The control of the pH of this aqueous solution also determines which dibasic acids will be resolved. We have made use of these methods in our laboratory for the study of positional isomerization of unsaturated fatty acids and have found them to be very excellent methods.

Another good example of partition chromatography was described in a recent publication (14). This chromatographic system employed a methyl cellosolve-water ammonia system on a silicic acid support as the stationary phase and petroleum ether-butyl ether mixtures as the mobile phase. Monocarboxylic acids from 14- to 2-carbon chain lengths are resolved by the column, using petroleum ether as the solvent. Dibasic acids from 22 carbons down are eluted with butyl ether-petroleum ether. Samples of only a few milligrams of the mixed acids are used, and the mono- and dicarboxylic acids can be resolved in one column. This chromatographic method should find increasing use in the analysis of fatty acids.

Fatty acids may also be separated by the technique known as gas-liquid chromatography. This method makes use of the partitioning of the fatty acids between a mobile gas phase and a liquid stationary phase. As described by James and Martin (7), the apparatus consists of a long column packed with a Celite-silicone oil mixture. The column is surrounded by a heating jacket so that the vapor of a boiling liquid surrounds the column and controls the temperature of the apparatus. The gas phase, a stream of nitrogen, carries the vaporized acids through the column into a titration cell, where they are absorbed in water and titrated. The method gives a very good resolution of fatty acids from formic to lauric and will also separate some isomeric acids such as isovaleric, methyl ethyl acetic, and n-valeric acids. The temperature of the column, which is maintained by a boiling liquid, depends on the vapor pressure of the acids to be separated. This should be between 10 and 1,000 mm. of mercury. For example, cellosolve, which boils at 137°, is suitable for the separation of acids from valeric to lauric. This method permits automatic titration of the acids as there is no large volume of liquid-eluting solvent to collect.

This presentation has only touched on a very few of the procedures that have been described for chromatographic separations in the field of lipids. In view of the infinite experimental procedures that are possible using this technique, it is indeed a very fertile field of research both for preparative work and analysis.

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Distillation

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DISTILLATION is a separation process—one of four included in the 1955 Short Course. The others are chromatography, crystallization, and solvent extraction. All four have the common characteristic that distribution occurs between two phases that are mechanically separable:

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distillation
vapor-liquid
chromatography
fluid-stationary
crystallization
liquid-solid
extraction
liquid-liquid

Separation is possible only when one component is enriched in one phase and depleted in the other relative to another component. In practice these separation processes are not sharply differentiated. Distillation may be combined with extraction to yield the familiar extractive distillation process. Gas chromatography combines some of the characteristics of distillation and extraction as well as the chromatographic technique.

For present purposes the subject of "Distillation" is limited in scope to "analytical techniques." Techniques of vacuum analytical distillation applicable to fatty oils and their derivatives include equilibrium distillation and molecular distillation. We will consider the mixture, the apparatus, and the procedure and will briefly outline distillation theory.

Mixtures of greatest interest to members of the American Oil Chemists' Society are animal and vegetable fats and waxes. The fats are glycerol esters of fatty acids, and the waxes consist mainly of higher alcohol esters of fatty acids. Derived from these by saponification are mixtures of alcohols or acids that, in turn, are the raw materials of a growing chemical industry. Some of the mixtures, such as lard, tallow, cottonseed, soybean, or marine oils, have great economic value. Others, like human hair oil or the bacterial waxes, have little commercial value but may be interesting scientifically.

Apparatus suitable for distilling such a range of mixtures includes equilibrium stills and molecular stills. The choice for a particular analysis will depend primarily on the vapor pressure and thermal stability of the sample. Multistage equilibrium stills are widely used for fractionation of free fatty acids, esters of fatty acids with low boiling alcohols, ni-

triles, fatty alcohols, and the like. At reduced pressures their vapors can exist in true equilibrium with the liquid state. Molecular stills are useful for mixtures having low thermal stability or for stable materials with extremely low vapor pressures.

The question of how completely a mixture needs to be separated depends on the use to be made of the data. How completely a mixture can be separated depends on the properties of the mixture, particularly the relative volatilities of the components, and on the characteristics and operation of the apparatus. Where sample size is the limiting factor, the analyst has a choice of miniature apparatus or the use of added components to spread the mixture (24). With few exceptions the fatty acids and alcohols of natural origin belong to homologous series whose members differ by two carbons. Separation is easy because the boiling points are widely spaced.

Equilibrium Distillation

Equilibrium distillation at reduced pressure differs from molecular distillation in the respect that the presence of noncondensable gases resists evaporation. For distillation to occur the vapor must push away the noncondensables to reach the condensing surface. This causes many of the vapor molecules to be reflected back to the liquid surface where they exchange their latent heat of vaporization with the liquid phase. True equilibrium is thereby approached but actually reached only when no vapor is withdrawn.

