suspension while Micronex (as well as other carbon blacks) forms relatively large aggregates in water. These aggregates are partially dispersed by vigorous agitation in a Waring Blendor but are more completely dispersed by rotation in the Launder-Ometer in the presence of fabric, yarn, or fiber of cotton or other textile material. The influence of hard water and of other inorganic salts on suspending power data is discussed. The need for careful selection of type of soil and fabric and for careful interpretation of data is stressed.

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[Received May 18, 1959]

Preparation of Pure Fatty Acid Methyl Esters by Countercurrent Distribution[1]

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COUNTERCURRENT DISTRIBUTION constitutes a useful physical tool for the isolation and preparation of pure fatty acids and methyl esters because of its inherently mild fractionating-conditions, high resolving power, and comparatively large sample-capacity. Like chromatographic methods and fractional solvent crystallization, it separates unchanged the naturally occurring isomers of unsaturated acids. In contrast, chemical methods involving the polybromides give products containing some unnatural isomers, and urea fractionation usually gives products containing at least small amounts of other fatty acids.

Various solvent combinations have been used for the separation of fatty acids and of methyl esters. Ahrens and Craig (1) studied systems formed by mixing heptane with acetic acid, methanol, and either formamide or acetonitrile for the countercurrent distribution of the higher fatty acids. Cannon, Zilch, and Dutton (3) reported the use of a nitromethane, nitroethane, pentane-hexane system for the separation of methyl esters.

This communication describes the use of acetonitrile and pentane-hexane for the analytical countercurrent distribution of methyl ester mixtures as well as the preparative separation of methyl esters of pure fatty acids. Acetonitrile is selective for esters of different degrees of unsaturation. It has a low boiling-point and forms azeotropes with the hydrocarbon solvents so that it is easily removed by evaporation. It is stable under the conditions used, and when the fractions are removed in portions of upper pentane-hexane layer by the single withdrawal procedure (4), the lower layer may be re-used. Thus operations are considerably simplified, and it is not necessary to empty the apparatus and refill with fresh solvent for each successive batch.

[1] Presented at the 50th meeting, American Oil Chemists' Society, New Orleans, La., April 20–22, 1959.

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Experimental

Methyl Linolenate. Since preparations of this ester have frequently been required in this laboratory, experience with this ester and the acetonitrile-pentane-hexane system is most complete. Because the weight of esters that can be fractionated per batch is limited by the size of the tubes of the countercurrent distribution apparatus, it is advantageous to start with esters having as high a concentration of methyl linolenate as possible. For this reason a methyl linolenate concentrate, prepared by the urea-complex procedure that was devised by Parker and Swern (10), was used as the starting material. This concentrate contained 84.7% methyl linolenate, 15.4% methyl linoleate, and 0.2% methyl oleate as measured by gas chromatography.

Countercurrent distribution was carried out in a 200-tube apparatus which contains 40 ml. of lower layer in each tube. To study separation under optimal conditions, only 10.0 g. of methyl linolenate concentrate were used. The concentrate was dissolved in 200 ml. of lower phase and 50 ml. of upper phase of the solvent system and was placed in the first five tubes of the instrument. Seven hundred transfers were applied, using 10-ml. portions of upper phase for each transfer. After the first 200 transfers the upper layers were withdrawn from the apparatus according to the single withdrawal procedure. These fractions were caught, one in each tube of the collector, and were evaporated in tared flasks. Because nothing was found in fractions corresponding to transfer 200 through 300, these fractions are not

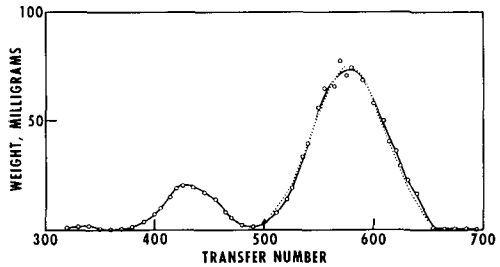

FIG. 1. Countercurrent distribution of methyl linolenate concentrate. Solid line—weight curve. Dotted line—calculated theoretical curve for methyl linolenate.

