol in the solution was determined spectrophotometrically.

The distribution coefficient, K_d, was calculated as follows:

$$K_d = \frac{\text{mg of substance in resin phase/g of dry resin}}{\text{mg of substance in solution phase/ml of solution}}$$

The pH values of each equilibrated solution were also recorded.

Analysis

The nitrosonaphthols were determined by UV spectrophotometry using a quartz cell of 1.00 cm path length. As the absorption spectra of the nitrosonaphthols varied according to the pH value of the solution, the absorbances were measured at the isosbestic points, *i.e.* 280.0 nm for α-nitroso-β-naphthol and 294.0 nm for β-nitroso-α-naphthol. The relationship between pH value and absorptivity (extinction coefficient) at 280.0 and 294.0 nm is shown in Fig. 1. The leakage of ferric ion into the column effluent was tested by evaporating 500 ml portions of effluent to 50 ml and determining the ferric ion colorimetrically as the thiocyanate complex.

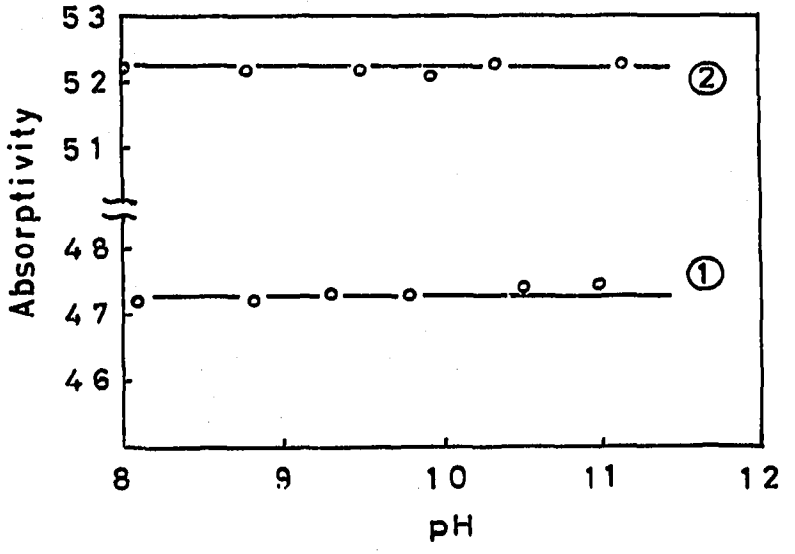


Fig. 1. Relationship between pH and absorptivity. (1) α-Nitroso-β-naphthol at 280.0 nm, concn. = 6.928 μg/ml; (2) β-nitroso-α-naphthol at 294.0 nm, concn. = 6.928 μg/ml.

Preparation of the column

Two or more volumes of dry resin (200–400 mesh, Fe^{3+} form) per volume of the chromatographic tube were placed in a large beaker. An appropriate volume of water or 50 % ethanol was poured into the beaker and the resin was stirred with a magnetic stirrer. After the resin had been swollen, concentrated aqueous ammonia was added dropwise using a pipet and the pH value of the solution was adjusted to the required value using a pH meter. After complete equilibration between the resin and the solution phases had been attained, the resin was separated from the solution by decantation and poured into a glass chromatographyc tube (11 mm diam. × 140 mm long) fitted with a stopcock. The equilibrated solution obtained by the above procedure was used as the developing solution.

In general, the resin used in the ligand-exchange technique must previously be equilibrated with the developer, because, otherwise, some water molecules coordinated with the ferric ion in the resin phase would be displaced by ammonia molecules or hydroxyl ions coming from the aqueous ammonia, and so the addition of a few drops of concentrated ammonia would cause little or no change in the pH value of the solution. It would be time-consuming to pack the column with resin that has been swollen in water alone and to equilibrate the resin by washing with a developer prepared independently.

