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The Separation of Hexacyanoferrate(II) and Hexacyanoferrate(III) Ions by Reversed-phase Partition Chromatography

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The separation of hexacyanoferrate(II) and (III) ions with a TBP-HCl extraction system has been carried out by reversed-phase partition column chromatography. TBP adsorbed on Kel-F powder was used as the stationary phase in the column. Elution was performed with hydrochloric acid of various concentrations and with water, both having been saturated with TBP. The actual separation of hexacyanoferrate(II) and (III) ions from each other was successfully achieved, even in a large volume of a sample solution, by carrying out the elution with 3N HCl. The dependencies of the distribution ratio on the acidity of the solution and on the concentration of TBP in the stationary phase were examined on the basis of the elution curves under various conditions. The liquid-liquid batch extraction was also carried out under the same conditions; it was found an identical relation of distribution between the two phases held in both column and batch extractions. The principal extracted species might be assumed to be a large ion-pair hydrated and solvated.

Reversed-phase partition column chromatography is closely related to the liquid-liquid batch extraction of the same system; the former makes possible the effective separation of each component in a mixture even when the separation factor obtained in the latter is very low, because multiple extraction is carried out in the column. Therefore, this chromatographic technique has been applied to inorganic ions difficult to separate, such as rare earths and transition metals.

In the preceding papers, one of the present authors described the liquid-liquid extraction of alkali metal ions as their poly-iodides in nitrobenzene;[1] this extraction was later developed to reversed-phase partition column chromatography, in which Kel-F powder was used to support the organic phase of nitrobenzene-iodine-iodide.[2] The liquid-liquid extraction of alkaline earth metals as their TTA-chelates in MIBK was also investigated;[3] these results were then further applied to the reversed-phase chromatography, in which the stationary phase was a MIBK solution of TTA held on Kel-F.[4] These chromatographic investigations revealed that valuable information concerning reversed-phase partition column chromatography could be given by a study of the identical liquid-liquid extraction system.

In this paper, the separation of hexacyanoferrate(II) and hexacyanoferrate(III) ions by the reversed-phase partition chromatography will be described; a TBP-(Kel-F) column will be used. It has been reported by Kiba *et al.* that these anions can be

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[1] I. Akaza, This Bulletin, **39**, 465 (1966).

[2] I. Akaza, *ibid.*, **39**, 585 (1966).

[3] I. Akaza, *ibid.*, **39**, 971 (1966).

[4] I. Akaza, *ibid.*, **39**, 980 (1966).

extracted from their hydrochloric acid solution by liquid-liquid extraction with TBP.⁵⁾ The present research aims at a more effective separation of these two ions by using reversed-phase chromatography; the relation between the chromatography and the liquid-liquid extraction will also be investigated.

It is noteworthy that reversed-phase partition chromatography has never before been applied to the separation of common anions.

Experimental

Apparatus. *Column.* A glass column, 0.92 cm in diameter and 30 cm long, and with a coarse frit at the bottom, was used during this study. A separating funnel was fitted at the top of the column as a reservoir of the eluting solution.

Fraction Collector. A fraction collector (Model SF 200A of the Toyo Roshi Co.) holding 200 tubes was employed.

Photoelectric Photometer. A photoelectric photometer, Hitachi EPW-4, was employed, along with a 1-cm cell.

Reagents and Materials. *Kel-F.* Kel-F (polytrifluorochloroethylene), a trade-name low-density 300 moulding powder, was imported from the Minnesota Mining and Manufacturing Co., U. S. A. The material was ground with a mixing grinder and sieved through screens; particles between 42 to 80 mesh were collected for use.

TBP. Commercially-available TBP was purified by washing it twice with a 5% sodium carbonate solution of an equal volume in order to remove any dibutyl- or monobutyl phosphate, and by then distilling the solvent at about 178°C and at 25 mmHg.

All the other chemicals used were of an analytical reagent grade.

