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# Analytical separations of metals by column chromatography on microcrystalline cellulose

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ANALYTICAL SEPARATIONS OF METALS BY  
COLUMN CHROMATOGRAPHY ON MICRO-  
CRYSTALLINE CELLULOSE.

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**ANALYTICAL SEPARATIONS OF METALS  
BY COLUMN CHROMATOGRAPHY ON MICROCRYSTALLINE CELLULOSE**

by

**Mark Anthony Peters**

A Dissertation Submitted to the  
Graduate Faculty in Partial Fulfillment of  
The Requirements for the Degree of  
**DOCTOR OF PHILOSOPHY**

**Major Subject: Analytical Chemistry**

**Approved:**

Signature was redacted for privacy.

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**1968**

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## INTRODUCTION AND LITERATURE SURVEY

The technique of inorganic cellulose column chromatography was introduced in the early fifties. This technique accomplished some new and exciting inorganic separations and demonstrated considerable potential. Relatively few metal mixtures and eluents were studied, however, before research into uses of the technique began to diminish after the mid-fifties. The object of this work is to investigate further the potential of cellulose column chromatography and present some useful separations. In addition a new cellulose, a microcrystalline form, is employed.

In early research in cellulose column chromatography, the pulp or filter paper was activated with acid. The acid attacks the amorphous areas of the cellulose which link the microcrystals together. This acid hydrolysis breaks some of the molecular hinges and reduces the fiber to crystallites, which retain their orientation in the fiber. The method of preparation for microcrystalline cellulose is the same as the one described above except that after a severe acid hydrolysis, the water slurry is subject to mechanical agitation. This agitation releases the microcrystals from their fibrous structure with the production of colloidal cellulose. Spray-drying then produces an entirely new fine-structure pattern, and a spongy, porous material results that is suitable for column work. This new form of cellulose can be purchased and used without further

preparation. It was introduced in 1962 by Battista, and additional information can be found in his papers (1,2).

Although microcrystalline cellulose has been used successfully in thin-layer work (3,4), no applications have been reported in the field of inorganic column chromatography. It is believed that the separation of metal ions reported in this thesis are the first use of microcrystalline cellulose in the field of inorganic column chromatography.

In this project the cellulose used requires no preliminary treatment or activation. It is merely slurried with the eluent (usually a mixture of a mineral acid and an organic solvent) and packed into a chromatographic column. The sample mixture is then placed at the top of the cellulose column, and each component is selectively eluted. The separation is realized because of the difference in the rate at which each component moves down the column. This rate depends upon the degree to which the solute is extracted by the stationary phase on the cellulose. The technique is called liquid-liquid partition chromatography with elution development.

Columns of cellulose in this work are used to separate the alkaline earths from each other and from the rare earths, the alkali metals from each other, traces of aluminum from rare earths, and some of the transition metals from each other. The reasons for developing these separations are three-fold: (1) to simplify and increase the accuracy of

of subsequent analyses, (2) to develop analytical and perhaps industrial methods for the production of pure salts, (3) to develop simpler and better separation procedures.

The history of cellulose column chromatography began with a paper by Burstall et al. (5). They separated some transition metals from each other on cellulose columns with ketone-hydrochloric acid eluents and applied the procedure to the analysis of steel. Burstall and Wells (6) separated uranium from monazite sands and refractory ores on cellulose columns with an ethyl ether-nitric acid eluent. Compound columns of alumina and cellulose have been used to separate uranium from arsenic and molybdenum (7). Compound columns have been used for separation of uranium and thorium in ores (8,9). The uranium is eluted in 400 ml. with a 1% nitric acid-ether eluent, and the thorium is eluted with a 12.5% nitric acid-ether eluent. Kember (10) demonstrated a similar separation on a cellulose column. Cellulose columns with ether-nitric acid eluents have also been used to purify uranium (11,12,13). Arden (11) purified triuranium octaoxide from pitchblend. A large amount of uranium (500 g.) has been separated from the rare earths (12), which permits their determination down to the parts per billion level. In a more recent paper Muzzarelli (13) demonstrated a separation of submicrogram amounts of thirteen metals from 50 g. of uranium on cellulose columns with a nitric acid-ether eluent.

In a series of papers (14,15,16) Burstall and Williams presented the first column separations of niobium and tantalum. The tantalum is eluted from the cellulose column in 250 ml. with ethyl methyl ketone. The column is then washed with 400 ml. of this ketone-1% hydrofluoric acid eluent to arrest the movements of titanium, tin, and zirconium; and finally the niobium is eluted with 500 ml. of the same ketone containing 12.5% hydrofluoric acid. The method has been applied (17,18) to other ores.

A partial zirconium-hafnium separation and other early cellulose column separations can be found in a United States patent (19).

Columns of cellulose have been used to separate gold (20) from other metals and for separations in connection with the analyses of steel (21,22,23). Ghe and Fiorentini (21) separated and determined molybdenum in special steels and Venturullo and Ghe (22) separated most of the important elements in special steels on cellulose columns. Cellulose columns have also been used in aluminum alloy analyses (24) and to separate tin and copper in titanium alloys with a butanol-hydrochloric acid eluent (25).

Rees-Evans et al. (26) investigated the separations of the platinum metals; and Ghe (27) demonstrated a rapid separation of antimony from germanium, by eluting the germanium with



pyridine and the antimony with water.

In a series of recent papers, Muzzarelli (13,28,29,30, 31,32,33,34) studied the movement of numerous metals with non-aqueous eluents on both columns of natural fibrous and substituted celluloses. The papers are fairly brief and demonstrate only limited separations. Muzzarelli also published an excellent review of inorganic chromatography on columns of natural and substituted celluloses (35).

In this thesis a method which is superior to existing alkaline earth separation procedures is developed. Eluents used in this project are simple mixtures which do not interfere in analyses. Excellent separations of the alkaline earths from each other are accomplished on cation-exchange resins with eluents such as ammonium lactate (36), diaminocyclohexanetetracetic acid (37), or  $\alpha$ -hydroxyisobutyrate (38); however, the separations have the disadvantage that these eluents complicate the subsequent analysis. Fouarge (39,40) studied alkaline earth separations on cellulose columns. He investigated only some of the alkaline earths and only at tracer level concentrations.

The alkali metals, sodium through cesium, were separated from each other in this project on cellulose columns with a phenolic eluent; lithium, sodium, and potassium were separated with a methanol-hydrochloric acid eluent. Only two papers have appeared in the literature which have studied alkali

metal separations on cellulose columns. Burma (41) separated the hydroxides of lithium, sodium, and potassium with an ethanol-water eluent. Fouarge (42) separated cesium from rubidium and from fission products. Fast and clean separations of the alkali metals from each other were demonstrated by Kraus et al. on zirconium phosphate (43) and zirconium tungstate (44). These inorganic cation exchangers, however, have the disadvantage of being slightly soluble in the eluents.

Some of the mixtures employed as eluents in this project have been used as developing agents in paper chromatography (45,46,47,48). A formula was developed which relates column and paper chromatographic parameters, and thus allows one to determine the feasibility of a cellulose column separation from paper chromatographic data.

Cellulose is an excellent support for column chromatography. The material does not shrink or swell with a change of eluent. The microcrystalline cellulose requires no activation or preliminary preparation. It produces sharp symmetrical elution bands and has excellent plate characteristics. Elution development on microcrystalline cellulose permits rapid and quantitative multi-component mixture separations, even at carrier-free, tracer-level concentrations. In addition the material is a pure white powder, containing only a few trace impurities (49).

## THEORY

## General Column Partition Chromatography

A polar phase in column chromatography is sorbed onto an inert support and placed in a chromatographic column. This polar phase is called the immobile or stationary phase. As the non-polar mobile phase or eluent is percolated through the column, the solutes partition themselves between the stationary and mobile phases. The solutes move down the column at a rate which is inversely proportional to the batch distribution coefficient,  $D$  (ratio of concentration of solute in immobile phase vs mobile phase). Solutes having different distribution coefficients form distinct bands as they move down the column and are eventually separated.

Reversed-phase column chromatography is exactly the same as discussed above except the immobile phase is now non-polar and the mobile phase is polar. These techniques are called liquid-liquid partition column chromatography or extraction chromatography.

The following equation is valid if the mechanism in column elution chromatography is one of pure partitioning:

$$\bar{v} = V_m + DV_s \quad (1)$$

where,  $\bar{v}$  = retention volume, or volume of effluent collected to reach solutes peak concentration.

$V_m$  = volume of mobile phase (interstitial volume)

$V_s$  = volume of immobile or stationary phase

$D$  = batch distribution ratio or ratio of solute concentration in the immobile phase vs the mobile phase.

Therefore, an experimental determination of  $V_m$ ,  $D$ , and  $V_s$  allows a determination of the retention volume,  $\bar{v}$ .

The plate theory of Martin and Synge (50) and Glueckauf (51) allows one to calculate the efficiency of a column. The column is divided into a number of theoretical plates, similar to the theoretical plates of a distillation column. A theoretical plate is the length of column in which the solute reaches equilibrium between the mobile and immobile phases. The number of theoretical plates in a column can be calculated from experimental data with the Glueckauf (51) equation,

$$N = 8 \left( \frac{\bar{v}}{\beta} \right)^2 \quad (2)$$

where,  $N$  = number of theoretical plates

$\bar{v}$  = retention volume

$\beta$  = width of the elution curve at the point,

$$c = 0.368 c_{\max.}$$

which is explained in Samuelson's (52) book.

The H.E.T.P. (height equivalent to a theoretical plate) is then calculated by dividing the column length by the H.E.T.P. Efficient columns, therefore, have a low H.E.T.P.

and consequently produce sharp elution bands. The magnesium (II) elution band presented in Figure 1 has been used to calculate the H.E.T.P. The 23.4 cm. cellulose column contains 417 theoretical plates and has a H.E.T.P. of 0.56 mm., which is a very good height.

#### Paper and Cellulose Column Chromatography

Paper chromatography is a technique whereby a large number of samples can be investigated on a single sheet of paper. The sample or mixture is spotted near the edge of the paper sheet and placed in a closed container containing the developing agent. The developing agent, which is similar to the mobile phase in column chromatography, is allowed to run over the spot and up the paper by capillary action. The distance the solute moves depends upon its partitioning, in the widest definition of the term, between the cellulose phase and the mobile phase.

Consden, Gordon and Martin (53) considered the filter paper as an inert support of an aqueous stationary phase and explained the observed separations as a result of continuous partitions of the solute between the aqueous stationary phase and the water-immiscible organic solvent flowing up the paper. They then developed the following equation:

$$\alpha = \frac{A_m}{A_s} \left( \frac{1}{R_f} - 1 \right) \quad (3)$$

where,  $\alpha$  = bulk partition coefficient of solute between the organic and aqueous phase

$A_m$  = cross-sectional area of mobile, organic phase

$A_s$  = cross-sectional area of stationary, aqueous phase

$R_f$  = ratio of solute distance travel vs solvent front travel.

Chalkley (54) used Equation 3 to prove that the mechanism of separation on cellulose is one of simple partition for an iron, aqueous hydrochloric acid, ethyl ether system.

Fouarge and Duyckaerts (55) also have shown that a cesium, rubidium separation on paper with a phenolic eluent is a partitioning process.

A pure partitioning mechanism has been criticized (1,56, 57,58) by some authors. If separations are due to partitioning between two solvents, it should be impossible to use water miscible eluents to effect separation, when only one phase exists. Numerous separations (22,24,32,51), however, have been achieved with water-miscible eluents.

