CHROMATOGRAPHY OF METHYL STEARATE, METHYL OLEATE, METHYL LINOLEATE, AND METHYL LINOLENATE: A CONCEPT OF AMPLIFIED CHROMATOGRAPHIC SEPARATIONS

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INTRODUCTION

A survey of methods available for isolating individual members of the C_{18}-series of fat acids is given below. It is felt that the limitations of these methods are sufficiently serious that none could be relied upon for obtaining a pure specimen of any member of this series which might be present as a minor constituent of a mixture of these acids. The investigation reported in this thesis was undertaken to provide a more effective method for isolating such individual acids from natural products.

Nonchromatographic Methods

Bromination-debromination methods. Of several possible procedures the most commonly used is that of Rollett[1]. While the yields are far from quantitative it can be used to obtain quite pure specimens of linoleic- and linolenic acids but is not generally applicable to the isolation of pure oleic- or stearic acids. This procedure has the serious disadvantage of producing a mixture of geometrical isomers[2].

Distillation. The individual members of this series show only small differences in their boiling points[3]. As a consequence, while they can be separated as a group from other fat acids by fractional distillation, preferably of the methyl esters, they are difficult to separate from one another by this means. Morris and Terry[3], using a column rated at 55 theoretical plates, distilled a mixture of equal parts of methyl stearate and methyl oleate. The first fractions contained about 95 percent methyl oleate and the last fractions about

95 percent methyl stearate. When a mixture of equal parts of methyl oleate and methyl linoleate was distilled the first fractions contained about 60 percent methyl linoleate and the last fractions about 57 percent methyl oleate.

Methods based on solubility of salts. A partial separation of stearic acid from the unsaturated acids of this group can be obtained by any of a number of methods based upon the relative insolubility of its barium-, magnesium-, lead-, or thallium salt in alcohol, ether, or other solvent[4]. With the lead-salt ether method the stearic acid may be contaminated with as much as 5 percent or even more of unsaturated acids[4]. The solubility of lead stearate, which is low in pure ether[4], is increased by the presence of lead salts of unsaturated acids and, so, the unsaturated acids are also contaminated with stearic acid. The use of salts in separating individuals of the unsaturated acid group leads to less satisfactory results than are obtained in the separation of stearic acid from unsaturated acids.

Low-temperature crystallization methods. This method, which can be applied to the free acids or to their esters, was first reported by Brown[5, 6]. By its use Wheeler and Riemenschneider[7] obtained methyl oleate which they considered to be 99 percent or better in purity. A yield of 296 g. of this material was obtained by 6 crystallization steps from 701 g. of starting material containing 512 g. of methyl oleate, 146 g. of methyl linoleate and 44 g. of saturated esters. For preparing the more unsaturated acids this method has proved less effective.

Frankel and Brown[8] prepared linoleic acid which they considered pure in about 20 percent yield from starting material containing 90 percent

linoleic acid. Shinowara and Brown[9] have reported a maximum concentration of 88 percent linolenic acid obtained by crystallization procedures.

Chromatographic Separations by Others

The methods listed above all show one or more of the following limitations: (1) partial isomerization of the acids, (2) low to zero yields of pure acids, (3) a laborious and time-consuming technique, (4) an inapplicability to all members of the group. This situation has stimulated a number of investigations of the application of chromatography to the isolation of the individual acids. This work is critically reviewed below in the same order as its chronological appearance.

Kondo[10] adsorbed a mixture of 0.5 g. of oleic acid and 0.5 g. of stearic acid on a column of alumina 20 cm. x 1.2 cm. and developed it with 500 ml. of benzene. On the basis of the solubility of the lead salts in ether he concluded that the top 6 cm. of the column contained only oleic acid, the next 6 cm. a mixture of the two acids and the next 6 cm. preponderately stearic acid. No attempt was made to find the most suitable conditions for the separation and the data do not form a basis for estimating the degree of separation obtained.

Kaufmann[11] has reported partial separations of mixtures involving different combinations of the C_{18}-acids. He used alumina, silica gel, and carbon as adsorbents. His purpose appeared to be to find the relationships between degree of unsaturation, nature of the adsorbent, and the adsorption sequence of the acids rather than to obtain useful separations. In no instance do his data indicate the separation of a pure compound.

Mamunta[12] has reported a serious attempt to separate stearic- and palmitic acids from a mixture of stearic, palmitic, and oleic acids. A mixture containing 0.3 g. of each of the acids was adsorbed on a 60 cm. x 3 cm. column of magnesium sulfate .1/2 H_2O and developed with petroleum ether. Despite a procedure which involved fractional crystallization and rechromatographing the best fractions no evidence is presented from which it could be concluded that any part of the material represented a single acid.

Graff and Skau[13] developed a system by means of which the movement of the acids on the column could be followed by color changes of an indicator, methyl red, which had been preadsorbed on magnesium oxide. A mixture of 100 mg. of stearic acid, m.p. 69.8°, and 100 mg. of oleic acid, iodine value 86.4 (theory 89.7) was adsorbed on a column of approximately 20 cm. x 1.2 cm. and developed with petroleum ether for 17 days. The column was cut into 4 sections as indicated by the color and from top to bottom the following materials recovered: (1) 110.2 mg., iodine value 1.6, m.p. 68-69°; (2) 21.6 mg., iodine value 72.8; (3) 71.3 mg., iodine value 91.6; (4) 1.4 mg. The separation cannot be assessed quantitatively because of uncertainties regarding the composition of the starting materials. The data indicate, however, a very good recovery of stearic acid of a purity slightly higher than 98 percent and a good recovery of oleic acid of an uncertain purity.

Swift, Rose, and Jamieson[14] chromatographed the methyl esters of cottonseed oil on alumina with a Brockmann activity of IV. In a total of four experiments, 16 g. of esters were added to 1600 g. of alumina and developed with petroleum ether. The best fraction recovered from the

eluate weighed 5.2 g. and had an iodine value of 159.5. Of this fraction, 5.0 g. was adsorbed on another column of 400 g. and likewise developed with petroleum ether. From the eluate were recovered 3.2 g. of methyl linoleate with an iodine value of 170.4. This corresponds to a purity of 97.7 percent, assuming methyl oleate to be the only impurity, and a yield of 37 percent, based on a 54.1 percent methyl linoleate content of the starting mixture.

Dutton[16] has chromatographed a mixture of 500 mg. each of oleic- and stearic acids on 40 g. of Darco. The column was developed with, successively, 1000 ml. of 0.5 percent amyl alcohol in Skellysolve B, 710 ml. of 1.0 percent amyl alcohol in Skellysolve B, and 390 ml. of diethyl ether. The time required for the experiment is not stated definitely but it appeared to be about 2 working days. About 64 percent of the oleic acid was recovered in those fractions containing 1 percent or less of stearic acid. The highest purity reported for stearic acid is 92.3 percent.

A. Santos Ruiz and M. Sanz Munoz[17] adsorbed 20 g. of acids obtained from linseed oil on a 50 cm. column of alumina and developed with 150 ml. of petroleum ether. The iodine values of the eluates from four sections of the column, starting at the top, and the filtrate were, respectively, 129, 153, 176, 182, and 185. These data give no indication that a pure acid had been separated.

In an investigation of the separation of oleic- from stearic acid, Tous and Pizarro[18] have reported that alumina is unsatisfactory because of chemical reaction with the acids. Development with 250 ml. of benzene of a mixture of 218.4 mg. of oleic acid and 58.6 mg. of stearic acid on a 65 cm. x 1.2 cm. column of silica over a period of 3 days produced no

pure fraction.

Mazumdar and Geswami [19] obtained very little separation of oleic- from stearic acid by adsorbing 1 g. of a mixture of the two acids on 10 g. of alumina and developing with 200 ml. of benzene in one experiment and 200 ml. of Skellysolve F containing 5 percent methanol in another experiment. The following iodine values were obtained with the material eluted from the top, middle, and bottom sections respectively: experiment 1- 57, 54.3, 50.1; experiment 2- 65, 56, 40.

Dutton and Reinbold[20] have examined the separation of the ethyl esters of the C_{18}-acids. Their experimental method involved the adsorption of a mixture of 10 g. of each component of an ester pair on 700 g. of alumina and development with 1.75 percent diethyl ether in petroleum ether. It is not possible to calculate either the time required for a separation or the recovery of an ester in any particular fraction from the data given. The maximum purity obtained for each ester in the various separations is as follows: (1) ethyl stearate, 88.9 percent—ethyl oleate, 83.7 percent; (2) ethyl oleate, 91.2 percent—ethyl linoleate, 78.2 percent; (3) ethyl linoleate, 83.5 percent—ethyl linolenate, 58.5 percent.

The most effective separations seem to be those of Riemenschneider et al.[21]. They used a column 6.3 cm. in diameter packed to a height of 35 cm. with 455 g. of an intimate mixture of 8 parts silicic acid and 2 parts filter aid. The column was heated, prior to the separation, to 70-75° for 3 hours while being flushed with an inert gas. An elaborate system of valves and connections was provided to permit the addition of the solution and developing solvent without permitting access of air to the column. The methyl esters (16.1 g.) obtained from the C_{18}-acids of

tobaccoseed oil were adsorbed and developed with 15 liters of petroleum ether at 700 ml./hr. The 13th liter of eluate contained 3.36 g. of methyl linoleate which was pure within the limits of accuracy of the analytical methods used. This fraction first appeared in the eluate after about 17 hours. The fraction immediately preceding it contained 88 percent methyl linoleate, on the basis of contamination by methyl oleate. The fraction immediately following it contained 94 percent methyl linoleate, on the basis of contamination by methyl linolenate. The 3.36 g. of pure methyl linoleate represent a recovery of about 27 percent calculated from analyses of tobaccoseed oils by Roberts and Schuette[22]. From a similar experiment with the methyl esters of the C_{18}-acids of linseed oil the two best fractions contained about 32 percent of the methyl linolenate of somewhat less than 98 percent purity. This material first appeared in the eluate after about 45 hours. By rechromatographing this material a net yield of about 10 percent of methyl linolenate with a theoretical iodine value was obtained.

Silica gel, as will be shown later, accelerates oxidation of the unsaturated esters. Presumably this is also true of other active adsorbents. In view of the prolonged development which has generally been found necessary in order to obtain good separations it is of interest to note that, without differentiating between its components, Taylor[23] has reported that air is held more tenaciously by silica gel than is water. This indicates the difficulty of providing completely air-free conditions on the column. Thus, to minimize oxidation as well as to save time, it is desirable to complete the development in as short a time as practicable. The esters isolated by Riemenschneider et al.[21] were from mixtures in which they occurred as the major components. The

extension of the chromatographic method to the isolation of minor constituents from mixed esters would be facilitated by, and might necessitate, the use of conditions which would give more efficient separations. It was, therefore, decided to examine the chromatographic process with this application in mind.

This work has led to more effective separations of the C_{18}-acids, judged on the basis of completeness and time involved, than those previously reported. A consideration of the totality of the data has led to a concept of chromatography which includes the prediction that the proper employment of a third solute with an intermediate adsorption affinity will result in a degree of separation otherwise unobtainable. This prediction has been verified experimentally. The similarities and differences between this process and carrier displacement chromatography, a related process which came to the author's attention subsequent to the completion of his work, are briefly discussed in the section on amplified chromatographic separations.

MATERIALS AND METHODS

Materials

Solvents.

Petroleum ether. Skellysolve F was distilled and the fraction, b.p. 35-50°, collected.

Benzene. A.C.S. thiophene-free benzene was washed repeatedly with water, dried over anhydrous potassium carbonate, filtered, distilled, and the middle fraction, b.p. 80°, collected.

Methanol. C.P., A.C.S. 99.5 percent methanol was used as purchased.

Solutes.

Stearic acid. Eastman-grade acid was crystallized 3 times from acetone and air-dried in thin layers on filter paper; m.p. 68.5°, neut. equiv. 284.0 (theory 284.3).

Methyl stearate. To each 10 g. portion of purified stearic acid 100 ml. of methanol and 2.5 ml. of concentrated sulfuric acid were added. The mixture was heated under reflux for 5 hours, cooled and dissolved in an equal volume of ether. To the solution, in a separatory flask, was added crushed ice and ice-water. The ether solution was washed twice more with water, once with a 1 percent solution of sodium carbonate and once more with water. During the washings additional ether was added as necessary to maintain its original volume. The washed ether solution was dried with anhydrous sodium sulfate, filtered, and the ether removed on a steam bath. The residue of methyl stearate was crystallized from methanol, distilled, and the fraction b.p. 155° at 1 mm. pressure, collected; m.p. 38.0°, acidity, calculated as stearic

acid, 0.2 percent.

Methyl caproate. The ester was prepared from Eastman-grade caproic acid by the procedure outlined for methyl stearate, except for the omission of the crystallization from methanol. It was distilled and the fraction, b.p. 149.0-149.5°, collected. N_D^{20} 1.4048.

Methyl myristate. The ester was prepared from Eastman-grade myristic acid by the procedure outlined for methyl caproate and the fraction, b.p. 137-138° at reduced pressure, collected. N_D^{20} 1.4368. In order to obtain a uniform sample for the experiment recorded in Table IX, 6 ml. of the ester was adsorbed on 40 g. of silica gel and developed with petroleum ether at 300 ml./hr. The following fractions were collected: (1) 178 mg., N_D^{20} 1. 4372; (2) 1031 mg., N_D^{20} 1.4369; (3) 599 mg., N_D^{20} 1.4369; (4) 511 mg., N_D^{20} 1.4369; (5) 376 mg., N_D^{20} 1.4366. Material from fraction 3 was used for the experiment.

Methyl oleate. Two preparations were used. (1) The ester used in obtaining the data of Fig. 1b and Tables I-III was prepared from olive oil by the method of Wheeler and Riemenschneider[24]; iodine value 85.3 (theory 85.7), thiocyanogen iodine value 85.6 (theory 85.7), N_D^{20} 1.4522. (2) The ester used in other experiments was purchased from the Hormel Foundation, Austin, Minn.; iodine value 85.4, N_D^{20} 1.4522.

Methyl linoleate. The ester, which was purchased from the Hormel Foundation, had been prepared from debrominated tetrabromostearic acid. Iodine value 172.6 (theory 172.4), N_D^{20} 1.4616.

Methyl linolenate. The ester, which was purchased from the Hormel Foundation, had been prepared from debrominated hexabromostearic acid. Iodine value 260.0 (theory 260.4), N_D^{20} 1.4711.

All melting points recorded above are corrected, all boiling points uncorrected. Refractive indices were measured at 20° ± 0.5° and corrected to 20.0° by the temperature coefficients of Mattil and Longenecker[25]. Each of the unsaturated esters was chromatographed and the refractive indices of the middle fractions, representing the bulk of each sample, were found to be identical with the values recorded above.

Adsorbents. Adsorbents in Table I were all used as purchased.

Silica gel. Unless otherwise specified, gel of 200-325 mesh was used. The gel was extracted with acetone by slow percolation through a column of the gel until no more soluble material was obtained. A quantity of acetone 20 times that of the gel was usually found to be sufficient, but the amount depends upon the rate of filtration. The extracted column was dried by drawing air through it, washed with distilled water, and activated by heating in thin layers of not over 0.5 cm. at 175° for 18 hours. This constitutes the most highly activated gel. It was used unless otherwise indicated. It was found that unless the gel was treated to remove the acetone prior to activation a certain amount of charring resulted. Partially deactivated gels were prepared by leaving the gel in contact with the vapors of the deactivating agent (a closed system was used with methanol) until the desired increase in weight was obtained.

Methods

All experiments were conducted at 20° unless otherwise noted. The Wijs method[4] (30 min.) for determining iodine values was used on all material containing methyl linoleate or methyl linolenate. Otherwise, the Wijs--or the Hanus method[4] (30 min.) was used interchangeably. The

standard methods were modified to the extent of using smaller volumes of solutions to correspond to smaller samples. The preparation and use of each solution are briefly described below. Calculations, as necessary, are given to illustrate each method.

Iodine value.

Potassium iodide solution. Fifteen g. of Merck Reagent-grade potassium iodide were dissolved in 100 ml. of distilled water the same day the solution was to be used.