RESULTS AND DISCUSSION

Effect of the ammonia concentration on adsorption

The distribution coefficients of α-nitroso-β- and β-nitroso-α-naphthols in aqueous ammonia on Amberlite CG-120 in the Fe^{3+} form are shown in Fig. 2 as a function of the pH value of the solution.

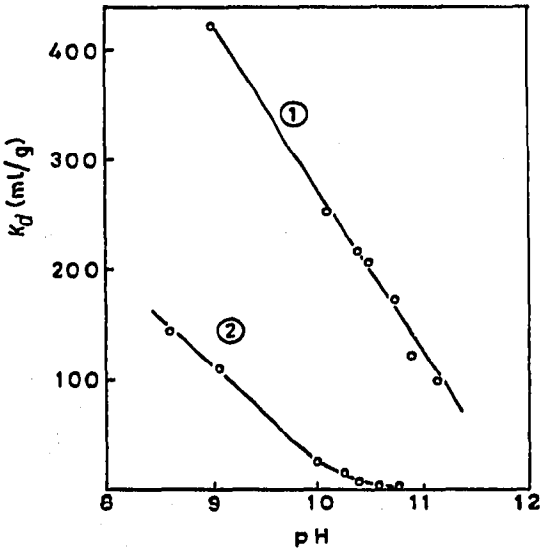


Fig. 2. Effect of pH (ammonia concentration) on the distribution coefficients of nitrosonaphthols. (1) α-Nitroso-β-naphthol; (2) β-nitroso-α-naphthol; resin: Amberlite CG-120, Fe^{3+} form.

It is obvious that the K_d values of these two substances, especially that of α-nitroso-β-naphthol, are quite large in neutral or very dilute ammonia solution and that they decrease with increasing pH value. The separation factor is large enough to permit a quantitative separation of the isomers in the pH range 8.0 to 11.0. The results shown in Fig. 2 also suggest that the formation constant of the Fe(III)–α-nitroso-β-naphthol complex is greater than that of the β-nitroso-α-naphthol complex.

Furthermore, the results are in good agreement with the generalization for ligand exchange that the ligands of higher coordination valency have a higher affinity to the metal ion in the resin phase than those of lower coordination valency, if the concentrations of ligands such as OH^-, NH_3 (monodentate) or nitrosonaphthol (bidentate) are low.

Effect of the ionic form of the resin on adsorption

The distribution coefficients of the nitrosonaphthols between resin in the Cu^{2+} form and aqueous ammonia are shown in Table I. Although the relationship between K_d and pH was nearly the same as that found for the resin in the Fe^{3+} form, the selectivity for nitrosonaphthol isomers followed the order β-nitroso-α- > α-nitroso-β-naphthol. This order was the reverse of that found for the resin in the Fe^{3+} form. The separation factor was too low to permit separation of the isomers by using the resin in the Cu^{2+} form.

Effect of ethanol on adsorption

In order to study the effect of ethanol on the adsorption of nitrosonaphthols,

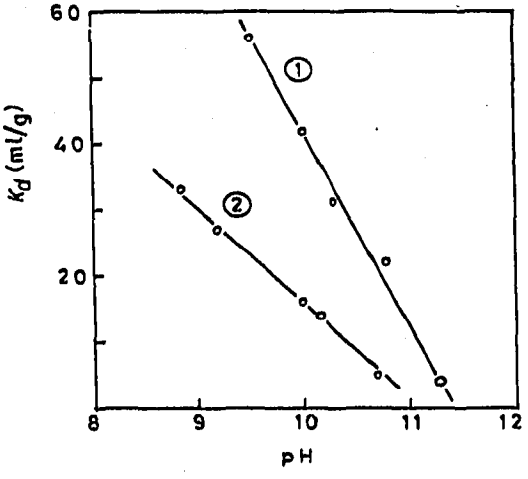
TABLE I

K_d VALUES OF NITROSONAPHTHOLS ON THE RESIN IN THE Cu^{2+} FORM AT VARIOUS pH VALUES

Substance	*pH value*[a]				
	9.0	*9.5*	*10.0*	*10.5*	*11.0*
α-Nitroso-β-naphthol	160.9	105.2	59.0	38.5	26.4
β-Nitroso-α-naphthol	203.3	126.1	73.9	48.2	38.1

