1) By means of a dipper or heated buret add .26 g fat to 1½ in. x ½ in. Terry cloth swatches (1½ in. x 1 in. for most liquids).
2) Age swatches one half hr before using.
3) Set Tergotometer at 75 rpm.
4) Add sample to respective beakers.
5) Fill a 2000-ml flask with water at 120F and adjust hardness.
6) Measure 400 ml water from the flask into each beaker.
7) After two min mixing time, add 1 swatch every 15 sec.
8) Continue adding swatches until foam disappears.
9) Number of swatches added equal number of dishes.

Summary

A Tergotometer has been modified so that foam studied can be made on laundry products. Using sebaceous soil and a Polaroid camera, a photographic record of foam breakdown can be made which fortells performance in practical laundry situations.

A simulated dishwashing procedure has been used with the same set-up as a control method once it has been correlated with a given product. It is hoped that eventually changes can be made so that there will be complete correlation with any dishwashing test and then it can double as a screening procedure for dishwashing operations.

ACKNOWLEDGMENTS

Assistance in doing much of the experimental work by R. Roga and P. Leisentritt. Help in redesigning the Tregotometer by the mechanical engineering department.

REFERENCES

1. Barnett, G. and D. H. Powers, Proc. of Sci. Sec. Toilet Goods Assoc. *24*, 24 (1955).
2. Bickerman, J. J., J. M. Perri, R. B. Booth, and C. C. Currie, "Foams," Reinhold Pub. Corp. New York, 1953, p. 9.
3. Kind, W., and O. Oldenroth, Wascheri-Tech-u-Chem No. 1 (1948).
4. Leenerts, L. O., and J. M. Myers, JAOCS *34*, 361 (1957).
5. Machemer, H., and K. Heinz, "Determination of Detergent Foaming Power," First World Congress on Surface Active Agents, Paris, p. 278, 1954.
6. McBain, M., and E. Hutchinson, "Solubilization and Related Phenomena," Academic Press Inc., New York, 1955.
7. Peper, H., J. Colloid Sci. *13*, 199 (1958).
8. Powe, W. C., and W. L. Marple, JAOCS *37*, 136 (1960).
9. Reich, H. E., J. T. Patton, and C. V. Francis, "Foam Measurement by a Dynamic Method," ASTM D-12, March 1961, New York.
10. Ross, J., and G. D. Miles, Oil & Soap *18*, 99 1941.
11. Smith, W. B., and A. Taylor, J. Soc. Cos. Chem. *6*, 96 and 239 (1955); *7*, 413 (1956).
12. Spangler, W. G., C. Buck, and A. J. Frantz, "Hand Dishwashing Test for Evaluating Detergents," ASTM D-12, March 1959, New York.
13. Weeks, L. E., J. C. Harris, and E. L. Brown, JAOCS *21*, 254 (1954).
14. Miles, G. D., and J. Ross, Ind. Eng. Chem. *35*, 1298 (1943).

[Received October 21, 1963—Accepted December 24, 1963]

Use of Rubber Columns for the Chromatographic Separation of Triglycerides and Other Non-Polar Compounds

J. R. TROWBRIDGE, A. B. HERRICK,[1] and R. A. BAUMAN, Research and Development Laboratories, Colgate-Palmolive Company, New Brunswick, New Jersey

Abstract

Some applications of rubber powder as a stationary phase in the chromatographic separation of relatively nonpolar compounds are described. Evidence of a substantial degree of separation of triglyceride components in coconut oil and linseed oil is obtained using a mixture of methanol and acetone as the mobile phase. Separations of trilaurin from mono and dilaurin, of methyl esters of coconut oil fatty acids and of components in Oil of Petitgrain are also accomplished.

Introduction

A FEW YEARS AGO Boldingh (1) described a technique for the separation of saturated normal fatty acids. He prepared a column with rubber powder as the immobile phase and used benzene-saturated mixtures of 3 parts methanol, 1 part acetone, and decreasing amounts of water as successive developing solvents. By this reversed-phase procedure, the components were eluted from the column in order of decreasing polarity or increasing chain length of the acids. The process may be more comparable to liquid-liquid partition than to liquid-solid adsorption as the rubber was reported to be an excellent solvent for fatty materials and considerable swelling of the rubber was observed.

We investigated rubber in a search for a suitable method of separating hydrocarbons and other relatively non-polar compounds. Since we did not have on hand the "Mealorub" (vulcanized rubber powder) used by Boldingh, we prepared our powder from laboratory gum rubber tubing. Other laboratories may find useful applications for these conveniently prepared columns in addition to those which are described here.