Hanes and Iskerwood (56), Moore and Stein (57), and Martin (59) believe that water exists in the cellulose with a large degree of structural organization - a cellulose-water complex. The solute molecules become incorporated, competitively with water and perhaps alcohols, into the water-cellulose complex. They also point out that the cellulose-water complex may be looked upon as a hydrated cellulose, a strong solution of glucose or a polysaccharide phase. A strong solution of glucose will indeed form two phases with aqueous

propanol - the glucose-water phase and the propanol phase.

Some water is absorbed with a high heat of sorption and high apparent density if dry cellulose is exposed to water vapor. With the absorption of more water, the heat of sorption and apparent density of water change, becoming more like liquid water. Burma (58) points out that some of the water in cellulose shows reduced activity, i.e., reduced dissolving capacity. Burma believes there are three different kinds of water in cellulose, two of which extract solutes by pure partitioning, and the other which extracts by an adsorption mechanism.

Therefore, the mechanism of separations with water-miscible developing agents in paper chromatography is still unsettled. It is perhaps only a matter of definition of terms whether the mechanism is called adsorption on, or a partition in, the water-cellulose complex.

The mechanism of separations on cellulose columns closely parallels that of paper chromatography. There are, however, some very important differences. In paper chromatography the samples are spotted and perhaps dried, whereas in column techniques, liquid samples are added directly to the column. The double solvent front often seen in paper chromatography is eliminated in column work. The washing of the cellulose in the column with the eluent probably produces a homogeneous stationary phase. Weinberg (60) passed absolute ethanol

through columns of cellulose containing 10% water by weight. Early effluent fractions contain water which is removed from the cellulose by the mobile, ethanol phase. The percentage of water in effluent fractions after 20 ml. reaches a low, constant value, suggesting that the system is at equilibrium. In this thesis because of the symmetry of many of the elution bands and also to simplify the discussion, the mechanism of separations on the cellulose columns is called partitioning.

#### Derivation of Relation between Column and Paper Chromatography

During the experimental work of this thesis a relationship was found to exist between cellulose column and paper chromatography. The derivation of this relationship follows:

Equation 1 (p. 8) is rearranged to yield:

$$D = \frac{\bar{v} - V_M}{V_S} \quad (4)$$

Equation 3 (p. 10) is rewritten to yield:

$$D = \frac{V_M}{V_S} \left( \frac{1}{R_f} - 1 \right) \quad (5)$$

if we assume that  $\alpha \equiv D$ ,  $A_M \equiv V_M'$ , and  $A_S \equiv V_S'$ . Equating Equations 4 and 5, we get:

$$\frac{\bar{v}}{V_S} - \frac{V_M}{V_S} = \frac{V_M'}{V_S' R_f} - \frac{V_M'}{V_S'}$$



If we assume that the ratios of the volumes of mobile and stationary phase are equal for cellulose column and paper chromatography (i.e.  $V_m/V_S = V'_m/V'_S$ ); then the preceding equation reduces to:

$$\bar{v} = \frac{V_m}{R_f V'_S} \cdot V_S .$$

If the volumes of the stationary phases are equal for cellulose column and paper chromatography (i.e.  $V_S = V'_S$ ), the preceding equation reduces to:

$$\bar{v} = \frac{V_m}{R_f} . \quad (6)$$

Equation 6 is the desired relation between the retention volume,  $\bar{v}$ , and  $R_f$ . The volume of the mobile phase,  $V_m$ , can easily be determined experimentally. We thus can predict retention volumes and separation factors for metal and perhaps organic mixtures from the  $R_f$  values found in the many papers and books on paper chromatography. Equation 6 is tested throughout this thesis by comparing the calculated and experimental retention volumes for different metals and various eluents. Excellent agreement is found in all cases.

The H.E.T.P., calculated from the elution bands of this thesis with Equation 2, for various metals and different eluents on cellulose columns is close to the average value of 0.6 mm. For a given column, therefore, the number of theoretical plates can be calculated. Knowing this number

and also the retention volume,  $\bar{v}$  from Equation 6, the width of the elution band can be calculated with Glueckauf's equation, Equation 2.

Therefore, from literature  $R_f$  values, both the retention volume,  $\bar{v}$ , and band width,  $\beta$ , can be calculated for the various metals in a mixture. Thus the feasibility of a separation can easily be determined before any experimental work need be done.

## EXPERIMENTAL

## Reagents

Microcrystalline cellulose preparation

The cellulose for most of the separations was obtained from the F.M.C. Corporation, American Viscose Division. It is their Avicel Technical Grade, a white free-flowing powder. The fines were removed by slurring the cellulose in the eluent, allowing the mixture to settle a few minutes, and removing the milky supernatant liquid with suction. This slurry was then carefully added to the glass column and washed with the eluent.

Eluents

All eluents were prepared by mixing the proper proportions of reagent grade acid, organic and distilled water. The phenolic eluent, for alkali metal separations, was prepared by shaking solid phenol crystals with aqueous hydrochloric acid (20%), and separating the resulting phases.

Metal ion solutions

Most metal ion stock solutions were prepared by dissolving reagent grade chlorides in distilled water. Rare earth solutions were prepared from the oxide.

The radioisotopes, barium<sup>133</sup>(II), strontium<sup>85</sup>(II), beryllium<sup>7</sup>(II) and cesium<sup>134</sup>(I), were obtained from Oak Ridge, and radium<sup>226</sup>(II) from New England Nuclear Corporation. The radioisotopes sodium<sup>24</sup>(I), potassium<sup>42</sup>(I), rubidium<sup>86</sup>(I),

barium<sup>139</sup>(II), and strontium<sup>87</sup>(II), were prepared at the Ames Laboratory research reactor. The radioisotope stock solutions contained enough acid to prevent hydrolysis and absorption.

### EDTA

All standard EDTA solutions were prepared from the reagent grade disodium salt, and standardized with standard zinc(II) using naphthyl azoxime S(NAS) as indicator (61).

## Apparatus

### Columns

The chromatographic columns used for the separations were of the following types: coarse frit, 1x15 cm. or 1x30 cm.; glass columns with Teflon stopcocks; glass columns, 1.1 cm. i.d. with Fisher-Porter 1 mm. straight-bore Teflon stopcocks; a Teflon straight union reducer attachment fitted with a Teflon stopcock attached to a 60x1.15 cm. glass column; conical glass column with a Teflon stopcock (0.7 cm. at base, 2.4 cm. at a distance 20 cm. up from the base).

For columns requiring pressure, the air flow was regulated and filtered with a Johnson regulator and Koby air filter.

### Spectrophotometer

A Bausch and Lomb Spectronic 600 recorded the optical spectra. A modified (62) Beckman Model B was used for spectrophotometric titrations.

### Atomic absorption spectrophotometer

A Perkin-Elmer atomic absorption spectrophotometer, model 303, was used to determine lithium and non-radioactive sodium and potassium.

### Gas chromatographic

An F&M, model 500, gas chromatographic unit was used to determine the ratio of organic/water before and after mixing with dry cellulose. A Porapak Q column, 4.0'x0.25", at 110°C was used.

### pH meters

A Beckman Zeromatic or Corning Model 12 pH meter, fitted with the proper calomel and glass electrodes, was used for all pH measurements.

### Neutron source

The Ames Laboratory, 5 megawatt, heavy-water cooled, research reactor was the source of neutrons for all irradiations. It has a flux of  $3.5 \times 10^{13}$  neutrons/cm.<sup>2</sup>-sec. in the R-5 and R-3 tubes.

### Multi-channel analyzer

An RIDL, model 34-26, 256-channel gamma analyzer was used to check the purity of irradiated stock solutions and effluent concentrations determined by neutron activation.

### Scintillation counter

An RIDL, anti-walk single channel analyzer, model No. 27352, with a thallium activated 3"x3" sodium iodide crystal

was used to isolate and count the gamma rays of the radioisotopes.

### Analytical Procedures

#### Column separations

The metal ion stock solutions were pipeted into 10-ml. beakers, heated to reduce the volume, and quantitatively transferred to the cellulose column with a dropping pipette. The sample volume was 1 to 4 ml. Some columns required a pressure of up to 7.0 lbs./in.<sup>2</sup> to maintain a flow-rate of 0.4 to 2.0 ml./min. Even though no deterioration of the cellulose was noticed, the cellulose was not used for more than four separations.

#### Determination of distribution coefficients

The distribution of the alkalies between phenol and 20% hydrochloric acid was determined by pipetting a radioisotope and carrier into a separatory funnel. Equal volumes of organic and aqueous phases were added, shaken for 10 min., separated, and the gamma emissions counted.

#### Determination of stationary phase

Gas chromatography studies      A gas chromatographic technique was used to determine whether water is preferentially absorbed by cellulose from an eluent mixture. A weighed amount of dry cellulose was slurried by a batch technique with the eluent mixture; after filtering, a constant amount of the supernatant liquid was injected into the gas chromatograph.

The resolved peaks were cut out and weighed. Standards went through the same procedure and conditions except for the addition of cellulose. Preferential absorption was then determined quantitatively by an absolute method.

Determination of cellulose capacity      A weighed amount of cellulose was slurried with a 70-30 (v/v) methanol-hydrochloric acid mixture and added to a glass column. The eluent was changed to a calcium(II)-saturated 70-30 methanol-hydrochloric acid mixture, and elution continued until the effluent calcium(II) concentration was the same as the eluent. After removing the mobile phase with either cyclohexane or air pressure, the stationary phase was removed with an aqueous hydrochloric acid eluent (pH = 2). The concentration of calcium, hydrogen, and chloride ion was determined and the cellulose capacity calculated. The hydrogen-ion capacity of cellulose was also determined for eluents containing no calcium(II).

Determination of stationary phase weight      Columns were prepared, as directed above, for various eluents with and without calcium. After removal and measurement of the mobile phase, the stationary phase weight was calculated by difference from the weight of the column.

Determination of stationary phase volume      The volume of the stationary phase on the cellulose was determined by subtracting from the bed volume, the volume of the cellulose

and the interstitial volume. The volume of the cellulose was determined by adding the eluent from a burette to a volumetric flask containing a weighed amount of dry cellulose. The difference between the amount of eluent added to reach the mark in the empty flask and the one containing the cellulose is the volume of the cellulose in the flask.

#### Determination of metal ions

Titrimetric procedure Magnesium(II), calcium(II), strontium(II), and barium(II) were determined at pH = 10 with EDTA (63). Aluminum(III), lanthanum(III), and erbium(III) were determined by addition of excess EDTA at pH = 2 and back-titration with copper(II) at pH = 6.0 using NAS (61) indicator.

Spectrophotometric procedures Trace amounts of aluminum(III) were determined with chrome azural S (64).

Barium(II) was titrated spectrophotometrically with EDTA using arsenazo I as indicator at 555 m $\mu$ . at pH = 10.

Radiometric procedures Radioactive effluents were collected in test tubes and the gamma emissions measured. Elution curves were drawn and the percentage recovery was determined by the ratio of total sample activity to a standard. In all cases the same pipette transferred standards and samples. The absolute error for radiometric recoveries is probably about 3% because any slight error in the counting of a single effluent fraction may be increased when these counts are added to give the total sample activity.



