each other have not been changed, but there is insufficient evidence to decide whether there is a greater or lesser spread between any two curves at the higher or lower temperatures.

In applying the data reported here to adsorption analysis it may be reasoned that, assuming no influence of the acids upon the adsorbabilities of each other in a mixture, the acid with a greater adsorbability as indicated by its adsorption isotherm will form a zone above that with a lesser adsorbability in the adsorption column.[3,4] This follows, too, from the theory of Wilson, as he has pointed out.[9] This assumption is, however, one which should be made with caution.

It is particularly interesting that adsorbents of the same type (*i. e.*, carbons) have been obtained on which the relative adsorbabilities of lauric and stearic acids are reversed, for this gives a means of comparing the positions of adsorption on the columns of these two acids, as well as their separabilities, with their adsorption isotherms on these adsorbents. These points will be considered in a later communication.

The decrease which is found in adsorption from ligroin indicates that this solvent might have useful properties as an eluent or desorbing liquid for fractional removal of adsorbed acids from portions of adsorption columns.

I am indebted to Professor R. J. Anderson, who provided some of the materials used in this work.

Summary

As a first step in the systematic study of adsorption analysis as a means of separating higher fatty acids it has been shown by comparisons of the adsorption isotherms that lauric, myristic, palmitic and stearic acids differ markedly in their adsorption behaviors toward carbons, aluminas, magnesium oxide, active clay and silica gel.

NEW HAVEN, CONN. RECEIVED JUNE 19, 1940

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[9] Wilson, THIS JOURNAL, **62**, 1583 (1940).

[CONTRIBUTION FROM THE STERLING CHEMISTRY LABORATORY, YALE UNIVERSITY]

Adsorption Analysis. III. Relation between Adsorption Isotherm and Position on the Adsorption Column[1]

BY HAROLD G. CASSIDY

In a previous paper[2] the adsorption behaviors of lauric, myristic, palmitic and stearic acids in petroleum ether solutions were compared toward a number of different types of adsorbents. This was a first step in a systematic attempt to find the most suitable adsorbent for the separation of these fatty acids from their mixtures. In the course of that investigation adsorbents were found some of which (a) adsorbed the higher molecular weight acids better than the lower, others of which (b) adsorbed the lower molecular weight acids better than those of higher molecular weight, and still others of which (c) did not differentiate appreciably between the different acids.

Availability of adsorbents with these different behaviors made it possible to examine the following problem. If the simple assumption may be made that there is little influence of each of the acids in a mixture upon the adsorbabilities of the others, then it would be expected that the acid with the greater adsorbability, as indicated by its adsorption isotherm, would form a zone above that of the acid with the lesser adsorbability when a mixture of the two is chromatographed, and the differences in adsorbabilities as judged from a comparison of the respective isotherms would give an indication of the ease of separability of the two acids. If this assumption were valid, or approximately so, the search for suitable adsorbents for making separations such as this one would be greatly simplified, for isotherms of this type are comparatively easily obtained.

The following data show that adsorption isotherms obtained for these higher fatty acids from their pure solutions are of uncertain value as criteria of separability of the acids by the method of chromatographic adsorption analysis.

Materials and Solvents.—These were the same as those described previously.[2]

Methods.—The Tswett adsorption method was used. The column of adsorbent was placed in a glass tube. It was supported on a small pad of cotton which in turn was

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[1] Part of the data is taken from the dissertation submitted by H. G. Cassidy to the Faculty of the Graduate School, Yale University, 1939, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Cassidy, THIS JOURNAL, **62**, 3073 (1940).

held by a perforated porcelain disk which rested on a constriction in the tube. Sometimes the adsorbent, following the very useful method of Strain,[3] was mixed with a filter aid to give more uniform packing and freer flow. Figure 1 shows that neither of the filter aids[4] nor the cotton acted as adsorbents toward lauric acid. Mechanical retention accounts for the small amounts of acid held by these substances.

