Crystallization of Fatty Acids

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CRYSTALLIZING is a well established and time-honored procedure in organic chemistry but with fatty acids it was applied for a long time only to the saturated ones. A change set in about 25 years ago when J. B. Brown and coworkers fractionated cottonseed and corn oil acids (1) and prepared oleic acid from olive oil (2) by crystallization at temperatures much lower than those customarily used. Brown states in a review that it was chiefly the availability of dry ice that made the technique possible (3). One may add that his initiative opened a new avenue for research on lipids and furthered its development.

Crystallization of fatty acids has a wide range of applications. On an industrial scale, it serves for crude separations of saturated and unsaturated acids, while on kilogram as well as milligram scale it is used in the laboratory for preparation of acids in high purity. The latter applications will be discussed here. They require that crystallization be combined with other separation techniques to be of preparative value.

Difference in solubility of the acids is, of course, the prerequisite for their separation by crystallization and great effort has been taken to provide the basic information. The solubilities of fatty acids increase with higher unsaturation and decrease with longer chain lengths. Unsaturated acids in *cis* configuration are more soluble than their *trans* isomers, and so are branched acids when compared with their straight-chain isomers. Because esters are more soluble than their corresponding acids, the fractionation of unsaturated esters by crystallization in many cases becomes impractical. The solubilities of fatty acids and esters increase with temperature and are largely reflected by their melting points: high melting point substances are less soluble than low melting point substances. These characteristics of the lipids agree well with those stated for nonelectrolytes in general (4). They are substantiated in numerous publications which are easily accessible and have been abstracted in several reviews (3,5,6,7,8). A reference index is given in Table I.

Only the most common acids have been investigated thoroughly. Although they represent merely a small fraction of the great number of known acids, they do include the most abundant ones. Data are lacking on the fatty acids now attracting the interests of research workers. For example, data are rather scarce on the conjugated unsaturated and oxygenated acids which are encountered in the search for new types of industrial oils. Similarly, data on highly unsaturated acids of importance in lipid metabolism do not go beyond linoleic and linolenic acids. Undoubtedly, numerous incidental observations concerning crystallization of less common acids have been made but they seldom appear in the literature.[1]

The systematic studies on solubility and crystallization of the closely related fatty acids had practical importance and contributed to theoretical aspects. Of foremost interest here is the rule that with homologs the logarithms of solubilities, when expressed as mole fractions, increase by constant increments (9). Solubilities of "missing" compounds can be calculated, or others checked, by colligating the values of members of the same series. However, with normal saturated fatty acids different sets of solubility values are encountered for even- and odd-numbered members. This is a consequence of their different types of crystal structure which bring about also the alternating of melting points and heats of fusion.

Solubility data of individual fatty acids or esters are not fully valid when mixtures are to be separated by crystallization. Solid solutions or crystalline compounds form with the closely related fatty acids and mutual solubilization of the acids occurs in solvents. Table II gives a reference index for investigations of binary mixtures of acids in solvents, a situation which is still far removed from practical conditions. Esters do not associate as strongly as the acids (10).

The reduced separation efficiency, which can be foreseen on these grounds is aggravated by incomplete removal of mother liquor from the crystalline phase. So it is understandable that only general rules have been established for fractionation by crystallization in contrast to the rather strict patterns of separation which are common in chromatographic procedures.

The technique of crystallization is simple when compared with other procedures of fatty acid separation. An inverted filtration apparatus, as described by Brown (6), is standard in many laboratories. Figure 1 specifies an apparatus of greater capacity, holding about 45 liters, which is in use at this institute. In most cases the suspension of crystals passes freely through the spigot, but they are still coarse enough for fast filtration in a table-top Büchner funnel. Rapid filtration is necessary since temperature and humidity are not controlled.

Although autoxidation is seldom a critical factor, a rather inert atmosphere can easily be provided when necessary. More elaborate devices have been designed for crystallizing and filtering small amounts (11,12, 13), where changes of temperature and condensation of water would occur rapidly when filtering in an open system. Many crystallizations of 10–100 mg. of acids have been carried out in this laboratory using the device described in Figure 2. Recrystallizations were often done in the same funnel without removing the crystals, and the apparatus is conveniently handled but it does not provide the precision which is required for measurement of solubilities.

The isolation of several fatty acids with emphasis upon their crystallization, has been described in conjunction with studies on the solubility of individual acids (3,6).

Preparation of pure oleic acid is a classical example, and details of a current procedure follow (14a).

Olive oil is interesterified in methanol with sodium methoxide and the methyl esters washed free of alkali. After drying, nearly all of the C_{16} esters is then removed by distillation in a Podbielniak hypercal col-

[1] For example, Fulco and Mead (J. Biol. Chem., *234*, 1411–1416, 1959) mention that, unexpectedly, eicosatrienoic acid could not be separated from octa- and hexadecenoic acids by crystallization.

TABLE I

Solubilities of Acids
(Literature Index)