shown in the weight curve given in Figure 1. In this figure there are well-separated bands corresponding to the elution of linolenate, linoleate, and oleate of the starting material. Partition coefficients calculated from the position of the maximum of the weight curve for each component (4) are $K_{linolenate} = 2.1$, $K_{linoleate} = 3.5$, and $K_{oleate} = 5.9$. The calculated theoretical curve (4) for linolenate indicated by the dotted line corresponds closely to the experimental curve and indicates "ideal" conditions of operation. The material represented by transfers 490 through 650 was combined to give 6.24 g. of methyl linolenate.

To prepare larger amounts of methyl linolenate, runs were made with 40-g. portions of linolenate concentrate placed in the first 20 tubes. After 200 transfers the fractions were collected by combining the eluate from two transfers in each collector tube. A weight curve for such a run is shown in Figure 2.

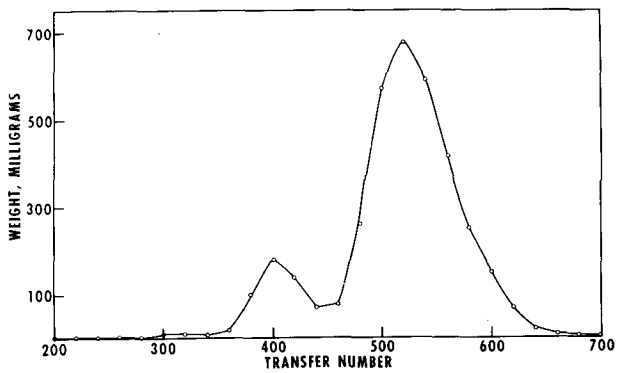

FIG. 2. Preparative scale countercurrent distribution of methyl linolenate concentrate.

Fractions from 480 to 640 were combined and comprised the methyl linolenate preparation. Another 40-g. sample of linolenate concentrate was then placed in the first 20 tubes, and the procedure was repeated, using the same lower layer. Additional lower layer was passed through the instrument ahead of the sample to insure re-establishment of interfaces in the proper position. Upper layer was also passed through to insure that the phases were mutually saturated. From 328 g. of linolenate concentrate processed in eight such runs, 238 g. of methyl linolenate were recovered. This was distilled to give 214 g. of product, which analyzed 100% methyl linolenate by alkali isomerization (2) and contained only about 0.01% impurity as measured by gas chromatography. It has an iodine value of 258.7 and $n^{30/D}$ 1.4675.

Methyl Linoleate. A similar procedure has been used to prepare methyl linoleate except that no prior enrichment by urea fractionation was required. A 40.1-g. sample of safflower methyl esters containing 77.7% linoleate was put in the first 20 tubes of the apparatus. Distribution was carried out as described for the linolenate except that four transfer samples were combined in each collection tube. The weight curve is shown in Figure 3. Fractions were combined

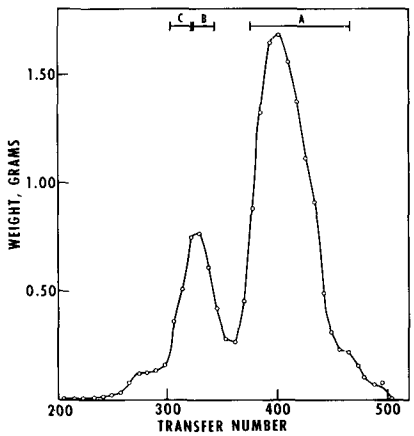

FIG. 3. Countercurrent distribution of safflower oil methyl esters.

as indicated in the figure and analyzed by gas chromatography. Fraction A consisted of 22.8 g. of methyl linoleate of greater than 99.5% purity by gas chromatography. Alkali isomerization indicated 100% linoleate. Iodine value was 172.8 and $n^{30/D}$ 1.4578. Fraction B contained 70.4% methyl oleate and 26.9% methyl palmitate while fraction C contained 55.5% oleate and 44.5% palmitate. While oleate and palmitate are found in the same group of tubes, there is a partial separation. If it is desired to recover pure oleate, removal of palmitate prior to countercurrent separation could readily be performed by distillation or urea crystallization.