Starch solution[26]. Five g. of soluble starch were mixed with 10 mg. of red mercuric iodide and a small amount of cold water. This paste was poured slowly into 1 liter of boiling distilled water while stirring and boiled for 2 minutes. The solution was cooled, filtered and stored in a glass-stoppered bottle.

Standard potassium iodate solution. Merck Reagent-grade potassium iodate, 1.7835 g., was dissolved in distilled water and diluted to 500 ml. to make a solution which was 0.10000 normal to iodine.

Standard sodium thiosulfate solution. Twenty five g. of sodium thiosulfate pentahydrate and 0.1 g. of sodium carbonate were dissolved in 1 liter of water and boiled gently for 5 minutes. The solution was transferred to a recently steamed storage bottle and held 1 day before standardization. One g. of potassium iodide and 10 ml. of 1 N hydrochloric acid were added to 25 ml. of standard potassium iodate solution. The resultant solution was titrated with sodium thiosulfate solution from a 10 ml. burette, which had been calibrated by the National Bureau of Standards, to a pale yellow color. One ml. of starch solution was added and the titration continued to a colorless endpoint. Toward the end of the titration split drops as small as 0.01 ml. were added by

touching the tip of the burette to the inside of the neck of the flask and rinsing into the solution with distilled water. In triplicate titrations the thiosulfate equivalent of 25 ml. of potassium iodate solution was found to be 24.58 ml., 24.59 ml., and 24.58 ml. for an average value of 24.583 ml. The normality was calculated to be (25.000/24.583) x 0.1 or 0.1070.

Wijs solution[4]. Thirteen g. of powdered, resublimed Baker's C.P. iodine were dissolved in 1000 ml. of Merck Reagent-grade glacial acetic acid conforming to the Dichromate Test by warming on the steam bath. The solution was cooled and filtered through a sintered glass filter to insure freedom from undissolved iodine. Chlorine gas, washed successively with water and concentrated sulfuric acid, was passed into the solution until the halogen content was nearly doubled, care being taken to avoid an excess of chlorine.

Hanus solution[4]. Powdered iodine, 13.8 g., was dissolved in 825 ml. of glacial acetic acid by heating on a steam bath. The solution was cooled, filtered, and to it was added a sufficient quantity of a 1.5 percent solution, by volume, of bromine in glacial acetic acid to double the concentration of halogens.

Thiocyanogen solution.[4] Lead thiocyanate was precipitated by the addition of an aqueous solution of lead acetate to an aqueous solution of potassium thiocyanate. The precipitated lead thiocyanate was washed and dried by the successive treatment with distilled water, alcohol, ether, air, and phosphorus pentoxide in a desiccator. A 1 percent solution of bromine in anhydrous acetic acid (prepared from glacial acetic acid and acetic anhydride) was added to an excess of a 10 percent suspension of lead thiocyanate in anhydrous acetic acid. The excess lead

thiocyanate and the lead bromide were removed by filtration. This solution was used within 1 week of its preparation.

Iodine value by the Wijs method. A 0.1095 g. sample was dissolved in 10 ml. of chloroform in a 125 ml. Erlenmeyer flask provided with a ground glass stopper. Ten ml. of Wijs solution were added from a burette. The stopper was moistened with potassium iodide solution and the stoppered flask placed in the dark for 30 minutes. To the contents of the flask were added 6 ml. of potassium iodide solution and 50 ml. of distilled water, the additions being made in such a manner as to rinse down the stopper and the inside of the neck of the flask. The contents of the flask were then titrated with the standard sodium thiosulfate solution of which 8.35 ml. were required. Duplicate blank determinations each required 18.75 ml. of solution. From the net consumption of 10.40 ml. of thiosulfate solution the iodine value was calculated by the formula: iodine value = (wt. of iodine adsorbed x 100)/ (wt. of sample), which, for this determination, was (0.12692 x 10.40 x 0.1017 x 100)/ 0.1095 or 122.6. A duplicate determination using 0.1068 g. of sample showed a net consumption of 10.10 ml. of thiosulfate solution which corresponded to an iodine value of 122.5. The average value of 122.4 was recorded as the iodine value of the sample and used in further calculations.

Iodine value by the Hanus method. Determinations of the iodine value by this method were made in the same manner as those by the Wijs method with the exception that the Wijs solution was replaced by the Hanus solution.

Thiocyanogen iodine value. Determinations of the iodine value by this method were made in the same manner as those by the Wijs method

with the following exceptions: a carefully controlled reaction temperature of 20° was used, a reaction time of 24 hours was used, and the potassium iodide solution was added as rapidly as possible.

Duplicate determinations of the iodine value were made when sufficient sample was available. Otherwise it was necessary to rely on single determinations. In some instances, as for the data in Table V, it was found to be advisable to use reduced quantities of reagents. The data in this table were obtained by recovering the esters in a 20 ml. weighing bottle, adding 1 ml. of Hanus solution and using correspondingly reduced quantities of other reagents. The detailed procedure for determining the methyl oleate content of fractions as recorded in this table follows. The solute was recovered from the eluate in a preweighed 20 ml. weighing bottle by evaporation of the solvent on the steam bath under carbon dioxide, cooling in a vacuum desiccator, weighing the residue of esters, adding 1 ml. of chloroform and 1 ml. of Hanus solution and determining the iodine value with correspondingly reduced quantities of other reagents. The amount of methyl oleate present was calculated by the formula: wt. of methyl oleate in the sample = (iodine value of sample x wt. of the sample)/(theoretical iodine value of methyl oleate). For example, a sample weighing 4.3 mg. required 0.94 ml. of 0.01017 N sodium thiosulfate solution. So the iodine value was (0.12692 x 0.94 x 0.01017 x 100)/0.0043 or 28.2. The methyl oleate content of the sample was (28.2 x 4.3 mg.)/85.7 or 1.4 mg.

Refractive index. The determination was made in the following manner. The sample was placed in an Abbe refractometer in a 20° constant temperature room. The prisms were washed with water which had come to a temperature equilibrium with the room and readings were taken at 20.0° ± 0.5° until a constant N_D^{20} value was obtained. These values were

calculated from the measured values by means of the temperature coefficients reported by Mattil and Longenecker[25]. The correction factor used was 0.0004 units of refractive index per degree C. The composition of a mixture was calculated as follows, using data from Table VI. The N_D^{20} value of a sample weighing 13.0 mg. was 1.4529. The value for pure methyl oleate was 1.4522 and for pure methyl linoleate 1.4616. Therefore, the weight of methyl linoleate in the sample was (1.4529 - 1.4522)/(1.4616 - 1.4522) x 13.0 mg. or 0.07 mg. It was reported as 0.1 mg.

Titrations with alkali.

Standard sodium hydroxide solutions. A concentrated carbonate-free solution was first prepared by dissolving 50 g. of C.P. sodium hydroxide in 50 ml. of distilled water. This solution was protected from the air by a soda lime tube and held overnight after which the solution was rapidly filtered through a sintered glass filter with the aid of suction. An approximately N/10 solution was prepared by adding 40 g. of the concentrated solution to 5 liters of distilled water in a storage bottle protected from the air by a soda-lime tube and thoroughly mixing the contents. This solution was standardized and an approximately N/100 solution was prepared from it, the day of use, by diluting 25 ml. to 250 ml. with distilled water.

Standardization of N/10 sodium hydroxide solution. The solution was standardized with acid potassium phthalate, using phenolphthalein as the indicator. The solution was delivered from a burette calibrated by the National Bureau of Standards. A solution of 0.2149 g. of acid potassium phthalate required 8.53 ml. of sodium hydroxide solution for neutralization. The normality was calculated to be equal to (0.2149)/(0.20414 x 8.53) or 0.1234. In check determinations, 0.2035 g. required 8.08 ml. of alkali for a normality of 0.1234 and 0.2109 g. required 8.38 ml.

of alkali for a normality of 0.1233. So the normality was recorded as 0.1234. Dilution of this solution as described above resulted in a solution with a normality of 0.01234.

Neutralization equivalent. A sample of stearic acid weighing 0.6052 g. was dissolved in 50 ml. of 95 percent ethanol, containing 3 drops of a 1 percent solution of phenolphthalein in the same solvent, by warming on a steam bath. This solution required 17.40 ml. of 0.1234 N sodium hydroxide solution to produce a faint pink color which was maintained for 10 seconds while swirling the contents of the flask. Titration of an equal volume of solvent and indicator under the same conditions resulted in the consumption of 0.12 ml. of alkali. The net consumption of alkali was 17.28 ml. The neutralization equivalent was calculated by the formula: neut. equiv. = (mg. of sample)/(normality of solution x ml. consumed) and found to be 283.8. Titration of 0.5998 g. of stearic acid resulted in a net consumption of 17.10 ml. of alkali, corresponding to a neutralization equivalent of 284.2. The average value of 284.0 was reported as the neutralization equivalent of the acid.

Stearic acid-content of a sample. Two methods were used for determining the stearic acid-content of a sample. In the first method, which was used for most of the determinations, the sample was titrated as in a determination of the neutralization equivalent, and the weight of stearic acid calculated by the formula: mg. of stearic acid = ml. of standard alkali consumed x normality of solution x 0.2843. The other method consisted of rapidly titrating both the blank and the sample but using one more drop of solution for the sample than was used for the blank and comparing the color of the resultant solutions immediately after the addition of alkali. This method was used in obtaining the

data recorded in Table XI, experiment No. 4. The purpose of this variation was to obtain a maximum value for the stearic acid which would involve no uncertainty regarding an identical color of the end-points.

Determination of K-values. In this thesis K is defined as equal to k_1/k_2 in which k_1 is the distribution coefficient of the more strongly adsorbed solute between the adsorbent and the solution, and k_2 is that of the less strongly adsorbed solute when the equilibrium is established in the presence of both solutes. Three different procedures have been employed in the determination of K-values.

First method. A petroleum ether solution of 0.861 g. of methyl stearate and 0.859 g. of methyl oleate per 100 ml. was prepared. Three g. of silica gel were added to 100 ml. of the solution contained in a 250 ml. Erlenmeyer flask. Equilibration was obtained by holding at 20° for 1 hour with frequent shaking. The flask was stoppered with a glass stopper during this period to prevent evaporation of the solvent. The contents of the flask were then poured into a sintered glass Buchner funnel and rapidly filtered with the aid of a mild suction. The filtrate was added in portions to a weighed 25 ml. Erlenmeyer flask and the solvent was removed on a steam bath under a current of carbon dioxide. Heating was continued for 15 minutes after the solvent appeared to have been all removed. Separate experiments had shown that this procedure ensured a complete removal of petroleum ether. When benzene or methyl caprate was present this period of heating was extended to 30 minutes. The flask and its contents were then brought to room temperature in a vacuum desiccator and weighed. The esters weighed 1.080 g. Duplicate iodine value determinations were 36.64 and 36.71. The methyl oleate content, calculated as previously described, was found to be 0.462 g. By difference,

the methyl stearate content was found to be 0.618 g. The weight of methyl oleate adsorbed on the gel was 0.859 — 0.462 or 0.397 g. The weight of methyl stearate adsorbed on the gel was 0.861 — 0.618 or 0.243 g. $K = k_1/k_2 = (0.397/0.462)/(0.243/0.618)$ or 2.185. In this instance the K-value was checked by analysis of the adsorbed esters which were eluted with 100 ml. of 2 percent methanol in petroleum ether. The recovered esters weighed 0.640 g. and had duplicate iodine values of 53.02 and 52.99. The methyl oleate content, calculated from the iodine values as above, was found to be 0.396 g. The methyl stearate was calculated as 0.640 — 0.396 or 0.244 g. Substitution of these values in the formula for K gave a K-value of 2.171.

Second method. This method, essentially a modification of the first method, has refinements for minimizing errors resulting from evaporation of the solvent during filtration and mechanical retention of solution by the gel and the filtration apparatus. The additional refinements are described below.

A filter tube was constructed by sealing a sintered glass filter plate between the ends of, and normal to the long axis of, a straight glass tube provided on each end with a 24/40 standard taper ground glass male joint. The dimensions of the finished tube were approximately 7 x 2 cm. The air in a 250 ml. Erlenmeyer flask provided with a 24/40 ground glass female joint was displaced by boiling off a few ml. of petroleum ether on a steam bath. One end of the filter tube was inserted into the neck of a similar flask containing the equilibrated gel and solution and then into the inverted air-freed flask. The relative positions of the two flasks were interchanged and the air-freed flask immediately plunged into cold water. The desired filtration then took place without evaporation of the solvent. Corrections for the solution

which was retained mechanically by the filtration system were made in the following manner. First, completely deactivated silica gel was prepared by placing 20.40 g. of fully activated gel in a humidor. The following weights were recorded as adsorption of water proceeded: 22.20 g. at 16 hours, 24.10 g. at 22 hours, 27.70 g. at 4 days, and 27.73 g. at 5 days. The gel had adsorbed moisture corresponding to 35.8 percent of its weight so that 1.36 g. of deactivated gel were equivalent to 1.00 g. of fully activated gel. In each of three 250 ml. flasks were placed 125 ml. of petroleum ether containing 0.3380 g. of methyl stearate. To the first flask were added 1.36 g. of deactivated silica gel corresponding to 1.00 g. of fully activated gel. To the second were added 4.07 g. and to the third 6.79 g. of deactivated gel, corresponding to 3.00 g. and to 5.00 g. respectively of activated gel. After equilibration for 1 hour at 20°, 25 ml. of solution were pipetted from each flask without disturbing the gel which had been allowed to settle. The ester recovered from the samples weighed 0.0684 g., 0.0685 g., and 0.0682 g. respectively. Compared to an original concentration of 0.0676 g. per 25 ml., these results indicated that there was no loss of ester from the solutions due to adsorption by the gel. The 100 ml. of solution remaining in each flask were then filtered by the procedure outlined above. From the flask containing 1.36 g. of gel there was recovered 0.2680 g. of methyl stearate which corresponds to a loss of 1.2 ml. of solution during the filtration. From the flask containing 4.07 g. of gel there was recovered 0.2642 g. of methyl stearate which corresponds to a loss of 2.6 ml. of solution during the filtration. From the flask containing 6.79 g. of gel there was recovered 0.2598 g. of methyl stearate which corresponds to a loss of 4.2 ml. of solution

during the filtration. The data can be represented by the following equation: loss of solution (ml.) 0.5 + 0.7 x in which x equals the number of grams of fully activated silica gel present in the equilibrated mixture. In the experiments performed according to this method the weight of esters recovered from the equilibrated solution was corrected by means of this equation. For example, if 5 g. of silica gel had been equilibrated with 100 ml. of solution and from the filtrate there were recovered 96 mg. of esters, the corrected value would be given as 100 mg. The loss in filtration 0.5 ml. + (0.7 ml.)(5) or 4.0 ml. of solution. Therefore, 96 mg. recovered from 96 ml. of solution is equivalent to 100 mg. in 100 ml. of solution.

Third method. This method consists in passing a solution of mixed esters of known concentrations over a convenient weight of silica gel in a tube until the adsorbate is in equilibrium with the solution. The adsorbent is freed as completely as possible from solution by one of two methods. If a traditional-type filter tube has been used, carbon dioxide pressure is used to force the solution past the adsorbent. If a modified centrifuge tube, such as shall be described later, is used, the adsorbent is freed from solution by centrifuging. The adsorbate is then eluted by passing 100 ml. of 2 percent methanol in petroleum ether over the gel. The subsequent treatment of the eluate is the same as that applied to the equilibrium solutions in methods 1 and 2. The quantities of adsorbent and of wash-solution required are indicated by the data recorded in Tables II and III. This method has certain disadvantages. It requires large amounts of materials and a great deal of time for each determination. It is also to be expected that, unless precautions were taken to eliminate oxygen, the more highly unsaturated esters would suffer

considerable oxidation from the prolonged exposure to the silica gel. However, by this method K-values can be obtained for predetermined concentrations of each ester. Also it was found that, in practice, more accurate determinations of the methyl oleate-methyl stearate equilibrium could be made by using this method. The experiments can always be designed so that there is ample material available for analysis even when dilute solutions are used. Also, there is no uncertainty regarding the concentration of either ester in the equilibrated solution.

