



Chromatographic separation of the high molecular weight straight chain fatty acids
by Thomas E Shellenberger

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree
of Master of Science in Chemistry

Montana State University

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Abstract:

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THOMAS E. SHELLENBERGER

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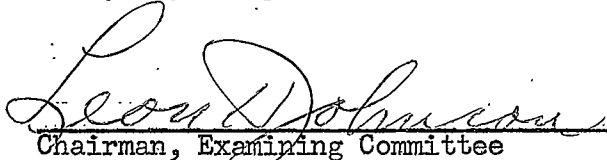
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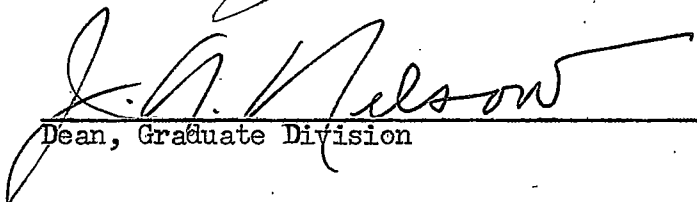
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ABSTRACT

An attempt has been made to find a chromatographic system which will effectively separate high molecular weight straight chain fatty acids. Charcoal, alumina, magnesium oxide, urea, nylon, paper, and silicone coated paper were tested as column materials. The columns were developed using various alcohols in combination with acetone, chloroform and saturated hydrocarbons. Analysis of the effluent was accomplished by titration and all results were expressed graphically to observe elution peaks of the fatty acids. No effective separation was found with these systems.

INTRODUCTION

One of the many problems in biochemistry is the quantitative isolation of the high molecular weight fatty acids present in natural waxes and fats. The acids present in any material may vary from readily volatile to nonvolatile acids and from completely saturated to highly unsaturated acids. In most cases, the acids present are members of a homologous series whose chemical and physical properties vary only slightly from one another. This makes sharp separations of the acids or their derivatives difficult.

The techniques usually employed for the separation and isolation of fatty acids are divided into three general classes: (1) distillation, (2) solubility, and (3) chromatography (20). This work is concerned only with chromatography.

HISTORICAL REVIEW

The use of chromatography as an analytical tool has progressed far from the first paper absorption procedure originated by Schöbein or the columnar adsorption method perfected by Tswett (33). Since then, chromatography has had wide application in the field of inorganic chemistry and has been successfully applied to separations of amino acids, carbohydrates, and lipids. The application of chromatography to the separation of fatty acids, on the other hand, has just begun to be investigated to any extent.

Much of the work in identification of fatty acids by paper chromatography has been done by H. P. Kaufmann and J. Budwig (13, 14, 15, 16) although other investigators (3, 11, 12, 18, 29) used paper and paper-

partition chromatography to great advantage. The work of Kaufmann deals mainly with the identification of different fatty acids, $C_3 - C_{18}$, or their metal soaps by colored spot tests. Inouye and Noda (11, 12) have applied paper chromatography to saturated $C_4 - C_{22}$ and unsaturated fatty acids $C_{12} - C_{22}$ by developing a descending chromatogram with a methanol: petroleum hydrocarbon bp 140-70 mixture, as the solvent system. Long, Quayle, and Stedman (18), Reid and Lederer (29) and Duncan and Porteous (3) have used alcohol-ammonium hydroxide mixtures to obtain partition separations of the lower fatty acids, $C_2 - C_7$, on paper.

Column chromatography has been investigated much more, possibly because it can be made to give an easy quantitative as well as a qualitative measure. R. T. Holman, in conjunction with J. G. Hamilton and W. T. Williams (4, 6, 7, 8, 9) have used columns of 1 part Darco G-60 with 2 parts Hyflo Supercel to effect separations of $C_{12} - C_{18}$ saturated fatty acids. He and his associates used modified Tiselius-Claesson interferometric absorption analysis apparatus to detect differences in carrier displacement separations of fatty acids.

Much work has been done to separate the fatty acids by partition chromatography on columns of silicic acid and silica gels. Muzumdar and Boswami (22) used a silica gel column in their separation of stearic and oleic acids. They also used alumina, magnesium oxide and activated carbon for column materials. Ramsey and Patterson, in two of their publications (27, 28) reported separations of all fatty acids from $C_5 - C_{19}$ on columns of silicic acids using methanol and 2,2,4,-trimethyl pentane as solvent

for $C_5 - C_{10}$ and a mixture of furfuryl alcohol and 2 aminopyridine with hexane for $C_{11} - C_{19}$. Nijkamp (23, 24, 25) has also worked out a separation of the fatty acids from $C_4 - C_{20}$ on silica gel columns using isooctane saturated with an excess of 95% methanol as the solvent. A reversed-phase partition method advanced by Howard and Martin (10) was reported successful using kieselguhr treated with dichlorodimethylsilane vapor to render it "unwetttable". They found the most suitable solvent systems for acids from lauric to stearic were aqueous methyl alcohol-octane or aqueous acetone-medicinal paraffin. This same reversed phase system was employed by Silk and Hahn (32) to separate mixtures of $C_{16} - C_{24}$ fatty acids. Harris and Wick (5) have presented a procedure designed to give a silica gel suitable for chromatography.

Alumina (Al_2O_3) has been used extensively in columns for the separation of the fatty acids. Asahara and associates (1) used petroleum ether system to obtain separations while Ruiz and Munoz (30) found that the saturated acids accumulate in the upper parts of these columns. The former also used magnesium oxide and calcium oxide mixed with ethanol as adsorbents for column chromatography. Acetone was employed by DiModica and Rossi (2) to elute the unsaturated acids linolenic, linoleic, and oleic from a cold alumina column.

A micro method has been worked out by Mai (19) to separate the fatty acids from $C_8 - C_{18}$ by partition chromatography on nylon thread. The solvent he employed was a 1:1:1 mixture of ethanol:water:acetone.

Separations of steroids by Kritchevsky and Tiselius (17) was ac-

complished by reversed phase partition chromatography on silicone treated paper. A two phase solvent system resulted from his solvent system of ethanol, water, and chloroform. The chromatograms were developed with the polar phase after saturating the paper with the non-polar solvent. This publication, although not dealing directly with fatty acids, was reviewed for possible application to fatty acid separation.