	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}	C_{17}	C_{18}	Oleic
n-Pentane	....	....	....	....	....	....	....	....	....	....	a	....
2-Methylbutane	....	....	....	....	....	....	....	....	....	....	a	....
n-Hexane	....	....	....	....	....	....	....	....	....	....	....	e
2-Methylpentane	....	....	....	....	....	....	....	....	....	....	a	a
2,2-Dimethylbutane	....	....	....	....	....	....	....	....	....	....	a	a
2,3-Dimethylbutane	....	....	....	....	....	....	....	....	....	....	a	a
n-Heptane	....	....	....	....	....	....	....	....	a	....	a	a
Skellysolve B	....	....	....	....	b	....	b	....	b	....	b	b
2,2,4-Trimethylpentane	....	....	....	....	....	....	....	....	....	....	a	a
Cyclohexane	c	c	c	c	c	c	c	c	c	c	c	e
Methylcyclohexane	....	....	....	....	....	....	....	....	....	....	a	a
Carbontetrachloride	c	c	c	c	c	c	c	c	cf	c	cf	ef
Chloroform	c	c	c	c	c	c	c	c	c	c	c	e
1,2-Dichloroethane	d	....	d	....	d	....	d	....	df	....	df	ef
1,1-Dichloroethane	....	....	....	....	....	....	....	....	b	....	....	b
Trichloroethylene	....	....	....	....	....	....	....	....	f	....	f	f
Benzene	h	h	h	h	h	h	h	h	h	h	h	e
Toluene	d	....	d	....	d	....	d	....	abd	....	ad	ab
o-Xylene	d	....	d	....	d	....	d	....	d	....	d	e
Chlorobenzene	d	....	d	....	d	....	d	....	d	....	d	e
Ethyl ether	....	....	....	....	....	....	....	....	ab	....	a	abe
Dioxane	....	....	d	....	d	....	d	....	d	....	d	e
Methyl acetate	....	....	....	....	....	....	....	....	b	....	....	b
Ethyl acetate	c	c	c	c	c	c	c	c	ac	c	ac	ae
Butyl acetate	c	c	c	c	c	c	c	c	c	c	c	e
Acetone	hg	h	hg	h	bhg	h	bhg	h	bha	h	bha	abe
2-Butanone	....	....	h	h	h	h	h	h	h	h	h	e
Methanol	c	c	c	c	bc	c	bc	c	abc	c	abc	abe
Ethanol, 99%	....	....	....	....	h	....	h	....	h	h	h	....
Ethanol, 95%	h	h	h	h	h	h	h	h	h	h	h	e
Isopropanol	c	c	c	c	c	c	c	c	c	c	c	e
n-Butanol	c	c	c	c	c	c	c	c	bc	c	c	be
Acetic acid	....	....	h	h	h	h	h	h	h	h	h	....
Acetonitrile	c	c	c	c	c	c	c	c	c	c	c	e
Nitromethane	....	....	d	....	d	....	d	....	d	....	d	e
Nitroethane	c	c	c	c	c	c	c	c	c	c	c	e
Nitrobenzene	d	....	d	....	d	....	d	....	d	....	d	e
Furfural	d	....	d	....	d	....	d	....	d	....	d	e
Sulfur dioxide	....	....	....	....	i	....	i	i	i	....	i	i
Dimethyl formamide	....	....	....	....	....	....	....	....	....	....	a	....
Carbon disulfide	....	....	....	....	....	....	....	....	b	....	....	b
Solubilities of Methyl Esters												
Cyclohexane	....	....	....	....	....	....	j	....	j	....	j	....
Carbontetrachloride	....	....	....	....	j	....	j	....	j	....	j	....
Chloroform	j	....	....	....	j	....	j	....	j	....	j	....
Benzene	....	....	....	....	....	....	j	....	j	....	j*	....
Ethyl acetate	j	....	....	....	j	....	j	....	j	....	j*	....
Butyl acetate	j	....	....	....	j	....	j	....	j	....	j	....
Acetone	gj	....	g	....	gj	....	gj	....	gj	....	gj*	g
Methanol	j	....	....	....	j	j	j	....	j	....	j	....
Ethanol, 95%	j	....	....	....	j	j	j	....	j	....	j*	....
n-Butanol	j	....	....	....	j	j	j	....	j	....	j	....
Acetonitrile	j	....	....	....	j	j	j	....	j	....	j*	....
Sulfur dioxide	....	....	....	....	j	....	i	....	i	....	i	i

*Solubilities of ethyl, n-propyl, and n-butyl stearates are found in j.

umn at 5 mm. pressure. When the head temperature of the column rises from about 166° to 182°, C_{18} esters begin to distill essentially free of C_{16} esters. At this point the distillation is interrupted and the undistilled C_{18} and possibly higher esters are subjected in 600-g. lots (12-l. flasks) to the crystallizations listed in Table III. The recovery of oleate of >98% purity is about 50% in one such sequence, and it can be improved by recycling enriched fractions with the next batch. Precipitate P4 is distilled in batches of 3.5 kg. for final purification at 5 mm. in a hypercal column. A small forerun containing palmitate, about 200 g., is separated from the major portion, 2.8–3.0 kg., I.V. 85.5. The residue, besides containing small amounts of longer-chain fatty esters, is enriched with methyl stearate, traces of which are not removed from oleate by crystallization. Therefore, the distillation of the oleate should not be carried to the extreme end of the C_{18} fraction.

The relatively low solubility of oleate permits crystallization as ester and subsequent distillation without chemical conversion. This is not the case with linoleate. In the preparations at this institute (see below), the acid is crystallized first with and then without urea and subsequently esterified for distillation (14b).

The preparation of petroselinic (15) and of α- and β-eleostearic acids (16) are other recent examples for crystallization procedures. A scheme for purification of palmitoleic acid is shown in Table IV, using a C_{16} fraction of menhaden fatty acid methyl esters. The starting material contained about 45% palmitate, 45% palmitoleate, 8% higher unsaturated C_{16} esters, and 2% pentadecanoate and unidentified material. The GLC recordings of the pertinent fractions are given in Figure 3. Precipitate 3 was pure enough for the particular purpose of this preparation. It appears that the separation of palmitic and palmitoleic acids does not involve the difficulties encountered with oleic and stearic acids.