After the column was prepared it usually was saturated with dry petroleum ether. The fatty acids dissolved in 100 cc. of dry petroleum ether were then added just as the previously applied liquid passed into the column. This was followed by the addition of petroleum ether as the developer liquid. Care was taken that the top of the column did not become dry. The liquid passing out of the column was collected usually in 100-cc. portions. After development the column was either extruded from the tube, sectioned, and the sections eluted, or else the eluent was added directly to the column in the tube. If the column was sectioned the sections were numbered from the bottom of the column upward. The lower numbers thus indicate lower adsorbabilities whether they refer to percolate fractions or to fractions of acid from elution of sections of adsorption columns.

The liquids were forced through these columns usually under a small air pressure. If unknown substances or unstable ones are being handled nitrogen should be used, and indeed the use of nitrogen is now standard practice in this Laboratory as a safety measure.

Elutions were carried out with petroleum ether containing about 5% of acetone or 2% of methanol, or benzene containing about 5% of acetone. No attempt was made in most cases to obtain quantitative elution. It should be noted that when the elutions are not very complete the less well adsorbed parts of the adsorbate will predominate in the eluted mixture. This must be taken into account in interpreting the results of inefficient elution. This does not seriously affect the data presented in this paper, however, for in all cases the developer liquid washed enough material out of the columns to show the courses of the separations and the results of the elutions needed to be of a confirmatory nature only, which they were.

Average equivalent weights of fractions were determined by titrating samples of the fractions as obtained from the columns after removal of solvent, and without recrystallization. Micro or semimicro samples were weighed, dissolved in 2 cc. of boiling neutralized 95% alcohol, and titrated to the phenolphthalein end-point with approximately 0.02 *N* alcoholic sodium hydroxide. Not more than 1% error was found in titrating similar samples of known acids such as lauric and stearic.

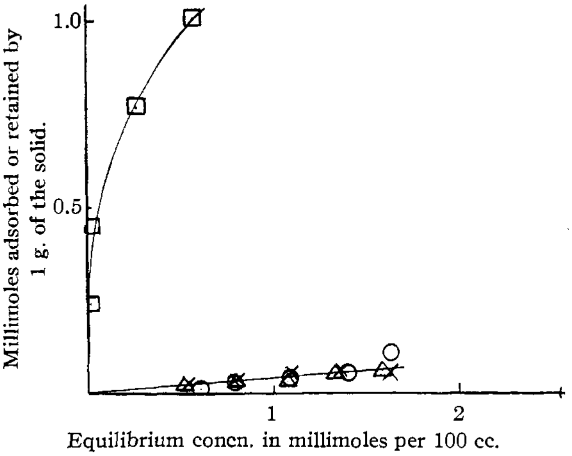

Fig. 1.—Retention of lauric acid by: ×, Hyflo Super-Cel filter aid; △, Speedex Dicalite filter aid; ○, Cotton; □, Comparison curve of adsorption on Carbon I. The solvent in all cases is petroleum ether.

Results. (a) Adsorbent which Adsorbs the Higher Molecular Weight Acids More Strongly than Those of Lower Molecular Weight. Carbon I.—Carbon I[2] shows a regular increase in strength of adsorption of the fatty acids from lauric to stearic with increase in molecular weight (even-numbered acids). A number of experiments have shown that Carbon I will separate a mixture of lauric and stearic acids almost quantitatively, the lauric acid appearing almost pure in the percolating liquid at the bottom of the column, or remaining on the column at the bottom, and the stearic acid remaining at or near the top of the column. One such experiment is reported in some detail below.

One gram of Carbon I was packed in a tube to form a column 1.3 cm. in diameter and 3 cm. in length. Lauric acid, 0.1506 g. (0.75 millimole) and 0.1538 g. of stearic acid (0.54 millimole) were dissolved in 100 cc. of petroleum ether and added to the column immediately after it was prepared. The column became warm at the region where the advancing front of the liquid moved down it. Air pressure of about 20 cm. of mercury was used in developing the column. Data for this experiment are shown in Table I. Total amount of acid

TABLE I

Percolate in cc.	Acid present, g.	M. p. (cor.), °C.	Equiv. wt.	Nature of fraction
100	0.0251	42–42.8	198.2 204.2	Almost pure lauric acid
100	.0954	43.6	198.3 199.2	Pure lauric acid
100	.0160	43.3–43.6		Pure lauric acid
100	.0079	41.5		Impure lauric acid
300	.0178	57–58	241.2	Almost half stearic

Petroleum ether containing 2% methanol was added at this point as eluent:

Percolate in cc.	Acid present, g.	M. p. (cor.), °C.	Equiv. wt.	Nature of fraction
100	0.1084	69–70	282.8 281.0	Pure stearic acid
100	.0121	68–68.2		

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(3) Strain, *J. Biol. Chem.*, **105**, 523 (1934).