Adsorption isotherms. These determinations were made by the second method for K-values with the exception that there was no need for analyzing the weighed solute recovered from the equilibrated solution.

K′-values. These were calculated from the adsorption isotherms by means of the following relationship: $K' = k_1' / k_2'$ in which the letters have the same meanings as were defined for K. The primes indicate that equilibrium coefficients are calculated from equilibria of single solutes in contrast to those of K-values which are calculated from equilibria of mixed solutes. As with K-values (with the exception of equation 2, page 30, which involves fractional exponents) any units of weight and of concentration may be used.

Following is a sample calculation of a K′-value. Let us assume that a K′-value is wanted for a concentration of 100 mg./ 100 ml. of methyl oleate and the same concentration of methyl stearate. Reference to Fig. 2 shows that methyl oleate, the more strongly adsorbed solute, is adsorbed to the extent of 150 mg. per unit weight of adsorbent at this concentration. Therefore, $k_1' = 150 / 100$. At this same concentration,

$k_2' = 130 / 100$ so $K' = (150/100) / (130/100)$ or 1.15.

Chromatographic separations. Two methods were used.

First method. A coarse-grade sintered glass filter plate was sealed into a Pyrex tube with a 23 mm. inner diameter. The tube extended 63 cm. above the filter plate and 2 cm. below the plate. At this point it was sealed onto a drip tube 5 cm. long with an 8 mm. outer diameter and a 2 mm. inner diameter. The lower end of the tube was sealed with rubber tubing and a clamp during filling operations. It was then nearly filled with solvent. Sufficient anhydrous sodium sulfate was added to form a layer which extended above the nonuniform portion of the tube. This material was manipulated to obtain as flat a surface as possible. The desired amount of adsorbent was added and its top surface was likewise flattened. Several hundred ml. of solvent were then passed through the column for the purpose of obtaining a more dense packing. Another layer of anhydrous sodium sulfate, about 2 cm. thick, was added. The solution was added when the solvent had drained to the top surface of the sodium sulfate. When the solution, in turn, had drained to the same position the inside of the column was rinsed with a few ml. of solvent. When the rinse-solvent had drained to the same position, solvent was added to the column until the pressure was sufficient to produce the desired flow-rate. This rate was maintained by further additions of solvent as required. Samples were collected in graduates.

Second method. In this method the column was packed by centrifuging the adsorbent in a 20-ml. test tube. It was found, by using dye solutions, that the most uniform movement of the dyes appeared to take place in columns packed by this method. These columns are less convenient to prepare and operate than conventional columns, but permit comparisons

with a minimum of interference from variations in packing. The test tube was prepared by drilling a small hole in the bottom of the tube. This was covered with a circle of hardened filter paper which was held to the tube with linen tape containing a hole intermediate in size between the diameter of the filter paper and the hole in the test tube. The tube was packed by filling with petroleum ether, adding successively, (1) sufficient anhydrous sodium sulfate to cover the constricted lower portion of the tube, (2) the desired weight of silica gel, and (3) another layer of sodium sulfate, and centrifuging at 2,000 r.p.m. for 15 to 20 minutes after each addition of material. The centrifuge provided a distance from the center of rotation to the bottom of the tube of approximately 8 in. It was often found advisable to loosen a thin layer of material and recentrifuge in order to improve the planarity of a boundary. The packed tube was connected, by means of a rubber stopper, to a metal tube which, in turn, was connected to a reservoir of solvent. The solvent was forced through the adsorption tube by means of nitrogen pressure. Nitrogen was found to be more satisfactory than carbon dioxide for this purpose. During experiments it was found necessary at times to interrupt the filtration in order to change solvents or to lower the level of the solvent in the adsorption tube (the solvent-level, which tended to rise because of leakage around the stopper, must be kept at a safe distance from the stopper to avoid contaminating the solvent). When, for this purpose, the pressure was released dissolved gas would come out of solution and sometimes disrupt the column of adsorbent. This tendency seemed to be considerably less with nitrogen than with carbon dioxide. The pressures needed to maintain the desired flow-rates varied from about 1 to 15 pounds, with 1 or 2 pounds usually being sufficient. The necessary

pressure can be controlled to a large extent by means of the size of the hole in the bottom of the test tube.

Although the system which has been described proved to be satisfactory it is felt that for a more permanent installation a modification would be desirable. The proposed modified tube would be provided with a standard taper female joint, and the metal connecting tube with a metal standard taper male joint. A leak-proof connection could probably then be made if the joints were lubricated with a material such as Nonaq. This system would also eliminate the possibility of contamination from a rubber stopper. A further suggested alteration is the use of a sealed-in-sintered glass filter plate. Such a plate has successfully withstood the pressure developed by the centrifuging procedure described above. A limited number of experiments with colloidal silica gel have indicated the desirability of using higher centrifugal speeds for this adsorbent. If such were used it might be found necessary to use thicker filter plates and / or special methods for sealing the plates to the tubes to enable them to withstand the higher pressures.

RESULTS AND DISCUSSION

Martin and Synge[27] have developed a mathematical expression, verified experimentally for partition chromatography, by means of which the movement on a column of each component of a mixture can be related to its partition coefficient between the nonmobile- and the mobile liquid phases and to the number of theoretical plates in the column. A knowledge of these values, therefore, enables one to predict the effectiveness of a separation under the conditions for which the values were obtained. Mayer and Tompkins[28] have developed a similar expression which has been verified for ion-exchange columns. Williamson and Craig[29] have also, for a discontinuous countercurrent distribution in tubes, developed a similar expression which they verified experimentally for this process. In the development of each of these mathematical equations there was the fundamental postulate of a constant distribution coefficient. In separations of the compounds studied here by what shall be called normal chromatography, as contrasted to partition- and to ion-exchange chromatography, the distribution coefficient of a solute between the adsorbent and the liquid phase depends on both its own concentration and on that of each other solute present and, therefore, varies throughout the separation. This fact precludes the use of distribution coefficients and column efficiency data for quantitative predictions by any of the equations cited above. It does not alter the basic importance of these values, however, in determining the effectiveness of a separation.

In this thesis K is defined as equal to k_1/k_2, k_1 representing the distribution coefficient of the more strongly adsorbed solute between the

adsorbent and the liquid phase and k_2 that of the less strongly adsorbed solute when the equilibrium is established in the presence of both solutes. K' is defined as equal to k_1' / k_2' in which the primes indicate that constants are calculated from adsorption isotherms of single solutes. Since a K-value of one represents a condition under which no separation would take place, a comparison of different systems can be made more easily by using the terms $(K - 1)$ and $(K' - 1)$.

The values in Table I were calculated on the basis of an arbitrary value of 100 assigned to standard silica gel at each point on its (K-1)-concentration curve (Fig. 1, curve 1b) which corresponded to the concentration of combined esters in the solution equilibrated with the adsorbent. The K-values of silica gel, as will be shown, vary inversely with the concentration of combined esters in solution and inversely with the ratio of the concentration of methyl oleate to that of methyl stearate. Since, in general, each of the other adsorbents showed less adsorptive capacity as well as a smaller K-value than did silica gel, the values recorded here show smaller differences in K-values than would be obtained by direct comparisons of the separations obtained by adding equal weights of each adsorbent to aliquots of the solution and greater differences than would be obtained in separations in which the equilibrated solutions contained identical concentrations of each ester. Each of the carbons adsorbed methyl stearate more strongly than methyl oleate, while each of the other adsorbents preferentially adsorbed methyl oleate. On the basis of these data the choice of an adsorbent seemed to lie between one of the aluminas and standard silica gel. It was found that a considerable proportion of esters adsorbed on alumina could not be removed whereas adsorption on silica gel was completely reversible, as shown in Table II.

TABLE 1

COMPARISON OF VARIOUS ADSORBENTS[a]

Adsorbent	Relative (K-1)-Value
Standard silica gel	100
High-temperature silica gel	62
Silicic acid (Merck)	31
Alumina (Fisher)	90
Alumina (Brockmann)	86
Magnesium oxide	12
Darco	70
Coconut charcoal	24
Wood charcoal	22
Nuchar	14
Norit-A, neutral	2

(a) Relative (K - 1)-values determined at 20° in the system: methyl oleate-methyl stearate-petroleum ether-adsorbent.

TABLE II

REVERSIBILITY OF ADSORPTION ON SILICA GEL[a]

Experiment[b] No.	Vol. of wash-solution ml.	Methyl oleate in adsorbed esters %
1	0	----
1	25	12.4
1	100	21.3
1	200	22.5
1	300	22.8
2	0	100.0
2	300	23.1
2	400	23.0

(a) Equilibrium composition of adsorbed esters obtained by washing silica gel (2 g.) with a petroleum ether solution of 0.21 g. of methyl oleate and 1.86 g. of methyl stearate per 100 ml. of solution; temp. 20°; flow-rate, 60 ml./hr.

(b) Experiment (1), no initial adsorbate on silica gel; experiment (2), 0.2 g. of methyl oleate adsorbed per g. of silica gel prior to washing.

From the data of Table III the following empirical relationships were derived in which c is expressed in mg./100 ml., m is the weight of solute adsorbed per unit weight of adsorbent, and the subscript (1) designates the more strongly, and (2) the less strongly adsorbed solute[a]:

$$(1)\quad m_1 / m_2 = 3.192\,(c_1^{0.8976} / c_2^{0.9556})$$

$$(2)\quad K = 3.192\,(c_2^{0.0444} / c_1^{0.1024})$$

A test of the relationships was made, using a solution of 161 mg. of methyl oleate and 415 mg. of methyl stearate per 100 ml. of solution. An equilibrium value of 49.5 percent methyl oleate in the adsorbed esters was found in comparison with a calculated value of 49.0 percent. It is likely that with more complete and more accurate data it would be found that additional terms were needed to express the relationships completely.

Values calculated from equation (1) represent the maximum ratios of methyl oleate to methyl stearate that would be obtained on a column during the predevelopment adsorption. The minimum ratio, i.e. the maximum methyl stearate to methyl oleate ratio, obtainable during the adsorption stage can be shown indirectly by this equation to have no limit. This has been realized experimentally as recorded in Fig. 7. From equation (2) it is seen that high K-values are favored by both a low total concentration of esters and a low ratio of methyl oleate to methyl stearate. It can be concluded that during the development stage of a chromatographic separation, as during the adsorption stage, methyl stearate can be obtained completely free from methyl oleate but that methyl oleate cannot be obtained completely free from methyl stearate. Enrichment of methyl oleate would stop when a K-value of 1 was reached. This can be

(a) See Appendix III for derivations of these relationships.

TABLE III

RELATIONSHIP OF (K - 1)-VALUES TO EQUILIBRIUM CONCENTRATION OF ESTERS

Concn. of methyl oleate mg./100 ml.	Concn. of methyl stearate mg./100 ml.	(K - 1)
848	856	1.16[a]
187	1573	1.59[a]
18.7	157.3	1.96[b]

(a) Each equilibrium was established by washing 2 g. of silica gel with 450 ml. of a petroleum ether solution of mixed esters at 20° at 60 ml./hr.

(b) The equilibrium was established by washing 2 g. of silica gel, first with 15 ml. of the solution used in the preceding experiment, and then with 800 ml. of the same solution diluted with petroleum ether to the recorded concentration.

be shown to occur when $c_1 = 3.192^{9.7656} \times c_2^{0.4336}$ or, alternatively, when $c_2 = c_1^{2.3063} / 3.192^{22.5221}$.

A concentration of 6 mg./100 ml., as was obtained for methyl oleate in its separation from methyl stearate (see Table V), would require a c_1 / c_2 equilibrium ratio of about 2×10^{10}. While it is realized that the data cannot be used to extrapolate to such an extent with any expectation of accuracy in the result, it is, perhaps, justifiable to conclude that the limiting ratio is large enough to permit the isolation of what would normally be considered pure methyl oleate.

It is of interest to examine the effect of an additional component on the separability of methyl stearate from methyl oleate. In Fig. 1, the data are strictly comparable only within each section. The experiments recorded in the two sections were made at different times and with different materials and concentrations. Partial deactivation with water, as is generally known, lowers the adsorptive capacity of the gel. In addition, it decreases the selectivity of the gel as shown by lower K-values, and in proportion to the amount of adsorbed water. The same effect is shown by methanol. Each of these substances is held more strongly by the adsorbent than is either methyl stearate or methyl oleate. With a view to finding the effect of an additive which is adsorbed more strongly than petroleum ether, but less strongly than either of the esters, benzene was investigated. It proved to be too loosely held on the gel to permit reproducible results when used in the same manner as water or methanol. Curve 5b shows its depressing effect on the K-values when used as the solvent. This corresponds to the greatest "deactivation" of the gel possible with benzene. Smaller concentrations of benzene also lower

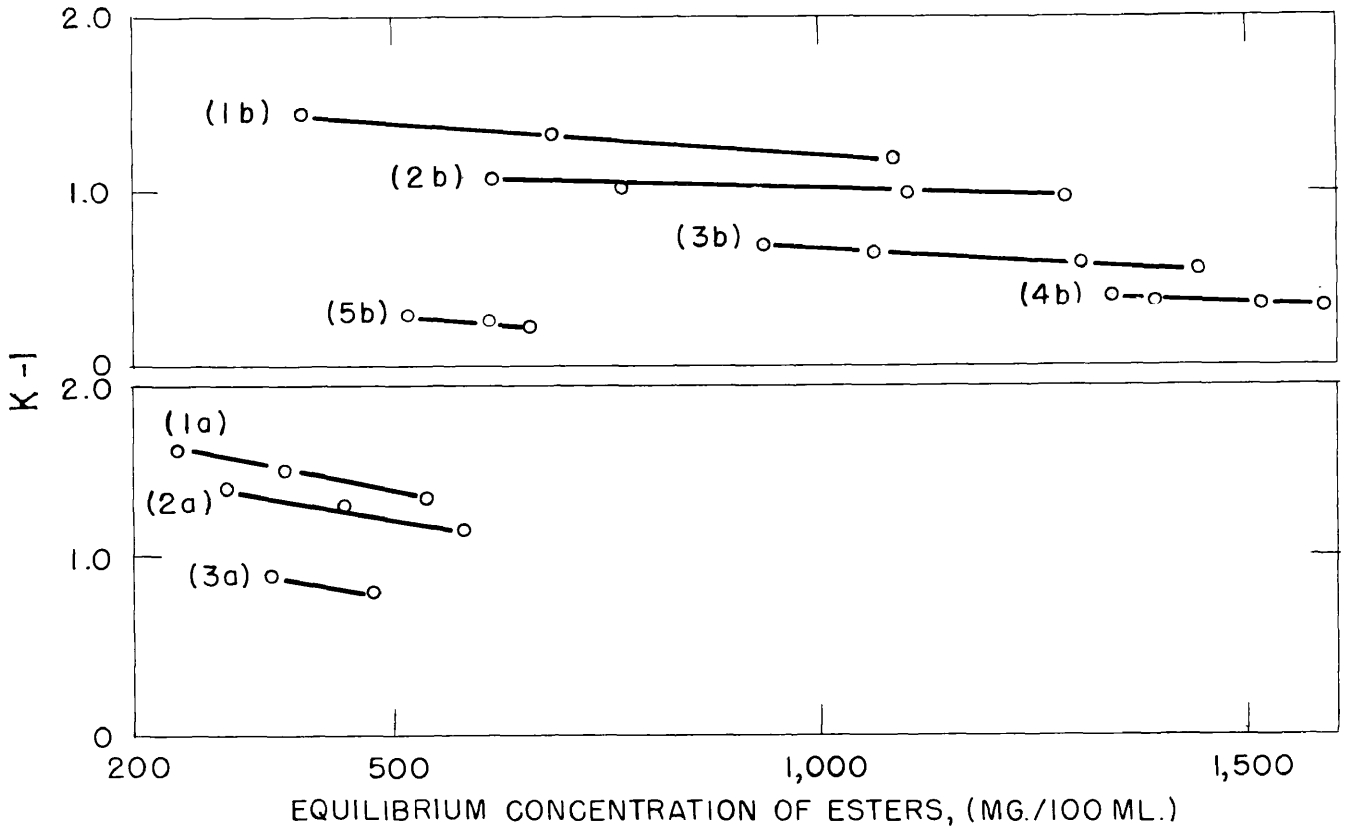

Fig. 1.--Effect of partial deactivation on (K-1)-values in the system: silica gel-methyl oleate-methyl stearate-solvent.

