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The Separation of Alkali Metals as Poly-Iodides by Reversed-Phase Partition Chromatography

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The liquid-liquid extraction of alkali metal ions as their poly-iodides had previously been investigated by the present author. In order to attain a good separation of each metal, this extraction system has now been extended to a reversed-phase partition chromatography in which Kel-F powder was used to support the organic phase of nitrobenzene-iodine-iodide. Column chromatographic separation was carried out in the presence of relatively large amounts of the metals by elution with water and 1 N or 6 N hydrochloric acid - eluting solutions. Chromatographic behavior of each metal ion was investigated and the elution from the column was found to be in the order of Li-Na-K-Rb-Cs. The distribution ratio of the metal ions varied with the quantity of the ions loaded on the column; the smaller the quantity of the ions used, the larger was the distribution ratio obtained. The actual separation of individual metal ions from the mixture was successfully achieved; a minute amount of cesium could be particularly isolated from large amounts of other alkali metals, even in a large volume of the sample solution. Elution curves of various amounts of metal were obtained as an aid to a discussion of the partition on the column. A comparison of the distribution ratio in the liquid-liquid extraction with that in the column chromatography showed that the extraction of the same mechanism proceeded in both cases.

Previously the author studied the liquid-liquid extraction of alkali metal ions as poly-iodides; the method presented consisted of the conversion of various kinds of alkali salts into iodides by double decomposition with ammonium iodide in an aqueous solution and the batch extraction of the iodide with nitrobenzene containing free iodine. The details of the investigation were described in a preceding paper.[1] It was found to be very difficult to separate alkali metals from one another by a batch extraction because the distribution ratios of the poly-iodides were too

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

close together in this extraction system. The low separation factor between each pair of metals of the alkali group led the author to seek other convenient methods which would be more efficient. Reversed-phase partition chromatography is closely related to liquid-liquid extraction, except that the water-insoluble extractant is immobilized on some stationary, inert support. In a column extraction of this type an aqueous solution containing the solute under consideration is always in contact with the fresh organic solvent on the column, and a multistage batch extraction can be carried on through the process; therefore, effective separation can be achieved even between substances with a low separation factor. Moreover, a larger quantity of material can be treated by this method.

A summary of the investigations of this method up to the end of 1964 has already been given by the present author,[2] so further description will be omitted here.

The author tested the qualifications of many substances which had been recommended for use as inert solid supporting materials to see if they were suitable for the poly-iodide extraction. Some of them could not retain nitrobenzene containing poly-iodide, while others were affected by the solvent system. However, the Kel-F (polytrifluorochloroethylene) moulding powder recommended by Hamlin et al.[3] was found to be very suitable because of its large absorptivity and capacity for the solvent and its strong resistivity against chemical agents. Therefore, this substance was finally chosen for this investigation.

Reversed-phase chromatography has never been applied in the separation of alkali metals, excepting the work of O'Laughlin and Banks[4] who separated sodium from lithium using bis(di-*n*-hexyl-phosphinyl)methane. Recently, however, Cerrai and his co-workers[5] attempted the paper chromatographic method with di-(2-ethylhexyl) orthophosphoric acid, HDEHP; the R_f value for each metal showed a good prospect of separation.

This paper will describe a column method of the reversed-phase chromatographic separation of alkali metals, using iodine - ammonium iodide - nitrobenzene as the stationary phase supported on Kel-F. The results of the investigation of the liquid-liquid extraction of poly-iodides of alkali metals described in a preceding paper[1] will be fully utilized. The elution of individual ions and their separation from their mixtures were both successfully achieved by the column method; the correlation between this chromatographic separation and the liquid-liquid extraction indicated by the detailed data obtained will be discussed.

Experimental

Apparatus. — *Column.*—A glass chromatographic column, 0.98 cm. in diameter and 23 cm. in length, and with a coase frit at the bottom, was used during this study; in order to keep the temperature constant, a water jacket was fitted so as surround the column tube, and water from the tap or a water-tank of a constant temperature was made to flow through it. A separating funnel as a reservoir of the eluting solution was fitted to the top of the column tube, a polyvinyl chloride tube 1 cm. in diameter was coiled around the funnel, and the constant-temperature water was allowed to flow through it before entering the water-jacket.

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

Flame Photometer.—A spectrophotometer, Hitachi EPU-2, provided with a flame spectrophotometry accessory, H-2, was employed, hydrogen being used as the fuel.

Scintillation Counter.—A counting set made by the Kōbe Kōgyo Co. (Model EA-14, NaI(Tl) crystal and Model SA-250 scaler) was employed.

Pulse-height Analyzer.—A 100-channel pulse-height analyzer, model AN-100, of the Kōbe Kōgyo Co. was employed.

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. When the material was ground with a mixing grinder and sieved through screens, particles between 42 to 80 mesh were collected for use.

Chemical Reagents.—The ammonium iodide, iodine, hydrochloric acid, nitrobenzene and alkali metal chlorides and sulfates were all of analytical reagent grade. Radio isotopes, ^{86}Rb as chloride and ^{137}Cs as nitrate, were purchased from the Radiochemical Center, Amersham, England. No diluted stock solutions were prepared; the original solutions were used as they were.

Organic Solvent as the Stationary Phase in the Column.—6.1 g. of iodine, 1 g. of ammonium iodide and 40 ml. of nitrobenzene were placed in a separatory funnel. Then there was added 40 ml. of an aqueous solution which had been selected from among distilled water, 6 N hydrochloric acid and 1 N hydrochloric acid, according to the kind of eluting solution to be used in the eluting process. After thirty minutes' shaking, the two phases were separated. The organic solvent thus treated contained iodine and iodide in the molar ratio of about 3 : 1; this ratio had been found in a preceding paper[1] to be the optimum for the extraction of alkali metal ions. The composition of the aqueous phase after the shaking should hold also in the eluting solution. Therefore, the amounts of iodine and iodide in the aqueous solution were determined and the same composition was adopted in the preparation of each eluting solution.

Eluting Solution.—*1) Aqueous Eluting Solution.*—Nitrobenzene was put in water in a separatory funnel

2) T. Kiba and I. Akaza, *Kagaku* (Chemistry), **19**, 984 (1964).
3) A. G. Hamlin, B. J. Roberts, W. Laughlin and S. G. Walker, *Anal. Chem.*, **33**, 1547 (1961).
4) J. W. O'Laughlin and C. V. Banks, *U. S. A. E. C. Rep.*, **IS-737**, 145 (1963).
5) E. Cerrai and G. Ghersini, *J. Chromatog.*, **13**, 211 (1964).

and shaken to produce a saturated solution, and the excess nitrobenzene was rejected through the stem of the funnel. The water thus treated was called nitrobenzene-saturated water in this investigation. On the other hand, 1.4 g. of iodine and 1.5 g. of ammonium iodide were dissolved in a small volume of the nitrobenzene-saturated water, and then the solution was diluted to 1 l. with the water.