[a] pH values were adjusted with aqueous ammonia.

the distribution coefficients were measured in an ethanolic ammonia solution. As can be seen in Fig. 3, the tendency of the results was similar to that observed in Fig. 2 when a 50 % v/v ethanolic ammonia solution was used instead of aqueous ammonia, but the K_d values and the separation factors were both much lower. This is probably due to the greater solubility of nitrosonaphthols in an ethanolic solution, rather than to the complex formation of ethanol with the ferric ion, since the absorption spectra of aqueous and 50 % ethanolic solutions of ferric ion (4×10^{-5} *M*), adjusted to pH 9.5 were identical in the range from 210.0 to 400.0 nm.



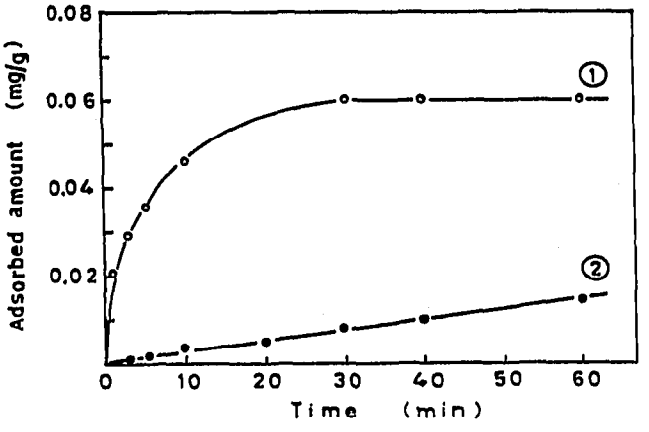


Fig. 3. Effect of ethanol on the distribution coefficients of nitrosonaphthols. (1) α-Nitroso-β-naphthol; (2) β-nitroso-α-naphthol. Resin: Amberlite CG-120, Fe^{3+} form; solvent: 50 % ethanolic ammonia solution.

Fig. 4. Rate of adsorption of nitrosonaphthols in aqueous ammonia at 23°. (1) α-Nitroso-β-naphthol; (2) β-nitroso-α-naphthol. Resin: Amberlite CG-120, Fe^{3+} form; solvent: aqueous ammonia (pH 9.6).

Rate of adsorption

The results of an examination of the sorption equilibria between nitrosonaphthols and the resin in the Fe^{3+} form are shown in Fig. 4: the amount of α-nitroso-β-naphthol adsorbed reaches its maximum within 30 min in aqueous ammonia at pH 9.6 and 23°, but that of β-nitroso-α-naphthol still tends to increase even after 60 min or more. These facts show that the rate of adsorption of α-nitroso-β-naphthol is comparatively slow, and that of β-nitroso-α-naphthol is even slower.

In general, a successful separation by ligand exchange requires not only a large

difference in the formation constants of the ligand complexes to be separated, but also a fast rate of equilibration. In order to carry out elution under equilibrium conditions when separating nitrosonaphthol isomers, the flow rate of the developer must be kept as slow as possible, within the limits between which no appreciable peak broadening occurs, owing to diffusion.

According to TAUBE[8], a d^5 metal ion, like Fe^{3+}, can form a high-spin labile complex as well as a low-spin inert complex. In view of the slow adsorption rate of nitrosonaphthols, the Fe(III)–nitrosonaphthol complex is probably a low-spin inert complex, containing OH^-, H_2O, NH_3 and nitrosonaphthol as its ligands.

Although the cause of the difference in adsorption rates between α-nitroso-β- and β-nitroso-α-naphthols is not quite clear, it can probably be attributed to the difference of the π-electron density of the nitroso group at the α- and β-positions: the electron density of a nitroso group in the β-position is higher than that in the α-position, so that it produces a stronger crystal field splitting.