EXPERIMENTAL TECHNIQUE AND PROCEDURE

The chromatographic techniques employed in this investigation were (1) direct adsorption of acid to column material, (2) partition separation between a polar and non-polar solvent system, where the polar solvent is the immobile phase, and (3) reversed phase partition chromatography where the non-polar solvent is the immobile phase. Regardless of the technique used, the column materials were packed in glass chromatographic tubes with sintered glass plates at the bottom and the acids eluted from the column using proper solvents or solvent systems. By employing an automatic fraction cutter, fractions of nearly equal volumes of effluent were collected and analyzed for the fatty acid content of each. The acids were identified by an increase in titer when the amount of acid present in each fraction was graphed versus the total volume of effluent. In this manner, several column materials and solvent systems were tested in an attempt to find a combination that would give an effective separation of fatty acids from myristic to arachidic.

The method of packing the columns varied with the kind of material used, the size of particles in the material, the solvent or solvents em-

ployed, the diameter of tube, and the height to which the tube was packed. In general, it was found that a thick slurry of the column material and solvent system should be suspended in the chromatographic tube to obtain best results. This slurry can be packed to give a homogeneous column by tamping or by using 5-10 lb. pressure from a pressure tank. It was also found that the longer columns did not require as tight packing as the shorter columns. The small diameter columns also required less packing. The amount of packing required for different column materials varied considerably. When using nylon for the column material, a much tighter packing was necessary to give a reasonable flow rate than with paper and its modifications. The solvents used with the paper columns had an effect on flow rate but no prediction as to which system would require a loose or tight pack could be made. Column packing is a developed art and reasonably reproducible columns can only be achieved after acquiring some experience in packing methods.

The flow rate of any column type was dependent upon (1) the height of the column, (2) the tightness of pack, (3) the material in the column, (4) the solvent system employed, and (5) the height of the solvent level above the top of the column. The first four criteria were decided when the columns were packed but the latter was varied after development of the column had commenced. For best results, a flow rate of .5 to 3 cc./15 min. should be maintained by raising or lowering the solvent reservoir. (In some cases, a reduced head of pressure was necessary to give a reasonable flow; that is, top of the solvent level was below the top of the column.)

The fraction cutter was made in this laboratory and consisted of a rotating turntable timed to move a glass receiving tube under the chromatographic column every 15 minutes. The turntable has an 80 tube capacity.

The effluent was analyzed for fatty acids by titration with .01N potassium hydroxide. This was used since the potassium salt of a fatty acid is slightly more soluble than the sodium salt in an alcohol solution. All titrations were carried out in an alcohol media and titrated to a phenolphthalein end point. The graphs were made by plotting volume potassium hydroxide on the vertical axis against total volume of effluent on the horizontal axis. The position of the acid as it elutes from a particular column system is characteristic for each acid. The term "elution position" is used to indicate the volume of effluent that has emerged from the column prior to elution of the fatty acid under examination.

The fatty acids were applied to the columns in solutions containing 10 mg. of the fatty acid in one of the constituents of the solvent system under investigation.

RESULTS

The first column material utilized was 1 part Darco G-60 mixed with 3 parts Hyflo supercel. The solvent system employed was ethanol and ethanol:water mixtures. Stearic acid, when developed with ethanol on charcoal, eluted from the column at 12 cc., just behind the solvent front. Single acids were used in cursory examinations of column materials. This was done so as to observe the placement of individual acid peaks. When the

solvent system was changed to 80% ethanol, stearic acid was not eluted from the column. A mixture of methanol, isopropanol, and water was next tried on a charcoal column and stearic acid again had not eluted from the column at 150 cc. At this point the flow rate dropped and the column began to dry and pull away from the side of the chromatographic tube. No success was apparent so the use of this material was abandoned.

Aluminum oxide (Al_2O_3) was tried next for possible separation properties. Stearic acid again was developed with 95% ethanol in a cursory examination. A sharp increase in titer was noted at a point corresponding to about 700 cc. of effluent. Normal propanol and butanol were employed as solvents in an attempt to bring this peak closer to solvent front but no improvement was noted. Stearic acid had not eluted from the column at 900 cc. using butanol as solvent. In these trials, when using butanol and propanol, the solvent was evaporated and ethanol added before attempting the titration. Because of the large volume of effluent required, it was felt that columns using alumina would be difficult to duplicate; therefore, alumina was rejected as a column material.

Next attempts at acid separations were made on nylon columns. The columns were packed using ground Zytel nylon resin provided by DuPont. The nylon was kept cold with dry ice during grinding to minimize shredding due to heat produced during the grinding process. The nylon was ground until it would pass a 1/2 mm. screen. This was still too coarse and resulted in a high flow rate. The solvents used were combinations of ethanol, acetone, and water. The column diameter in all trials was 1.1 cm.

and the column lengths were always approximately 9-10 inches (23-25.5 cm.). Table I shows the relative positions of the fatty acids with different solvent combinations. Trials I-J and K-L are shown as complete plots on figures 1 and 2.

TABLE I
EFFECT OF SOLVENT COMPOSITION ON ELUTION OF FATTY ACIDS

TRIAL	ETHANOL:ACETONE:WATER RATIO	ACID	ELUTION POSITION
A	1:1:1	C ₁₈	11.5
B	1:1:1	C ₁₈	12.0
C	0:1:1	C ₁₈	No elution
D	1:1:0	C ₁₈	Front
E	1:0:1	C ₁₈	No elution
F	4.5:1:4.5	C ₁₈	No elution
G	2:1:2	C ₁₈	No elution
H	1:1:1	C ₂₀	Front
I	3:2:3	C ₁₆	15.5
J	3:2:3	C ₁₆	12.5
K	5:4:5	C ₁₄	12.0
L	5:4:5	C ₁₈	12.0

In all trials on nylon columns, the higher homologs come out of the column before the next lower acid indicating a reversed phase chromatographic system in operation. The nylon columns showed some tendency to affect separations of fatty acids. All attempts to move the acid fronts back further from the solvent front did not succeed so this material was rejected as a column material.