(4) Thanks are due to The Dicalite Company and to Johns-Manville for samples of their filter aids.

recovered was about 93%, of which a small middle fraction, about 8%, consisted of mixed acids.

From this experiment it may be seen that the behaviors of the fatty acids on the column are consistent with their adsorption isotherms. That is, stearic acid is better adsorbed than lauric under both conditions.

(b) **Adsorbents which Adsorb the Lower Molecular Weight Acids Better than Those of Higher Molecular Weight. Silica Gel.**—According to the adsorption isotherms, Silica Gel I[2] adsorbs lauric acid to a greater extent, mole for mole, than it does stearic acid. The question was whether or not a mixture of these two acids would be separated on an adsorption column of silica gel and whether or not stearic acid would take the lower position in the column.

Five grams of silica gel and 1 g. of filter aid were mixed and packed in a glass tube to form a column 1.3 cm. in diameter and 6.3 cm. long. The column was wet down with 100 cc. of petroleum ether and became warm at the region of the advancing face of the solvent. Lauric acid, 0.3011 g. (1.5 millimoles), and 0.4126 g. of stearic acid (1.45 millimoles) were dissolved in 100 cc. of petroleum ether and applied to the column. Results of this experiment are shown in Table II.

TABLE II

Fraction	Percolate, cc.	Acid present, g.	M. p. (cor.), °C.	Equiv. wt.	Nature of fraction
1	100	0.0089	66.5–67.5		More stearic than lauric acid
2	100	.1263	64–67	270.7	
3	100	.0627	63–64	260.6	
4	100	.0422	63–64	257.3	
5	100	.0309	63		
6	100	.0236	61–61.5	251.5	
7	100	.0190	61	250.7	

The column was extruded, sectioned and the sections eluted

Fraction	Section	Solids eluted, g.	M. p. (cor.), °C.		
8	Bottom	0.1081	49–51	230.2	
9	Middle	.1347	44.5–45	225.6	More lauric than stearic acid
10	Top	.0920	43–44	223.2	

The results of this experiment indicate that the separation of the two acids was not very complete. It is perfectly clear, however, that the lower position in the column was being taken by the stearic acid, and this is consistent with the relative positions of the adsorption isotherms.

Carbon VIII.[5]—According to the adsorption isotherms of lauric acid and stearic acid on carbon VIII[2] lauric is better adsorbed than stearic from pure solutions of the two acids. The behavior of a mixture of these acids was investigated in the following experiment.

Five grams of carbon VIII was mixed with 5 g. of filter aid and packed in a tube to form a column 1.6 cm. in diameter and 10.5 cm. long. As soon as the column was packed, 100 cc. of petroleum ether was added to it under an air pressure of about 10 cm. of mercury. The column became warm in the region of the advancing face of the liquid. Lauric acid 0.3000 g. (1.5 millimoles) and 0.4107 g. of stearic acid (1.44 millimoles) were dissolved in 100 cc. of petroleum ether and applied to the column. The results of the experiment are summarized in Table III.

TABLE III

Fraction	Percolate, cc.	Acid present, g.	M. p. (cor.), °C.	Equiv. wt.	Nature of fraction
1	100	0.0003			
2	100	.0155	43–44	These three mixed 203.8	Lauric acid
3	100	.0116	43–44		
4	100	.0090	43–44		
5	100	.0062	43–44		

At this point the column was extruded, sectioned, and the sections eluted. Complete elution was not obtained,[6] but since lauric acid was present in the first percolates this was sufficient to the object of the experiment. It showed that lauric acid was the less well adsorbed from its mixture with stearic acid on Carbon VIII in the adsorption column. This relation between the two acids is the opposite of that found in a comparison of the adsorption isotherms obtained for the pure acids.