The use of crystallization for isolation of minor components has been demonstrated masterfully by Hansen, Shorland, and their coworkers. The unsaturated acids of a C_{18} fraction of butterfat had been concentrated by repeated crystallization at low temperature. It was expected that oxidation, with $KMnO_4$ in acetone, of the oil retained in the mother liquor, would leave behind only stearic or possibly other common saturated acids. However, after oxidation, the residual long-chain acids, although saturated, were not compatible with straight chain acids (17). When C_{18} acids of lard were subjected to the same procedures only the conventional acids were found, indicating a genuine difference in the two materials. In further pursuit, the isolation of the unknown acids was improved by hydrogenating the concentrate instead of oxidizing it. More than 4 kg. of C_{18} methyl

TABLE I (Concluded)

Solubilities of Acids
(Literature Index)

	Elaidic	Petroselinic	Petroselaidic	Linoleic	Linolenic	Stearolic	C_{20}	Eicosenoic	C_{22}	Erucic	Brassidic
n-Pentane	....	....	....	....	....	....	....	....	....	....	....
2-Methylbutane	....	....	....	....	....	....	....	....	....	....	....
n-Hexane	....	....	....	e	....	....	....	....	....	....	....
2-Methylpentane	....	....	....	....	....	....	....	....	....	....	....
2,2-Dimethylbutane	....	....	....	....	....	....	....	....	....	....	....
2,3-Dimethylbutane	....	....	....	a	....	....	....	....	....	....	....
n-Heptane	a	a	a	a	....	a	a	a	a	a	a
Skellysolve B	....	....	....	b	b	....	b	b	b	b	....
2,2,4-Trimethylpentane	....	....	....	....	....	....	....	....	....	....	....
Cyclohexane	....	....	....	e	....	....	....	....	....	....	....
Methylcyclohexane	....	....	....	a	....	....	....	....	....	....	....
Carbontetrachloride	....	....	....	e	....	....	....	....	....	....	....
Chloroform	....	....	....	e	....	....	....	....	....	....	....
1,2-Dichloroethane	....	....	....	....	....	....	....	....	....	....	....
1,1-Dichloroethane	....	....	....	....	....	....	....	....	....	....	....
Trichloroethylene	....	....	....	....	....	....	....	....	....	....	....
Benzene	....	....	....	e	....	....	....	....	....	....	....
Toluene	a	a	a	....	....	a	a	a	a	a	a
o-Xylene	....	....	....	....	....	....	....	....	....	....	....
Chlorobenzene	....	....	....	....	....	....	....	....	....	....	....
Ethyl ether	a	a	a	....	....	a	a	a	a	a	a
Dioxane	....	....	....	....	....	....	....	....	....	....	....
Methyl acetate	....	....	....	....	....	....	....	....	....	....	....
Ethyl acetate	a	a	a	ae	....	a	a	a	a	a	a
Butyl acetate	....	....	....	....	....	....	....	....	....	....	....
Acetone	a	a	a	abeg	bg	a	ab	ab	ab	ab	a
2-Butanone	....	....	....	e	....	....	....	....	....	....	....
Methanol	a	a	a	abe	be	a	ab	ab	ab	ab	a
Ethanol, 99%	....	....	....	....	....	....	....	....	....	....	....
Ethanol, 95%	....	....	....	e	....	....	....	....	....	....	....
Isopropanol	....	....	....	e	....	....	....	....	....	....	....
n-Butanol	....	....	....	e	....	....	....	....	....	....	....
Acetic acid	....	....	....	....	....	....	....	....	....	....	....
Acetonitrile	....	....	....	e	....	....	....	....	....	....	....
Nitromethane	....	....	....	....	....	....	....	....	....	....	....
Nitroethane	....	....	....	e	....	....	....	....	....	....	....
Nitrobenzene	....	....	....	....	....	....	....	....	....	....	....
Furfural	....	....	....	....	....	....	....	....	....	....	....
Sulfur dioxide	i	....	....	i	....	....	....	....	....	....	....
Dimethyl formamide	....	....	....	....	....	....	....	....	....	....	....
Carbon disulfide	....	....	....	b	....	....	....	....	....	....	....
Solubilities of Methyl Esters											
Cyclohexane	....	....	....	....	....	....	....	....	....	....	....
Carbontetrachloride	....	....	....	....	....	....	....	....	....	....	....
Chloroform	....	....	....	....	....	....	....	....	....	....	....
Benzene	....	....	....	....	....	....	....	....	....	....	....
Ethyl acetate	....	....	....	....	....	....	....	....	....	....	....
Butyl acetate	....	....	....	....	....	....	....	....	....	....	....
Acetone	....	....	....	g	g	....	....	....	....	....	....
Methanol	....	....	....	....	....	....	....	....	....	....	....
Ethanol, 95%	....	....	....	....	....	....	....	....	....	....	....
n-Butanol	....	....	....	....	....	....	....	....	....	....	....
Acetonitrile	....	....	....	....	....	....	....	....	....	....	....
Sulfur dioxide	....	....	....	....	....	....	....	....	....	....	....

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e. Hoerr, C. W., and Harwood, H. J., J. Phys. Chem., *56*, 1068–1073 (1952).

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g. Privett, O. S., Breault, E., Covell, J. B., Norcia, L. N., and Lundberg, W. O., J. Am. Oil Chemists' Soc., 35, 366–370 (1958).

h. Ralston, A. W., and Hoerr, C. W., J. Org. Chem., *7*, 546–555 (1942).

i. Schlenk, H., and Ener, M. A., J. Am. Oil Chemists' Soc., *36*, 145–149 (1959).

j. Sedgwick, R. S., Hoerr, C. W., and Harwood, H. J., J. Org. Chem., *17*, 327–337 (1952).

ester concentrate were subjected to three crystallizations from acetone at −30°. The acetone-soluble material was hydrogenated and again fractionated by repeated crystallization to eliminate newly formed stearate. The portion then still soluble in acetone was repeatedly distilled and appropriate fractions combined according to their physical properties. Further crystallizations in the form of acids led finally to the isolation and identification of *iso-* and *anteiso*-heptadecanoic acids (18).

The unexpected result drew more attention than the skillful use of the crystallization method. The occurrence of isomeric acids has subsequently been established for many natural fats (19) and more recent work still exemplifies the use of crystallization. Isolation of *cis*-9,10-heptadecenoic acid involved 18 recrystallizations as part of the intermediate enrichments (20).

