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Fractional Solvent Crystallization

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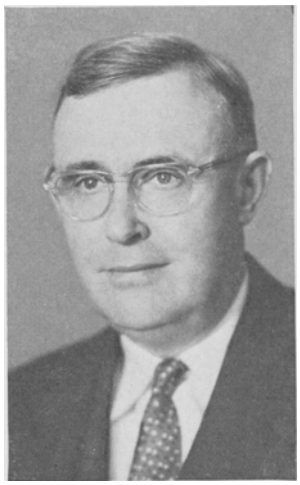
THE POSSIBILITIES of crystallization from solvent as a convenient procedure for separating fatty acid mixtures were first pointed out in a series of papers from this laboratory, beginning in 1937. Since then, there has been a rapidly increasing volume of literature from this and other laboratories, further 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.



J. B. Brown

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-

ing steps: a) the preparation of a crystal fraction of corn or cottonseed oil fatty acids, coming down between —50° and —70°, approximately 90% linoleic acid; b) the product dissolved in petroleum ether (65 g./l.) is cooled to —48°, giving a crystal fraction, about 95% linoleic acid; c) to remove the remaining oleic acid (5%) advantage is taken of the fact that at —60° the solubility of linoleic acid is only 4–5 times that of the oleic whereas the mixture is actually 19–1 linoleic-oleic. Thus the 95% acid is dissolved in sufficient petroleum ether to hold all of the oleic acid in solution. Cooling this solution then to —60° should, and does, produce crystals of pure linoleic acid. This was the first time this acid had been prepared in a pure state by physical procedures.

The second important early result of the crystallization procedure was the isolation of pure linoleic and linolenic acids by repeated crystallization of the corresponding α-acids, prepared by debromination (20). From this work it was shown that the α-acids were heterogeneous and, in fact, mixtures of the natural *cis* acids with isomers of the same which had been formed during bromination-debromination. The separation was possible because solubilities of the *cis* acids were lower than the contaminating isomers.

General Solubility Considerations in Relation to Practical Separations

Perhaps the most important single factor in applying solvent crystallization to separating fatty acids is solubility or solubility differences. Most of the data on pure compounds have appeared since 1940 from the laboratories of the Research Division of Armour and Company and of the Ohio State University. For reviews of these data the reader is referred to Ralstan's book (24) and to the second review previously cited (3). For purposes of quick inspection certain typical examples of solubilities are shown in Table I.

TABLE I

Typical Solubilities of Fatty Acids at —20°

	Acetone	Methanol	Skellysolve B
Lauric	17.4[a]	17.0[a]	3.79[a]
Myristic	4.33[a]	3.44[a]	1.31[a]
Palmitic	1.34[a]	0.63[a]	0.12[a]
Stearic	0.05[b]	0.11[b]	.....
Oleic	52.0[b]	40.2[b]	22.5[b] (heptane)
Linoleic	1470[c]	2330[c]	1700[b] (hexane)

[a] g./1,000 g. solution (10).
[b] g./1.000 g. solution (18).
[c] g./1,000 g. solvent (15).

In acetone at —20°, for example, taking stearic acid as unity, the relative solubilities are: palmitic, 27; myristic, 86; lauric, 348; oleic, 1,040; and linoleic, 29,400. Innumerable other relative solubilities can be worked out from the curves in Figure 1, which are taken from data in the recent paper by Kolb and Brown (18).

If the solubilities of individual fatty acids in mixtures even approximated those of the pure acids, fabulous separations would be possible. However Waentig and Pescheck (30) as early as 1919 showed important intersolubilizing effects in a series of saturated fatty acids in chloroform and benzene, but not in ethanol, ethyl ether, or ethyl acetate. More recently, Singleton (29) found that small amounts of oleic acid crystallized out with stearic acid, even at temperatures and conditions which did not theoretically permit oleic acid to precipitate, He also reported that oleic acid appreciably increased the solubility of palmitic and stearic acids. On the contrary, stearic acid had little effect on the solubility of oleic acid. The formation of eutectics between oleic and stearic in the ratio of 98–2 and the general possibility of eutectic formation in fatty acid mixtures are always limiting factors in effecting separations. Mixed crystal formation and/or hydrogen bonding may be expected to work against attaining theoretical results. Further examples of intersolubilization, eutectic formation, and mixed crystal formation will be evident from the examples cited later.

