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The Separation of Carboxylic Acids by Fractional Distribution

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I am submitting herewith a thesis written by Clyde Orr Jr. entitled "The Separation of Carboxylic Acids by Fractional Distribution." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Chemical Engineering.

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We have read this thesis and recommend its acceptance:

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Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

August 2, 1948

To the Committee on Graduate Study:

I am submitting to you a thesis written by Clyde Orr, Jr. entitled "The Separation of Carboxylic Acids by Fractional Distribution." I recommend that it be accepted for fifteen quarter hours of credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Chemical Engineering.

Major Professor

We have read this thesis
and recommend its acceptance:

Alvin H. Nielsen

H. J. Garber

R. M. Boarts

Accepted for the Committee

E. G. Waters
Dean of the Graduate School

THE SEPARATION OF CARBOXYLIC ACIDS BY FRACTIONAL DISTRIBUTION

A THESIS

Submitted to
The Committee on Graduate Study
of
The University of Tennessee
in
Partial Fulfillment of the Requirements
for the degree of
Master of Science

by

Clyde Orr, Jr.

August 1948

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TABLE OF CONTENTS

PAGE

LIST OF TABLES

LIST OF FIGURES

FIGURE PAGE

THE SEPARATION OF CARBOXYLIC ACIDS BY FRACTIONAL DISTRIBUTION

SUMMARY

The quantitative separation of substances by fractionally distributing them between two liquid phases has only recently become of interest; previously the method has been used for the identification of substances. This investigation was undertaken to determine if fractional distribution by a column of adsorbent material on which one of the liquid phases is immobilized and through which the other liquid phase perfuses is a method suitable for large scale development.

Partial separation of the similar substances succinic acid and fumaric acid was obtained without using what was considered an excessive quantity of solvent or requiring an inordinate length of time; however, the separation was shown to be improved by increasing the time for flow through the column. Much more complete separation was obtained by using columns in series.

Silica in either of two forms was found to be superior to all other of the seven adsorbents tested.

Agreement between theory and experiment was found adequate to indicate that column action may be calculated from the physical properties of the column.

The methods of column separation are believed applicable in the field of fine chemicals.

THE SEPARATION OF CARBOXYLIC ACIDS BY FRACTIONAL DISTRIBUTION

INTRODUCTION

Any process for the separation of mixtures by means of the relative distribution of the constituents between two liquid phases has become known as "countercurrent extraction." A more descriptive term for the process employed in this work and one in keeping with other engineering nomenclature, e.g., "fractional crystallization" and "fractional distillation," is "fractional distribution." Fractional distribution is defined in this work as a discontinuous process for the separation of very similar solutes by means of their relative distribution between two liquid phases. It offers an effective means for the separation of biological materials which are otherwise difficult to separate, is not limited to volatile substances as is the analogous fractional distillation, and may be applied to almost any substance.

Review of Previous Apparatus

The application of the countercurrent principle to the distribution of substances between two liquid phases would appear to necessitate a multichamber apparatus similar to a plate column or a diffusion battery through which one or both liquids could be circulated. An apparatus of Martin and Synge (1), employed in studying the distribution of acetamino acids between chloroform and water, consisted of forty stirring and settling tubes. Perforated disk type stirrers were driven with such a motion that, in addition to stirring, the countercurrent circulation of both liquids was achieved. The acetamino acid under investigation was usually fed in a tube near the middle of the apparatus. The apparatus was bulky, cumbersome, and expensive; therefore the investigators turned to another line of development described in the subsequent discussion.

A column with provisions for stirring and settling was designed and studied by Cornish, Archibald, Murphy, and Evans (2). The column consisted of a series of cylinder blocks and spacers mounted in a thin-walled tube with a central stirring shaft. A thermostatically controlled liquid was circulated in a jacket surrounding the column. Two immiscible solvents were circulated countercurrently through the column. A mixture of vitamins to be fractionated was injected in the middle of the column. By proper adjustment of the solvent flow rates the desired purified vitamin was recovered from the central section of the column.

An assembly designed by Craig (3) has proved useful as an analytical tool when it is desirable to make intimate examination of the fractionation at any stage of the analysis. The apparatus is best described by likening it to two turning chambered breeches of a revolver mounted on the same axis with their bases in contact and the other ends of the chambers sealed. Each cylinder in the Craig apparatus contained twenty chambers. When the chambers were filled with the proper quantities of two solvents, turning one cylinder one-twentieth of a revolution served to separate the liquid phases at the interface and bring the solvents into countercurrent contact. The phases were mixed by inverting the entire apparatus.

Technical difficulties preclude designing a compact apparatus for the quantitative separation of substances along the lines suggested above. Martin and Synge (4) have reduced the problem of fractional distribution to a simple process by immobilizing one liquid in the interstices of a column of an inert adsorbent through which the second solvent may be perfused. The adsorbent acts merely as a mechanical support for one phase, and any separation attained depends on the distribution between two liquid phases and not on differences in adsorption between liquid and solid phases.

Published information to the writer's knowledge has dealt exclusively with the application of the percolation column technique to the identification of substances, and not to their quantitative separation. However, certain industries, notably, the pharmaceutical, are known to be investigating the process of column percolation as a means of large scale separation and purification. The purpose of this investigation is to make available data on the separation of the components of representative systems by one method suitable for practical development. It will be shown that fractional distribution by a column offers such possibilities.

THEORY OF FRACTIONAL DISTRIBUTION

The Distribution Column

The behavior of a packed distillation column is described in terms of theoretical plates on each of which perfect equilibrium between the two phases exists. The height of such a theoretical plate is known as the height equivalent to one theoretical plate or the H.E.T.P. The following theory of a packed distribution column, as developed by Martin and Synge (4), is based on a H.E.T.P. defined as the height of a section of the column such that the solution issuing from it is in equilibrium with the mean concentration of solute in the stationary phase throughout that section of the column.

For a column of many plates, assuming that diffusion between plates does not exist and that at equilibrium the distribution ratio of each solute between the two phases is independent both of its concentration and the presence of the other solutes, let,

h = the H.E.T.P.,

A_s= the cross sectional area of the stationary phase,

A_m= the cross sectional area of the mobile phase,

A_i= the cross sectional area of the inert solid,

A_c= the cross sectional area of the column ($A_c = A_s + A_m + A_i$),

v = the volume of solvent used in eluting the column,

α = the distribution ratio, i.e., at equilibrium,

$$\frac{\text{grams of solute per cc. of stationary phase}}{\text{grams of solute per cc. of mobile phase}},$$

V = volume of mobile phase on each plate, or $h(A_m + \alpha A_s)$,

$$R = \frac{\text{movement of position of maximal concentration of solute}}{\text{movement of eluting solution in empty part of column}},$$

r = serial number of plate measured from bottom of column upward,

Q_r= total quantity of solute in plate r.

If unit mass of a single solute is put into the first plate and is then followed by pure solvent, after the first infinitesimal volume of mobile phase, dv, has passed, the quantity of solute in the first plate will be 1 - dv/V, and the quantity in the second plate will be dv/V. Expressing this by the equation $[(1 - dv/V) + (dv/V)]^n$ and expanding by the binomial theorum, the quantity on any plate after n successive volumes of solvent, dv, have passed is

$$Q_r = \frac{n(n-1)(n-2) \ldots (n-r+2)(1-dv/V)^{n-r+1}(dv/V)^{r-1}}{(r-1)!} .$$

Rearranging, one obtains

$$Q_{r+1} = \frac{n(n-1)(n-2) \ldots (n-r+1)(1-dv/V)^{n-r}(dv/V)^{r}}{r!} ,$$

$$Q_{r+1} = \frac{n(n-1)(n-2) \ldots (n-r+1)(1-dv/V)^{n-r}(ndv/V)^{r}}{r!\, n^r} .$$

But if n is large in comparison to r,

$$Q_{r+1} = \frac{1}{r!} (1-dv/V)^n (ndv/V)^r ,$$

and by a binomial expansion

$$(1-dv/V)^n = 1 - n(dv/V) + \frac{n(n-1)(dv/V)^2}{2!} - \frac{n(n-1)(n-2)(dv/V)^3}{3!} + \ldots$$

$$(1-dv/V)^n = 1 - n(dv/V) + \frac{n^2(dv/V)^2}{2!} - \frac{n^3(dv/V)^3}{3!} + \ldots .$$

By an expansion into a Maclaurin series

$$e^{-ndv/V} = 1 - n(dv/V) + \frac{n^2(dv/V)^2}{2!} - \frac{n^3(dv/V)^3}{3!} + \ldots .$$

By substitution

$$Q_{r+1} = \frac{1}{r!} (ndv/V)^r (e^{-ndv/V}).$$

But ndv is the total volume of solvent put through the column, or ndv = v, and

$$Q_{r+1} = \frac{1}{r!}\,(v/V)^r\, e^{-v/V}.$$

For moderate values of r little error will be introduced by using Stirling's approximation, or

$$r! = \sqrt{2\pi}\, r^{r+\frac{1}{2}}\, e^{-r}.$$

Therefore one obtains the equation

$$Q_{r+1} = \frac{1}{\sqrt{2\pi r}}\,(v/rV)^r\, e^{r-v/V}.$$

When $v/rV = 1$, Q_{r+1} is a maximum and equals $\frac{1}{\sqrt{2\pi r}}$ so that the position of maximal concentration has moved a distance, v/rV, directly proportional to the volume of solvent used in eluting the column, and, since $v/rV = hv/V$,

$$R = \frac{hv/V}{v/A_c},$$

$$R = \frac{hA_s}{V},$$

$$R = \frac{A_c}{A_m + \alpha A_s},$$

$$R = \frac{A_s + A_m + A_i}{A_m + \alpha A_s},$$

$$\alpha = \frac{A_c}{RA_s} - \frac{A_m}{A_s}. \qquad \text{(Equation 1)}$$

<u>Finite Number of Equilibrium Chambers</u>

By examination of calculated tables for the distribution of solutes between equal quantities of two solvents in his apparatus, Craig found (3) that the migration of the maximal concentration was given by the equation

$$N = n\left(\frac{\alpha}{\alpha+1}\right), \text{ or } \quad \alpha = \frac{N}{n-N}, \qquad \text{(Equation 2)}$$

where α = the distribution ratio,

N = number of the chamber of maximal concentration,

n = total number of chambers involved.

The agreement between values of α found by equation 1, equation 2, and by direct experiment is shown in Table I on page 24.

EXPERIMENTAL WORK

For this investigation succinic acid, fumaric acid, and β-naphthoic acid were chosen as solutes, and water and n-butyl alcohol were chosen as solvents. Succinic acid is about equally soluble in both n-butyl alcohol and water, fumaric acid is somewhat more soluble in n-butyl alcohol than in water, and β-naphthoic acid is much more soluble in n-butyl alcohol than in water.[1] Water and n-butyl alcohol are relatively immiscible.

A picture and a schematic diagram of the column apparatus are given by Figures 1 and 2 respectively.

The quantity of acid in all fractions was determined by titration with standard base.