The procedure for neutron activation determinations was as follows: the effluent containing the metal was concentrated by evaporation, nitric acid added, and concentrated again to remove chloride ion. The solution was quantitatively transferred to a volumetric flask and an aliquot pipetted into a small polyethylene vial. Standards were also prepared according to the same procedure. Standard and sample vials were sealed and irradiated at the Ames Laboratory reactor in the same "rabbit". Gamma emissions were measured directly without further sample preparation for metal concentrations at the milligram level. The determination of barium (II) at tracer-level concentrations ( $10^{-7}$  g.) requires much longer irradiation times; and, therefore, irradiated impurities present much more of an interference. These interferences were removed partially by a precipitation technique. The solutions in the irradiated vials were quantitatively transferred to a beaker and carrier added (barium(II)); the barium<sup>139</sup> sulfate was filtered and the gamma emissions recorded. Unknown concentrations were then determined from a plot of activity vs. concentration.

The gamma activities were counted at the following energies:

<u>Isotope</u>	<u>Energy (MeV)</u>	<u>Isotope</u>	<u>Energy (MeV)</u>
Na <sup>24</sup>	1.368	Be <sup>7</sup>	0.478
K <sup>42</sup>	1.52	Sr <sup>85</sup>	0.515
Rb <sup>86</sup>	1.077	Sr <sup>87</sup>	0.391
Cs <sup>134</sup>	0.6047	Ba <sup>133</sup>	0.080
Eu <sup>152-154</sup>	1.12	Ba <sup>139</sup>	0.166
		Rn <sup>226</sup>	2.2

## RESULTS AND DISCUSSIONS

## Selection of the System

Solvent system

Alcoholic (methanol or ethanol) hydrochloric acid mixtures were chosen as eluents for many of the separations. These eluents, which can easily be removed, do not interfere in subsequent analyses of effluents; and, because of low viscosity, permit reasonable flow-rates. In addition, the eluents produce good separations, symmetrical peaks, and are inexpensive, pure, and non-toxic. Some of the eluents had been chosen with the help of Equation 6 and published  $R_f$  values.

Cellulose

The American Viscose microcrystalline cellulose used as a support in most of the separations is a pure white inexpensive, free-flowing powder. One of the main advantages of microcrystalline cellulose is the direct relationship between cellulose column characteristics and paper chromatography data. The  $R_f$  data in the literature can be used to predict the retention volume ( $\bar{v}$ ) of metals on microcrystalline cellulose columns; and, consequently, separation factors can be calculated. Other reasons for using cellulose are: no volume change is detected with a change in eluent; trace concentrations are easily separated and eluted with no hold-up; and plate-height values are very good.

## Separations

Alkaline earths

Mutual separations of alkaline earths at the milligram level The alkaline earths were separated from each other and from other elements on cellulose columns. Table 1 shows data for alkaline earth separations at the 1 mg. level. The cellulose columns were shorter than subsequent columns, and only one eluent was used throughout the entire separation. An elution curve for a magnesium(II)-calcium(II) separation is presented in Figure 1. The elution curve was prepared by titrating 1 ml. effluent fraction with EDTA. The height equivalent to a theoretical plate (H.E.T.P.), calculated using the method on page 9, was 0.56 mm. for the bell-shaped magnesium(II) peak and 1.1 mm. for the slight-tailing calcium (II) peak for the curves of Figure 1. These values are considered very good, and the symmetry of the peaks indicates that microcrystalline cellulose is a good support for column chromatography.

In order to separate greater concentrations of the alkaline earths and increase the separation factors, longer columns of the microcrystalline cellulose were employed. Table 2 shows these separations. To speed the removal and prevent tailing of strontium(II) and barium(II), the eluent was changed during the separation.

In Tables 1 and 2 all of the metal-ion effluents were

Table 1. Column separations of alkaline earths

Column: microcrystalline cellulose  
 Eluent: methanol-hydrochloric acid (70-30)

Exp. No.	Metal	$\bar{v}$ (ml.)	Band width (ml.)	Concentration added (mmoles)	Concentration found (mmoles)	% Recovery	Column dimensions
1	Mg(II)	18.5	17-22	0.0746	0.0746	100.0	25.3x1.0 cm.
	Ca(II)	29.5	27-34	0.0374	0.0374	100.0	
2	Mg(II)	24.5	20-28	0.0660	0.0659	99.8	26.7x1.0
	Ca(II)	35.0	32-42	0.0374	0.0372	99.5	
	Sr(II)	54.0	49-77	0.0180	0.0181	100.4	
3	Mg(II)	21	16-29	0.0660	0.0669	101.4	29.0x1.0
	Ca(II)	38	30-45	0.0374	0.0364	97.3	
	Sr(II)	63	50-83	0.0180	0.0187	103.7	
	Ba(II)	115	---	0.00929	0.00940	101.2	

Metal	Average % recovery
-------	--------------------

Mg	100.4
Ca	98.9
Sr	100.8

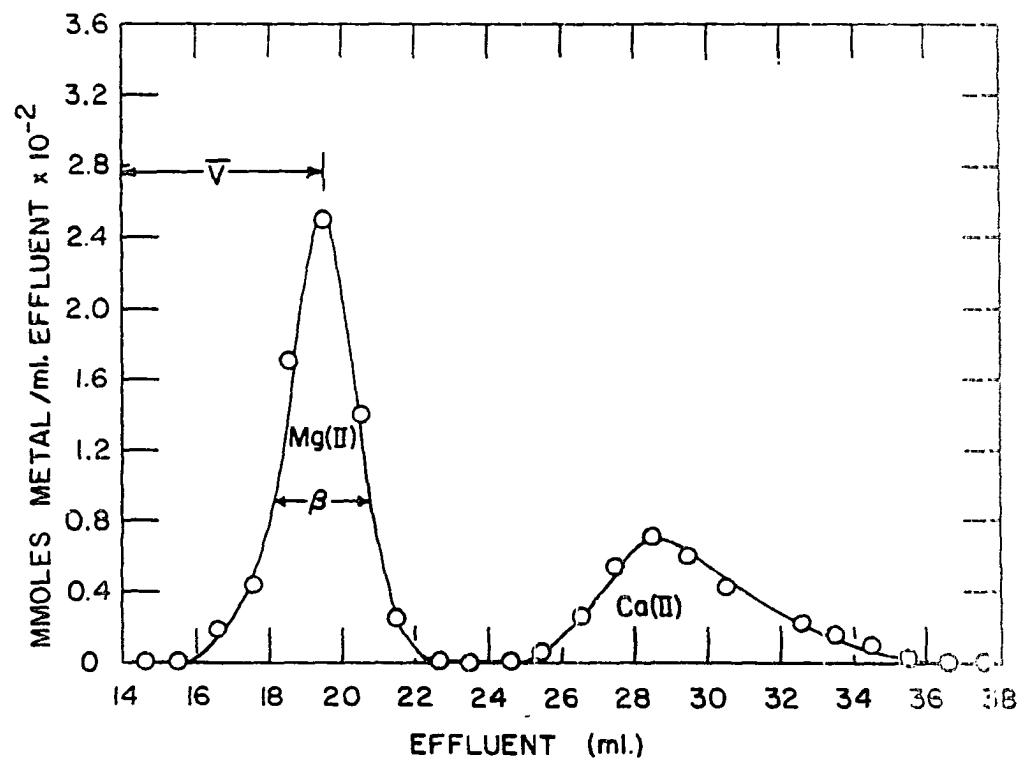


Figure 1. Elution curves for a magnesium(II)-calcium(II) column separation  
 Column: microcrystalline cellulose (23.4x1.0 cm.)  
 Eluent: methanol-hydrochloric acid (70-30 v/v), flow-rate =  
 0.5 ml./min.  
 Metal ion concentration: Mg(II) = 0.0746 mmoles, Ca(II) = 0.0374 mmoles  
 Sample volume: 1.4 ml.

Table 2. Column separations of alkaline earths

Column: microcrystalline cellulose  
 Metal ion concentration added (mmoles):  
 Mg(II) = 0.109 , Sr(II) = 0.0375  
 Ca(II) = 0.0793 , Ba(II) = 0.0340

Exp. No.	Metal	$\bar{v}$ (ml.)	Band width (ml.)	Concentration found (mmoles)	% recovery	Column dimensions	Eluent
1	Mg	46.0	35-52	0.109	100.0	46.4x1.15	MeOH-HCl(70-30)
	Ca	70	65-87	0.0792	99.8		
2	Mg	46	41-52	0.1098	100.7	44.0x1.15	MeOH-HCl(70-30)
	Ca	67	52-79	0.0789	99.5		MeOH-HCl(95-5)
	Sr	108	94-126	0.0	(poor e.p.)		MeOH-H <sub>2</sub> O(95-5)
	Ba	140	127-185	0.0341	100.3		
3	Mg	51	40-62	0.1098	100.0	50.1x1.15	Same
	Ca	80	71-104	0.0801	101.0		
	Sr	140	121-169	0.0387	103.2		
	Ba	230	213-248	0.0329	96.7		
4	Mg		- 56	0.109	100.3	49.1x1.15	Same
	Ca		65-103	0.0795	100.2		
	Sr		116-169	0.0379	101.1		
	Ba		200-243	0.0335	98.4		

Metal	Average % recovery	Number of determinations
Mg(II)	100.3	4
Ca	100.1	4
Sr	102.1	2
Ba	98.5	3

analyzed by EDTA titrations. These tables show clean and quantitative separations of magnesium(II), calcium(II), strontium(II), and barium(II) from each other at equal weight ratios in less than 250 ml. of effluent. The flow rates were between 0.5 and 1.0 ml./min. up to the calcium(II) peak, and between 1.0 and 2.0 ml./min. for the elution of strontium(II) and barium(II).

Mutual separations of alkaline earths with strontium(II) at the parts per million level      The analyses of strontium (II) and especially barium(II) by the Eriochrome Black T-EDTA method were very difficult. For this reason the radioisotopes, strontium<sup>85</sup>(II) and barium<sup>133</sup>(II), were employed to determine the percentage recovery and also the elution band shapes. In addition, these carrier-free radioisotopes permitted separations of trace concentrations. Table 3, Experiment 1 and Figure 2 show an alkaline earth separation with barium(II) at the parts per million level, and Experiment 2 with strontium(II) at the parts per million level. Therefore the alkaline earths can be separated from each other at weight ratios equal to unity, or in mixtures of the four containing either strontium(II) or barium(II) at tracer-level concentrations.

Mutual separations of alkaline earths-determination of strontium(II) and/or barium(II) by neutron activation      In order to investigate the technique of neutron activation



Table 3. Column separations of alkaline earths  
 [Column: microcrystalline cellulose]

Exp. No.	Metal	$\bar{v}$ (ml.)	Band width (ml.)	Concentration Added (mmoles)	Concentration Found (mmoles)	% Recovery	Weight ratio <sup>a</sup>	Eluent MeOH-H <sub>2</sub> O (V-V-V)	Column dimensions (cm.)
1	Mg	-	25-62	0.0971	0.0969	99.8	..	70-30	50.9
	Ca	-	69-104	0.0741	0.0739	99.7	..	90-10	x1.1
	Sr <sup>85</sup>	140	123-204	0.0344	0.0345 <sup>b</sup>	100.3	..	80-5-15	
	Ba <sup>133</sup>	-	299-349	1.26x10 <sup>-7</sup>	1.26 x10 <sup>-7b</sup>	100.0	0.48		
2	Mg	-	25-59	0.0971	0.09640	99.3		70-30	49.8
	Ca	-	70-105	0.0741	0.0726	98.0		90-10	x1.1
	Sr <sup>85</sup>	172	129-210	1.70x10 <sup>-7</sup>	1.67x10 <sup>-7b</sup>	98.2	0.63	80-5-15	
	Ba <sup>133</sup>	220	210-310	0.0304	0.03230 <sup>b</sup>	106.1			
3	Mg	-	25-63	0.0971	0.09620	99.1		70-30	50.0
	Ca	-	68-106	0.0741	0.0711	96.0		90-10	x1.1
	Sr <sup>87</sup>	-	112-202	0.0115	0.0115	100.0		80-5-15	
	Ba <sup>139</sup>	-	205-325	0.0101	0.0103 <sup>b</sup>	101.9			
4	Sr <sup>85</sup>	45	40-96	0.115	0.120 <sup>b</sup>	104.8		95-5	19.4x
	Ba <sup>133</sup>	-	165-190	1.26x10 <sup>-7</sup>	1.22x10 <sup>-7b</sup>	96.8	0.59	0-0-100	conical

<sup>a</sup>Weight ratio =  $\frac{\text{Total weight M(II)}}{\text{Weight of trace M(II)}} \times 10^{+6}$ .

