Determining the Density of Triglycerides and Esters by the Falling Drop Method[1]

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THE time required for a sphere of known size to fall through a measured depth of medium has often been used to determine the density of the sphere or, conversely, the viscosity of the medium. An early application of this method was the determining of the density of blood and plasma (1, 2). Use of the method to measure deuterium oxide in water in concentrations of parts per million has been reviewed by Cohn (3). In these procedures a drop of aqueous solution was allowed to fall through a hydrocarbon medium. The converse has been tried, *i.e.*, a drop of mineral oil falling through a solution of aqueous ethanol (6). Although the procedure was much less elaborate than those used by earlier workers, the density of mineral oils was determined with an accuracy of ±0.001. The purpose of the present investigation was to determine whether the falling drop method could be used to measure the density of micro quantities of triglycerides and their esters.

Apparatus and Materials

A commercial "Falling Drop Apparatus" (Figure 1) was available for the present investigation. The media in the tubes were kept at 25 ±0.002°C. by pumping distilled water through the jacket from a constant temperature bath. A Beckmann thermometer was used to determine drift in the setting of the thermoregulator. The constant temperature bath was also used to bring the samples of oil to the same temperature as the medium before rate of fall was determined.

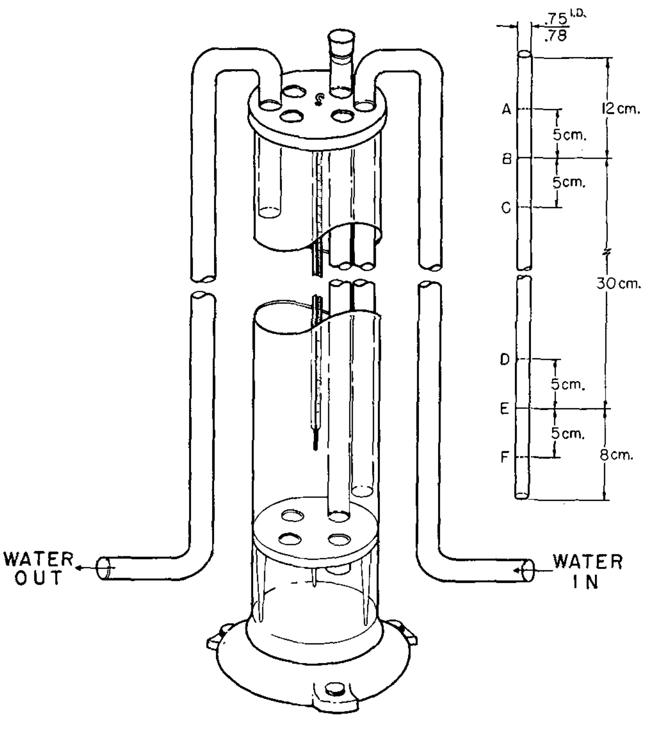

FIG. 1. Apparatus for determining density by the Falling Drop Method.

A micro pipette, graduated to read 0.005 ml. and provided with a Guthrie manipulator (5), was used to deliver the drops. Grinding the pipette tip to a sharp point allowed the drops to be released more readily. The time of fall of a drop between the selected sighting marks was measured by a stopwatch reading to 0.1 sec.

Aqueous ethanol and aqueous acetonitrile were the media employed in this investigation. The empirical relation $\eta + 5.79d_0 = 5.09$, derived from Stokes' Law, was used as a guide in selecting density and viscosity combinations suitable for drops of 0.01 ml. volume and density of 0.88.

Solutions of ethanol in water were made up on a percentage by volume basis. The densities were then determined by pycnometer at the temperature of the water jacket. From the densities, exact composition of the solutions on a weight basis and their viscosities were determined from standard tables. The viscosity and density of the acetonitrile solution were determined directly. The properties of the media are listed in Table I.

TABLE I

Properties of the Media

Medium	Density 25/4	% Composition		Viscosity c.p., 25°C.
		By vol.	By wt.	
A: ethanol-water	0.9269	48	42.2	2.376
B: ethanol-water	0.9045	58	52.5	2.370
C: ethanol-water	0.8995	61	54.6	2.340
D: ethanol-water	0.8592	77	71.3	2.002
E: acetonitrile-water	0.8501	....	63.0	0.667

The drops used were methyl esters of fatty acids or triglyceride oils. Triolein, for triglycerides, and methyl erucate, for esters, were selected as reasonably stable reference materials. The methyl esters were either prepared in the laboratory or purchased from the Hormel Institute. Except for triolein, the triglyceride oils were samples of thermally polymerized linseed oil.

