Chromatography of lipids on silicic acid: infrared spectrophotometric elution curves

Direct spectrophotometric measurements on eluates greatly facilitate preparative column chromatography. They give elution curves that are often as useful as gravimetric elution curves, and make it possible to isolate compounds constituting specific peaks rapidly, under chemically mild conditions.

Measurement of electronic absorption is not appropriate for lipid eluates because most lipids only absorb selectively at frequencies at which the eluents are opaque. But measurement of vibrational absorption is potentially more useful, if eluent composition is constant or not subject to abrupt change. Thus we have been able to obtain spectrophotometric elution curves for silicic acid columns developed[1,2] with a continuous concave gradient of methanol in chloroform.

Results of measurements at 1745 cm^{-1} (the approximate fundamental frequency of C:O stretching in esters) were very satisfactory: most features of gravimetric curves were reproduced qualitatively until just before lecithins were eluted (see Fig. 1). Rising eluent opacity, caused by the gradient of methanol concentration, tended to submerge peaks in the spectrophotometric elution curves. The tendency was not very significant in the experiment described here, but it was much more significant in an experiment in which less lipids and less silicic acid were used with the same elution device, *viz.*, when lipid concentrations were roughly quartered. Attempts were made to correct for eluent opacity by subtracting functions (D_{eluent}) of the fraction number (n) from each absorbance reading (D). Several functions of the form, $D_{eluent} = An + Bn^2 + Cn^3$, were tried, but one of the simplest, $D_{eluent} = 10^{-4}n^2$, proved quite satisfactory.

Other spectrophotometric curves were plotted for hydrocarbon and peptide absorptions (2850 cm^{-1}, 1540 cm^{-1}). The results were not so good as those for ester absorption, though they were of limited use: a sterol peak, for example, was located by its hydrocarbon absorption.

Method

A sample cell of path length 2.5 mm, with barium fluoride windows, was used*. The spectrophotometer (Perkin-Elmer Infracord, Model 137E) was fitted with a logarithmic chart reading absorbance, and was adjusted to the polystyrene peak at 1745 cm^{-1}. The reference beam was attenuated with sufficient polyethylene film to balance roughly the absorption of the sample cell filled with the initial eluent (pure chloroform).

Tubes were removed from the fraction collector up to 60 h after they had been filled. The contents of each in turn were stirred with a small glass piston, and samples were removed by syringe for measurement of D. Since chart readings of D greater than 0.6 are very inaccurate it was sometimes necessary to "subtract" a part of D by putting more polyethylene film in the reference beam and then restoring sensitivity

* Sodium chloride windows are eroded by $>10\%$ methanol in chloroform.

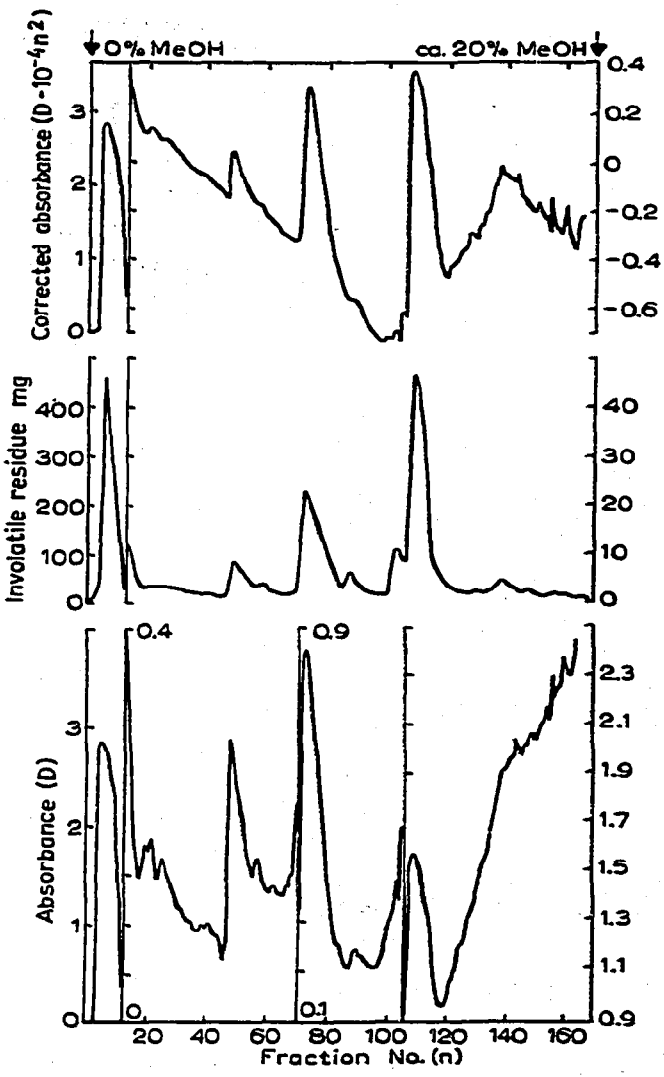

Fig. 1. Continuous, concave gradient elution of a wheat flour lipid preparation (6 g) from a column of silicic acid (170 g, diameter 33 mm). Lower curve: spectrophotometric (1745 cm^{-1}). Middle curve: gravimetric (every third 20 ml fraction taken). Upper curve: spectrophotometric, simple correction applied for eluent opacity.

by increasing the slit width; then two readings on a single sample were necessary to show the increment subtracted. It was sometimes necessary to reverse this procedure at the tail of a large peak.

Eventually, when they contained about 20% methanol, eluates became too opaque for measurement. The nominal values of slit width and D were then 450 μ and 2.5 respectively.

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