<sup>b</sup>Based on counting of added radioisotope; all counts are between 5,000 and 30,000 c/1 min.

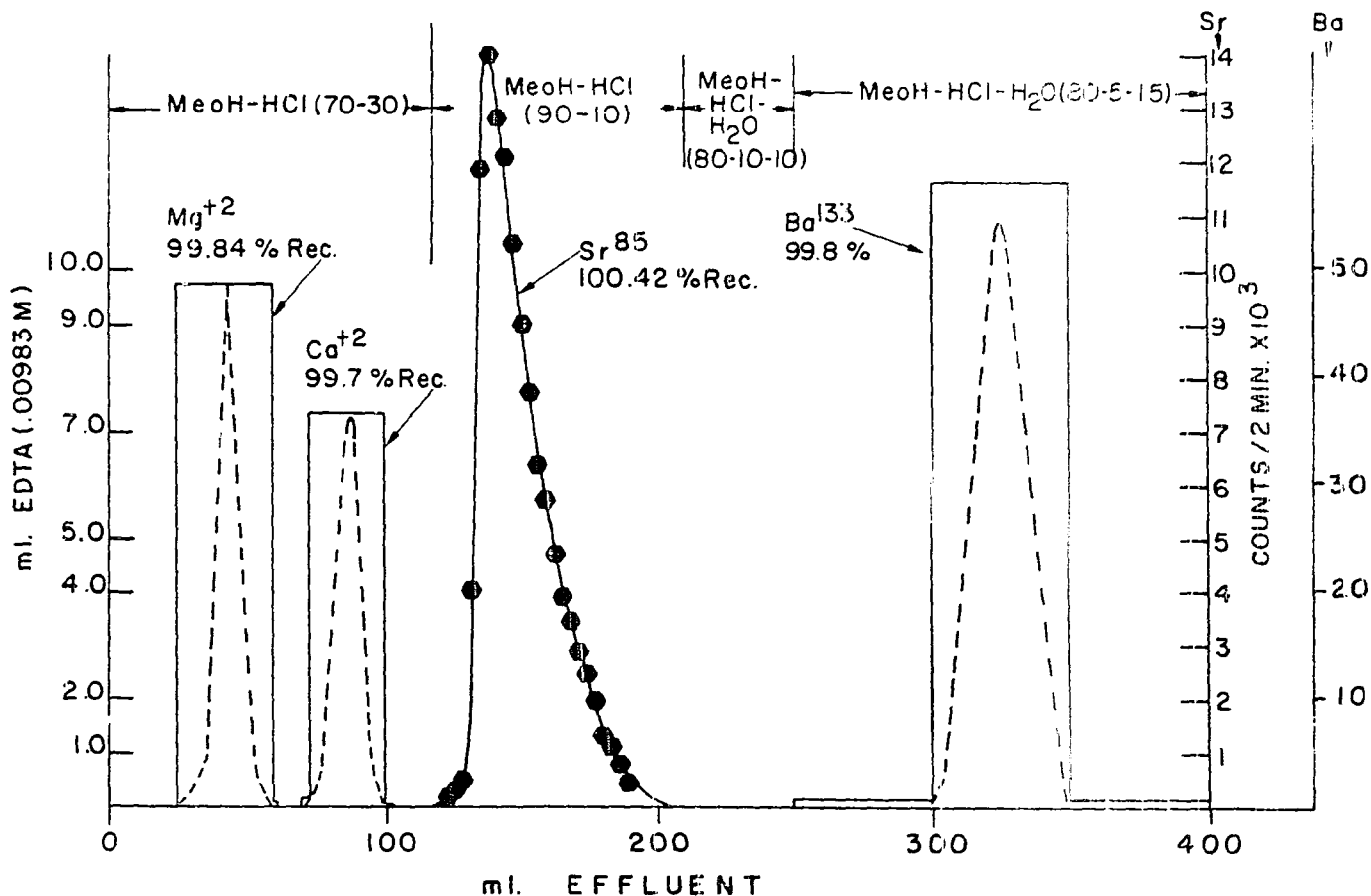


Figure 2. Column separation of the alkaline earths (Table 3, Exp. 1).  
 Column: microcrystalline cellulose (50.9x1.1 cm.),  
 Metal ion concentrations: Mg(II) = 0.0971 mmole, Ca(II) = 0.0741 mmole  
 Sr(II) = 0.0344 mmole, Ba<sup>133</sup>(II) =  
 1.73x10<sup>-8</sup> gm.

Weight ratio:  $\frac{\text{total metal ion weight}}{\text{Ba(II) weight}} = 0.48 \times 10^{+6}$ .

analysis and combine it with separation technology, strontium (II) and barium(II) were determined by this method after separation. Mixtures of the alkaline earths were separated, the magnesium(II) and calcium(II) fractions were determined by EDTA, and the strontium(II) and barium(II) fractions were collected and treated as directed in analytical radiometric procedures. The data appear in Table 3, Experiment 3. The polyethylene vials containing the strontium(II) fraction and standards were irradiated for 1.0 min. at a flux of  $3.5 \times 10^{13}$  neutrons/cm.<sup>2</sup>-sec. After the gamma-ray spectra purity was checked on the multichannel analyzer, the sample and standards were counted on a single channel analyzer and the percentage recovery calculated. The barium(II) fraction was treated the same, except only a 20-second irradiation time was necessary.

In order to investigate more fully neutron activation analysis, a trace (70 ppm) of barium(II) was determined by this technique after its separation from strontium(II). The separation procedure was established with the radioisotopes, strontium<sup>85</sup>(II) and barium<sup>133</sup>(II). The data for this separation are presented in Table 3, Experiment 4. The column used for this and subsequent experiments involving neutron activation is conical. The advantage of this column over a cylindrical column will be discussed later.

Preceding actual separations, conditions for standard curve preparation had to be established. Polyethylene vials

containing  $1.15 \times 10^{-7}$ ,  $4.62 \times 10^{-7}$ ,  $6.92 \times 10^{-7}$  g. of barium(II) were irradiated for 25 min. at a flux of  $3.5 \times 10^{13}$  neut./cm.<sup>2</sup>-sec. The method outlined in analytical, radiometric procedures was then followed. The gamma emissions of the trace of barium<sup>139</sup>(II) in the carrier, barium sulfate, in the Whatman No. 42 filter paper were measured; but were found to have no relationship to the barium(II) concentration. The gamma spectra revealed that the interference was tungsten. The trace of tungsten came from contaminated volumetric flasks. This interference was removed by plotting the log activity of the samples and standards versus time. A typical decay curve for a mixture of short and long-lived isotopes resulted because of the difference in half-lives (barium<sup>139</sup>(II) = 83.min., tungsten<sup>187</sup> = 24.hr.). Extrapolation of the straight portion of the curve caused by tungsten<sup>187</sup> activity back to zero time gave the tungsten<sup>187</sup> activity at zero time. Subtraction of this tungsten<sup>187</sup> activity from the total activity gave the true barium<sup>139</sup>(II) activity. A plot of this activity versus barium(II) concentration resulted in a straight line.

The Baker's Analyzed strontium(II) used in these experiments contained a trace of barium(II) (0.002%). This trace of barium(II) was separated from the strontium(II) (10.04 mg.) on the conical column. The barium(II) fraction (140 to 250. ml. of effluent), stripped off with water, was concentrated by evaporation and treated the same as the standard curve

solutions. The purified strontium(II) was run through the column again; and the effluent between 140 and 250 ml., analyzed by neutron activation, was found to still contain some barium(II) activity. This barium(II) activity was considered a "blank" and subtracted from all determinations presented in Table 4. A trace of barium(II) was added to the "purified strontium(II)", separated, and the percentage recovery determined. All of these results are presented in Table 4.

Table 4. Alkaline earth separations

Determination of barium(II) by neutron activation	
Column: Cellulose, conical, 19.4 cm. long	
Eluent: (1) methanol-hydrochloric acid (95-5 v/v) for strontium(II)	
(2) water for barium(II)	
Metal ions: Sr <sup>85</sup> (II): concentration = 10.04 mg.	
Sr <sup>85</sup> (II): $\bar{v}$ = 42 ml.	
Sr <sup>85</sup> (II): average % recovered = 101.6%	
Ba(II) fraction: 140-250 ml.	

Weight of barium <sup>139</sup> (II) found per 10.04 mg of Baker's strontium(II)	$3.22 \times 10^{-6}$ g. $\frac{0.67 \times 10^{-6}}{1.95 \times 10^{-6}}$ g. $1.95 \times 10^{-6}$ g.
Weight of barium(II) reported on Baker's strontium(II)	$0.61 \times 10^{-6}$ gm. Ba/10 mg. Sr
Weight of barium <sup>139</sup> (II) added to "purified" strontium(II)	$0.69 \times 10^{-6}$ g.
Weight of barium <sup>139</sup> (II) found	$0.77 \times 10^{-6}$ g.

Even though the results of Table 4 are not quantitative, the technique of separation of one or more trace components followed by neutron activation analysis shows great promise. The determination of a trace of barium(II) in strontium(II) could not be done by neutron activation without a separation

step. This general technique could easily be extended to other systems requiring trace determinations and could perhaps go down to  $10^{-9}$  g. levels or lower.

Separations of strontium(II) and barium(II) As a prelude to the study of column shapes and packings, separations of strontium(II) and barium(II) on cylindrical, cellulose columns were studied. Table 5 presents the results of these separations. It should be noted that Table 5, Experiment 2, presents a separation of two carrier-free radioisotopes; and even though the strontium<sup>85</sup>(II) band tails a bit, the separation is almost clean. The change in concentration of strontium(II) from the low milligram to the  $10^{-8}$  g. level shown in Table 5, Experiments 1 and 2, resulted in a shift of retention volume ( $\bar{v}$ ) from 25 ml. to 58 ml. Because of the slight difference in column length and the rather large concentration shift, this  $\bar{v}$  change is not considered large.