Theory. Vapor-liquid equilibrium data for the methyl ester series in contrast with the free fatty acids show that behavior is nearly ideal, *i.e.*, Raoult's law is followed closely (15). The ideal behavior of mixtures of methyl palmitate and methyl stearate at a pressure of 4 mm. is illustrated by Figure 1. Since ester distillation figures so importantly in fat analysis, a brief discussion of ideal mixtures and the definition of the theoretical plate are pertinent.

Dalton's law states that the total pressure of a vapor is equal to the sum of the partial pressures of the components. The partial pressure of a component in the vapor above an ideal liquid mixture is, according to Raoult's law, equal to its vapor pressure at that temperature multiplied by its mole fraction in the liquid:

$$\overline{p}_A = p_A x_A$$

The composition of the vapor in equilibrium with liquid mixtures of methyl palmitate and methyl stearate calculated according to Raoult's law from known vapor pressures is represented by the solid line in Figure 1. Agreement with the experimental results is very good.

To define the theoretical plate for ideal systems Rose (22) uses the term "volatility" interchangeably with "vapor pressure." Thus the single-stage separation factor, α, relative volatility or relative vapor pressure of the two pure components, is an indication of the degree of separation obtainable with one theoretical plate. Where "A" is the lower boiling of two components, α is defined by the following ratios:

$$\alpha = p_A/p_B = v_A/v_B = [A/B]_{vapor}/[A/B]_{liquid}$$

The number of stages, n, to make a given separation is

$$\alpha^n = [A/B]_{distillate}/[A/B]_{pot.}$$

Binary mixtures of free fatty acids show non-ideal behavior. Vapor-liquid equilibrium data for the myristic acid-lauric acid system plotted in Figure 2 deviate significantly from the dashed line representing a Raoult's law calculation. For such systems a correction factor called the "activity coefficient" theoretically relates the vapor pressure to the volatility.

$$v_A = p_A\gamma_A \ (\gamma = 1 \text{ in ideal systems})$$

Since γ often varies with the composition, it is common practice to determine the plate requirement for such non-ideal mixtures by stepping off the stages of separation on the experimentally determined vapor-liquid equilibrium curve.

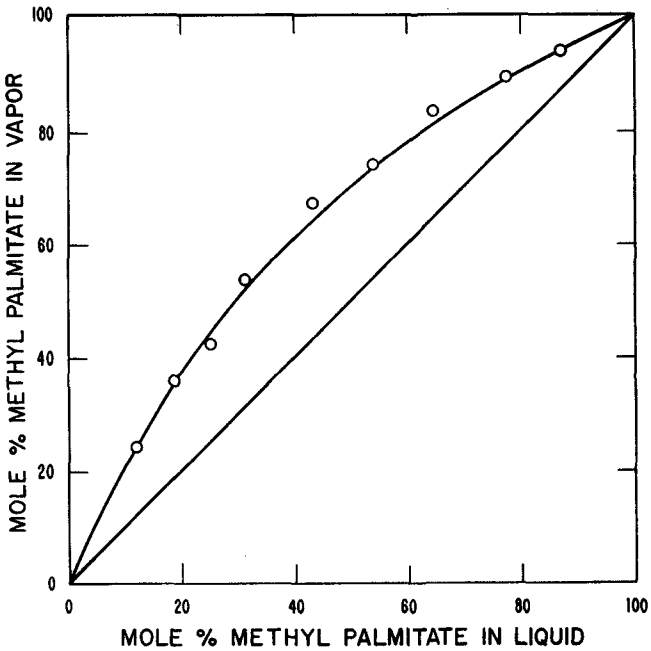

FIG. 1. Vapor composition vs. liquid composition for methyl palmitate-methyl stearate at 4-mm. pressure (15).

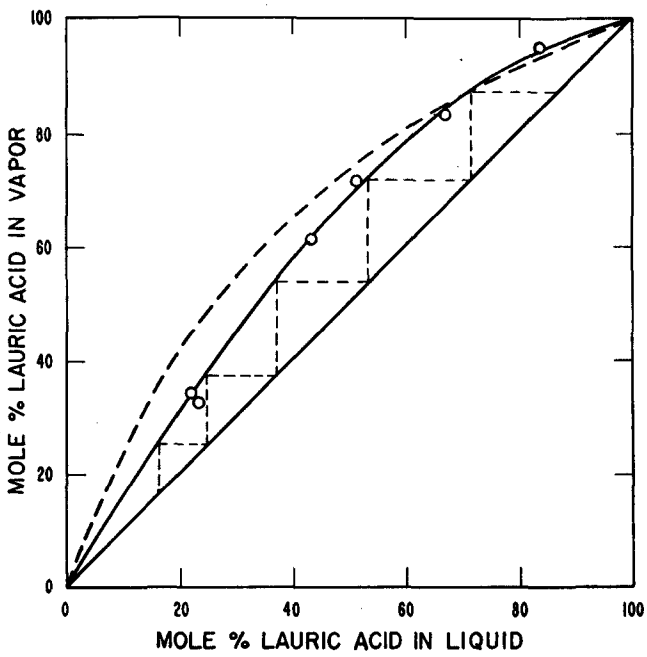

FIG. 2. Vapor composition vs. liquid composition for lauric acid-myristic acid at 4-mm. pressure (15).

