Phase Investigations of Fats

I. Apparatus and Techniques for Fat-Solvent Systems at Low Temperatures

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THE technique of fractional crystallization from solvents—for the resolution of mixtures of fatty acids or their derivatives—is of fundamental importance in the analysis of fats and the laboratory preparation of pure fatty compounds. Lately it has also begun to assume some industrial importance.

Present solubility data on single fatty acids and related substances are of limited value in predicting the behavior of mixtures of these substances in solution, for the intersolubilizing effects of different components in the mixtures are considerable. Consequently, the fractional crystallization of natural mixtures of fatty acids or other fatty materials is now a largely empirical operation. A beginning toward placing the process on a completely scientific basis can be expected only after complete phase diagrams have been constructed for fat-solvent systems containing more than one fatty component.

That such diagrams are not as yet available may probably be ascribed in part to the considerable experimental difficulties involved in equilibrating and separating mixtures of fats and volatile solvents at relatively low temperatures. This communication will describe the construction and use of an apparatus which has been successfully employed in carrying out these operations.

The Constant-Temperature Bath

A photograph and a very brief description of the constant-temperature bath used have been published previously (1).

The bath proper consists of a square tank made from sheet copper, 16 inches in each dimension. It is filled to within about 2 inches of the top with ethyl alcohol. The sides and bottom of the tank are insulated with 6 inches of cork board, and the top is closed with a 2-inch cork board cover. All outer cork surfaces are coated with an asphalt paint to make them impervious to moisture. The bath is stirred by two 2½-inch, 3-bladed opposed impellers on a vertical shaft, which is coupled directly to a 1/20-horse power electric motor.

The bath is cooled by chilled trichloroethylene circulated through approximately 50 feet of ⅜-inch O. D. copper tubing in the form of a box coil around the inner sides of the tank. From the upper coil of tubing the trichloroethylene goes into an open reservoir of approximately 0.1-gallon capacity. The reservoir empties through a ¼-inch gate valve into the suction of a cast iron centrifugal pump. The pump is driven by a ⅛-horse power motor. The pump, valve, and reservoir are grouped together in an insulated box attached to one side of the bath and are elevated slightly above the cooling coil in the bath.

The discharge side of the pump is connected to the bottom end of a circular 135-foot coil of ½-inch O. D. copper tubing placed in the chilling chamber of a —120° F. Cascade Deepfreeze unit. This coil is wound in such a manner that it lies closely against the walls of the circular chilling chamber; it thus decreases the storage capacity of the chamber very little.

Temperature control of the bath is obtained by on-off operation of the circulating pump through an electronic relay circuit which includes a special toluene-filled thermoregulator. A diagram of the relay circuit is shown in Figure 1. This diagram is self-

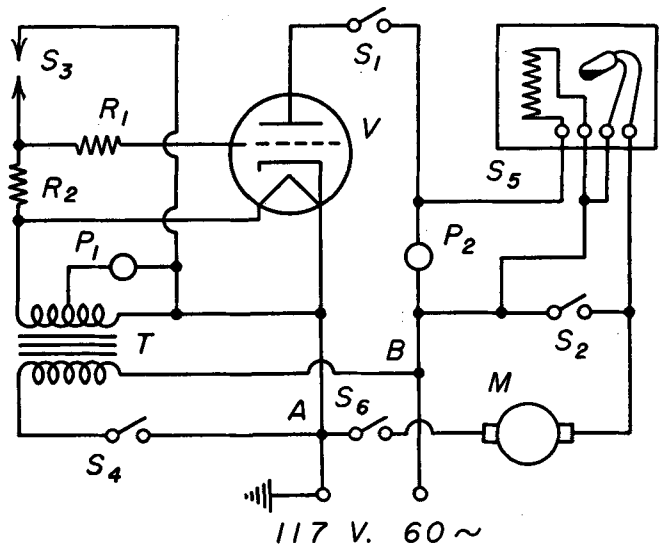

FIG. 1. Wiring diagram, temperature control system S_1, S_2, S_4, S_6, toggle switches, 3 a., 125 v.; S_3, thermoregulator; S_5, Mercoid switch, pilot circuit, 30 v., D. C., 0.25 a., load circuit, 115 v., 0.25 a.; R_1, R_2, resistors, 1 megohm, A; 1 w.; P_1, pilot lamp, 2.5 v.; P_2, pilot lamp, 115 v., 5 w.; T, transformer, 5 v., 5 a., center tap; M, pump motor, ⅛ h.p.; V, G. E. Thyratron, FG-57.