The next trials were made on columns packed with Whatman Cellulose Powder Standard Grade #21480 Cellulose Powder for Chromatography. This was an attempt to adapt the paper separation method of Inouye and Noda

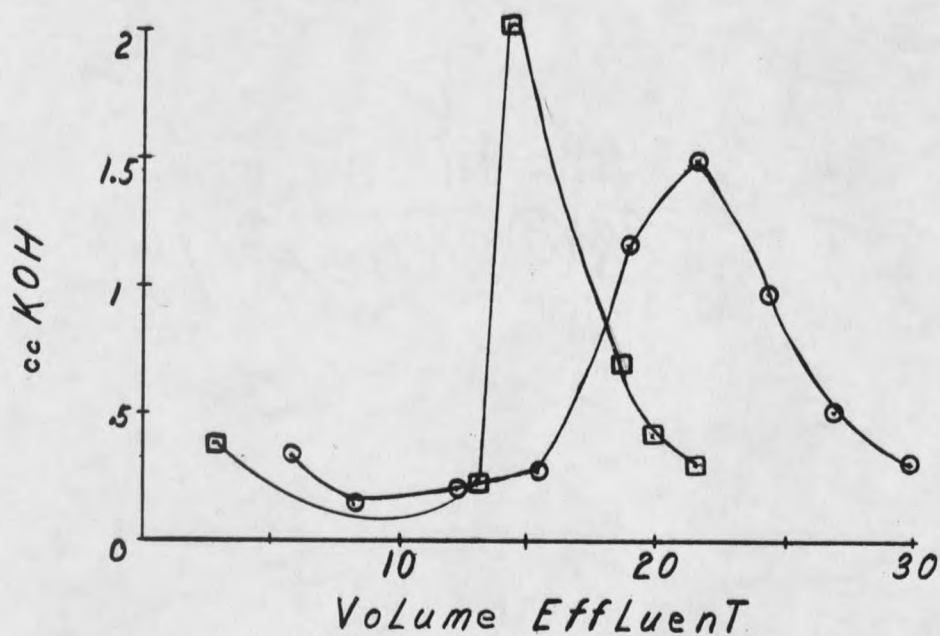


Figure 1. Nylon column. Solvent: ethanol:water:acetone in ratio of 3:3:2. $\circ$ - Myristic acid $\square$ - Palmitic acid

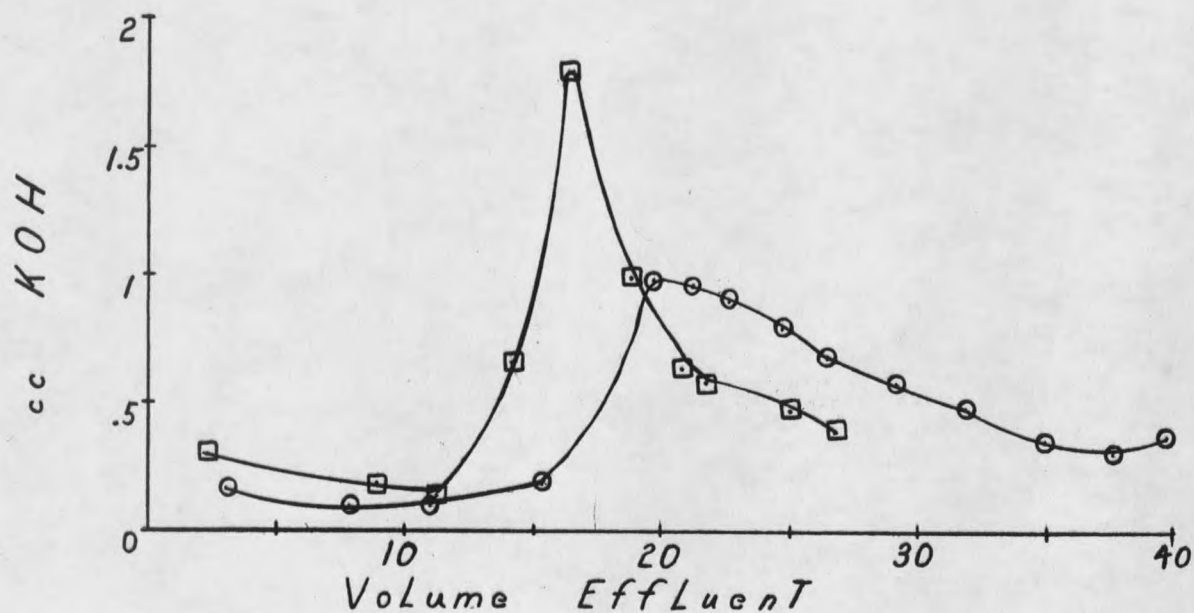


Figure 2. Nylon column. Solvent: ethanol:water:acetone in ratio of 5:5:4. $\circ$ - Myristic acid. $\square$ - Palmitic acid

(11, 12) to column procedures. Figure 3 shows the results when stearic, arachidic, and a mixture of stearic and arachidic acids are developed on a paper column using a 1:1 mixture of methanol:Skellysolve B. The column was 1.1 cm. wide and was 14.5 inches long. Stearic acid shows an elution position of 18 cc. and arachidic has an elution position of 20 cc. The acids showed a slightly different elution position which also can be seen by the sudden increase in the titrations curve for the mixture. It was hoped that a longer column would resolve the acids better. With this in mind, a column .9 cm. wide and 35 1/2 inches long was set up and the acids were developed with the same solvent system. The results are shown in figure 4. As indicated on the graph, the elution position of both acids was the same.

As a result of the data illustrated in figures 3 and 4, the ratio of methanol to Skellysolve was changed in further trials. Figure 5 shows the results obtained with a solvent composition of 3 parts methanol to 1 part Skellysolve. The acids again showed a definite tendency to overlap and the solvent combination was changed to 4:1 methanol:Skellysolve. The results obtained with this combination showed overlapping of the acid peaks with their position directly behind the solvent front. The peaks obtained with the 3:1 combination were set back from the front about 10 cc.

A solvent combination of 3 parts methanol to 1 part heptane was tried on a paper column. Palmitic and stearic acids were used in this development. Both acids peaked very well but eluted from the column right behind the solvent front.

