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The Separation of Alkaline Earth Metals as Their TTA-Complexes by Reversed-Phase Partition Chromatography

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The author's preceding work, the liquid-liquid extraction of alkaline earth metals with TTA-MIBK, has now been extended to a reversed-phase partition chromatography in order to attain a good separation of the metal ions. The method was carried out with relatively large amounts of the metals through a column of Kel-F on which the organic phase of 1.5 M TTA - MIBK was supported. Elution was performed with 0.5 M buffer solutions, of various pH values, consisting of acetic acid, ammonium acetate and aqueous ammonia. The actual separation of each metal ion from the mixture of alkaline earth metals was achieved; the results are shown in several figures. The dependence of the distribution ratio on the pH of the solution and on the concentration of TTA in the stationary phase was examined from the elution curves; it might be considered that the same relation held as in the case of the liquid-liquid extraction system. Other experiments were then carried out in order to clarify the behavior of the TTA-chelates in the presence of a large amount of TTA; it was found that all of the TTA in the system participated in the effective extraction of the metal ion. The steps in the formation of the higher TTA-chelate in the column are depicted by the break-through curve of the chromatographic process.

Previously the present author studied the liquid-liquid extraction of alkaline earth metal ions with TTA (2-thenoyl trifluoroacetone) in methyl isobutyl ketone (abbreviated as MIBK); the details of that investigation have been described in a preceding paper.[1] As a consequence of that study it was found that the separation of alkaline earth metals from one another by batch extraction was very difficult because the distribution ratios of the TTA-chelates of the metals between methyl isobutyl ketone and an aqueous solution of a different pH value were so close together. The lower separation factors among the metal ions induced the author to seek other more convenient and efficient methods based upon extraction with TTA - MIBK. Reversed-phase partition chromatography is closely related to liquid-liquid extraction, except that in the former the water-immisible extractant is immobilized on a stationary inert support. In the column extraction of this type an aqueous solution containing the solute to be extracted is always in contact with the fresh organic solvent on the column, and multiple extraction may be carried out throughout the process; therefore, effective separation may be achieved between the substances of a lower separability. Moreover, a larger quantity of metal ions may be treated by this method.

In this investigation Kel-F (polytrifluorochloroethylene), presented by Hamlin et al.,[2] was chosen as the inert solid support from among the

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[1] I. Akaza, This Bulletin, **39**, 971 (1966).

[2] A. G. Hamlin, B. J. Roberts, W. Laughlin and S. G. Walker, *Anal. Chem.*, **33**, 1547 (1961).

various kinds of material that had been recommended. Reversed-phase partition chromatography has never been applied for the separation of alkaline earth metals in the column method, excepting the work by O'Laughlin and Banks³⁾ who separated barium, magnesium and calcium from each other using bis(di-*n*-hexyl-phosphinyl)-methane. Recently, however, Cerrai and Ghersini⁴⁾ attempted the paper chromatographic method with di-(2-ethylhexyl)orthophosphoric acid, HDEHP; the R_f value for each metal showed a capability of separation.

This paper will describe a column method of reversed-phase chromatography in which the stationary phase is a solution of TTA in MIBK supported on Kel-F, and the mobile phase is a 0.5 M buffer solution consisting of acetic acid, ammonium acetate and aqueous ammonia. The results of the liquid-liquid extraction of TTA-chelates of alkaline earth metals described in a preceding paper¹⁾ will be fully utilized. The elution of the individual ions and the isolation of each metal could be achieved successfully by the column method. The relation of the chromatography to the liquid-liquid extraction will be discussed in detail.

Experimental

Apparatus.—The column, the fraction collector and the flame photometer were the same as those described in previous paper,⁶⁾ but the inner diameter of the column tube was 1.2 cm. and the length was 20 cm.

Reagents and Materials.—Kel-F (polytrifluorochloroethylene), TTA (2-thenoyltrifluoroacetone) and MIBK (methyl isobutyl ketone) were the same as those used by the author in previous investigations.¹,⁶⁾

The Organic Solvent Solution Used for The Stationary Phase.—In 20 ml. of MIBK placed in a separating funnel, dissolve 8.888 g. of TTA; the volume of the solution thereby becomes 27 ml. Adjust the pH of a 0.5 M ammonium acetate solution saturated with MIBK to the value which is preferred for each eluting process. Put 27 ml. of this solution into the separating funnel, and shake the vessel until the two phases are equilibrated enough. Use the organic phase for adsorbing on Kel-F powder, as will be described below. This method of preparation was employed in many cases in this research and is referred to as the 1.48 M TTA-MIBK solvent solution. In some cases, however, MIBK solutions with different amounts of TTA were also used.

Eluting Solution.—The eluting solution in each case should have a composition similar to that of an aqueous solution which has been equilibrated with the organic solvent solution it comes in contact with in the column. To save the trouble of actually equilibrating the two phases, the following method should be employed: First, prepare a solution containing a definite amount of TTA in MIBK. Next, prepare a 0.5 M ammonium acetate - acetic acid or aqueous ammonia buffer solution of a definite pH which is saturated with MIBK. Place equal volumes of the TTA-MIBK solution and the buffer solution in a separating funnel, and equilibrate the two phases by shaking. Take out a definite volume of the organic phase and pour it on the Kel-F powder in order to prepare a stationary phase of the column. Determine the amount of TTA present in the aqueous phase by the author's method.⁵⁾ Weigh the appropriate amount of TTA as calculated from the value and dissolve it in the buffer solution in the manner shown below.