2) 1 N Hydrochloric Acid Eluting Solution.—Nitrobenzene was dissolved to saturation in 1 N hydrochloric acid in the manner described in the case of nitrobenzene-saturated water; this solution was called nitrobenzene-saturated acid. In a small volume of the acid 0.78 g. of iodine and 0.61 g. of ammonium iodide were dissolved, and the solution was made up to 1 l. with the acid.

3) 6 N Hydrochloric Acid Eluting Solution.—Nitrobenzene-saturated 6 N hydrochloric acid was prepared in the way described above. In the 6 N acid 1.51 g. of iodine and 0.04 g. of ammonium iodide were dissolved, and the solution was made up to 1 l. with the same acid. It happened occasionally that iodine and ammonium iodide did not readily dissolve in the acid; shaking in a separatory funnel was resorted to in order to overcome this trouble. It is essential to keep the temperature constant both in the preparation of an organic solvent for the stationary phase and of eluting solutions for the mobile phase, and in carrying out the chromatographic separation.

Sample Solution.—It is often desirable, though not in all cases, that the sample solution has a composition alike to that of the eluting solution; for example, the correct amount of alkali metal salt was dissolved in a definite volume of the eluting solution mentioned above in order to make a sample solution to be tested. Three milliliters of the solution was usually employed for the chromatographic separation in the column method, but as much as 50 ml. of the solution was employed in the separation of trace amounts of ^{137}Cs from the mixture of sodium, potassium, rubidium and cesium salts.

Experimental Procedure.—*The Preparation of the Stationary Phase.*—Place in an Erlenmyer flask 6 g. of the Kel-F powder which had been dried at 80°C and stored in a calcium chloride desiccator, and then pour on it 4.5 ml. of the organic solvent solution prepared above. After stoppering the flask tigthly, shake it by hand until the content appears homogeneous and then let it stand over three hours. Add a small volume of a suitable eluting solution, stir gently to get rid of air bubbles, and pour the slurry little by little into the chromatographic column tube, taking care so as not to include any air bubbles. It is desirable to keep the temperature constant during the preparation by passing water of a constant temperature through the jacket fitted to the glass tube. Every time a portion of slurry is added, gently press the column bed with a glass rod in order to make it about 17.5 cm. in depth; meanwhile about 50 ml. of the eluting solution is passed through the column in order to remove any contaminant such as sodium that may be present in the column bed. The upper surface of the column should be below the surface of the eluting solution contained in the glass tube lest it becomes dry.

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 through the column at the rate of about 0.25 ml. per minute; then wash the inside of the glass tube above the top of the column bed twice with small portions of the eluting solution, letting them flow down at the same rate.

On the top of the glass tube, set a separatory funnel from which an eluting solution of a definite composition is supplied into the glass tube; then carry out the elution at the rate of 0.5 ml./min. During the preparation of the column and the elution of the material, the temperature should be kept constant— in this experiment 18°C was preferred. If the volume of the sample solution is large, the separatory funnel may be employed for the loading of the sample. Collect the effluent from the bottom of the column in suitable fractions with a fraction collector, and determine the content of alkali metal ions in each fraction by flame spectrophotometry. When a radioisotope was used, the activity in each fraction was counted by a scintillation counter and the nuclides separated were identified by γ-ray spectrometry.

Results and Discussion

The Characteristic Behavior of the Column.—*1) The Choice of Column Material.*—The application of the reversed-phase partition chromatography technique depends on the availability of a suitable supporting material. Several examples which had been reported were reviewed and discussed by the present author.[2] In the present work the organic phase consisted of nitrobenzene and poly-iodide; therefore, a more resistant material was sought.

The first choice was Kel-F moulding powder, previous experience having shown that the particular grade used had a large capacity for stationary phase and resistance against chemical agents.[3] A number of tests were made to find out whether other polymers or inorganic materials were equally suitable. Inorganic materials, such as alumina and silica gel, were found to be useless, as they failed to retain any nitrobenzene even after being treated with dimethyldichlorosilane. On the other hand, organic polymers such as cellulose powder, porous polyethylene chloride, and the polymer of tetrafluoroethylene (Teflon) had the disadvantages of reacting with iodine and of requiring difficult techniques in order to release the organic phase from them. The outstanding behavior of Kel-F moulding powder is presumably due to the large surface area of the aggregate and the resistance of the substance to chemical agents, but this has not been explained by organic chemists.

2) The Amounts of Nitrobenzene Adsorbed by Kel-F.—In an Erlenmyer flask fitted with a glass stopper, 45 ml. of nitrobenzene and 10 g. of Kel-F powder were left together overnight; the nitrobenzene unadsorbed was then drained through a small glass filter into a measuring cylinder placed in a slightly-evacuated vessel. The flask was washed with one milliliter of nitrobenzene, which was then poured

on the Kel-F powder, all of the excessive solvent being drained through the filter. The difference between the volume of nitrobenzene initially taken and that measured in the cylinder showed the volume of the solvent adsorbed by Kel-F; this was found to be 7.4 ml. per 10 g. of Kel-F powder.

3) The Volume of the Mobile Phase in the Column.—A column was packed with Kel-F loaded with nitrobenzene; a solution of sodium chloride saturated with nitrobenzene was poured onto the column and allowed to flow down to the bottom until the effluent became of the same concentration as the solution originally poured. After having been washed with water the inside of the glass tube below the fritted glass filter, the sodium chloride solution retained in the column was completely washed down by passing through water from the top of the column. The amount of sodium chloride contained in the washings was estimated argentimetrically. From this amount the volume of the sodium chloride solution in the column was computed as is usually done in chromatographic investigations. It was found that the volume of the mobile phase in the column was 4.68 ml. per 6 g. of Kel-F. The amount of nitrobenzene originally adsorbed on the Kel-F column was 4.5 ml., corresponding to the ratio of 7.4 ml./10 g. obtained in the experiment in 2); hence the volume ratio of the stationary phase to the mobile phase was 1 : 1 in this case. This indicates that the elution process consists of multiple extraction between equal volumes of the organic and the aqueous phases. The author's method of preparing the stationary phase and the eluting solution was built up consciously in order to maintain the above relation between the two phases during the elution.