Method

Measurement of Rate of Fall. When the approximate density of the test material is known, a tube is filled with the appropriate medium and suspended in the cylinder. Several tubes, containing different media, may be required if the density of the drop is unknown. The cylinder is then set vertically, using the thermometer as a plumb bob, and the tubes are checked to ensure that they are hanging freely.

When the test material and medium are at bath temperature, a pipette is filled with the drop substance and its tip inserted ¼ in. into the medium. Pressure is then applied slowly and evenly to form a drop which is released by a slight upward movement of the pipette. The time for the drop to pass the sighting marks is measured.

Because of the viscosity of triglyceride oils, care should be taken to squeeze the drop out sufficiently

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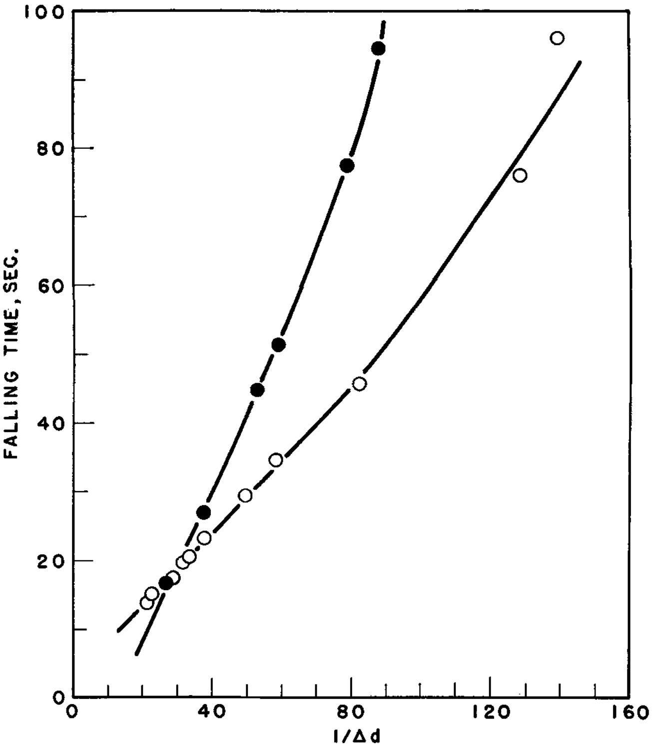

Fig. 2. Arithmetic plot of time of fall *vs.* reciprocal of density difference.

○ triglycerides drop volume 0.01 cm.^{3}; ● esters drop volume = 0.005 cm.^{3}

slowly for the pipette to drain. To ensure constant density, fresh media should be used every day.

Calculation of Density. The data may be treated in several ways:

a) If the density of the medium is not known directly as in most of the earlier work, the medium must be calibrated by determining the time of fall of a reference drop. Then, on the assumption that the density of the medium has not changed, the density of the test material is determined by comparing its falling time with that of the reference substance. A calibration curve like Figure 2 can be used to estimate the density difference between the medium and the reference and test materials.

b) If the data fall on the linear portion of the falling time-reciprocal density difference curve, the density of the sample can be calculated directly, using the relation $(d_1 - d_o)/(d_2 - d_o) = t_2/t_1$.

c) If the temperature of the bath and the density of the medium are known with certainty and kept constant, reference material is unnecessary after the original calibration. One way of using the calibration data is to employ the method of least squares to determine the "best" calibration curve and the upper and lower limits of the line (Figure 3).

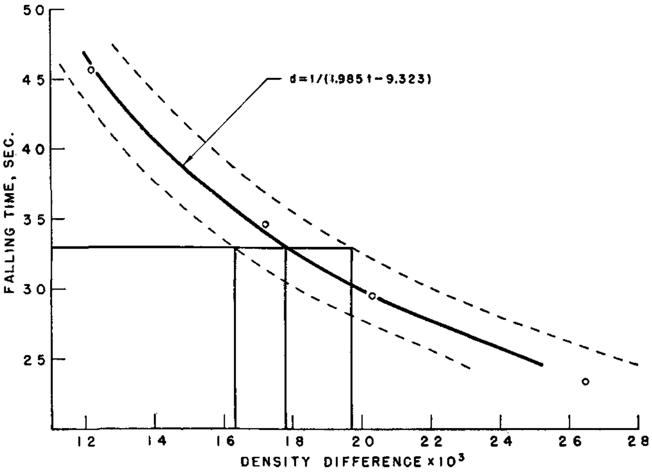

Fig. 3. Plot of "best" line obtained by least squares calculation showing limit of error for 95% probability.