Eight-Chamber Countercurrent Distribution

The distribution of each acid by equilibrium chambers applied countercurrently was first determined. Eight flasks each containing 10 cc. of n-butyl alcohol saturated with water, and eight flasks each containing 10 cc. of water saturated with n-butyl alcohol were prepared. The water phase in

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[1] A table of relative solubilities is given in the appendix, page 51.

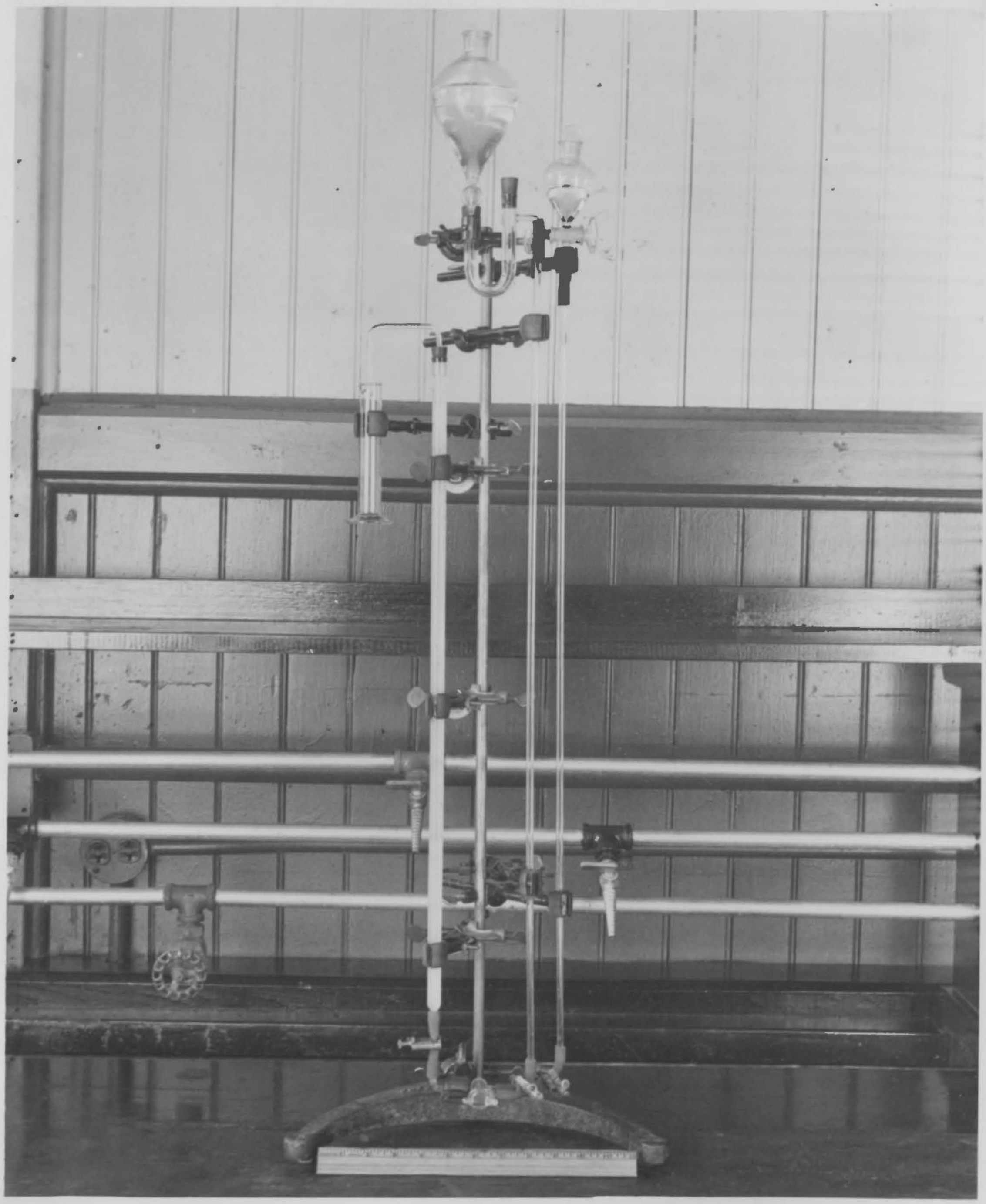

FIGURE I

THE COLUMN DISTRIBUTION APPARATUS

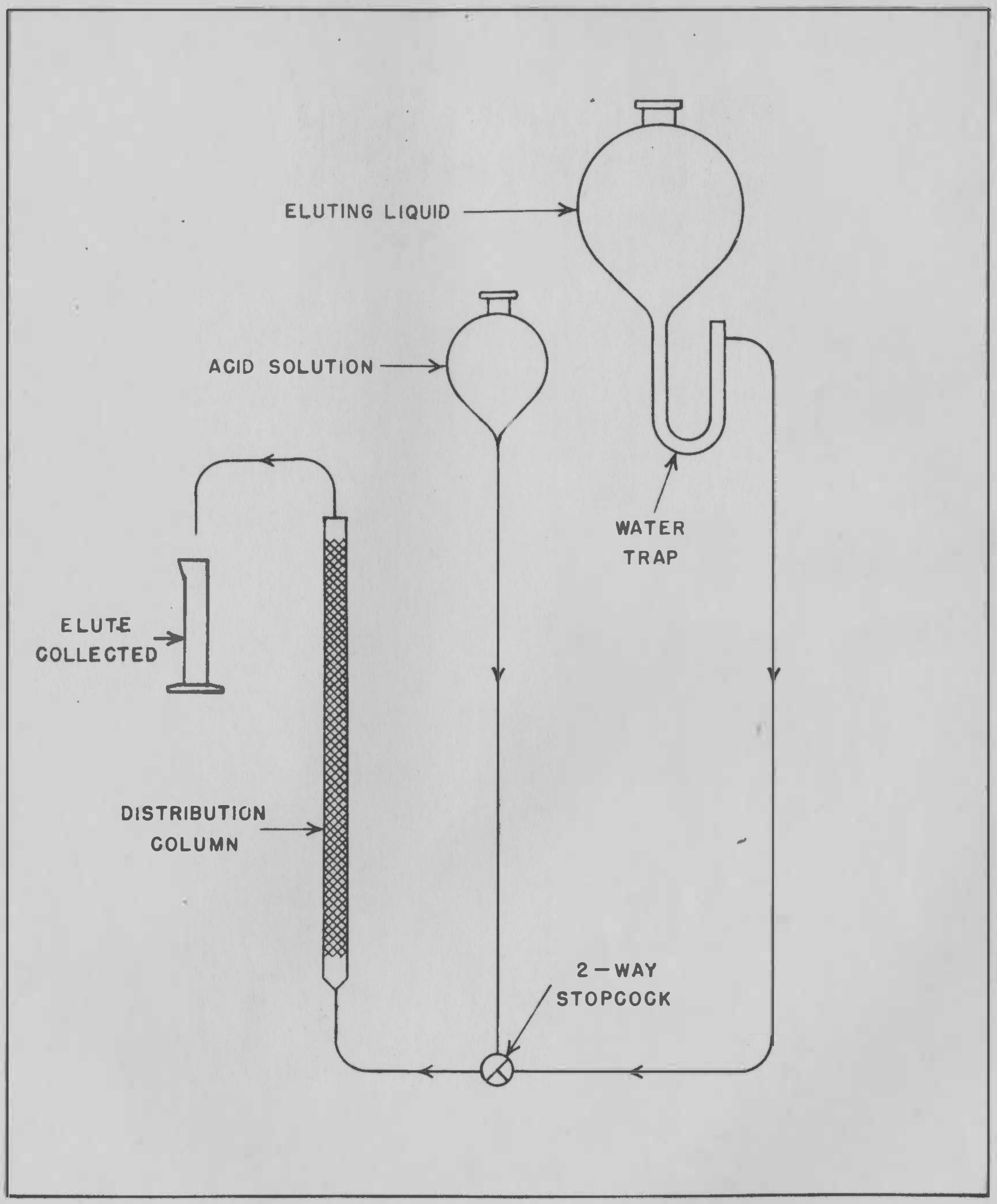

FIGURE 2

SCHEMATIC DIAGRAM OF COLUMN APPARATUS

one flask was combined with the alcohol phase in one flask, and a quantity of acid was distributed between the two phases by 5 minutes of vigorous shaking followed by a period of calm to allow the phases to separate. The phases were then discretely separated; the alcohol phase was combined with another 10 cc. of water phase, and the first water phase was combined with another 10 cc. of alcohol phase. This was continued for eight steps with the fresh phase always being added in the first flasks. The results are given by Figures 3, 4, and 5. Examination of the figures reveals that β-naphthoic and succinic acids are readily separable, and that partial separation of fumaric and succinic acids can be achieved provided each acid does not interfere with the distribution of the other.[2]

Column Distribution

The most important factor in column distribution aside from the solvents and solutes is the adsorbent. It was arbitrarily decided that a practical adsorbent should be about 10-mesh material, or equivalent, so as not to offer excessive resistance to flow and require undue time for a separation. Previous identification columns used 200- to 300-mesh (Tyler) adsorbent, required days for substances to distribute downward through the column, and required a packing to contain the adsorbent; by using a loosely packed, coarser adsorbent and upward flow a packing was not needed, and the time for a substance to move through the column was reduced to a few hours.

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[2] Evidence that such interference is negligible in this case is given in the appendix, page 30.