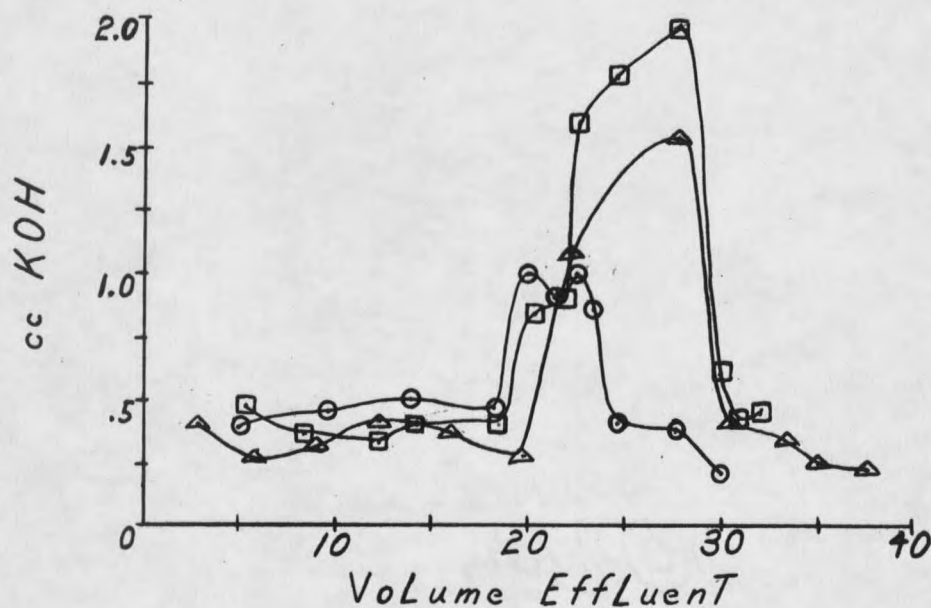


Figure 3. Paper column. Solvent: methanol:Skellysolve B in ratio of 1:1
 ○ - Stearic acid. ▲ - Arachidic acid. □ - Mixture of
 stearic and arachidic acid.

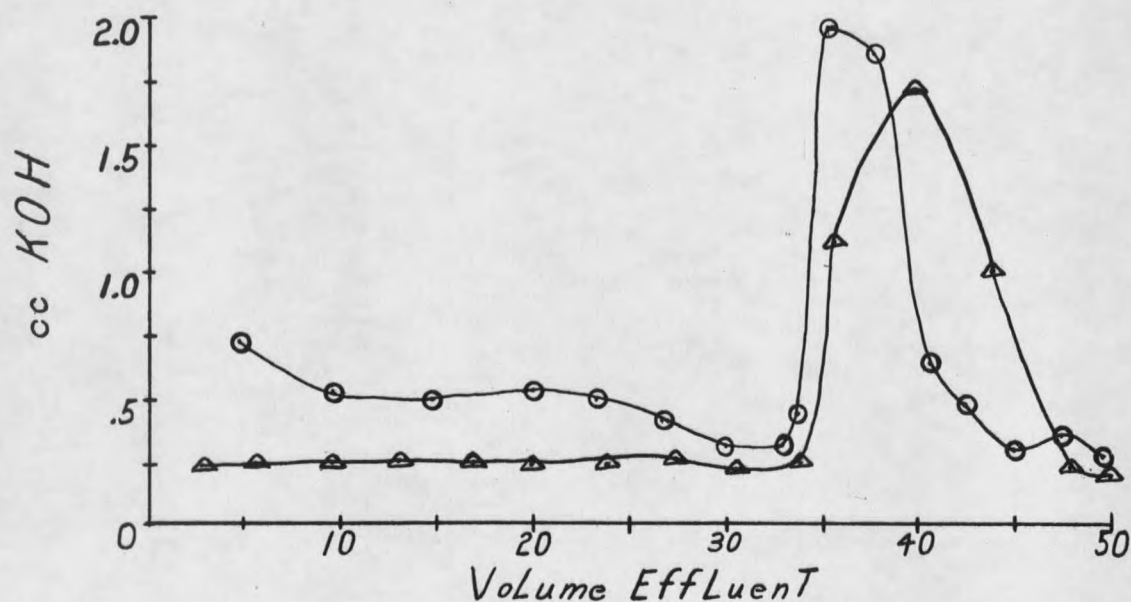


Figure 4. Paper column. Solvent: methanol:Skellysolve B in ratio of 1:1.
 ○ - Stearic acid. ▲ - Arachidic acid.

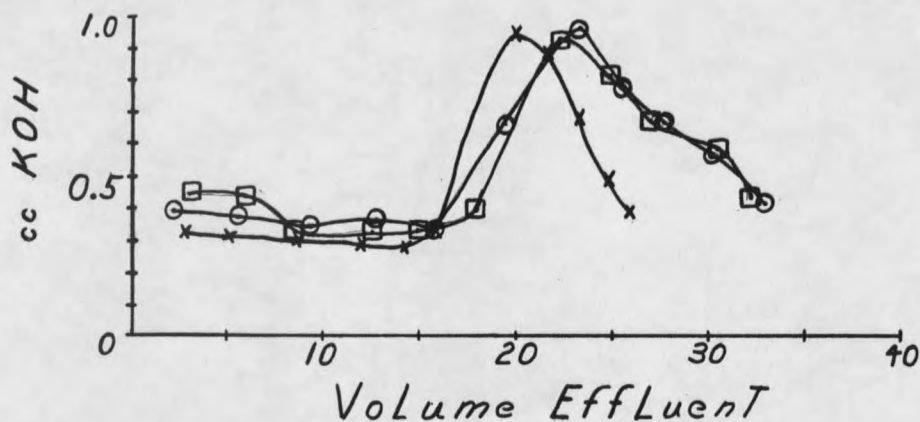


Figure 5. Paper column. Solvent: methanol:Skellysolve B in ratio of 3:1. x - Palmitic acid. $\odot$ - Stearic acid. $\square$ - Arachidic acid.