Separation of isomers

From Figs. 2 and 3, it may be expected that the separation of the isomeric nitrosonaphthols would best be effected by stepwise elution using 50 % v/v ethanolic ammonia at different pH values, since their K_d values in aqueous ammonia are too large for their elution at a practical rate, even if a shorter column is employed.

When a mixture of these two isomers was eluted through a column (11 × 150 mm) using a 50 % v/v ethanolic ammonia solution, pH 9.5, at a flow rate of 0.25 ml/min, α-nitroso-β-naphthol was quantitatively retained on the column, and could be easily separated from β-nitroso-α-naphthol. After the complete elution of β-nitroso-α-naphthol with 100 ml of the above developer, the pH value of the developer was changed to 12.0 to elute the α-nitroso-β-naphthol rapidly. A typical elution curve is shown in Fig. 5. Both peaks show extreme tailing because of the slow adsorption rate of nitrosonaphthols: they are eluted before the sorption equilibrium has been attained at each plate of the column. The smaller retention volume of β-nitroso-α-naphthol, as compared with the value to be expected from Fig. 3, may be explained on the same grounds. The average recovery for α-nitroso-β- and β-nitroso-

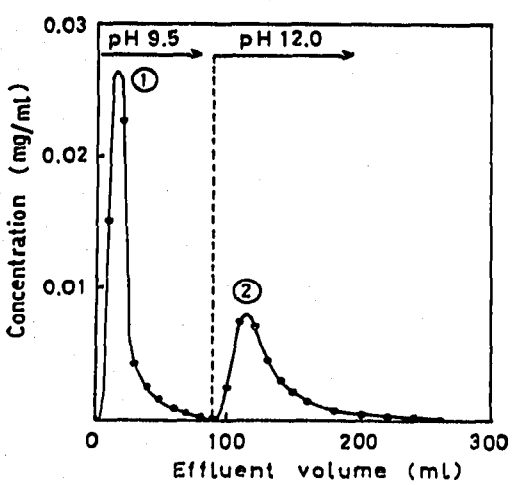


Fig. 5. Elution curve: (1) β-nitroso-α-naphthol; (2) α-nitroso-β-naphthol. Column size: 11 × 150 mm; developer: 50 % ethanolic ammonia solution; flow rate: 0.25 ml/min.

α-naphthol calculated from the results of three runs were 90.0 % and 96.0 %, respectively.

Composition of the complex

When three or more moles of nitrosonaphthol were present per mole of ferric ion in a weakly acidic or neutral solution, a water-insoluble black complex was formed from either nitrosonaphthol. In both cases, the composition of the complex, *i.e.* the molar ratio of ferric ion to nitrosonaphthol, was found to be 1:3 by the following method: the unaltered species were removed by treatment with 50 % ethanol, the complex was dissolved in absolute ethanol, conc. hydrochloric acid was added, and then ferric ion and nitrosonaphthol were determined by means of the thiocyanate method and UV spectrophotometry, respectively. The results are shown in Table II.

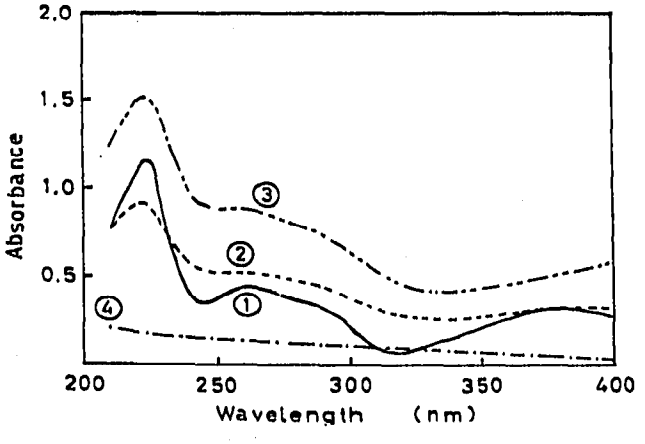
When the molar ratio of ferric ion to nitrosonaphthol was 1:1 or 1:2, no water-insoluble complex was formed, but the UV absorption spectra of the solution, shown