Hydroxamic acids, which can be easily obtained from fatty acid esters, have been used for fractional crystallization (21). The hydroxamates of oleic, linoleic, and linolenic acids have m.p. 61°, 41–42°, and 37–38°, respectively. They are easier to handle in crystallizations than the acids or esters and purification of oleic acid by this procedure has been described (22). However, the hydroxamic acid method did not find broad application, probably because of the chemical conversions required.

Saturated, oleic or elaidic acids each form a com-

TABLE II

Solubilities of Binary Mixtures of Fatty Acids

	Palmitic+stearic	Palmitic+oleic	Stearic+oleic
Skellysolve B	....	c	b
Carbon tetrachloride	....	d	....
Dichloroethane	....	d	d
Trichloroethylene	....	....	d
Benzene	a	....	....
Acetone	a	c	b

a) Ralston, A. W., and Hoerr, C. W., J. Org. Chem., *10*, 170–174 (1945).
b) Singleton, W. S., J. Am. Oil Chemists' Soc., *25*, 15–20 (1948).
c) Singleton, W. S., J. Am. Oil Chemists' Soc., *26*, 332–336 (1949).
d) Preckshot, G. W., and Nouri, F. J., J. Am. Oil Chemists' Soc., *34*, 151–155 (1957).

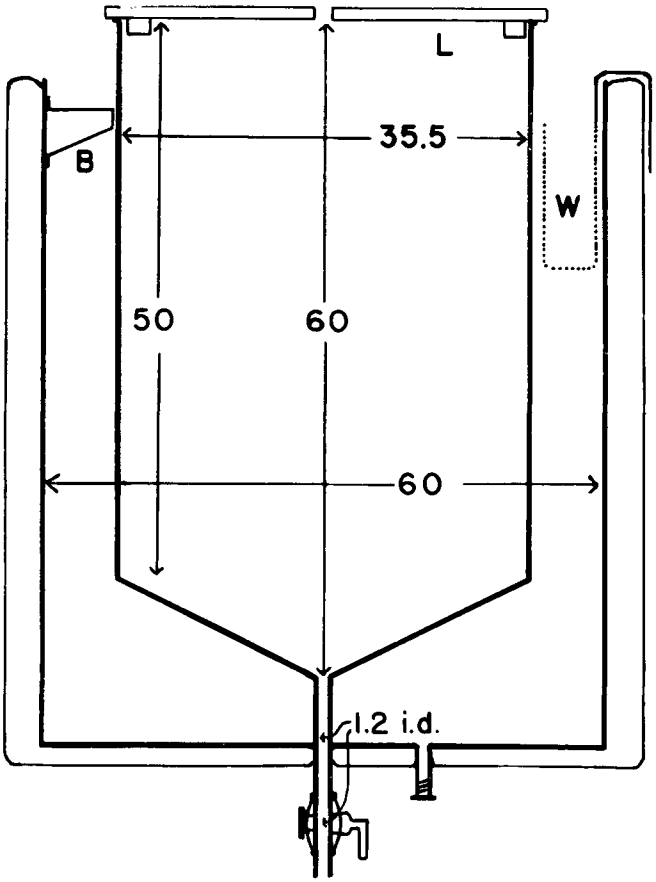


FIG. 1. Crystallization apparatus for large amounts at low temperature (dimensions in cm.): stainless steel, capacity of the inner container about 45 l.; segmented wooden lid; two or three wire gauze baskets, W, to facilitate feeding of dry ice into organic coolant; brackets, B, to center the inner container; insulated with sheets of fiberglass covered with asbestos paper. The container is mounted on a tripod with angle iron feet 30 cm. high, welded to rings on top and bottom. Explosion proof motors drive stainless steel stirrers in the bath and in the solution. The hole in the spigot must have the same diameter as the tubing. When crusts of lipids form on the wall they are scraped off and the bath is cooled more slowly.

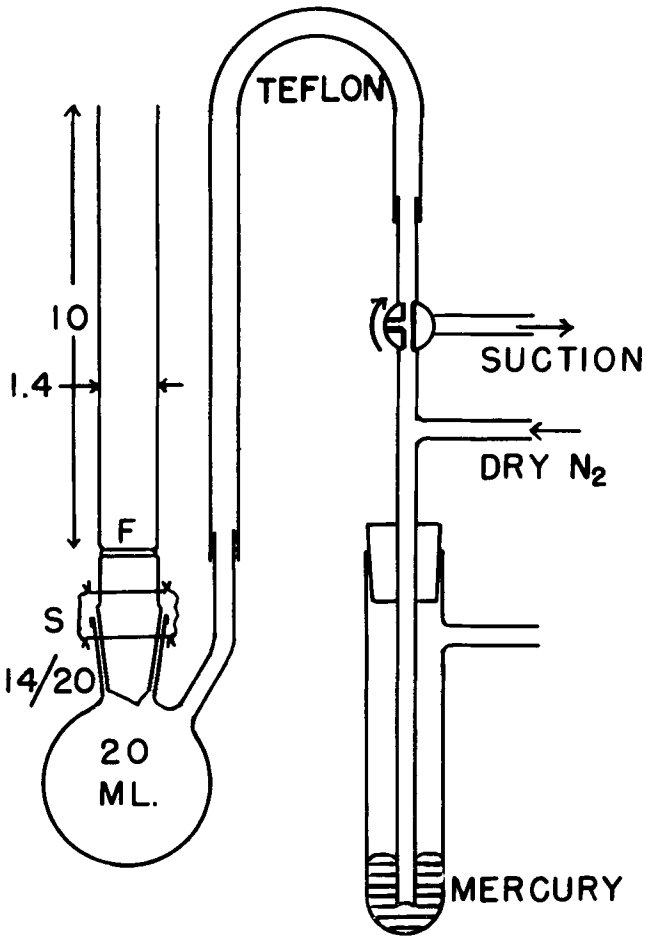


FIG. 2. Crystallization apparatus for small amounts at low temperature: medium porosity sintered glass filter, F; springs, S. The flow of N_2 is regulated to bubble slowly through the mercury so that the solvent is held over F by a slight overpressure. Bulb and funnel with solvent are immersed into a transparent Dewar flask with organic coolant, observed for leakage, and then the sample is added. After mixing, a drying tube is placed on top of the funnel and the temperature lowered slowly.