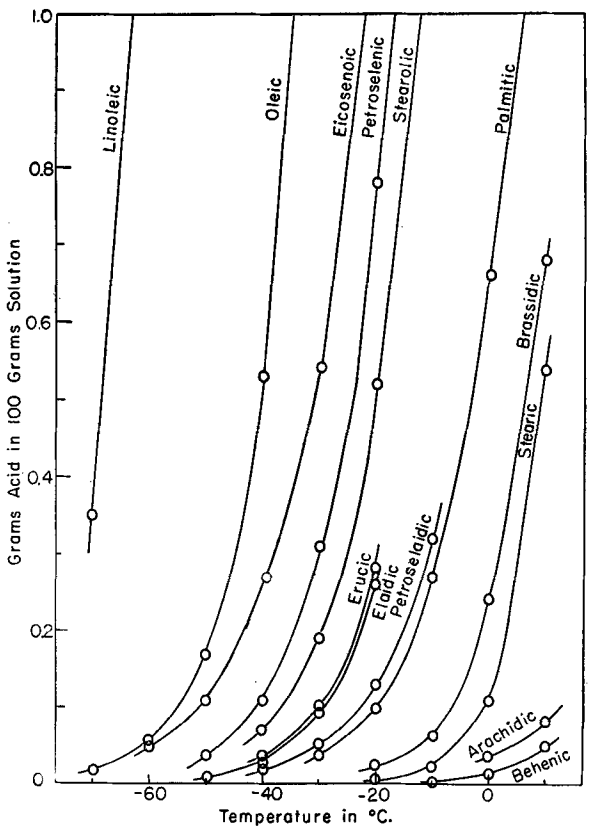


FIG. 1. Solubility of fatty acids in acetone (18).

Association in solution seems to be minimized with ester mixtures. Consequently it is often advisable as well as convenient to employ the esters, especially the methyl esters, in solvent crystallization because these are often prepared in connection with ester distillation. Furthermore the availability of main ester fractions after such distillation simplifies the mixture to be crystallized. Thus with olive oil the C_{16} fraction is entirely methyl palmitate and hexadecenoate, while the C_{18} fraction is stearate, oleate, and linoleate. The use of esters necessitates appreciably lower temperatures in effecting given separations. Typical solubilities of esters, part of an extended series of data by Sedgwick *et al.* (26), are shown in Table II.

Laboratory Apparatus for Low Temperature Crystallization

In our laboratory we use a simple apparatus for solvent crystallization, the principal features of which are illustrated in Figure 2 (3). The crystallizing chamber is a standard Pyrex cylinder, 6 in. in diam-

TABLE II

Solubilities of Alkyl Esters in Acetone (26)

Ester	Grams of Ester per 100 g. of Solvent						
	−40.0°	−30.0°	−20.0°	−10.0°	0.0°	10.0°	20.0°
Me caprylate	380	∞	∞	∞	∞	∞	∞
Me laurate	0.9	3.6	12.5	67	645	∞	∞
Me myristate	....	0.5	2.1	7.1	28.7	248	∞
Me palmitate	....	0.1	0.4	1.3	5.2	21.0	250
Me stearate	....	....	....	0.1	0.7	3.6	19.9
Et stearate	....	....	....	0.4	1.5	5.2	40.5
n-Pr stearate	....	....	....	0.9	2.0	6.0	185
n-Bu stearate	....	....	....	1.2	2.4	6.7	345

eter and 12 in. tall, which will hold conveniently 4.5 l. of solution. It is weighted with four lead bars to prevent capsizing during filtration. The jar is placed in a copper can surrounded by glass wool insulation and partially filled with waste solvent, which is cooled with dry ice. The solution to be crystallized is brought gradually to temperature with slow stirring with a Monel paddle. Usually the solution is kept at the desired temperature for at least an hour, then the crystals are allowed to settle, and suction filtration is applied through a special 5½-in. filter stick cone, perforated with 5-mm. holes, and covered with filter paper. The filter stick is lowered gradually during filtration so that most of the crystals remain below it and can be pressed by the stick. Filtrate is collected in a 4-l. suction flask, or, in our larger apparatus, accommodating an 18-in. cylinder (7 l.), in a 12-liter balloon flask.