Soybean Methyl Esters. The fractionation of a more complex mixture is illustrated by the countercurrent distribution of soybean methyl esters. A 10.97-g. sample was placed in the first five tubes, and the distribution was performed as described above, collecting the fractions corresponding to each transfer in separate tubes. The weight curve is shown in Figure 4. Fractions were combined, as indicated in the figure, and were analyzed by gas chromatography (Table I). The first fractions are largely C_{20} and C_{22} saturated esters followed by methyl stearate. Methyl palmitate and methyl oleate appear together in subsequent fractions but are partially separated as we observed before with safflower esters. Analysis by gas chromatography shows that the methyl linoleate contains a small amount of impurity, probably palmi-

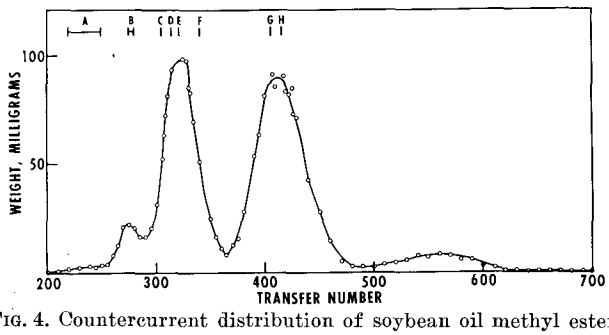

FIG. 4. Countercurrent distribution of soybean oil methyl esters.

TABLE I

Composition of Soybean Methyl Ester Fractions

Fraction	Composition, %		
A	51.3 Behenate	32.6 Arachidate	16.1 Stearate
B	1.8 Arachidate	93.6 Stearate	3.67 Palmitate
C	56.1 Palmitate	43.9 Oleate	
D	39.1 Palmitate	60.7 Oleate	
E	25.2 Palmitate	74.5 Oleate	
F	6.1 Palmitate	92.3 Oleate	
G	96.2 Linoleate		
H	97.1 Linoleate		

toleate. The last component withdrawn from the countercurrent distribution instrument is linolenate.

Methyl Arachidonate. To investigate the feasibility of this procedure for preparation of methyl arachidonate, one countercurrent distribution was made of methyl esters from hog liver lipids. Difficulty was anticipated because of the expected similarity of the partition coefficients of arachidonate and linolenate. While arachidonate has one more double bond than linolenate, it also has two more carbon atoms. The effect of increasing polarity by adding a double bond has been found to be nearly equal in effect to that of subtracting two methylene groups. For this reason the two esters would be expected to have similar partition coefficients and to emerge from the apparatus in the same fractions and in a way similar to the oleate and palmitate.

The crude lipids were prepared from hog liver by the alcohol-extraction procedure of Holman (6). After the fatty acids were isolated by saponification, they were esterified by refluxing with methanol and sulfuric acid and were distilled, giving a product containing 9.3% methyl arachidonate. Fortunately these esters were found to contain only a trace of linolenate. However to establish the location of linolenate in the distribution, 31 mg. of C^{14} labeled methyl linolenate (5) were added to 15.1 g. of the liver esters used for the distribution. This sample was placed in the first five tubes of the instrument, and 1,000 transfers were applied by using 10-ml. portions of upper layer and combining two fractions in each collector tube. The weight curve and the measurement of radio-activity are shown in Figure 5. The maximum for radio-

