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NASA TECH BRIEF

Ames Research Center

"Dry-Column" Chromatography of Plant Pigments

The primary differences between dry-column chromatography and conventional wet column chromatography are that the solid adsorbent is placed in the column without the aid of a solvent and that the development of the column is normally terminated when the solvent front has just reached the bottom of the column. More important, however, is that a dry-column procedure can be derived directly from the results of preliminary experiments with the techniques of thin-layer chromatography.

It has been found that the separation of plant pigments which can be accomplished on thin-layer silica plates with a mixture of petroleum ether, a halocarbon, acetone, and a polar solvent can be readily translated into a dry-column technique that yields reproducible chromatograms after elution in the fashion of liquid chromatography with a fluorimeter as a detector. The best solvent system, which provides clean separations and does not produce double-banding in silica-packed columns, was found to be a mixture of 22 parts by volume of petroleum ether (30—60°), 3 parts dichloromethane, 3 parts acetone, and 2 parts ethyl acetate.

A satisfactory sorption column for analytical separation of plant pigments contains 160 mm of silica gel (4.5 grams) drawn by suction into a 7.8-mm (I.D.) stainless steel tube, and the silica gel is held firmly packed and in position against a 180-mesh screen disc by a spring pressing on another screen located in the top fitting of the column. A vessel containing the elution fluid under helium pressure is connected to the top of the analytical column; a short link of 0.5-mm I.D. (20-mil) polytetrafluoroethylene tubing is used to join the bottom of the analytical column to the bottom of a fluorimeter flow cell. The flow cell is a 3-mm I.D. quartz tube with ends of reduced diameter; it has an active volume of 100 micrometers, and is appropriately mounted in a fluorimeter. The top of the fluorimeter cell is attached by tubing to another pressure vessel that is connected to a packed column; the combination acts as a suppressor stage and prevents formation of gas bubbles in the fluorimeter cell.

The analytical column is conditioned or regenerated by passing through anhydrous acetone followed by acetone with 7% water, and finally drying with a stream of helium. Samples contained in 10 to 50 microliters of a nonpolar solvent such as petroleum ether are deposited on the top screen of the analytical column. Then, the elution solvent is allowed to enter the column; gradually, helium pressure is applied to cause a flow of about 20 ml per hour and the fluorimeter detector is set to record the elution chromatogram.

Note:

Requests for further information may be directed to:

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Patent status:

NASA has decided not to apply for a patent.

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Category 04