4) Blank Test for the Column.—If alkali metal ions could be retained on the column of Kel-F without poly-iodide ions, separation by this method would not be satisfactory because of the complication caused by the adsorption on the stationary phase. To test the above phenomenon, a radioactive cesium tracer was passed through the column (cesium poly-iodide had been found by a preliminary experiment to be the most easily retained species). Six grams of Kel-F powder containing 4.5 ml. of nitrobenzene which had been pre-equilibrated with water was used for the column, while a trace of cesium-137 nitrate in 3 ml. of water pre-equilibrated with nitrobenzene was charged on the column. Elution was carried on by nitrobenzene-saturated water, and the effluent was collected in 10 ml. fractions. Strong radioactivity was found as early as in the first fraction, and the maximum appeared in the second. This showed that if poly-iodide ion was absent in the organic phase on Kel-F, no alkali metal ion will be retained on the column; there will be only a distribution caused by the poly-iodide ion in the chromatographic process.

5) Column Life and Stability.—After loading the column with 0.5 mg. of potassium, elution with an aqueous eluting solution was carried out; the usual elution curve was obtained. This experiment was repeated five times through the same column during a month's period; the band width and position of the peak were also examined. At the end of the five runs, the total volume of the eluting solution, including the preliminary washing with 1000 ml., was as large as 3070 ml. Table I shows the results. We can see that the column can stand the repeated uses, though a slight delay was observed at the front of the band and at the peak of the curve. As the nitrobenzene and poly-iodide of which the stationary phase consisted are tolerably soluble in an aqueous solution, the eluting solution and sample solution should have the same composition, as equilibrated with the organic solvent of the stationary phase, in order to prevent the degradation of the column during the continuous use.

TABLE I. VARIATION OF BAND WIDTH AND PEAK OF ELUTION CURVE DURING REPEATED USES OF THE COLUMN

Number of time	Band width shown as volume of effluent ml.	Peak of the curve shown as volume of effluent, ml.
1	243.8—413.4	300
2	249.1—408.1	300
3	265.0—402.8	307
4	254.4—413.4	315
5	242.5—388.0	291

6) The Recovery of Kel-F Powder.—The Kel-F powder could be recovered by the following method from a column degraded by long service; take out the Kel-F supporter from the column tube into a beaker, wash it with plenty of water, and filter it through a glass filter under suction. Put it back in the beaker, and pour a 30% ethanol-water mixture on it to remove roughly the nitrobenzene and poly-iodide remaining on the powder. Repeat this treatment if necessary, and finally wash it with water in order to drive off the ethanol. Stir 10 M nitric acid into the powder in order to separate the solid iodine from the poly-iodide, as in the case of the back extraction of alkali metal ions described in the preceding paper.[1] Filter the mixture of Kel-F powder and solid iodine under suction, and wash the residue with water in order to wash off the nitric acid. Transfer the residue into a beaker, and wash again with a 30% ethanol-water mixture to dissolve the iodine. Filter off the iodine solution. After preliminary washing with water, treat this Kel-F again with nitric acid and follow the serial procedure indicated, repeating the process three times. Wash the powder thus obtained by stirring it well with warm absolute alcohol in the beaker. Filter the powder under suction, and repeat the washing until the color of iodine disappears. Dry the white powder at 80°C before storing.

7) The Capacity of the Column.—The capacity of the column used was estimated by loading it with potassium salt and then examining its break through, shown in Fig. 1, in which the sharp rise of the curve indicates that the transfer rate of potassium ions is very high at the surface of and within the stationary phase. The capacity was estimated to be 17.$_1$ mg. of potassium when a solution containing 0.2 mg. of potassium per ml. was used as the feed solution, and 30.$_6$ mg. when a solution containing 5.0 mg. of potassium per ml. was used. As a matter of fact, the capacity in this case is distinctly different from that in the case of ion-exchange chromatography; the former merely depends on the partition of the substance between two phases, and its value varies remarkably with the concentration of the feed solution.

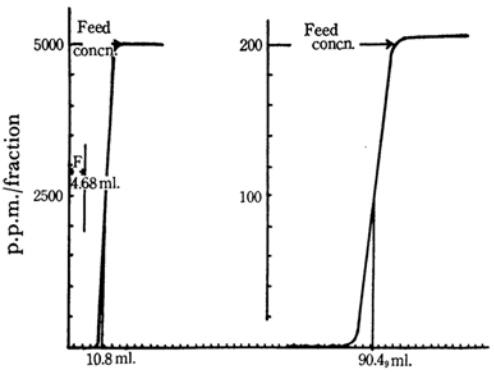


Fig. 1. Break-through curve of potassium.
Feed: 5 mg. potassium per ml. and 0.2 mg. potassium per ml. in aqueous eluting solution.
Flow rate: 0.2 ml. per minute.
F: Interstitial volume of the column.

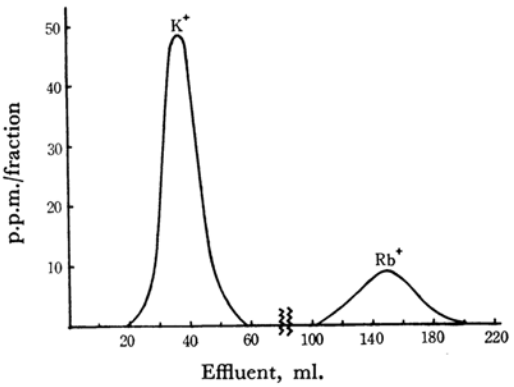


Fig. 3. Elution curves of potassium and rubidium. (Experiments with K and Rb conducted separately)
Loaded: 0.5 mg. of the element
Eluting solution: 1 N hydrochloric acid eluting solution

The Chromatographic Behavior of Alkali Metal Ions.—The chromatographic behavior of alkali metal ions is to be expected from the results obtained in the liquid-liquid extraction of alkali poly-iodides described in the preceding paper.[1] Their distribution ratios were found to depend on the ratio of free iodine to iodide ion in the system, on the acidity of the solution, and on the amounts of metal to be treated. Moreover, the temperature was also found to affect the distribution ratios. Because a marked effect will appear in the chromatographic process unless the temperature is kept constant, all the author's experiments were carried out at 18°C, except for the data shown in Figs. 2, 4, and 12, and in Table I. Since the distribution