TABLE II

COMPOSITION OF Fe(III)-NITROSONAPHTHOL COMPLEXES (WATER-INSOLUBLE)

Complex	*Sample No.*	*Amounts in 50 ml of decomposed solution*		*Composition Fe(III) : ligand*
		Ferric ion[a] *(μmole)*	*Ligand*[b] *(μmole)*	
α-Nitroso-β-naphthol complex	1	0.42	1.21	1:2.92
	2	0.65	1.67	1:2.73
β-Nitroso-α-naphthol complex	1	0.70	1.96	1:2.80
	2	0.62	1.97	1:3.18

[a] Determined by the thiocyanate method.
[b] Determined by UV spectrophotometry.



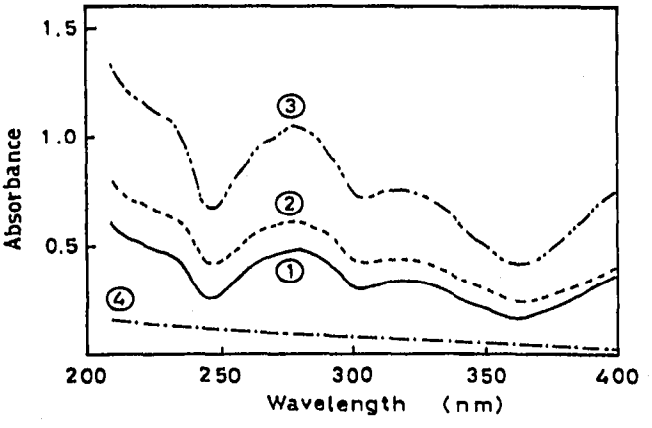


Fig. 6. Absorption spectra of Fe(III)–α-nitroso-β-naphthol complex. (1) α-Nitroso-β-naphthol (4×10^{-5} *M*); (2) Fe(III) (4×10^{-5} *M*) + ligand (4×10^{-5} *M*); (3) Fe(III) (4×10^{-5} *M*) + ligand (8×10^{-5} *M*); (4) Fe(III) (4×10^{-5} *M*).

Fig. 7. Absorption spectra of Fe(III)–β-nitroso-α-naphthol complex. (1) β-Nitroso-α-naphthol (4×10^{-5} *M*); (2) Fe(III) (4×10^{-5} *M*) + ligand (4×10^{-5} *M*); (3) Fe(III) (4×10^{-5} *M*) + ligand (8×10^{-5} *M*); (4) Fe(III) (4×10^{-5} *M*).

in Figs. 6 and 7, strongly suggest that some water-soluble complex or complexes are formed in solution.

In the ligand-exchange reaction, the ferric ions loaded on to the Amberlite CG-120, which had been equilibrated with aqueous ammonia at pH 9–10, must have formed either hydroxo–aquo or hydroxo–aquo–ammine mixed complexes: the IR spectrum of the resin equilibrated with aqueous ammonia at pH 9.5 was distinctly different from that equilibrated with sodium hydroxide solution at the same pH, especially at 3300–2800 cm^{-1} and at 1500–1400 cm^{-1}, suggesting the presence of the latter complex in the resin phase. Although an accurate composition of the complex formed in the resin phase is not known, it is evident that more than two moles of hydroxyl ion cannot have coordinated with one mole of ferric ion: the coordination of more than two moles of hydroxyl ion would make the complex neutral or negatively-charged and cause leakage of the ferric ion into the external solution, which in fact was not observed in the equilibrated solution. When a nitrosonaphthol solution is added to the resin under these conditions, one or two moles of the ligands that have coordinated with ferric ion will be displaced by nitrosonaphthol by ligand-exchange reaction: more than two moles of nitrosonaphthol cannot be adsorbed, because nitrosonaphthol acts as a univalent anion in alkaline solution and in addition the quantity is very small (1 μmole) compared with the amount of ferric ion in the resin phase. For the same reason, the sum of nitrosonaphthol and hydroxyl ions should never exceed two moles. Instead of a water-insoluble 1:3 complex, a 1:1 soluble complex might possibly be formed in the resin phase as well as in solution.