explanatory; however, mention should be made of certain precautions which are necessary in operating the circuit. A new Thyratron tube should be heated 30 minutes before it is made to conduct current. In other words, switch S_1 should not be closed until 30 minutes after switch S_4 is closed. Even an old tube must be heated 15 to 20 minutes. If the tube does not conduct or glow after it has been properly heated and the circuit completed by closing switches S_1 and S_4 and the thermoregulator contacts, the leads to the primary of the transformer should be interchanged at points A and B.

The construction of the thermoregulator is shown in Figure 2. This instrument consists essentially of two Pyrex glass bulbs A and C connected by a length of 1 mm. capillary tubing in which has been inserted a stopcock B. Just below the stopcock a U-shaped side

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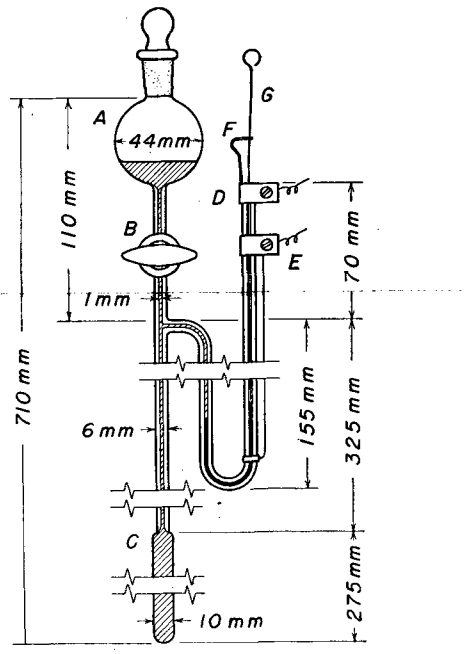

FIG. 2. Equilibration-filtration apparatus, including support and electromagnet.

arm of 1 mm. capillary tubing is attached. A platinum wire is fused into the glass near the bottom of the "U" so that one end projects into the capillary; the other end is brought up to the terminal *E*. An adjustable chromel wire *G*, which is inserted into the open end of the side arm, is held in place by a phosphor bronze spring *F*. The terminal *D* is used for electrically connecting the wire into the relay circuit. This circuit is opened or closed as mercury in the side arm makes or breaks contact with the sharpened end of the wire.

With the assembly at room temperature, stopcock *B* is closed and bulb *A* filled with dry toluene. The system is evacuated through the open end of the U-tube and completely filled with toluene by opening the stopcock *B*. Stopcock *B* is again closed and bulb *C* heated, forcing toluene out of the open end of the U-tube. By placing this open end below the surface of a supply of mercury and discontinuing the heating of bulb *C*, mercury will flow into the U-tube as the toluene cools. When the U-tube is approximately half full of mercury, stopcock *B* is opened, the headspace in bulb *A* is filled with dried air, and the system is sealed by inserting the stopper at the top of the bulb.

In use, the thermoregulator is placed in the bath with the stopcock open until thermal equilibrium has been attained. The stopcock is then closed, and fine adjustment of the operating temperature is obtained by sliding wire *G* up or down the open end of the U-tube.

To avoid drifting of settings through slow separation of dissolved moisture it is essential to dry the toluene thoroughly and to keep the apparatus sealed against moisture in the atmosphere. Drying is satisfactorily accomplished by placing the toluene under reduced pressure and allowing a portion to boil off.