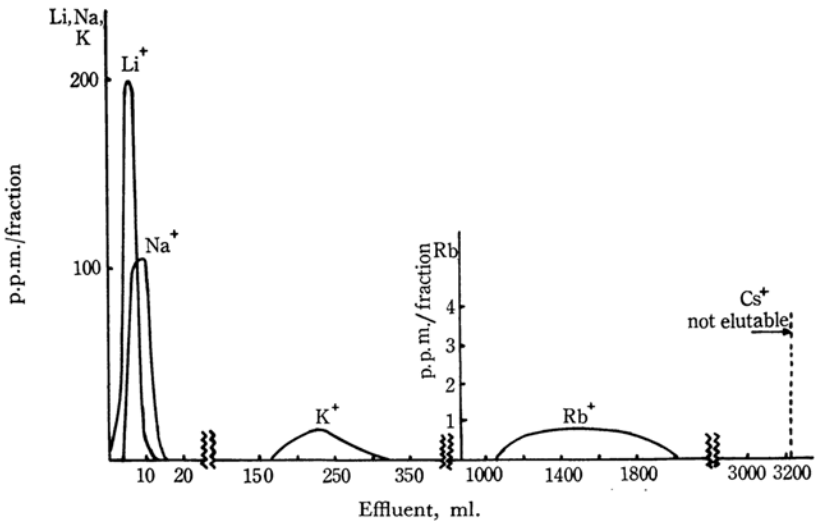


Fig. 2. Elution curves of individual ions.
Loaded: 0.5 mg. of the ion in each case Eluting solution: Aqueous

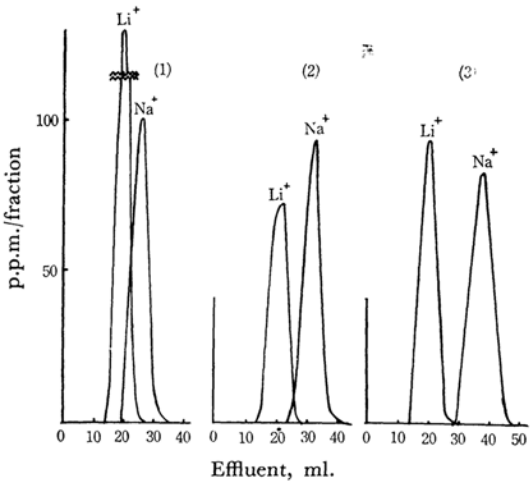


Fig. 4. Elution curves of lithium and sodium.
Stationary phase: 42—80 mesh Kel-F, 17 g. and 13 ml. of the organic solvent of nitrobenzene-iodine-iodide.
Column height: 49 cm.
Loaded: 0.5 mg. of the element.
(1) Composition of the organic solvent: Same as discribed in procedure.
Eluting solution: Aqueous.
(2) Organic solvent: 18.3 g. of iodine, 3 g. of ammonium iodide and 40 ml. of nitrobenzene shaken with 40 ml. of water.
Eluting solution: Aqueous eluting solution prepared by dissolving 2.54 g. of iodine and 2.91 g. of ammonium iodide in 1 l. of water saturated with nitrobenzene.
(3) Composition of organic solvent: A mixture of 24.4 g. of iodine, 4 g. of ammonium iodide and 40 ml. of nitrobenzene was used after shaking with 40 ml. of water.
Eluting solution: 3.4 g. of iodine and 4.0 g. of ammonium iodide dissolved in 1 l. of nitrobenzene-saturated water.

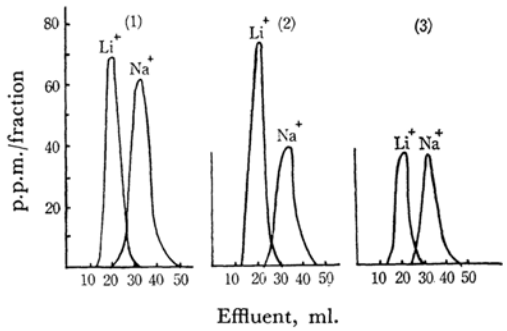


Fig. 5. Separation of lithium from sodium.
Loaded: (1) Li: 0.25 mg., Na: 0.25 mg.
(2) Li: 0.25 mg., Na: 0.1 mg.
(3) Li: 0.1 mg., Na: 0.1 mg.
Eluting solution: Aqueous

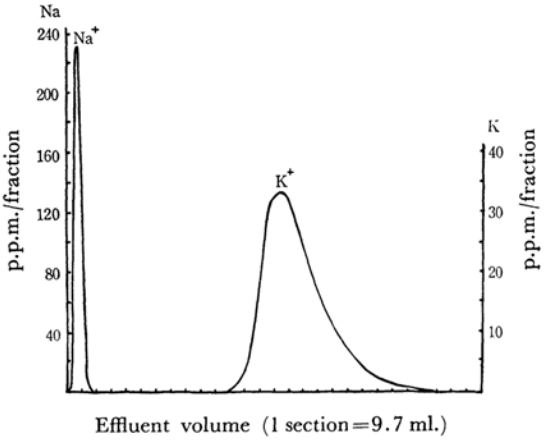


Fig. 6. Separation of sodium from potassium.
Loaded: Na: 1 mg., K: 1 mg.
Eluting solution: Aqueous

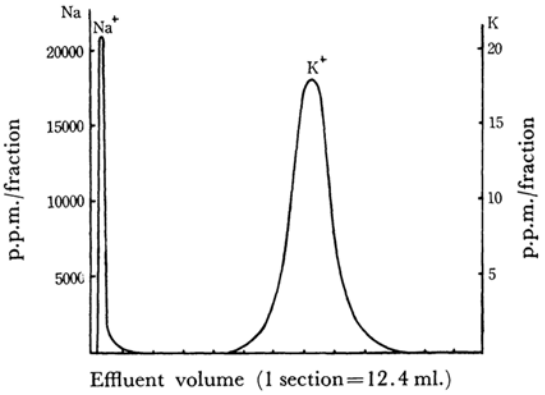


Fig. 7. Separation of potassium from sodium.
Loaded: Na: 100 mg., K: 0.5 mg.
Eluting solution: Aqueous

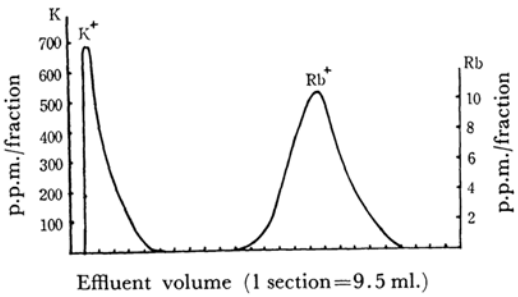


Fig. 8. Separation of potassium from rubidium.
Loaded: K: 12 mg., Rb: 0.5 mg.
Eluting solution: 1 N-hydrochloric acid eluting solution

ratio of individual alkali poly-iodide between nitrobenzene and an aqueous solution increased in the order of Li-Na-K-Rb-Cs, the elution from the column of Kel-F/nitrobenzene-iodine-iodide will show the expected resolution of alkali metals in

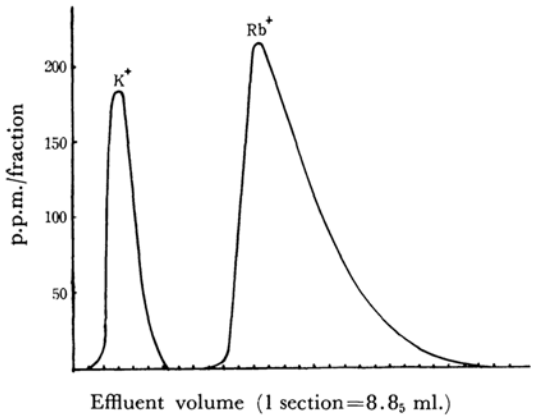


Fig. 9. Separation of potassium from rubidium.
Loaded: K: 0.5 mg., Rb: 10 mg.
Eluting solution: 1 N hydrochloric acid eluting solution.