Experimental Results

Reproducibility of Time of Fall. Table II shows the reproducibility with which the time of fall could be measured at different drop velocities. The coefficients of variation suggest that maximum accuracy was obtained with a 45-sec. time of fall, corresponding to a drop velocity of 0.67 cm. per sec. In preliminary work, where the temperature was controlled to only ±0.16°C., the coefficients of variation were almost doubled.

TABLE II
Precision of Measurement of Falling Time

	Time in sec. for triolein falling through medium of density[a]		
	0.8592	0.8995	0.9045
Mean of 10 measurements	11.45	45.14	96.12
Coefficient of variation	0.460	0.114	0.433
Confidence limit, P = 0.99	±0.172	±0.169	±1.352
Corresponding range of densities	0.0020	0.0001	0.0001

[a] Cf. Table I.

Influence of the Medium. Unless the medium contained more than 70% ethanol or 60% acetonitrile by weight, esters fell too rapidly for accurate measurement of velocity. Because solutions of such high solvent content might dissolve the ester, the phase diagrams for the systems, methyl oleate-ethanol-water and methyl oleate-acetonitrile-water, were determined at 25°C. The data (Figure 4) show that, at equilibrium, two phases will appear when a 71% by weight solution of ethanol has dissolved 2% methyl oleate and that methyl oleate is less soluble in acetonitrile-water mixtures.

Therefore to determine if the medium affected the rate of fall, additional sighting marks were put on a tube 5 cm. above and below the original lines (Figure 1). The rate of fall through each of the intervals was determined for methyl oleate in aqueous aceto-

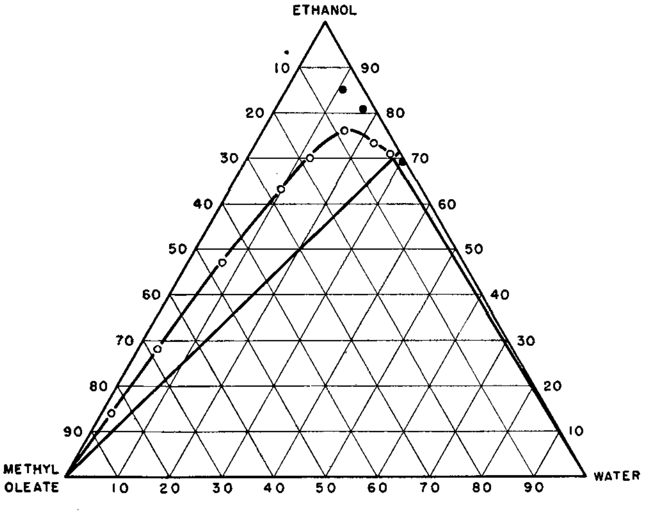

Fig. 4. Phase diagram for the systems: methyl oleate, ethanol, water; methyl oleate, acetonitrile, water at 25°C.
○ ethanol; ● acetonitrile.

TABLE III

Interval c.f. Fig. 1	Rate of fall, cm./sec.			
	Triolein	Methyl oleate		
	Ethanol-water	Acetonitrile-water	Ethanol-water	Ethanol-water [a]
AB	0.64	1.39	0.98	0.99
BC	0.64	1.22	0.93	0.97
CD	0.64	0.98	0.88	0.95
DE	0.63	0.76	0.81	0.92
EF	0.64	0.66	0.78	0.89

[a] Saturated at 25°C. with methyl oleate.

nitrile and in aqueous ethanol (Table III). Triolein was included in this experiment as a reference substance, and aqueous ethanol saturated with methyl ester was tried as a medium in which diffusion out of the drop would not occur. As expected from solubility considerations, the drop of triglyceride fell at a constant rate. In contrast, the rate of fall of the methyl ester was observed to decrease with depth, especially when the medium was aqueous acetonitrile. Saturation of the medium reduced this effect.

Calibration of Apparatus. The apparatus was calibrated by determining with pycnometer the densities of a variety of oils and esters and the densities of the media (Table IV). Then the time for a drop of each material to fall through 30 cm. of medium was measured (Table IV). With triglycerides a drop volume of 0.01 cm.^{3} was used, but with methyl esters, the volume was 0.005 cm.^{3} The falling times have been plotted as arithmetic functions of reciprocal of density differences (Figure 2).