A lubricant resistant to solution in toluene must be used on the stopcock and on the ground glass stopper which closes bulb *A*. "Citriteg" (2), a citric acid-tetraethylene glycol resin, has been found a very suitable lubricant. The stopper in bulb *A* may be sealed in permanently. The stopcock, however, must be opened before the thermoregulator can be removed from the bath and must be opened and closed each time any considerable change is made in the temperature adjustment. It is essential that there be provided for the toluene an expansion bulb which can be shut off from the operating part of the system. An expansion bulb within the system must necessarily be exposed to atmospheric temperatures, and even in an air-conditioned room sufficient fluctuations in volume will occur to render control of the bath temperature inaccurate.

In operation, the bath is cooled to the desired temperature, the thermoregulator is set, and the valve on the suction of the pump is throttled so that the pump runs about 15 seconds each time that the circuit is closed. The temperature control is then fully automatic, and the temperature will remain constant within about ± 0.02-0.03° C. Somewhat closer control could doubtless be achieved, if desired, by reducing heat capacity of the cooling coils.

The Equilibrium-Filtration Apparatus

Equilibration of the fat-solvent system and separation of the solid and liquid phases is carried out in the Pyrex glass apparatus shown in Figure 3. Parts

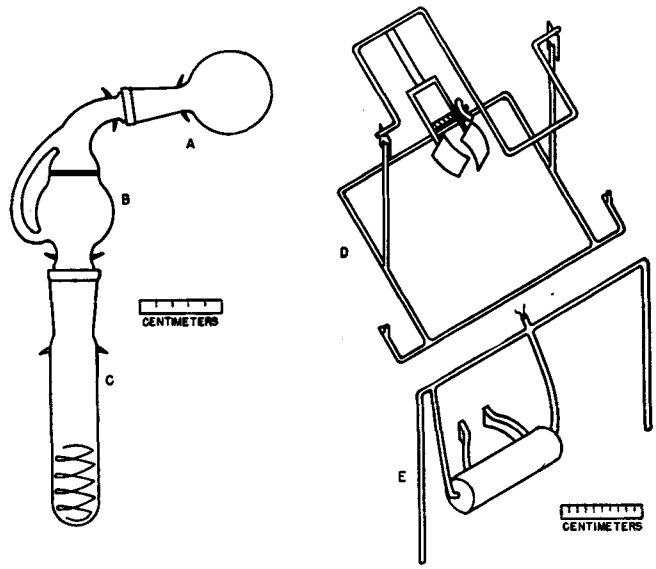

FIG. 3. Thermoregulator.

A, *B*, and *C* are individual components which when assembled contain the solvent-fat mixture. *A* is a round-bottom flask of 75-100 ml. capacity with a 19/38 standard taper ground joint. Part *B* is a bulb to which has been sealed a glass Gooch-type crucible with a coarse grade fritted glass disc. A pressure-equalizing tube by-passes the filtering disc. *C* is a glass tube, joined to *B* by a 24/40 ground joint. Both joints are given a special polishing with jeweler's rouge. Contained in part *C* is a free-moving stirrer coil. The coil is made of No. 18 B & S gauge iron thermocouple lead wire. It has 7 or 8 turns and is tin-dipped to make it rust-proof and resistant to the corrosive action of fatty acids.

Part *D* supports the assembled glass apparatus in the bath, and part *E* furnishes the motive power for the stirrer coil. *D* is constructed of rigid copper tubing; it consists of a base with two perpendicular arms, upon which rests a free-turning support to which the

glass apparatus is clamped. Part *E* is an electromagnet supported by a frame of rigid copper tubing. The round iron core of the magnet is 5/8 in. by 2¾ in.; it is tapped and threaded at each end and wound evenly with No. 26 AWG insulated copper wire, to a resistance of 14 ohms (approximately 343 feet). The coil is sealed liquid-tight within a length of large-size copper tubing and the projecting poles of the magnet, made of ¾ in. by ¾ in. flat iron stock and bolted to the ends of the core, are tinned. Lead wires from the winding are brought out through one of the supporting arms. The allowable carrying capacity for the winding is about 0.6-1.0 amp. Current is supplied by two 6-volt storage batteries connected in series.

