

## Chromatography of lipids on commercial silica gel loaded filter paper

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**SUMMARY** Commercial silica gel loaded filter paper has been found to give good separations of neutral lipids and phospholipids. Unidimensional or two-dimensional chromatography can be used. Rat liver lipids and  $C^{14}$ -algae lipids were studied. An advantage over thin-layer chromatography is that several spot tests can be applied on the same chromatogram.

**KEY WORDS** silica gel · paper chromatography  
neutral lipids · phospholipids · silica gel loaded paper

THE SEPARATION of lipids on silicic acid impregnated filter paper has found wide application for the study of lipids (1-5). The development of thin-layer chroma-

tography has also found extensive use in lipid analysis (5-8). The relative amount of time and degree of difficulty in preparing the impregnated papers or the chromatoplates may be the factors determining which method is preferred. I wish to report the availability of commercial silica gel loaded filter paper which has given excellent separation of neutral lipids and phospholipids. The Whatman silica gel loaded filter paper (grade SG-81) is available from H. Reeve Angel and Co., Inc., Clifton, N.J. This paper is uniformly impregnated and has higher adsorptive properties than the silicic acid impregnated papers prepared by the method of Marinetti (1). It is therefore necessary to modify the solvent system used for lipids (1). The commercial papers also can handle a higher lipid load.

The chromatograms and autoradiographs in Figs. 1-3 show some separations achieved on the commercial silica gel loaded filter paper. The phospholipids were separated by two solvent systems, namely diisobutyl ketone-acetic acid-water 40:25:5 (v/v) (DAW) and chloroform-methanol-water 65:25:4 (v/v) (CMW).

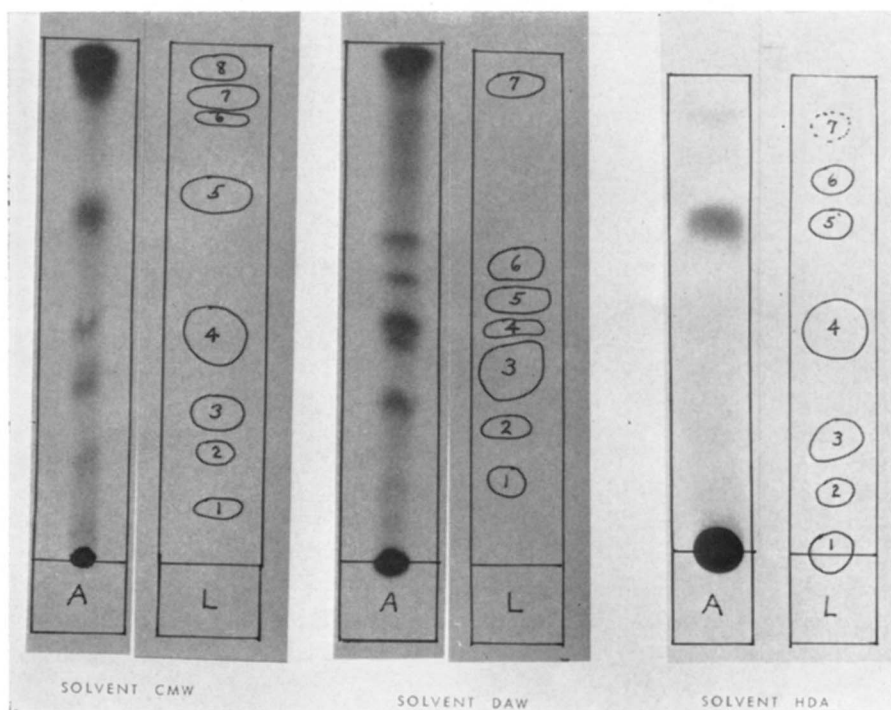


FIG. 1. One-dimensional chromatograms and autoradiographs of rat liver lipids and  $C^{14}$ -algae lipids. The solvent systems are given in the text. A = algae lipids, L = liver lipids. The identification of the liver lipids is as follows: solvent CMW, 1 = lysolecithin, 2 = inositol phosphatide, 3 = sphingomyelin + phosphatidyl serine, 4 = lecithin, 5 = phosphatidyl ethanolamine, 6 = cardiolipin, 7 = diglycerides + cholesterol, 8 = other neutral lipids (triglycerides, cholesterol esters); solvent DAW, 1 = lysolecithin, 2 = sphingomyelin + inositol phosphatide, 3 = lecithin, 4 = phosphatidyl serine, 5 = phosphatidyl ethanolamine, 6 = cardiolipin, 7 = combined neutral lipids; solvent HDA, 1 = phospholipids plus monoglycerides, 2 = diglycerides, 3 = free cholesterol, 4 = free fatty acids, 5 = triglycerides, 6 = unidentified, 7 = cholesterol esters (minor component). The exposure time for the autoradiograph on Kodak X-ray film was 3 days.