Organic Solvent Useful for Both the Stationary Phase of the Column and the Organic Phase of the Batch Extraction. The TBP used in each experiment had been pre-equilibrated with hydrochloric acid of the same concentration as that for the mobile phase or the aqueous phase. No diluent such as benzene was used except when, in some experiments, it was necessary to use TBP of varying concentrations.

Eluting Solution of the Column Extraction and Aqueous Phase Solution of the Batch Extraction. Hydrochloric acid of various concentrations and water were used as the eluting solutions; both had been saturated with TBP or, in some experiments, with TBP-benzene. They were also used as the aqueous phases of the batch extraction.

Test Solution. Hydrochloric acid solutions of potassium hexacyanoferrate(II) and potassium hexacyanoferrate(III) in the range of 10^{-1}M to 10^{-4}M were prepared; the concentration of hydrochloric acid had been made up to equal that in the eluting solution of the aqueous phase solution in each case. A mixed test solution was prepared by mixing equal volumes of these solutions.

Experimental Procedure. All the experiments were performed in rooms from which sunlight was kept out and the temperature, except in the experiment of Table 2, was always about 6—10°C.

The Preparation of the Stationary Phase. Six grams of Kel-F were placed in an Erlenmyer flask fitted with a stopper, and then 6 m*l* of an organic solvent prepared as above was poured in. The volume, 6 m*l* to 6 g of Kel-F, was derived from the amount of TBP adsorbable on Kel-F, an amount which had been determined by other experiments.²,⁴⁾ The stoppered flask was shaken gently by hand until the contents became homogeneous, and then it was left for over three hours as it was. A small volume of an eluting solution was added, and the slurry was poured little by little into the chromatographic column, taking care not to include any air bubbles. Every time a small portion of slurry was added, gentle press for the column bed was done with a glass rod; the bed length eventually reached about 17.4 cm. About a 50-m*l* portion of the eluting solution was then passed through the column in order to remove any contaminant.

The volume of the mobile phase in the column was found to be 3.6 m*l*. The amount of TBP originally adsorbed on the Kel-F column was 6 m*l*; hence, the volume ratio of the stationary phase to the mobile phase was 6 : 3.6.

The Elution and Separation of Hexacyanoferrate(II) and Hexacyanoferrate(III) Ions. Three milliliters of the test solution were poured onto the upper surface of the column and were then percolated through the column at the rate of about 0.25 m*l* per minute; then the inside of the glass tube above the top of the column bed was washed twice with small portions of the eluting solution, letting them flow down at the same rate. On the top of the glass tube, a separatory funnel was placed; then the elution was carried out with eluting solutions of various concentrations of hydrochloric acid supplied from the funnel at the rate of 0.5 m*l*/min. The effluent was collected in suitable fractions with a fraction collector, and the hexacyanoferrate content in each fraction was determined.

A 3-m*l* portion of the test solution was usually employed, but a larger volume, such as 50 m*l*, was also employed in some cases.

Determination of Hexacyanoferrate(II) and Hexacyanoferrate(III) Ions. The $[Fe(CN)_6]^{3-}$ content in each fraction was determined by measuring the absorbance at 420 mμ directly. On the other hand, the concentration of $[Fe(CN)_6]^{4-}$ was determined absorptiometrically after oxidizing it to $[Fe(CN)_6]^{3-}$ by the addition of hydrogen peroxide to the acidic solution. This new determination method will be described here for the first time: Hydrochloric acid and 2% H_2O_2 were added to a solution of $[Fe(CN)_6]^{4-}$, and the mixture was diluted to a definite volume; the concentrations of HCl and H_2O_2 had to be about 1.5N and 0.26% respectively. After the vessel had then been placed at 20°C for 20 min, the yellow color was measured at 420 mμ; its absorbance was invariable, even 5 hr later. The working curve of the $[Fe(CN)_6]^{4-}$ solution coincided exactly with that of $[Fe(CN)_6]^{3-}$, and in both cases the curve could be used up to 5.5×10^{-4} mmol/m*l*; when the concentration of the ions exceeded 5.5×10^{-4} mmol/m*l*, the slope of the curve became gentle.

