• *Letter to the Editor*

A Fraction Collector for Preparative Gas Liquid Chromatography

EFFLUENTS EMERGE AS AEROSOLS from a gas liquid chromatographic column operated at high temp. Several devices and techniques have been described (1,2) to break these aerosols or fogs and allow the efficient collection of the desired fractions. These techniques have been unsatisfactory in our hands for the collection of long-chain fatty esters from a preparative gas chromatograph when the sample size of a single component exceeds 50 mg. We have, however, made satisfactory collections of such samples with the aid of the device shown in Figure 1 which is a modification of the gradient cooling technique described by Schlenk and Sand (3). It consists of six nickel-chromium heating coils fastened to an asbestos sheet mounted on a plywood frame. The heating coils are segments of 750 w heating elements (E. H. Sargent & Co., S40715, Catalog 109). The coils are heated to a dull red glow by a variable output transformer. Four mm OD, 2 mm ID x 110 cm glass tubing is passed through the coils and into a silicone rubber gasket in the collection port of the chromatograph. The effluent emerging from the column condenses after each heating coil in successively decreasing amt. The collection tubes can be quickly removed and new ones inserted for the collection of succeeding fractions. The back of the fraction collector stand (Fig. 1) is used to hold the collection tubes. After the tubes have cooled sufficiently the collected fractions can be washed from them.

This fraction collector has been routinely used in this laboratory for the past two years for the isolation of fatty acid methyl esters by the use of Wilkens Instrument Co. Aerograph A-90-P gas chromatograph equipped with a thermal conductivity detector. Aluminum columns 17 ft x ⅜ in. packed with 30% SE 30 (Analytical Engineering Laboratories, Hamden, Conn.) on 45–60 mesh firebrick are used to separate methyl esters according to carbon number. Flow rates of 150–200 ml/min of He are usually used. The detector and the collection port are maintained a few degrees above the maximum temp of the column. On-column injection and temp programming from 100–290C in conjunction with the described fraction collector allows sample sizes of up to one ml of mixed

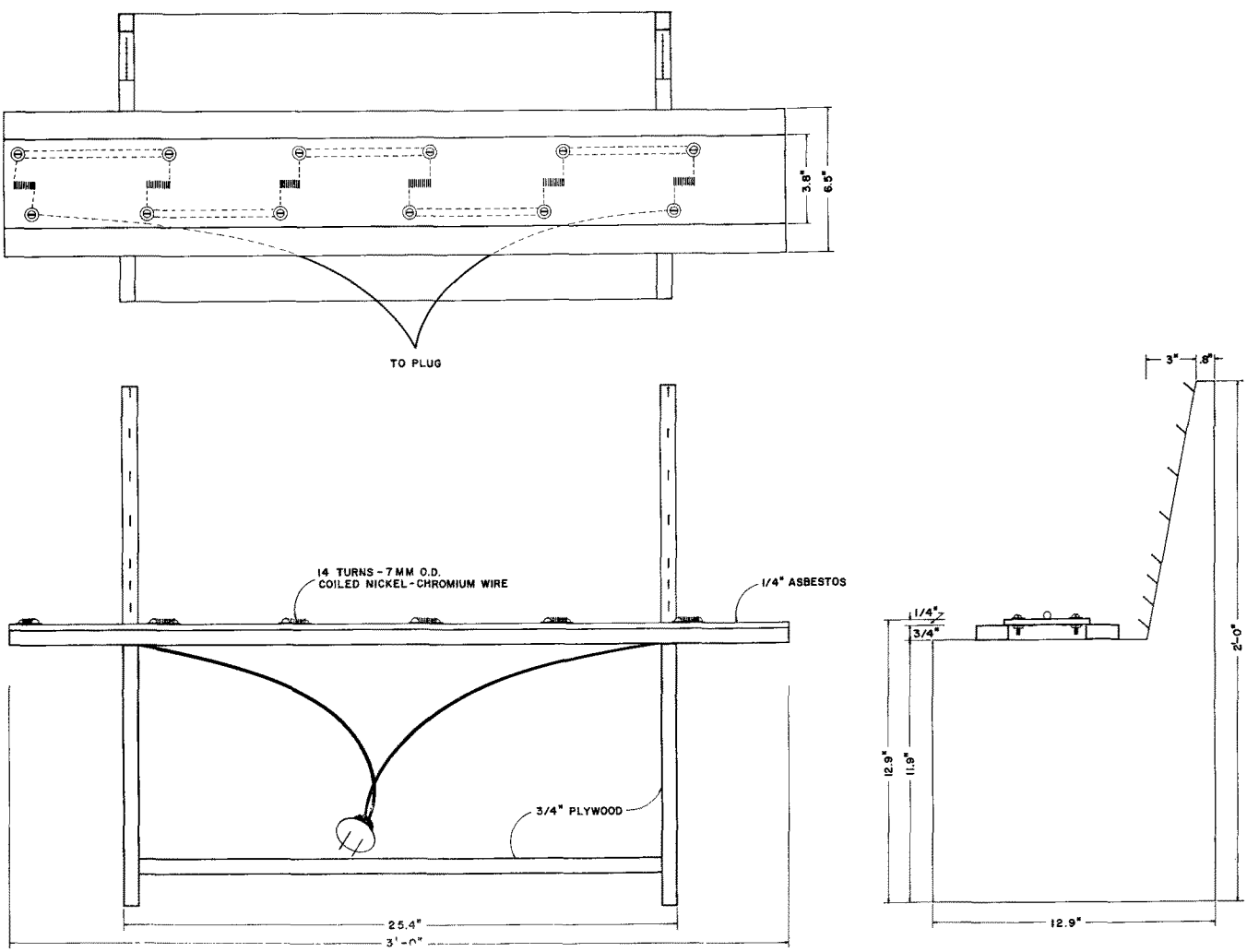


FIG. 1. A fraction collector for preparative GLC. Constructed to be used with a Wilkens Instrument Co. Aerograph A-90-P gas chromatograph for the collection of fatty acid methyl esters.

methyl esters, such as that from rat fat, to be separated and collected in less than 25 min. The sample size that can be efficiently separated and collected depends, of course, upon the number of components and the concentration of each sample. Samples that contain solvents or components with high vapor pressures make injections without loss of sample difficult because of the back pressure they create. In spite of these losses, recoveries in excess of 90% have been obtained from approx 200 μl injections of solvent-free methyl laurate in recovery tests.