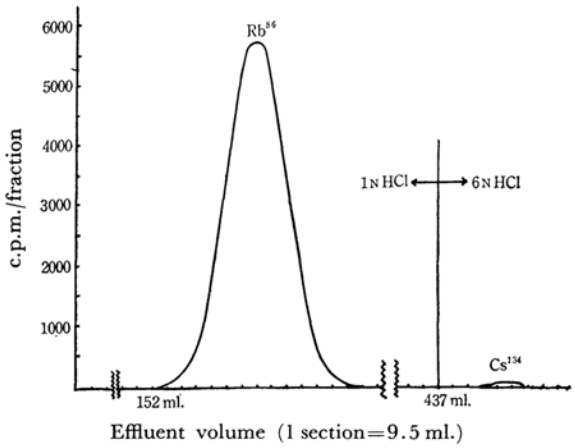


Fig. 11. Separation of cesium-134 from contaminated rubidium-86.
Loaded: A trace amount of rubidium-86.
Eluting solution: 1 N hydrochloric acid eluting solution followed by 6 N hydrochloric acid eluting solution.

the effluent, the lighter species appearing first and the heavier later.

1) The Elution Curve of Individual Ions.—In order to examine quantitatively the retention of individual ions on the column, a column prepared as described above was charged with 0.5 mg. of each ion; it was then eluted by a continuous stream of the aqueous eluting solution described above at a flow rate of 0.5 ml./min. The effluent was collected in 1.93-ml. fractions in the case of lithium and sodium, and in 9.86-ml. fractions in the case of heavier metals. The results are summarized in Fig. 2, in which it may be seen that lithium and sodium can easily be separated as a group from potassium, rubidium and cesium by this procedure. However, the later appearence of potassium and rubidium in the effluent, the plateau in their elution curves, and the failure of cesium to appear up to 3200 ml. of the effluent made it desirable to elute them faster in order to get a sharp peak on the curve. The acid eluting solution may serve for this purpose, because of the lower distribution ratio in an acid medium.

The other elution curves shown in Fig. 3 are those of potassium and rubidium eluted with a 1 N acid eluting solution; each has a relatively sharp peak. Therefore, this eluting solution is useful for the separation of potassium, rubidium and cesium from one another. On the other hand, the separation

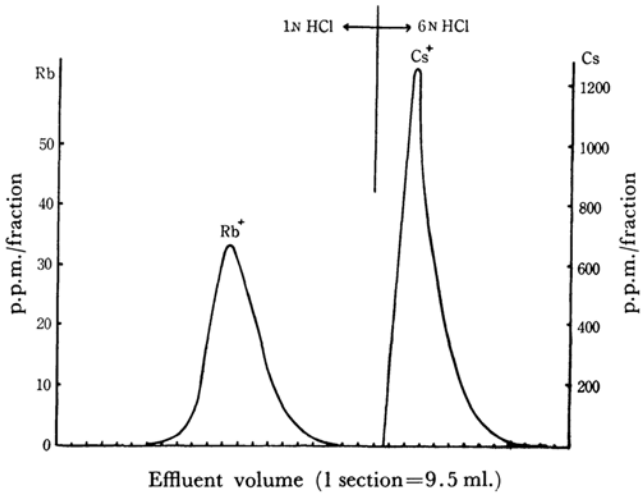


Fig. 10. Separation of rubidium from cesium.
Loaded: Rb: 0.5 mg, Cs: 25 mg.
Eluting solution: 1 N hydrochloric acid eluting solution followed by 6 N hydrochloric acid eluting solution.

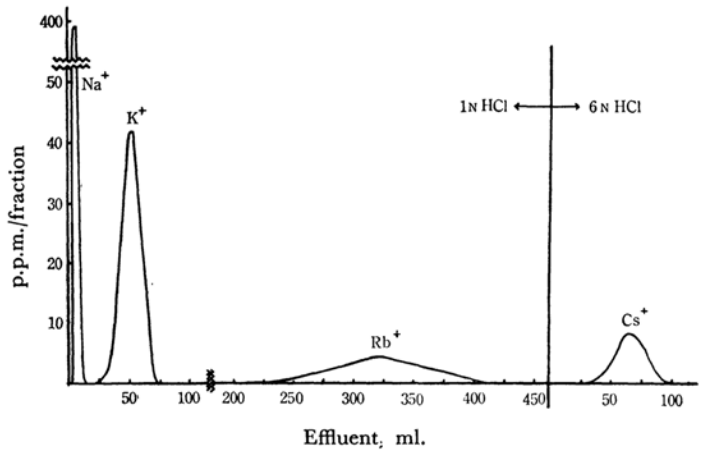
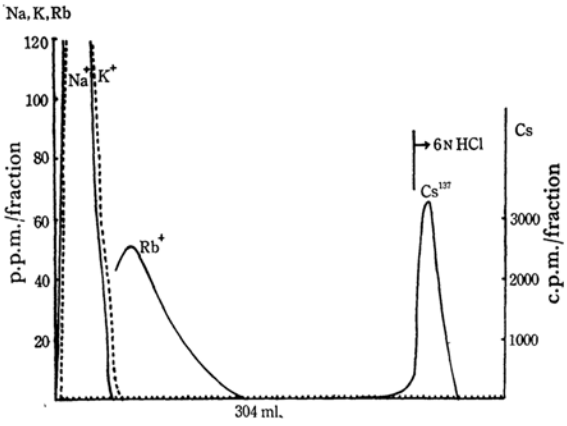


Fig. 12. Separation of Na-K-Rb-Cs.
Loaded: 0.5 mg. of each metal ion.
Eluting solution: 1 N hydrochloric acid eluting solution followed by 6 N hydrochloric acid eluting solution.



Effluent volume (1 section=9.5 ml.)