(c) **An Adsorbent which Does Not Differentiate Appreciably between Acids of Different Molecular Weights. Carbon V.**—According to the adsorption isotherms from petroleum ether[2] Carbon V[7] adsorbs lauric and stearic acids very strongly but does not appear to differentiate between them except as the shapes of the isotherms are slightly different. The following experiment was carried out to determine whether or not this carbon, since it adsorbed both acids almost equally strongly, would separate them from a mixture.

Two grams of Carbon V and 2 g. of filter aid were mixed and packed in a glass tube to give a column 1.3 cm. in diameter and 6.5 cm. in length.

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(5) Cliffchar R-Fine, Cliffs Dow Chemical Company.

(6) When Carbon VIII is shaken with initially dry petroleum ether solutions of fatty acids, white particles appear which sink less rapidly than the carbon and so are found on the surface of the settled adsorbate. These yield an alkaline residue on ignition. This complicates the problem of elution, but does not affect the conclusion from this experiment.

(7) Columbia activated carbon Grade 5P, Carbide and Carbon Chemicals Corporation.

This was wet down with 100 cc. of petroleum ether and became warm in the region of the advancing face of the liquid. Lauric acid, 0.3012 g. (1.5 millimoles), and 0.4107 g. of stearic acid (1.44 millimoles) were dissolved in 100 cc. of petroleum ether and applied to the column under an air pressure of about 10 cm. of mercury. The results of this experiment are summarized in Table IV.

TABLE IV

Fraction	Percolate, cc.	Acid present, g.	M. p. (cor.), °C.	Equiv. wt.	Nature of fraction
1	100	0.1283	41–43	218.6	Impure lauric acid
2	100	.1545	47–52	230.5	
3	100	.0304	45–47		
4	100	.0190	46–47		
5	100	.0127	46–48		
6	100	.0103	49–51.5		

The column was extruded, sectioned and the sections eluted

	Section	Solids eluted, g.			
7	Bottom	0.0557	53–56.5	236.5	
8	Middle	.0472	57.5–58.5	247.0	
9	Top	.0252	61–62.5	260.4	Impure stearic acid

It may be seen from the data of this experiment that a partial separation of the mixture of lauric and stearic acids had occurred.

Discussion of Results

The data reported above show that it is unsafe to predict, from a comparison of the adsorption isotherms of pure fatty acids, whether or not they will be separable from mixtures on adsorption columns. It is also evident from the data that the positions taken on the columns cannot with assurance be predicted from a comparison of the isotherms.

These observations indicate that when the acids are mixed in solution, each exerts an effect on the adsorbability of the other. These effects appear not to be negligible. Their magnitudes and directions are under investigation.

It is known that in most cases of adsorption from mixtures the adsorption of one solute is affected by the concentrations of the other solutes present. Thus the amount of lauric acid adsorbed from a mixture with stearic acid might be expected to change with changes in concentration of the stearic acid. It has been pointed out by Wilson,[8] in his development of a theory of chromatography, that there are at present no general rules for predicting such effects from the adsorption isotherms which apply to pure solutions of individual solutes.

It was the object of these experiments with different adsorbents to find out whether these effects were marked for such substances as lauric and stearic acids. If the influence of one acid on the adsorption of another in the mixture had not been very great, then it would have been possible to apply the simplified calculations advanced for such cases by Wilson.[8] (Such calculations may be found possible in the case of Carbon I when more data are available.)

It appears, however, that the influence of each acid upon the other may be very marked in some cases, thus precluding the use of the simpler (and more restricted) of Wilson's two formulations.

I am indebted to Professor R. J. Anderson for providing some of the materials used in this work.

Summary

It has been shown that the adsorption isotherms obtained for fatty acids from their pure solutions are of uncertain value as criteria of separability of the acids by the method of chromatographic adsorption analysis.

NEW HAVEN, CONN. RECEIVED JULY 25, 1940

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[8] Wilson, THIS JOURNAL, **62**, 1583 (1940).