We have been unable to satisfactorily separate large samples according to degree of unsaturation using diethylene glycol succinate polyester (DEGS) or other polar phases. This difficulty is circumvented by preliminary separation according to carbon number followed by separation according to degree of unsaturation by silver nitrate thin-layer chromatography (TLC).

RANDALL WOOD
RAYMOND REISER
Department of Biochemistry & Nutrition
Texas A&M University
College Station, Texas

ACKNOWLEDGMENTS

This work was supported in part by a grant (AM-06011) from the National Institutes of Health.

REFERENCES

1. Meinertz, A., and V. P. Dole, J. Lipid Res. *3*, 140 (1962).
2. Hajara, A. K., and N. S. Radin, *Ibid. 3*, 131 (1962).
3. Schlenk, H., and D. M. Sand, Anal. Chem. *34*, 1676 (1962).

[Received November 16, 1964—Accepted September 8, 1964]

Improved Techniques in Neutral Oil Determination

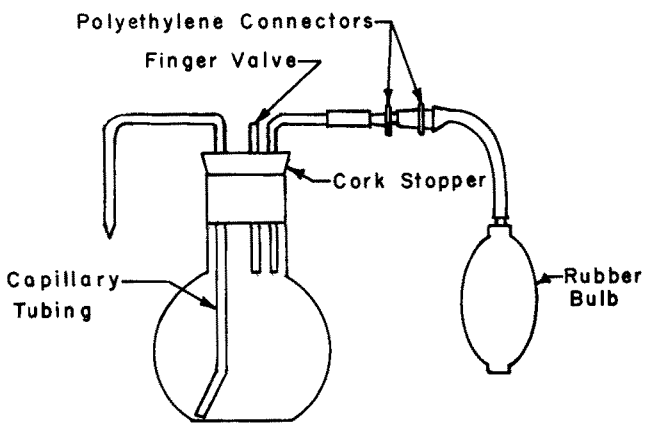
Because of the recent adoption of neutral oil as a basis for price settlement of crude soybean oil, this analysis is becoming routine in vegetable-oil laboratories.

The following techniques in preparing the alumina column and the transfer of oil sample into the chromatographic tube have been found, by this laboratory, to simplify and improve neutral oil determinations. These techniques have been used for some time and have been modified to accommodate the new glassware specified in AOCS Method Ca 9f-57.

A. Preparation of the Alumina Column

Activated alumina (20 g $\pm$ 1 g) is carefully poured through a small bore funnel into a 125 ml separatory funnel which has been half-filled with ether-methanol solvent. The alumina is allowed to settle to the bottom of the separatory funnel before dispensing into the solvent-filled chromatographic tube. The chromatographic tube is maintained at near-full solvent level by adjustment of the funnel and tube stopcocks. The top of the alumina column is made level by closing the tube stopcock and tapping the tube. A full head of solvent in the chromatographic tube aids this adjustment.

This technique simplifies transfer of the alumina and improves the formation of the alumina column. Heat of reaction between the alumina and the ether-methanol solvent is sometimes responsible for formation of ether bubbles which tend to rise and break up the alumina column. This heat is initially dissipated in the solvent contained in the separatory funnel. Further heat dissipation and release of ether bubbles in the alumina slurry occurs as the slurry is allowed to fall through the solvent in the chromatographic tube.



Oil-sample transfer flask

B. Transfer of Sample

A weighed sample of crude oil is transferred quantitatively onto the alumina column by means of a pressure flask as shown in diagram.

The transfer device consists of a cork of suitable size, for a 100 ml Soxhlet flask, fitted with three pieces of glass tubing.

1. A delivery tube; capillary bore.
2. Pressure inlet tube; fitted with a short piece of rubber tubing and polyethylene connector.
3. Finger valve tube.

A polyethylene connector is attached to the tubing end of a rubber atomizer bulb (pressure type with two valves).

The crude oil sample is weighed into the flask and dissolved with 10 ml ether-methanol solvent. The transfer device is placed in the flask and the delivery tube is adjusted to reach the bottom and side of flask. The flask is then positioned above the alumina column so that the stream from the delivery tube will be directed well inside and on the side of the chromatographic tube. Forefinger is placed over the glass valve and the rubber bulb is gently squeezed and pressure maintained until the flask is empty. Delivery tube is rinsed inside column and the rubber bulb is disconnected.

The flask is rinsed by adding 10 ml of solvent and washing down the sides of the flask with solvent from a squeeze bottle. The flask is rinsed three times into the chromatographic tube and the remaining 100 ml of solvent is added using the 50 ml burette.

Results of the neutral oil analysis of a sample of crude soybean oil with and without the improved techniques were compared. Twelve determinations were made, six by the regular AOCS Method, and six using the outlined techniques.

The mean recovery of neutral oil was 0.21% higher using the improved techniques, with a standard deviation of $\pm$ 0.11% as compared to $\pm$ 0.14% by the AOCS Method.

JAMES VELASCO
Field Crops and Animal Products Branch
Agricultural Marketing Service
U. S. Department of Agriculture
Washington, D. C.