Effect of physical adsorption on ligand-exchange adsorption

It is known that most water-soluble aromatic compounds are adsorbed on ion-exchange resin by van der Waals' interaction between the solute and the resin matrix. In order to study the effect of any such adsorption which might occur during ligand-exchange adsorption, the retention volumes of the nitrosonaphthols were measured using a resin in the cationic form which had no coordination sites. The NH_4^+ form of Amberlite CG-120, 200–400 mesh, was chosen to avoid an ion-exchange reaction occurring between a cation loaded previously on to the cation exchanger and the ammonium ion in the external solution. The eluting conditions, such as column length, flow rate of developer, etc., were the same as those used in ligand-exchange chromatography.

As can be seen from Fig. 8, the retention volumes of two nitrosonaphthols, especially of β-nitroso-α-naphthol, were evidently greater than the column hold-up volume (about 10 ml) when they were eluted with aqueous ammonia at pH 9.5. These results suggest that the effect of physical adsorption cannot be neglected in ligand-exchange adsorption of nitrosonaphthols.

Determination of the coordination site of the nitroso group

The nitroso group is potentially capable of coordinating with a metal ion through either its oxygen or its nitrogen atom. According to FEIGL[9], the bond between α-nitroso-β-naphthol and a metal ion can be regarded as being formed through the nitrogen atom to give a five-membered chelate ring instead of a six-membered ring through the oxygen atom. Studies of the electronic configuration of the Co(II) complex also suggest that the bond is favoured through nitrogen, rather than oxygen[10].

On the other hand, CHATTERJEE[11] has reported that, in the case of the Cu(II) chelate of α-nitroso-β-naphthol, the bond is formed through the oxygen atom, in agreement with the generalization that a six-membered ring is more stable than a five-membered one when two double bonds are present in a chelate ring.