Eluting Solution of pH 5.5.—Put into a 1-l. separating funnel 3.15 g. of a TTA reagent and 900 ml. of a 0.5 M ammonium acetate solution which has been saturated with MIBK; then shake the vessel for 30 min. in order to dissolve the reagent completely. Then adjust the pH of the solution to 5.5 by adding 0.5 M acetic acid. Make the total volume of the solution to 1 l. with a solution of 0.5 M ammonium acetate saturated with MIBK. Employ this solution for elution.

The amounts of TTA in the various buffer solutions which have been determined are as follows; the same amount of TTA should be dissolved for the preparation of each eluting solution:

Organic phase contacted	pH of the buffer solution	TTA g./l.
1.48 M TTA - MIBK	5.5	3.15
1.48 M TTA - MIBK	6.5	3.2
0.2 M TTA - MIBK	5.5	0.2
0.2 M TTA - MIBK	6.5	0.42
0.2 M TTA - MIBK	7.5	0.59

These eluting solutions can be stored without decomposition for a month in a dark place between 0°C and 3°C.

In the above case the amount of TTA to be dissolved in each eluting solution differs with the temperature, so the temperature during the preparation of the eluting solution and the elution of a solute from the column should be kept constant. Changes in temperature affect the solubility of MIBK in the eluting solution; therefore, the solubility of TTA also varies.

Sample Solution.—Barium chloride dihydrate, strontium nitrate tetrahydrate, calcium chloride dihydrate, and magnesium chloride hexahydrate, all of which are analytically-pure reagent grade materials, were employed. It is desirable, but not in all cases, that the sample solution have a composition like to that of the eluting solution. Three-milliliter portions of the sample solution were used for the column experiment.

Experimental Procedure.—*Preparation of the Stationary Phase.*—In a glass-stoppered Erlenmyer flask, put 8.0 g. of the Kel-F powder which has been dried at 80°C and stored in a calcium chloride desiccator; pour on it 7.3 ml. of an organic solvent solution prepared as above. After stoppering the flask, shake it by hand in order to homogenize the contents, and leave it in an ice-box for at least three hours. Add a small volume of a suitable eluting solution, and pour the slurry little by little into the chromatographic column, taking care not to let any air bubbles in, and gently pressing the slurry down with a glass rod each time. After pouring in all

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3) J. W. O'Laughlin and C. V. Banks, U.S.A.E.C. Rep., **IS-737**, 145 (1963).

4) E. Cerrai and G. Ghersini, *J. Chromatog.*, **13**, 211 (1964).

5) I. Akaza, M. Kosaka and T. Imamura, *Bunseki Kagaku* (*Japan Analyst*), **14**, 825 (1965).

6) I. Akaza, This Bulletin, **39**, 585 (1966).

the slurry, press the column bed with a glass rod in order to make it height about 18 cm. The upper surface of the column should be covered by the eluting solution.

The Elution, Separation and Determination of Metals.—Pour 3 ml. of the sample solution on the upper surface of the column and allow it to percolate into the column at the rate of 0.2 ml. per minute; then twice pour in little portion of the eluting solution in order to wash inside of the glass tube above the upper surface of the column, letting it flow down the column at the same rate. Set on the top of the glass tube a separating funnel from which the eluting solution of a definite composition is allowed to flow through the column, and carry out the elution at the rate of 0.5 ml. per minute. Collect the effluent in suitable fractions by a fraction collector, and determine the amount of metal in each fraction by flame-photometer. In order to get a satisfactory separation of an ion from the others with reasonable speed, a different eluting solution should be used for each ion.

Results and Discussion

The Characteristic Behavior of the Column. —*The Choice of Column Material.*—The author preferred Kel-F moulding powder for the same reason as was stated in a preceding paper.[6]

The Amount of Methyl Isobutyl Ketone that can be adsorbed by Kel-F.—In a glass-stoppered Erlenmyer flask 10 g. of Kel-F powder was placed; 50 ml. of MIBK was then poured on the powder, and the vessel was put aside for overnight after having been gently shaken. The unadsorbed solvent was drained through a small glass filter into a measuring cylinder under a slight suction. The inside wall of the flask was washed with 1 ml. of a fresh solvent in order to transfer all the Kel-F powder to the filter. The difference between the volume taken and that measured in the cylinder indicated the volume of the solvent adsorbed on Kel-F. It came to 9.0 ml. of the solvent for 10 g. of Kel-F powder.

The Volume of the Mobile Phase in the Column.—The column bed was prepared as follows: On to 8.0 g. of Kel-F powder, 7.2 ml. of MIBK saturated with water was poured. The ratio of these amounts was fixed at that obtained in the above experiment. The powder was then allowed to adsorb the liquid by standing overnight. A 0.1 M sodium chloride solution saturated with MIBK was added to the powder, and the resulting slurry was poured into the glass tube in order to make a column bed 18 cm. deep. Plenty of 0.1 M sodium chloride solution-saturated MIBK was allowed to flow down until the effluent showed the same concentration of sodium chloride as the original solution. After careful washing, with water, the inside of the tube below the fritted glass filter, water-saturated MIBK was poured on the column in order to wash down the 0.1 M sodium chloride solution occupying the pores of the column. The amount of sodium chloride was determined in the washing liquor by titrating it with a 0.1 M silver nitrate solution as usual; the volume of the pores of the column was then obtained from this value. That turned out to be 7.27 ml; hence, the volume ratio of the stationary phase to the mobile phase was found to be 1 : 1 in this case. This showed that the elution process consisted of multiple extractions between equal volumes of the two phases.