TABLE IV

Material	Density of drop [a]	Density of medium [a]	Difference in density	Time of fall, sec.
Triolein	0.9117	0.9045	0.0072	96.1
9 hr. linseed	0.9347	0.9269	0.0078	76.0
Triolein	0.9117	0.8995	0.0122	45.7
15 hr. linseed	0.9441	0.9269	0.0172	34.6
21 hr. linseed	0.9472	0.9269	0.0203	29.5
0 hr. linseed	0.9260	0.8995	0.0265	23.4
3 hr. linseed	0.9295	0.8995	0.0300	20.7
6 hr. linseed	0.9310	0.8995	0.0315	19.8
9 hr. linseed	0.9347	0.8995	0.0352	17.7
15 hr. linseed	0.9441	0.8995	0.0446	15.1
21 hr. linseed	0.9472	0.8995	0.0477	13.8
Me. eicosenoate	0.8706	0.8592	0.0114	94.7
Me. oleate	0.8720	0.8592	0.0128	77.6
Me. erucate (A)	0.8764	0.8592	0.0172	51.3
Me. erucate (B)	0.8784	0.8592	0.0192	44.8
Me. linoleate	0.8861	0.8592	0.0269	27.0
Me. linolenate	0.8957	0.8592	0.0365	17.0

[a] By pycnometer at bath temperature.

Discussion

The curvature in the plots (Figure 2) indicates that the experimental conditions assumed in the derivation of Stokes' Law have not been met in this investigation. The effects of the dimensions of the tube, the size, shape, and velocity of the drop and the influence of the medium on drop velocity are being reported elsewhere.

The data in Table II suggest that density may be measured by the falling drop method to ±0.0001 under optimum conditions. However when a reference material is used, the falling time must be determined for both the unknown and the reference substance. Therefore the error in any one density measurement combines the errors in the two determinations. Standard deviation for the density of triglycerides and esters was 0.0003, representing the precision of measurement by the differential method employed in this investigation.

With the differential method, as used by earlier workers, the density of the medium does not enter directly into the calculation. Nevertheless the temperature of the medium must be known accurately and be kept constant. Since fresh supplies of media were used each day and no density change could be detected in the reserve supplies over a period of months, determining density without using a reference material appears feasible. When no reference drop is employed, the precision is greater (Figure 3) because only one measurement is used.

The highest accuracy, in contrast to precision, will be attained with a graphical method because use is made of a mean line. Densities calculated from the relation between pairs of points will be correspondingly less accurate.

When the density of many like samples is to be measured, the greatest precision and accuracy is obtained by a non-comparative method, using a graph like Figure 3. When density is to be determined on only a few samples, use of a comparative method makes a calibration curve unnecessary. However because of the marked influence of oxidation on density, the density of the reference material cannot be assumed constant, unless stored under nitrogen at low temperatures. The magnitude of this influence has suggested density measurements as a means of estimating extent of oxidation when the sample is small and is to be recovered.

Because error in timing and drop size are of a general nature and have been adequately treated elsewhere (2, 4), only the effect of temperature will be discussed here. Since the coefficient of density change over the range of 15°C. to 25°C. is essentially the same for esters, triglycerides, and the solutions of alcohol and water used in this investigation, the effect of variation in temperature on density difference was negligible. Since the temperature of the bath in which the tube hung was controlled to ±0.002°C., the maximum change in viscosity caused by variation in temperature would have been 0.002 c.p. The effect of such a change on the falling time is however only 0.05%; i.e., about the same magnitude as that of the lowest coefficient of variation (Table II).

Because, in this investigation, density difference was unaffected by temperature, velocity of fall, as a function of drop size, varied as the 0.66 power of the change in density or temperature of the drop substance. Differentiation of these relations and application of the appropriate numerical factors has shown that temperature fluctuations of 0.1°C. have a negligible effect on the velocity of a drop.

Determination of density by the Falling Drop Method, as described here, is less accurate but more rapid than by other micro methods. Unlike the use of micro weighing pipettes, the procedure involves weighing only during calibation. In contrast to equilibrium methods it lends itself to rapid routine use.

Summary

The densities of triglyceride oils and their esters have been determined by the Falling Drop Method to within ±0.0003 when the behavior of two drops is compared and to ±0.0002 when only one drop is employed. Triolein and methyl erucate have been used as reference materials and solutions of ethanol in water as media. Errors due to variation in temperature have been analyzed for the systems and apparatus employed.