Curve No.	Solvent	Adsorbent
1a	petroleum ether	fully activated silica gel
2a	" "	silica gel deactivated with 1.4% water
3a	" "	silica gel extracted with hydrochloric acid and reactivated
1b	" "	fully activated silica gel
2b	" "	silica gel deactivated with 4.0% water
3b	" "	silica gel deactivated with 12.0% water
4b	" "	silica gel deactivated with 12.0% methanol
5b	benzene	fully activated silica gel

the K-values as seen in Table IV. Curve 3a in Fig. 1 was obtained with a gel which had been pretreated with 30 percent hydrochloric acid and could not be restored to its original activity. The partial deactivation is likely due to adsorbed chlorides and represents the effect of a very strongly held additive.

In Table IV, the $(K' - 1)$-values were calculated from the data shown in Fig. 2, k_1' and k_2' being determined for the same concentration of each ester as was used in calculating the corresponding $(K - 1)$-values in the preceding column. The significance of the $(K' - 1)$-values will be discussed in the section on amplified chromatographic separations. The $(K - 1)$-values, using benzene-petroleum ether as solvent, were taken from those points on the $(K - 1)$-concentration curve corresponding to the same concentrations of combined esters as were used in calculating the corresponding $(K - 1)$- values determined in petroleum ether. It is to be noted that, in contrast to the trend found with methyl stearate and methyl oleate, with both the methyl linoleate-methyl oleate combination and the methyl linolenate-methyl linoleate combination and in both petroleum ether alone and combined with benzene, the $(K - 1)$-values increase with an increase in the ratio of the more highly adsorbed- to the less highly adsorbed solute. The analytical problems involved with the more highly unsaturated esters and their greater susceptibility to oxidation with the resultant introduction of an additional species with a strong affinity for the adsorbent combine to lower the accuracy of the K-values, particularly in comparison with those of methyl oleate and methyl stearate determined by the washing-method used in obtaining the data of Tables II and III. It is felt, therefore, that pending confirmatory data, this trend should be accepted with reservation. The trend, as recorded here, indicates no limit to the enrichment of the more strongly

TABLE IV

SEPARABILITY OF ESTER-PAIRS UNDER VARIOUS CONDITIONS[a]

Concentration of ester mg./100 ml.		K - 1	K - 1	K′ - 1
Component 1	Component 2	Petroleum ether-benzene solution	Petroleum ether solution	Petroleum ether soln.
Me oleate	Me stearate			
25	48	——	1.73	1.05
193	235	——	1.12	0.25
386	427	——	0.88	0.12
54	373	——	1.57	4.32
193	235	——	1.12	0.25
352	79	——	0.71	(-)0.66
Me linolenate	Me oleate	3% benzene-97% petroleum ether		
19	40	1.10	1.59	0.84
175	238	0.85	1.33	0.37
364	434	0.55	0.92	0.23
54	372	0.75	1.15	4.45
175	238	0.85	1.33	0.37
316	87	0.93	2.03	(-)0.64
Methyl linolenate	Methyl linoleate	6% benzene-94% petroleum ether		
165	223	1.04	1.40	0.35
354	408	0.83	0.85	0.16
50	347	0.86	1.28	5.37
165	223	1.04	1.40	0.35

(a) The first group under each ester-pair illustrates the effect of concentration on their separability, with the ratio of the two esters relatively constant — the second group, the effect of their ratio with concentration relatively constant.

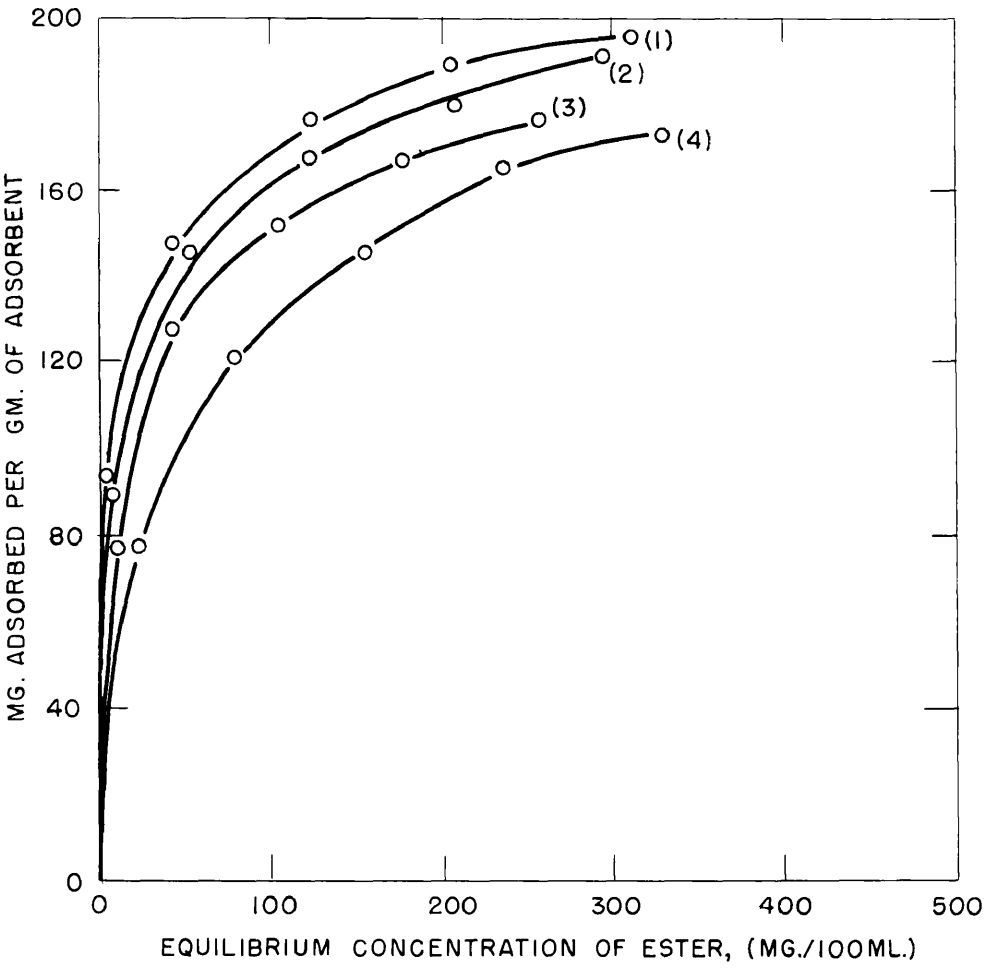

Fig. 2.--Adsorption isotherms at 20° C. in the system: silica gel-petroleum ether-ester.

(1) methyl linolenate
(2) methyl linoleate
(3) methyl oleate
(4) methyl stearate

adsorbed solute but a limit, dependent on its concentration, to the enrichment of the less strongly adsorbed solute. As before, this limiting ratio would be large enough that what would normally be considered pure solute could be separated. The significance of these trends will be discussed further in the section on amplified chromatographic separations.

The temperature effect on K-values was found to be small. Compared with an arbitrary (K - 1)-value of 100 at 25° the value at 14° was 107 and at 2° was 111. Relative to actual separations this trend would be opposed by an expected decrease in column efficiency at lower temperatures.

Figs. 3-6 show the expected relationships between the separation achieved and particle-size, flow-rate, and adsorbent-solute ratio. It is noteworthy, as shown in Figs. 4 and 5, that, as conditions are made more favorable, the separations improve rapidly at first but quickly reach a plateau from which but little further improvement is obtained. A rationalization of this observation will be attempted in the section on amplified chromatographic separations.

Fig. 8 shows the extent to which a separation depends on the relative amounts of each solute present. As the ratio of stearic acid to methyl oleate is increased the latter has to travel over a greater length of column before it can separate from the more strongly adsorbed stearic acid.

It has been generally assumed that better separations can be obtained when the predevelopment adsorption is made from dilute- rather than concentrated solutions. This view is supported by the relationship shown in this thesis between K-values and solute concentration. Fig. 7

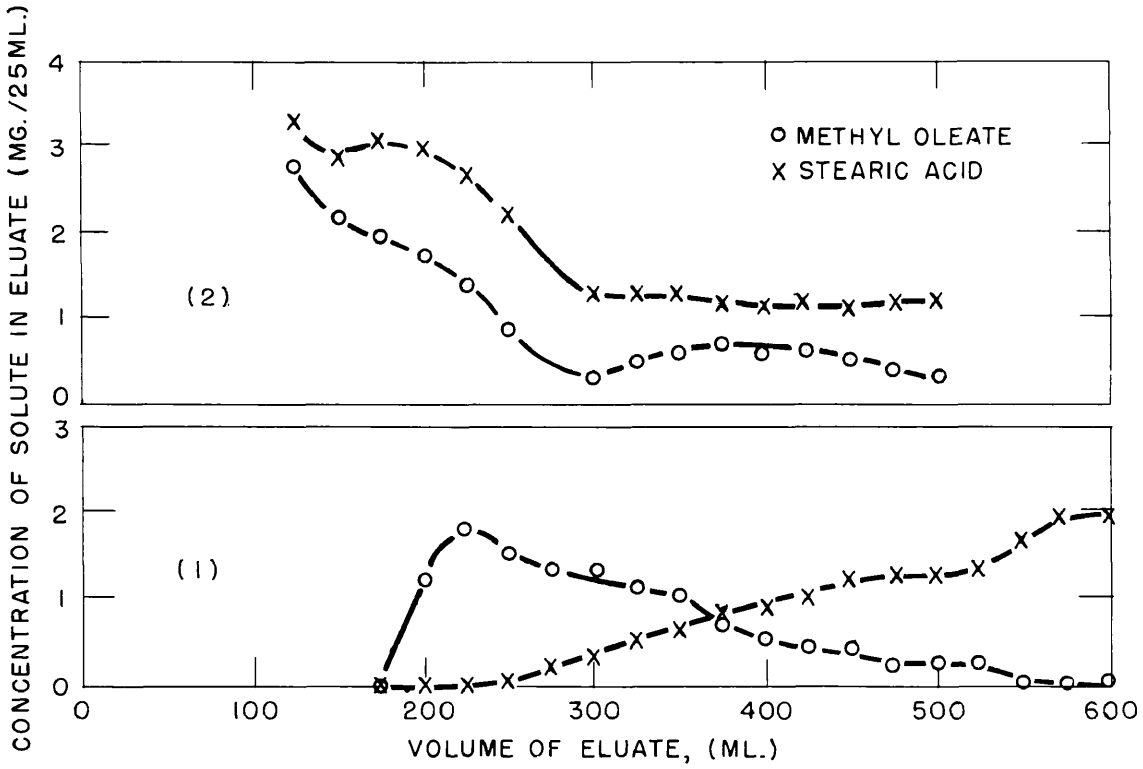

Fig. 3--Relationship of particle-size to separation.

Curve No.	Wt. of SiO_2 gm.	Particle size mesh	Flow-rate ml./hr.	Wt. of methyl oleate mg.	Wt. of stearic acid mg.
1	1.400	200-325	170	13.8	78.2
2	1.400	12-200	160	13.8	78.2

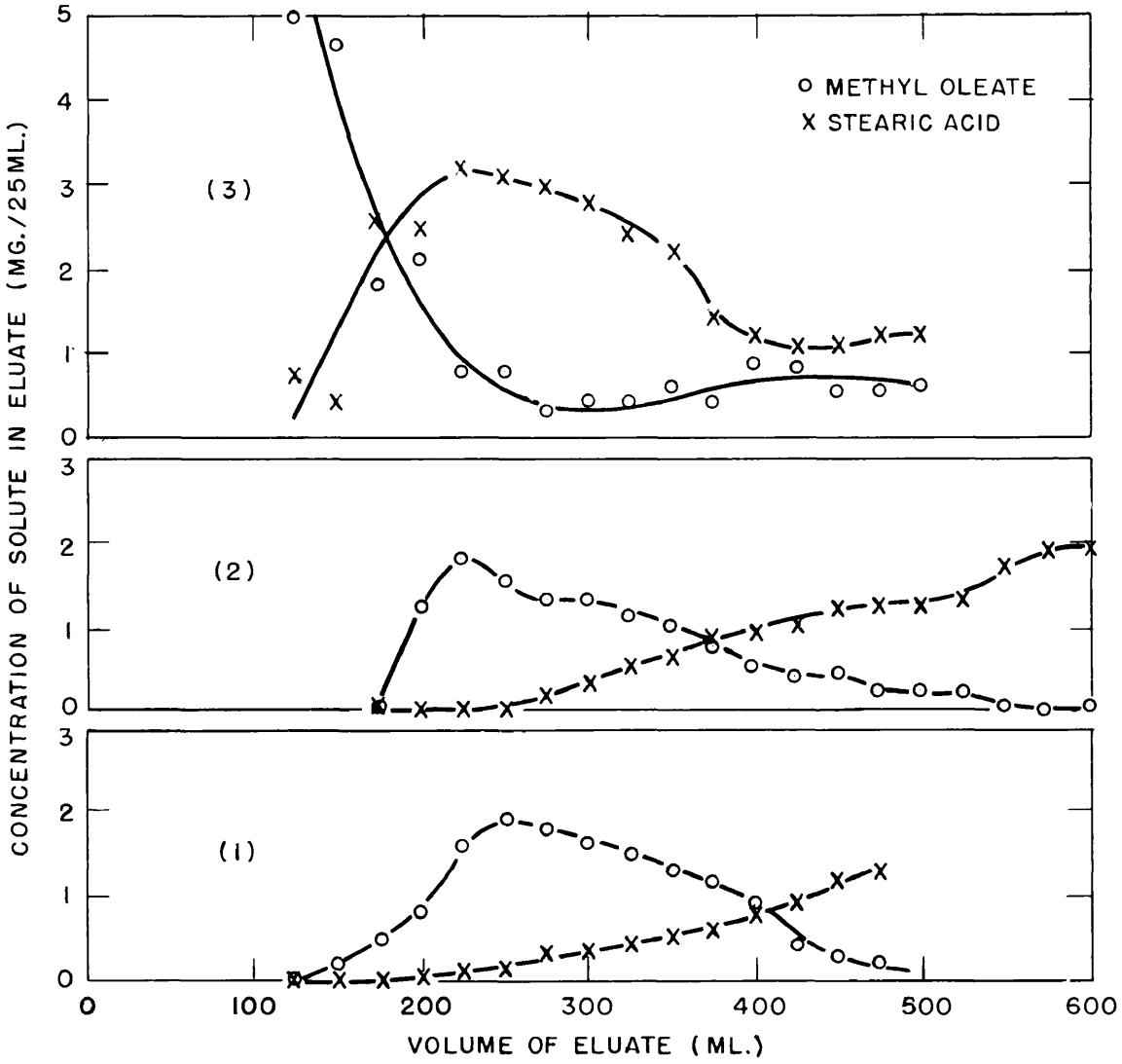

Fig. 4.--Relationship of flow-rate to separation.