A continuously recording differential refractometer (Phoenix Model R-1000-T), sensitive to a change of 4×10^{-6} refractive index units, was used to follow changes in the composition of the eluent stream and greatly aided in the search for suitable column phases by providing an immediate record of the degree of separation achieved.

Experimental Procedure

Preparation of Rubber Columns. The rubber powder was prepared from amber "pure gum" surgical tubing manufactured by the Davol Rubber Co., Providence, R. I. With the exception of a yellow latex tubing of another manufacturer, we have not investigated the suitability of other sources of rubber. The latter tubing was inferior for our purpose as it yielded a somewhat sticky powder which produced a column of low resolving power.

The end of a length of tubing (½ in. diam and ⅛ in. wall thickness) was frozen in a bath of methanol and dry ice. One or two inches was then ground off on a 6 in. diam motor-driven grindstone and the tubing was refrozen in the bath. By using several lengths of tubing in rotation, over 500 g powder

[1] Present address: Grocery Products Div., Armour & Co., Chicago, Ill.

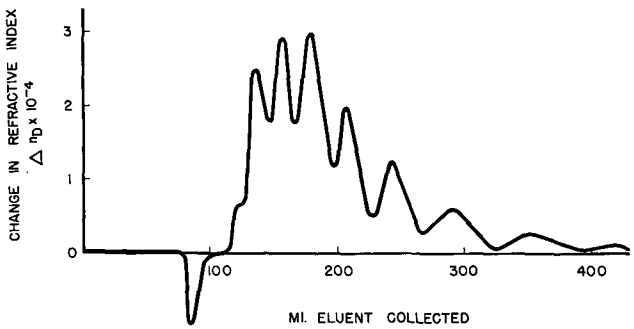

FIG. 1. Coconut oil: 35 ml/hr flow rate through a 1.2 x 95 cm column, 0.291 g sample.

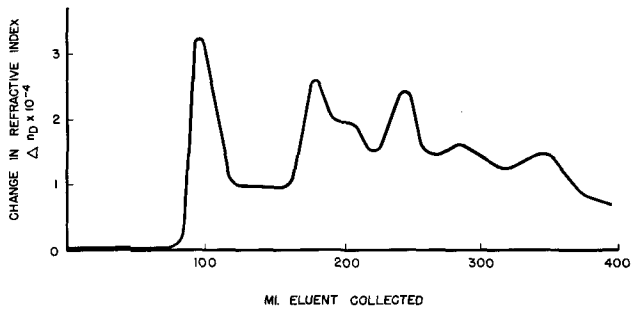

FIG. 2. Linseed oil: 112 ml/hr flow rate through a 1.2 x 95 cm column, 0.253 g sample.

could be obtained within 2 hr. The ground rubber was then continuously extracted for 24 hr with methanol in a Soxhlet apparatus. The extracted powder was slurried with ethyl ether and poured through a No. 20 U. S. standard sieve to remove any large pieces of rubber. The excess ether was removed by filtration and the powder further dried under reduced pressure.

To prepare a column, the dried rubber powder was slurried in methanol (ca. 10 g/100 ml) with the aid of a small high-speed metal stirrer until the suspension acquired a uniform appearance. A portion of freshly agitated slurry was poured into the column which was fitted with a coarse sintered glass plate at the bottom or simply with a plug of glass wool retained by a constriction at the base of a narrow diameter column. The packing was compressed by forcing a rapid flow of methanol through the column with compressed air. Additional quantities of slurry were added and the compressing process repeated. As the length of the compressed section increased and the flow rate diminished, the upper section of the column tended to be more loosely packed. This section was further compressed with a plunger made by fastening a strip of cotton cloth to the end of a rod, the strip being folded in front of the rod to form a close-fitting plug. This arrangement allowed the withdrawal of the plunger without excessively distributing the upper portion of the packing. Too much compression of the packing may produce too slow a rate of flow. The packing was judged sufficiently compressed when large voids were absent and it presented a uniform appearance to the eye. This was accomplished with a packing density of ca. 0.4 g dry rubber powder/cc column volume. When the desired length of packing was obtained, several circles of filter paper, cut to the diam of the column, were added to the top of the column and held in place by a layer of glass beads.