The single-stage separation factors for pairs of saturated methyl esters are plotted in Figure 3 over the range of 1- to 10-mm. pressure. The α's get larger as the pressure is lowered but decrease with increasing molecular weight. The ester pairs of greatest interest to this group have α's in the range of 2 to 4.

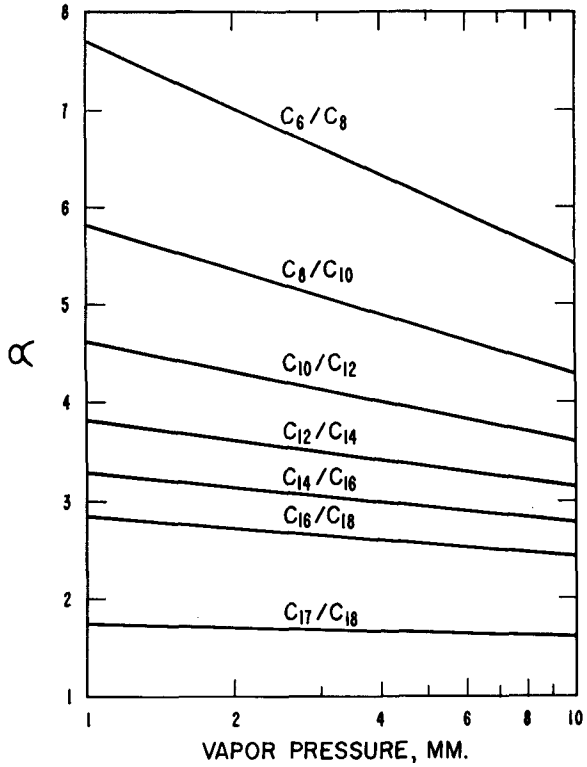

FIG. 3. α-Values for methyl esters.

The number of plates needed to achieve a given separation is plotted as a function of the separation factor in Figure 4. The separation of methyl palmitate from methyl stearate, each in 99.9% purity, requires only about 15 to 20 theoretical plates over the range of 1- to 10-mm. pressure. The adjacent homologues, methyl margarate and methyl stearate, with half the difference in boiling point ($\triangle T = 10°C.$) would take twice as many plates. Over small ranges of pressure or molecular weight, the number of stages of separation, n, will be approximately inversely proportional to $\triangle T$. Hence such difficult separations as methyl oleate or methyl isostearate from methyl stearate, $\triangle T$ about 3°C., for which precise equilibrium data are not available, would need upwards of 100 plates.

In packed columns the length of packed section that produces one plate of separation at total reflux is called the height equivalent to a theoretical plate, or H.E.T.P. The effectiveness of packed columns for analytical purposes depends on how the H.E.T.P. is affected by changes in throughput and reflux ratio. An efficiency factor obtained by dividing throughput by holdup per plate compares columns with respect to how well they retain their separating power when throughput is increased.

Reflux ratio is the proportion of condensate returned to the column as reflux relative to that taken off as product. The separating power increases with increasing reflux ratio, but in practice little is gained by having the reflux ratio exceed the number of theoretical plates. Low holdup aids separation, particularly at high reflux ratio or low throughput.

Apparatus. The development of apparatus for equilibrium distillation probably began with simple direct-fired retorts for the concentration of ethyl alcohol. Similar apparatus was applied to ester fractionation when vacuum pumps became available. As recently as 25 years ago Claisen flasks and Willstätter bulbs were used. However some analysts doubted that the distillate fractions were simple binary mixtures and questioned the validity of the customary method of calculating composition on the basis of saponification equivalents. During that period such non-existent fatty acids as margaric and daturic were often reported. Some major components were missed. Thus the C_{20} acid comprising 10–15% of rapeseed oil was regularly overlooked until about 10 years ago.

The early work on packed columns at Pennsylvania State University provided some fairly efficient packing materials and defined some of the important operating variables. Results obtained on a column packed with single-turn glass helices established ester distillation as an analytical technique (11). This still was equipped with a reflux controller and variable heaters for control of column temperature. It probably had a separating power near 20 plates. Although crude by modern standards, it produced spectacular separations of the methyl esters of the fatty acids of butter fat. For the first time composition could be estimated from boiling point data. Distillation curves, such as the one shown in Figure 5, gave positive evidence of the presence of each homologue reported.