Curve No.	Wt. of SiO_2 gm.	Particle size mesh	Flow-rate ml./hr.	Wt. of methyl oleate mg.	Wt. of Stearic acid mg.
1	1.400	200-325	78	13.8	78.2
2	"	"	170	"	"
3	"	"	280	"	"

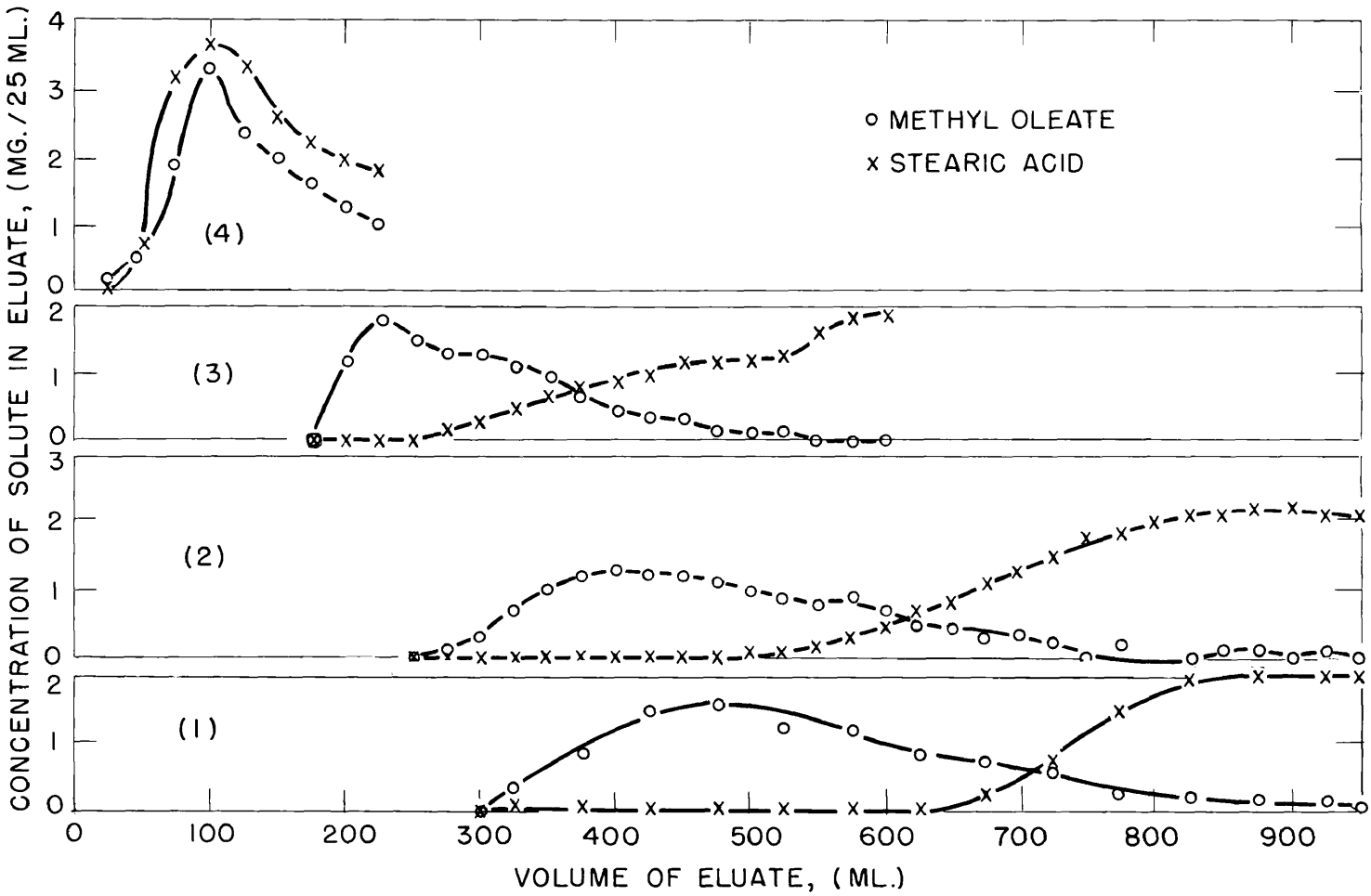

Fig. 5.--Relationship of adsorbent-solute ratio to separation.

Curve No.	Wt. of SiO_2 gm.	Particle size mesh	Flow-rate ml./hr.	Wt. of methyl oleate mg.	Wt. of stearic acid mg.
1	1.800	200-325	187	13.8	78.2
2	1.600	"	154	"	"
3	1.400	"	170	"	"
4	1.000	"	178	"	"

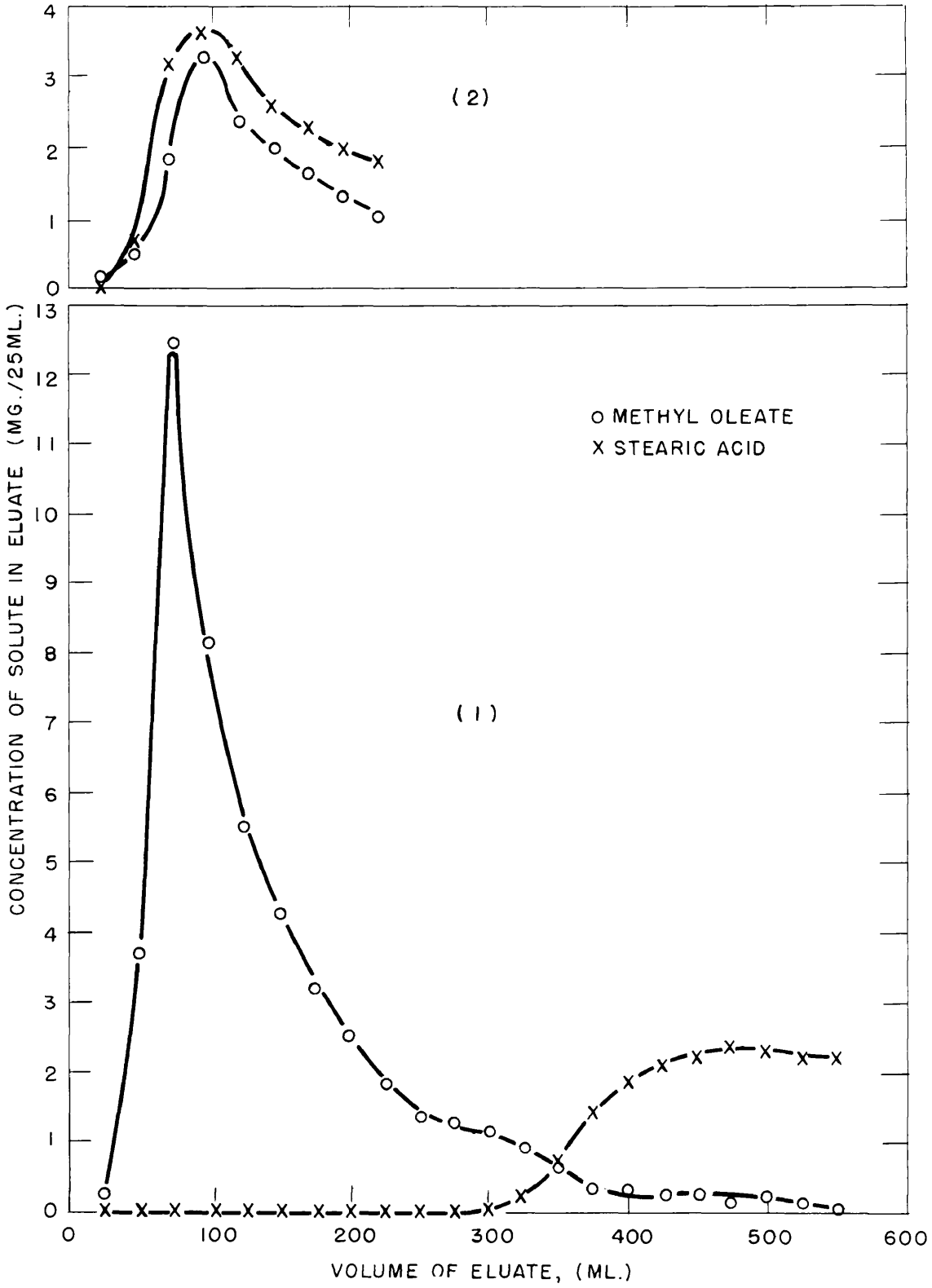

Fig. 6.--Relationship of component-ratio to separation.

Curve No.	Wt. of SiO_2 gm.	Particle size mesh	Flow-rate ml./hr.	Wt. of methyl oleate mg.	Wt. of stearic acid mg.
1	1.000	200-325	125	46.0	46.0
2	"	"	178	13.8	78.2

shows, however, that with the same ratio of adsorbent to solute, the mixed-ester zone is larger and requires a longer time before its appearance when the adsorption is made from a dilute solution than when made from a more concentrated one. The shapes of the two curves in the mixed zone do suggest, however, that if the adsorbent-solute ratio were sufficiently increased a more complete separation would be obtained eventually from the dilute solution than from the concentrated one, although at the expense of a further increase in the time required for the separation. The explanation of this result would seem to be that the head of the more strongly adsorbed solute travels a considerable distance toward the bottom of the column during a long predevelopment adsorption while at the termination of this stage it is still contaminated with a considerable quantity of the less strongly adsorbed component at the top of the column. Thus, although the separation takes place under conditions of more favorable K-values during the subsequent development, the tail of the less strongly adsorbed solute has a greater distance to travel before it has reached the head of the more strongly adsorbed solute, and the latter a shorter distance before it has reached the bottom of the column than when the adsorption was made from a more concentrated solution.

Assuming the most favorable conditions have been used for a separation, in order to obtain comparable results with a larger quantity of solute without increasing the experimental time, it is necessary to collect a greater volume of solution per unit of time. One obvious way to do this is to operate several columns of the same dimensions in parallel. Fig. 8 shows the results obtained by varying the dimensions of a single column. Curves (1) and (2) show nearly identical separations while

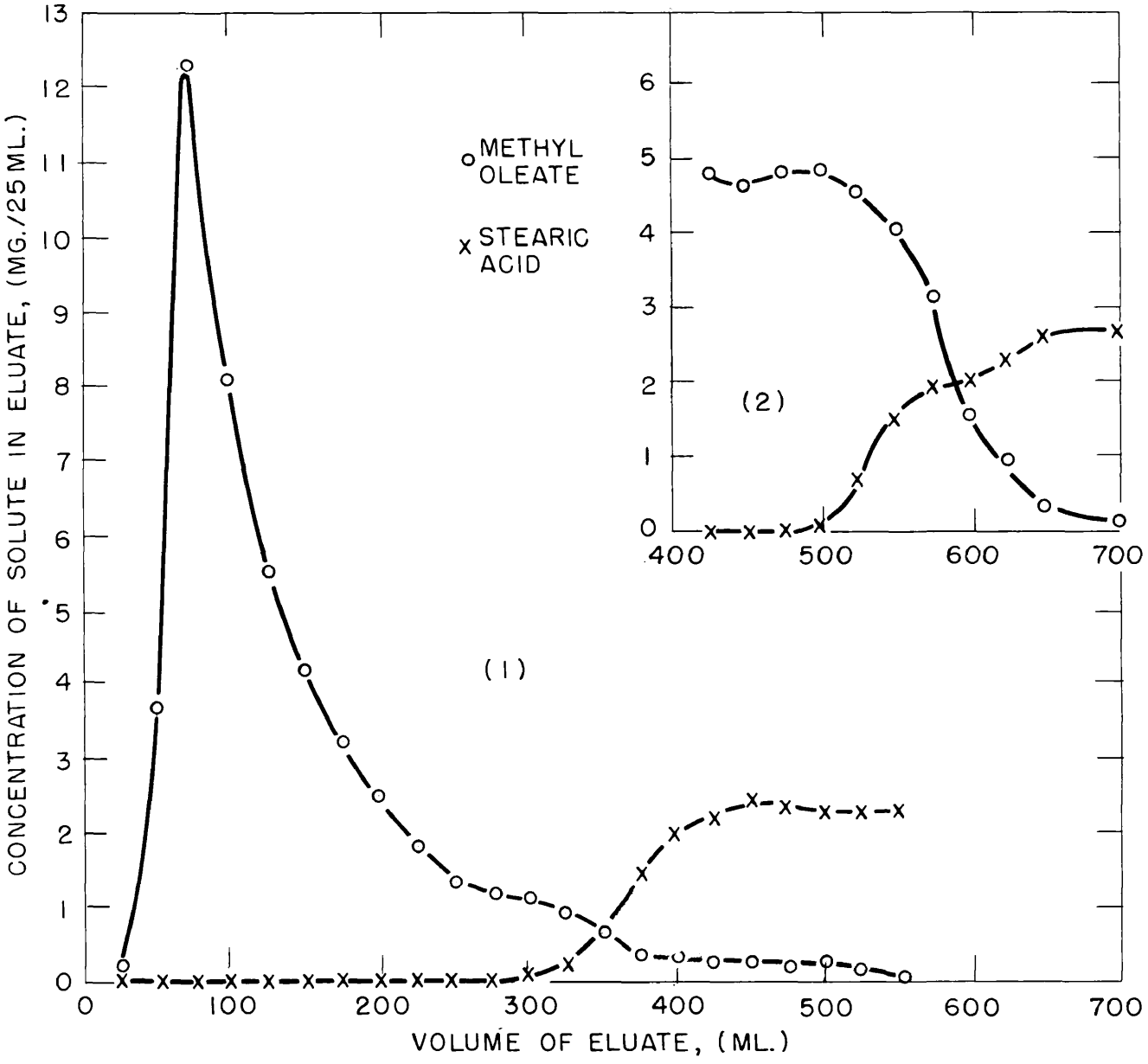

Fig. 7.--Relationship of solute-concentration to separation.

(1) Predevelopment adsorption from a solution of 46 mg. of methyl oleate and 46 mg. of stearic acid in 15 ml. of 3% benzene in petroleum ether. Development with same solvent.

(2) Predevelopment adsorption from a solution of 46 mg. of methyl oleate and 46 mg. of stearic acid in 500 ml. of 3% benzene in petroleum ether. Development with same solvent.

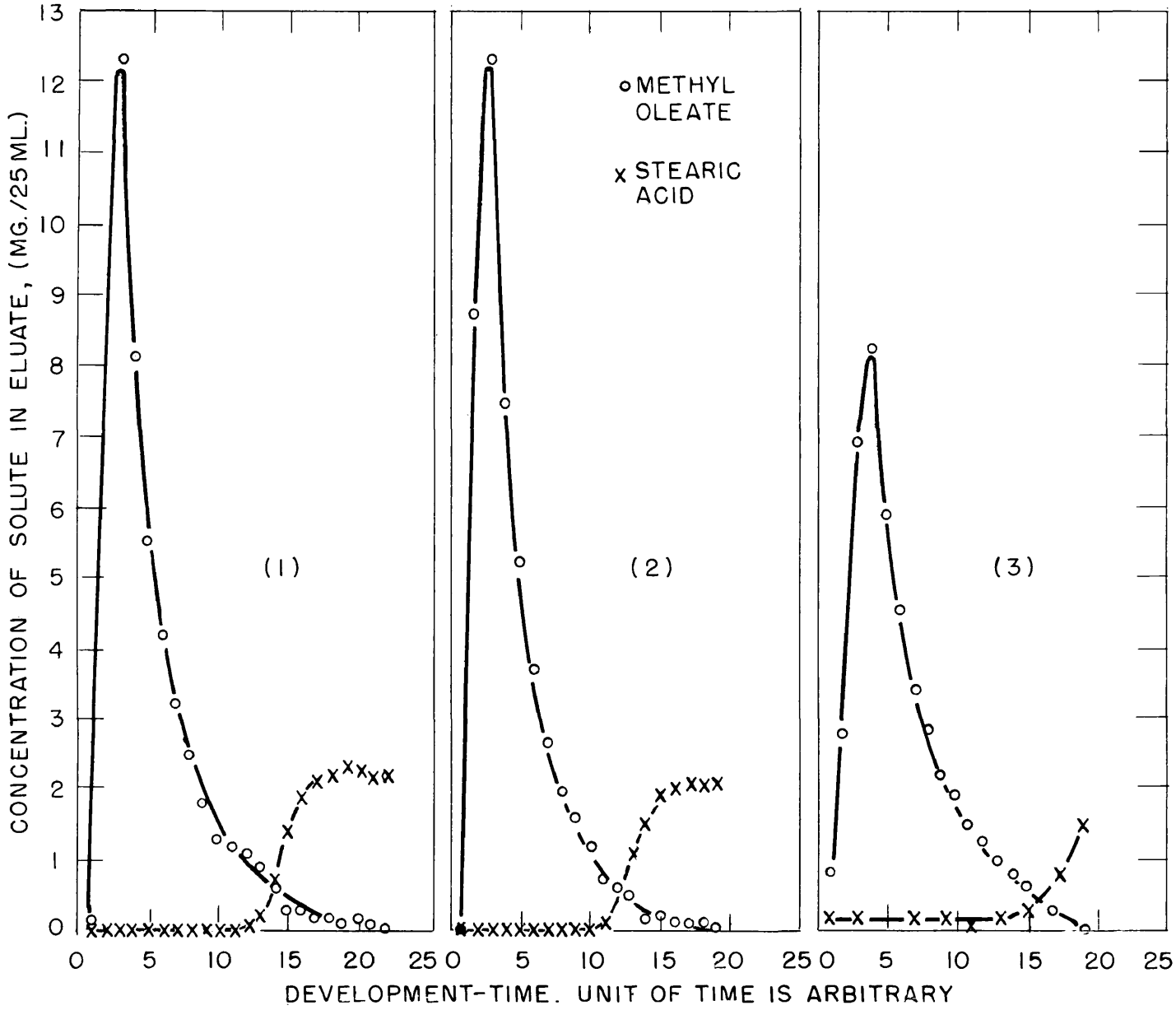

Figure 8.--Comparison of methods of separating different quantities of solutes with separation-time constant.