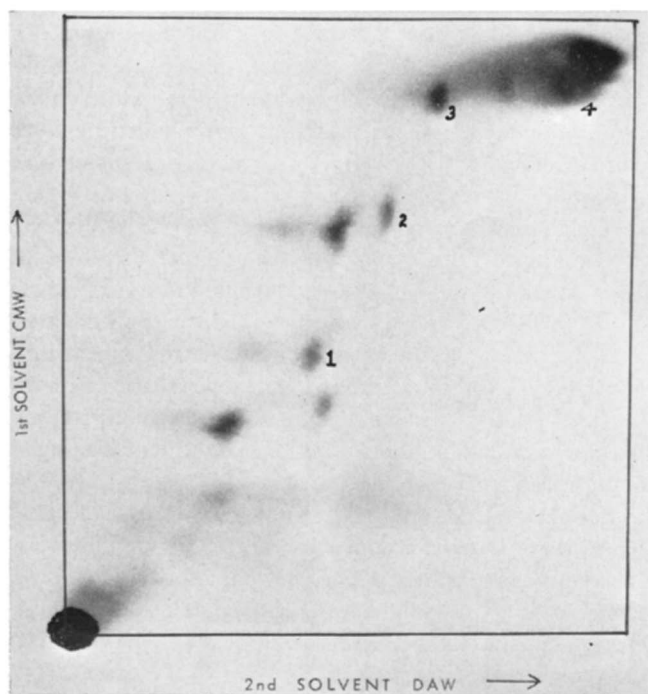


FIG. 2. Two-dimensional autoradiography of  $C^{14}$ -algae lipids. The solvent systems are given in the text. The spots include phosphatides, glycolipids, and neutral lipids. The exposure time was 3 days. 1 = lecithin, 2 = phosphatidyl ethanolamine, 3 = cardiolipin (or phosphatidic acid), 4 = combined neutral lipids.

The neutral lipids are resolved by use of *n*-heptane-diisobutyl ketone-acetic acid 85:15:1 (v/v) (HDA). All solvent systems contained 0.05% butylated hydroxytoluene (California Corporation for Biochemical Research, Los Angeles, Calif.) as antioxidant to minimize lipid peroxidation.

Chromatography was carried out at 23° in wide-mouthed 2 quart Mason jars using papers measuring 20 × 20 cm. The time required for chromatography was as follows: solvent HDA, 1.5 hr; solvent CMW, 2.5–3 hr; solvent DAW, 4–5 hr. The relative humidity of the room was 70%.

The separation of the liver lipids and  $C^{14}$ -algae lipids (Applied Science Labs., State College, Pa.) was very good. Identification of the liver lipids was possible since sufficient material (300  $\mu$ g of total lipid applied to the paper in a 30  $\mu$ l volume) was available for spot tests. The algae lipids could be detected only by autoradiography since a total of 5  $\mu$ g of lipid was applied to the paper.

The separation of the phospholipids was achieved by both one- and two-dimensional chromatography. The autoradiograph in Fig. 2 reveals many spots, representing phosphatides, glycolipids, and neutral lipids. The tracing shown in Fig. 3 is of a similar separation of the liver

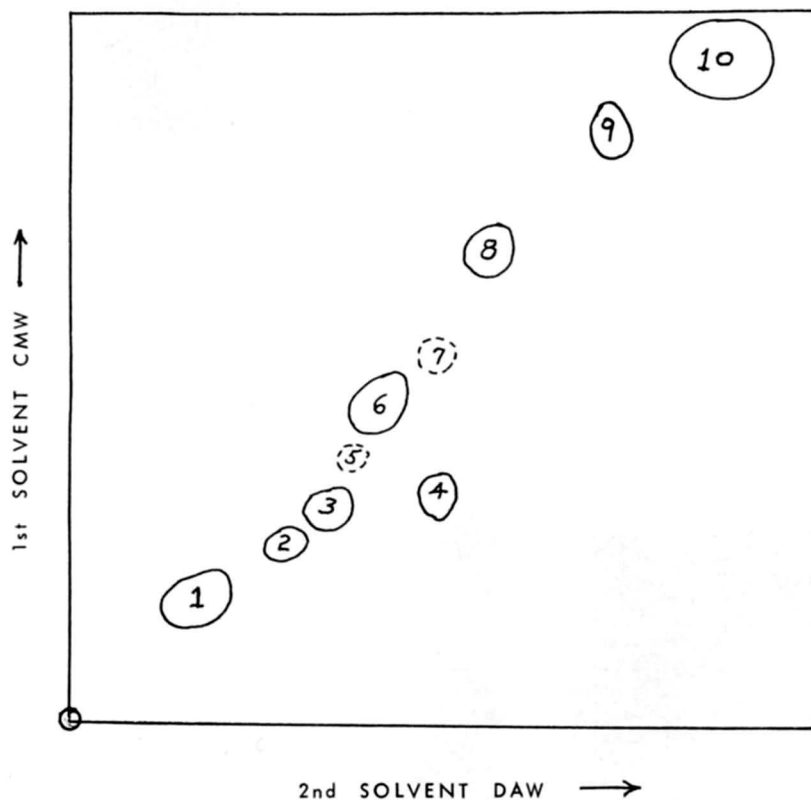


FIG. 3. Two-dimensional chromatogram of rat liver lipids. The solvents are given in the text. 1 = lysolecithin, 2 = inositol phosphatide, 3 = sphingomyelin, 4 = phosphatidyl serine, 5 = unidentified (possibly lysophosphatidyl ethanolamine), 6 = lecithin, 7 = unidentified (possibly cerebroside), 8 = phosphatidyl ethanolamine, 9 = cardiolipin, 10 = combined neutral lipids.

lipids. If the order of solvents for two-dimensional chromatography is reversed, equally good separations can be obtained.

A comparison of these silica gel loaded papers with thin-layer chromatography on glass plates coated with silica gel reveals several similarities. The  $R_f$  values of the lipids, the shape of the spots, the degree of resolution, and the loading capacity are about the same. A major advantage of the silica gel loaded filter papers is that a variety of spot tests is easy to perform on the same chromatogram.

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