pound with acetamide of a component ratio 1 acid: 1 acetamide (23,24). Phase diagrams of the eleostearic acids with acetamide failed to indicate such compound formation, and it must be concluded that fatty acids of unsaturation higher than one double bond do not undergo complex formation (25). The compounds of saturated acids with acetamide can be recrystallized from concentrated solutions and their use for purification has been demonstrated (26). The acids can be recovered by washing with water.

When discussion about the structure of the natural glycerides began (27), crystallization was the only separation technique for these components. Although oxidation procedures and countercurrent distribution

TABLE III

Oleate by Crystallization from Acetone

C_{18} esters, 7%, −60°
- – – – – ML_1 (removal of mainly linoleate)

P_1, I.V. 81 — 84, 10%, −37°
- – – – – P_2 (removal of mainly stearate)

ML_2, I.V. 86 — 90, 7%, −60°
- – – – – ML_3 (removal of linoleate)

P_3, I.V. 84 — 87, 7%, −60°
- – – – – ML_4 (recycled)

P_4, I.V. 84.5 — 85.5 (oleate)

TABLE IV

Palmitoleic Acid by Crystallization from Acetone

130 g. C_{16} esters
10%, −25°
- – – – – 60 g. P_1 (palmitate)

70 g. ML_1 → 64 g. acids
8%, −38°
- – – – – 5 g. P_2

ML_2, 15%, −62°
- – – – – ML_3

20 g. P_3 (palmitoleic acid)

have now become prominent tools in such investigations, crystallization is still used with the more saturated triglycerides. A modification of the conventional crystallization technique has been applied recently to triglycerides and is discussed in the following.

A column filled with glass beads about 0.1 mm. in diameter serves as a filter bed so that diffusion along the column is prevented while local equilibration of crystals and solution can proceed. The column is placed into a closely fitting metal block or heavy-wall tube which is insulated outside, but heated at the top and cooled at the bottom. A rather linear temperature gradient prevails over the length of the column. The initial solvent chosen is a "poor" one for the sample, and the solubility is raised by gradient mixing with "good" solvent. As components are dissolved at the top of the column by improved solvent they are carried into a colder part where they crystallize again, and so on. They migrate according to their solubility in the balance of solvent composition *vs.* temperature, and the procedure has some features in common with column chromatography. It has been classified as such (28) or as thermal gradient crystallization (29).

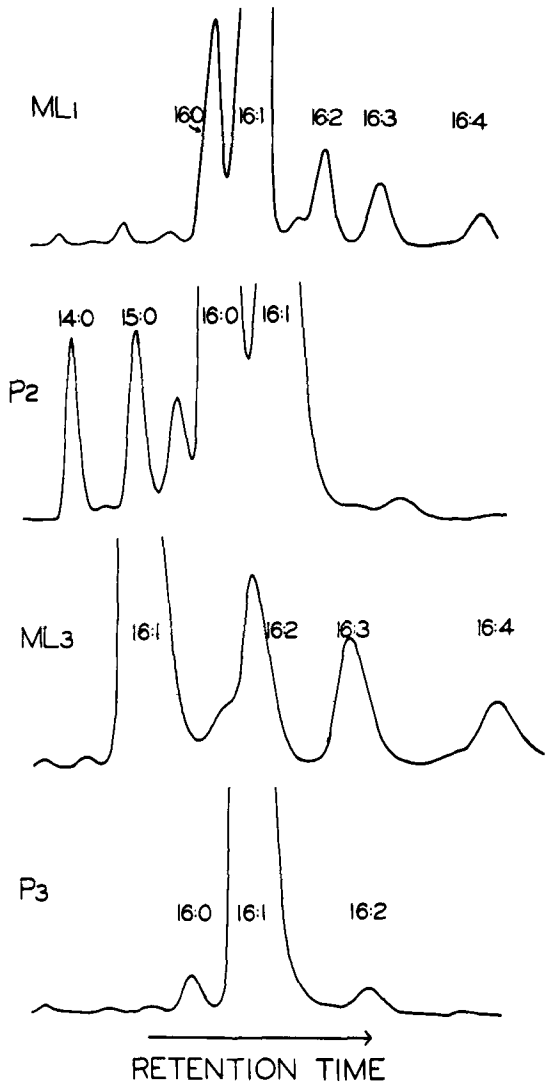


FIG. 3. Palmitoleic acid by crystallization from acetone. GLC analysis of the fractions encircled in Table IV.

This principle was used first for fractionation of polystyrenes (28) but has since been applied also to triglycerides (29). In the latter case, acetone and Skellysolve B were the poor and good solvents, respectively, and temperature differences of 30° have been employed, in a range between −29° and +35°. At least one hour and often longer is considered necessary for equilibrating crystals and solutions of lipids. Therefore, the flow rates must be held very low. Separations with model mixtures of triglycerides were sometimes in agreement with predictions, which had to consider eutectica. In several cases, however, the expected efficiency was not reached. Besides model mixtures of triglycerides, cocoa butter has served for testing this method (30).