Curve No.	Wt. of methyl oleate mg.	Wt. of stearic acid mg.	Wt. of SiO_2 gm.	Length of SiO_2 column cm.	Flow-rate ml./hr.
1	46	46	1.000	1.1	125
2	92	92	2.000	2.3	255
3	138	138	3.000	1.0	360

curve (3) indicates a "leaking" of stearic acid through a column that was too short for its diameter.

It is felt that, particularly when dealing with column-labile compounds, it is more significant in judging the effectiveness of a procedure to relate the separation obtained to the time required rather than to the amount of adsorbent used or to the length of the column. It would appear that, on this basis, the most effective separation would be obtained when the following conditions were met: (1) an adsorbent and solvent giving the highest R_f and K -values was used; (2) the particle-size of the adsorbent was the smallest that could be retained in the column and with which the desired flow-rate could be obtained; (3) the most uniform and densest packing of the adsorbent obtainable with plane boundaries normal to the direction of flow of the solution be used, again with the limitation that the desired flow-rate be obtainable; (4) the optimum ratio of column length to diameter be used; (5) the optimum ratio of adsorbent to solute be used; (6) the optimum rate of flow of the solution and of the solvent be used; (7) the optimum concentration of solutes be used.

Each of the conditions, 4-7, requires an optimum value. In addition to irregularities within a column, deviations from a plane surface normal to the direction of flow of the solution which occurred at the upper and lower boundaries of the column would require the solution to pass through varying quantities of adsorbent in different sections of the column with a resultant decrease in effectiveness of the separation. Although the effect would depend on the operator's skill in packing the column, it would become relatively more important as the ratio of length to diameter decreased. At the boundary between

the column and the wall of the tube diffusion toward the tube would not result in adsorption with the result that the solute would move more rapidly than in the interior. This effect would become relatively less important with a decrease in the ratio of length to diameter. Conditions 5 and 6 are based on the findings that after a certain degree of separation has been achieved, the additional time required with more adsorbent or a slower flow-rate becomes less and less rewarding in terms of more effective separations. Condition 7 has been discussed above.

Tables V-VII show the results obtained in separations of the esters. The refractive index was used as the criterion for judging the appearance of a pure ester in the eluate. The accuracy obtained by this method can only be conjectured but it is considered likely that the correct refractive index of a single fraction represents a purity of 99-100 percent while the correct value for a series of fractions represents a purity considerably nearer the upper limit, particularly for the nonterminal members of the series. Lot No. 18, Table V, represents the only instance of a single fraction with a correct refractive index. Analysis, by determination of the iodine value, indicates a purity of 99.7 percent. This deviation from 100 percent very nearly represents the probable error in the determination so that this fraction can be judged to have a purity of 99.4-100 percent. In the following summary of the results obtained in these separations, use of the term "pure ester" is made within the limitations indicated above. In the separation of methyl oleate from methyl stearate, 71 percent of the former and 60 percent of the latter was obtained pure. The first appearance in the eluate of the more strongly adsorbed solute, methyl oleate, in a pure condition, occurred 6.5

TABLE V

SEPARATION OF METHYL STEARATE FROM METHYL OLEATE[a]

Lot No.	Vol. of eluate ml.	Wt. of esters mg.	Analysis ML. 0.01017 N $Na_2S_2O_3$	Wt. of Me stearate mg.	Wt. of Me oleate mg.
1	300	49.8	0.00	49.8	0.0
2	75	18.5	0.00	18.5	0.0
3	75	14.7	0.00	14.7	0.0
4	75	11.2	0.47	10.5	0.7
5	75	9.6	0.43	8.9	0.7
6	75	7.5	0.65	6.5	1.0
7	75	6.7	0.64	5.8	0.9
8	75	5.8	0.50	5.1	0.7
9	75	4.9	0.57	4.0	0.8
10	82	4.3	0.94	2.9	1.4
11	102	5.5	1.36	3.5	2.0
12	75	3.2	1.12	1.5	1.7
13	75	3.3	1.37	1.2	2.1
			N_D^{20}		
14	75	3.5	1.4495	1.2	2.3
15	75	3.7	1.4502	1.0	2.7
16	110	6.8	1.4504	1.6	5.2
17	98	5.9	1.4506	1.2	4.7
18	100	6.7	1.4517	0.4	6.3
19[b]	50	99.2	1.4522	0.3[c]	98.9

(a) 138.3 mg. of methyl stearate plus 139.1 mg. of methyl oleate were dissolved in 20 ml. of petroleum ether and adsorbed on 3.00 g. of silica gel. Development with the same solvent at 20° at 280 ml./hr.

(b) Benzene added to column.

(c) Calculated from an iodine value of 85.4.

TABLE VI

SEPARATION OF METHYL OLEATE FROM METHYL LINOLEATE[a]

Lot No.	Vol. of eluate ml.	Wt. of esters mg.	Analysis n_D^{20}	Wt. of Me oleate mg.	Wt. of Me linoleate mg.
1	100	0.0	----	----	----
2	100	14.7	1.4522	14.7	0.0
3	100	41.8	1.4522	41.8	0.0
4	100	32.7	1.4522	32.7	0.0
5	100	23.4	1.4522	23.4	0.0
6	100	20.1	1.4526	19.2	0.9
7	100	13.0	1.4529	12.0	1.0
8	200	24.3	1.4547	17.8	6.5
9	200	19.6	1.4569	9.8	9.8
10	200	14.6	1.4579	5.7	8.9
11	300	21.0	1.4589	5.6	15.4
12	300	34.4	1.4602	5.1	29.3
13	300	25.7	1.4616	0.0	25.7
14[b]	300	38.1	1.4616	0.0	38.1
15	300	30.3	1.4616	0.0	30.3
16	300	24.0	1.4621	---	---

(a) 202.3 mg. of methyl oleate plus 191.3 mg. of methyl linoleate were dissolved in 20 ml. of petroleum ether and adsorbed on 4.00 g. of silica gel (column was 4.6 cm. x 1.3 cm.). Development with 2% benzene in petroleum ether at 20° at 500 ml./hr.

(b) Concentration of benzene in the solvent was increased to 3%.

TABLE VII

SEPARATION OF METHYL LINOLEATE FROM METHYL LINOLENATE[a]

Lot No.	Vol. of eluate ml.	Wt. of esters mg.	Analysis N_D^{20}	Wt. of Me linoleate mg.	Wt. of Me linolenate mg.
1	100	0.0	----	----	----
2	100	27.2	1.4615	27.2	0.0
3	100	40.6	1.4616	40.6	0.0
4	100	31.7	1.4616	31.7	0.0
5	100	23.7	1.4616	23.7	0.0
6	100	17.8	1.4619	17.2	0.6
7	100	14.6	1.4630	12.5	2.1
8	100	11.6	1.4640	8.7	2.9
9	200	21.4	1.4660	11.6	9.8
10	200	18.0	1.4672	7.5	10.5
11	300	24.8	1.4687	6.5	18.3
12	300	30.4	1.4697	4.7	25.7
13	300	30.0	1.4711	0.0	30.0
14	300	28.8	1.4711	0.0	28.8
15	400	35.0	1.4711	0.0	35.0
16	300	9.3	1.4711	0.0	9.3
17	300	4.0	----	----	----

(a) 202.2 mg. of methyl linoleate plus 199.3 mg. of methyl linolenate were dissolved in 20 ml. of petroleum ether and adsorbed on 4.00 g. of silica gel (column 4.8 cm. x 1.3 cm.). Development with 4% benzene in petroleum ether at 20° at 500 ml./hr.

hours from the start of the experiment. In the separation of methyl oleate from methyl linoleate, 56 percent of the former and 49 percent of the latter was recovered pure. The pure methyl linoleate appeared in the eluate within 4 hours. In the separation of methyl linoleate from methyl linolenate, 61 percent of the former and 52 percent of the latter was obtained pure. Pure methyl linolenate appeared in the eluate within 4 hours.

Further improvement in the separations could be expected from the following modifications of the procedures which were used in obtaining the above results. None of these requires a more effective adsorbent-solvent combination than silica gel-petroleum ether. The use of oxygen-free silica gel should result in an improved recovery of the unsaturated ester. Table VIII shows the extent to which the unsaturated esters are oxidized when in contact with silica gel. The data concerning methyl linoleate show that the esters recovered from the equilibrated solution have retained their full unsaturation. This must result from a very strong affinity of the oxidized species for the adsorbent. The practical result of this behavior is that the oxidation decreases the yield of esters, but the oxidized esters tend to remain on the column rather than to appear in the eluate as a contaminant. More effective separations could also be expected by the use of colloidal silica gel packed by means of higher centrifugal speeds, by the use of columns with a greater ratio of length to diameter and, probably, by the omission of benzene from the solvent used to develop the unsaturated esters. The omission of benzene for this purpose would require a reduction in the quantity of adsorbent in order to keep the experimental time the same. This, in turn, would require the use of narrower tubes to maintain the same ratio of column

TABLE VIII

DETERIORATION OF ESTERS ON SILICA GEL

Ester	Time of contact	Iodine value of ester		
	hr.	theory	adsorbate	solution
methyl oleate [a]	2	85.7	83.9, 83.6	83.9, 84.0
methyl linoleate	2	172.5	169.6, 170.0	172.8, 173.4
methyl linoleate	22	172.5	153.3, 154.2	172.6, 172.5
methyl linolenate[b]	2	260.6	255.0, 255.4	———

(a) The iodine value of the ester, which was contaminated with methyl stearate, was 84.0.

(b) The experiment was conducted under conditions which led to nearly complete adsorption.

length to diameter. If the tubes were provided with sintered glass filter plates, the use of narrower tubes would require higher pressures to maintain the same flow-rate. This sequence of requirements illustrates the nature of the mechanical changes in design and operation which would be indicated as necessary for a more optimum procedure than that employed by the author. It is to be expected, however, that separations under optimum conditions would still be less satisfactory than would be obtained by the method which shall be referred to as amplified chromatographic separations. This method, which requires the use of a suitable third solute, is discussed in the next section.

The Concept of Amplified Chromatographic Separations

Schwab and Jockers[30] have reported instances of increased separability of inorganic ions in the presence of a third ion which is adsorbed between them. Strain[31] has stated that similar effects have been observed in separations of extracts of plant and animal materials. Tiselius[32], in a discussion of his method of displacement chromatography, stated that each zone of solute represents a pure material. This conclusion seems to be based on his premise, stated in the terminology used in this thesis, that when two solutes are competing for the same adsorption site, K must always have a value greater than one, regardless of how small the concentration of the less readily adsorbed solute has become. In this connection, he recognised the existence of systems in which the less strongly adsorbed solute could not be completely displaced. In the same paper, as a logical extension of these views, he suggested that the contiguous zones should be separable by the inclusion, in the sample, of solutes with intermediate adsorption affinities. He has since implemented this suggestion by the use of such solutes in separations

of amino acids and peptides[33]. The new procedure, named "carrier displacement chromatography", has been applied by Holman to the separation of saturated fatty acids[34]. The data of Holman, however, show a continuous elution of acids instead of the expected acid-free areas. Synge and Tiselius[35] have mentioned the possibility that some phenomena observed in the elution of adsorption columns with successively different solvents might be interpreted as resulting from this type of displacement.

The following discussion, which leads to the same conclusion regarding the effect of an intermediately adsorbed solute in normal chromatography as was reached by Tiselius for displacement chromatography, is independent of any theoretical considerations regarding the adsorption process. It is based on a quantitative study of the adsorption coefficients of the C_{18}- esters. The amplifying effect is demonstrated with an ester-pair for which these coefficients had been determined and is studied quantitatively with a solute-pair whose separability on columns had previously been studied extensively. The presentation of the concept is followed by a brief comparison of the amplifying effect in normal- and in displacement chromatography.

In Fig. 9a the solid lines represent a completed chromatographic separation of two compounds under ideal column conditions such that no internal mixing has taken place, the flow-rate has been uniform throughout the column and each unit volume of solution has passed over the same effective mass of adsorbent. The vertical fronts used to represent the elution curves conform to the theory of Glueckauf[36].

During the separation the movement of each solute in mixed-solute zones on the column has been governed by the relationships found for K-values; in the zones where pure solutes have separated by those for K -

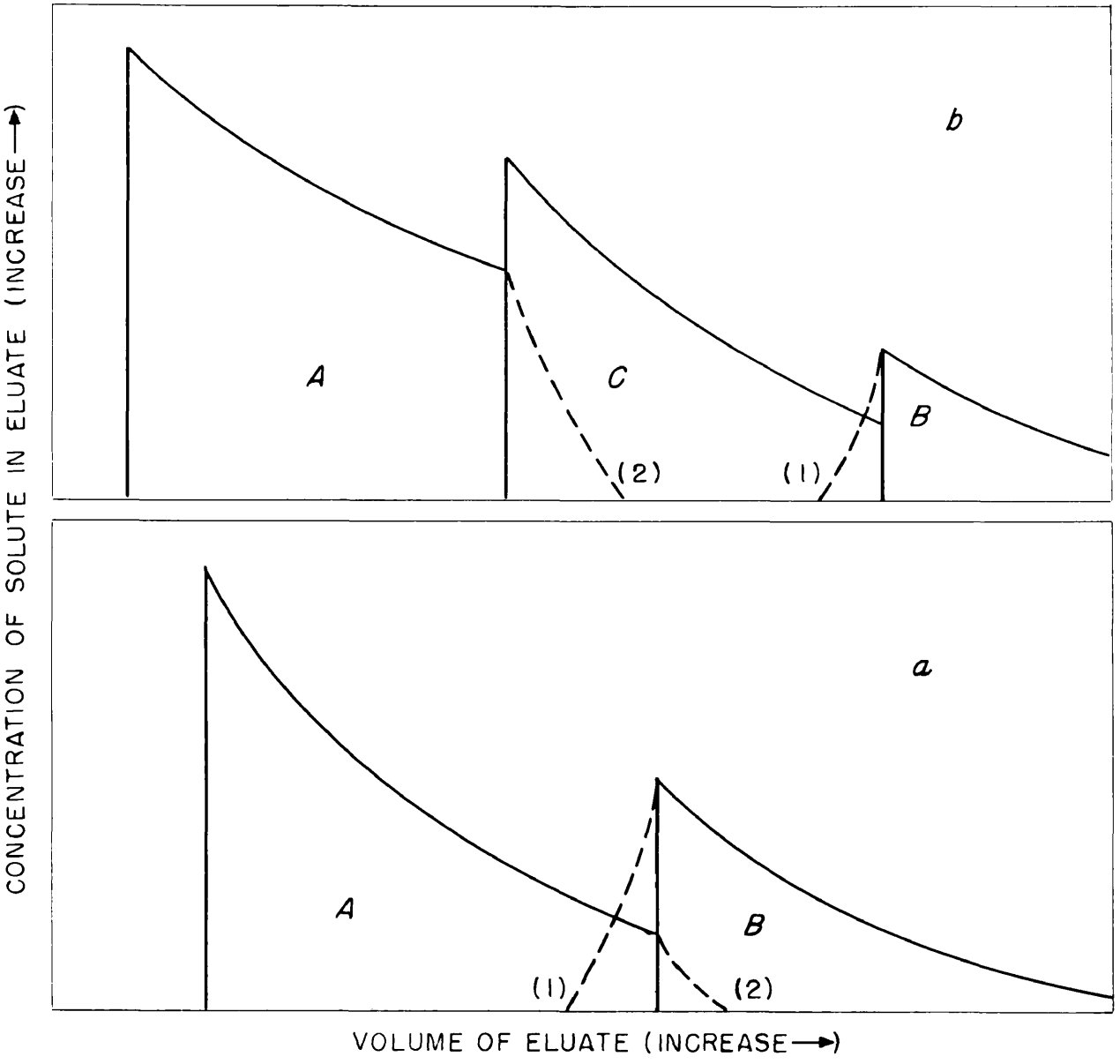

Fig. 9.--Representation of the concept of amplified chromatographic separations.