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5) T. Kiba, K. Terada and N. Kisaka, Preprints for the Annual Meeting of the Chemical Society of Japan (April, 1962), No. 16407; (April, 1963), No. 3105.

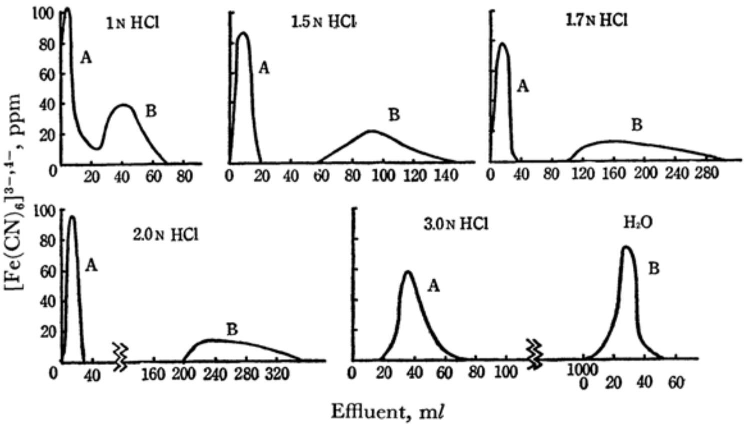

Fig. 1. The elutions at various concentration of hydrochloric acid.
Loaded: mixture of 7.5×10^{-3} mmol each ion
A: $[Fe(CN)_6]^{3-}$ B: $[Fe(CN)_6]^{4-}$

Results and Discussion

The Chromatographic Behavior of Hexacyanoferrate(II) and Hexacyanoferrate(III) Ions. The chromatographic behavior of the ion may be expected from the results of its liquid-liquid extraction. According to the results obtained in the liquid-liquid extraction of $[Fe(CN)_6]^{3-}$ and $[Fe(CN)_6]^{4-}$ by Kiba *et al.*,[5] the distribution ratios of both ions increase with the concentration of hydrochloric acid, and the difference in the distribution ratio between the two ions is large in the range from 1N to 3N of hydrochloric acid. Therefore, the authors' experiments were attempted at concentrations of hydrochloric acid within this range.

The States of the Elutions at Various Concentration of Hydrochloric Acid. Solutions of 5×10^{-3}M $[Fe(CN)_6]^{3-}$ and $[Fe(CN)_6]^{4-}$ in hydrochloric acid of various concentrations were prepared. Both of them, in the same concentration of hydrochloric acid and in equal volumes, were then mixed together. A 3-m*l* portion of the mixed solution was then eluted with acid of the identical concentration. The results are summarized in Fig. 1. It was found that $[Fe(CN)_6]^{3-}$ and $[Fe(CN)_6]^{4-}$ cannot be separated from each other by elution with 1N HCl, but can be easily separated with an acid of higher concentration; also, in all cases $[Fe(CN)_6]^{3-}$, which has the smaller distribution ratio, is first eluted. Moreover, the position of the peak of the elution curve shifts backward on the abscissa with an increase in the concentration of HCl—as is clearly shown in the case of $[Fe(CN)_6]^{4-}$; namely, the distribution ratio of the ion increases with the concentration of HCl. In the case of 3N HCl, all the $[Fe(CN)_6]^{4-}$ remains in the column even after an eluting solution of 1*l* has flowed down, but as soon as the 3N HCl eluting solution is replaced by a water eluting solution, $[Fe(CN)_6]^{4-}$ is eluted immediately.

TABLE 1. THE STATES OF THE ELUTIONS AT VARIOUS AMOUNTS OF $[Fe(CN)_6]^{3-,4-}$

Concn. of HCl N	Loaded, mmol $[Fe(CN)_6]^{3-}$	Loaded, mmol $[Fe(CN)_6]^{4-}$	Elution range, m*l* $[Fe(CN)_6]^{3-}$	Elution range, m*l* $[Fe(CN)_6]^{4-}$	
2.0	1.5×10^{-2}	7.5×10^{-3}	0—34	186—395	max. at
2.0	7.5×10^{-3}	1.5×10^{-2}	0—34	167—499	
1.5		15×10^{-4}		52—112	82
1.5		15×10^{-3}		43—171	93
1.5		3×10^{-2}		40—207	106