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Acetin Fats. I. Products Made from Mixed Acetin Fats

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THE introduction of acetyl groups into glyceride molecules results in the formation of fats with interesting and unusual properties (1-5). These properties permit the designing of products that are either improved versions of existing types or represent new materials. The present report deals with fats which contain both the possible types of mixed acetyl glycerides, namely, mono- and diacetyl triglycerides, beside the normal triglycerides. The terms "monoacetins" and "diacetins," respectively, are used in this paper for these monoacetyl and diacetyl triglycerides. The general term "acetin fats" is proposed for these mixed acetyl glycerides regardless of melting point. The preparation and use of acetin fats containing predominantly oleic as the high molecular weight fatty acid have been described by Feuge *et al.* (6).

The most useful effect of the introduction of acetyl groups into glyceride molecules is the pronounced lowering of the melting point. Heretofore the use of a low melting fat called for an unhydrogenated or only slightly hydrogenated oil. Such highly unsaturated oils, *e.g.*, cottonseed or soybean oil, do not have the oxygen stability or keeping quality of partially hydrogenated oils, a fact which has limited their current use in the production of plastic shortenings. Existing commercial shortenings are made principally from a partially hydrogenated oil or basestock which is stiffened by the addition of a low I.V. hydrogenated fat or hardstock to give the so-called all-hydrogenated type shortening. Improved techniques of hydrogenation and plasticizing have significantly improved the overall plasticity of these products, but they still do not approach the plastic range of the so-called compound or blended type shortenings which consist of hardstock in oil and which show relatively little change in plasticity from a refrigerator temperature of between 40° to 50°F. to a room temperature of up to about 100°F. The aim of equal or increased oxygen stability and wider plastic range, in comparison with conventional shortenings, was accomplished in plastic fats made from acetin fat basestocks by virtue of the lower melting points of the component acetyl glycerides. As an illustration of the potentialities of this new type of fat, the properties of acetin fat shortenings will be described.

Preparation of Acetin Fats

The mixed acetin fats may be prepared by a number of methods. The two methods most often employed involve interesterification of normal fats and oils with triacetin or acetylation of a superglycerinated fat. In the first method a low-temperature interesterification catalyst is added to a mixture of triacetin and conventional triglycerides composed of fatty acids of the desired molecular weight range and degree of unsaturation. The usual refining, bleaching, and deodorization steps as well as inactivation of the catalyst must be instituted somewhere in the processing. Deodorization of acetin fats yields a bland product and removes the small quantity of triacetin present. A more detailed description of a preparation is given below.

To a refined and dried or deodorized oil or fat in the liquid state is added the selected quantity of triacetin. The amount of triacetin[1] is chosen on the basis of the content of acetyl groups desired in the final interesterification product. To the mixture of oil or melted fat and triacetin is added about 0.3% of a low-temperature interesterification catalyst, such as sodium methoxide or sodium metal, as a suspension in an inert solvent such as xylene. With agitation the two-phase mixture rapidly becomes homogeneous as

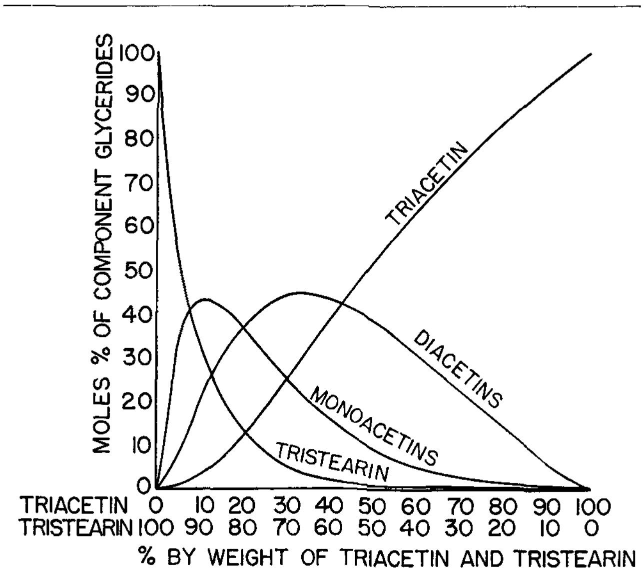

Fig. 1. Composition of component glycerides at equilibrium of interesterified tristearin-triacetin, mixes, calculated in moles %.

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[1] Figures 1 and 2 express the molar and weight concentrations of the various triglycerides present after random interesterification of triacetin and tristearin. Figure 3 shows the weight percentage concentrations of the component glycerides in the triacetin-free fats.