A few years later, with the advent of Stedman screen-cone packing, a still with more than 60 plates was constructed (25). It was used for the analysis of many samples of hydrogenated sardine oil and animal fats and was efficient enough to separate the odd- and even-numbered normal acids of human hair fat (26). A more difficult separation that was accomplished was the separation of the odd- and even-numbered branched acids from the normal acids of wool fat (23).

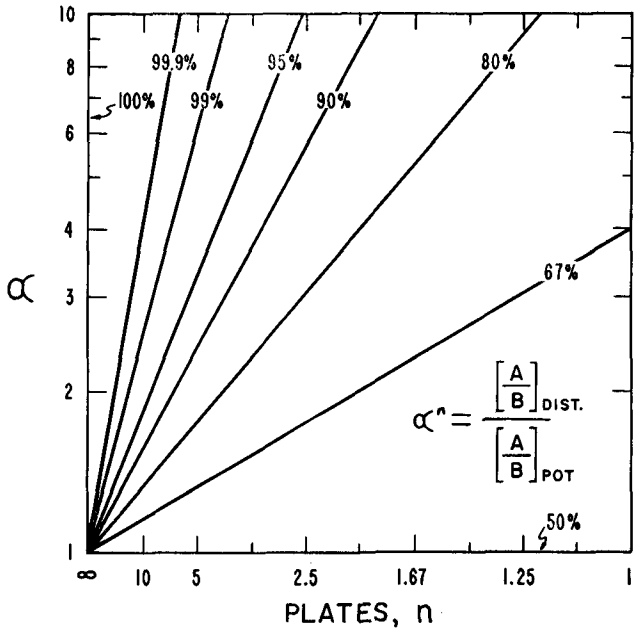

FIG. 4. Plates vs. α for a given purity of distillate and bottoms.

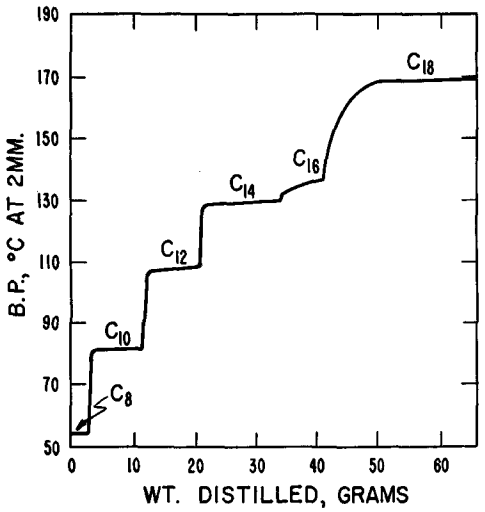

FIG. 5. Distillation of butterfat "liquid esters" (11).

A period of commercial development of laboratory stills followed. Podbielniak vacuum-jacketed Heligrid columns equipped with automatic controls were used for precise analysis of fatty acid mixtures (19). The characteristic feature of the Heligrid packing and the Stedman screen cones is that they are precisely formed according to a geometric pattern designed to give maximum vapor-liquid contact and freedom from channelling. Recent advances in column packings have combined the convenience of randomness with the advantage of carefully designed capillary spaces. Examples of some excellent "dump" packings are the Podbielniak Heli-pak and Octa-pak and the Pennsylvania State protruded distillation packings. Their operating characteristics have been thoroughly studied (21, 3).

Packed columns have two limitations. They require fairly large samples, and they have appreciable pressure drops. Large samples are not always available. The high temperatures resulting from a pressure drop of even a few mm. may cause decomposition of the C_{24} and higher alcohols and acids that are common constituents of natural waxes.

Spinning-band columns were investigated as a possible solution to these problems (16). By careful attention to rotor design, H.E.T.P. values comparable to good packed columns were obtained. Because of the low holdup per plate and low pressure drop it was possible to fractionate the acetates of n-alcohols up to C_{34} at 1-mm. pressure. Sample sizes were 50 g. or less, depending on the number of components to be separated.

Miniature columns, both spinning band and packed, have been developed for petroleum fractionation (27). Sample sizes were 15-50 ml. The scaled-down operation required much more precise control of throughput and reflux ratio. Voltage fluctuations in the power supply to the flask heater had to be less than 0.1 volt, or column equilibrium would be seriously disturbed. Remixing of the tiny distillate fractions due to liquid holdup in the still head was minimized by use of a specially designed needle valve. Vacuum work was facilitated by use of an enclosed magnetic drive for the rotor.