The entire apparatus is so designed that it can be completely immersed in the bath liquid; there are no projecting parts to transmit heat to the fat-solvent mixture, and the complete operation of equilibration and filtration can be carried out without removing any part of the apparatus from the bath. Previous experience has shown that it is extremely difficult to maintain the contents of a vessel exactly at the temperature of a liquid bath in which it is immersed if any portion projects into the atmosphere and if there is a considerable temperature difference between the bath and the atmosphere.

Under working conditions the glass apparatus is clamped into position by the holder on part *D*, with the tube *C* in a vertical position as shown and the flask *A* extending back over the clamp. The electromagnet is then placed in position with the legs of *E* in the sockets provided for them on the base of *D*. The arms of the magnet will then straddle the glass tube and be at about the level of the top of the stirrer coil.

Stirring of the fat-solvent mixture in *C* is accomplished by the upward motion of the stirrer coil when attracted by the magnet and its fall when the current is interrupted. The circuit is alternately opened and closed by a timing device [2] operating on a cycle of about 5 seconds.

In practice, parts *B* and *C* are connected and the *B-C* assembly and flask *A* are both stoppered and weighed. The fatty materials and the solvent are successively weighed into the stoppered tube *C*, which is removed from *B* for this purpose. Assembly of the apparatus is then completed, the different parts being held together with spring coils. The ground glass joints are lubricated with a very thin film of a silicone stopcock grease.

The contents of *C* are heated until all solid materials are in solution, and the complete apparatus is cooled, with the stirrer in operation, in an air bath approximately 20° C. below the desired temperature of operation. After a suitable cooling period the system is equilibrated overnight in the constant-temperature bath. The electromagnet is then removed, and without removal from the bath, the apparatus is rotated through an angle of 180° (in a clockwise direction according to Figure 3) so that the material falls from tube *C* into bulb *B*. The greater part of the liquid phase will then drain by gravity through the fritted filter into flask *A*. After a drainage period amounting usually to about one hour, the apparatus is removed from the bath. Flask *A* is then immediately disconnected, and both this flask and the *B-C* assembly are stoppered. After they have come to room temperature, they are weighed to determine the proportions of the original charge in the filtrate- and precipitate-portions. Portions of the charge remaining in part *B* are then washed into *C* with solvent and the solvent is removed from the fatty materials in flask *A* and tube *C* by heating under a current of hydrogen or other inert gas, and finally applying a vacuum. *A* and *B-C* are then weighed to determine the amount of fatty material in each, the amount of solvent in each being taken by difference.

[2] A Sangamo Flasher, Model 3, Form 61ST, 115 v., 15 a.

Efficiency of Recovery of Materials

In order to carry out the calculations to be described later, it is necessary to determine the amount of the liquid phase entrained in the crystals of the separated precipitate-fraction. This can be done accurately only if there is no considerable loss of either fatty material or solvent during the course of the experiment.

With proper manipulation of the apparatus, losses of material are very small. In the typical experiment on which detailed data are given later in this paper, the recovery of materials in the separated fractions was as follows (in grams):

	Original	Recovered	% Recovery
Fatty acids	1.499	1.497	99.87
Solvent	17.080	17.069	99.94
Acids plus solvent	18.579	18.566	99.93

Small differences in figures for the amounts of material charged to the apparatus and the amounts recovered can arise from loss of solvent vapors, condensation of water in the apparatus, failure to strip the fatty material quite free of solvent, etc. However, differences of the order of magnitude indicated above will in general give rise to errors in calculating the composition of the two phases which are much smaller than experimental errors in determining iodine or thiocyanogen values or in carrying out other chemical analyses of the materials.

Calculation of Composition of Solid and Liquid Phases

Methods for calculating the composition of the solid and liquid phases may be best illustrated by an example. The following data were obtained in an actual experiment on the system stearic acid-oleic acid-commercial hexane (Skellysolve B). All quantities are given in terms of grams.