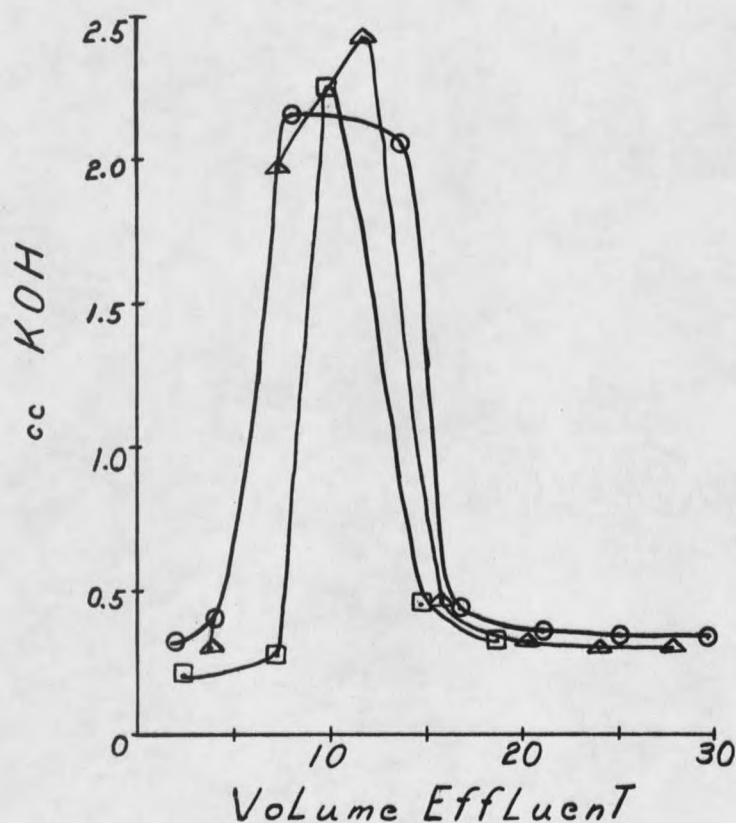


Figure 6. Paper column. Solvent: n-propanol:Skellysolve B in ratio of 3:1. $\odot$ - Palmitic acid. $\triangle$ - Stearic acid. $\square$ - Arachidic acid.

Different alcohols in combination with Skellysolve B were tried next on paper packed columns. N-propyl, allyl, and benzyl alcohols in a ratio of 3:1 with Skellysolve B were used to try to move the acids back from the solvent front. The curve obtained with n-propyl:Skellysolve B as a solvent is shown in figure 6. Palmitic, stearic, and arachidic acids were not moved back from the solvent front and did not show any tendency to separate. The same types of curves were obtained with allyl and benzyl alcohols. The results indicated on paper columns were not promising so the material was rejected as unlikely to affect fatty acid separations.

A 1:1 mixture by weight of magnesium oxide and Hyflo supercel was utilized next for column material using a solvent combination of Skellysolve B and acetone. This procedure is used by this laboratory in the routine analysis of plant material for carotene and it was hoped that fatty acids could also be separated by the system. Several trials were made using a mixture of acids. Elution peaks were noted in one trial but these results were not duplicated in repeat columns. This seems to indicate that any results obtainable on a magnesium oxide-Hyflo supercel column would be unpredictable and make this type of column very difficult to use.

In 1940, Bengen discovered the phenomena of urea complex formation with normal aliphatic compounds. Schlenk and Holman (31) have utilized this for a method of separating and stabilizing fatty acids. An attempt was made to adapt this method to column chromatography. Ethanol and isopropanol were used as solvents after saturation with urea. The columns

were packed using a slurry of urea in the alcohols. The acids came out with the solvent front but it was noted that only a small proportion of the applied acids emerged with the effluent solvent. It was further noted that as the length of the carbon chain of the acid increased the amount of acid eluted from the column diminished. This effect can be seen in figure 7.

The titer required for equivalent amounts of stearic acid was less than that for palmitic acid and the titer required for palmitic acid was less than that needed for myristic acid.

Attempts to adapt the method of Kritchevsky and Tiselius (17) for steroid separations to fatty acid separations gave the best indication of a system suitable for this type of chromatography.

Dow Corning Antifoam A was used as the silicone source. The paper was coated by suspending the antifoam in diethyl ether and mixing in sufficient quantities of the paper. The ether was then evaporated to leave the paper evenly coated with the silicone. The solvent system originally employed was ethanol:chloroform:water in the ratio of 10:10:6. This formed a two phase system. The coated paper was packed in the chromatographic columns using the lower phase. After addition of the acids by an alcohol solution, the columns were developed using the upper phase. It was found that a new column had to be prepared for each new run since the chloroform tended to remove the silicone from the paper. The first column was short, 7 1/2 inches long, and the mixture of acids showed a tendency to separate as shown in figure 8. This result indicated the need for a

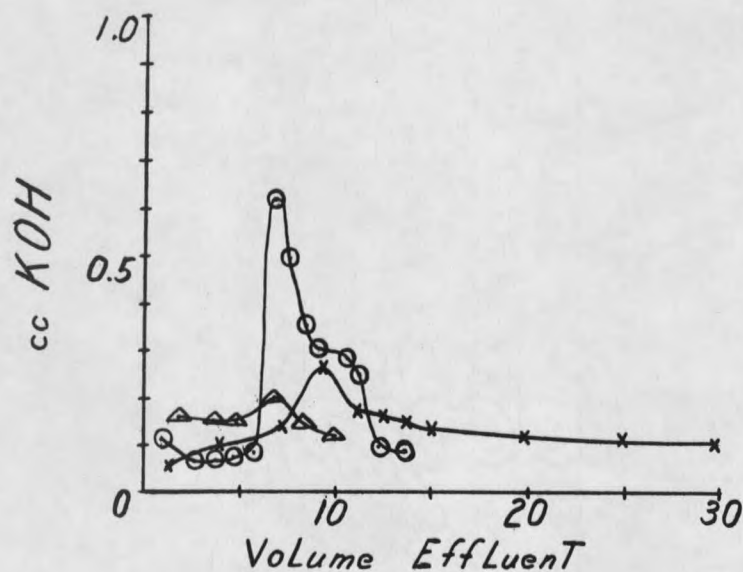


Figure 7. Urea column. Solvent: Isopropyl alcohol. $\circ$ - Myristic acid. $\times$ - Palmitic acid. Δ - Stearic acid.