Comparison of American Viscose and Whatman cellulose

The American Viscose microcrystalline cellulose used in this project was compared to the microgranular Whatman brand CC-31. The basis of comparison was a strontium(II)-barium(II) separation under similar conditions on the two brands of cellulose. This comparison appears in Table 6 and Figure 3. The Whatman cellulose has a higher density (g./bed volume) and requires more pressure to produce a reasonable flow rate than the American Viscose. For these reasons and because of the

Table 5. Column separations of strontium(II)-barium(II)  
 [Column: microcrystalline cellulose]

Exp. No.	Metals	$\bar{v}$ (ml.)	Band width (ml.)	Concentration (mmoles)		% Recovery	Column dimensions (cm.)	Eluent (MeOH-HCl-H <sub>2</sub> O)
				Added	Found			
1	Sr	25	16-32	0.0375	0.0372	99.2	24.9x1.0	95-5-0
	Ba	35	33- 64	0.0340	0.0343	100.9		95-5-0
2	Sr <sup>85</sup>	58	43-86	1.70x10 <sup>-7</sup>	1.69x10 <sup>-7a</sup>	99.52	26.2x1.0	93-7-0
	Ba <sup>133</sup>	--	+tail 134-	1.26x10 <sup>-7</sup>	1.39 x10 <sup>-7a</sup>	110.3		90-0-10
3	Sr <sup>85</sup>	33	22-72	0.0125	0.0124 <sup>a</sup>	99.2	27.1x1.0	95-5-0
	Ba <sup>133</sup>	120	87-	1.26x10 <sup>-7</sup>	1.35x10 <sup>-7a</sup>	106		95-5-0

<sup>a</sup>Based on counting of added radioisotopes; all counts are between 30,000 and 80,000 counts/min.

sharper bands for American Viscose cellulose shown in Table 6 and Figure 3 for the system studied, the American Viscose cellulose is recommended for chromatographic work.

Table 6. Comparison of American Viscose and Whatman cellulose

Cellulose	Metal	$\bar{v}$	Band width	% Recovery <sup>a</sup>
American Viscose	Sr <sup>85</sup> (II) Ba <sup>133</sup> (II)	33 ml. 120	22-57 ml. 87-	99.2 106
Whatman CC-31	Sr <sup>85</sup> (II) Ba <sup>133</sup> (II)	49.0 325	36-164 239-389	100.6 103.8

Separation of strontium<sup>85</sup>(II)-barium<sup>133</sup>(II)  
 Column dimensions: 27.1x1.0 cm.  
 Eluent: methanol-hydrochloric acid (95-5 w/v)  
 Metal ion concentrations: Sr(II) = 0.0125 mmoles  
 Ba(II) = 1.26x10<sup>-7</sup> mmoles

<sup>a</sup>Based on counting of added radioisotope.

Comparison of cylindrical and conical shaped columns

Slahr et al. (65) in a recent paper pointed out that conical columns produce tighter bands than cylindrical chromatographic columns. This idea was tested with a strontium<sup>85</sup>(II)-barium<sup>133</sup>(II) separation on microcrystalline cellulose. The same weights of cellulose, prepared according to the directions in the experimental section, were added to cylindrical and conical chromatographic columns. Mixtures of strontium<sup>85</sup>(II) and barium<sup>133</sup>(II) were then separated on the two columns under identical conditions and the comparison is presented in



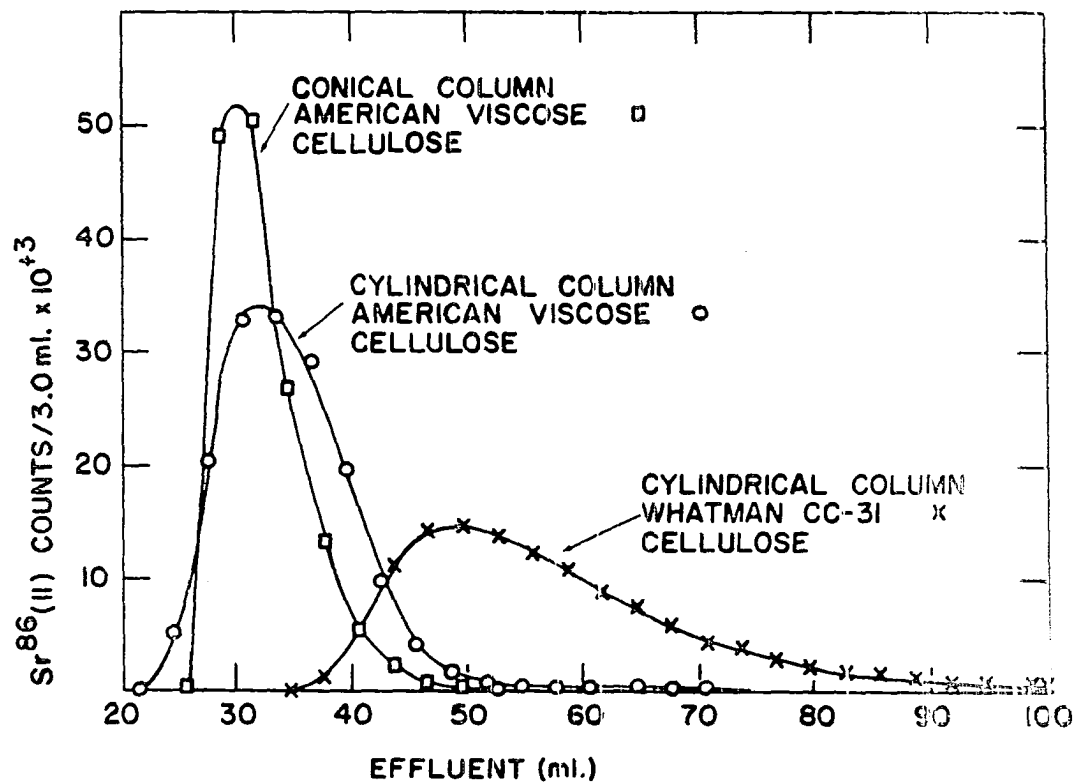


Figure 3. Band shape of strontium<sup>85</sup>(II) for different column shapes and celluloses. The same weight of cellulose was used in the three experiments

Eluent: methanol-hydrochloric acid (95-5 v/v)  
 Sr(II) concentration = 0.0125 mmoles

Table 7 and Figure 3. The results show no improvement in the separation factor ( $\bar{v}_{Sr}/\bar{v}_{Ba}$ ) for strontium(II) and barium(II).

Table 7. Comparison of cylindrical and conical chromatographic columns

Column shape and dimension	Metal	$\bar{v}$ (ml.)	Band width (ml.)	Effluent volume between bands (ml.)
Straight 27.1x1.0 cm.	Sr <sup>85</sup> (II)	33	72-22=50	15
	Ba <sup>133</sup> (II)	120	87-	
Conical 14.6 cm. long	Sr <sup>85</sup> (II)	30.5	57-24=33	25
	Ba <sup>133</sup> (II)	100.0	82-...	

Column packing: American Viscose microcrystalline cellulose  
 Eluent: methanol-hydrochloric acid (95-5 v/v)  
 Metal ion concentration: Sr<sup>85</sup>(II) = 0.0125 mmoles  
 Ba<sup>133</sup>(II) = 1.26x10<sup>-7</sup> mmoles

The strontium<sup>85</sup>(II) peak is, however, much narrower for the conical column, producing a better separation than was possible for the cylindrical column. The conical column also gave a reasonable flow rate with no pressure while the cylindrical column required 5 lb./in.<sup>2</sup> for the same flow-rate. Similar comparisons for a barium<sup>133</sup>(II)-radium<sup>226</sup>(II) separation (Table 8, Experiment 4) also show that the conical column produces superior separations. Therefore, for the systems discussed here the conical shape appears to have a definite advantage over the cylindrical chromatographic columns. For this reason, conical columns were used for the neutron activation analysis experiments discussed earlier.

Table 8. Column separations of barium<sup>133</sup>(II)-radium<sup>226</sup>(II)

$$\left[ \text{Mole ratio } \frac{\text{Ba(II)}}{\text{Ra}^{226}\text{(II)}} = 1.4 \times 10^{+4} \right]$$

Exp. No.	Metals	(ml.)	Band width (ml.)	Amount (mmoles)		% Recovered <sup>a</sup>
				Added	Found	
1	Ba <sup>133</sup>	27	24-57	0.00997	0.00963	96.6
	Ra <sup>226</sup>	84	42-..	$7.08 \times 10^{-7}$	$6.64 \times 10^{-7}$	93.8
2	Ba <sup>133</sup>	45	30-81	0.00997	0.01005	100.83
	Ra <sup>226</sup>	--	85-130	$7.08 \times 10^{-7}$	$6.24 \times 10^{-7}$	88.1
3	Ba <sup>133</sup>	36	36-127	0.00997	0.00941	94.4
	Ra <sup>226</sup>	176	98-..	$7.08 \times 10^{-7}$	$6.34 \times 10^{-7}$	89.5
4	Ba <sup>133</sup>	61	39-135	0.00997	0.00956	95.9
	Ra <sup>226</sup>		128-..	$7.08 \times 10^{-7}$	$6.78 \times 10^{-7}$	95.8

Exp. No.	Metals	Column dimensions cm.	Eluent (MeOH-H <sub>2</sub> O-HCl)	Flow rate
				ml./min.
1	Ba <sup>133</sup> Ra <sup>226</sup>	11.4x1.0	85-5-10	0.3
2	Ba <sup>133</sup> Ra <sup>226</sup>	14.8x1.0	85-5-10 0.100-0	0.1
3	Ba <sup>133</sup> Ra <sup>226</sup>	16.8x1.1	85-5-10 70-20-10	0.6
4	Ba <sup>133</sup> Ra <sup>226</sup>	11.4 cm, long (conical shape)	85-5-10 70-20-10 0-100-0	0.6- 0.75

<sup>a</sup>Determined by counting of added radioisotope.

Separation of barium<sup>133</sup>(II)-radium<sup>226</sup>(II) and beryllium<sup>7</sup>(II)-magnesium(II) In order to make the alkaline earth separation study more complete the last in the series, radium<sup>226</sup>(II), was studied. The mixture, barium<sup>133</sup>(II)-radium<sup>226</sup>(II) was separated on a cellulose column with the eluent methanol-water-hydrochloric acid (85-5-10 v/v/v). These separations are presented in Table 8. The percentage recovery of radium<sup>226</sup>(II) was low in all cases. This is due to the separation of a very short lived daughter or impurity from the radium<sup>226</sup>(II), which appears in the effluent before the barium<sup>133</sup>(II) peak. Because of the very short half-life of this impurity, no correction factor was applied to the radium<sup>226</sup>(II) recovery. It should be noted in Table 8 that Experiments 3 and 4 contained the same weight of cellulose. The conical column in Experiment 4, however, produced a much better separation than the cylindrical column of Experiment 3. In Experiments 1, 3, and 4 the barium<sup>133</sup>(II) peak tailed a bit and resulted in a very slight overlap of peaks. The slower flow rate of Experiment 2, however, eliminated the overlap of peaks and this separation is shown in Figure 4.