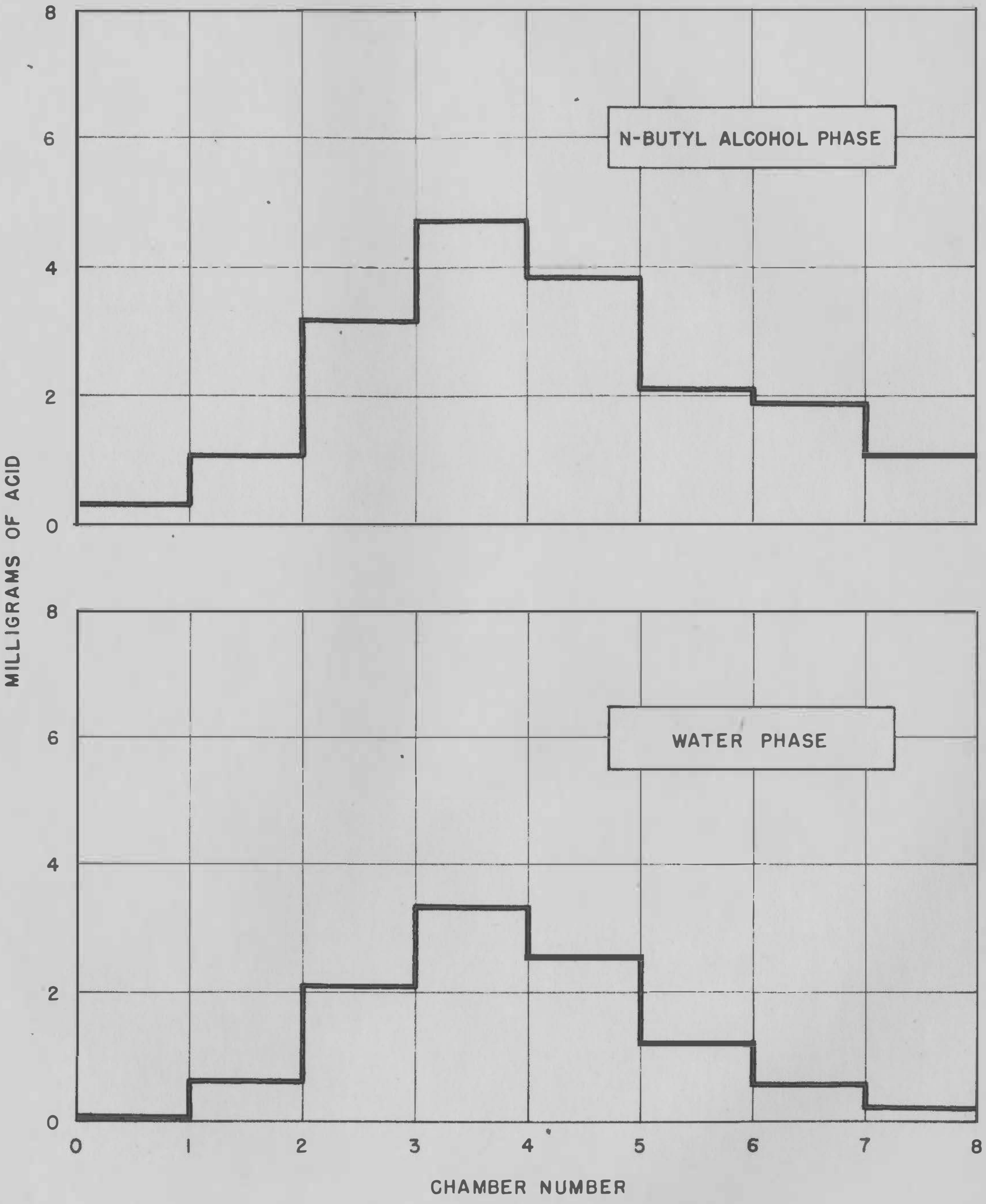

FIGURE 3

EIGHT-CHAMBER COUNTERCURRENT DISTRIBUTION

OF SUCCINIC ACID

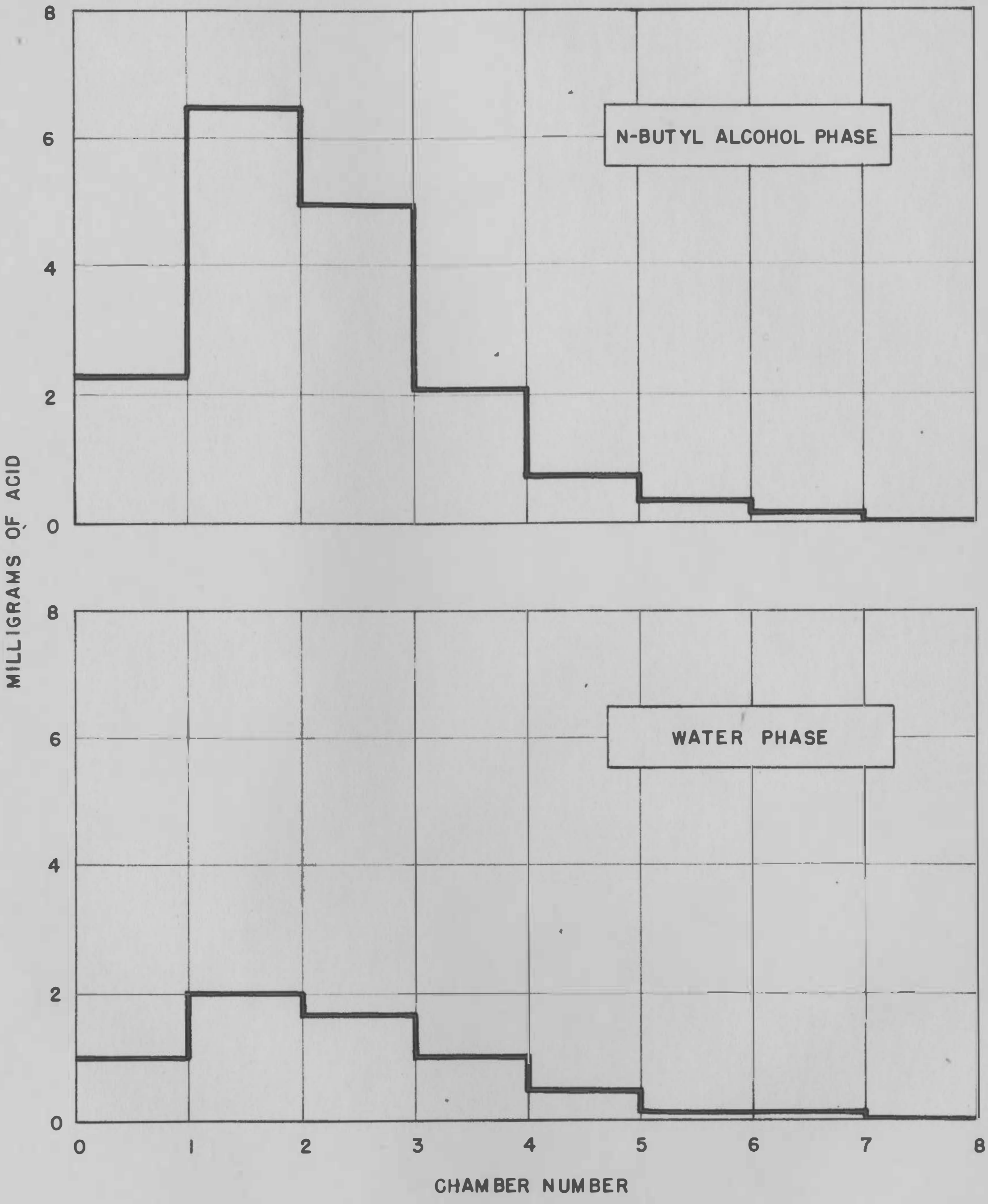

FIGURE 4

EIGHT-CHAMBER COUNTERCURRENT DISTRIBUTION

OF FUMARIC ACID

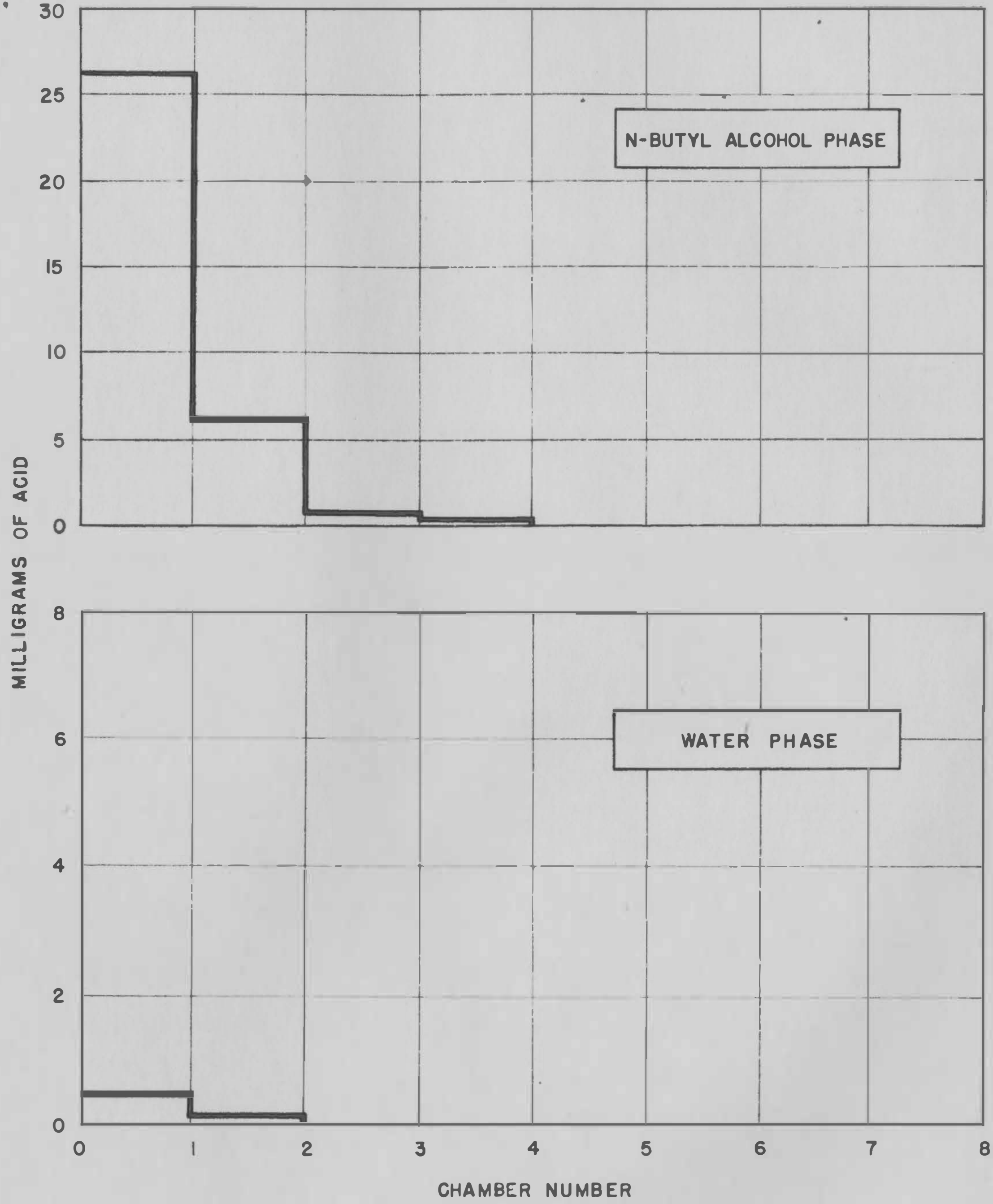

FIGURE 5

EIGHT-CHAMBER COUNTERCURRENT DISTRIBUTION

OF β-NAPHTHOIC ACID

Seven adsorbents were tested; two of them, cellulose acetate and cotton duck, were entirely unsuitable because each exhibited little preferential adsorptivity for either solvent. To secure the advantages of a finely divided adsorbent while retaining good fluid flow characteristics, a column was made by filling a 100 cc. burette with minus 9- to plus 20-mesh (Tyler) ground glass and then slowly circulating a water suspension of a siliceous material[3] through the column until deposits of the suspended material had built up on horizontal portions of the ground glass. An adsorbent material was made by mixing more of the siliceous material with just enough sodium silicate to form a coherent mass, baking at 250°F for 12 hours, crushing, and retaining the minus 9- to plus 20-mesh (Tyler) material. Minus 9- to plus 14-mesh (Tyler) plaster of Paris, $CaSO_4 \cdot \frac{1}{2}H_2O$, minus 6-mesh (Tyler) catalytic beads,[4] and minus 9- to plus 14-mesh (Tyler) silica gel[5] were also investigated as adsorbents.