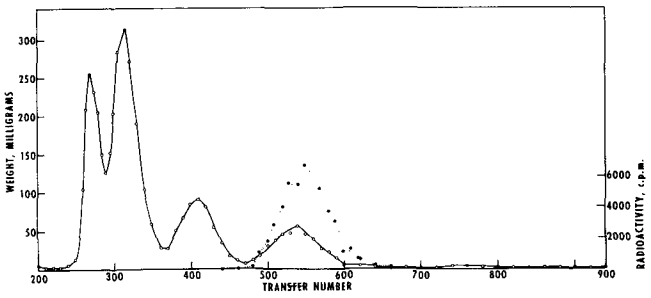

FIG. 5. Countercurrent distribution of hog liver lipid methyl esters. Solid line—weight curve. Dotted line—radio-activity caused by adding C^{14} methyl linolenate.

activity of the C^{14} methyl linolenate appears in the same fractions as the maximum weight in the arachidonate band; therefore little separation of linolenate and arachidonate would have occurred in this system had linolenate been present.

Composition of the fractions was investigated by gas chromatography and by alkali isomerization, using 21% potassium hydroxide in ethylene glycol (2). Gas chromatography indicated that the composition of some of the fractions was quite complex, each with several minor unidentified components, but that fractions in the region from transfer 510 to transfer 570 contained about 90% methyl arachidonate with two other components. Alkali isomerization indicated the presence of a pentaenoic ester. From its position in the countercurrent distribution curve this would be presumed to be methyl docosapentaenoate. It appears that if liver lipid methyl esters were first distilled to give a 20-carbon acid fraction, as was done by Montag *et al.*, (8) pure methyl arachidonate could be prepared with this type of solvent system.

Small amounts of material were also recovered by combining fractions corresponding to transfers 720 through 820 and 840 through 940. Based on ultraviolet absorption after alkali isomerization and their position on the distribution curve, it is believed that, in addition to unidentified materials, Fraction 720–820 contained methyl eicosapentaenoate and docosahexaenoate and Fraction 840–940 contained methyl eicosahexaenoate.

Discussion

Countercurrent distribution employing an acetonitrile and pentane-hexane solvent system is a practical procedure for separating pure "natural" methyl esters of fatty acids in quantities sufficient for many laboratory uses. Since weight curves are quite reproducible from run to run, it is necessary to check only the general shape and position of the bands before combining samples. In recent preparations this location of bands is being done by following the refractive index change of the solutions issuing from the apparatus with a recording differential refractometer, thus obviating the need to evaporate and weigh the fractions. Addition of automatic refractometric monitoring of eluates to the already automatic operation of the countercurrent distribution apparatus eliminates much tedious labor, which has discouraged the use of countercurrent distribution methods in the past. Approximately 40 g. of methyl esters can be processed in each four days of automatic operation, using a 200-tube countercurrent distribution apparatus (80-ml. capacity tubes) and applying 650 transfers.

Although this acetonitrile-pentane-hexane solvent system separates methyl esters on the basis of unsaturation (isologous series) and to some extent on chain length (homologous series), it has little selectivity for some other mixtures encountered. For example, a mixture of methyl linolenate and methyl pseudoeleostearate (10 *trans*, 12 *trans*, 14 *trans*-octadecatrienoate) gave only one maximum for the two compounds in its weight curve. According to spectroanalysis, the maxima for the two components were separated by not more than 10 transfers. Also as described in another paper (11), distribution of esters of cyclic monomers from alkali-isomerized linolenic acid gave only one band although ultraviolet absorption indicated incomplete fractionation had taken place.

Application of this solvent system is described for a 200-tube automatic apparatus, but the system should also be useful in smaller instruments. Suitable conditions would have to be established for each separation, but the following illustrates the type of results anticipated. Consider the separation of linoleate and linolenate. If the volume of upper phase in each tube is one-fourth that of the lower phase and if the sample