The eluent-line from the column was then connected with 2 mm diam Teflon tubing to the inlet of a Phoenix recording differential refractometer where it passed through the heat exchanger and the solution side of the optical cell, the reference side of the cell having been filled with the desired solvent. The solvent was allowed to flow through the column until a steady base line was observed on the recorder, several column volumes generally being required for freshly prepared columns. The sample was applied to the column as a 5% solution after allowing the solvent to drain to the top of the packed section and was followed by two or three washings with smaller volumes of solvent. A fairly constant flow rate was then maintained during the rest of the experiment by keeping a nearly constant head of liquid above the column by means of a siphon leading from a large capacity solvent reservoir. Variations in flow rate could be obtained by adjusting the height of the exit tube from the refractometer. In extended runs it was advisable to protect the solvent from atmospheric moisture by drying tubes to prevent a gradual shift in base line.

When not in operation, the columns were stored filled with solvent and were sometimes used repeatedly over a period of several months without any change in operating characteristics being noted. In changing solvents, the new solvent was placed in the reference cell and allowed to flow through the column until no further change in refractive index was noted.

Applications

Triglycerides. The use of rubber as a stationary phase and a mixture of methanol and acetone as the developing solvent appears to have great promise as a tool for investigating the glyceride distribution in vegetable oils. There are indications of separations comparable to those which have been achieved by Dutton and his co-workers who have made notable progress in this field through use of the Craig apparatus for counter-current distribution (3).

Figures 1 and 2 show the elution curves which we obtained from the chromatography of coconut oil and linseed oil. A mixture of equal parts by volume of methanol and acetone was used as the developing solvent. The addition of acetone was required to obtain reasonable solubility of the oils and to reduce the retention time on the column. In Figure 1, the initial change of refractive index recorded is in a negative direction (i.e., the eluent has a lower refractive index than the original solvent). We believe this "negative peak" which appears with the solvent front is caused by a perferential displacement of methanol from the rubber when the sample is initially applied to the top of the column. This produces a methanol-rich zone of lower refractive index which travels with the solvent front. A similar peak did not appear with the solvent front in Figure 2 in which an old sample of linseed oil was chromatographed. Its appearance was probably masked by the initial large peak containing oxidized degradation products of the oil and free fatty acids. The chromatogram in Figure 2 was also interrupted before all of the material had been eluted from the column.

In the case of coconut oil (Fig. 1) the sample was one that had been freshly refined and contained less than 0.1% free acid as lauric acid. The chromatogram indicates that partial separation of at least nine triglycerides in the oil has been achieved, counting the eight maxima and the shoulder which appears just prior to the initial maximum.

Methyl Esters. The methyl esters of coconut oil fatty acids, obtained by the alkali-catalyzed methanolysis of the original oil, were chromatographed on another substantially larger diam rubber column using 100% methanol as the mobile phase. The degree of separation achieved shows in Figure 3. The positions of the maxima, as indicated, correspond to those observed with known samples of saturated fatty acid methyl esters and methyl oleate, the latter ester showing the same retention as observed for methyl palmitate. The initial peak probably represents traces of more polar impurities which appears with the solvent front. No certain explanation can be offered to explain the change in base line observed during this run, although it may have been due to a gradually changing composition of the solvent. The refractive index of methanol varies with amount of dissolved air and a freshly warmed sample from which dissolved gases have been expelled shows a greater refractive index.

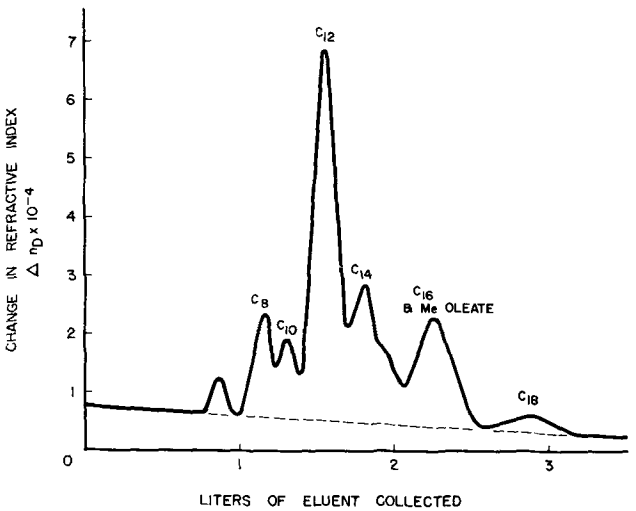

FIG. 3. Methyl esters of coconut oil fatty acids: 690 ml/hr flow rate through a 6.0 x 46 cm column, 2.50 g sample.

Hirsch (6) has recently described more extensive work on the complete separation of fatty acid methyl esters using a rubber column and an aqueous acetone solvent.