Comparative curves[6] obtained when each adsorbent was used in a column are given in Figures 6 and 7. The procedure employed was as follows: the adsorbent was saturated with water which was saturated with n-butyl alcohol before the column was packed, and after packing, n-butyl

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[3] "Filter-Cel" manufactured by the Johns-Manville Corporation, New York, N. Y.

[4] Cracking grade, 41 activity index, manufactured by the Socony-Vacuum Oil Company, Inc., Paulsboro, N. J.

[5] Manufactured by The Davidson Chemical Corporation, Baltimore, Md.

[6] As shown in the appendix, the effect of the presence of one acid on the distribution of the other acid by a column is entirely negligible for the concentrations used, so superimposing the separate distributions as is done in these figures and others is justifiable.

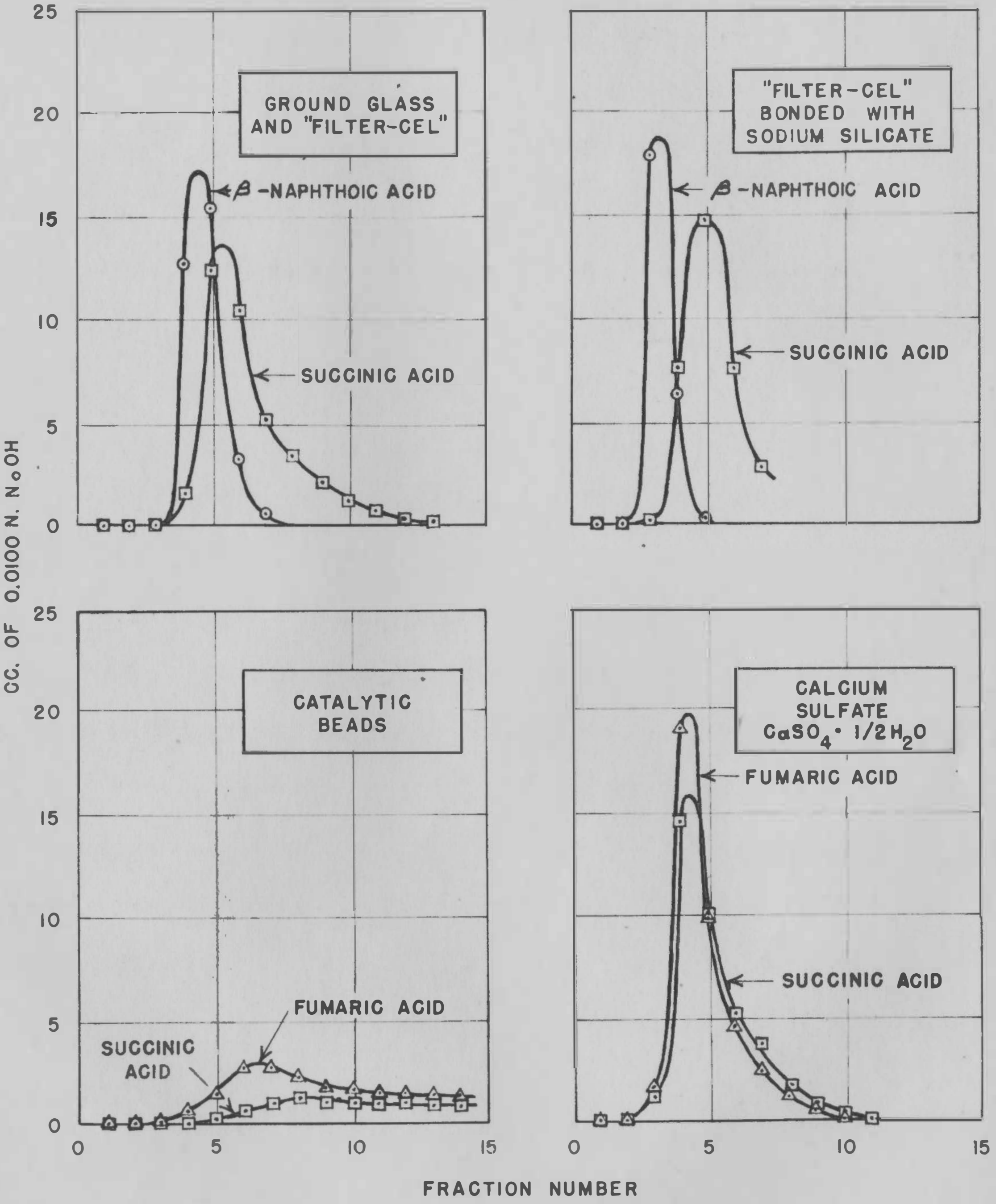

FIGURE 6

RELATIVE DISTRIBUTION GIVEN BY FOUR ADSORBENTS

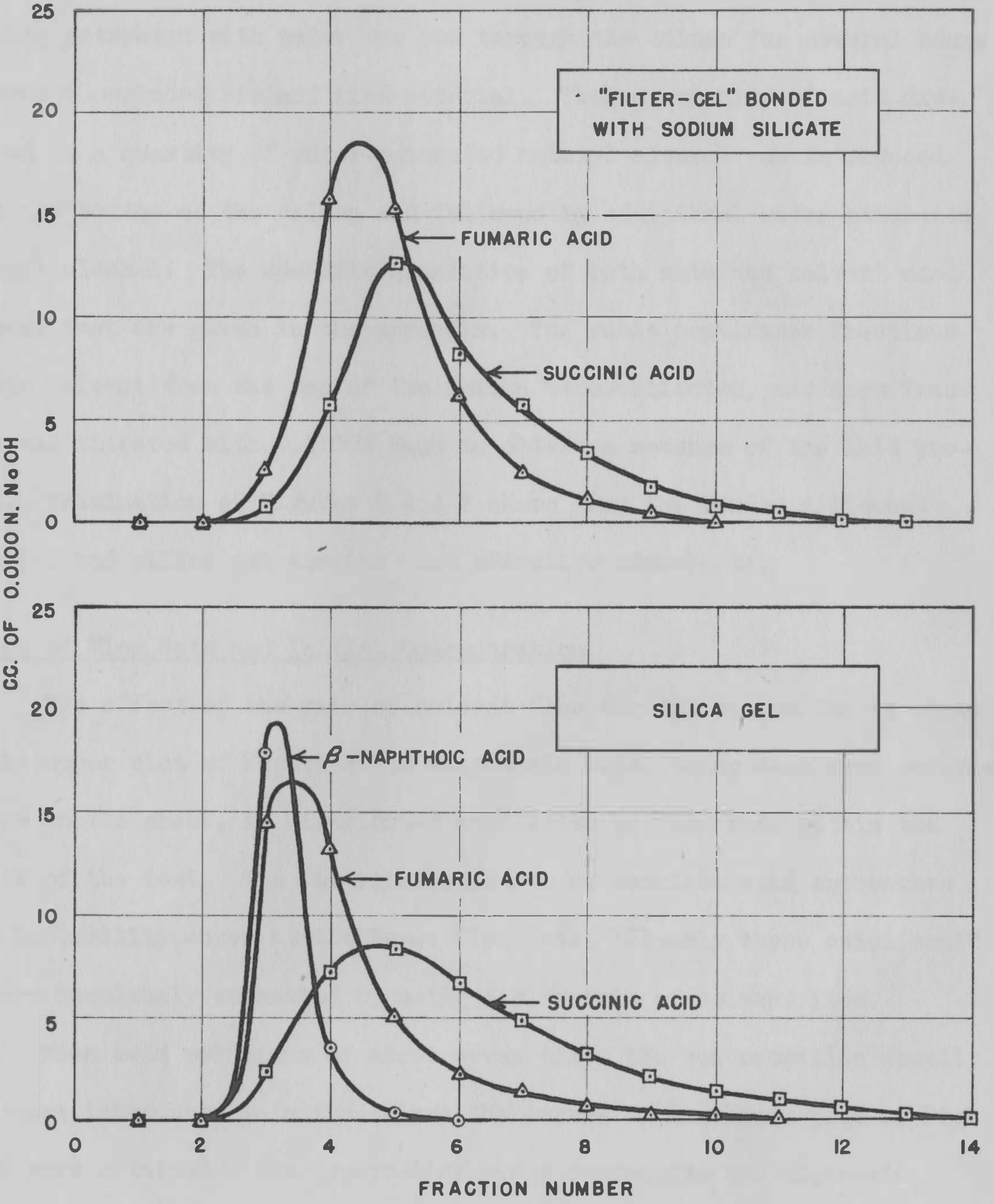

FIGURE 7

RELATIVE DISTRIBUTION GIVEN BY TWO ADSORBENTS alcohol saturated with water was run through the column for several hours to remove occluded air and fine material. Then a quantity of acid dissolved in a quantity of water-saturated n-butyl alcohol was introduced into the bottom of the column and followed by additional water-saturated n-butyl alcohol. The specific quantities of both acid and solvent used in each test are given in the appendix. Ten cubic centimeter fractions of the solvent from the top of the column were collected, and each fraction was titrated with 0.0100N NaOH to obtain a measure of the acid present. Examination of Figures 6 and 7 shows that the bonded siliceous material and silica gel are the most effective adsorbents.

Effect of Flow Rate and Initial Concentration

The effect of the rate of solvent flow through one column is shown by the upper plot of Figure 8. β-Naphthoic acid, being much more soluble in the mobile phase, is distributed unaffected by flow rate within the limits of the test. The distribution curve of succinic acid approaches a true probability curve at the lower flow rate. Clearly these acids could be more completely separated by using a slow rate of solvent flow.