The separation of beryllium<sup>7</sup>(II)-magnesium(II) was studied on a 27.0x1.1 cm. cellulose column with a methanol-hydrochloric acid (70-30 v/v) eluent. Only a partial separation was achieved - with considerable overlap of peaks. The

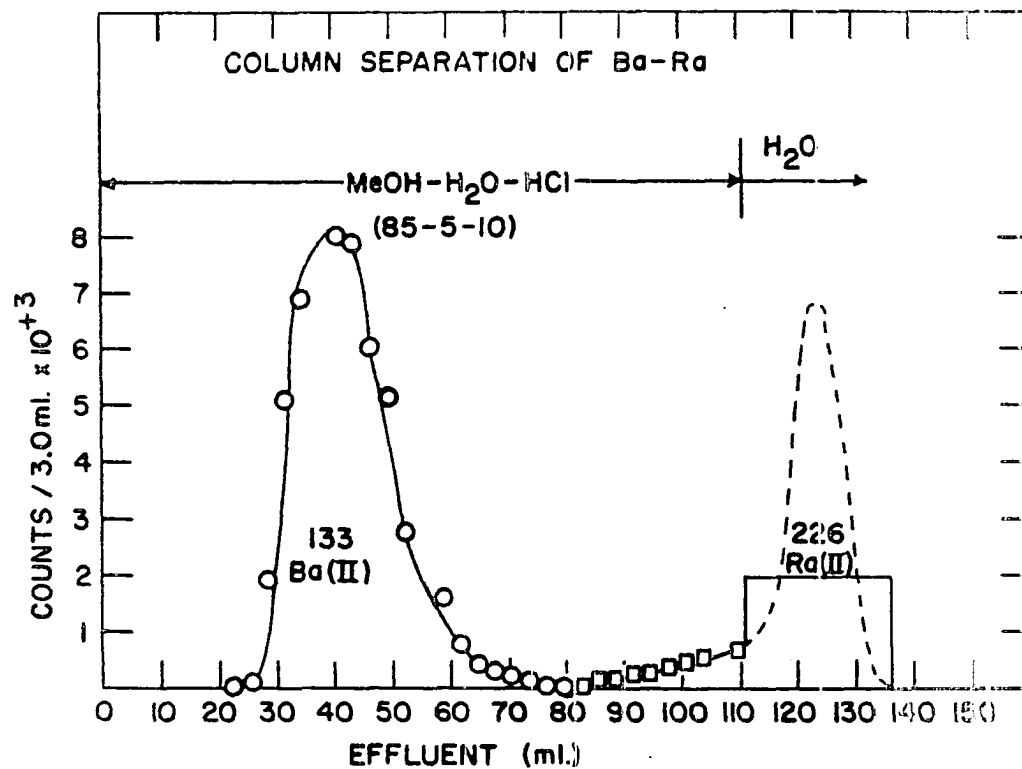


Figure 4. Column separation of barium<sup>133</sup>(II)-radium<sup>226</sup>(II)  
 Column: microcrystalline cellulose (14.8x1.0 cm.)  
 Eluent: methanol-water-hydrochloric acid (85-5-10);  
 flow rate = 0.1 ml./min.  
 Metal ion concentration: Ba(II) = 0.00997 mmoles  
 Ra(II) = 7.08x10<sup>-7</sup> mmoles

beryllium<sup>7</sup>(II) peak preceded the magnesium(II) peak retention volume ( $\bar{v}$ ) by only a few ml. No complete separation was achieved, but a longer column might resolve the peaks.

Separation of alkaline earths from rare earths. A plot of the ionic radius for the alkaline earths, magnesium(II), calcium(II), strontium(II), and barium(II) versus the retention volumes ( $\bar{v}$ ) from Table 1, Experiment 3 resulted in a smooth curve. Interpolation of the europium<sup>152-154</sup>(III) ionic radius into the plot showed that europium<sup>152-154</sup>(III), which had approximately the same ionic radius as calcium(II), should be eluted with calcium(II) between magnesium(II) and strontium(II). To prove a relationship between retention volume ( $\bar{v}$ ) and ionic radius a separation of magnesium(II), europium<sup>152-154</sup>(III), strontium<sup>85</sup>(II), and barium<sup>133</sup>(II) was attempted. The data in Table 9, Experiment 1 show that the europium<sup>152-154</sup>(III) peak does indeed appear between magnesium(II) and strontium(II) and lends support to the retention volume ( $\bar{v}$ )-ionic radius relationship.

Because alkaline earths and rare earths often appear together and their separation is difficult or time consuming, this separation was studied further. A rapid separation of a representative rare earth, erbium(III), from barium<sup>133</sup>(II) is presented in Table 9, Experiment 2. These elements were separated by Nascutiu (66) by paper chromatography. Retention volumes ( $\bar{v}$ ) for erbium(III) and barium<sup>133</sup>(II) were calculated

Table 9. Column separations of alkaline earths-rare earths  
 [Column: microcrystalline cellulose]

Exp. No.	Metals	$\bar{v}$		Band width (ml.)	Concentration (mmoles)		% Recovery	Column dimensions	Eluent (v/v)
		Calc.	Exp.		Added	Found			
1	Mg			25-63	0.0971	0.096	99.2		MeOH-HCl (70-30)
	Eu <sup>152-154</sup>		93	77-124	0.0277	0.027 <sup>a</sup>	99.6	50.1 x1.1	
	Sr <sup>85</sup>		147	121-214	0.0344	0.0348 <sup>a</sup>	101.2		MeOH-HCl (90-10)
	Ba <sup>133</sup>			230-304	0.0304	0.0322 <sup>a</sup>	105.8		
2	Er	26	33	25-45	0.0870	0.0870	100.6	26.5	4N HNO <sub>3</sub> -EtOH (1:2 v/v)
	Ba <sup>133</sup>	93	75	58-90	1.26x 10 <sup>-7</sup>	1.22x 10 <sup>-7</sup>	96.5	x1.1	

from Mascutin's  $R_f$  values using the equation,  $\bar{v} = V_m/R_f$ , which is presented on page 14. The calculated and experimental retention volumes ( $\bar{v}$ ) are considered to be in agreement. The height equivalent to a theoretical plate (H.E.T.P.) was calculated for the bell-shaped barium(II) peak. Using the formula of page 9, the H.E.T.P. is 1.2 mm. for this system.

#### Alkali metal separations

Methanolic eluents Sommer (45) separated the alkali metals lithium(I), sodium(I), potassium(I) by the method of paper chromatography. Retention volumes ( $\bar{v}$ ), calculated from the equation,  $\bar{v} = V_m/R_f$ , using Sommer's  $R_f$  values were far enough apart for the alkali metals to warrant a cellulose column study. Between 3 and 6 mg. of each of the alkali metals, lithium(I), sodium(I), and potassium(I) were separated on cellulose columns using the same eluent as Sommer, methanol-hydrochloric acid-water (75-10-15 v/v/v). The data appear in Table 10. All of the metals were determined by atomic absorption spectroscopy. Table 10 not only shows alkali metal separations but also the excellent agreement between the calculated and experimental retention volumes ( $\bar{v}$ ). Studies of rubidium(I) and cesium(I) under similar conditions on cellulose columns showed that they appeared in the effluent with the potassium(I) fraction. These metals cannot, therefore, be separated by this system.



Table 10. Alkali metal separations

Column:	microcrystalline cellulose
Eluent:	methanol-hydrochloric acid-water (75=10=15)
Metal ion concentration:	Li(I) = 0.904 mmoles
	Na(I) = 0.156 mmoles
	K(I) = 0.111 mmoles

Exp. No.	Metal	$\bar{v}$ (ml.)		Band width (ml.)	% Recovery	Column dimensions
		Calcu.	Exp.			
1	Li(I)	9.6	12.5	18-17	98.5	19.5x 1.0 cm.
	Na(I)	19.4	21	18-28	94.4	
	K(I)	38.8	48	37-69	107.5	
2	Li(I)	11.8	17	9-23	100.3	24.4x 1.0 cm.
	Na(I)	23.9	27	23-37	98.6	
	K(I)	47.8	47	37-61	95.5	

Average % recovery

Li(I) = 99.4

Na(I) = 96.5

K(I) = 101.5

Phenolic eluents      The separation of the alkali metals cesium<sup>134</sup>(I), rubidium<sup>86</sup>(I) and potassium<sup>42</sup>(I), was accomplished on a cellulose column with the eluent, phenol-hydrochloric acid-methanol (230 g.-80 ml.-90 ml.). This eluent was used as a developing agent by Magee and Headridge (47) to study alkali metal movement on paper. Their  $R_f$ 's were used to calculate retention volumes ( $\bar{v}$ ). The data for this separation are presented in Table 11. The calculated retention volume ( $\bar{v}$ ) of 54.7 ml. for sodium<sup>24</sup>(I) on the same length column was determined experimentally to be 54 ml. Therefore sodium(I) cannot be separated from potassium(I) by this system.

Table 11. Alkali metal separations

Column:	cellulose (16.0x1.0 cm.)
Eluent:	Phenol=hydrochloric acid=methanol (230 gm.-80 ml.-90 ml.)
Metal ion concentration:	Cs(I) = 2.95 mg, 0.0222 mmole Rb(I) = 4.4 mg., 0.0515 K(I) = 1.01 mg., 0.0258 Na(I) = 10.06 mg, 0.437

Metal	$\bar{v}$ (ml.)		Band width (ml.)	Activity added (c/2.0m)	Activity found (c/2.0m)	% Recovery
	Exp.	Calc.				
Cs <sup>134</sup> (I)	19	18.3	15-26	400,820	394,141	98.3
Rb <sup>86</sup> (I)	32	38.2	27-42	11,589	11,976	103.3
K <sup>42</sup> (I)	51	60.8	45-66	11,613	11,505	99.1

The alkali metals, cesium<sup>134</sup>(I), rubidium<sup>86</sup>(I), potassium<sup>42</sup>(I) and sodium<sup>24</sup>(I), were separated from each other on cellulose columns with the eluent prepared by shaking phenol crystals with 20% hydrochloric acid. The data for these separations are presented in Table 12. A slight difference in cellulose column "preparation" produced different elution curves (compare Table 12, Experiments 1 to 4). The column of Experiment 1 was prepared by slurring the cellulose in the phenolic eluent and pouring into the glass column. The columns of Experiments 2, 3, 4 and 5 were prepared by slurring the cellulose with the equilibrated aqueous phase from the eluent preparation. This slurry was poured into a column, washed with the phenolic eluent, and then removed and re-packed with the phenolic eluent. Columns, prepared in this

Table 12. Column separations of the alkali metals

Column: microcrystalline cellulose

Metal ion concentrations (mmoles): Cs(I) = 0.0222, Rb(I) = 0.0515,

K(I) = 0.0256, Na(I) = 0.437

Eluent: phenol shaken with 20% hydrochloric acid

Exp. No. (Col. dem.) cm.	Cellulose was slur- ried with:	Metal	$\bar{v}$ (ml.)		Band width (ml.)	Activity added (counts/ 2 min.)	Activity found (counts/ 2 min.)	% Recov- ery
			Exp.	Calc.				
1. (17.4x1.0)	Phenolic phase	Cs	19	16.0	15-26	406,418	404,500	99.5
		Rb	33	38.1	29-44	8,689	8,911	102.6
		K	57.5	57.2	47-65	221	234	105.9
		Na	83	98.0	68-109	9,261	9,164	99.0
2. (15.4x1.0)	Equili- brated aqueous phase (20% HCl)	Cs	23	14.8	20-31	400,131	400,131	99.9
		Rb	46.5	35.3	39-55	8,689	9,020	103.9
		K	72	52.98	62-88	53,045	52,928	99.8
		Na	108	90.8	93-156	86,244	87,458	101.4
3. (19.4x1.1)	Same	Cs	36	21.1	28-49	404,804	383,743	94.8
		Rb	83	50.3	70-98	8,154	7,981	97.8
		K	128	75.4	107-151	2,712	2,544	93.8
		Na	207	129.3	171-252	57,002	59,993	103.8
4. (18.4x1.0)	Same	Cs	25		18-36	404,804	409,649	101.2
		Rb	56.0		46-71	8,182	8,281	101.2
		K	92.0		75-105	2,835	2,751	97.0
		Na	140		117-248	61,594	64,063	104.0

Table 12. (Continued)

Exp. No. (Col. dem.) cm.	Cellulose was slur- ried with:	Metal	$\bar{v}$ (ml.)		Band width (ml.)	Activity added (counts/ 2 min.)	Activity found (counts/ 2 min.)	% Recov- ery
			Exp.	Calc.				
5. (17.8x1.1)	Equili- brated aqueous phase (20% HCl)	Cs	31		22-48	393,989	394,772	100.2
		Rb	72		59-88	8,400	9,016	107.3
		K	114		94-140	8,579	8,669	101.1
		Na	170		141-188	109,627	108,120	98.6
			<u>Metal</u>	<u>Avg. % recovery</u>				
			Cs	99.1				
			Rb	102.5				
			K	99.5				
			Na	101.3				

way, gave better separation factors and more effluent volume between bands. The elution curve for Table 12, Experiment 2 is presented in Figure 5. These strikingly symmetrical curves show that a clean and accurate separation of the alkali metals is possible in less than 200 ml. of effluent. The H.E.T.P. was calculated from the curves in Figure 5 using the technique discussed on page 9. The H.E.T.P. calculated from the cesium(I), rubidium(I), potassium(I), and sodium(I) peaks is respectively 1.47, 0.58, 0.52, 0.46 mm.