One of the principal difficulties encountered in this procedure is efficient removal of entrained filtrate in the crystal fraction. Sometimes it is advantageous to repeat the crystallization, but often good results can be achieved by simply adding a quantity of fresh solvent, cooled 15–20° below the crystallization temperature to the crystals, brief stirring, and filtration. Mixed crystal formation is not avoided by this manipulation, but the second washing improves the overall efficiency of the operation.

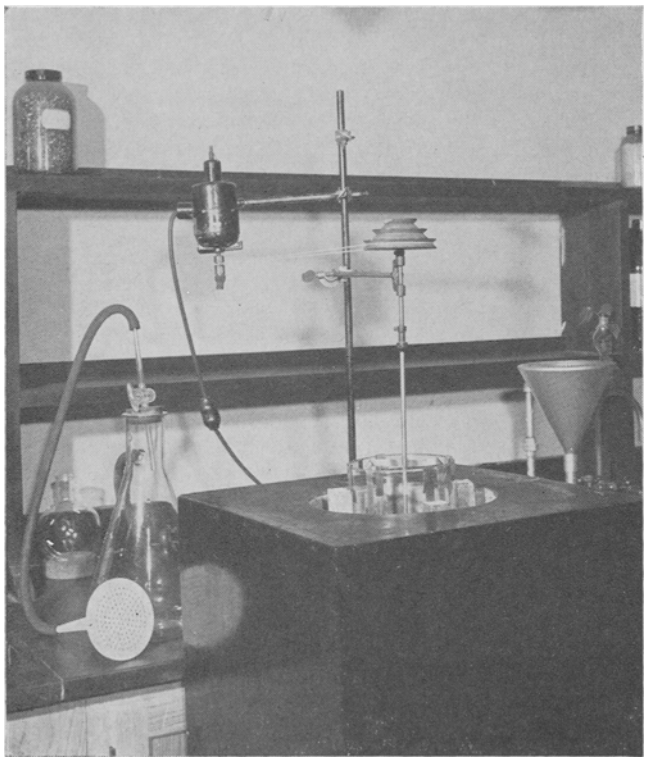


FIG. 2. Laboratory apparatus for crystallization at low temperatures (3).

Typical Uses of Solvent Crystallization in the Separation of Fatty Acids and Esters

It is beyond the scope of this paper to more than cite examples which typify some of the separations which are possible by solvent crystallization. In so doing, it should be pointed out that when working with very low temperatures, the number of solvents suited to the technic is strictly limited. A list of these is shown in Table III. Relatively few of these have been tried.

TABLE III

Solvents Suitable for Solvent Crystallization at Low Temperatures

	M.P.°C.	B.P.°C.
Acetone	− 94.6	56.5
Allyl alcohol	−129.0	96.6
Carbon disulfide	−108.6	46.3
Carbon tetrachloride	− 22.6	76.8
Chloroform	− 63.5	61.2
Ethyl ether	−116.3	34.6
Ethyl acetate	− 82.4	77.1
Ethyl alcohol	−112.0	78.4
n-Heptane	− 90.6	98.4
n-Hexane	− 94.0	69.0
Methyl alcohol	− 97.8	64.7
Methyl acetate	− 98.7	57.1
Octane (iso) (2,2,4 trimethyl pentane)	−107.4	99.3
Pentane	−127.7	36.3
Pyridine	− 42.0	115.6
Toluene	− 95.0	110.8
Ethylene Trichloride ClCH:CCl_2	− 73.0	87.2

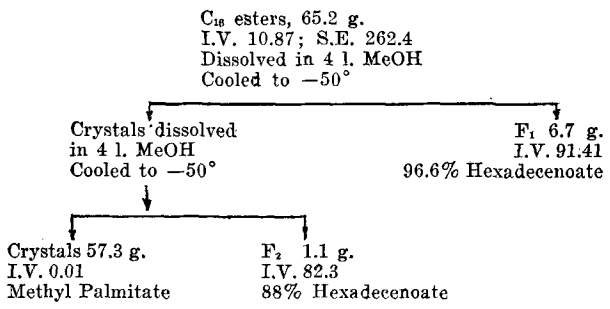
Generally speaking, the order of solubilities with any series of acids or esters will be similar in different solvents, but actual solubilities may differ considerably. Also, solubility will generally parallel melting point qualitatively, but not exactly. Thus knowing the melting point gives some clue to relative solubility behavior.