Fig. 13. Isolation of radioactive cesium from large amounts of sodium, potassium and rubidium.
Feed solution: 100 mg. each of Na, K, Rb and a trace amount of Cs-137 in 50 ml. of 1 N hydrochloric acid eluting solution.
Loaded: 50 ml. of the solution.
Eluting solution: 1 N hydrochloric acid eluting solution followed by 6 N hydrochloric acid eluting solution.

factor between lithium and sodium is so small that the separation of the two metals was found to be extremely difficult, as may be expected from the results of their liquid-liquid extraction. An attempt to increase the separability of these two metal ions was made by the elongation of the column bed in order to increase the plate number of the extraction. However, the experiment with a long column bed failed to give a good separation, as is shown in Fig. 4, in which (1) shows the results with a long column bed, and (2) and (3), with a stationary phase containing concentrated poly-iodide.

2) The Actual Separation of Metal Ions.—a) The Separation of Li-Na.—As has been described above, the separation of lithium from sodium seemed to be difficult, but the elution was performed for mixtures of various amounts of metal chlorides, with the results shown in Fig. 5. In these experiments the same stationary phase as in Fig. 4 (3) was employed, and the peaks of elution curves were easily distinguishable from each other, even though complete separation could not be achieved.

b) The Separation of Na-K.—The column prepared as described in the general procedure was loaded with 3 ml. of an aqueous solution containing 1 ml. each of sodium and potassium as chlorides, and elution was carried out with the aqueous eluting solution. Good separation was achieved as is shown in Fig. 6. Elution for a sample solution containing 100 mg. of sodium and 0.5 mg. of potassium was attempted in the same way with the results shown in Fig. 7.

c) The Separation of K-Rb.—Three milliliters of a 1 N hydrochloric acid eluting solution containing 12 mg. of potassium and 0.5 mg. of rubidium, and another 3 ml. of the solution containing 0.5 mg. of potassium and 10 mg. of rubidium were separately fed through the column, and then eluted with a 1 N hydrochloric acid eluting solution. The results are shown in Figs. 8 and 9.

d) The Separation of Rb-Cs.—Three milliliters of a 1 N hydrochloric acid eluting solution containing 0.5 mg. of rubidium and 25 mg. of cesium was fed through the column, and eluted first with a 1 N hydrochloric acid eluting solution and then with a 6 N hydrochloric acid eluting solution. The results are shown in Fig. 10.

e) The Separation of Cesium-134 from Contaminated Rubidium-86.—Radioactive rubidium-86 chloride

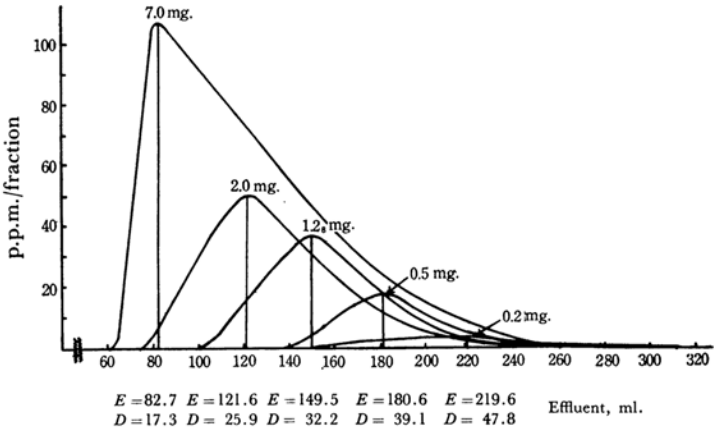


Fig. 14. Elution curve of various amounts of potassium loaded.
Potassium loaded: Given in mg. above each peak.
E, and D calculated from Eq. 1 are noted below each peak.

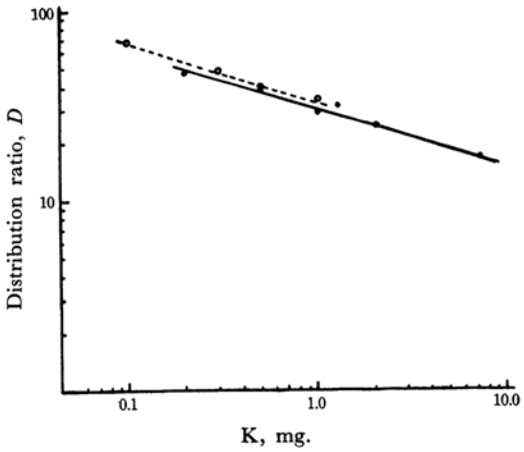


Fig. 15. Relation of potassium loaded to distribution ratio.
○----Refference data from liquid-liquid extraction under the same condition.

is sometimes contaminated with radioactive cesium derived from impurities in the target material. By eluting trace amounts of rubidium-86 chloride through the column, according to the standard procedure, with a 1 N hydrochloric acid eluting solution, the following fact was revealed. As is shown in Fig. 11, the proper elution curve was drawn up to 437 ml. of the effluent, and none of the activity seemed to remain in the column. Nevertheless, when a 6 N hydrochloric acid eluting solution was subsequently fed through the column, a minute amount of radioactivity was detected in the effluent after about 30 ml. of the new eluent had been spent. The radioactivity was shown by a γ-ray spectrometer to be the γ-activity of cesium-134. In the γ-ray spectrum no activity from rubidium was found; therefore, it was also revealed that the complete elution of rubidium had been accomplished with the 1 N hydrochloric acid eluting solution. Thus, this elution technique is useful for removing the

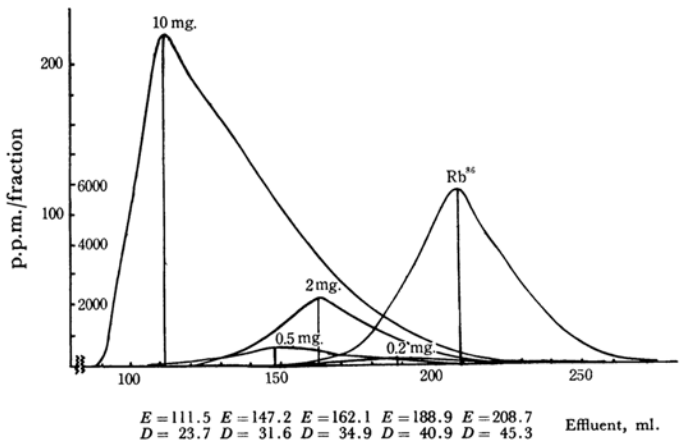


Fig. 16. Elution curve of various amounts of rubidium loaded.
Rubidium loaded: Noted in mg. above each peak.
E, and D calculated from Eq. 1 are noted below peak.