The pre-equilibration between the organic solvent and eluting solution of equal volumes, described in the preceding section, was necessary to maintain the condition of actual equi-volume extraction. However, they were not always prepared by the actual equilibration of the two phases, but usually by putting a calculated amount of the reagent in an aqueous solution.

Column Life and Stability.—After loading 0.09 mmol. of calcium on the column, elution with a solution of pH 5.5 was carried out; the elution curve was thus obtained as usual. The same experiment was repeated three more times in succession with the same column. The volume of eluent spent before the emergence of the calcium ion at a maximal concentration, as denoted by *E*, in each case is shown in Table I. In these experiments the temperature was kept at about 12°C, and the column was kept covered with black paper in order to exclued sunlight. In each elution about 420 ml. of the eluent was used, so the total volume came to as much as 1.7 l. On the other hand, two elutions carried out under the same conditions with the same column at an interval of two weeks gave a satisfactory result each time. Therefore, the column seems to remain capable of satisfactory use for a week, if the temperature is kept below 12°C and if light is excluded.

TABLE I. COLUMN LIFE REVEALED BY THE SHIFTING OF ELUTION PEAK

Experiment in order	Date	Volume of effluent before peak of maximum elution
1	April 22	158.9 ml.
2	April 23	161.9 ml.
3	April 24	174.9 ml.
4	May 1	126.8 ml.

The Recovery of Kel-F.—The Kel-F powder could be recovered from the degraded column by the following method: The powder used was washed with water repeatedly, and with 1 M hydrochloric acid several times. It was then put in water containing 40% alcohol, which had been found to be a good wetting solvent for Kel-F, and stirred well in order to remove the MIBK from the powder. This treatment was repeated several times until the solution became neutral and the powder, pure white. The powder was then washed again with 99% alcohol and the washings were removed with a glass filter under suction. Some ethyl ether was poured on the powder. The powder was dried under strong suction in order to remove the ether completely. The powder of Kel-F thus treated could be used again after drying at 80°C for one hour.

Column Capacity.—The capacity of the column prepared as described in the procedure was estimated by loading it with a calcium salt of a known concentration. The breakthrough curve obtained was not as sharp nor as smooth as usual. This may be due to the mechanism of the extraction described in a foregoing paper[1]; the meaning of an irregular curve will be discussed later.

The Chromatographic Behavior of Alkaline Earth Metals.—The chromatographic behavior of each alkaline earth metal ion can be predicted from the previously-descrived results obtained in the liquid-liquid extraction of their TTA-chelates with MIBK.[1] Their distribution ratios were found to depend on the hydrogen ion concentration of the aqueous phase, the concentration of TTA in the organic phase, and the quantity of metal ion to be treated. At any definite pH value of the aqueous phase, the distribution ratio of each metal ion was in the desending order: magnesium, calcium, strontium and barium. From these results it was expected that, in the column chromatography, barium could be eluted from the column faster than the others. Taking each extraction curve obtained previously into account, the elution curves of each metal ion were obtained at pH 6.5 and 5.5.

The Elution Curve of Individual Ions.—To examine quantitatively the retention on, and the elution from, the column, 0.025 mmol. of each metal ion was placed in the column and eluted with an eluting solution of pH 6.5. The results are shown in Fig. 1, which shows that the elution at this condition may be useful for the separation of the two metals. When a similar elution was carried out at pH 5.9, the two curves overlapped with each other. The elution curves of strontium and calcium at pH 5.5 are shown in Fig. 2. They show the possibility of using this condition for the separation of the above two metals. Magnesium, however, was precipitated as the insoluble TTA-chelate over the pH range mentioned above.

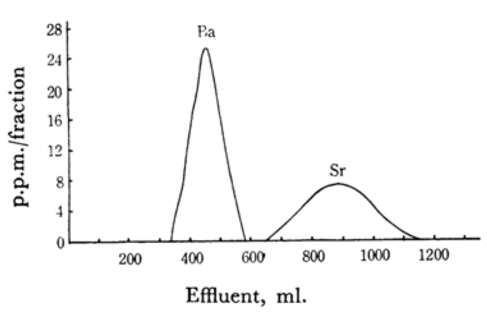


Fig. 1. Elution curves of barium and strontium. (Experiments with Ba and Sr conducted separately)
Loaded: 0.025 mmol. of the element
Eluting solution: pH 6.5

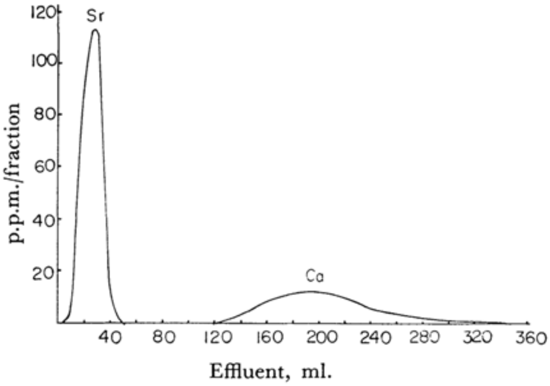


Fig. 2. Elution curves of strontium and calcium, individually.
Loaded: 0.025 mmol. of the element
Eluting solution: pH 5.5

The Separation of Metal Ions.—a) The Separation of Barium and Strontium.—Various amounts of