a) *Separation of Saturated and Unsaturated Acids.* In the usual type of fat the saturated and unsaturated acids can be easily separated by cooling 5% solutions of the acids in acetone, methanol, or petroleum ether to −20°, filtering and washing the precipitated acids once or twice with fresh solvent cooled to −35 to −40°. Iodine values below 10 or even 5 or less are easily attained for the saturated fraction. When the mixed fatty acids of the fat are employed, it is to be expected that small amounts of palmitic acid may pass into the unsaturated fraction, most of the myristic, and all of the lauric acid. With such fats as coconut oil and butterfat poor separations may be expected because the lower saturated acid solubilities approach that of oleic acid. It may perhaps be advisable to lower somewhat the temperature of the crystallization with certain fats, and under some circumstances to crystallize actually twice (cf. Earle and Milner) (8). In case a refrigerated centrifuge is available, the separation may be carried out conveniently by centrifugation. Unusual oils of the rapeseed type present a problem because the erucic acid comes down at appreciably higher temperatures than oleic acid so that the optimum crystallization temperature may be as high as 0°. Actually each fat presents an individual problem. However, once having considered the composition of the mixture and perhaps performed two or three trial runs, it is comparatively simple to work out a more effective and convenient procedure than the official lead soap method.

b) *Preparation of Monoethenoic Acids (or Esters).* The first step in isolating a monoethenoic acid is always to prepare a pure single carbon series fraction by ester distillation. To assure absence of adjacent carbon series it may be advantageous to distill twice. The resulting fraction is likely to be very simple in composition, C_{12}, C_{14}, and C_{16} fractions being usually binary mixtures of saturated and monoethenoic esters. The usual C_{18} ester fraction will contain at least three components, stearate, oleate, and linoleate, and many will, in addition, contain linolenate. Tetraethenoic ester may be present in the C_{18} fractions from fish oil. Similar fractions from animal fats are usually found to contain appreciable amounts of iso-unsaturated acids.

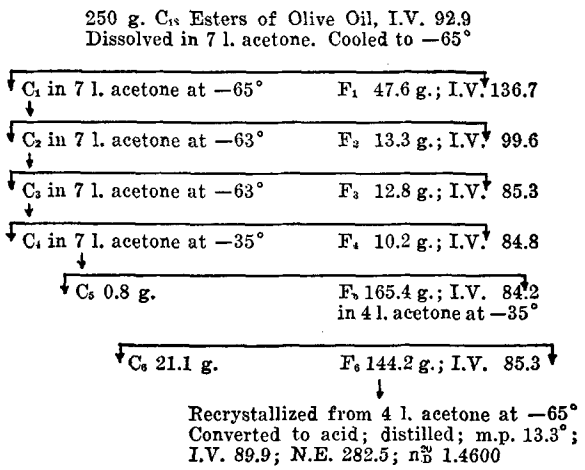
The one-step isolation of 96.6% hexadecenoate from human milk fat is described in Chart 1 (4). No further purification was attempted.

CHART 1
Crystallization of C_{16} Esters Human Milk Fat



Because of its importance, details of the preparation of highly pure oleic acid are described in Chart 2 (17). A similar technic has been used by Foreman and Brown (10) and others so that the method may now be regarded as relatively standard. For example, Millican (22) used it suitably modified in our laboratory to prepare oleic acid from linseed in which this acid is a minor component.

CHART 2
Scheme for Isolation of Oleic Acid from Olive Oil

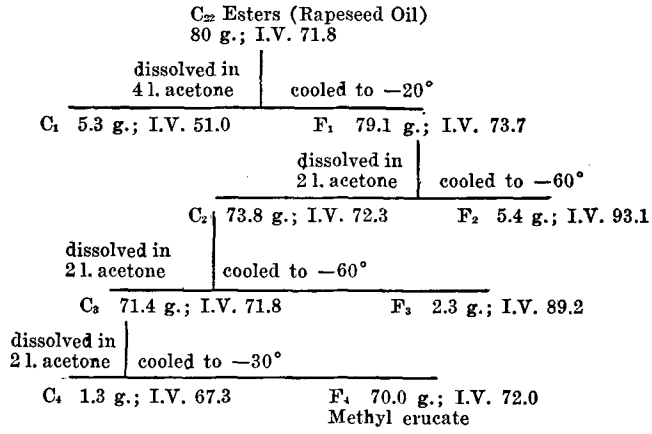


The isolation of pure methyl erucate is shown in Chart 3 (17).