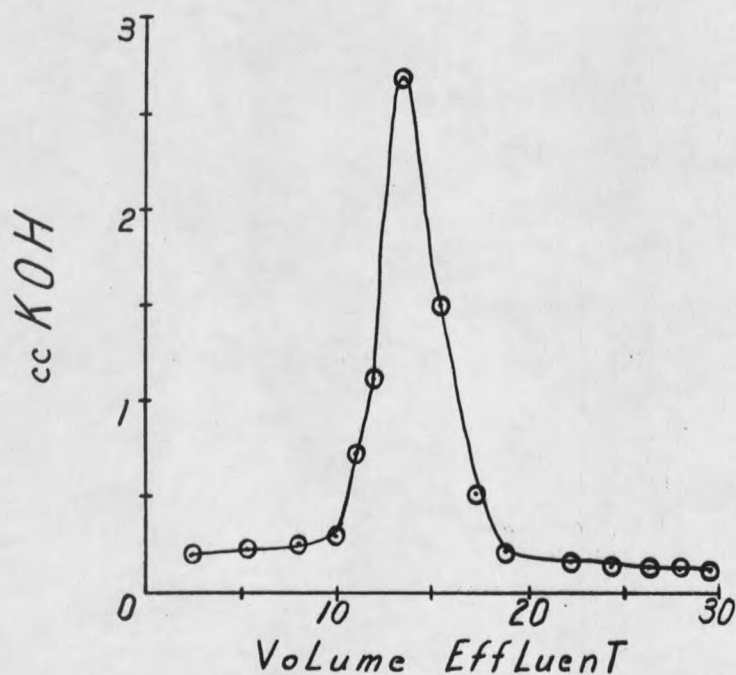


Figure 8. Silicone coated paper column. Solvent: ethanol:chloroform: water in ratio of 10:10:6. $\circ$ - Mixture of stearic and arachidic acids.

longer column. Figure 9 shows the curve obtained with a column 28 1/2 inches long. The resolution was good enough to show a drop in titer before the next acid eluted from the column. The order of acid elution was established by developing a column with 20 mg. stearic acid and 10 mg. arachidic acid.

Figure 10 shows the resulting curve. Again the resolution was good enough to show two distinct peaks. The area under the second peak showed conclusively that stearic acid eluted from the column after arachidic acid. The positions of the peaks in figure 10 were 10 cc. behind the peaks observed in figure 9. This was attributed to the 6 inch difference in column length and the amount of silicone coating on the paper. The amount of silicone present was found to affect the degree of separation. A trial column with no silicone present gave no resolution of C₁₈ and C₂₀ on a column 36 inches long. A trial was made using Skellysolve B instead of chloroform and no separation was indicated. Methanol, in place of ethanol, was tried. The column was difficult to pack and the flow rate was too slow to show any results. Isopropyl alcohol in the solvent system showed no improvement in the degree of separation of the fatty acids from that of the solvent system containing ethanol. These results indicated the need for further work with other silicones in conjunction with ethanol, chloroform, and water as the solvent system.

DISCUSSION

All attempts to affect separations of the higher molecular weight straight chain fatty acids by chromatographic techniques have given very

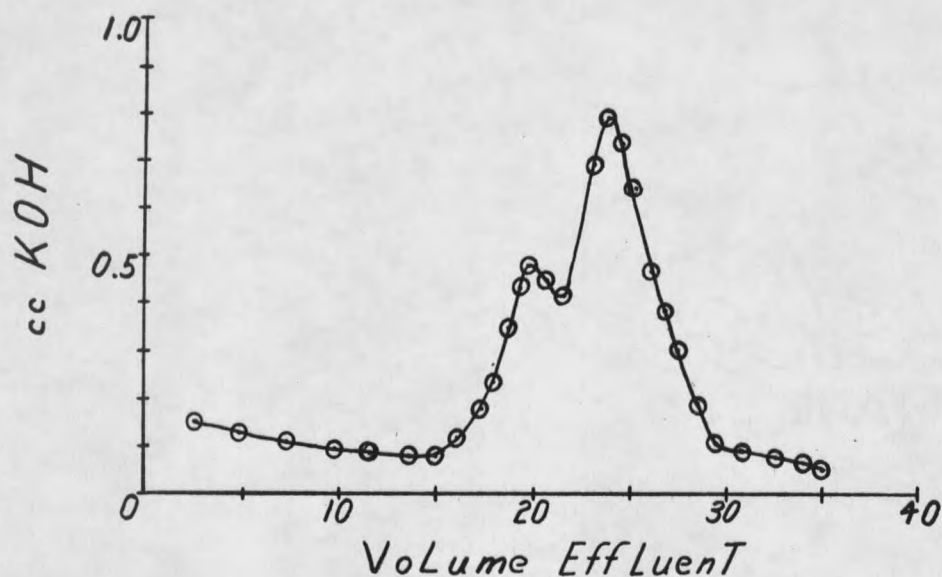


Figure 9. Silicone coated paper column. Solvent: ethanol:chloroform: water in ratio of 10:10:6. $\odot$ - Mixture of stearic and arachidic acids.