The States of the Elutions at Various Amounts of the Ions. The following two experiments were carried out, one at varying ratios of $[Fe(CN)_6]^{3-}$ to $[Fe(CN)_6]^{4-}$ in 2N HCl, and the other at varying amounts of only $[Fe(CN)_6]^{4-}$ in 1.5N HCl. The results of elution are shown in Table 1. The situation of the peak of each elution curve seems practically unchangeable, though there is a slight shift backward with an increase in the amount of the ions loaded; this is contrary to the cases of alkali polyiodides and alkaline earth-TTA chelates, in which the distribution ratios decrease with an increase in the amount of the substance loaded. This discrepancy may be attributed to the difference between the types of extraction mechanisms: in this extraction system of hexacyanoferrate ions, the extraction is not affected by the relative amounts of the ion and the reactant because the ions transfer, without any reactant, into the TBP phase as their solvated free acids.

The Separation of Hexacyanoferrate(II) and (III). Eluting solutions of from 2N to 3N hydrochloric acid may be useful in separating these two substances, as is shown in Fig. 1. Among these solutions, the 3N solution was preferred. The sample solution of 3N HCl was loaded, and then an eluting solution with the same HCl concentration was flowed in order to elute $[Fe(CN)_6]^{3-}$. In order to elute the $[Fe(CN)_6]^{4-}$ rapidly, the eluting solution was replaced with water. A satisfactory separation was attained, as is shown in Fig. 2.

Quantitative Separation of $[Fe(CN)_6]^{3-}$ and $[Fe(CN)_6]^{4-}$ from a Mixed Test Solution of a Large Volume. To show the practical usefulness of the method and a quantitative separation of the ions, a simple process was carried out with a test solution of a 50-m*l* portion of a 3N HCl solution containing each of the two ions in 5×10^{-2} mmol (10.59 mg), and without a fraction collector.

This experiment was carried out at 20°C under a flow rate of 1 m*l*/min, faster than in the other experiments. The effluent of 3N HCl was placed successively in 100-, 50-, and 25-m*l* measuring flasks; the portions were named the first, second, and third fractions respectively. As a result of this process $[Fe(CN)_6]^{3-}$ should flow down. In order to examine if all of the $[Fe(CN)_6]^{4-}$ was left on the column, a new eluting solution of 1.5N HCl-H_2O_2*[1] was supplied while about 2 m*l* of the preceding 3N HCl eluting solution still remained on the top of the column; the elution of $[Fe(CN)_6]^{4-}$ was then continued, with the fourth and fifth fractions placed in measuring flasks of 50 m*l* and 25 m*l* respectively. By this elution the $[Fe(CN)_6]^{4-}$ in the column was oxidized to $[Fe(CN)_6]^{3-}$. The absorbancy of each fraction was measured at 420 mμ. Furthermore, the absorbancy of the first and second fractions was measured again after the addition of H_2O_2. This treatment is necessary to certify the absence of $[Fe(CN)_6]^{4-}$ in the fractions of $[Fe(CN)_6]^{3-}$.