c) *Isolation of Linoleic Acid.* The only dienoic acid to which the solvent crystallization technic has been applied in its isolation is linoleic acid. The method of Frankel and Brown, previously cited (11), has been employed in a number of laboratories in the isolation of linoleic acid from those vegetable oils in which it occurs to the extent of 50% or more. In our laboratory linoleic acid has been prepared from wheat germ and tobaccoseed oils (1), soybean phosphatides (12), and soybean oil (9), the latter two in spite of the fact that the problem is complicated by the presence of small amounts of linolenic acid. In Chart 4 is described the full procedure for isolating linoleic acid from safflower seed oil (24).

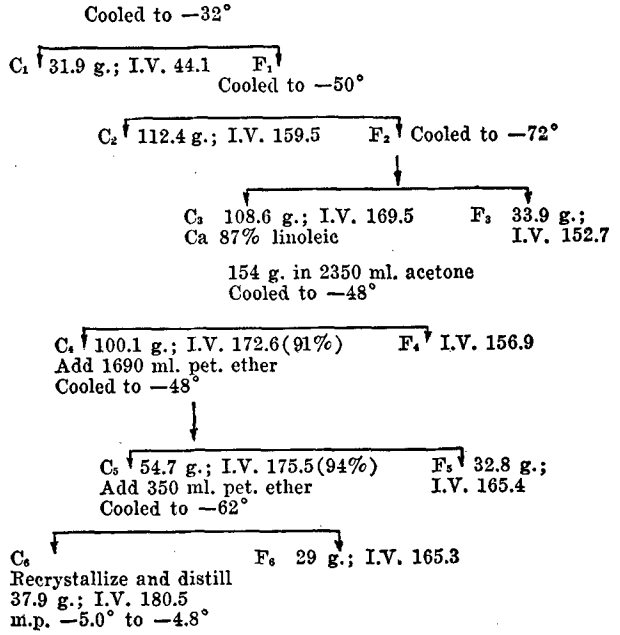
Attempts to prepare pure linoleic acid by this method from certain animal fats, such as human milk and depot fats and butterfat, have been unsuccessful because of the presence of considerable amounts of isolinoleic acid. The net results are eutectic of linoleic acids with oleic.

CHART 3 (17)
Preparation of Methyl Erucate by Crystallization of the C_{22} Methyl Esters of Rapeseed Oil



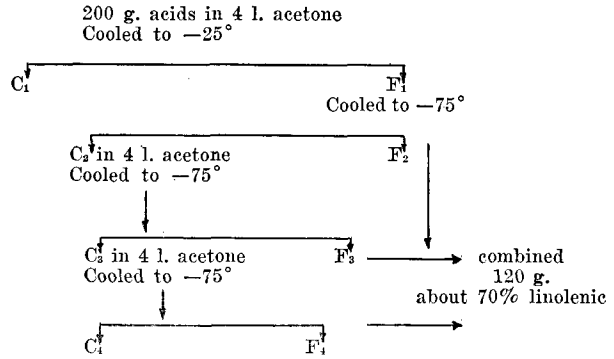
d) *The Isolation of Polyethenoic Acid (or Ester) Concentrates.* While it is comparatively simple to

CHART 4
The Isolation of Linoleic Acid from Safflower Seed Oil (24)
297 g. F.A. Safflower Oil (I.V. 148.4) in 4 l. acetone



prepare pure saturated and monoethenoic acids by solvent crystallization and relatively easy to isolate pure linoleic acid, it has so far not been possible to apply the method satisfactorily for preparing pure linolenic, arachidonic, and other liquid polyethenoic acids, the last particularly in the highly complex mixtures as found in fish oils. Two obstacles, met in attempting to do this, are eutectic formation (probable) and high solubility even at the lowest practical temperatures. On three separate occasions in this laboratory we have tried to isolate pure linolenic acid from linseed oil. While it is possible to separate linolenic acid from linoleic as these occur in soybean oil, the removal of the last few per cent of linoleic from linolenic acid is practically impossible. Shinowara (28) prepared linolenic acid concentrates (84–88%) by several combinations of solvent crystllization. Mrs. Guy (13) prepared a concentrate of linolenic acid as noted in Chart 5. By repeated crystallization of the

