A COLORIMETRIC PROCEDURE FOR THE DETERMINATION OF SMALL AMOUNTS OF FATTY ACID

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The basis of the method is the measurement of the color change produced by the reducing action of fatty acid or cholesterol on a sulfuric acid-dichromate mixture. This reaction has been used in several ways for the determination of fatty acids and other organic material. Bang (1) made it the basis of a micromethod by titrating the excess of dichromate with starch-iodine and thiosulfate. In this laboratory (2) the procedure of Bang was modified to make the oxidation complete. Van Slyke and Folch (3) used the reagent by collecting and measuring the carbon dioxide evolved in the oxidation. As far as can be determined, no one has reported the use of the color change in the reagent as a means of measurement of fatty acids or other organic material, and, since it was found that the change was closely proportional to the amount of fatty acid oxidized and that the actual determination was rapid and easy to carry out, a procedure was developed and reported (4). Since the reagent is sensitive to organic matter of all kinds, it is necessary to isolate the fatty acids before measurement. The procedure for the isolation has been worked out by previous investigators and need not be reported here. The change in color cannot be measured in the Duboscq or similar visual colorimeters, but it can be determined by the photoelectric type of instrument. For greater sensitivity a depth of liquid greater than in the ordinary photoelectric colorimeter was desirable and a special colorimeter was devised and is described below.

Reagent—The Nicloux type of sulfuric acid-dichromate reagent was used. It is made by dissolving 10 gm. of AgNO₃ and 10 gm. of K₂Cr₂O₇ separately in water, mixing the solutions, and purifying the precipitated silver dichromate by centrifuging and washing the precipitate two or three times with water in the centrifuge. 100 cc. centrifuge tubes were found convenient for mixing and washing. The separated precipitate was dissolved in 500 cc. of concentrated H₂SO₄ and kept in a glass-stoppered bottle.

Solvent—Either purified petroleum ether or a mixture of 7 parts of petroleum ether to 1 of chloroform can be used. The latter is more satisfactory, since it does not easily form emulsions in extracting lipide from either acid or alkaline solutions.
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Procedure

The material to be determined (fatty acid or cholesterol), dissolved in petroleum ether or petroleum ether-chloroform (7:1), was made to a volume such that 1 cc. contained about 0.1 mg. of the substance to be measured. From this solution an aliquot containing 0.4 to 0.5 mg. was measured into a 50 cc. beaker and the same volume of solvent alone was measured into another beaker. The solvents were evaporated by immersion in boiling water, after which 3 cc. of the reagent were measured into the beakers and the mixture digested for 15 minutes in a closed vessel set in boiling water. At the end of the period 3 cc. of distilled water were added and the mixture set aside for 10 minutes to cool, after which it was transferred to the calorimeter cups and readings made. The values were determined by the use of a calibration curve. Care was taken during the evaporation of the solvent not to expose the tiny residues to heat and air longer than necessary to remove the solvent completely, since even the higher fatty acids are appreciably volatile and oxidizable under these conditions. Slight changes occur in the diluted mixture on standing, so that it is advisable to make the readings near the 10 minute point. Measurement of the dichromate reduced and hence of the amount of lipide present can be made by titration with thiosulfate and starch in the usual way. By this means it was found that, by the procedure given, the fatty acid or cholesterol was oxidized to the extent of about 88 per cent of the theoretical value, and the amount of reagent used constituted an excess of about 4 times that needed for this oxidation.

Calibration Curves for Fatty Acid or Cholesterol For this purpose fatty acid or cholesterol was dissolved in petroleum ether or petroleum ether-chloroform (7:1) and made to volume such that 1 cc. of the solution contained 0.1 mg. of the standard substance.

Either palmitic, stearic, or oleic acid may be used as standard, since the differences in values are within the limit of error of the method. As cholesterol does give significantly higher values, a separate curve was run for it. For the calibration, from 1 to 8 cc. of the standard solutions was measured into one of the 50 cc. beakers and the same amount of solvent into another. After evaporation of the solvent the residues were treated as directed in the procedure and readings were made. Eight or ten determinations were made at each level and the positions marked on coordinate paper. The results are shown on the calibration chart. The accuracy of the measurements was about ±5 per cent. The curves obtained are shown in Fig. 1.

Special Colorimeter—In most of the photoelectric colorimeters at present available the intensity of color is measured by passing the light across small tubes (about 25 mm. in diameter). The sensitivity may be increased by the use of a greater thickness of solution, such as is obtained in the
Fig. 1. Calibration curves for cholesterol and fatty acid

Fig. 2. Diagram of the special colorimeter

cups used in the visual colorimeters. Such cups are used in the instrument described below. No claim can be made for originality in idea and very
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little in device for this instrument, since a similar one has already been described (5), but the simplicity of construction, ease of use, and versatility of the present instrument are believed to warrant its description.

A diagram of the special instrument is shown in Fig. 2. It consists of an oblong box about $4\frac{1}{2} \times 5 \times 7$ inches with a light-tight door. The working parts (holders) are made of 3/32 inch bakelite or similar material, sliding in and out of grooves in the inside of the box. The light system is a modification of that described by Evelyn (6) and consists of a 6 volt flashlight bulb with a 6 volt automobile battery as the source of current. In the present work it was necessary to keep the battery fully charged; so that a permanent connection with a charger is desirable. The colorimeter cups are carried in a slide mounted on another holder which has an opening directly over the light source. The slide is operated from outside the box and is so constructed that first the cup containing the blank and after adjustment the cup containing the solution to be measured are centered over the light. A light filter and the flashlight bulb are mounted in similar holders beneath the cups, as shown in Fig. 2. (In the present work no filter was found which gave better results than the unfiltered white light.)

Situated a short distance above the colorimeter cups is the photoelectric cell (Weston model 594, type 2, 668-0) connected to a galvanometer of the ordinary student type (Leeds and Northrup type P).

In operation the colorimeter cups containing the blank and the solution to be measured are mounted in the sliding plate and the cup containing the blank solution is pushed over the light. The galvanometer is adjusted by means of the variable resistances $R$ and $R_1$ to a convenient point on the scale and let stand for a minute or two to come to rest; then after final adjustment the cup containing the solution to be tested is pushed into place and readings made. The difference between this reading and the reading set for the blank is the reading for the solution being tested. The values are then read off the calibration curve.

**Summary**

A method is described for the colorimetric determination of small amounts of fatty acid or cholesterol, making use of a special type of photoelectric colorimeter.

**Bibliography**

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