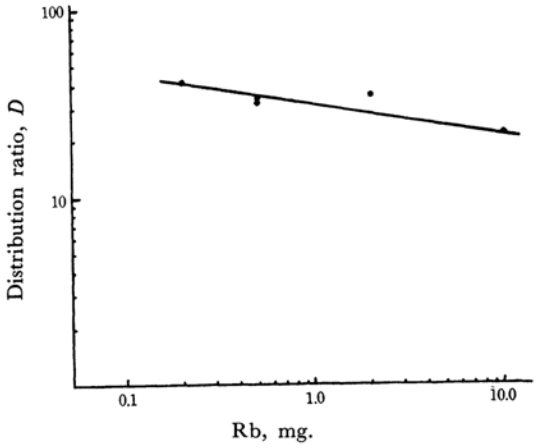


Fig. 17. Relation of potassium loaded to distribution ratio.

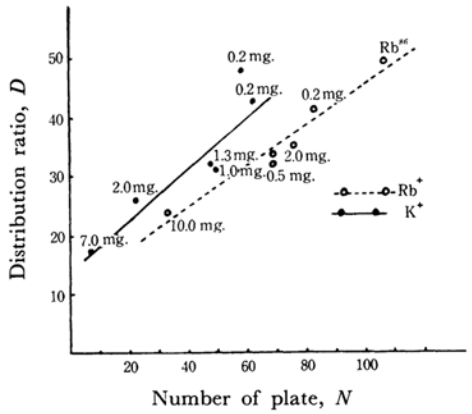


Fig. 18. Relation of distribution ratio to number of theoretical equivalent plates.

radioactive contaminants from rubidium-86, as well as for the determination of the amount of the contaminant.

f) *The Separation of Na-K-Rb-Cs.*—Three milliliters of a 1 N hydrochloric acid eluting solution containing 0.5 mg. each of the alkali metal ions together was fed to the column and eluted with a 1 N hydrochloric acid eluting solution up to 460 ml. of effluent and then further eluted with a 6 N hydrochloric acid eluting solution. The separation of the metal ions was achieved as shown in Fig. 12.

g) *The Isolation of Radioactive Cesium from Large Amounts of Sodium, Potassium and Rubibium.*—A mixed solution containing 100 mg. each of sodium, potassium, and rubidium, as well as a trace amount of radioactive cesium, was prepared with the 1 N hydrochloric acid eluting solution so as to bring the whole volume up to 50 ml. The entire amount was then fed to the column and eluted with the same eluting solution and then with a 6 N hydrochloric acid eluting solution. The results are shown in Fig. 13, in which it may be seen that a minute amount of cesium can be isolated from large amounts of other alkali metals, even in as large a volume of the solution as 50 ml.

h) *The Separation and Recovery of Individual Ions from the Na-K-Rb-Cs Mixture.*—The preceding experiments indicate that the extraction rate and the selectivity of the Kel-F/nitrobenzene-iodine-iodide column is adequate for the separation of an individual ion from other alkali metal ions. To demonstrate the practical usefulness of the method and to devise a convenient method, a typical simple separation was carried out without a fraction collector. In a few milliliters of the aqueous eluting solution, 847.28 mg. sodium chloride, 317.57 mg. potassium chloride, 9.42 mg. rubidium chloride and 9.07 mg. cesium sulfate were dissolved. Some of the nitrobenzene separated out from the solution because of the salting-out effect of the above added salts. To redissolve it, some water was added, little by little, to the above solution in a 100-ml. volumetric flask; finally, the aqueous eluting solution was added to bring it up to the mark. Three milliliters of this solution, containing 10 mg. of sodium, 5 mg. of potassium, 0.2 mg. of rubidium and 0.2 mg. of cesium, was placed in the column and eluted with the eluting solutions. Five fractions of the effluent were collected, without the help of a fraction collector, in five measuring flasks of the proper capacities. The first and second fractions, 50 ml. and 250 ml. respectively, were collected using the aqueous eluting solution; the third and fourth, 50 ml. and 250 ml. respectively, using 1 N acid eluting solution as the eluent, and the fifth, 100 ml., when the 6 N acid eluting solution was used. Each of the first three fractions was shaken well and submitted to flame photometry, at 589 mμ for sodium in the first fraction, and 768 mμ for potassium in the second. The fourth and fifth fractions were found too dilute for the determination of the amounts of rubidium and cesium, and so each fraction was evaporated to dryness under an infrared lamp and the residue was re-dissolved in 25 ml. of water. The solution thus obtained were submitted to flame photometry at 780 mμ for rubidium and at 852 mμ for cesium.

A known amount of each metal ion was dissolved in the same eluting solution as was used in the sample, and the solution was treated as above with the exception of the column process. The flame photometric value of the solution was compared with the value obtained from each fraction. Table II gives the results obtained in this separation process; each alkali metal ion was quantitatively recovered in each proper fraction without contamination.

The metal separated in each fraction could be recovered by evaporating the solution to dryness under an infrared lamp and in an electric furnace and by then treating the residue with a suitable

Table II. Recovery of alkali metal ions from their mixed solution by reversed-phase partition chromatography

Fraction of effluent No.	Eluting solution	Effluent volume ml.	Metal ion recovered mg.	Metal ion taken mg.
1	water	50	Na 10.03	Na 10.11
2	water	250	K 5.06	K 5.03
3	1 N HCl	50	—	—
4	1 N HCl	250	Rb 0.26	Rb 0.26
5	6 N HCl	100	Cs 0.20	Cs 0.20

The values in the last column were obtained from a reference solution of the identical composition.

acid to make its alkali salt, while the nitrobenzene and iodine contained in the effluent were all driven off by the heating with the strong acid.

The Relation to the Liquid-Liquid Extraction.—The reversed-phase partition chromatography is related to the liquid-liquid extraction of alkali poly-iodide into nitrobenzene described in the preceding paper.[1] The partition on the column, based on the theory of Martin and Synge,[6] seems to be equivalent to a counter current system consisting of a series of plates.

1) Distribution Ratio.—As has been described, the distribution ratio of alkali metal ions between the nitrobenzene-iodine-iodide solvent and an aqueous phase decreases with the increase in the number of metal ions; therefore, the effect of the amount of metal ions on the column upon the elution curve was also critically examined, especially in the case of potassium ions. The results shown in Fig. 14 reveal that the position of the peak of the elution curve (E in ml.) proceeds forward on the abscissa with the increase in the amount of metal ions loaded; this phenomenon seems to be caused by the decrease in the distribution ratio, which was calculated, from the data obtained by column chromatography, by the following equation[6,7];

$$D = A/RA_S - A_L/A_S = (E-F)/B \qquad (1)$$

where D is the 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; F, the volume of the mobile phase, with empty space in the column; A, the cross-sectional area of the column; A_S, the cross-sectional area of the non-mobile phase (B/column length); A_L, the cross-sectional area of the mobile phase (F/column length), and R, the ratio of (the movement of the position of the maximum concentration of the solute) to (the simultaneous movement of the surface of the developing fluid in the empty part of the above column) (column length/E/A).