Zone Refining

Fractional freezing of a crystallizable liquid without solvent has been used in several instances for purification of organic compounds (31). The present interest in such procedures originates mainly from inorganic chemistry where the melting and freezing process, when carried out repeatedly and in directed manner, led to remarkable achievements in purification of metals. A prerequisite for such a process is that the components of the melt do not form solid solutions or eutectica, or will do so only to a very minor extent. Therefore, it can be expected that this method will have only limited application to the traditional separations of palmitic, stearic, oleic, and linoleic acids. It is briefly discussed here although there are only a few reports on zone refining of lipids. The automatic and continuous technique may balance some of the limitations, and attempts to separate lipids in this fashion are undoubtedly being made in many laboratories.

Zone refining is carried out in a long tube or trough which holds the sample and is kept at a temperature below the freezing point of the material. The tube passes slowly through one or several hot zones repeatedly in one direction (or the hot zone moves along the tube) to melt impure material at the front and to crystallize it in better purity at the end of the melted area. The components which are rejected by the newly forming crystalline phase are carried with the liquid zone along the tube. Figure 4 shows the principle of the process.

A zone melting refiner for samples of 1 to 50 ml. is commercially available (32) and several other designs, some of them for smaller amounts, have been described in the literature (33,34,35,36). Obviously zone melting is more easily applied at temperatures higher than room temperature. Lower temperatures, however, will be necessary for many lipids. Besides, it has been experienced that high melting fatty acids undergo some decomposition in zone refining although they are normally considered as perfectly stable.

Zone refining has been applied to a C_{16} fraction of menhaden esters. The major component was palmitate, containing 7% palmitoleate and probably very small amounts of higher unsaturated esters. After 24 passes, palmitoleate had accumulated to a concentration of 15% at one end of the tube (37). About 5 g. were used in these experiments, but the technique has also been applied to centigrams of fatty alcohols. Thirty mg. of a mixture of C_{20} and C_{26} alcohols yielded nearly 10 mg. of the latter in purity but the former was not obtained in pure form (38).

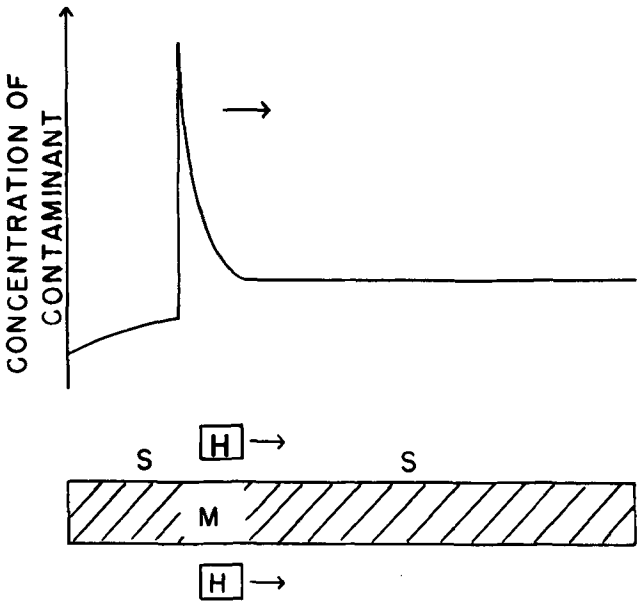


FIG. 4. The principle of zone refining. The ring-shaped heater, H, moves slowly to melt the zone M of the solid material S. The rate of movement must take into consideration the growth rate of the crystals and the diffusion rate of the contaminants in the melt. The reverse movement to the origin is rapid to avoid melting before start of the next pass.

Other examples were C_{16} and C_{18} alcohols, waxes, synthetic 3-methyl-1,2-cyclopentanophenanthrene (33), and phenols (39).

The purification of benzene from thiophene is an example of zone refining at lower temperature (36). In this publication it is also pointed out that zone melting can supplement, and in some respects excel, the technique of freeze-drying. Very diluted aqueous solutions of ascorbic acid and of certain quinones could be greatly enriched, in the latter case without involving loss due to volatility, as would be the case by the freeze-drying method.

The efficient applications of zone refining to lipids may be in areas quite different from those traditional for separations by crystallization. Monographs on zone refining are mainly devoted to metals and inorganic compounds (40). A monograph announced for the near future (38) may emphasize its use in organic chemistry.

Urea Inclusion Compounds

Although the crystallization of fatty acids with urea has not found application on an industrial scale, it is often used in the laboratory. Oleic, linoleic, and other unsaturated acids or esters can be crystallized in the presence of urea at more convenient temperatures than is possible without the auxiliary crystal structure of urea. On the other hand, the composition of the crystals is about 3 parts urea : 1 part lipid. This enlarges the amount which must be handled and an additional operation is necessary to remove the urea even when the desired compound remains unbound in the mother liquor.

The urea compounds [2] of fatty acids have been reviewed (41), and the possibility of their application on industrial scale has been discussed (42). There are newer reviews on inclusion compounds but they are not devoted to fatty acids specifically (43).

[2] Attention is called to a brief history of the initial findings, by F. Bengen, the discoverer of the urea complexes [Angew. Chem., *63*, 207–208 (1951)].

Urea crystallizes from solvents in a tetragonal system of closely packed molecules. When crystallizing from solvents containing straight-chain solutes of more than 4 to 6 carbon atoms, urea forms a hexagonal structure in which the molecules are packed more loosely. The solute molecules are incorporated inside the channels of the hexagons. Although urea as the "host" determines the crystal structure, its hexagonal form exists only when filled with "guest" molecules. The dimensions of the structure are not variable and from Figure 5 the selectivity of the urea reaction can be understood (44,45). n-Heptane is readily included, 3-methylheptane and substituted benzene rings may be included under certain conditions, while 2,2,4-trimethylpentane is not included.