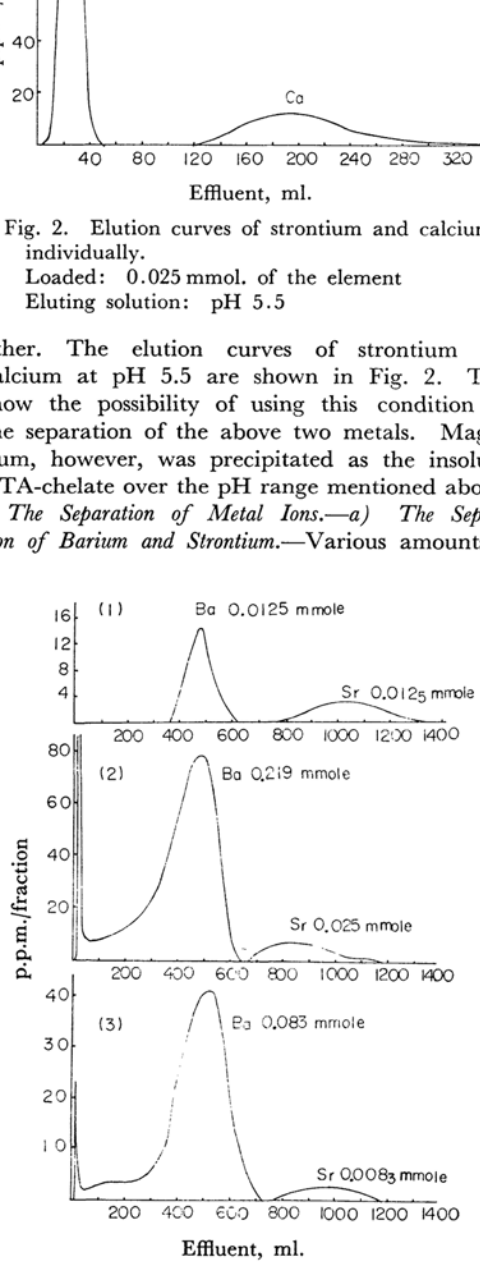
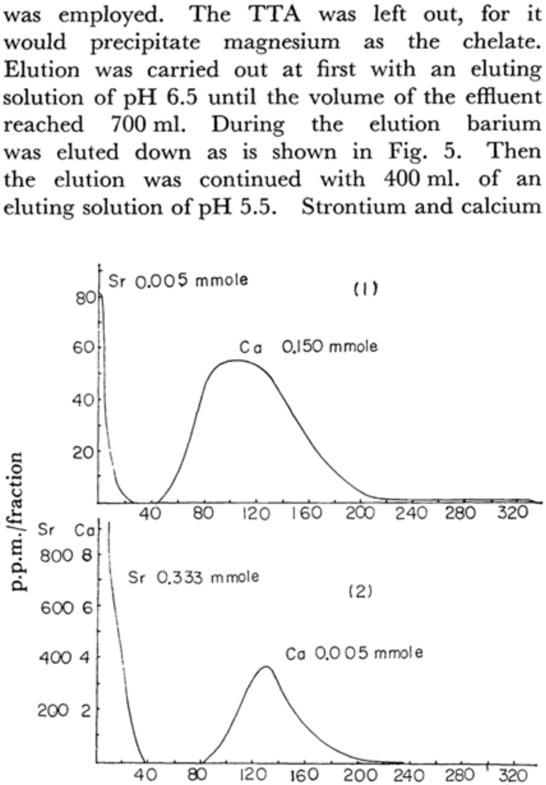


Fig. 3. Separation of barium from strontium.
Loaded: (1) 0.0125 mmol. Ba and 0.0125 mmol. Sr
(2) 0.219 mmol. Ba and 0.025 mmol. Sr
(3) 0.083 mmol. Ba and 0.008_3 mmol. Sr
Eluting solution: pH 6.5

barium and strontium ions were mixed in an eluting solution of pH 6.5 to make three kinds of a sample solution: (i) 0.0125 mmol. barium and 0.0125 mmol. strontium; (ii) 0.219 mmol. barium and 0.025 mmol. strontium and (iii) 0.083 mmol. barium and 0.0083 mmol. strontium, each in 3 ml. portions. Three milliliters of each sample solution were placed in the column, and the elution was carried out with the eluting solution of pH 6.5. The results obtained are shown in Fig. 3, from which it may be seen that the two metals could be separated from one another, even if they were present in widely different ratios.

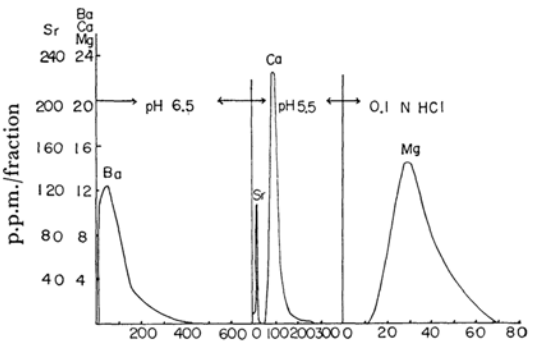
b) The Separation of Strontium and Calcium.—In the column prepared as described in the procedure there was placed 3 ml. of a sample solution containing 0.333 mmol. strontium and 0.005 mmol. calcium and then another 3 ml. containing 0.005 mmol. strontium and 0.15 mmol. calcium. The elution was carried out with an eluting solution of pH 5.5. A satisfactory separation could be achieved, as is shown in Fig. 4.

c) The Separation of Barium, Strontium, Calcium and Magnesium.—A 3 ml. sample solution containing 0.0125 mmol. barium, 0.0125 mmol. strontium, 0.025 mmol. calcium and 0.0125 mmol. magnesium was employed. The TTA was left out, for it would precipitate magnesium as the chelate. Elution was carried out at first with an eluting solution of pH 6.5 until the volume of the effluent reached 700 ml. During the elution barium was eluted down as is shown in Fig. 5. Then the elution was continued with 400 ml. of an eluting solution of pH 5.5. Strontium and calcium appeared in the effluent. At last the magnesium retained on the column was eluted down with 0.1 M hydrochloric acid. Thus the separation of alkaline earth metals from their mixture was achieved.



