A SILICA GEL IMPREGNATED GLASS FIBER FILTER PAPER AND ITS USE FOR THE SEPARATION OF CHOLESTEROL, TRIGLYCERIDES AND THE CHOLESTERYL AND METHYL ESTERS OF FATTY ACIDS*

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The separation of cholesterol from cholesteryl esters on silicic acid impregnated glass fiber filter paper was first observed by Dieckert and Reiser (1954). The use of silicic acid-coated glass paper for the separation of cholesterol from cholesteryl esters of serum led to the observation that frequently there was some separation of cholesteryl esters which differed in their fatty acid component. On an occasional paper a separation was seen which was recognized as being similar to the separation of cholesteryl esters obtained by Klein and Janssen (1959) on silicic acid columns. This report describes the preparation of an impregnated glass fiber paper which will consistently and reproducibly separate cholesteryl and methyl esters of fatty acids.

Impregnation of glass paper with "silica gel". Glass fiber paper (No. 934 AH, obtained from H. Reeve Angel Co., Clifton, N. J.) is coated with a supersaturated silicic acid solution which subsequently gels. The supersaturated silicic acid solution is prepared by mixing 30 ml. of NH_4Cl solution (5% W/V in water) with 100 ml. of potassium silicate solution (approximately 2% aqueous, prepared by making a 15 to 1 dilution of "Potassium Silicate-Electronics 200", Electrochemicals Dept., E. I. DuPont, Wilmington, Del.). Sheets of glass paper, 19 x 20 cm. are

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dipped singly into the coating mixture for about 10 seconds, wiped free of excess solution and air dried. The coating mixture sets to a gel in about 15 minutes. This gelation time can be shortened or lengthened by increasing or decreasing, respectively, the volume of 5% NH_4Cl solution added to the potassium silicate solution. After drying, the gel papers are freed of salts by washing in a tray with three changes of distilled water. The gel-papers are then air-dried, with the help of a hot plate after most of the water has evaporated. The dried papers are wrapped in aluminum foil and stored until needed. Alternately, the NH_4Cl is removed by sublimation instead of by washing; in this case, the papers are air-dried for 45 minutes and then hung in a furnace at 500°C for 20 minutes.

Chromatographic separation of cholesteryl and methyl esters of fatty acids. The samples containing cholesteryl or methyl esters are spotted approximately 2.0 cm from the bottom of the impregnated paper and developed by ascending chromatography in isooctane (2,2,4-trimethylpentane, Phillips Petroleum Co., Bartlesville, Okla.) which has been purified by passing through a column of alumina. The chromatograms are allowed to develop until the solvent front reaches a height of 15 cm., which takes 20 minutes. The chromatogram is dried in air at room temperature, sprayed with concentrated H_2SO_4 and heated to produce carbon from the compounds. All of the compounds give grey-black spots on a white background. As little as 0.2 microgram is readily detectable except for methyl esters of saturated fatty acids which are less easily oxidized to carbon. Detailed chromatographic procedures have been previously described by Swartwout et al. (1960).

The R_f values of cholesteryl and methyl esters vary with the relative humidity. In Table 1 R_f values are shown at a relative humidity of 70%, the humidity zone which permits an optimal separation of neutral lipids on the gel paper. At low relative humidities (60% or lower) the addition of benzene (up to 10%, v/v) to the isooctane gives a useful separation of esters. If the relative humidity is above 80% drying the papers over

TABLE I

R_f Values of Lipids on Silica Gel Impregnated Glass Paper.

Lipid	R_f	Lipid	R_f
Cholesteryl palmitate	0.75	Methyl palmitate	0.80
Cholesteryl stearate	0.75	Methyl stearate	0.80
Cholesteryl oleate	0.66	Methyl oleate	0.70
Cholesteryl linoleate	0.57	Methyl linoleate	0.61
Cholesteryl linolenate	0.46	Methyl linolenate	0.51
Cholesteryl arachidonate	0.43	Methyl arachidonate	0.48
Cholesterol	0.10		
Triglycerides	0.02		

silica gel gives desirable separations. Both cholesterol and triglycerides have low R_f values in these solvent systems and are clearly separated from the esters. The similarity of the R_f values of cholesteryl and methyl esters of the same fatty acid is noteworthy. A difference of one double bond is sufficient for an adequate separation; however, decreasing the chain length by about six carbons atoms gives a resolution of fatty acids similar to the addition of one double bond to a fatty acid of the C_{18} series. This accounts for the inadequate separation of linolenate and arachidonate esters on 15 cm chromatograms whereas this pair of esters is adequately separated on chromatograms developed to a height of 30 cm.