Comparison of the miniature columns with macro columns revealed equivalent separating power but no saving of time (18). The spinning-band columns had the lowest holdup and pressure drop. Tight-fitting rotors were best. Optimum rotor speed was about 2,000 r.p.m. At higher speeds vibration was hard to control, and frictional heat affected the throughput.

Amplified Distillation. The amplified-distillation technique (24) is useful for the analysis of ester samples that are too small to be distilled in miniature columns or for the isolation of minor components of large samples. In this method the sample is diluted with a mineral oil of the same boiling range in an amount appropriate for the distillation apparatus at hand. The course of the separation is followed by determining saponification numbers on distillate fractions. This technique was originally applied to methyl esters of fatty acids and later extended to acetates of fatty alcohols (17). Both classes of esters form nearly ideal mixtures with mineral oils. The elimination curves, examples of which are reproduced in Figures 6 and 7, are symmetrical, and their peaks are at the boiling points of the pure esters. Excellent separations can be obtained with any efficient still because it is always possible to add enough oil to spread a given ester component over enough plates to do the job. After saponification the acids are quantitatively recovered from the oil by extraction, the alcohols by adsorption on alumina.

Free fatty acids form minimum boiling azeotropes with mineral oil. As the oil is distilled, the acid appears in the distillate as much as 40°C. below its boiling point. As distillation progresses, the concentration of acid in the distillate increases and then

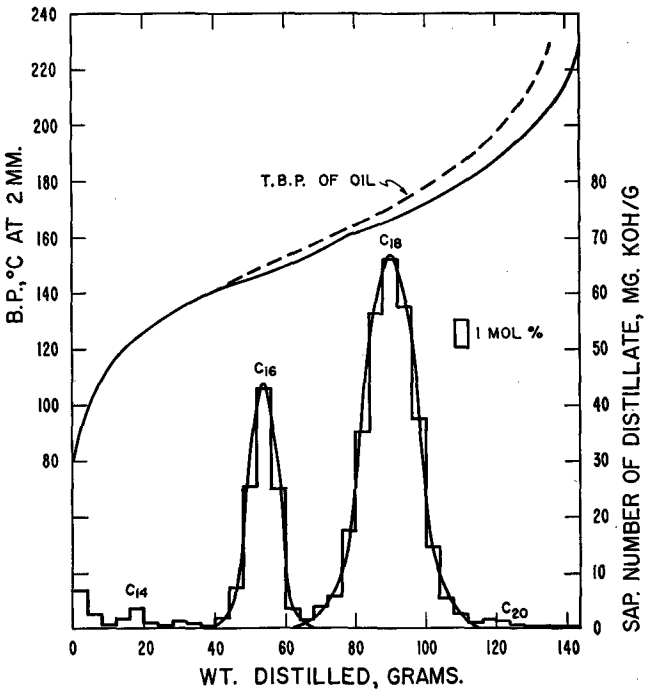

FIG. 6. Amplified distillation of cottonseed methyl esters (24).

drops abruptly as the supply is depleted. The typical elimination curve shown in Figure 8 is lopsided, and the peak is below the boiling point of the pure acid. This is characteristic of azeotropic distillation. The elimination curve covers such a broad temperature range that adjacent homologues overlap. Obviously, azeotropic distillation is of little use for separating homologous free acids although it is a powerful aid

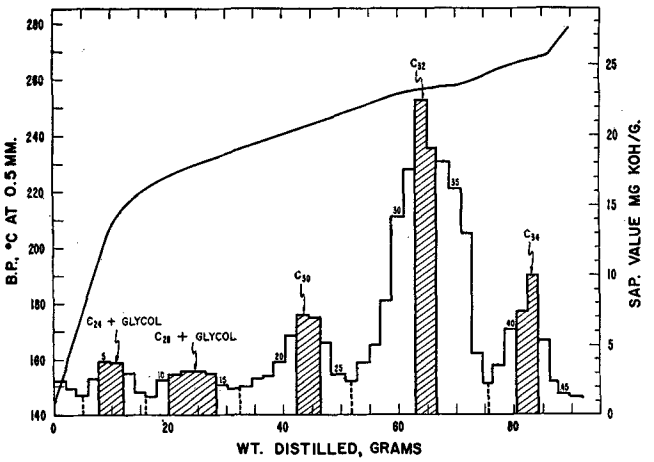

FIG. 7. Amplified distillation of the acetylated wax alcohols (17).

in the separation of the close-boiling pairs of dissimilar substances that occur naturally in petroleum and in the products of chemical reaction.