CHART 5
SEPARATION OF LINSEED OIL ACIDS (13)

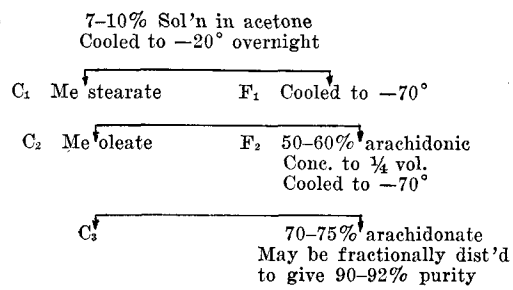


70% acid from petroleum ether she finally isolated small amounts of product of I.V. 265.5, which is a binary mixture of linoleic and linolenic acids containing 91% of the latter acid. Very recently we have repeated this work and on one occasion were able to get a product of 92% purity. Use of methyl or ethyl esters in the final stages to avoid association of components was without result. Ethyl linolenate is so soluble at −75° as to make its crystallization impractical. Finally it is possible that lowering of the temperature to −90 to −100° might be effective. Also, as noted later, urea adducts can be used in place of the free acids to give crystalline compounds separable at higher temperatures.

A 70% concentrate of the polyethenoic methyl esters of adrenal phosphatides, mainly methyl arachidonate, but containing also small amounts of pentaenoic acids and others, has been described by Shinowara and Brown (29) and Mowry, Brode, and Brown (23). The general procedure is shown in Chart 6 (25). Procedures similar to that described in Chart 6 can be applied directly to fish oil mixed acids (or esters) whereby they can be separated into saturated, monoethenoic, and polyethenoic fractions, the last with an I.V. of close to 300.

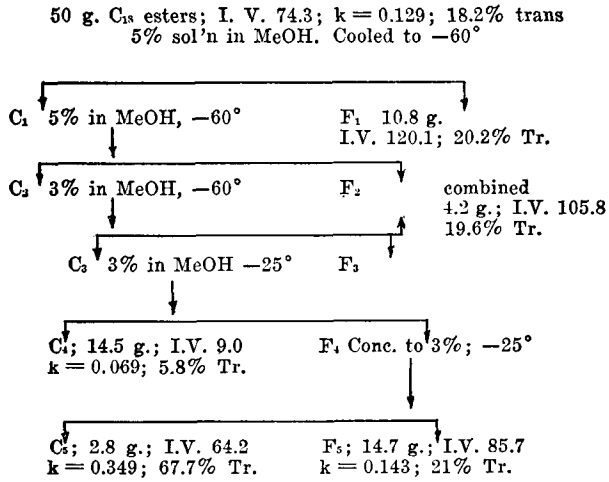
e) *The Separation of Cis-trans Mixtures of Fatty Acids.* The *trans* component of a given *cis-trans* pair of acids melts higher than the *cis* acid and possesses an intermediate solubility between the saturated member of the series and the *cis* acid. For

CHART 6
Me Esters Adrenal Phosphatides (23)



this reason it has been common practice for many years to separate such acids as oleic and elaidic by simple repeated crystallization from cold alcohol. We have used solvent crystallization to resolve partially the C_{18} esters of summer butterfat containing considerable amounts of *trans* component(s). Such a scheme is shown in Chart 7 (7). In Chart 7 about three-fourths of the octadecenoate in fraction C_5 is *trans*. The total *trans* content of the C_{18} esters, about 18.2%, is unevenly distributed throughout all of the fractions, indicating the probable presence of several *trans* octadecenoates as well as certainly some *trans* octadecadienoates. Incidentally, attempts to carry out similar separations by use of the free acids in the same solvents were unsuccessful. We have had similar experiences in trying to fractionate the fatty acids of margarines and shortenings, where the C_{18} acids are still more complicated in composition.