Effluent, ml.

Fig. 4. Separation of strontium from calcium.
Loaded: (1) 0.005 mmol. Sr and 0.15 mmol. Ca
(2) 0.333 mmol. Sr and 0.005 mmol. Ca
Eluting solution: pH 5.5



Effluent, ml.

Fig. 5. Separation of barium, strontium, calcium and magnesium.
Loaded: 0.0125 mmol. Ba, 0.0125 mmol. Sr, 0.025 mmol. Ca and 0.0125 mmol. Mg
Eluting solution: As mentioned in the graph

A Blank Test with the Solvent without TTA.—If the absence of the chelating agent TTA in MIBK causes a retention of alkaline earth metal ions by the stationary phase on the column, the method will be unsatisfactory for the separation of these ions. To test whether such retention really occurs, 7.3 ml. of MIBK which had been equilibrated with a 0.5 M buffer solution of pH 6.5 saturated with MIBK was allowed to be adsorbed on 8.0 g. of Kel-F powder and then the column was prepared. The elution of calcium ions was then carried out. Calcium ions appeared as early as in the first fraction of the effluent, and reached a maximum in the third. Therefore, the calcium ions placed in the column could not be retained at all without the presence of TTA in the organic solvent.

Some Relation to the Liquid-liquid Extraction.—*The Dependence of the Distribution of Metal Ions on the pH of the Solution.*—In a preceding paper[1] the present author stated that the dependence of the distribution ratio upon the hydrogen ion concentration of the aqueous phase could be represented by a straight line with a slope of about one to two in the graph of log$D$ vs. pH. The slope of the line was sharper when the quantity of metal ions was smaller, as well as when the stability of the chelate of the metal was higher; a higher concentration of TTA in the organic phase also made the slope sharper. In the present study, the relation between the distribution ratio of metal and the pH was investigated in the extraction process in the column. Each 3-ml. portion of sample solutions of varying pH values contained 0.005 mmol.

of strontium. This 3 ml.-portion was placed in the column; elution was then carried out with eluting solutions of the same pH. The elution curve was obtained as usual, and the distribution ratio of the metal in each case was calculated by Eq. 1, which is identical with the one generally used in partition chromatorgraphy:

$$D = (E - F)/B \qquad (1)$$

where
D: distribution ratio; E: 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, viz., the pores in the column.

The value of D calculated in each case is plotted in a graph of log D vs. pH in Fig. 6. In these experiments the pH of the eluting solution scarcely varied from the initial value. The relation between log D and pH is shown as a straight line with a slope of 2.3.

For comparison with the above results, a series of liquid-liquid extractions was carried out with 7.3 ml. of the same organic solvent as had been adsorbed on Kel-F powder and 21.9-ml.* portions of several eluting solutions containing 0.1 mmol. of calcium ions; the calcium ion content in the aqueous phase was determined by flame-photometry. This was repeated with samples of different pH values; the distribution ratio of calcium was calculated as usual. The data obtained are shown in Fig. 7 as a graph of log D vs. pH.

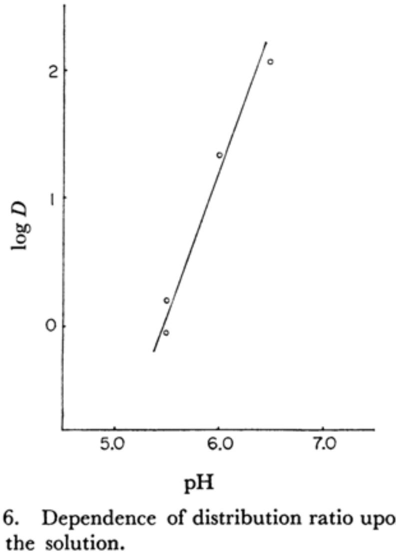


Fig. 6. Dependence of distribution ratio upon pH of the solution.
The values of D were calculated from respective elution curves.

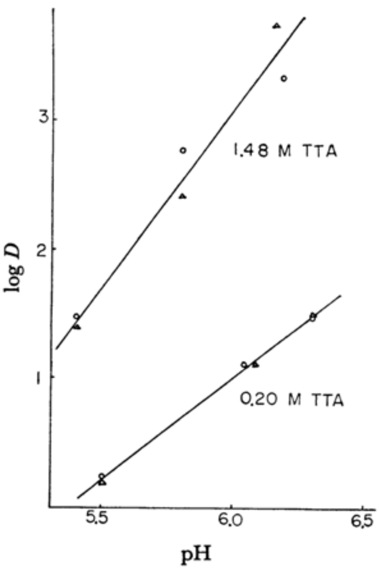


Fig. 7. Dependence of distribution ratio of calcium upon pH of the solution. Obtained from a batch extraction between each 7.3 ml. of solvent and 21.9 ml. of aqueous solution.
Loaded 0.1 mmol. Ca
△ Liq.-liq.(on Kel-F) batch extraction
○ Liq.-liq. batch extraction

Another series of extractions was carried out with the organic solvent solution adsorbed on Kel-F powder. The organic solution and the aqueous solution, as well as their volumes, were identical in this experiment and in that described just above. In a 100-ml. glass-stoppered Erlenmyer flask 8.0 g. of Kel-F powder was placed, and then an organic solvent solution was poured in. After the vessel had stood for more than three hours, each eluting solution containing calcium ions was introduced into it. The vessel was gently shaken for a while under tightly-stoppered conditions. The content was poured on a coarse glass filter, and the aqueous phase was allowed to drain by gravity into another vessel. The calcium ion content in the solution was determined by flame-photometry. The data obtained are shown in Fig. 7, in which the pH is that of the aqueous solution after the extraction.