The alkali metals discussed above were studied by Steel (48) on paper with the same phenolic eluent used in Table 12. The calculated retention volumes ( $\bar{v}$ ) are in close agreement with those determined experimentally for Table 12, Experiment 1; but Experiments 2, 3, 4, 5 show some disagreement. This is to be expected because in Experiments 2 to 5 the cellulose was first slurried with something other than the eluent.

The cesium<sup>134</sup>(I) was obtained pure from Oak Ridge, and the rubidium<sup>86</sup>(I), potassium<sup>42</sup>(I), sodium<sup>24</sup>(I) nitrate salts were irradiated at the Ames Laboratory reactor. The potassium<sup>42</sup>(I) and sodium<sup>24</sup>(I) were free of any interfering activity, but the rubidium<sup>86</sup>(I) salt contained about 1% by weight cesium(I). This cesium<sup>134</sup>(I) impurity was separated from the rubidium<sup>86</sup>(I) (Table 13) and used to correct the percentage recovery of both cesium<sup>134</sup>(I) and rubidium<sup>86</sup>(I) in all experiments. The cesium<sup>134</sup>(I) activity separated from the

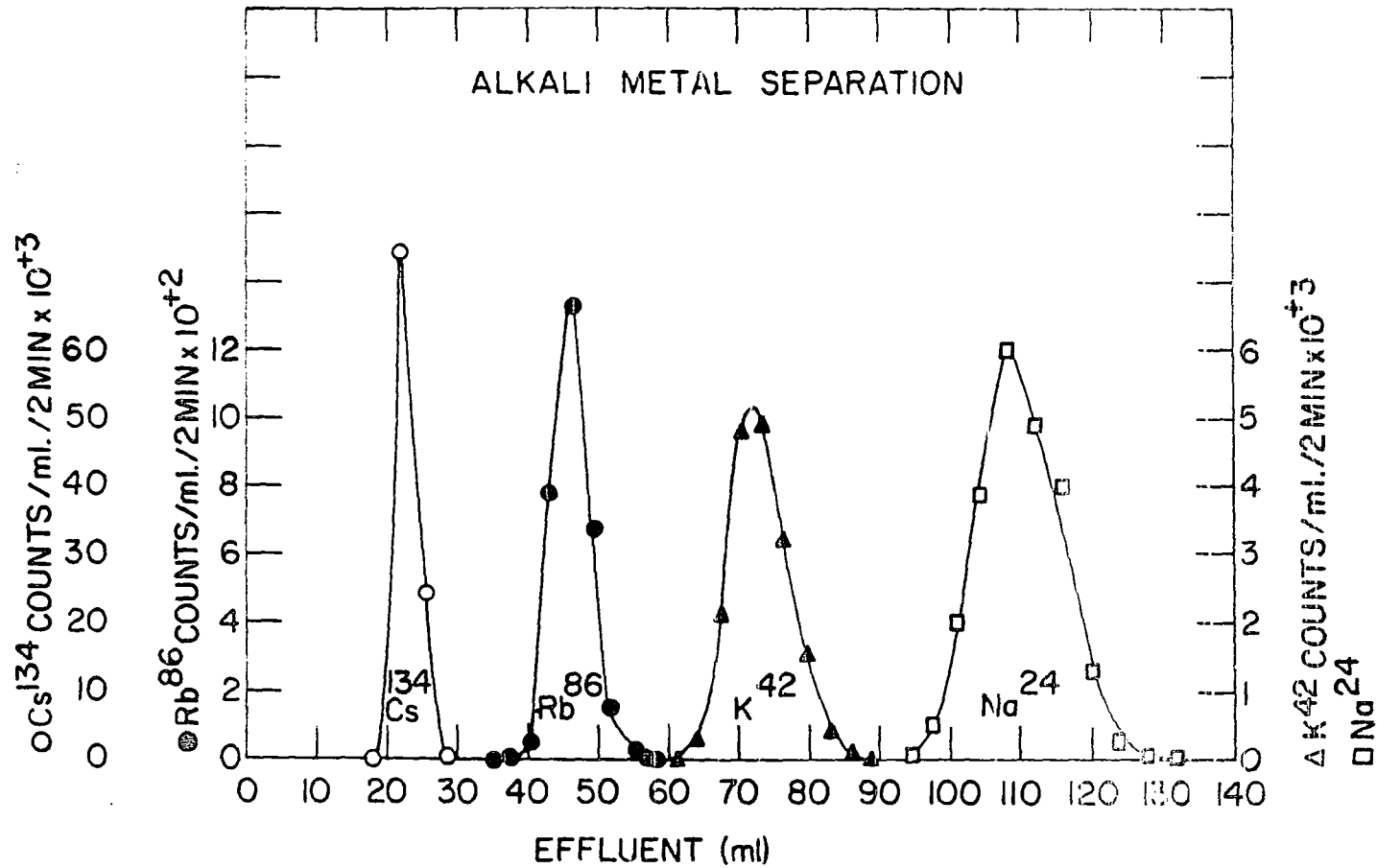


Figure 5. Column separation of the alkali metals  
 Column: microcrystalline cellulose (15.4x1.0 cm.)  
 Eluent: phenol equilibrated with 20% HCl, flow rate = 0.5 ml./min.  
 Metal ion concentration (mmoles): Cs = 0.022, Rb = 0.052,  
 K = 0.026, Na = 0.438

rubidium<sup>86</sup>(I) was added to the stock cesium<sup>134</sup>(I) activity before percentage recoveries were calculated. The separated rubidium<sup>86</sup>(I) activity was plotted versus time and the true rubidium<sup>86</sup>(I) activity at the time of each experiment read from this plot.

Table 13. Determination of cesium impurity in rubidium stock solution

Column:	microcrystalline cellulose
Eluent:	(1) phenol-hydrochloric acid-methanol (2) phenol equilibrated with 20% hydrochloric acid
Metal ion concentration:	Cs(I) = 0.04 mg. Rb(I) = 4.4 mg.

Exp. No.	Metal	$\bar{v}$ (ml.)	Band width (ml.)	Eluent	Activity found per 1.0 ml. Rb(I) stock solution counts/2.0 min.	Column dimensions
1	Cs	19	13-25	(1.)	4,518	15.2x
	Rb	34	31-49		11,885	1.0 cm.
2	Cs	18	14-24	(2.)	5,472	15.3x
	Rb	36	29-44		5,915	1.0 cm.
3	Cs	14	11-19	(2.)	4,992	10.6x
	Rb	36	28-45		6,565	1.0 cm.

Average Cs<sup>134</sup> activity per 1.0 ml. Rb stock solution = 4,994. c/2.0 min.

Distribution coefficients for some of the alkali metals were determined between the phases, phenol and 20% hydrochloric acid. The concentration of each metal was the same as that used in the separations presented in Table 12. The data appear in Table 14.

Table 14. Determination of distribution coefficients for alkali metals

Aqueous phase:	20% hydrochloric acid
Organic phase:	phenol
Volume ratio:	1

Metal	$D = \frac{\text{Activity in organic phase}}{\text{Activity in aqueous phase}}$
Cs <sup>134</sup> (I)	0.229
Rb <sup>86</sup> (I)	0.113
Na <sup>24</sup> (I)	0.041

The mechanism of the column separation is probably extraction of the different alkali metal to varying degrees by the phenolic, mobile phase from the immobile cellulose phase. The agreement between the experimental retention volumes and those calculated from the equation,  $\bar{v} = V_m + DV_s$  discussed on page 8, is only fair. The difficulty in accurately determining the stationary volume ( $V_s$ ) can easily produce an error in the calculated  $\bar{v}$ .

#### Aluminum(III)-rare earth(III) separations

Aluminum(III) and the rare earths appear together in some ores and alloys. Analysis of a trace of aluminum(III) in a rare earth matrix by emission spectrographic techniques is difficult because of the complex rare earth spectra. A rapid and clean aluminum(III)-rare earth separation would therefore simplify spectrographic, and also spectrophotometric and volumetric analyses. Table 15 shows aluminum(III)-



Table 15. Column separation of aluminum-rare earths

Column: microcrystalline cellulose  
 Eluent: 80% ethanol in 2N hydrochloric acid removes aluminum  
 methanol-water (90-10) removes the rare earth  
 Flow rate: 0.5 to 2.0 ml./min.

Exp. No.	Metal	Band width (ml.)	Weight ratio <sup>a</sup>	Concentration		̄ Recovery	Column dimensions (cm.)
				Added (mmoles)	Found (mmoles)		
1	Al(III)	0-48	10.18	0.03059	0.03069	100.33	13.9x1.0
	La(III)	55-100		0.06082	0.06048	99.44	
2	Al	0-58	53.9	0.0204	0.0202	99.02	24.5x1.0
	La	73-132		0.2135	0.2103	98.5	
3	Al	0-63	53.9	0.0204	0.0207	101.47	29.6x1.0
	La	95-165		0.2125	0.2114	99.5	
4	Al	25-75	1.01x	0.001107	0.000982	89.1	29.9x1.1
	La	130-200	10 <sup>+3</sup>	0.2125	0.2110	99.3	
5	Al	25-85	1.01x	0.001107	0.00114	104.66	29.0x1.1
	La	125	10 <sup>+3</sup>	0.2125	0.2120	99.76	
6	Al	0-75	2.07x	0.001107	0.001121	101.3	28.6x1.1
	La	75-197	10 <sup>+3</sup>	0.865	not determined	-	

$$^a \text{Weight ratio} = \frac{\text{weight rare earth(III)}}{\text{weight aluminum(III)}}$$

lanthanum(III) and aluminum(III)-erbium(III) separations on cellulose columns at various weight ratios from 10 to 1000. Lanthanum(III), erbium(III) and the higher concentrations of aluminum(III) were determined with EDTA. The traces of aluminum(III) (30  $\mu$ g.) were determined spectrophotometrically with chrome azural S (64). No rare earth was found in the separated aluminum(III) fractions by emission spectroscopy.