The distinction between straight and cyclic or branched compounds is not by an all-or-none rule (44). A considerable number of the latter type molecules have been included (46) but the branches are not a prominent feature of their shape. The stability of these complexes is much lower than that of comparable straight compounds. Apparently, the low stability of an obstructed area can be compensated for by another area of the channel which contributes great stability. Such compensation can also occur intermolecularly, for example, when 3-methylheptane is "trailed" into the structure by n-decane although the former alone would not be included under equal conditions. The same must be expected for Substituents like OH, Br, and others enter similarly into the consideration of steric compatability when they are attached to the secondary carbon atoms of fatty acids of different tendency to react with urea. a chain. When in primary position, that is, at the end of the molecule, they contribute to the stability of the complexes, but their influence is not great with aliphatic chains of 12 or more carbon atoms.

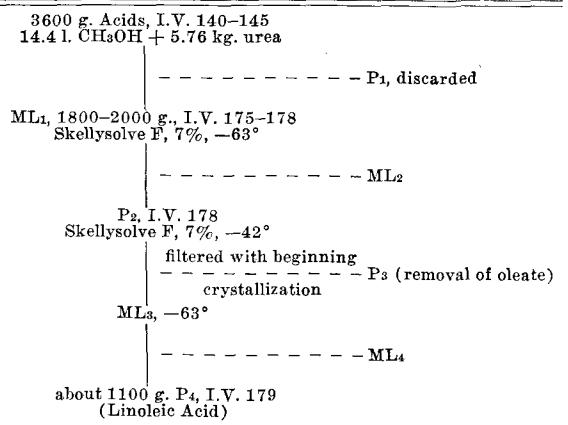
The formation of the complexes can be described by the overall equation m Urea + n Acid $\rightleftharpoons$ $Urea_m \cdot Acid_n$ (crystalline). The longer the included chain, the more the equilibrium tends toward the solid phase, but unsaturation shifts the equilibrium towards dissociation. Already one methyl branch causes a marked change towards disassociation but crystallization is not ruled out completely. Interaction becomes practically nil with heavily branched or otherwise bulky molecules.

Separation of fatty acids concerns mainly chain length and unsaturation, while branched acids are seldom involved. Urea is rather inefficient in separation by chain length, at least with palmitic, stearic, and similar acids. Urea fractionation according to unsaturation, however, has often replaced the direct crystallization. The difference in complex formation is particularly pronounced with oleic and linoleic acids. The complex of linolenic acid is prepared with good yield only at high concentration of the components and at low temperature (47), but arachidonic acid can still be bound (48).

Fatty acids with 5 or 6 double bonds have very little tendency to react with urea. However, their methyl esters are bound much more easily than the increase in chain length by the additional methyl group would warrant (49). In cases so far reported the fatty acids are dimers in the channel, probably by hydrogen bonding between the carboxyl groups (50). Complexes of methyl esters, to the author's knowledge, have not been investigated in this respect,

TABLE V

Crystallization of Linoleic Acid



but one can speculate that, as in solutions, there is little if any interaction between them in the channel. This would free forces of the ester group to stabilize the urea structure while in the case of the acids, they are bound to the neighboring carboxyl group.

According to the reaction of oleic, elaidic, and stearic acids with urea, *cis* unsaturated compounds are less stabile than their *trans* isomers, which in turn are less stabile than the corresponding saturated compounds. Since a molecule of elaidic acid can be perfectly straight just like one of stearic acid, the lower stability of the former cannot be explained by steric reasons such as are brought forward to explain the lower stability of oleic acid-urea. The eleostearic acids react with urea much more readily than does linolenic, but again not as easily as stearic acid.

The preparation of methyl linoleate is given as a typical example of a urea separation (14b). A 24-l. flask, containing 14.4 l. of methanol and equipped with a motor-driven stirrer is heated on a steam bath. Altogether 5.76 kg. of urea is added in portions, while N_2 is passed first through, then over the liquid. Heating is stopped when all urea is dissolved and 3.6 kg. of safflower acids, preheated to about 50°, is added in a slow stream. A precipitate forms immediately and good agitation is necessary until the temperature falls to 20–25°. Crystallization is completed by standing overnight at room temperature. The mixture is filtered through a Büchner funnel and the filtrate washed to remove the unreacted urea in 2-l. portions using the equal volume of 5% aqueous HCl in a 6-l. separatory funnel. The recovered acids are washed several times with water and then dried for further crystallization. The complete scheme is given in Table V. Precipitate P_4 is esterified and distilled in a Podbielniak hypercal column to obtain linoleate of a purity of >99%.

Similarly, preparations of linolenic (51) and arachidonic esters (56) involve a urea precipitation for removal of the more saturated portion of the starting materials. Numerous examples to this end are found in the literature. One has to keep in mind, however, that normally the urea procedure alone cannot yield pure individual acids or esters from natural source mixtures.

Some less known applications of urea to lipid separations are mentioned briefly in the following.

Vinyl esters of fatty acids have been precipitated with urea to purify them from cross-linked polymers and other contaminants (53). Peroxides of oxidized unsaturated acids have been concentrated from the non-autoxidized portion by binding the latter (54). The reactions of mono- and diglycerides with urea have been investigated: it appears that under certain conditions diglycerides are precipitated preferentially (55,56), but the contrary has also been reported (57); the α-monopalmitin is selectively precipitated from its mixture with β-monopalmitin (58); the withdrawal of the α-compound from the solution is apt to bring about the further conversion of β- into α-palmitin (58); furthermore, methanolysis occurs during adduct formation of monoglycerides (59).