Extractive distillation resembles azeotropic distillation except that the added component is nonvolatile and must be circulated by pumping. It has the same limitations as azeotropic distillation in dealing with mixtures of fatty acids

Molecular Distillation

Molecular distillation differs from equilibrium distillation in the important respect that the vapor is not in equilibrium with the evaporating liquid. The difference arises from the absence of residual noncondensable gas, which greatly increases the mean free path of the molecules in the vapor phase. At atmospheric pressure a molecule of a certain size and shape might travel an average of only one ten-thousandth of a millimeter between collisions. Since the mean free path is inversely proportional to the absolute pressure, such a molecule would travel an average of 7.6 cm. between collisions at 10^{-3} mm. Thus if a condensing surface is placed within a few cm. of the evaporating surface, the majority of molecules with energy enough to break away from the liquid will be projected onto the condenser without having collided with any other molecules.

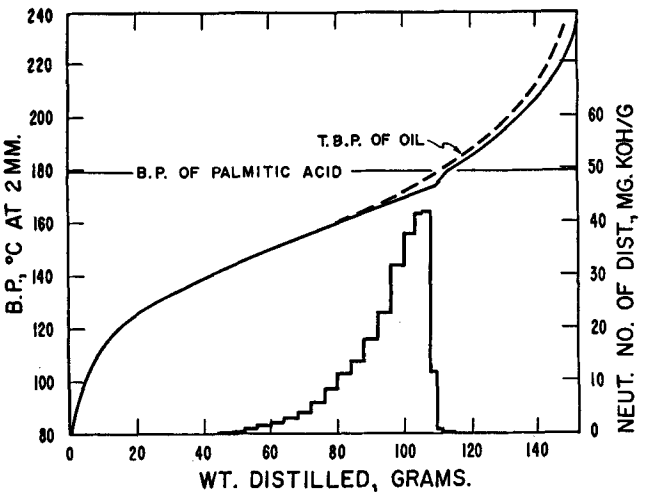

FIG. 8. Amplified distillation of palmitic acid (24).

Molecular distillation is applicable to substances having a vapor pressure of about 10^{-3} mm. in the range of 25 to 350°C. Such substances would have molecular weights ranging from about 150 to 1,200.

Theory. Composition of the distillate in molecular distillation depends on relative rates of evaporation but, in equilibrium distillation, only on the relative volatilities of the components. The difference arises from a molecular weight effect. According to Langmuir's equation (10), the rate of evaporation of a pure substance in a vacuum is proportional to the vapor pressure and varies inversely with the square root of the molecular weight. Raoult's law of partial vapor pressures enters the calculation of the relative rates of evaporation of the components of a mixture. Hence the relative quantities of components A, B, etc., distilling are given by:

$$p_A x_A/\sqrt{M_A},\ p_B x_B/\sqrt{M_B},\ \text{etc.}$$

Accordingly the more volatile components will tend to pass into the distillate first, followed by the successively less volatile ones. A plot of the concentration of any given component in a series of fractions of the distillate will show a rise to a maximum and then a falling off. The elimination curves shown in Figure 9 are similar in shape to those obtained in

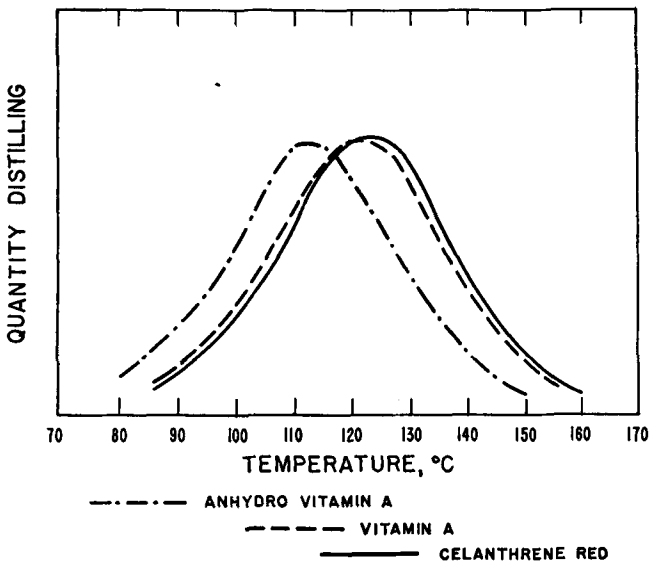

FIG. 9. Comparative elimination curves of vitamin A-like materials (8).

amplified distillation but extend over a much broader temperature range because single-stage still was used. Hickman (8) employed the position of the peak of the elimination curve as an identifying characteristic of the substance, much as the boiling point is used in equilibrium distillation. To locate the peak he distilled the substance in the presence of a "constant-yield" oil. This oil yielded constant increments of distillate when distilled for a definite time interval at each of a series of temperatures. The component in question will be distilled in its proper turn relative to the components of the constant-yield oil, and therefore its concentration will always peak at the same position in that particular carrier oil.