The results shown in Table 2 show that all of the $[Fe(CN)_6]^{3-}$ was eluted and recovered in the first 125-m*l* effluent, and $[Fe(CN)_6]^{4-}$ did not appear in this fraction, since the $T\%$ of the first and the second fractions after oxidation by H_2O_2 remained the same as before the oxidation. None of the ions could be detected in the next, third fraction. The results from the fourth and fifth fractions showed that $[Fe(CN)_6]^{4-}$ was left in the column quantitatively. This means that comparatively large amounts of $[Fe(CN)_6]^{4-}$ ions can be caught in the column quantitatively from the test solution of a larger volume, even at quite a fast flow rate. In this experiment the water-eluting solution was intentionally not employed for $[Fe(CN)_6]^{4-}$, unlike the ordinary elution presented in Fig. 2. A reason for this treatment is as follows. It was found in a preliminary test that $[Fe(CN)_6]^{4-}$ separated itself, as white, free acid $H_4[Fe(CN)_6]$, when a little TBP was added to a solution containing a large amount of $[Fe(CN)_6]^{4-}$ at a high concentration of hydrochloric acid. On the other hand, in the above column experiment, the concentration of $[Fe(CN)_6]^{4-}$ was be raised remarkably in a small upper part of the column, since adequate extraction conditions, such as 3N HCl, were kept; moreover, a large amount of $[Fe(CN)_6]^{4-}$ was handled here. The spontaneous separation of the free acid could be expected, as in the preliminary test. This free acid seemed unstable in water, and indeed it at once became blue when the hydrochloric acid-eluting solution was displaced with water. Hence, the water-eluting solution was omitted. The fact that $[Fe(CN)_6]^{4-}$ remained near the top of the column, has also the advantage that the separation from $[Fe(CN)_6]^{3-}$ can be achieved successfully without any consideration of the volume of the sample solution and the flow rate of elution. If one desires to take out the $[Fe(CN)_6]^{4-}$ as it is, not according to above procedure, it should be washed down to-

TABLE 2. QUANTITATIVE SEPARATION OF $[Fe(CN)_6]^{3-}$ AND $[Fe(CN)_6]^{4-}$ FROM MIXED TEST SOLUTION OF LARGE VOLUME

Fraction number	Eluant	Effluent collected m*l*	Found mg	Taken mg
1	3N HCl	100	10.38 ($[Fe(CN)_6]^{3-}$)	10.59
2	3N HCl	25		
3	3N HCl	25	0.0	
4	1.5N HCl-H_2O_2	50	10.41 ($[Fe(CN)_6]^{4-}$)	10.59
5	1.5N HCl-H_2O_2	25		

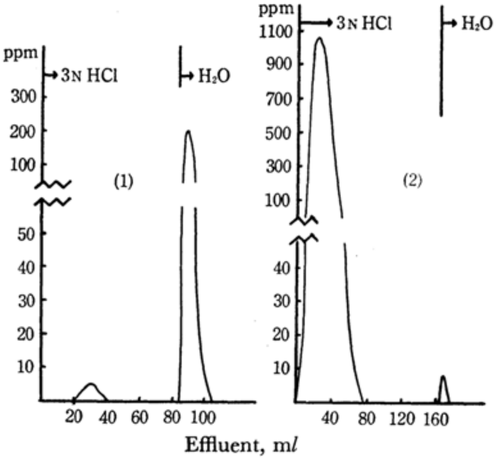

Fig. 2. The separation of $[Fe(CN)_6]^{3-}$ and $[Fe(CN)_6]^{4-}$.
Loaded: (1) 3.0×10^{-4} mmol $[Fe(CN)_6]^{3-}$
1.5×10^{-2} mmol $[Fe(CN)_6]^{4-}$
(2) 1.5×10^{-1} mmol $[Fe(CN)_6]^{3-}$
3.0×10^{-4} mmol $[Fe(CN)_6]^{4-}$

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*[1] In preparing the eluting solution of HCl-H_2O_2, 13.5 m*l* of 2% H_2O_2 were added to 50 m*l* of 3N HCl, and then the solution was made up to 100 m*l* with water.

gether with the stationary phase of TBP by passing 50 m*l* of a 1 N HCl - 50% alcohol solution (2 N HCl : $C_2H_5OH=1:1$) through the column.

Some Relation to the Liquid-Liquid Extraction. The mechanism of reversed-phase partition chromatography is very similar to that of the liquid-liquid extraction of the same system, has been clarified by one of the present authors. Now, the extraction of $[Fe(CN)_6]^{4-}$ with TBP was preferentially examined.