When acid solutions of about seven times the concentration usually used were introduced into the column, the curves of the lower plot of Figure 8 were obtained. The broken-line curve represents the algebraic addition of the curves for each acid distributed individually, while the heavy-line curve was obtained by distributing the same quantity of each acid concurrently. The lack of congruity for the more concentrated region indicates mutual interference of the acids either directly or

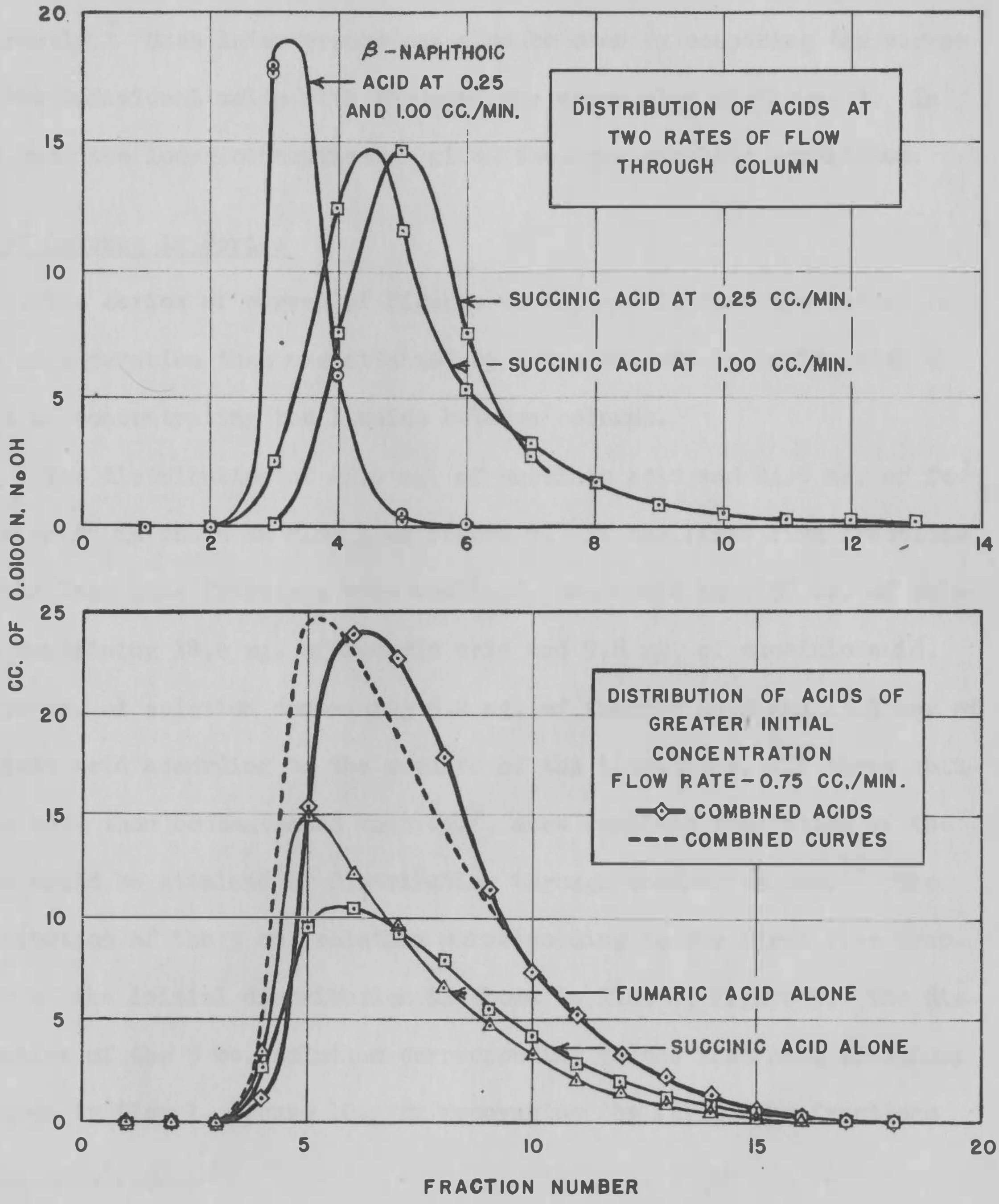

FIGURE 8

EFFECT OF TWO VARIABLES ON THE DISTRIBUTION

OF ACIDS BY A BONDED "FILTER-CEL" COLUMN indirectly.[7] Such interference can also be seen by comparing the curves for the individual acids with those of the upper plot of Figure 7. In this case the lower concentration gives the more complete separation.

Use of Columns in Series

The series of curves of Figures 9, 10, and 11 show the actual degree of separation that was attained by using columns in series with a means of concentrating the liquids between columns.

The distribution of 25.0 mg. of succinic acid and 24.6 mg. of fumaric acid[8] is shown in Plot 1 of Figure 9. If the first five fractions and the last nine fractions were combined, one would have 50 cc. of solution containing 18.6 mg. of fumaric acid and 9.8 mg. of succinic acid, and 90 cc. of solution containing 5.2 mg. of fumaric acid and 15.3 mg. of succinic acid according to the results of the titrations. If these solutions were then concentrated to 5 cc.[9], more complete separation of the acids would be attained by distribution through another column.[10] The distribution of the 5 cc. solution corresponding to the first five fractions of the initial distribution is shown in Plot 2, Figure 9. The distribution of the 5 cc. solution corresponding to the last nine fractions is shown by Plot 1, Figure 10. By recovering the first four fractions

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[7] See the suggestion on page 25.

[8] This is 0.000212 gram mol of each acid.

[9] Instead of evaporating these solutions to 5 cc., identical solutions of 5 cc. were prepared.

[10] The same column was again used.

from the distribution of the fumaric acid-rich solution, one would have 41 per cent of the fumaric acid 89 per cent pure; or by recovering the first five fractions of the same distribution, one would have 58 per cent of the fumaric acid 79 per cent pure. By recovering the last nine fractions of the distribution of the succinic acid-rich solution, 56 per cent of the succinic acid 80 per cent pure would be obtained.

Greater purity could be obtained by combining only the first four fractions of the original equimolar distribution, concentrating to 5 cc., and redistributing through a column. The results of such a process are shown in Plot 2 of Figure 10. By this method 24 per cent of the fumaric acid 94 per cent pure could be obtained. By combining the last nine fractions of the original equimolar distribution, concentrating to 5 cc., redistributing through a column, again collecting the last nine fractions, concentrating to 5 cc., and redistributing, 28 per cent of the succinic acid would be recovered 89 per cent pure. The results of such a process are shown in Figure 11. In the continuous operation of either procedure uncollected fractions could be concentrated and recycled so that no acid need be lost.

Agreement of Results with Theory

Pertinent data for the silica gel column is given in Table XX. From this, assuming the density of silica gel to be 2.3 g./cc., A_c = 1.00 cm.^{2}, A_m = 0.39 cm.^{2}, A_1 = 0.16 cm.^{2}, and A_s = 0.61 cm.^{2}. From Equation 1 and the peaks of the curves of the lower plot of Figure 7 and Plot 1, Figure 9, the distribution ratio may be calculated by