The data obtained from the liquid-liquid batch extraction and those from the batch extraction between the liquid and a stationary phase on Kel-F are in good agreement. This shows that Kel-F did not affect the extraction. The slope of the line in log D vs. pH is 2.7 in the case of 1.48 M TTA-MIBK and 1.5 in the case of 0.2 M TTA. As in Fig. 9 of a preceding paper,[1] in which similar behavior of the relation log D vs. pH had been shown in this case the slope of the line increased with the concentration of TTA.

The column extraction and the batch extraction of metal ions were found to give lines of the same

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[*] The reason for taking 21.9-ml. portions of the solution is as follows: If equal volumes (7.3 ml. each) of the solution and the organic solvent were used, the volume would be too small to separate the aqueous phase from the extraction system in the batch extraction with the organic solvent adsorbed on Kel-F described below. The author carried out the extraction at the volume ratio of 3 : 1.

slope, as is shown in Fig. 6 and Fig. 7, though the ion treated was strontium in the former and calcium in the latter. However, the slopes of the lines were 2.3 and 2.7 respectively; that is, they were larger than the slope (2.0) of the lines illustrated in a preceding paper,[1] in which a 0.2 M TTA solvent solution was used. Since the number of TTA attachable to a calcium ion with ionic linkage is two, the slope must also be two. In this case, however, the excess over two seems to indicate the formation of some anionic species of chelate in a higher concentration of TTA, such as 1.48 M.

The Dependence of the Distribution Ratio on the Concentration of TTA in the Solvent.—The relation of log$D$ to log(TTA) was obtained by the column chromatographic method, with a stationary solvent phase containing varying amounts of TTA. A definite volume of MIBK containing various amounts of TTA was equilibrated with an equal volume of the eluting solution of pH 5.5. The organic phase was used for the stationary phase on Kel-F powder. Several eluting solutions were prepared in accordance with the amounts of TTA in the organic phase in each case. Then, 0.02 mmol. of the calcium ion was loaded to the column and elution was carried out with the proper eluting solution. The dependence of log D on log (TTA) was obtained from the effluent volume of E. The results are shown in Fig. 8.

In a glass-stoppered centrifugal tube, liquid-liquid extraction was carried out with 7.3 ml. of the same organic solvent used in each stationary phase in the column chromatography, and with 21.9 ml. of the same eluting solution, which contained 0.1 mmol. of calcium ion. After centrifugation in order to separate the phases, the aqueous phase was taken out for the determination of calcium. The distribution ratio of calcium was calculated as usual.

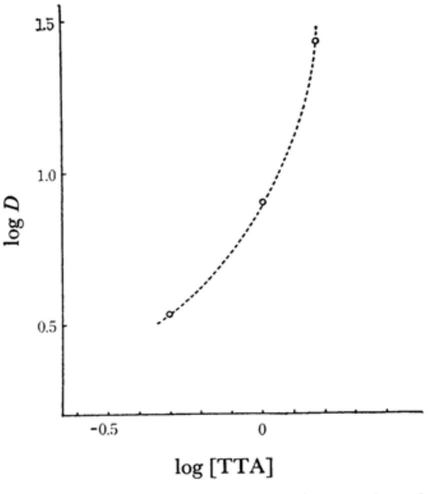

Fig. 8. Dependence of distribution ratio of calcium upon the concentration of TTA in the organic phase. The values of D were obtained from the elution curves for different concentrations.
Loaded: 0.02 mmol. Ca
Eluting solution: pH 5.5

Another batch extraction was carried out with the same organic solvent placed on Kel-F powder and with each eluting solution used in the above extraction. In a 100 ml.-Erlenmyer flask 7.3 ml. of the organic solvent solution was poured onto 8.0 g. of Kel-F powder. After the vessel had stood more than three hours, 21.9 ml. of the eluting solution, containing 0.1 mmol. of calcium ion, was poured onto the powder. The vessel was then stoppered and shaken gently for ten minutes. Calcium ion content in the aqueous phase was determined. This was repeated with solvents containing different amounts of TTA. The data obtained are shown in Fig. 9 as a graph of log$D$ vs. log(TTA). The data obtained from the liquid-liquid extraction and those from the batch extraction with the stationary phase placed on Kel-F are found to be in good agreement on the curve. This shows again that Kel-F powder does not affect the extraction mechanism.

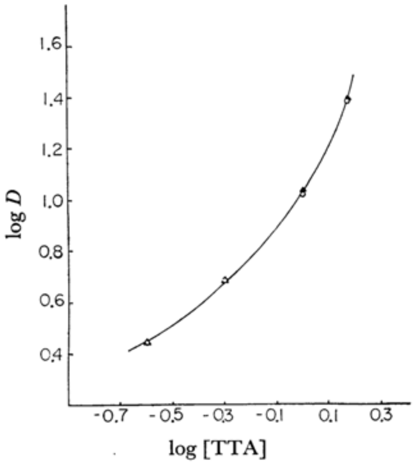

Fig. 9. Dependence of distribution ratio of calcium upon the concentration of TTA in the organic solvent.
○: D was obtained from liq.-liq. extraction.
▲: D was obtained from batch extraction with Kel-F.
Taken: 7.3 ml. of organic solvent, 21.9 ml of aqueous solution of pH 5.5, and 0.1 mmol. Ca

The column extraction and batch extraction resemble each other, as is shown in Figs. 8 and 9. In the former, however, the distribution ratio is a little lower in the low concentration of TTA. Because when the concentration of TTA is low the rate of chelate formation may be very small, in the column fresh eluting solution seems to be running through without reaching a satisfactory equilibrium. But in the batch extraction complete equilibrium of the two phases is achieved upon longstanding contact.