The equation for the extraction of $[Fe(CN)_6]^{4-}$ with TBP can be written simply as:

$$n\mathrm{H}^+_{(a)} + \mathrm{A}^{n-}_{(a)} + m\mathrm{TBP}_{(o)} = \mathrm{H}_n\mathrm{A}\cdot m\mathrm{TBP}_{(o)} \qquad (1)$$

where A^{n-} is the hexacyanoferrate ion. The distribution ratio, *D*, for this process can be written as:

$$D = K[\mathrm{H}^+]^n[\mathrm{TBP}]^m \qquad (2)$$

where *K* is the thermodynamic extraction constant. Therefore, the distribution ratio, *D*, depends on the activity of both TBP and hydrogen ions. These activities can, for convenience, be displaced by the concentration of an acid and TBP.

The distribution ratio in the column extraction by reversed-phase partition chromatography was calculated, by using Eq. (3), from the elution curve obtained:

$$D = (E - F)/B \qquad (3)$$

where *E* is the volume of the effluent before the emergence of the solute in a maximal concentration; *B*, the volume of the stationary phase, and *F*, the volume of the mobile phase.

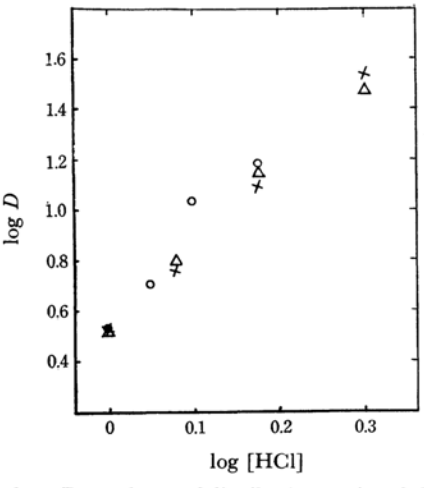

Fig. 3. Dependence of distribution ratio of $[Fe(CN)_6]^{4-}$ upon the concentration of HCl.
○ Column chromatography
△ Liq.-liq. (on Kel-F) batch extraction
× Liq.-liq. batch extraction

First, the dependence of the distribution of $[Fe(CN)_6]^{4-}$ on the concentration of hydrochloric acid was examined. Three-m*l* portions of a 5×10^{-3}M $[Fe(CN)_6]^{4-}$ solution with various concentrations of hydrochloric acid were fed into the column; elution was then carried out with a hydrochloric acid-eluting solution of the same concentration. The distribution ratio of $[Fe(CN)_6]^{4-}$ in each case was calculated by putting a value of *E* obtained from an elution curve into Eq. (3), and plotted on a graph of log*D* *vs.* log[HCl] as is shown in Fig. 3.

For comparison with the above results, a series of batch extractions was carried out between (Kel-F)-TBP and an aqueous phase of the eluting solution. In a 100-m*l* glass-stoppered Erlenmyer flask, (Kel-F)-TBP was prepared with TBP pre-equiliburated with HCl of varying concentrations in the manner described above. After the solution had stood for more than three hours, 9 m*l**2 of each eluting solution of a definite acidity and 3 m*l**2 of a 5×10^{-3}M solution of $[Fe(CN)_6]^{4-}$ were introduced into the flask. The stoppered flask was gently shaken for a while and then set aside. The contents were poured on a coarse glass filter, and the aqueous phase was allowed to drain before it was submitted to photometry for $[Fe(CN)_6]^{4-}$. The results are shown in Fig. 3.

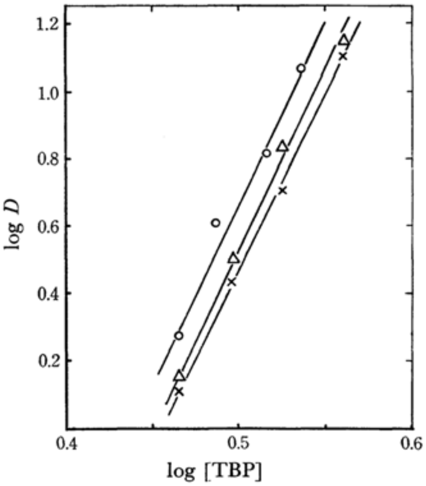

Fig. 4. Dependence of distribution ratio of $[Fe(CN)_6]^{4-}$ upon the concentration of TBP.
○ Column chromatography
△ Liq.-liq. (on Kel-F) batch extraction
× Liq.-liq. batch extraction