Original Charge:		
Stearic acid	0.591	
Oleic acid	0.908	
Total fatty acids		1.499
Solvent	17.080	
Total charge		18.579
Separated Portions:		
Filtrate-fraction:		
Fatty acids	0.771	
Solvent	13.838	
		14.609
Precipitate-fraction:		
Fatty acids	0.726	
Solvent	3.231	
		3.957
Iodine value of filtrate-acids		85.1
Iodine value of precipitate-acids		21.9

The composition of the acids in the precipitate- and filtrate-portions can be calculated from the iodine values of the two portions to be as follows: filtrate-acids, 5.2% stearic acid and 94.8% oleic acid; precipitate-acids, 75.6% stearic acid and 24.4% oleic acid. In these calculations the iodine values of pure oleic and stearic acids are taken as 89.9 and 0, respectively.

Since there was 0.771 g. of fatty acids associated with 13.838 g. of solvent in the recovered filtrate, it can be calculated that the entire amount of solvent, 17.080 g., must have contained 0.952 g. of fatty acids. The entire body of liquid, including that entrained in the solids, then consisted of the following:

Stearic acid	0.049 g. or 0.272%
Oleic acid	0.902 g. or 5.003%
Solvent	17.080 g. or 94.726%

The composition of the solid-phase, free of liquid is given by the difference between the original amounts of stearic and oleic acids and the amounts of these acids in the liquid phase, as indicated above. The figures obtained are as follows:

Stearic acid	0.542 g. or 98.91%
Oleic acid	0.006 g. or 1.09%

Although the above method provides all essential data, more reliable figures for the composition of the solid phase are probably obtained if this composition is calculated directly from data on the acids in the precipitate-fraction. The method is as follows:

According to their iodine value, the acids in the precipitate-fraction consisted of the following:

Stearic acid	0.549 g. or 75.6%
Oleic acid	0.177 g. or 24.4%

However, from the analysis of the filtrate it can be calculated that the 3.231 g. of solvent entrained in the precipitate carried with it 0.180 g. of fatty acids, consisting of 0.171 g. of oleic acid and 0.009 g. of stearic acid. If these amounts of entrained acids are deducted from the above amounts of total fatty acids in the precipitate, the following figures are obtained for the composition of the liquid-free crystals:

Stearic acid	0.540 g. or 98.90%
Oleic acid	0.006 g. or 1.10%

It is to be noted that calculations by the two methods provide a check on the accuracy of the chemical analyses. The two sets of calculated values will agree only if there has been no substantial alteration in the chemical characteristics of the fatty material during the course of the experiment. If, for example, the oleic acid had been permitted to oxidize during the course of the above experiment, some reduction in the iodine value might have resulted. This would have had the effect of making the amount of oleic acid in the crystals too high, as calculated by the first method, but too low as calculated by the second method.

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Abstracts

Oils and Fats

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M. M. PISKUR and SARAH HICKS

THE FATTY ACIDS OF HUMAN MILK FAT. J. B. Brown and B. M. Orians (Ohio State Univ., Columbus). *Arch. Biochem. 9,* 201-19 (1946). A large specimen of human milk fat has been examined quantitatively with reference to the amounts and nature of its component fatty acids. No evidence could be found for the presence of more than traces of acids below C_{10}. As a result of distillation of 2 specimens of Me esters, prepared from this fat, and applying low temperature crystallization technics to the main fractions, a number of pure esters and acids have been isolated for the first time and identified. This specimen of fat is much more similar to human body fat than it is to a typical milk fat.

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THE RATE OF AUTOXIDATION OF MILK FAT IN ATMOSPHERES OF DIFFERENT OXYGEN CONCENTRATION. P. S. Schaffer, G. R. Greenbank, and G. E. Holm (Bur. Dairy Ind., U.S.D.A., Washington). *J. Dairy Sci. 29,* 145-50 (1946). Data have been presented which indicate that autoxidation of milk fat with 0.80% by volume of O_2 is sufficient to render it inedible. In the atmosphere of a container of dried milk this is equivalent to 0.20% of O_2. This level of O_2 concen-