The Variation in the Elution Curve due to the Variation in the Quantity of Metal Ions in the System.—It was described in a preceding paper[1] that, in the liquid-liquid extraction of the TTA-chelate of metal, the distribution of the metal ion decreases with the increase in the metal-ion content in the aqueous phase. Now the author has tried to ascertain if this is also the case in column extraction.

A sample solution, 3 ml., of pH 5.5, containing a known quantity of calcium ions, was placed on the column, and elution was carried out with the same eluting solution. This was then repeated with different amounts of calcium. The results are summarized in Table II, which shows that the peak of the elution curve is displaced forward with the increase in the quantity of metal ions loaded. This phenomenon seems to be caused by the decrease in the distribution ratio of the metal, as has been described previously.[1] The number of theoretical equivalent plates, N, was also calculated from the usual equation:

TABLE II. DISTRIBUTION RATIO OBTAINED FROM THE ELUTION CURVES AT VARIOUS CONCENTRATION OF CALCIUM

Amount of calcium loaded mmol.	Effluent volume before maximum elution, ml. (E)	Distribution ratio calculated (D)	Number of plates (N)
0.0125	220.0	29.1	39.6
0.03	204.7	27.0	22.2
0.09	174.9	22.9	20.1
0.15	170.9	22.4	19.2

$$N = E(E-F)/s^2 \qquad (2)$$

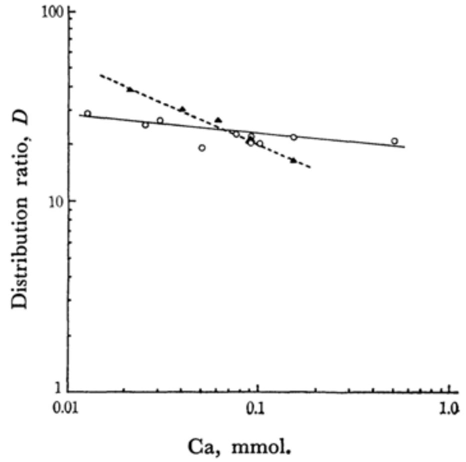

Fig. 10. Dependence of distribution ratio of calcium upon the concentration of calcium taken.
○ Obtained from elution through the column
▲ Obtained from liq.-liq. extraction

For comparison with the above column extraction, batch extraction was carried out between 7.3 ml. of the organic solvent and eluting solutions of pH 5.5 containing various quantities of calcium ions. The organic solvent was exactly the same as that held on Kel-F powder in the column. The relation observed in the batch extraction is shown in Fig. 10 with ▲ marks. In the batch extraction the decrease in the distribution ratio due to the increase in metal ion content appeared remarkably larger than in the column extraction. This inequality may be caused by the differences in the contact times of the two phases.

The Breakthrough Curve and the Capacity of the Column.—A column was prepared as described in the procedure; 500 ml. of a feed solution containing 0.00406 mmol. of calcium per ml. was also prepared by diluting 8 ml. of a stock solution of calcium salt with an eluting solution of pH 5.5 in a 500-ml. measuring flask. The solution was then fed on the column continuously at the rate of 0.05 ml. per minute. The breakthrough curve obtained is very irregular, as is shown in Fig. 11. It is neither sharp nor smooth over the whole range of the effluent, unlike most other breakthrough curves. The part of the curve shown by the dotted line merely shows the tendency of the curve, because the deviation of the measured results was too large for a perfect curve to be drawn. Let us attempt to account for the irregularity of the breakthrough curve. The calcium ion coming into the column at first forms its lowest TTA-chelate on the stationary phase, and then it changes to a higher TTA-chelate, which has a higher distribution ratio. Therefore, the increase in the amount of calcium in the effluent becomes slower, and the curve becomes less steep. When the stationary phase is saturated with calcium chelate of the highest distribution ratio, higher chelates begin to be degraded

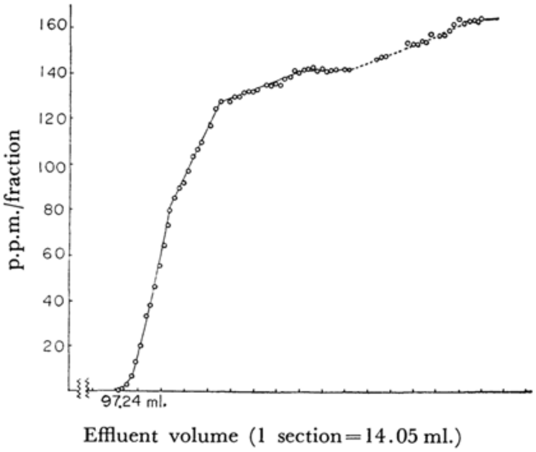

Fig. 11. Breakthrough curve of calcium.
With eluting solution of pH 5.5 containing 0.0040_6 mmol. Ca per ml.

to lower ones by the influx of new calcium ions into the phase, followed by a gradual decrease in the distribution ratio. The curve thus begins to become steeper again, and eventually the concentration in the calcium ion in the effluent reaches the constant value possessed by the initial feed solution. It may be assumed that in the column process, unlike as in the batch extraction, a solution passes through the column without reaching a satisfactory state of phase-equilibrium because of the small formation rate of the higher TTA-chelate. Consequently, the breakthrough curve may be thought to depict the steps of the formation of TTA-chelates as they really are. The amount of calcium retained on the column from the beginning to the breakthrough point was estimated to be 0.36 mmol., while the amount of calcium retained on the column throughout the interval represented in the curve was estimated to be 0.58 mmol.