CHART 7
Partial Separation of C_{18} Esters of Butterfat (7)

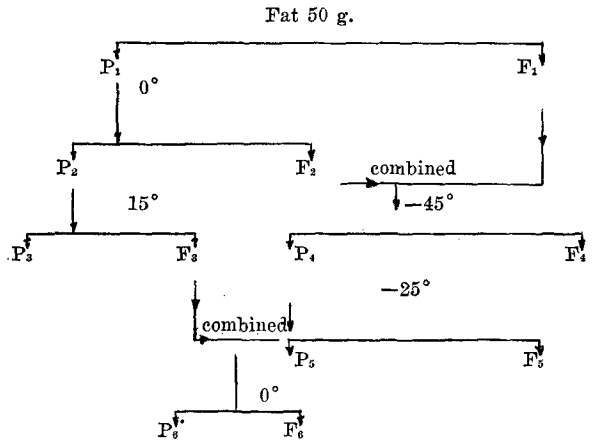


f) *The Partial Resolution of Natural Glyceride Mixtures.* The natural fats are complex mixtures of mixed triglycerides. Four solubility types may occur in any fat, namely, the fully saturated triglycerides, GS_3, m.p. 45–60°; the disaturated-unsaturated, GS_2U, m.p. 30–45°; the saturated-diunsaturated, GSU_2, m.p. 0–10°; and the triunsaturated, GU_3, m.p. below 0° For many years Hilditch and co-workers have used solvent crystallization for the partial separation of these mixtures in connection with their work on glyceride structure of the fats. Thus crystallization from

acetone at 0°–10° will partially separate the mixture into least soluble, intermediate soluble, and most soluble fractions. With liquid fats similar separations may be attained by use of temperatures down to —60°. For various modifications of the procedure the reader is referred to Hilditch's book (14) or better still to the numerous literature references to this work. In our previous reviews several examples were cited where oils such as cottonseed and corn and other fats have been rather exhaustively treated by this procedure. For purposes of illustration a recent study by Luddy *et al.* (19) of the glyceride composition of several fats follows. The scheme in Chart 8

CHART 8

Separation of Fat Into Simplified Glyceride Fractions by Crystallization from Acetone (18)
(All Concentrations, 30 ml. solvent/g.)



separates the fat into six simplified fractions, no one containing allegedly more than two of the glyceride types noted above, for example, GS_3–GS_2U, etc. Results of the study are shown in Table IV.

TABLE IV

Glyceride Composition of Lard, Chicken Fat, Palm Oil, and Cottonseed Oil (19)

Type of Glyceride	Lard	Chicken Fat	Palm Oil	Cottonseed Oil
	mol.%	*mol.%*	*mol.%*	*mol.%*
GS_3	2.8	2.3	9.4	0.0
GS_2U	27.4	17.9	48.1	14.5
GSU_2	54.8	49.2	37.3	51.0
GU_3	15.0	30.4	3.2	34.5

Solvent Crystallization of Hydroxamic Acids and Urea Adducts

During the past few years two new types of compounds of the fatty acids have been proposed for separation by solvent crystallization, namely, the hydroxamic acids and urea inclusion compounds. Space limitations here permit only brief discussion of each.

Treating the methyl esters of the fatty acids with hydroxylamine under suitable conditions results in the formation of the hydroxamic acids. The hydroxamic acids of the unsaturated acids melt much higher than the corresponding fatty acid and are considerably less soluble in solvent. Inoue and Yukawa (16) have claimed that these derivatives are especially suitable for separating fatty acids by solvent crystallization and that the procedure can be used for preparing pure unsaturated acids. Our experience with these compounds has been an unhappy one, and we have been unsuccessful in our attempts to use them in solvent fractionation. Moreover the technic is cumbersome since it involves preparation of the derivative, fractional crystallization of the product, and, finally, regeneration of the fatty acid in question. It is difficult to see how the method will come into general use.

In contrast to the cumbersome hydroxamic acid technic, the use of urea adducts of the fatty acids and their esters involves only one complicating factor, the addition of a third variable phase to the solute-solvent system, but, to more than balance this complication, the urea adducts provide certain specificities in the solvent fractionation method and are especially useful when working with polyethenoic acids. In view of the fact that there has been a very recent review of this subject by Schlenk (26), only the principle of the method and some of its accomplishments in this field will be mentioned here.