If the same type of carrier oil having a different distribution of components was used, the fraction number corresponding to the peak concentration of

the added component will vary, but its position on the temperature scale will be the same, provided the time interval for each fraction is held constant. Changing the chemical nature of the carrier oil may alter the position of the peak, and for a series of dissimilar components might even change the order of elimination. Non-ideal behavior is not restricted to equilibrium distillation; in fact, the effects are likely to be exaggerated at the relatively lower temperatures of molecular distillations.

Apparatus. Single-stage molecular stills are either of the pot type or the thin-film type. The pot still has the advantage of simplicity but the disadvantage that the distilland must be heated for long periods of time. This limits its use for unstable materials because the thermal hazard depends on duration of heating as well as on the temperature. Fractionation will be less than one theoretical plate in pot stills because depletion of the more volatile component from the surface layer of the distilland is not completely prevented even by efficient mechanical mixing.

Thin-film stills are either of the falling-film type or the centrifugal type. The thermal hazard is greatly reduced because the distilland is at the temperature of the evaporator for only a few seconds in the falling film stills or for a small fraction of a second in the centrifugal stills. In thin films mixing due to flow is very efficient, and diffusion to the surface is not a limiting factor. In such stills each actual stage closely approaches a theoretical plate.

Ten years ago Hickman (8) wrote, "we are just consolidating our knowledge of how to make the molecule take a single leap into empty space from a thin film of distilland. It remains to train the same molecule to dance precisely through a succession of evaporators and condensers until it loses all competitors and emerges only in the company of its fellows." Since then multistage molecular stills have been constructed by combining several single-stage stills. Combinations of pot-type stills (14, 13) have the advantage that gravity can be used to transport distillate and reflux from one stage to the next. Combinations of falling-film stills (12) are very complicated to construct and operate because pumps are needed to lift distillate and reflux from the bottom of one stage to the top of the next.

Applications. The application of molecular distillation to natural fats began with the vitamin separation studies of Hickman (9). Such minor constituents as odors, vitamins, and natural antioxidants in fats are readily concentrated in the first distillate fractions. The concentrating of vitamins quickly became a commercial process and provided the incentive for extensive study of the theory and practice of molecular distillation.

Glycerides such as cottonseed or corn oil distill over the fairly narrow temperature range of 220–260°C., and little fractionation is possible in a single-stage still. Systematic redistillation, or preferably multistage distillation, permits some concentration of the palmitate esters in the first fractions (7).

The methyl esters of the heat-sensitive highly unsaturated acids of fish oil were fractionated by systematic redistillation in a falling-film still at temperatures below 110°C. (6). Relative distillation rates of adjacent carbon-number fractions correspond to $\alpha = 5$. The thermal hazard was so slight that it was possible for the first time to isolate a C_{22} hexaene acid with its original nonconjugated structure intact.

Linoleic and linolenic acids were obtained in spectroscopic purity by debromination of the tetrabromo- and hexabromostearic acids (20). This was possible only when oxygen was rigidly excluded and the final product was molecularly distilled. The distillation, carried out in a pot still at 113–116°C., avoided isomerization while removing traces of polymeric material.

Polymerized methyl linoleate was separated into monomer, dimer, and trimer fractions (1). The monomer was first taken off at 1-mm. pressure. The polymeric portion was separated into homogeneous fractions of dimer and trimer by two fractionations on a cyclic falling-film still at 160–290°C. and 2-micron pressure. There was no evidence of higher polymer.

Peroxidized methyl oleate was fractionally distilled in a falling-film still (5). Four passes at 65° removed most of the unreacted methyl oleate. Most of the monohydroperoxide was distilled in two additional passes at 91°. Loss by thermal decomposition was negligible.

Frontiers

Two additional techniques that depend on vapor pressure offer real possibilities for the rapid analysis of submicro samples. One is mass spectrometry, and the other is gas chromatography.

The mass spectrometer, equipped with a heated inlet system, is capable of analyzing a wide variety of high molecular weight or strongly adsorbed materials. Brown, Young, and Nicolaides (2) demonstrated the power of this instrument by determining the compositions of the C_{16} to C_{27} saturated and unsaturated aliphatic alcohols from human hair fat.

Gas chromatography depends on the distribution of a vaporized sample between a moving gas stream and a stationary solvent. The immobility of the solvent is the main characteristic that distinguishes it from azeotropic and extractive distillation. The analysis of mixtures of even-numbered homologues ranging from methyl laurate to methyl behenate has been reported (4). Sample sizes were 20 milligrams, and the time required for analysis was 40 min.