In order to clarify the above picture, liquid-liquid extraction was carried out. In particular, the saturation of calcium in the organic phase was examined under conditions similar to those used in the column process. Each 7.3 ml. of an organic solution and an aqueous solution of pH 5.5 containing various amounts of calcium ion were shaken for ten minutes and then centrifuged. The amount of calcium remaining unextracted in the aqueous phase was then determined. The ratio of the amount of calcium extracted from each sample to the amount of TTA in the organic phase was plotted against the total amount of calcium taken. In this case the amount of TTA had been estimated to be 10.7 mmol. by the author's method.⁵⁾ The relation is shown in Fig. 12, in which the graph is a curve, not a straight line. This may be due to the step-by-step change in the species of metal-chelate with varying amounts of metal ions, followed by the variation in the distribution ratio. With the increase in the amount of calcium taken into the system, the quantity of TTA attached to a calcium ion decreases steadily. The distribution ratio of the metal-chelate formed at that time may be assumed to be lower. Finally, the organic phase is saturated with calcium, and the calcium chelate begins to separate out. This behavior of saturation corresponds to that of the breakthrough curve in Fig. 11; moreover, the saturation of calcium ion is maintained when about 0.5—0.6 mmol. of calcium is present in the system, while the amount of calcium in the organic phase is estimated to be 0.31 mmol.

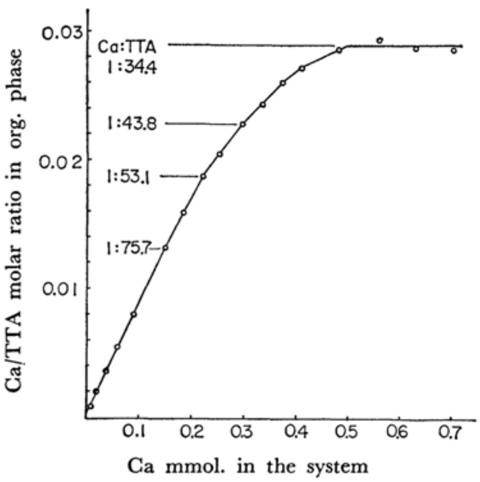

Fig. 12. Saturation of calcium in the organic phase.

Shown in the relation between the composition of the phase and the amount of calcium taken in the system.

When the elution is carried out, the amount of calcium loadable may be estimated to be approximately 0.3 mmol., that is, the value of the breakthrough point, though it is dependent on the flow rate, the concentration of metal ions, the particle size of Kel-F, the temperature, and so on. When 0.5 mmol. of calcium ion is fed into the column, the ion makes its appearance as early as in the first fraction, as has been confirmed by the author's experiment.

The picture of the process taking place in the column seems to be endorsed by these experiments.

Some Consideration of the Mechanism of the Extraction on the Column.—In liquid-liquid extraction the distribution ratio of alkaline earth metals depends upon the pH of the aqueous phase and on the concentration of TTA in the organic phase, as has been described in a preceding paper.¹⁾ The behavior of the column extraction seems to be similar to that of the liquid-liquid extraction described in the same paper, in view of the group of facts clarified by the author's experiments. In the column extraction, as well as the other experiments described in this paper, an organic solvent containing as high a concentration of TTA as 1.48 M was used. At higher concentrations of TTA, calcium ions combine substantially with TTA, but not with acetate ions or MIBK. Among these, the number of TTA molecules involved in the ionic linkage is two; the number attached to calcium, including the above number, increases remarkably, as shown in Figs. 8 and 9, with the increase in the concentration of TTA in the solvent. However, as is shown in Fig. 12, the molar ratio of TTA to a calcium ion is thirty-four at the saturation of calcium in the organic phase; this number is the minimum ratio of TTA to calcium ion. Even this minimum number seems to be far larger than the number of TTA molecules which, we may expect from the relation of $\log D$ vs. $\log(\text{TTA})$, are required by a calcium ion. Almost all the TTA in the system should be considered to participate in the extraction of calcium ions directly or indirectly, just as was described in a preceding paper.¹⁾

Summary

The author has extended the liquid-liquid extraction process of alkaline earth metal ions to a reversed-phase partition column chromatography, taking advantage of the results described in a preceding paper.[1] In the column, a methyl isobutyl ketone solution of TTA held on Kel-F powder has been used as a stationary phase. The separation of each metal ion from its mixture has been successfully achieved by carrying out the elution with eluting solutions of various pH values. It is noteworthy that as large an amount as several milligrams of a sample can be treated by this method. The relation between the method and the liquid-liquid extraction has been examined; no fundamental difference could be discovered between them. However, in the column chromatography, as well as in the batch extraction, described in this paper the organic phase contains a higher concentration of TTA than it does in the batch extraction in the liquid-liquid extraction described in a preceding paper. The experiments have also confirmed several facts which the batch method of the liquid-liquid extraction in a preceding paper[1] had seemed to suggest, regarding the behavior of TTA chelates in the presence of large amounts of TTA. Since the formation of TTA-chelate usually seems to require considerable time, in the column the eluting solution may pass through each plate while in an incomplete state of extraction equilibrium. The total distribution ratio of a metal ion as calculated from an elution curve is not much affected by the concentration of the metal in the system. The breakthrough curve of the column is thought to reflect the process taking place on the column.

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