The values of D calculated from Eq. 1 by putting, for E, the volume of eluent at the peak of each elution curve are given under the respective peaks in Fig. 14. The logarithmic value of D calculated above is plotted against the logarithm of the mg. of the metal loaded on the column in Fig. 15, in which the relation is shown as a nearly straight line; if the line is elongated upward, the value of D at any point may show the value of D at any lower concentration of metal ions loaded. On the other hand, all of the tail ends of the elution curves are situated so close together on the abscissa in Fig. 14 that they seem to coincide with one another in the range of permissible error in all the procedure. Moreover, the E shifts rightward on the abscissa with the decrease in the amount of metal ions loaded on the column; the position of E when a trace amount of metal is treated may be assumed to be close to the tail end of the elution curve at last. The distribution ratio can usually be calculated from the value of E, so the position of the tail end can be used to calculate the distribution ratio at a very low concentration of the metal ions that probably may be focused on a constant value. The volume of the effluent at the tail end is 316.6 ml. in the author's experiment, while the value of D calculated from this volume is 69.3. This value of D is situated on the elongated part of the straight line in Fig. 15.

When the straight line is extended downward, it will show the relation of D to various larger amounts of potassium loaded. When potassium ions are converted into their poly-iodide, the amount of iodide present in the system may decide the maximum amount of poly-iodide to be formed; in this case, 4.5 ml. of the organic solvent held on the Kel-F supporter corresponds to 28.5 mg. of potassium ions, as calculated from the amount of iodide present in the system. On the other hand, the amount of potassium retained on the column had been estimated, from the value of one of its break-through curves in Fig. 1, as being equivalent to 30 mg. of potassium. These two values, one from the calculated and the other from the break-through, agree well with each other, though incidentally. This value seems to be the maximum amount of potassium loadable on the column; this amount is also the upper limit of the concentration of potassium on the straight line shown in Fig. 15.

As the straight solid line in Fig. 15 shows the relation of D to the amount of potassium loaded on the column, if the amount of potassium loaded on the column is known, the value of D can be estimated from the figure. Putting this value of D into Eq. 1, we get E, the location of the peak of the elution curve on the axis of the effluent volume.

Further liquid-liquid extraction experiments

6) A. J. P. Martin and R. L. M. Synge, *Biochem. J.*, **35**, 1358 (1941).

7) H. B. F. Dixon, *J. Chromatog.*, **7**, 467 (1962).

were carried out between 4.5 ml. of the same solvent as the stationary phase held on Kel-F and 4.5 ml. of the eluting solution containing exactly the same amounts of potassium ion as in the column. The volume of the phase was 4.5 ml.; this amount is equal to the organic solvent held on the column. The distribution ratios obtained in these experiments are also shown in Fig. 15 as ○ marks. This relation fairly agrees well with the relation between the amounts of metal ions and D obtained in the column extraction.

Moreover, similar results were obtained in the case of rubidium, as is shown in Figs. 16 and 17. In Fig. 16, the value of E when a minute amount of the metal is treated is situated close to the tail end, as in the case of potassium, Therefore, the author calculated D at a very low concentration of the metal ions, using 233 ml. as the mean of the effluent volumes of the tail ends of many elution curves; he estimated the value to be 50.7. On the other hand, the D value calculated from the E of the elution curve of Rb-86 tracer was 45.3. The above two values seem almost to agree with each other when the difference in sensitivity between flame photometry and radioactivity measurement is taken into account. These values are also nearly equal to the value obtained by extraporating upward the straight line in Fig. 17.

The distribution was also measured by batch extraction between a Kel-F/nitrobenzene-iodine-iodide mixture and an aqueous phase of the eluting solution containing potassium ions as follows. In a glass-stoppered Erlenmyer flask 6.0 g. of Kel-F powder containing 4.5 ml. of a nitrobenzene-iodine-iodide mixture and 13.5 ml. of a water-eluting solution were placed together; the vessel was then shaken for a while. The contents of the flask were then poured onto a No. 3 glass filter, and the aqueous solution was allowed to drain into a vessel before it was submitted to flame photometry for potassium, The distribution ratio thus estimated coincided with the value obtained in the case of batch extraction with the organic solvent and the aqueous solution in the same volume ratio. From this it may be concluded that Kel-F did not participate in this reversed-phase partition chromatography at all.

2) Column Efficiency.—Column efficiency is usually presented as the number of plates of equivalent extraction carried out on the column. The author tried to calculate the number of plates, N, in this reversed-phase partition chromatography, even though the partition process was far from ideal, in which the partition coefficient remains constant. The author's tentative calculations, based on the elution curve of potassium in Fig. 14 and that of rubidium in Fig. 16, are summarized in Fig. 18, which reveals that the smaller the amount of sample loaded, the larger the plate number, N, for the distribution ratio increases with the decrease in the number of ions treated; the relation between N and D may be assumed to be roughly proportional.

Summary

Partition chromatography and liquid-liquid extraction are closely related to each other with regard to the distribution of solute between two phases. The previous investigation by the present author has clarified that an alkali metal ion can be extracted from an aqueous phase into nitrobenzene containing iodine and ammonium iodide, but the metals are so similar in distribution behavior into the organic phase that they cannot be separated from one another, even by repeated batch extraction. The author has attempted to extend this extraction method to a reversed-phase partition chromatography in which Kel-F powder was used to support the organic phase of nitrobenzene-iodine-iodide; column chromatography has been carried out by eluting with three kinds of eluting solutions. Such characteristics of the column as the volume of the stationary phase held on Kel-F, the volume of the mobile phase in the column, the column life and the capacity of the column have been investigated. The elution of alkali metals has also been carried out with each individual ion. Actual separations, between lithum and sodium, potassium and sodium, potassium and rubidium, rubidium and cesium have been successfully achieved, in addition to the separation of radioactive cesium from rubidium and from large amounts of other alkali metals and the recovery of individual metals from their mixture. The capacity of the column can be estimated as the amount of solute that the column can retain, but the amount largely depends upon the distribution of the solute in the organic phase. Elution curves of various amounts of metal have been obtained for use in discussing the partition of the metal on the column. A comparison of the distribution ratio obtained in the liquid-liquid extraction with that obtained in the column chromatography has shown that same mechanism holds in both cases. The column efficiency has also been calculated, employing the band width of the elution curves and by using the equation commonly adopted. The distribution ratio varies with the amount of solute placed in the column; the larger the distribution, the smaller the amount. In this experiment, however, the number of the plates of equivalent extraction and the distribution ratio have been found to have a linear relationship; this shows that only the partition phenomenon is predominant in this chromatographic process.

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