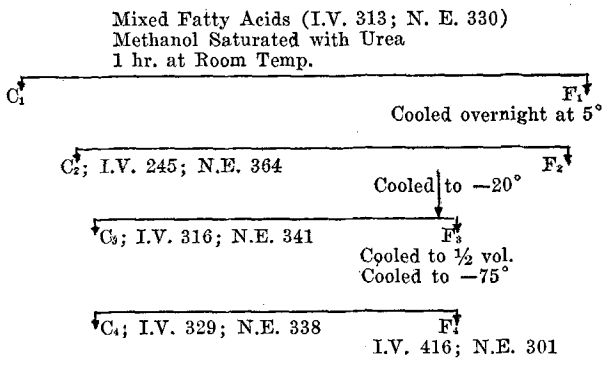
Linear organic compounds and, in the present instance, the fatty acids and their esters form definite inclusion compounds with urea by simply adding the acids or esters to an alcoholic solution of urea. The resulting crystalline compounds apparently contain only one molecule of the acid along with a specific number of molecules of urea. The adducts vary in solubility and gradual lowering of the temperature of the solution results in specific urea adduct fractions crystallizing from solution. Separation is effected according to chain length, degree of unsaturation, and branching of chain. Branched chain compounds rarely form adducts. Regeneration of the acids of a given fraction is accomplished by treatment with water and extracting the acid with immiscible solvent. In addition to the preparation of pure oleic acid, methyl oleate, methyl linoleate, and methyl ricinoleate, urea adducts have been used to separate the saturated and unsaturated components of many fats and oils, for the removal of free fatty acids from fats, and, in a number of instances, for the preparation of highly unsaturated fatty acid concentrates from marine fats. The urea adducts of the polyethenoic acids have the special advantage that the urea shell of the adduct effectively prevents easy access to oxygen. An excellent sample of separation of the polyunsaturated acids from bovine tissues is taken from the recent paper by McElroy *et al.* (21) and is shown in Chart 9. In the work described in Chart 9 it is worth noting that fractions rich in docosahexaenoic and arachidonic acids were obtained as solid complexes. The advantage of the adducts over the free acids in this instance is obvious.

General Summary

The preceding discussion has reviewed very briefly the development of solvent crystallization in the practical separation of fatty acid mixtures and certain of their derivatives. The simplicity and effectiveness of the procedure can be vouched for by those who have had occasion to use it, especially where suitable laboratory equipment is available and where dry ice is a laboratory commodity. So far the method has found only very limited commercial application, but in the research laboratory it is one of the most useful methods of lipid investigation. The method can be applied to the free fatty acids, certain of their esters, to their

CHART 9

Separation of Polyunsaturated Acids of Bovine Tissue (21)



hydroxamic acids, and to urea inclusion compounds. The urea adducts have been found in recent years to provide several very specific applications. The polyethenoic acid adducts can be crystallized at temperatures above −75° at which temperature the free acids remain liquid in solvent. Some of the mutually intersolubilizing effects observed in direct solvent crystallization of the fatty acids are apparently minimized with the urea adducts.

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The Analysis of Lipids by Countercurrent Distribution

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COUNTERCURRENT DISTRIBUTION is the name given to a particular type of liquid-liquid, multiple-stage extraction. Although this operation can be carried out as a separatory funnel procedure, the ease and labor-saving advantages of Craig's ingenious laboratory instruments makes these devices (6) and the countercurrent-distribution procedure synonymous in practice.

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The technique of countercurrent distribution holds particular advantage to the lipid chemist for a number of reasons. First, liquid-liquid extraction processes are inherently mild processes and adaptable to the study of instable lipids. In theory and usually in actual practice all of the material introduced into the fractionating device is recovered. Perhaps a most important advantage of this technique is its mathematical description which permits the prediction of solute behavior and of separability of solutes. Because of this mathematical basis both qualitative and quantitative data may be obtained from a simple weight curve. The partition coefficient which is fundamental to the calculations may be looked upon as being as characteristic a constant of the solutes as is refractive index, boiling point, melting point, and specific rotation. The partition coefficient however has the advantage over the usual constants in suggesting methods of isolation and of indicating structural features of a molecule.

Fundamentals and Useful Calculations of Countercurrent Distribution

Separation by countercurrent distribution depends upon the differences which exist in the differential solubility of individual chemical compounds when these compounds are distributed between two immiscible solvents. This differential solubility is described by the partition coefficient (K) and is defined by the equation

$$C_1/C_2 = K$$

where C_1 and C_2 are the concentrations of the given solute in the lighter upper (hyperphase) and the heavier lower (hypophase) solvent layers, respectively. At high solute concentrations and under other conditions where the ratio of associated and dissociated molecules varies, deviations from this law do occur. In those cases in which the shape of the countercurrent-distribution curve is important, an attempt is made to adjust the concentrations to the range of constancy of partition coefficient. Conditions