Chromatography of triglycerides (TG) and cholesterol (CH). From table 1 it can be seen that CH has a higher R_f value than TG when the lipids are chromatographed on silica gel paper in isooctane. This is unique in adsorption chromatography of these substances; generally the TG have an R_f value greater than CH (Dieckert and Reiser) and are eluted from a silicic acid column before CH (Borgstrom 1952). It was observed, however that addition of benzene or ether to the isooctane causes an increase in the R_f of TG which is greater than the simultaneous increase in R_f of CH. In fact the R_f of TG becomes greater than the R_f of CH and the order of the TG and CH becomes reversed. That such a reversal probably also takes place on silicic acid columns can be deduced from the study by Hirsch

and Ahrens (1959) on the adsorption isotherms of TG and CH on silicic acid.

These observations prompted a study of the influence of solvent composition on the chromatography of CH and TG. Two useful solvent systems were discovered during this investigation, namely, isopropyl acetate in isooctane and 1,2-dichloroethane in isooctane. In figure 1 the results are shown which were obtained with these two solvent systems, both of which exhibit this interesting reversal phenomenon. Thus it becomes possible to choose whether triglycerides will precede or follow cholesterol on a chromatogram. (Figure 1 shows that the isopropyl acetate solvent is to be preferred for making triglycerides precede cholesterol; dichloroethane is preferable for the other case.) Such a choice constitutes an advantage in the handling of materials which differ in the nature and in the proportions of their lipid constituents and increases the usefulness of two-dimensional chromatograms.

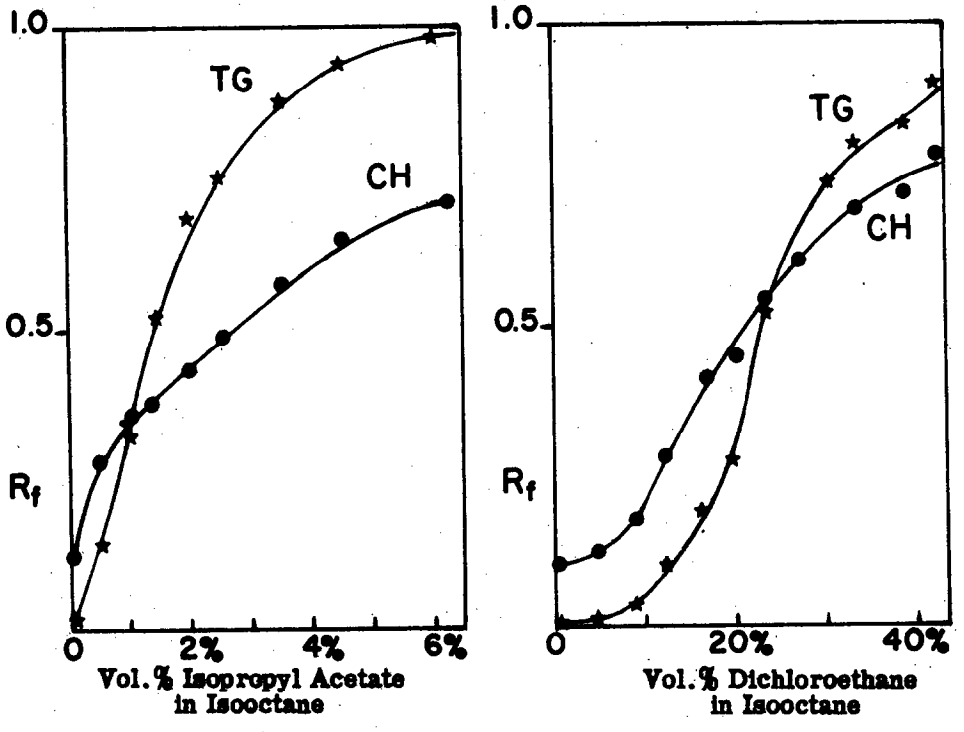

Figure 1. Influence of solvent composition on R_f value of triglycerides (TG) and cholesterol (CH).

Some separation of triglycerides based on the unsaturation of the fatty acids is obtained. Triolein has a lower R_f value than tristearin and can be clearly separated from tristearin. In a limited number of natural mixtures examined, no useful separation of triglycerides on 15 cm. chromatograms was observed. The complex mixture of triglycerides present in a natural mixture gives an elongated spot with the more unsaturated glycerides having the lower R_f value.

The general usefulness of "silica gel" impregnated glass paper for separation of compounds based on differences of unsaturation has been extended to sterols and will be reported in a separate publication. Also the quantitative determination of cholesteryl and methyl esters with this paper will be the subject of a future publication.

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