values. By referring to Table IV it is seen that if the trends noted for K-values in the methyl stearate-methyl oleate system have prevailed, area A represents a pure compound, while area B represents compound B contaminated with small amounts of A but it also can be considered pure. If the trends noted for the other ester pairs have prevailed, these relationships are reversed. If compounds A and B have shown K-values which are independent of the A/B ratio, then there is no limitation placed on the purity with which either can be obtained. The practical result is that under any of the three possibilities both A and B would normally be considered pure. Separation of the tail of A from the head of B must then proceed according to the relationships found for K′-values. Reference to Table IV shows the great dependence of K′-values on the relative concentration of the two solutes. In contrast to K-values, K′-values show a reversal at a methyl oleate to methyl stearate ratio of less than 5 and at a methyl linoleate to methyl oleate ratio of less than 4. Again referring to Fig. 9a, it becomes apparent that the tail of A cannot draw away from the head of B; it can only decrease to that concentration for which K′ - 1 is zero. In a nonideal column, nonuniform movement of the solutes in different vertical sections of the column would result in the plane of separation of the solutes in each of these sections appearing in the eluate at different times with a resultant remixing of the solutes. This effect is represented by the dotted lines in Fig. 9a. As K′-values have been found to vary inversely with concentration of a solute, the relative concentrations of B and A at their common boundary and the area of mixed solutes will also vary with concentration. With dilute solutions of compounds having large K′-values, the ratio of B to A at the common boundary could become large enough that the separation would be considered complete. However, with K′-values of the magnitude found for the esters studied in

this thesis an appreciable mixed-ester zone would appear at all reasonably high concentrations. If the vertical-front theory is incorrect, the zone of mixed esters could become much smaller, although a head-to-tail boundary would still be maintained. The picture of a common boundary maintained between A and B is in harmony with the theory of Glueckauf[56].

It was noted earlier in this thesis that, as conditions were made more favorable for a separation by using slower flow-rates or higher adsorbent-solute ratios, the improvement in the separation was at first rapid but became slower and slower. The discussion, above, forms a basis for rationalizing this behavior. With each decrease in the A/B ratio further separation takes place according to smaller K-values and approaches the limiting equilibrium-ratio imposed by a K-value of 1. The intermixing due to nonideal column conditions then superimposes on the separation the experimentally observed mixed zone which can become smaller only through the relatively slow process of lowering the concentrations of the solutes by prolonged development.

The action of a third solute, adsorbed between A and B is represented in Fig. 9b. In effect, C establishes conditions under which A and B can migrate according to the rules for K- rather than for K′-values. The greater eluting effect of C on A than on B is magnified by the greater concentration of C at the A-C boundary than at the B-C boundary. In this way a positive separation between points (1) and (2) on the column can be obtained with a resultant complete separation of A and B. If solute C is easily removable from A and B it would then be of practical value in effecting their separation.

In order to test this concept, methyl myristate was chosen as the third solute for separating methyl oleate from methyl stearate. It had

been established by separate equilibria with each of the other esters to have an intermediate affinity for silica gel. Its refractive index, 1.4369 at 20° lies below that of both methyl stearate, 1.4445 (calculated from its value at 40°), and that of methyl oleate, 1.4522, and so the appearance of this refractive index in recovered solute cannot result from any mixture of the three esters. Prior to its use in the experiment, the methyl myristate was chromatographed and a sample chosen from the middle of a series having this same refractive index. The results are given in Table IX, which shows that over 1000 ml. of eluate containing only methyl myristate were collected between the last sample containing methyl stearate and the first one containing methyl oleate. This demonstrates the "spreading" effect of solute C, but does not give any quantitative information about the separation. Methyl caproate, which can be volatilised on a steam bath, was considered, on the basis of trends noted with the higher saturated esters, as likely to be adsorbed between methyl oleate and stearic acid. The results obtained with a solution of these three compounds are recorded in Table X. Again it is noted that a middle zone free from either solute A or B has been obtained. However, a significant amount of A has remained associated with B.

Table XI records the results obtained by adsorbing a mixture of methyl oleate and stearic acid on the column, carrying out a predevelopment with controlled amounts of methyl caproate in petroleum ether and continuing the development with the solvent alone. The recovered methyl oleate was shown to be free from methyl caproate, n_D^{20} 1.4046, by its correct value of n_D^{20} 1.4522. The tests for contamination by stearic acid were made by rapidly titrating 5 ml. of 95 percent ethanol with 0.01234 N

TABLE IX

AMPLIFIED SEPARATION OF METHYL STEARATE FROM METHYL OLEATE WITH METHYL MYRISTATE AS THIRD SOLUTE[a]

Lot. No.	Solvent	Vol. of eluate ml.	Wt. of solute mg.	n_D^{20}
1	pet. ether	500	104	solid
2	"	100	44	1.4384
3	"	200	72	1.4375
4	"	200	53	1.4373
5	"	200	39	1.4371
6	"	200	33	1.4369
7	"	200	27	1.4369
8	"	200	21	1.4370
9	"	200	19	1.4369
10	"	280	22	1.4369
11	acetone	12	134	1.4422

(a) 501 mg. of methyl myristate, 95 mg. of methyl oleate, and 103 mg. of methyl stearate were dissolved in 40 ml. of petroleum ether and adsorbed on 7.00 g. of silica gel. Development at 200 ml./hr.

TABLE X

AMPLIFIED SEPARATION OF METHYL OLEATE FROM STEARIC ACID WITH METHYL CAPROATE AS THIRD SOLUTE ADDED TO SOLUTION PRIOR TO DEVELOPMENT[a]

Lot No.	Solvent	Vol. of eluate ml.	Wt. of solute mg. Me oleate	Wt. of contaminant mg. stearic acid[b]
1	pet. ether	500	51	0.0
2	"	500	59	0.0
3	"	500	59	0.0
4	"	500	19	0.0
5	"	500	3	0.0
6	"	1200	2	0.0
			stearic acid	Me oleate[c]
7	3% HOAc in pet. ether	100	0	0.0
8	0.5% HOAc in pet. ether	100	68	10.8
9	"	100	34	6.7
10	"	100	53	0.0
11	"	100	37	0.1
12	"	100	6	0.1
13	"	100	1	0.1

(a) 202.7 mg. of stearic acid plus 211.1 mg. of methyl oleate plus 1.9954 g. of methyl caproate were dissolved in 100 ml. of petroleum ether, adsorbed on 24 g. of silica gel and developed at 30° at 350 ml./hr.

(b) Determined by maintenance of color of neutralized alcoholic phenolphthalein solution when added to combined lots 1-6.

(c) Determined by addition of each sample to 1 ml. of Wijs soln. (30 min.) and titration with 0.0201 N thiosulfate soln.

TABLE XI

AMPLIFIED SEPARATION OF METHYL OLEATE FROM STEARIC ACID WITH METHYL CAPORATE AS THIRD SOLUTE ADDED TO DEVELOPING SOLVENT

Expt. No.	Lot No.	Vol. of eluate ml.	Wt. of Me oleate mg.	Contamination[e] by stearic acid %
1[a]	1	500	55.8	
	2	500	40.6	< 0.1
	3	500	0.3	
2[b]	1	500	73.0	< 0.1
	2	500	25.3	
	3	500	0.0	
3[c]	1	500	96.1	< 0.1
	2	500	4.2	
	3	500	0.0	
4[d]	1	1000	100.6	< 0.07
	2	500	0.0	
	3	500	0.0	

(a) 102.8 mg. of methyl oleate plus 104.2 mg. of stearic acid, dissolved in 100 ml. of petroleum ether, were adsorbed on 12.0 g. of silica gel. Predevelopment with 200 ml. of 0.5% methyl caproate in the same solvent and development with petroleum ether at 25° at 350 ml./hr.

(b) Same solute and adsorbent as in (a). Predevelopment with 300 ml. of 0.5% methyl caproate in petroleum ether and development with petroleum ether at 28° at 350 ml./hr.

(c) Same solute and adsorbent as in (a). Predevelopment with 500 ml. of 0.5% caproate in petroleum ether and development with petroleum ether at 25° at 400 ml./hr.

(d) 102.2 mg. of methyl oleate, 100.9 mg. of stearic acid, and 12.0 g. of silica gel were used. Predevelopment with 1000 ml. of 0.25% methyl caproate in petroleum ether. Development with petroleum ether at 25° at 400 ml./hr.

(e) Determined by titration of the combined lots of each experiment.

sodium hydroxide solution until the first detectable shade of pink, using phenolphthalein as an indicator, was obtained. The same volume of solvent, containing the same quantity of indicator, was added to each of the samples and to each was added one more drop of alkali than was required for the blank. In each instance the resultant color was deeper than that of the blank, so that the contamination by stearic acid, if any, was less than that recorded. In order to insure freedom from silica gel, each sample, prior to weighing and analysis, was filtered through a fine-grade sintered glass filter. Since, in the volatilization of methyl caprate, a small amount of methyl oleate was also volatilized, the recovery, as given, represents somewhat less than the quantity actually separated on the column. In experiment 4, Table XI, the recovery of methyl oleate was 98.4 percent and was obtained in 2.5 hours chromatographing. The data give no indication that either figure represents the best that can be obtained by this method.

Since compounds with the properties both of being adsorbed between solutes A and B and of being easily removable from them may, in many instances, be either nonexistant or difficult to find, it is of interest to consider the effect of a third solute which is either more strongly adsorbed than B or has an adsorption affinity between that of A and the solvent. It may be concluded that neither type could produce the positive separation of A from B obtainable with a solute of intermediate affinity. The more strongly adsorbed type, if too strongly adsorbed, would remain behind B and have no effect. Otherwise, it would transform the normal chromatographic separation into a displacement chromatographic separation. In this case it could be expected to interfere with the separation in the sense that, by producing a higher concentration of B

at the B/A interface, a smaller K′-value at this boundary would result in more intermixing of A and B. The less strongly adsorbed type of third solute would be expected to interfere with the separation in mixed solute zones because of smaller K-values resulting from its presence. Because of a lack of K′-data, a basis for judging its effect at the B/A interface must await further experimental work.

In comparing amplified chromatographic separations with those obtained by carrier displacement chromatography it can be said that in each type the separation of the tail of A from the head of B is initiated by the presence of C which permits the movement of both C relative to B and of A relative to C to proceed according to the relationships found for K-values. Thereafter, the situation is different. In carrier displacement chromatography the beneficial effect of C on the separation is restricted to its maintenance of K-value conditions. In amplified chromatographic separations the greater concentration of C at the A/C boundary than at the B/C boundary produces an "amplified" movement of A relative to B, hence the use of this word in describing the process. The data recorded in Table X indicate the "amplifying" effect of C may be large and, therefore, important in effecting otherwise difficult separations. This effect makes it essential that solute C be added to the column after the addition of A and B. The practice in carrier displacement chromatography is to add A, B, and C simultaneously to the column. Even in this method it would appear that in difficult separations, in which large quantities of C, relative to A and B, were required, the addition of C subsequent to A and B might prove advantageous, although not as essential as in amplified chromatographic separations.

The concept of nonamplified chromatographic separations, presented

earlier in this section, contains an implication the author would like to stress; namely, the extravagance of prolonged development as a means of greatly increasing the percentage yields of pure solutes. It would appear that for every system there is an optimum development. This would seem to be that required for producing a degree of separation which can be improved only through lowering the concentrations of the solutes at the A/B interface. At this point the more economical procedure would be to isolate the mixed solutes and rechromatograph them.

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APPENDIX I

KEY TO EXPERIMENTAL METHODS

Reference	Experimental method
Table I	First K-value method
Table II	Third K-value method of equilibration
Table III	Third K-value method
Table IV	Second K-value method
Table V	Second chromatographic method
Table VI	Second chromatographic method
Table VII	Second chromatographic method
Table IX	First chromatographic method
Table X	First chromatographic method
Table XI	First chromatographic method
Fig. 1, curves 1b-5b	First K-value method
curves 1a-3a	Second K-value method
Fig. 2	Second K-value method without analysis
Fig. 3	Second chromatographic method
Fig. 4	Second chromatographic method
Fig. 5	Second chromatographic method
Fig. 6	Second chromatographic method
Fig. 7	Second chromatographic method
Fig. 8	Second chromatographic method

APPENDIX II

PRIMARY EXPERIMENTAL DATA

Reference Table No.	Adsorbent Type	Wt. g.	Original Solution Me oleate mg.	Me stearate mg.	Equilibrated Soln. Esters mg.	Esters iodine value
I	standard SiO_2	Same data as for Fig. 1, curve 1b				
"	high-temp. SiO_2	6.0	622.7	807.3	900	37.7, 37.9
"	silicic acid	7.0	459.0	487.3	580.2	38.8
"	alumina (Fisher)	10.0	847.6	802.4	950	36.7
"	alumina (Brockmann)	9.0	777.2	762.8	920	37.0, 37.0
"	magnesium oxide	15.0	832.8	817.2	1120	42.3, 42.4
"	Darco	7.0	853.8	797.2	460	52.8, 52.9
"	cocoanut charcoal	5.0	827.7	812.3	850	46.1, 46.1
"	wood charcoal	7.0	853.8	797.2	951	46.0, 46.0
"	Nuchar	7.0	853.8	797.2	769	46.5, 46.7
"	Norit-A, neutral	7.0	868.1	851.9	780	43.4, 43.6

Reference Table No.	Experiment No.	Vol. of wash-solution ml.	Adsorbed esters iodine value
II	1	0	———
"	"	25	19.84
"	"	100	18.22
"	"	200	19.23
"	"	300	19.52
"	2	0	———
"	"	300	19.76
"	"	400	19.68

Reference Table No.	Equilibrated Solution Me oleate mg./100 ml.	Me stearate mg./100 ml.	Adsorbate iodine value
III	848	856	68.19
"	848	856	68.15
"	187	1573	20.09
"	187	1573	20.11
"	19.7	167.3	22.23
"	19.7	157.3	22.30

APPENDIX II (CONTINUED)

PRIMARY EXPERIMENTAL DATA

Reference Table No.	Original Solution: Me oleate mg.	Me stearate mg.	Solvent	Equilibrated Solution: Esters mg.	Iodine value
IV	102.0	100.0	petroleum ether	72.9	29.8
"	305.9	300.0	"	428.7	38.6, 38.6
"	509.8	500.0	"	813.1	40.6, 40.6
"	102.0	500.0	"	427.5	10.90
"	509.8	100.0	"	430.7	70.0, 70.0
	Me linolenate	Me linoleate			
	mg.	mg.			
"	288.0	291.4	"	388.2	209.9, 209.9
"	479.3	486.7	"	762.1	212.1, 214.6
"	95.9	486.7	"	396.8	183.5, 183.4
"	303.3	287.9	pet. ether-benzene	409.9	212.9
"	505.5	479.8	"	807.5	216.2
"	101.1	479.8	"	422.2	185.4
	Me linoleate	Me oleate			
	mg.	mg.			
"	97.0	102.0	petroleum ether	59.0	113.9
"	231.4	308.8	"	413.6	122.5, 122.3
"	486.6	509.7	"	798.8	125.3, 125.0
"	97.1	509.7	"	426.8	96.5, 96.7
"	486.7	102.0	"	402.1	154.0, 153.3
"	97.1	102.0	pet. ether-benzene	81.4	118.7
"	255.6	315.5	"	405.1	120.4, 120.7
"	485.7	509.8	"	801.0	126.1, 126.1
"	97.1	509.8	"	446.9	97.5, 97.7
"	485.7	102.0	"	423.8	155.1, 155.5