is placed only in the first tube, we may calculate from Nichols' equation 5 (9) that in a 50-tube instrument 123 transfers would be applied before the fraction which corresponds to the point of intersection of the curves, *i.e.*, the fraction in the distribution of a two-component system which contains equal amounts of each component, leaves the instrument. With a 100-tube instrument 245 transfers would be applied; with a 200-tube instrument, 490 transfers, which checks our experimental value in Figure 1. Then if equal amounts of linoleate and linolenate are present in the starting mixtures and if the fractions are divided at the intersection point, we may calculate from the equations of Lancaster *et al.* (7) that each component from the 50-tube instrument would contain 4.5% of the other component as impurity. Each component from the 100-tube instrument would contain 0.8% of the other component. Greater purity can be achieved by starting with material enriched in the desired component, by discarding the small fractions near the intersection point of the curves, or by using a smaller ratio of upper-to-lower-solvent volumes.

Summary

Acetonitrile-pentane-hexane makes a desirable solvent system for preparation of pure methyl esters because of its immiscibility, selectivity toward unsaturation, low boiling point, stability, and ease of recovery. Since separated esters are removed from the apparatus dissolved in the pentane-hexane layer, successive batches may be fractionated without removing the acetonitrile layer from the instrument. Applications have been illustrated for the preparation of methyl linolenate from an 85% linolenate concentrate, methyl linoleate from safflower esters, and methyl arachidonate from pig liver lipids.

This procedure provides a source of "natural" fatty acids with the double bond configuration unchanged, in contrast to those from the conventional bromination-debromination process. Automation of the process is completed by use of a recording refractometer which monitors concentration of solutions issuing from the extractor. Resolutions to be anticipated with lesser numbers of extraction tubes than 200 are calculated for an equal mixture of linoleate and linolenate.

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[Received June 2, 1959]

Theory of the Washing Process[1]

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THE SOIL usually occurring on the textiles to be washed is a complicated mixture of very different components. For the sake of simplicity, they may be subdivided into materials which are liquid at the washing temperature and others which are solid under the same conditions. Regarding the washing process theoretically, it is useful to consider the displacement of both types of soil separately because they are taken away from the textile fibers by quite different mechanisms.

Liquid soil, or as we may call it, oily soil is chiefly displaced by preferential wetting. It has been shown by microscopic observations that the oil originally spread over the fiber as a thin and nearly uniform layer is pushed together to form spherical droplets after immersion in the washing liquor. From a detailed study of this process it follows that the preferential wetting is governed by the interfacial tension σ_{AB} between the oil and the washing liquor as well as by the difference Δj between the adhesion tensions for the washing liquor/fiber and for the oil/fiber interfaces. If Δj is greater than σ_{AB}, the droplets are spontaneously detached. The work A_W done by the system during this process is given by the following formula derived by Kling and Koppe (6):

$$-A_W \simeq F\,(\Delta j - \sigma_{AB}), \qquad (1)$$

where F is the area exempted from the oil.

In most of the practical cases however Δj is smaller than σ_{AB}. The oily droplets attain equilibrium in the form of spherical segments with a definite contact angle. A "residual work of washing" A_R must then be done to detach the soil completely. For A_R the following equation has been derived (7):

$$A_R = y \cdot \sigma_{AB} \qquad (2)$$

y is a function of the contact angle, which in turn is correlated to Δj and σ_{AB} by the Young relation. The function is expressed by the following equation:

$$y = \sqrt[3]{4} - \sqrt[3]{2 - 3\,x_0 + x_0^3} \qquad (2a)$$

where $x_0 = -\Delta j/\sigma_{AB}$. The term A_R as calculated by the equations (2) and (2a) is related to an oil drop of the volume

$$v = 1/3\sqrt{\pi})\ \text{cm}^3$$

The equations (2) and (2a) permit expression of the effectiveness of a detergent in removing oily soil by physically defined and measurable quantities, *i.e.*, Δj and σ_{AB}. This may be demonstrated by measurements with a model system, namely, a foil made from poly-ε-caprolactam, liquid paraffin, and aqueous solutions of sodium dodecylsulfate. Figure 1 shows, as

[1] Presented at the 50th Anniversary Meeting, American Oil Chemists' Society, New Orleans, La., April 20, 1959.