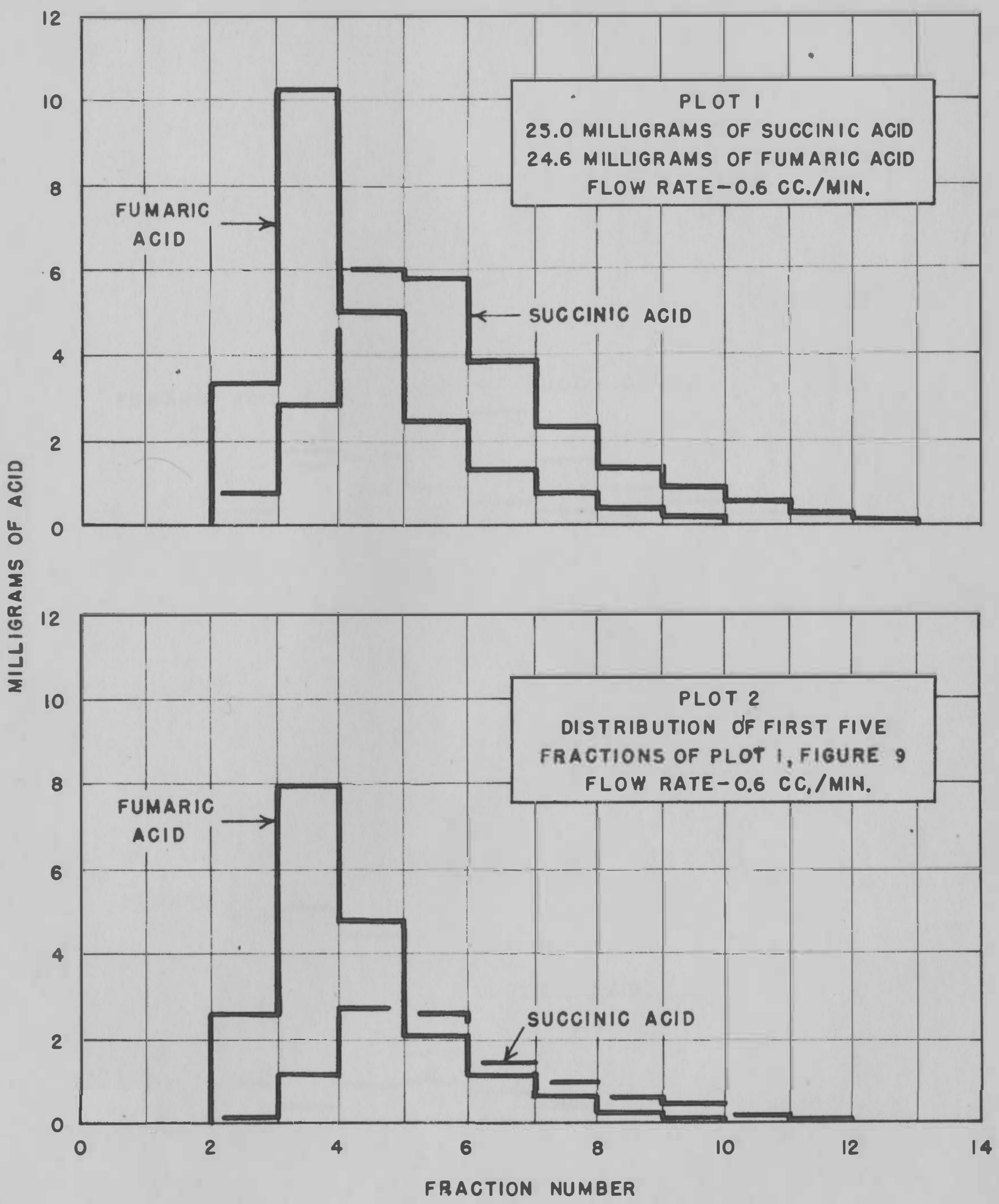

FIGURE 9

DISTRIBUTION OF SUCCINIC AND FUMARIC ACIDS

BY A SILICA GEL COLUMN

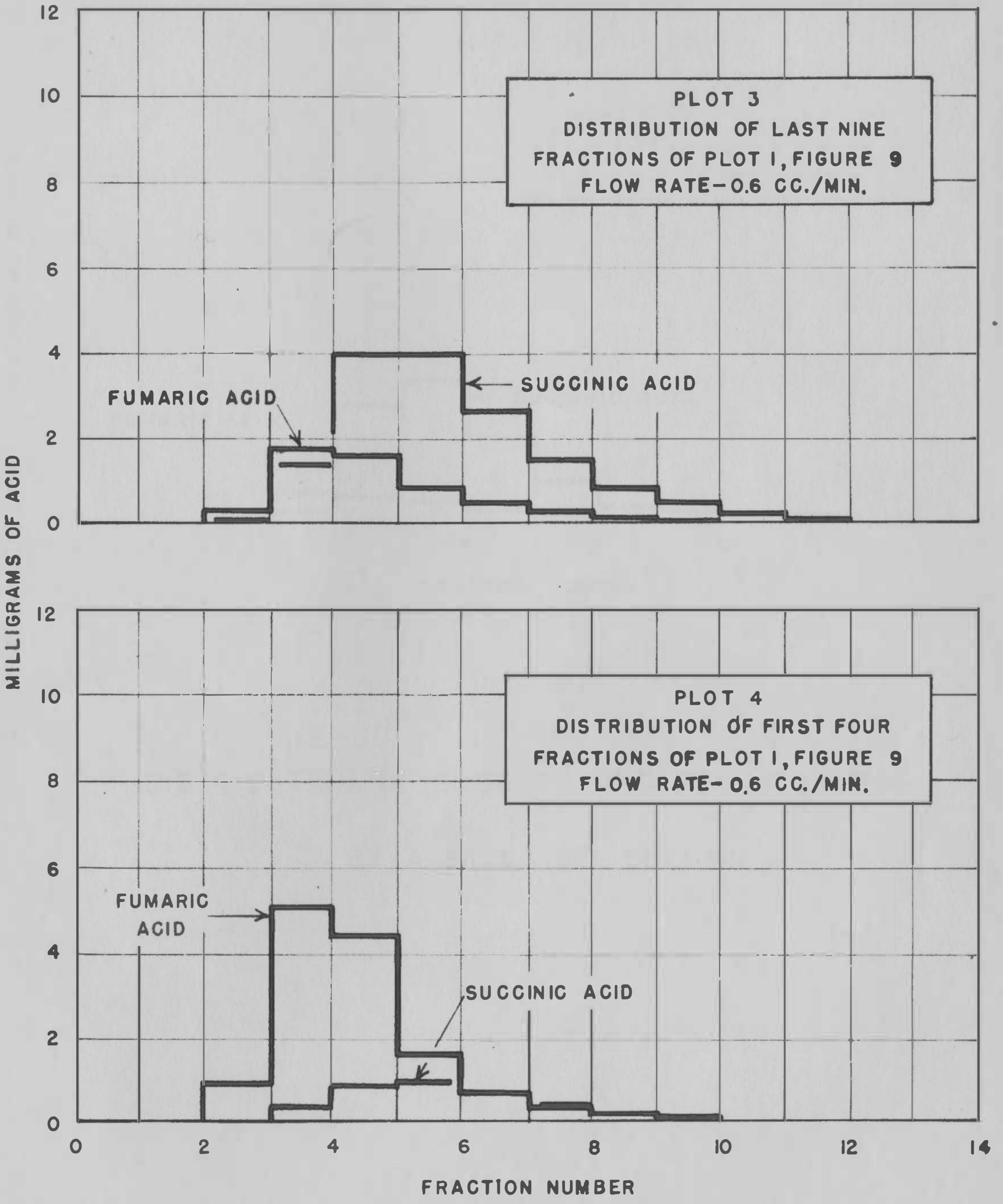

FIGURE 10

DISTRIBUTION OF SUCCINIC AND FUMARIC ACIDS

BY A SILICA GEL COLUMN

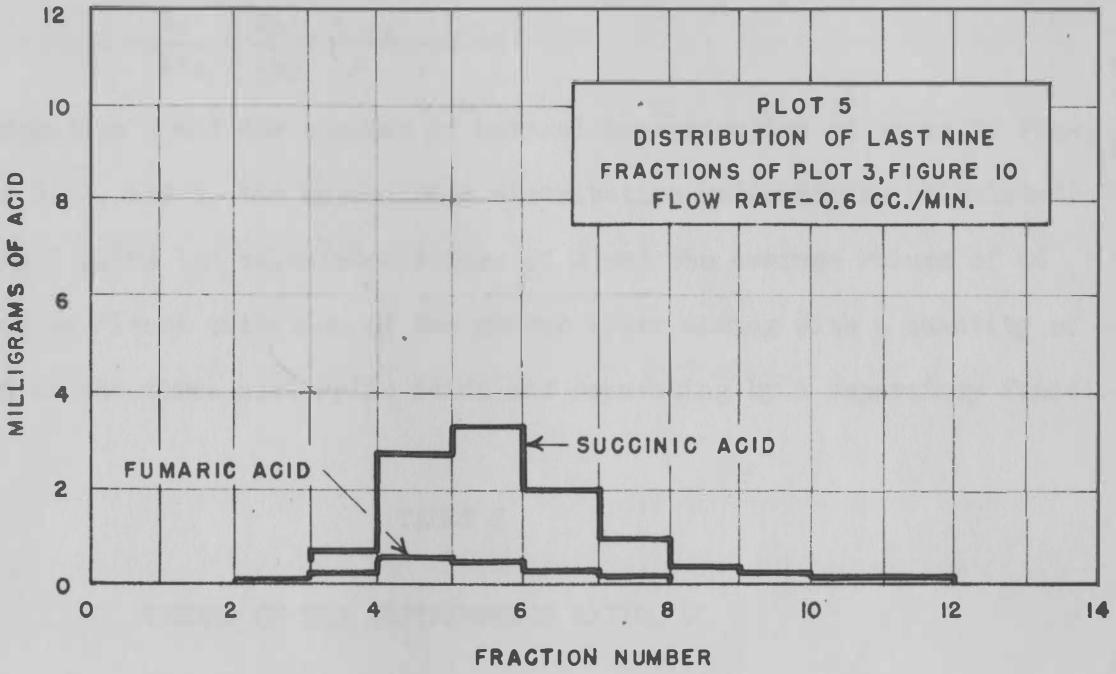

FIGURE 11

DISTRIBUTION OF SUCCINIC AND FUMARIC ACIDS

BY A SILICA GEL COLUMN

$$\alpha = \frac{A_c}{RA_s} - \frac{A_m}{A_s} = \frac{1.64}{R} - 0.64.$$

By Equation 2 and the chamber of maximal concentration as shown by Figures 3, 4, and 5, the approximate distribution ratio may be calculated. Table I gives the calculated values of α and the average values of α found by direct titration of two phases after mixing with a quantity of each of the three carboxylic acids and separating by a separatory funnel.

TABLE I

VALUES OF THE DISTRIBUTION RATIO, α

	α		
Carboxylic Acid	By Equation 1	By Equation 2	Direct Determination
succinic	0.85	1.0	0.91
fumaric	0.27	0.3	0.22
β-naphthoic	0.10	0.1	0.08

EVALUATION

This investigation has shown that (a) a column of relatively coarse adsorbent is sufficient to distribute similar substances so that partial separation of those substances may be realized in a reasonably short time using not too great quantities of solvent, (b) more complete separation is attainable by using columns in series, and (c) the effectiveness of an adsorbent column may be calculated from the physical properties of the column and the distribution ratios of the solutes.

There is no reason to expect, nor evidence to indicate, deterioation of a column through use so long as the mobile phase is kept saturated with the immobile phase. A tendency for the maximal concentration to move to the right is evident in the series of figures from Figure 9 to Figure 11. This is due to the effective lengthening of the column by an observable decrease in occluded gas with the continued use of the column.

The tables of data in the appendix give the quantity of acid indicated by the titration for comparison with the weighed quantity of acid involved when all the acid was apparently recovered. An error of 15 per cent is indicated for one test; however, since such an error occurs for a test in which only a gross comparison is desired, it is not considered significant. The discrepancy between the quantity of acid indicated by weighing and that indicated by titration is always less than 6 per cent in the series of tests concerning an actual separation. Such error is not extraordinary since it was necessary to titrate the n-butyl alcohol phase to a certain color rather than to the point of color change. No allowance was made for temperature change; all tests were run at room temperature.

Future Work

Aside from the application of the column technique to a great variety of specific separations, the general effect of column length and column diameter should be investigated. The use of buffers or other means should be studied to permit the largest amount of solute to be fractionated with the minimum volume of the two chosen phases. Charcoal, alumina, starch grains, etc. are adsorbents worthy of investigation. Much more solubility data are needed.

The use of ion-exchange materials in place of adsorbents offers wide possibilities.

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BIBLIOGRAPHY

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APPENDIX

APPENDIX

The solid curves of the upper plot of Figure 12 were obtained from the n-butyl alcohol phase distribution data for 25.0 mg. of succinic acid alone, 24.6 mg. of fumaric acid alone, and 25.0 mg. of succinic acid and 24.6 mg. of fumaric acid combined by an eight-chamber countercurrent distribution operation. The broken line represents the algebraic addition of the curves for each single acid. The substantial coincidence of the curves for the combined acids is good evidence that the distribution of one acid by an eight-chamber countercurrent operation is little affected by the presence of the other acid for the concentrations involved.

The solid lines of the lower plot of Figure 12 are a plot of the distribution by a silica gel column of 25.0 mg. of succinic acid alone, 24.6 mg. of fumaric acid alone, and of 25.0 mg. of succinic acid and 24.6 mg. of fumaric acid combined. As before, the broken line is the algebraic addition of the curves for the acids singly. The independence of distribution of each acid is again evident.

The quantities of acid were always dissolved in 5 cc. of mobile solvent for introduction into a column except where noted on the following tables.

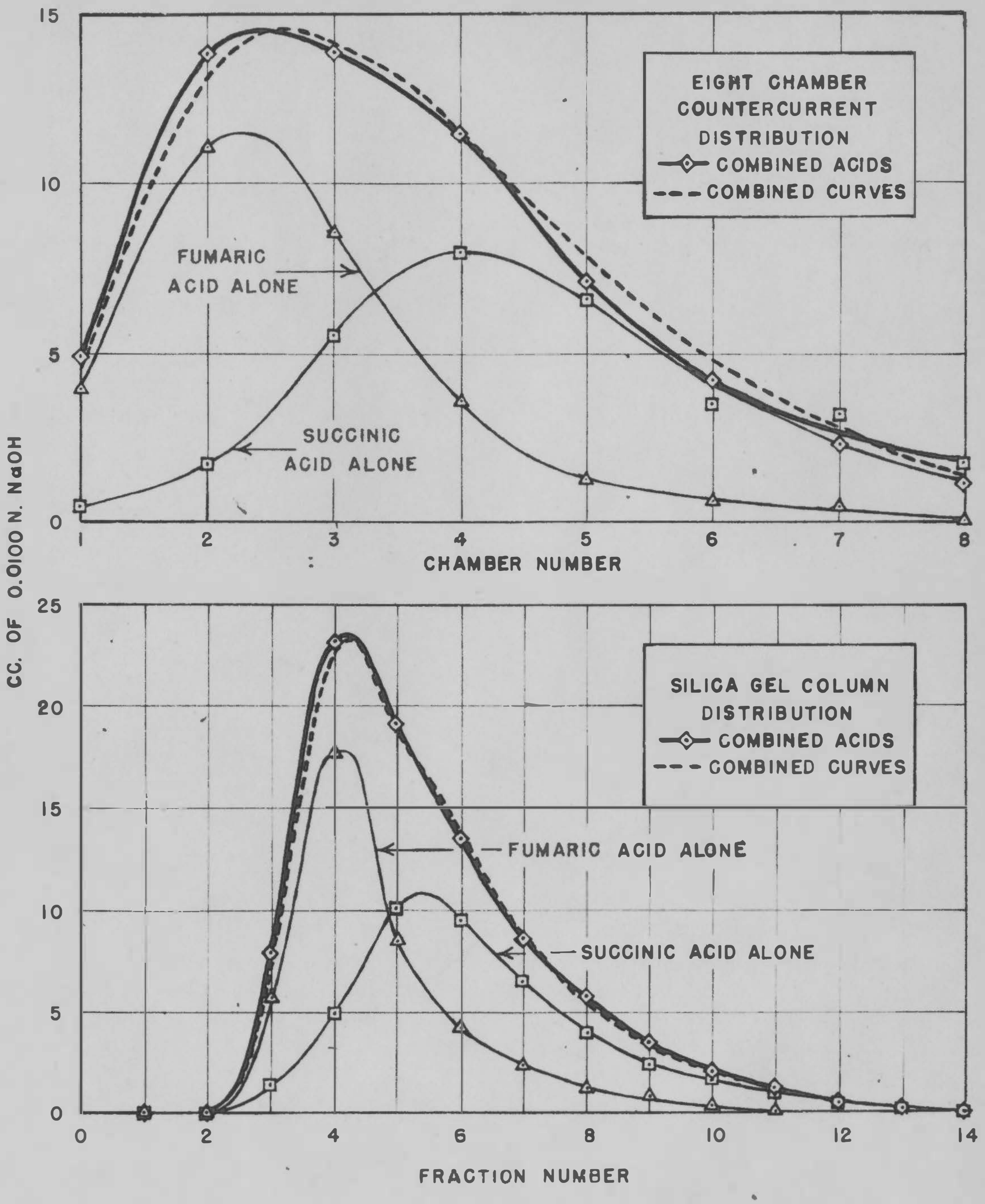

FIGURE 12

EVIDENCE THAT THE DISTRIBUTION OF ONE ACID IS LITTLE AFFECTED BY THE PRESENCE OF THE OTHER ACID FOR THE CONCENTRATIONS INVOLVED