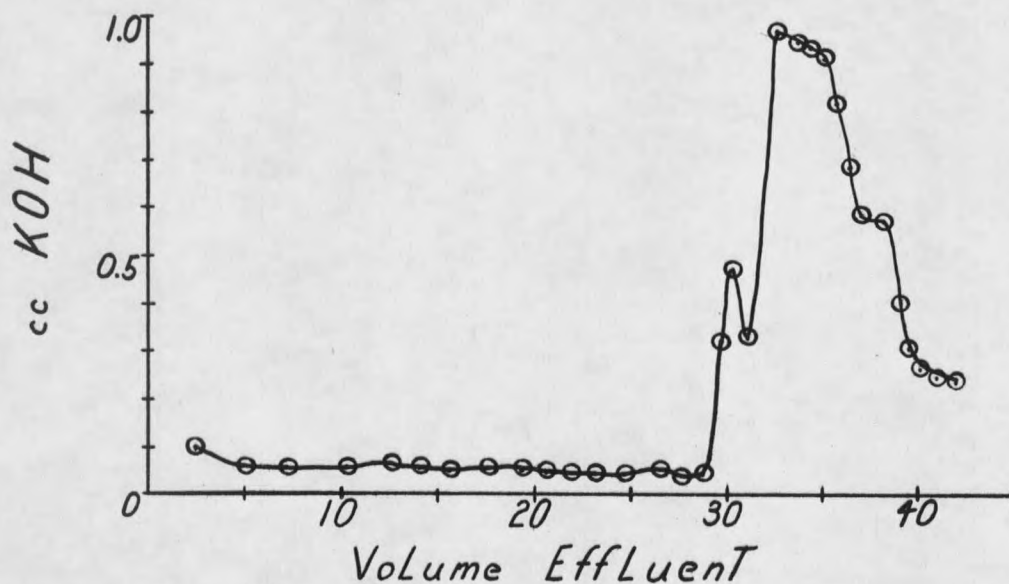


Figure 10. Silicone coated paper column. Solvent: ethanol:chloroform: water in ratio of 10:10:6. $\odot$ - Mixture with twice as much stearic acid as arachidic acid.

discouraging results.

Attempts to modify the charcoal method of Holman (4, 6, 7, 8, 9) for separations of the fatty acids did not succeed. The amount of water present in the solvent system had a tremendous effect on the rate of elution of stearic acid. The acid was eluted just behind the solvent front with absolute ethanol but did not appear at all when 80% ethyl alcohol was employed as the solvent. At 20° C., 2.25 grams of stearic acid are soluble in 100 grams of absolute ethanol while only 0.20 grams are soluble in 100 grams of 80.8% ethanol (20). In the previous trials 10 mg. of the acid was applied in one ml. of an absolute ethanol solution. When the developing solvent was absolute ethanol, the maximum solubility of stearic acid was not reached since one ml. of solvent will hold 22.5 mg. of the acid but 80% alcohol will hold only 2 mg. of the stearic acid which is much less than the amount applied to the column. This difference in solubilities with differing amounts of water would account for the vast differences in rates of elution providing that the degree of adsorption of the acid on the charcoal was the same in both cases. The experiments indicated that perhaps satisfactory results could be possible if the amount of water in the solvent system is carefully regulated. The separations affected by Holman always employed a carrier system of the methyl esters of the fatty acids present in the model mixtures. He found that the acids present in a system would elute from the column in front of the corresponding methyl ester. The elution of the methyl esters was followed by measuring the refractive index of the solvent with a modified Tiselius-Claesson adsorption

analysis apparatus as the solvent flowed from the column.

Holman (6) stated that the ratio of methyl esters to acids must remain high, probably in the order of 10:1 for best results since the acid reaches into the zone of the next lower methyl ester.

The results obtained from the alumina columns show a strong affinity of the alumina for the fatty acid. The solubility of stearic acid in 95% ethanol is appreciable, 1.13 gr./100 gr. solvent at 20° C. and this factor would not account for the great volume of effluent required before the acid eluted from the column. The adsorption product formed could be postulated as the aluminum soap of the fatty acid. The stability of such a product is so great that the solvent employed for column separations would not be able to reverse the reaction. This would mean that no separation of fatty acids would be possible on columns of aluminum oxide.

Nylon columns gave the first indication of a system that might separate fatty acids. In this series of columns, the higher molecular weight fatty acids came out first. The acetone constituent of the solvent seemed to be more strongly adsorbed of the nylon, thus this can be called a reversed phase partition system. The data from Table I shows the following interesting facts. When the per cent is below 20% (the remainder being 1:1 ethanol:water in all cases) no elution of acid occurs. When the per cent acetone is above 28% (remainder being 1:1 ethanol:water) elution occurs at about 12 cc. This is shown graphically in figure 11. It would seem that somewhere between 20% and 28% acetone, elution of stearic acid might occur between 80 and 100 cc., which, it seems, would be

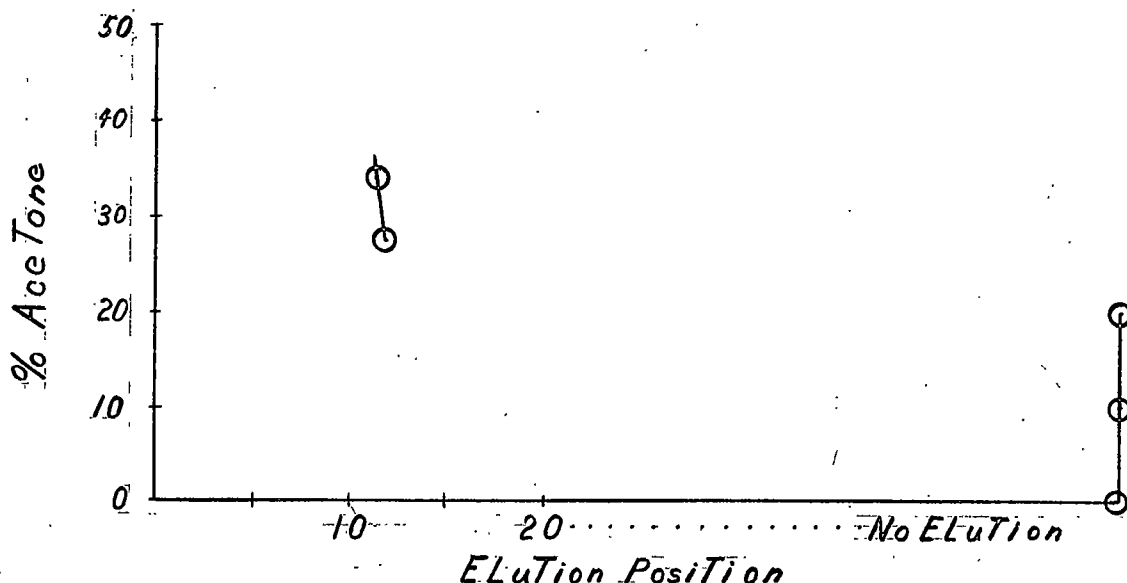


Figure 11. % acetone vs. elution position for stearic acid on nylon columns. Ratio of ethanol:water is 1:1.

a desirable position for separation of fatty acids. Whether or not this is the case is not known, since this position was not located in the few trials made. It seems more probable that no such position is possible and that there is either elution at a low volume or no elution at all, depending upon the acetone concentration. It appears more likely that a solvent system containing a higher ratio of ethanol:water, 8:1 or 9:1, with 20-30% acetone would be more favorable for elution of stearic acid in the region of 80-100 cc. Likewise, the use of other ketones might very likely give a system in which elution would occur in this range.