Separation of Trilaurin from Mono and Dilaurin. In the course of some other work, a procedure was required for the determination of mono, di and trilaurin (7). This was accomplished by chromatographic removal of the trilaurin from the mixture, determination of the monolaurin by periodate oxidation, and calculation of the dilaurin by difference. The mixture, dissolved in methanol, was passed through a short rubber column, 140 × 11 mm. The mono and dilaurin were not retained on the column and were eluted with the solvent front in a relatively narrow zone. Trilaurin was eluted later in the form of symmetrical peak. Evaporation of the methanol afforded a quantitative recovery of the separated fractions. Using a flow rate of 2.5 ml methanol/min, the initial appearance of the mono and dilaurin fraction was recorded within 6–7 min from the time the sample was applied to the column and the initial appearance of the trilaurin fraction was observed within 26–29 min in eight consecutive runs with different sample compositions in amounts ranging from 25–200 mg. The peak widths varied with the amount of solute

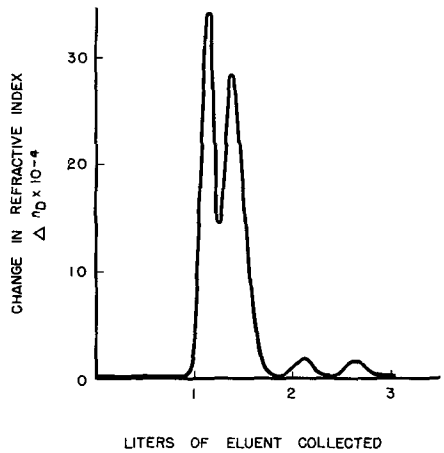

FIG. 4. Oil of Petitgrain: One 1/hr flow rate through a 6.0 x 46 cm column, 5.09 g sample.

present, 23 min being required for the elution of 18.4 mg trilaurin and 36 min for 82 mg.

The same column was also able to separate mixtures of mono and dilaurate esters of ethylene glycol, propylene glycol, and trimethylene glycol. In the case of these glycol esters, there was not such a wide separation between the two fractions, elution of the diester following immediately after completion of elution of the monoester. Although such separations were not attempted, it appeared that even on this short column mixtures of trilaurin and glycol dilaurates could have been resolved as their elution times were sufficiently far apart.

Removal of Terpenes from Oil of Petitgrain. Rubber columns provide a convenient means of separating terpenes from oxygenated components in essential oils. Figure 4 shows the elution curve which was obtained when a sample of Petitgrain Oil from Paraguay was chromatographed using methanol as the mobile phase. The two initial partially resolved peaks are chiefly linalool and linalyl acetate, which are major constituents of this oil (5). The first of the small peaks have a retention time near that which was observed for the aromatic terpene p-cymene whereas the last peak is in the region where the terpenes ocimene and limonene had been obtained previously.

This technique provides an excellent method for obtaining essential oil fractions free of terpenic hydrocarbons, particularly in those cases where the hydrocarbons are a minor fraction of the oil as described in recent patents (8). In the other chromatographic procedures using relatively polar adsorbents, the terpene-containing fraction is the first eluted from the column and the oxygenated components are then eluted by shifting to a more polar solvent (5). In some cases, there may be a danger of undesired chemical changes occurring during the process of adsorption or desorption from polar absorbents (2).

REFERENCES

1. Boldingh, J., Rec. trav. chim. *69*, 247 (1950).
2. Cassidy, H. G., Weissberger's "Technique of Organic Chemistry" Vol. 5, New York, Interscience Publishers Inc., 1951, p. 256.
3. Dutton, H. J. and J. A. Cannon, JAOCS *33*, 46 (1956). Scholfield, C. R., and M. A. Hicks, *Ibid.* *34*, 77 (1957). Dutton, H. J., C. R. Scholfield, and T. L. Mounts, *Ibid.* *38*, 96 (1961).
4. Guenther, E., "The Essential Oils" Vol. 3, D. Van Nostrand Co., Inc., New York, 1949, p. 225.
5. Kirchner, J. G., and J. M. Miller, Ind. Eng. Chem. *44*, 318 (1952).
6. Hirsch, J., Federation Proc. *18*, 246 (1959).
7. Bauman, R. A., and I. J. Krems, J. Am. Chem. Soc. *81*, 1620 (1959).
8. Patented as follows: U.S. 2,975,170; France 1,224,512; Germany 1,084,847; Great Britain 861,201; Switzerland (Pending).

[Received July 9, 1963—Accepted December 31, 1963]