TABLE II

DISTRIBUTION OF 25.0 MG. OF SUCCINIC ACID BY AN EIGHT-CHAMBER[a] COUNTERCURRENT OPERATION[b]

	Alcohol Phase		Water Phase	
Chamber No.	cc. of 0.0100N NaOH for Titration	Mg. of Acid	cc. of 0.0100N NaOH for Titration	Mg. of Acid
1	0.4	0.2	0.1	0.1
2	1.7	1.0	1.1	0.6
3	5.5	3.2	3.6	2.1
4	8.0	4.7	5.7	3.4
5	6.5	3.8	4.4	2.6
6	3.5	2.1	2.1	1.2
7	3.1	1.8	1.1	0.6
8	1.8	1.1	0.3	0.2

Total succinic acid indicated by titration, 28.7 mg.

---

[a] 10 cc. of n-butyl alcohol and 10 cc. of water, mutually saturated, in each.

[b] 5 minutes shaking, 3 minutes separating.

TABLE III

DISTRIBUTION OF 24.6 MG. OF FUMARIC ACID BY AN EIGHT-CHAMBER[a] COUNTERCURRENT OPERATION[b]

	Alcohol Phase		Water Phase	
Chamber No.	cc. of 0.0100N NaOH for Titration	Mg. of Acid	cc. of 0.0100N NaOH for Titration	Mg. of Acid
1	4.0	2.3	1.7	1.0
2	11.1	6.5	3.4	2.0
3	8.6	5.0	2.8	1.6
4	3.6	2.1	1.7	1.0
5	1.3	0.8	0.8	0.5
6	0.6	0.3	0.4	0.2
7	0.4	0.2	0.3	0.2
8	0.0	0.0	0.1	0.1

Total fumaric acid indicated by titration, 23.8 mg.

---

[a] 10 cc. of n-butyl alcohol and 10 cc. of water, mutually saturated, in each.

[b] 5 minutes shaking, 3 minutes separating.

TABLE IV

DISTRIBUTION OF 36.3 MG. OF β-NAPHTHOIC ACID BY AN EIGHT-CHAMBER[a] COUNTERCURRENT OPERATION[b]

	Alcohol Phase		Water Phase	
Chamber No.	cc. of 0.0100N NaOH for Titration	Mg. of Acid	cc. of 0.0100N NaOH for Titration	Mg. of Acid
1	15.3	26.4	0.3	0.5
2	3.7	6.4	0.1	0.2
3	0.4	0.7	0.0	0.0
4	0.2	0.3	0.0	0.0
5	0.0	0.0	0.0	0.0
6	0.0	0.0	0.0	0.0
7	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0

Total β -naphthoic acid indicated by titration, 34.5 mg.

---

[a] 10 cc. of n-butyl alcohol and 10 cc. of water, mutually saturated, in each.

[b] 5 minutes shaking, 3 minutes separating.

TABLE V

DISTRIBUTION OF 25.0 MG. OF SUCCINIC ACID AND 24.6 MG. OF FUMARIC ACID BY AN EIGHT-CHAMBER[a] COUNTERCURRENT OPERATION[b]

	cc. of 0.0100N NaOH for Titration	
Chamber No.	N-Butyl Alcohol Phase	Water Phase
1	5.9	1.7
2	13.8	4.3
3	14.0	6.1
4	11.4	6.9
5	7.1	5.9
6	4.2	3.4
7	2.4	1.0
8	1.3	0.0

[a] 10 cc. of n-butyl alcohol and 10 cc. of water, mutually saturated, in each.

[b] 5 minutes shaking, 3 minutes separating.

TABLE VI

DISTRIBUTION OF 25.0 MG. OF SUCCINIC ACID AND 50.0 MG. OF β-NAPHTHOIC ACID BY DEPOSITS OF "FILTER-CEL" ON GROUND GLASS IN A COLUMN[a]

Fraction[b] No.	cc. of 0.0100N NaOH for Titration	
	Succinic Acid	β-Naphthoic Acid
1	0.0	0.0
2	0.0	0.0
3	0.0	0.0
4	1.5	12.9
5	12.5	15.6
6	10.4	3.3
7	5.2	1.0
8	3.4	0.0
9	2.1	0.0
10	1.2	0.0
11	0.7	0.0
12	0.3	0.0
13	0.1	0.0
14	0.0	0.0

Rate of flow, 1.0 cc./min.

Total succinic acid indicated by titration, 22.1 mg.

Total β-naphthoic acid indicated by titration, 56.5 mg.

---

[a] Packed 100 cc. burette.

[b] 10 cc. fractions.

TABLE VII

DISTRIBUTION OF SUCCINIC ACID AND β-NAPHTHOIC ACID BY "FILTER-CEL" BONDED WITH SODIUM SILICATE[a] IN A COLUMN[b]

Fraction[c] No.	cc. of 0.0100N NaOH for Titration	
	Succinic Acid	β-Naphthoic Acid
1	0.0	0.0
2	0.0	0.0
3	0.1	18.0
4	7.6	6.4
5	14.8	0.2
6	7.5	0.0
7	2.8	0.0

Rate of flow, 0.25 cc./min.

---

[a] -9+20 mesh material.

[b] Packed 50 cc. burette.

[c] 10 cc. fractions.

TABLE VIII

DISTRIBUTION OF SUCCINIC ACID AND FUMARIC ACID BY A COLUMN[a] OF MINUS 6-MESH CATALYTIC BEADS

Fraction[b] No.	cc. of 0.0100N NaOH for Titration	
	Succinic Acid	Fumaric Acid
1	0.0	0.0
2	0.0	0.0
3	0.0	0.1
4	0.0	0.6
5	0.1	1.5
6	0.6	2.8
7	1.0	2.7
8	1.2	2.1
9	1.0	1.8
10	1.1	1.6
11	1.0	1.5
12	1.0	1.4
13	1.0	1.3
14	0.9	1.1

Rate of flow, 0.6 cc./min.

---

[a] Packed 100 cc. burette.

[b] 10 cc. fractions.

TABLE IX

DISTRIBUTION OF 25.0 MG. OF SUCCINIC ACID AND 24.6 MG. OF FUMARIC ACID BY PLASTER OF PARIS[a] IN A COLUMN[b]

Fraction[c] No.	cc. of 0.0100N NaOH for Titration Succinic Acid	Fumaric Acid
1	0.0	0.0
2	0.0	0.0
3	1.2	1.6
4	14.7	19.0
5	10.1	9.9
6	5.3	4.8
7	3.8	1.5
8	1.6	1.2
9	0.8	0.5
10	0.3	0.0
11	0.0	0.0

Rate of flow, 0.6 cc./min.

Total succinic acid indicated by titration, 22.3 mg.

Total fumaric acid indicated by titration, 22.3 mg.

---

[a] -9+14 mesh.

[b] Packed 50 cc. burette.

[c] 10 cc. fractions.

TABLE X

DISTRIBUTION OF 25.0 MG. OF SUCCINIC ACID AND 24.6 MG. OF FUMARIC ACID BY "FILTER-CEL" BONDED WITH SODIUM SILICATE[a] IN A COLUMN[b]

Fraction[c] No.	cc. of 0.0100N NaOH for Titration	
	Succinic Acid	Fumaric Acid
1	0.0	0.0
2	0.0	0.0
3	0.8	2.7
4	5.7	15.9
5	12.9	15.4
6	8.1	6.3
7	5.7	2.5
8	3.4	1.2
9	1.7	0.5
10	0.8	0.0
11	0.5	0.0
12	0.2	0.0
13	0.0	0.0

Rate of flow, 0.45 cc./min.

Total succinic acid indicated by titration, 23.5 mg.

Total fumaric acid indicated by titration, 25.8 mg.

---

[a] -9+20 mesh material.

[b] Packed 50 cc. burette.

[c] 10 cc. fractions.

TABLE XI

DISTRIBUTION OF 25.0 MG. OF SUCCINIC ACID, 24.6 MG. OF FUMARIC ACID, AND 36.3 MG. OF β-NAPHTHOIC ACID BY SILICA GEL[a] IN A COLUMN[b]

	cc. of 0.0100N NaOH for Titration		
Fraction[c] No.	Succinic Acid	Fumaric Acid	β-Naphthoic Acid
1	0.0	0.0	0.0
2	0.0	0.0	0.0
3	2.3	14.5	17.7
4	7.2	13.2	3.5
5	8.4	5.1	0.3
6	6.7	2.2	0.0
7	4.8	1.5	0.0
8	3.2	0.6	0.0
9	2.0	0.3	0.0
10	1.4	0.2	0.0
11	1.0	0.0	0.0
12	0.5	0.0	0.0
13	0.2	0.0	0.0
14	0.0	0.0	0.0

Rate of flow, 0.6 cc./min.

Total succinic acid indicated by titration, 22.3 mg.

Total fumaric acid indicated by titration, 21.8 mg.

Total β-naphthoic acid indicated by titration, 37.0 mg.

---

[a] -9+14 mesh.

[b] Packed 50 cc. burette.

[c] 10 cc. fractions.

TABLE XII

DISTRIBUTION OF SUCCINIC ACID AND β-NAPHTHOIC ACID BY "FILTER-CEL" BONDED WITH SODIUM SILICATE[a] IN A COLUMN[b]

Fraction[c] No.	cc. of 0.0100N NaOH for Titration	
	Succinic Acid	β-Naphthoic Acid
1	0.0	0.0
2	0.0	0.0
3	2.6	17.8
4	12.5	5.9
5	11.6	0.5
6	5.4	0.0
7	3.2	0.0
8	1.7	0.0
9	0.9	0.0
10	0.5	0.0
11	0.2	0.0
12	0.2	0.0
13	0.0	0.0

Rate of flow, 1.00 cc./min.