Attempts to separate the fatty acids by partition methods on paper columns did not give satisfactory results. The polar alcohol from the alcohol-Skellysolve or heptane solvent combination is more strongly adsorbed to the paper than the non-polar hydrocarbons. All the results ob-

tained from these trials with different alcohols; methyl, ethyl, propyl, allyl, and benzyl alcohols, and hydrocarbons, Skellysolve B and heptane, indicated that the fatty acids elute from the columns too close to the solvent front to give a satisfactory separation of the acids. All the previous trials were attempts to move the acids back from the solvent front and all were unsuccessful.

Another series of paper columns should be investigated using a solvent combination of acetone and absolute ethanol. The solubility of stearic acid in acetone is 1.54 gr./100 gr. solvent while its solubility in absolute ethanol is 2.25 gr./100 gr. solvent at 20° C. The fatty acids may move back from the solvent front if they are more soluble in the stationary ethanol phase than the moving acetone phase which was not the case under the experimental conditions. Although the solubilities of the fatty acids in Skellysolve or heptane are not known, they should be more soluble in these hydrocarbons than in the methanol since stearic acid is soluble in methanol only to the extent of 0.1 gr./100 gr. solvent at 20° C. (20). The paper columns did not effectively separate the fatty acids but this material cannot be discarded entirely until other solvent combinations have been tested.

This discussion will not include the work involving columns of magnesium oxide:Hyflo supercel and urea because of the small amount of data available. The only point of interest from these two systems is the decreasing amounts of acid that eluted from urea columns as the molecular length of the acid increased.

The phenomena of complex formation has been used for stabilization

of fatty acids (31) and the story of urea complexes with long chain compounds has been discussed elsewhere (21) so no time or space will be devoted here for such a discussion.

Silicone coated paper, when used as a column material, gave the best indication of a system capable of separating the fatty acids. The curves obtained with this system are shown in figures 8, 9, and 10. The silicone source used has been Dow Corning Antifoam A. Figure 12 shows the probable molecular arrangement of a cross-linked silicone resin (26).

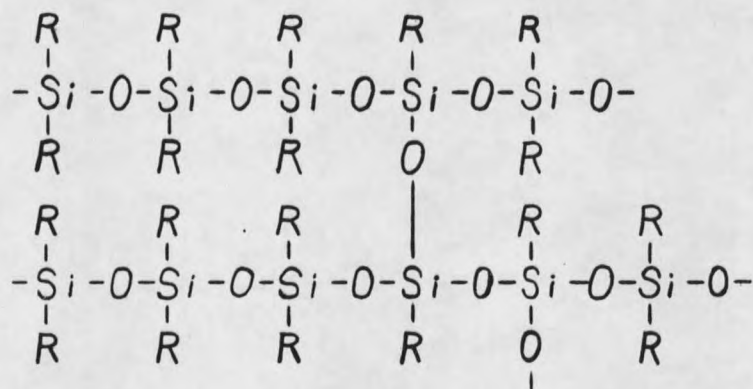


Figure 12. Probable arrangement of a cross-linked silicone resin.

The R groups will vary from resin to resin but are usually methyl or ethyl groups. The degree of cross-linkage will also vary between resins. The exact structure of the Antifoam A as to the degree of cross-linkage and the kind of R grouping are not known to me at the present time. The silicones are not affected by alcohols which is important when alcohols are present in the solvent system. The chloroform present in the solvent was the factor that removed the silicone from the paper and which necessitated a fresh column for each trial. The presence of the silicone was to render

the paper "unwetttable". The arrangement of the hydrophobic R groups in the resin adsorbed the more non-polar hydrocarbon constituents of the solvent system in preference to the hydrophylic alcohol portion. The solvent system was a 10:10:6 mixture of ethanol:chloroform:water which formed a two phase system, the upper phase of which was more polar than the lower. The column was packed by means of a slurry of coated paper and the lower phase. This essentially saturated the silicone with the non-polar phase which became the stationary phase when the column was developed with the more polar upper phase. The observed partial separation of the fatty acids depended upon the partition of the fatty acids between the non-polar stationary phase and the more polar moving phase of the solvent system. Skellysolve B was tried in place of the chloroform and no success was indicated showing that chlorinated hydrocarbons are preferred for the more non-polar phase of the solvent. Other chlorinated compounds, such as carbon tetrachloride, ethylene dichloride, ethylene tetrachloride, and ethylidene chloride, should be tested in place of the chloroform portion of the solvent. The alcohol portion of the solvent was changed but no better results were obtained with methyl and isopropyl than with ethyl alcohol so further modifications in this direction were terminated. Successful separations of the high molecular weight straight chain fatty acids by means of columns of paper coated with a silicone compound will not be possible until other sources of silicones have been tested with the abovementioned chlorinated hydrocarbons in combination with ethanol and water as the solvent systems.

SUMMARY

The possibility of chromatographic separation of the high molecular weight fatty acids has been studied. Several materials have been tested for possible application to this method of separation. The column materials were packed in long glass columns and the fatty acids applied on the top of the column by means of a solution containing 10 mg. of the acid in one of the constituents of the solvent system used in the development of this particular column material. The effluent was analyzed by titration with potassium hydroxide and the results were graphed by plotting volume potassium hydroxide required for the titration against the volume of effluent that passed through the column.

In this manner, several materials were tested. Charcoal and alumina were found to be ineffective for separations of the fatty acids studied. Nylon columns were successful only to a slight degree and should be tested further for possible separation properties. Paper columns were only partially successful and likewise should be tested further. Urea and magnesium oxide appear to be ineffective from the small amount of work done with them. Silicone coated paper columns gave only partial separation but this material appeared more promising for separation of these fatty acids. More work should be done on this method using different silicones and other solvent systems.

Complete separation of fatty acids was not accomplished but further work should be done before chromatographic methods are discarded since a partial separation of fatty acids was found to be possible and thus

further work is recommended on this method of separation.

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