Analysis of hydrolysis products by fractionation in equilibrium stills is well-advanced. The identities of most of the acids, alcohols, and hydrocarbons from the more abundant fats and waxes are known. More precise vapor-liquid equilibrium data are still needed for some of the closer-boiling pairs. Available apparatus is adequate for most purposes. Further developments in automatic controls, greater precision on small samples, and shorter distillation time are future possibilities.

The analysis of unhydrolyzed fats and waxes will be greatly facilitated when the minimum thermal hazard of the centrifugal molecular still is fully exploited in a multistage apparatus.

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Fractional Solvent Crystallization

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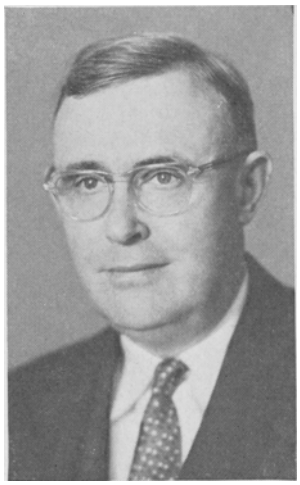
THE POSSIBILITIES of crystallization from solvent as a convenient procedure for separating fatty acid mixtures were first pointed out in a series of papers from this laboratory, beginning in 1937. Since then, there has been a rapidly increasing volume of literature from this and other laboratories, further describing the usefulness of the technic and showing that the method could be applied not only to the fatty acids but also to their methyl and glycerol esters, as well as to other natural lipids. Detailed reviews of the procedure up to about 1941 (2) and from 1941–1953 (3) have appeared. Because of time limitations the present discussion will narrate some of the milestones in developing the procedure in this laboratory, review solubility relationships as applied to the method, give a short description of laboratory technic, and finally illustrate typical separations which are possible through its application.



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Previous to the development of the low temperature crystallization method, the most important and, indeed, almost the only procedure for separating saturated and unsaturated acids was the classic, and presently official, lead soap ether (or alcohol) method. Those who have employed this technic over the years will only too well recall the trials and tribulations attendant on its use, the preparation of soap solutions in water, precipitation of the lead soaps, the difficulty of drying these soaps, and finally the tedious and often hazardous treatment with ether and subsequent filtration. Finally, there was the necessity of decomposing the lead soaps and working up the saturated and unsaturated fractions. Some of the hazards were overcome by using alcohol instead of ether.

Early Work in Developing Solvent Crystallization

Our work with solvent crystallization began about 1935. Frank Hartman came to Ohio State as chairman of the Physiology Department and, as part of his continuing work on cortin, installed in the basement below our fat laboratory two cold rooms, one in particular giving temperatures of —20 to —25°C. About this same time dry ice became available as a laboratory commodity. It occurred to us to explore the possibility of separating fatty acids by crystallization at low temperatures. Two graduate students at the time, G. G. Stoner and G. Y. Shinowara, undertook the separation of the fatty acids of cottonseed and olive oils. Their experiments were highly successful, and some of their data will be presented herein.

Oleic acid was prepared from olive oil fatty acids (5) by first crystallizing 225 g. acids from acetone (3,460 ml.) by standing overnight in the —25° room. The crystal fraction, mainly palmitic and stearic acids, was removed by suction filtration on a Büchner funnel in the same room. The resulting filtrate, consisting principally of oleic and linoleic acids, was then cooled to —60° in a dry ice bath; the crystal fraction this time was mainly oleic acid. This was removed likewise by suction filtration in the cold room and then subjected to three further —60° crystallizations, each time resulting in the removal of further amounts of linoleic acid. The final product was then redissolved in solvent and cooled to —35° to remove most of the remaining palmitic acid and also unavoidably some oleic acid. The filtrate allegedly was pure oleic acid. A refinement of this method, described later, has been used repeatedly to prepare highly pure oleic and other monoethenoic acids.

Stoner's work (6) was directed toward the purification of linoleic acid. Actually his results were considerably broader in implication. For example, in one series of experiments he described the separation of the saturated and unsaturated acids of cottonseed oil by cooling 10–12% solutions of the acids in acetone, petroleum ether, 95% ethanol, and methanol to —20°. Iodine value of most of the crystal (sat'd) fractions ranged from 3.4–7.2; values for the unsaturated fractions were 149.2–154.9, results which in our hands are quite superior to those obtainable by the lead soap procedure. Further cooling of the filtrate fractions was shown to separate partially the oleic and linoleic acids. For example, the crystal fraction coming down between —55° and —70° gave an iodine value corresponding to an 89–11 mixture of linoleic and oleic acids. Several preparations of linoleic acid were obtained of 85–93% purity.

Two other achievements of the crystallization procedure in the course of our work were the direct isolation of pure linoleic acid from vegetable oils by this technic alone and the isolation of pure linoleic and linolenic acids from the so-called α-acids prepared by debromination. The former, as described by Frankel and Brown (11), consisted of the follow-