---

[a] -9+20 mesh material.

[b] Packed 50 cc. burette.

[c] 10 cc. fractions.

TABLE XIII

DISTRIBUTION OF 35.0 MG. OF SUCCINIC ACID, 35.0 MG. OF FUMARIC ACID, AND 35.0 MG. OF SUCCINIC ACID AND 35.0 MG. OF FUMARIC ACID COMBINED BY "FILTER-CEL" BONDED WITH SODIUM SILICATE[a] IN A COLUMN[b]

Fraction[c] No.	cc. of 0.0100N NaOH for Titration		
	Succinic Acid	Fumaric Acid	Combined Acids
1	0.0	0.0	0.0
2	0.0	0.0	0.0
3	0.0	0.0	0.0
4	2.9	3.2	1.1
5	9.6	15.0	15.3
6	10.5	12.0	24.0
7	9.1	9.3	22.8
8	7.8	6.6	17.9
9	5.5	4.8	11.2
10	4.1	3.4	7.0
11	2.8	2.0	5.1
12	2.0	1.4	3.1
13	1.3	0.9	2.1
14	0.9	0.4	1.3
15	0.5	0.1	0.6
16	0.3	0.0	0.4
17	0.1	0.0	0.2
18	0.0	0.0	0.0

Acids dissolved in 1 cc. of mobile solvent.

Rate of flow, 0.75 cc./min.

Total succinic acid indicated by titration, 33.9 mg.

Total fumaric acid indicated by titration, 34.3 mg.

Total combined acids indicated by titration, about 66 mg.

---

[a] -9+14 mesh material.

[b] Packed 50 cc. burette.

[c] 10 cc. fractions.

TABLE XIV

DISTRIBUTION OF 25.0 MG. OF SUCCINIC ACID AND 24.6 MG. OF FUMARIC ACID BY SILICA GEL[a] IN A COLUMN[b]

	Succinic Acid		Fumaric Acid	
Fraction[c] No.	cc. of 0.0100N NaOH for Titration	Mg. of Acid	cc. of 0.0100N NaOH for Titration	Mg. of Acid
1	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0
3	1.3	0.8	5.8	3.4
4	5.0	3.0	17.6	10.2
5	10.1	6.0	8.6	5.0
6	9.8	5.8	4.2	2.4
7	6.6	3.9	2.3	1.3
8	3.9	2.3	1.3	0.8
9	2.4	1.4	0.8	0.5
10	1.6	0.9	0.3	0.2
11	1.0	0.6	0.0	0.0
12	0.5	0.3	0.0	0.0
13	0.2	0.1	0.0	0.0
14	0.0	0.0	0.0	0.0

Rate of flow, 0.6 cc./min.

Total succinic acid indicated by titration, 25.1 mg.

Total fumaric acid indicated by titration, 23.8 mg.

---

[a] -9+14 mesh material.

[b] Packed 50 cc. burette.

[c] 10 cc. fractions.

TABLE XV

DISTRIBUTION OF 9.8 MG. OF SUCCINIC ACID AND 18.6 MG. OF FUMARIC ACID BY SILICA GEL[a] IN A COLUMN[b]

	Succinic Acid		Fumaric Acid	
Fraction[c] No.	cc. of 0.0100N NaOH for Titration	Mg. of Acid	cc. of 0.0100N NaOH for Titration	Mg. of Acid
1	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0
3	0.2	0.1	4.4	2.6
4	2.0	1.2	12.8	7.4
5	4.0	2.4	7.3	4.2
6	3.9	2.3	3.7	2.1
7	2.5	1.5	2.1	1.2
8	1.7	1.0	1.2	0.7
9	1.1	0.6	0.6	0.3
10	0.8	0.5	0.2	0.1
11	0.3	0.2	0.0	0.0
12	0.2	0.1	0.0	0.0
13	0.0	0.0	0.0	0.0

Rate of flow, 0.6 cc./min.

Total succinic acid indicated by titration, 9.9 mg.

Total fumaric acid indicated by titration, 18.6 mg.

---

[a] -9+14 mesh material.

[b] Packed 50 cc. burette.

[c] 10 cc. fractions.

TABLE XVI

DISTRIBUTION OF 15.3 MG. OF SUCCINIC ACID AND 5.2 MG. OF FUMARIC ACID BY SILICA GEL[a] IN A COLUMN[b]

	Succinic Acid		Fumaric Acid	
Fraction[c] No.	cc. of 0.0100N NaOH for Titration	Mg. of Acid	cc. of 0.0100N NaOH for Titration	Mg. of Acid
1	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0
3	0.1	0.1	0.5	0.3
4	2.4	1.4	3.0	1.7
5	6.8	4.0	2.7	1.6
6	6.8	4.0	1.5	0.9
7	4.5	2.7	0.9	0.5
8	2.5	1.5	0.5	0.3
9	1.5	0.9	0.2	0.1
10	0.8	0.5	0.1	0.1
11	0.4	0.2	0.0	0.0
12	0.2	0.1	0.0	0.0
13	0.0	0.0	0.0	0.0

Rate of flow, 0.6 cc./min.

Total succinic acid indicated by titration, 15.4 mg.

Total fumaric acid indicated by titration, 5.5 mg.

---

[a] -9+14 mesh material.

[b] Packed 50 cc. burette.

[c] 10 cc. fractions.

TABLE XVII

DISTRIBUTION OF 3.8 MG. OF SUCCINIC ACID AND 13.6 MG. OF FUMARIC ACID BY SILICA GEL[a] IN A COLUMN[b]

Fraction[c] No.	Succinic Acid		Fumaric Acid	
	cc. of 0.0100N NaOH for Titration	Mg. of Acid	cc. of 0.0100N NaOH for Titration	Mg. of Acid
1	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0
3	0.0	0.0	1.6	0.9
4	0.6	0.4	8.8	5.1
5	1.5	0.9	7.6	4.4
6	1.7	1.0	2.8	1.6
7	1.2	0.7	1.2	0.7
8	0.6	0.4	0.6	0.3
9	0.3	0.2	0.3	0.2
10	0.2	0.1	0.1	0.1
11	0.1	0.1	0.0	0.0
12	0.0	0.0	0.0	0.0

Rate of flow, 0.6 cc./min.

Total succinic acid indicated by titration, 3.8 mg.

Total fumaric acid indicated by titration, 13.3 mg.

---

[a] -9+14 mesh material.

[b] Packed 50 cc. burette.

[c] 10 cc. fractions.

TABLE XVIII

DISTRIBUTION OF 9.9 MG. OF SUCCINIC ACID AND 1.9 MG. OF FUMARIC ACID BY SILICA GEL[a] IN A COLUMN[b]

	Succinic Acid		Fumaric Acid	
Fraction[c] No.	cc. of 0.0100N NaOH for Titration	Mg. of Acid	cc. of 0.0100N NaOH for Titration	Mg. of Acid
1	0.0	0.0	0.0	0.0
2	0.0	0.0	0.0	0.0
3	0.0	0.0	0.2	0.1
4	0.6	0.4	0.4	0.2
5	4.6	2.7	1.0	0.6
6	5.6	3.3	0.8	0.5
7	3.4	2.0	0.5	0.3
8	1.6	0.9	0.2	0.1
9	0.6	0.3	0.0	0.0
10	0.3	0.2	0.0	0.0
11	0.2	0.1	0.0	0.0
12	0.1	0.1	0.0	0.0
13	0.0	0.0	0.0	0.0

Rate of flow, 0.6 cc./min.

Total succinic acid indicated by titration, 10.0 mg.

Total fumaric acid indicated by titration, 1.8 mg.

---

[a] -9+14 mesh material.

[b] Packed 50 cc. burette.

[c] 10 cc. fractions.

TABLE XIX

DISTRIBUTION OF 25.0 MG. OF SUCCINIC ACID AND 24.6 MG. OF FUMARIC ACID COMBINED BY SILICA GEL[a] IN A COLUMN[b]

Fraction[c] No.	cc. of 0.0100N NaOH for Titration
1	0.0
2	0.0
3	7.9
4	23.2
5	19.1
6	13.6
7	8.7
8	5.7
9	3.5
10	2.0
11	1.2
12	0.5
13	0.2
14	0.0

Rate of flow, 0.6 cc./min.

---

[a] -9+14 mesh material.

[b] Packed 50 cc. burette.

[c] 10 cc. fractions.

TABLE XX

PHYSICAL PROPERTIES OF THE SILICA GEL COLUMN

Internal dimension of packed section of column

Length - 62 cm.
Diameter - 1.128 cm.

Weight of components in column

Dry silica gel - 22.56 g.
N-butyl alcohol phase - 19.4 g.
Water phase - 37.76 g.

TABLE XXI

DIRECTLY DETERMINED DISTRIBUTION RATIOS

	cc. of 0.0100N NaOH for Titration		Conc. in Water
Acid	N-Butyl Alcohol Phase	Water Phase	Conc. in N-Butyl Alcohol
succinic	9.4	8.2	0.87
succinic	39.6	34.0	0.86
succinic	59.8	56.4	0.94
succinic	74.5	72.3	0.97
fumaric	20.0	4.2	0.21
fumaric	45.5	10.1	0.22
β-naphthoic	4.1	0.2	0.05
β-naphthoic	9.8	0.7	0.07
β-naphthoic	28.9	3.4	0.12

SAMPLE CALCULATIONS

1. Conversion from cc. of 0.0100N NaOH for titration to milligrams of succinic acid.

$$\text{N solution} = \frac{\text{gram molecular weight}}{\text{hydrogen equivalent} \times 1000 \text{ cc.}}$$

$$37.4 \text{ cc. of soln.} \times \frac{0.0100 \text{ gram mol}}{2 \times 1000 \text{ cc. of soln.}} \times \frac{118.09 \times 1000 \text{ mg. succinic acid}}{\text{gram mol}}$$

$$= 22.1 \text{ milligrams of succinic acid}$$

2. Calculation of column cross section for stationary phase, A_s.

37.76 grams of water = 37.76 cc. of water

$$A_s = \frac{37.76 \text{ cc. of water}}{62 \text{ cm.}} = 0.61 \text{ cm.}^2$$

3. Calculation of R for fumaric acid.

The maximal concentration moved the length of the column, 62 cm., while 35 cc. of mobile phase was put into the column.[12] One centimeter of column height is equivalent to 1 cc. volume.

$$R = \frac{62}{35} = 1.8$$

4. Calculation of α for fumaric acid by equation 1.

$$\alpha = \frac{1.64}{R} - 0.64 = \frac{1.64}{1.8} - 0.64 = 0.27$$

5. Calculation of α for fumaric acid[13] by equation 2.

$$\alpha = \frac{N}{n-N} = \frac{2}{8-2} = 0.3$$

---

[12] See Plot 1, Figure 9.

[13] See Figure 4.