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*2 The reason for taking 12 m*l* as the sum of the two aqueous solutions is as follows: If the same volume ratio as in the column, organic phase: aqueous phase = 6 : 3.6, had to be kept, 3.6 m*l* of the aqueous volume would be too small to separate it from the organic solvent adsorbed on Kel-F. In this extraction system, the distribution ratio, *D*, did not differ upon a change in the volume ratio, so the data from the column extraction carried out at 6 : 3.6 and the batch extraction carried out at 6 : 12 are comparable.

Moreover, another series of liquid-liquid extractions, with Kel-F omitted from the system, was carried out. In this case, not only the organic solvent and the aqueous solutions, but also their volumes, were all identical to those described just above. These results are also shown in Fig. 3.

Next, the dependency of the distribution ratio on the concentration of TBP was examined by three kinds of experiments in the same manner as when the relation of $\log D$ to $\log[\mathrm{HCl}]$ was obtained. However, the concentration of hydrochloric acid was fixed at 1.5N in all these experiments, while that of TBP was varied by diluting the reagent with benzene. Organic solvents containing TBP in various concentrations were used for the stationary phase of the column chromatography, for the batch extraction with TBP-(Kel-F), and also for the liquid-liquid extraction. The data obtained are shown in Fig. 4 as a graph of $\log D$ *vs.* $\log[\mathrm{TBP}]$.

As Fig. 3 and Fig. 4, show all of the slopes showing the relation $\log D$ *vs.* $\log[\mathrm{HCl}]$ are about 3.5 and the dependency of D on the concentration of TBP is about 11 in every series of experiments. The data obtained in the column extraction chromatography and those in the liquid-liquid extraction are thus in good agreement. This shows that the extraction mechanism in the reversed-phase column chromatography is identical with that of the liquid-liquid extraction system.

In the former, however, the distribution ratio was found to be a little higher than in the liquid-liquid batch extraction; this might be caused by a small difference in temperature between the two experimental rooms,*3 not by the extraction mechanisms. Because the data obtained from the liquid-liquid batch extraction and those from the batch extraction with (Kel-F)-TBP are found to be in good agreement on the straight lines in Figs. 3 and 4, it can be said that Kel-F powder does not affect the extraction mechanism. Also, it was observed that this extraction system is readily affected by the temperature; *e.g.*, of the distribution ratios of $[\mathrm{Fe(CN)_6}]^{4-}$ at 20°C and 10°C, the latter has a value higher than the former by about 0.5 in logarithm.

The slopes of the lines in Figs. 3 and 4 presumably do not stand directly for n and m in Eq. (2). They only indicate the average reagent-dependence of the species present; furthermore, the experimental conditions are remarkably far from ideal for an infinite dilution system. As has been shown in the papers concerning the extraction mechanism of acids by TBP by Whitney and Diamond(6) and Biddle *et al.*,(7) the principal extracted species may be assumed to be a large species which is an associate of a hexacyanoferrate ion and about four trihydrated hydronium ions, each of the hydronium ions being solvated with three TBP's.

Summary

The present authors have developed a liquid-liquid extraction system of hexacyanoferrate(II) and (III) with TBP to a reversed-phase column partition chromatography. In the column, TBP held on Kel-F powder was used as the stationary phase. The separation of each ion from the mixture has been successfully achieved by carrying out the elution with an eluting solution of 3N HCl; tolerable amounts could thus be treated. The relation between the method and the liquid-liquid extraction has been examined; no fundamental difference could be found between them.

[*3] The chromatography was carried out in a room the temperature in which was kept at 6—10°C, while the extraction was done in an anteroom with a temperature slightly higher than the inner room.

6) D. C. Whitney and R. M. Diamond, *J. Phys. Chem.*, **67** 209 (1963).

7) P. Biddle, A. Coe, H. A. C. Mckay, J. H. Miles and M. J. Waterman, *J. Inorg. Nucl. Chem.*, **29**, 2615 (1967).