Partition and adsorption processes, when carried out in repetitious or continuous systems, achieve complete separations of fatty acids or esters. The results with urea in column procedures are somewhat disap-

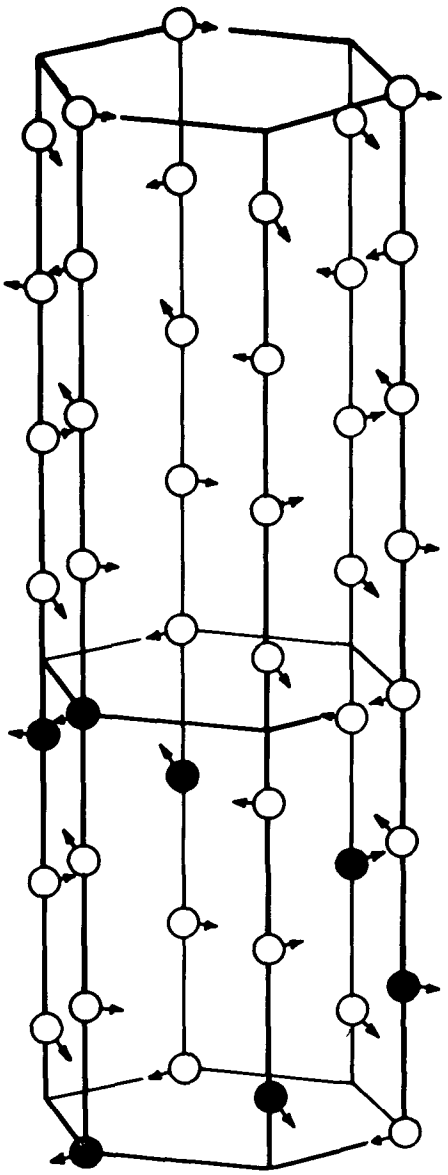


FIG. 5. The hexagonal structure of urea. The unit cell (lower part) is formed by 6 molecules of urea and has a length of 11.1 Å and a diameter of 8.2 Å. The diameter normally available for guest molecules is estimated to be about 4.5 to 5.5 Å. The maximum diameter of a cross section, vertical to the axis of the stretched molecules, is about 4.5 Å for n-heptane, 5.5 Å for 3-methylheptane, 6 Å for benzene in only one direction, and 6 Å for trimethylpentane in several directions.

pointing, and little has been reported on this. The separation of tuberculostearic (60) and of C_{27}-phthienoate (61) from related straight-chain compounds could be achieved in urea columns. It was not possible, however, to separate satisfactorily stearic and oleic acids.

A major distinction between urea and the conventional stationary phases is that the solute when bound by urea is not only in the surface layer, but also inside the solid phase. This may be expected to hinder equilibration. When provision is made for multiple and complete equilibration much better separations can be achieved. A countercurrent liquid-solid distribution has been described (62) with a urea-saturated liquid phase of methanol and ethyl acetate, 7:3, and a solid phase of urea and its complex. Figure 6 shows the separation of stearic, oleic, and palmitic acids and it is of particular interest that the standard pattern of liquid-liquid distribution is

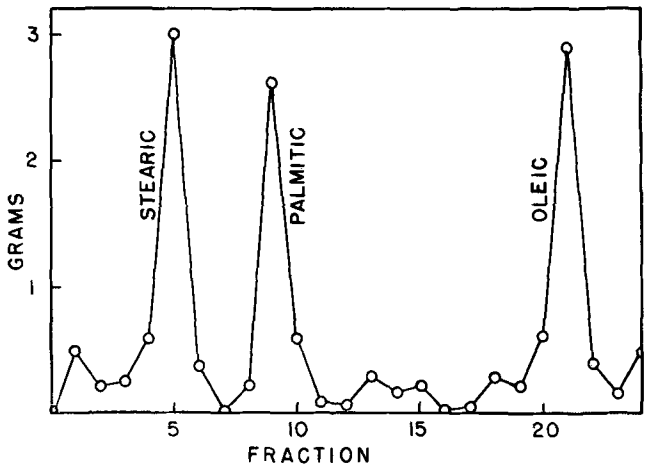


FIG. 6. Liquid-solid countercurrent distribution of fatty acids with urea as solid phase. Each tube contained 250 ml. of liquid and 10 g. solid phase. Twenty-four stages were carried out.

changed. Oleic acid moves here far ahead of palmitic acid while usually they migrate together. Elaidic and oleic acid have been separated by this method which also has been applied to salmon egg fatty acids. The equilibrations were carried out by heating and cooling (dissolving and crystallizing) in Erlenmeyer flasks and Büchner funnels were used for the phase separations. All operations were done by hand. The use of a more convenient device has not yet been reported for fatty acid separation.

Inclusion Compounds Other than Urea

The lack of a comprehensive review on inclusion compounds of fatty acids warrants noting that there are some complexes of fatty acids with host molecules other than urea.

Thiourea is able to form an inclusion structure similar to that of urea but it has wider dimensions. Branched or cyclic compounds like cyclohexane are bound tightly but the straight-chain fatty acids do not react. However, cyclohexyl esters of fatty acids are readily included by thiourea (63). The same esters are also able to react with urea, in which case the aliphatic portion of the molecules is the stabilizing factor (64). This permits comparison of thiourea and urea for fractionations of the very same compounds. Isobutyl esters react more readily with urea than with thiourea while the opposite applies to t-butyl esters. In all cases the saturated esters are bound preferentially and the same was found when reacting mixtures of fatty acids with deoxycholic acid to form "choleic acids," and when reacting fatty acids with cyclodextrins (Schardinger dextrins) (65).

The choleic acids have been reviewed (66) and numerous complexes of fatty acids with cyclodextrins have been described (67,68). When unsaturated fatty acids are bound in these channel-type complexes they are resistant to autoxidation as in the case of urea (63,69).

Fatty acids are solubilized in water by the presence of cyclodextrins due to inclusion association. The solubilization increases with chain length of the fatty acids but not enough to cancel completely the normal decrease of solubility with extending chain length (68).

None of the hosts besides urea have so far found practical importance for separation of fatty acids, although they could serve for such a purpose.

Acknowledgment

The author is thankful to O. S. Privett and J. D. Nadenicek for discussing and making available data on the preparations of oleate and linoleate.

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