APPENDIX II (CONTINUED)

PRIMARY EXPERIMENTAL DATA

Reference Fig. No.	Reference Curve No.	Adsorbent Wt. g.	Original Soln. Me oleate mg.	Original Soln. Me stearate mg.	Equilibrated Soln. Esters mg.	Equilibrated Soln. Esters Iodine value	Adsorbate Esters mg.	Adsorbate Esters I.V.
1	1b	5.0	859	861	1083	36.6, 36.7	640	53.0, 53.0
"	"	5.0	859	861	830	32.0	1030	50.0, 50.0
"	"	7.0	854	856	390	28.37	1320	47.1, 47.0
"	2b	2.60	886	906	1279	58.2, 58.4	512	52.6, 52.4
"	"	3.65	886	906	1100	56.7, 56.8	691	51.7, 51.5
"	"	5.72	886	906	769	53.7, 53.6	1022	49.2, 49.2
"	"	6.76	886	906	616	52.0, 52.0	1175	48.0, 48.0
"	3b	2.81	886	906	1457	40.5, 40.5	----	----
"	"	5.93	886	906	1311	39.75	----	----
"	"	6.17	886	906	1058	37.9, 37.9	----	----
"	"	7.29	886	906	1000	37.1, 37.6	----	----
"	4b	2.81	886	906	1591	41.6, 41.6	----	----
"	"	3.95	886	906	----	----	279	46.3
"	"		886	906	1392	40.7, 40.8	----	----
"	"	7.29	915	903	1341	41.2, 41.2	----	----
"	5b	10.0	431.7	432.4	656	41.5, 41.6	----	----
"	"	12.5	431.7	432.4	611	41.2, 41.2	----	----
"	"	15.0	431.7	432.4	518	40.7, 40.7	346	45.86
"	1a	2.0	459.0	467.3	641	35.93	----	----
"	"	3.0	459.0	467.3	380	29.98	----	----
"	"	4.0	459.0	467.3	247	26.99	----	----
"	2a	2.0	459.0	467.3	591	35.56	----	----
"	"	3.0	459.0	467.3	443	32.10	----	----
"	"	4.0	459.0	467.3	310	29.26	----	----
"	3a	3.0	459.0	467.3	481	35.44	----	----
"	"	4.0	459.0	467.3	362	33.36	----	----

APPENDIX II (CONTINUED)

PRIMARY EXPERIMENTAL DATA

References		Adsorbent	Original Solution	Equilibrated Solution
Fig. No.	Curve No.	Wt. g.	Wt. of water mg.	Wt. of water mg.
2	1	1.000	95.9	3.3
"	"	1.000	191.8	45.7
"	"	1.000	303.3	125.2
"	"	1.000	383.4	199.8
"	"	1.000	505.5	310.3
"	2	1.000	97.1	7.0
"	"	1.000	194.3	52.7
"	"	1.000	291.4	128.2
"	"	1.000	386.6	207.9
"	"	1.000	485.7	296.1
"	3	1.000	86.4	8.6
"	"	1.000	172.9	41.3
"	"	1.000	259.3	106.3
"	"	1.000	345.7	177.5
"	"	1.000	432.2	256.3
"	4	1.000	100.0	22.2
"	"	1.000	200.0	76.2
"	"	1.000	300.0	153.9
"	"	1.000	400.0	240.0
"	"	1.000	500.0	329.8

APPENDIX II (CONTINUED)

PRIMARY EXPERIMENTAL DATA

Reference		Eluate[a]			
Fig. No.	Curve No.	Lot No.	Volume ml.	Weight of solute mg.	Titration, 0.01238 N NaOH ml.
3	1	1	25	0.0	0.00
"	"	2	"	0.0	0.00
"	"	3	"	0.0	0.00
"	"	4	"	0.0	0.00
"	"	5	"	0.0	0.00
"	"	6	"	0.0	0.00
"	"	7	"	0.0	0.00
"	"	8	"	1.2	0.00
"	"	9	"	1.8	0.00
"	"	10	"	1.5	0.00
"	"	11	"	1.5	0.07
"	"	12	"	1.6	0.09
"	"	13	"	1.6	0.13
"	"	14	"	1.6	0.17
"	"	15	"	1.5	0.22
"	"	16	"	1.4	0.25
"	"	17	"	1.4	0.28
"	"	18	"	1.6	0.35
"	"	19	"	1.4	0.35
"	"	20	"	1.4	0.35
"	"	21	"	1.5	0.39
"	"	22	"	1.6	0.47
"	"	23	"	1.3	0.53
"	"	24	"	1.9	0.54
"	2	1	"	—	—
"	"	2	"	—	—
"	"	3	"	—	—
"	"	4	"	—	—
"	"	5	"	6.1	0.95
"	"	6	"	5.1	0.82
"	"	7	"	5.1	0.87
"	"	8	"	4.7	0.86
"	"	9	"	4.1	0.77
"	"	10	"	3.1	0.63
"	"	11	"	2.1	—
"	"	12	"	1.6	0.36
"	"	13	"	1.8	0.37
"	"	14	"	1.9	0.37
"	"	15	"	1.9	0.34

(a) The solutes were adsorbed and developed with 5% benzene in petroleum ether, Figs. 5 - 8.

APPENDIX II (CONTINUED)

PRIMARY EXPERIMENTAL DATA

Reference		Eluate[a]			
Fig. No.	Curve No.	Lot No.	Volume ml.	Weight of solute mg.	Titration, 0.01238 N NaOH ml.
3	2	16	25	1.8	0.34
"	"	17	"	1.8	0.34
"	"	18	"	1.4	0.30
"	"	19	"	1.5	0.31
"	"	20	"	1.5	0.35
4	1	1	25	0.0	0.00
"	"	2	"	0.0	0.00
"	"	3	"	0.0	0.00
"	"	4	"	0.0	0.00
"	"	5	"	0.0	0.00
"	"	6	"	0.2	0.00
"	"	7	"	0.5	0.00
"	"	8	"	0.8	0.00
"	"	9	"	1.7	0.02
"	"	10	"	2.0	0.08
"	"	11	"	2.1	0.08
"	"	12	"	1.9	0.09
"	"	13	"	1.9	0.12
"	"	14	"	1.8	0.14
"	"	15	"	1.8	0.17
"	"	16	"	1.7	0.22
"	"	17	"	1.3	0.26
"	"	18	"	1.5	0.34
"	"	19	"	1.5	0.38
"	"	20	"	1.8	0.46
"	"	21	"	2.0	0.53
"	"	22	"	1.8	0.55
"	"	23	"	2.0	0.57
"	"	24	"	1.9	0.56
"	"	25	"	2.0	0.58
"	2	— same data as for Fig. 3, curve 1.			
"	3	1	25	—	—
"	"	2	"	—	—
"	"	3	"	—	—
"	"	4	"	—	—
"	"	5	"	5.4	0.19

APPENDIX II (CONTINUED)

PRIMARY EXPERIMENTAL DATA

Reference			Eluate[a]		
Fig. No.	Curve No.	Lot No.	Volume ml.	Weight of solute mg.	Titration, 0.01238 N NaOH ml.
4	3	6	25	4.7	0.11
"	"	7	"	4.1	0.74
"	"	8	"	4.3	0.72
"	"	9	"	5.6	0.90
"	"	10	"	5.6	0.89
"	"	11	"	5.0	0.84
"	"	12	"	2.9	0.79
"	"	13	"	2.5	0.69
"	"	14	"	2.5	0.63
"	"	15	"	1.5	0.41
"	"	16	"	1.7	0.34
"	"	17	"	1.6	0.32
"	"	18	"	1.3	0.32
"	"	19	"	1.4	0.35
"	"	20	"	1.5	0.33
5	1	6	50	0.0	0.00
"	"	7	"	0.5	0.00
"	"	8	"	1.6	0.00
"	"	9	"	2.8	0.01
"	"	10	"	2.9	0.00
"	"	11	"	2.4	0.00
"	"	12	"	2.1	0.02
"	"	13	"	1.6	0.02
"	"	14	"	1.7	0.11
"	"	15	"	2.4	0.41
"	"	16	"	3.3	0.81
"	"	17	"	3.9	1.04
"	"	18	"	4.2	1.14
"	"	19	"	4.1	1.15
"	"	20	"	4.0	1.15
"	"	21	"	3.9	1.13
"	"	22	"	4.1	1.14
"	"	23	"	4.0	1.15

APPENDIX II (CONTINUED)

PRIMARY EXPERIMENTAL DATA

Reference				Eluate[a]	
Fig. No.	Curve No.	Lot No.	Volume ml.	Weight of solute mg.	Titration, 0.01238 N NaOH ml.
5	2	1	25	0.0	0.00
"	"	2	"	0.0	0.00
"	"	3	"	0.0	0.00
"	"	4	"	0.0	0.00
"	"	5	"	0.0	0.00
"	"	6	"	0.0	0.00
"	"	7	"	0.0	0.00
"	"	8	"	0.0	0.00
"	"	9	"	0.0	0.00
"	"	10	"	0.0	0.00
"	"	11	"	0.1	0.00
"	"	12	"	0.3	0.00
"	"	13	"	0.7	0.00
"	"	14	"	1.0	0.00
"	"	15	"	1.2	0.00
"	"	16	"	1.3	0.00
"	"	17	"	1.2	0.00
"	"	18	"	1.2	0.00
"	"	19	"	1.1	0.00
"	"	20	"	1.1	0.05
"	"	21	"	1.0	0.02
"	"	22	"	1.0	0.06
"	"	23	"	1.2	0.09
"	"	24	"	1.2	0.13
"	"	25	"	1.2	0.19
"	"	26	"	1.2	0.24
"	"	27	"	1.4	0.31
"	"	28	"	1.6	0.35
"	"	29	"	1.7	0.42
"	"	30	"	1.8	0.51
"	"	31	"	2.0	0.52
"	"	32	"	1.8	0.58
"	"	33	"	2.1	0.60
"	"	34	"	2.2	0.59
"	"	35	"	2.3	0.61
"	"	36	"	2.2	0.60
"	"	37	"	2.2	0.60
"	"	38	"	2.2	0.59
"	3 -- same data as for Fig. 3, curve 1.				

APPENDIX II (CONTINUED)

PRIMARY EXPERIMENTAL DATA

Reference					Eluate[a]
Fig. No.	Curve No.	Lot No.	Volume ml.	Weight of solute mg.	Titration, 0.01238 N NaOH ml.
6	4	1	25	0.2	0.01
"	"	2	"	1.2	0.19
"	"	3	"	5.1	0.91
"	"	4	"	7.0	1.06
"	"	5	"	5.7	0.94
"	"	6	"	4.6	0.75
"	"	7	"	3.9	0.66
"	"	8	"	3.3	0.57
"	"	9	"	2.8	0.50
6	1	1	"	0.2	0.00
"	"	2	"	3.7	0.00
"	"	3	"	12.3	0.00
"	"	4	"	8.1	0.00
"	"	5	"	5.5	0.00
"	"	6	"	4.2	0.00
"	"	7	"	3.2	0.00
"	"	8	"	2.5	0.00
"	"	9	"	1.8	0.00
"	"	10	"	1.5	0.00
"	"	11	"	1.2	0.00
"	"	12	"	1.1	0.01
"	"	13	"	1.1	0.07
"	"	14	"	1.3	0.20
"	"	15	"	1.7	0.39
"	"	16	"	2.2	0.53
"	"	17	"	2.3	0.59
"	"	18	"	2.4	0.63
"	"	19	"	2.5	0.69
"	"	20	"	2.5	0.65
"	"	21	"	2.3	0.63
"	"	22	"	2.2	0.63
"	2	-- same data as for Fig. 5, curve 4.			
7	1	-- same data as for Fig. 6, curve 1.			

APPENDIX II (CONTINUED)

PRIMARY EXPERIMENTAL DATA

Reference			Eluate[A]		
Fig. No.	Curve No.	Set No.	Volume ml.	Weight of solute mg.	Titration, 0.01238 N NaOH ml.
7	2	17	25	4.8	0.00
"	"	18	"	4.5	0.00
"	"	19	"	4.9	0.00
"	"	20	"	4.9	0.03
"	"	21	"	5.2	0.19
"	"	22	"	5.9	0.44
"	"	23	"	5.0	0.55
"	"	24	"	5.5	0.57
"	"	25	"	3.2	0.64
"	"	26	"	2.9	0.73
"	"	27	"	2.7	0.75
8	1	-- same data as Fig. 6, curve 1.			
"	2	1	50	0.1	0.00
"	"	2	"	17.6	0.00
"	"	3	"	24.5	0.00
"	"	4	"	15.0	0.01
"	"	5	"	10.3	0.00
"	"	6	"	7.3	0.00
"	"	7	"	5.4	0.00
"	"	8	"	4.0	0.00
"	"	9	"	3.1	0.00
"	"	10	"	2.3	0.00
"	"	11	"	1.6	0.06
"	"	12	"	2.1	0.26
"	"	13	"	3.0	0.61
"	"	14	"	3.3	0.86
"	"	15	"	4.3	1.10
"	"	16	"	4.3	1.12
"	"	17	"	4.3	1.17
"	"	18	"	4.3	1.16
"	"	19	"	4.3	1.18

APPENDIX II (CONTINUED)

PRIMARY EXPERIMENTAL DATA

Reference					Eluate[a]
Fig. No.	Curve No.	Lot No.	Volume ml.	Weight of solute mg.	Titration, 0.01238 N NaOH ml.
8	3	1	75	5.5	0.22
"	"	2	"	9.1	0.20
"	"	3	"	21.1	0.15
"	"	4	"	24.9	0.12
"	"	5	"	18.1	0.10
"	"	6	"	13.6	0.06
"	"	7	"	10.6	0.14
"	"	8	"	8.8	0.10
"	"	9	"	7.2	0.16
"	"	10	"	5.8	0.07
"	"	11	"	4.8	0.10
"	"	12	"	4.2	0.11
"	"	13	"	3.5	0.14
"	"	14	"	3.1	0.16
"	"	15	"	2.8	0.25
"	"	16	"	3.2	0.42
"	"	17	"	3.2	0.67
"	"	18	"	3.8	0.98
"	"	19	"	4.5	1.27

Ref.[b] page No.	Temp. °C	SiO_2 Wt. g.	Original Solution Na oleate mg.	Na stearate mg.	Equilibrated Solution Esters mg.	Analysis Iodine value
37	2	3.0	854.9	825.1	1000.0	36.89, 36.90
"	14	4.0	868.1	834.9	830.0	34.93, 35.04
"	25	5.0	860.0	830.0	660.0	33.70

(b) The K-values calculated from these data were compared with those corresponding to the same concentrations in Fig. 1, curve 1b. The ratios so obtained were each divided by that for 25° and multiplied by 100 to obtain the relative values reported on page 37.

APPENDIX III

MATHEMATICAL DERIVATIONS

Equations (1) and (2), page 30, were derived as follows. By dividing a Freundlich equation, $m = k_0 c^n$ for solute (1) by that for solute (2), the general equation, $m_1/m_2 = x(c_1^y / c_2^z)$, is obtained.

$m_1/m_2 = K (c_1/c_2)$, by definition

so $K = x(c_2^{1-z}/c_1^{1-y})$

Substituting K-values calculated from Table III,

(a) $2.16 = x(956^{(1-z)})/(848^{(1-y)})$; $0.3345 = \log x + 2.9325 - 2.9325 z - 2.9284 + 2.9284 y$

(b) $2.69 = x(1575^{(1-z)})/(187^{(1-y)})$; $0.4133 = \log x + 3.1967 - 3.1967 z - 2.2718 + 2.2718 y$

(c) $2.96 = x(157.3^{(1-z)})/(18.7^{(1-y)})$; $0.4715 = \log x + 2.1967 - 2.1967 z - 1.2718 + 1.2718 y$

The three constants in equations (1) and (2) are obtained from the solution of the above three simultaneous equations.