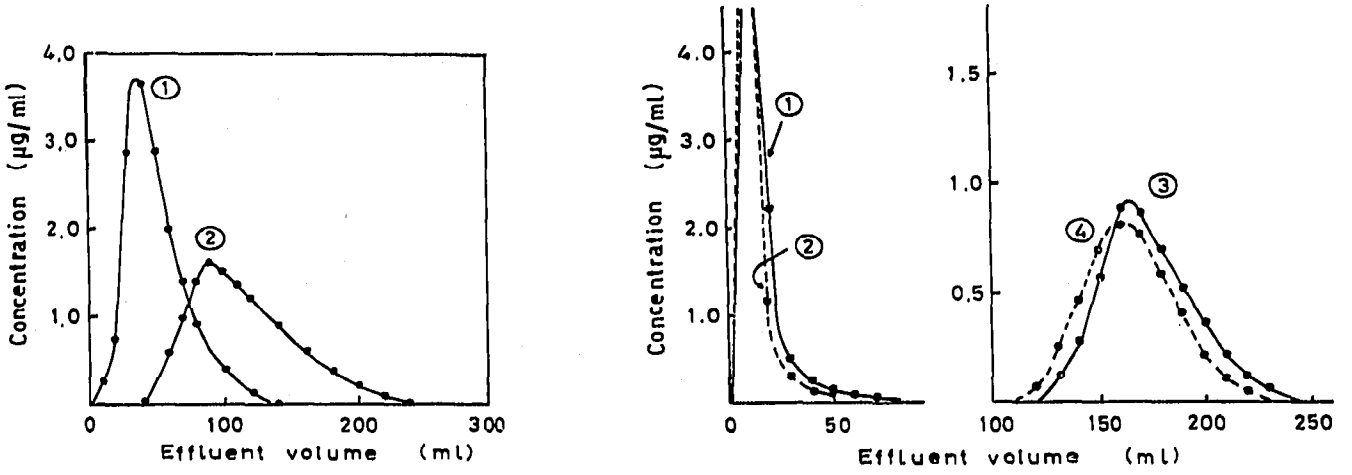


Fig. 8. Effect of physical adsorption on ligand-exchange adsorption. (1) α-Nitroso-β-naphthol; (2) β-nitroso-α-naphthol. Resin: Amberlite CG-120, NH_4^+ form; column size: 11 × 150 mm; developer: 50% ethanolic ammonia solution (pH 9.5); flow rate: 0.25 ml/min.

Fig. 9. Elution curves of hydroxynaphthoic acid and aminonaphthol. (1) 1-Hydroxy-2-naphthoic acid; (2) 2-hydroxy-1-naphthoic acid; (3) 1-amino-2-naphthol; (4) 2-amino-1-naphthol. Resin: Amberlite CG-120, Fe^{3+} form; column size: 11 × 150 mm; developer: aqueous ammonia (pH 9.5); flow rate: 0.25 ml/min.

In the present study, an attempt to determine the coordination site of the nitroso group was made by comparing the retention volume of hydroxynaphthoic acid and aminonaphthol with that of nitrosonaphthol. If the Fe(III) complex of nitrosonaphthol is formed through the oxygen atom in the nitroso group, the retention volume of nitrosonaphthol on the resin in the Fe^{3+} form would be similar to that of hydroxynaphthoic acid. On the other hand, if the complex is formed through the nitrogen atom, the retention volumes of nitrosonaphthol and aminonaphthol would be nearly the same. The retention volumes of 1-hydroxy-2- and 2-hydroxy-1-naphthoic acids and of 1-amino-2- and 2-amino-1-naphthols were measured, using a column of the same length as that used in ligand-exchange chromatography and are shown in Fig. 9: the hydroxynaphthoic acids were not adsorbed, whereas the aminonaphthols had a relatively large retention volume.

These results suggest that the Fe(III) complex of nitrosonaphthol is formed through the oxygen atom in the hydroxyl group and through the nitrogen atom in the nitroso group, to give a five-membered chelate ring. This view is also supported by the low-spin nature of the Fe(III) complex of nitrosonaphthol discussed above: the nitrogen atom having one pair of free electrons tends to produce a much stronger splitting of the crystal field of Fe(III) by concentrating the negative charge toward an e_g orbital of Fe(III), than the oxygen atom which has two of them.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. TEIICHI ANDO for his helpful advice and discussions.

REFERENCES

1 K. SHIMOMURA, L. DIEKSON AND H. F. WALTON, *Anal. Chim. Acta*, 37 (1967) 102.
2 R. BOCK AND A. MONERJAN, *Z. Anal. Chem.*, 230 (1967) 1.
3 K. SHIMOMURA AND H. F. WALTON, *Separ. Sci.*, 3 (1968) 493.
4 C. A. BURTIS AND G. GOLDSTEIN, *Anal. Biochem.*, 23 (1968) 502.
5 R. A. A. MUZZARELLI, A. F. MARTELLI AND O. TUBERTINI, *Analyst*, 94 (1969) 616.
6 W. FUNASAKA, K. FUJIMURA AND S. KURIYAMA, *Jap. Anal.*, 18 (1969) 19.
7 W. FUNASAKA, K. FUJIMURA AND S. KURIYAMA, *Jap. Anal.*, 19 (1970) 104.
8 H. TAUBE, *Chem. Rev.*, 50 (1952) 69.
9 F. FEIGL, *Spot Tests in Inorganic Analysis*, 5th ed., Elsevier, Amsterdam, 1958, p. 144.
10 D. D. PERRIN, *Organic Complexing Reagents*, Interscience, New York, 1964, p. 217.
11 K. K. CHATTERJEE, *Anal. Chim. Acta*, 20 (1959) 423.