#### Miscellaneous quantitative and qualitative separations

The movement of a number of common transition elements on cellulose columns was studied with various eluents. The data are presented in Table 16. The retention volumes were determined by spot tests. Most of the elements studied produced colored bands and if the band remained at the top of the column even after 50 ml. of effluent passed through the column, the words "no movement" (N.M.) were entered in the  $\bar{v}$  column of Table 16. The data of Table 16 were used to select eluents for the separations presented in Table 17. The qualitative separations of Table 17 were also followed by spot tests. The amount of each metal separated in Tables 16 and 17 was between 1.0 and 10. mg.

Quantitative separations of some of the transition metals on short columns of microcrystalline cellulose are presented in Table 18.

The results presented in this thesis point out that cellulose is an excellent support for column chromatography.

Table 16. Movement of metals on cellulose columns  
 [Column: microcrystalline cellulose (10.0x1.0 cm.)]

Eluent <sup>a</sup>	Retention volume $\bar{v}$ (ml.)								
	Cd(II)	Cu(II)	Cr(III)	Fe(III)	Co(II)	Ni(II)	Zn(II)	Al(III)	Hg(II)
1	7.8	9.0	NM	6.8	13.0	NM	13.0	NM	8.8
2	9.5	9.4	NM	7.5	25.5	NM	29.5	NM	10.5
3	NM	NM	NM	7.9	NM	NM	14.7	NM	9.0
4	NM	NM	NM	10.6	NM	NM	20	NM	8.95

<sup>a</sup>Eluent: 1 0.18M hydrochloric acid in acetone.  
 2 0.06M hydrochloric acid in acetone.  
 3 1.5% water in acetone.  
 4 acetone.

Table 17. Qualitative separation on cellulose column

Exp. No.	Metal	$\bar{v}$ (ml.)	Band width (ml.)	Spot tests	Column dimensions	Eluent
1	Cu(II) Co(II)	10.2 24.0	8.2-15. 17.-26.	$\alpha$ -Benzoin oxime KSCN-acid	10.0x 1.0 cm.	0.06M HCl in acetone
2	Fe(III) Cu(II)	10 42	0-20 35-50	$K_4Fe(CN)_6$ $NH_4SCN$	10x 1.0 cm.	acetone 0.03M HCl in acetone
3	Fe(III) Cu(II) Co(II) Cr(III) Ni(II)	19 87 96 130 220	-21 77-88.5 88.5-103. 110.-150. 216 -225.	diphenylcarbazide	21.5x 1.0 cm.	acetone 0.06M HCl in acetone 20% HCl in acetone 0.06M HCl in methanol
4	Na(I) K(I)	15.5 25.5	13-18 22-28	$ZnUO_2$ -acetate tetraphenylboron	18.5x 1.0	methanol
5	Li(I) Na(I)	13.5 18.3	13-17 17-20	Evaporation to salt	21.5x 1.0	methanol
6	Li(I) Na(I)	14.5 32.	13.-17. 30.-42.	Evaporation to salt	21.0x1.0	methanol-HCl. (90-10)

Table 18. Quantitative separations of transition metals  
 [Column: microcrystalline cellulose]

Exp. No.	Metal	Band width (ml.)	Concentration (mmoles)		% Recovery	Eluent	Column dimensions (cm.)
			Added	Found			
1	Cu(II)	0-45	0.1185 mm.	0.1176	99.2	Acetone+HCl	9.0
	Ni(II)	45-65	0.1924 mm	0.1934	100.51	MeOH	x1.0
2	Fe(III)	0-50	0.1771	.1751	98.85	0.08M HCl in acetone	5.5 x
	Ni(II)	50-70	0.1810	0.1807	99.8	0.8M HCl in MeOH	1.0
3	Fe(III)	0-100	.1903	0.1952	97.50	0.06M HCl in acetone	5.4 x
	Ni(II)	100-120	.1811	.1807	99.8	0.06M HCl in MeOH	1.0
4	Fe(III)	0-50	0.3113	0.3098	99.51	1M HCl in acetone	7.8 x
	Ni(II)	50-90	0.2698	0.2688	99.63	1M HCl in MeOH	1.0

It is pure white, has few impurities (49), and is inexpensive. The columns have a very low H.E.T.P. which is reflected in the excellent metal ion band shapes and separations presented. Cellulose columns do not swell or shrink with a change in eluent and have a fair capacity. In addition, a large change in metal ion concentration does not change the retention volume ( $\bar{V}$ ) drastically.

### Theory

#### Study of cellulose phase

Acetone eluents The data of Table 16, Experiment 4 and Table 17, Experiment 3 show that copper(II) has a high affinity for the cellulose phase when reagent grade acetone is used as eluent. The mechanism of this affinity has been further investigated. The data appear in Table 19. Reagent grade acetone which contains 0.33% water (Table 19, Experiment 2) produces a  $D_w$  of 16.2 for copper(II); and acetone containing 1.5% water (Table 19, Experiment 3) a  $D_w$  of 16.7. If we remove the water from the cellulose by heating and from the acetone with molecular sieves (Table 19, Experiment 1), the  $D_w$  for copper(II) drops to 0.56. Thus the system is very sensitive to water and only a very small amount of water is needed to produce a partitioning effect. The 4.3% copper(II) removed (Table 19, Experiment 1) by the cellulose from a dry acetone solution may be due to absorption. Addition of 1.5% hydrochloric acid to reagent grade acetone lowers the  $D_w$

(Table 19, Experiment 5) and allows one to elute the copper (II) from the column (Table 17, Experiment 3). The copper chloride probably is more soluble in the acetone phase than the water phase.

The gas chromatographic technique described in the experimental section shows that the water indeed enters the cellulose (Table 19, Experiments 2 and 4) and produces the necessary "aqueous second phase" needed for partitioning.

Table 19. Batch distribution coefficients of copper chloride-determinations of water in the stationary phase on cellulose

Weight of cellulose: 2.0 to 4.0 g.  
Volume of solution slurried with cellulose: 20 ml.  
Molarity of copper(II) in acetone: 0.125

Exp. No.	Solution studied	$D_w^a$	% Cu(II) remaining in solution	<u>g. water preferentially absorbed by cellulose</u> g. cellulose
1	Dry acetone	0.56	95.7	-
2	0.33% water in acetone	16.2	43.6	0.10
3	1.5% water in acetone	16.7	42.9	-
4	5.0% water in acetone		-	0.093
5	1.5% HCl in acetone	1.28	90.7	-

$$^a D_w = \frac{\text{mmoles Cu(II) in cellulose}}{\text{g. dry cellulose}} \bigg/ \frac{\text{mmoles Cu(II) in solu.}}{\text{ml. solution}}$$

Methanolic eluents Using the gas chromatography technique described earlier, a batch distribution method has been employed to determine preferential absorption or dehydration of water by the cellulose phase from various mixtures. The data are presented in Table 20. Most of the methanolic mixtures dehydrate the cellulose, which is the opposite effect seen earlier for acetone and other organic mixtures presented in the literature.

Table 20. Batch distribution gas chromatographic studies

Weight of cellulose: 3.0 to 4.0 g.  
Volume of mixture: 20 ml.

Exp. No.	Mixture studied (v/v)	g. water preferentially removed from cellulose per g. cellulose	gms. water sorbed on cellulose per g. cellulose
1	MeOH-H <sub>2</sub> O(92-8)	0.013 ≡ 1.3%	-
2	Anhydrous MeOH	0.031 ≡ 3.1%	-
3	MeOH-H <sub>2</sub> O(87.5-12.5)	0.036 ≡ 3.6%	-
4	MeOH-HCl(70-30)	-	0.020 ≡ 2.0%
5	MeOH-HCl(90-10)	0.052 ≡ 5.2%	-

The cellulose phase has been studied for the eluents which have been used to separate the alkaline earths (Table 20, Experiments 4 and 5). Table 21 also presents data on the mobile and immobile phase composition in cellulose columns for these same eluents. The data of Table 21 are summarized below:



Table 21. Study of stationary and mobile phase in cellulose columns

Exp. No.	Eluent (v/v)	Cellulose capacity <sup>a</sup>	Inter-stitial volume <sup>b</sup> (V <sub>m</sub> )	Acid concentration <sup>c</sup>	Stationary phase study			M <sub>HCl</sub>
					Weight <sup>d</sup>	Volume <sup>e</sup>	Chloride concentration <sup>f</sup>	
1	MeOH-HCl +Ca(II) (70-30)	1.13	0.431	4.33			6.31	
2	MeOH-HCl +Ca(II) (70-30)	1.042	0.345	4.29	0.896 (0.79 minus CaCl <sub>2</sub> weight)	1.14	6.15	3.62
3	MeOH-HCl +CaCl(II) (70-30)	1.09	0.399	4.75			6.92	
4	MeOH-HCl (70-30)	No Ca	0.316	5.49	0.82	1.26		4.2

<sup>a</sup> Mmoles Ca(II)/g. cellulose.

<sup>b</sup> Interstitial volume removal method: (1) air; (2) cyclohexane (ml./bed volume).

<sup>c</sup> Mmoles/g. cellulose.

<sup>d</sup> G./g. cellulose.

<sup>e</sup> ml./g. cellulose.

<sup>f</sup> Chloride concentration (mmoles/g. cellulose).

Table 21. (Continued)

Exp. No.	Eluent (v/v)	Cellulose capacity <sup>a</sup>	Inter-stitial volume <sup>b</sup> ( $V_m$ )	Acid concentration <sup>c</sup>	Stationary phase study		
					Weight <sup>d</sup>	Volume <sup>e</sup>	Chloride concentration <sup>f</sup>
5	MeOH-HCl (70-30)	No Ca	0.342	-	-		
6	MeOH-HCl (90-10)	No Ca	0.419	1.31	0.59	0.95	1.37
7	MeOH-HCl (90-10)	No Ca	.45				

- (1) The average capacity of cellulose for calcium(II) from a methanol-hydrochloric acid (70-30) mixture is 1.087 mmoles/g.
- (2) The average acid concentration of the stationary phase for a calcium(II) saturated methanol-hydrochloric acid (70-30) eluent was 4.46 mmoles/g. cellulose. With a methanol-hydrochloric acid (70-30) eluent with no calcium(II) the value increases to 5.49. This suggests that perhaps the calcium(II) (1.09 mmoles/g. cellulose) replaces some of the  $H^+$  ion (1.03 mmoles/g. cellulose) in the cellulose-water-acid complex.  
With a methanol-hydrochloric-acid (90-10) eluent the acid concentration of the cellulose stationary phase is 1.31 mmoles/g. cellulose.
- (3) The weight and volume of the stationary phase is greater with a methanol-hydrochloric acid (70-30) eluent than with a MeOH-HCl (90-10) eluent.
- (4) The molarity of the hydrochloric acid is slightly higher in the stationary phase than the mobile phase for both eluents studied.
- (5) The cellulose phase contains more water (2%) than the mobile phase for a methanol-hydrochloric acid (70-30) mixture; but less (5.2%) than a methanol-hydrochloric acid (90-10) mixture.
- (6) The calcium(II) in the stationary phase is associated

with two chloride ions.

The data indicate that the mechanism of separation of the alkaline earths on cellulose columns is probably not one of pure partitioning between two different liquid phases. The mobile and stationary phases in cellulose columns are not very different in composition, as shown; and thus the cellulose probably alters the stationary phase in order to produce a medium sufficiently different from the mobile phase so that the desired partitioning can be effected. The cellulose probably "complexes" the eluent to produce a cellulose-water-acid-methanol complex which can compete with the mobile phase for the solute.

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