CHEMISTRY LETTERS, pp. 103-104, 1978. Published by the Chemical Society of Japan

RAPID AND EASY SEPARATION OF CHLOROPHYLLS, THEIR DERIVATIVES, AND PLANT YELLOW PIGMENTS BY THIN-LAYER CHROMATOGRAPHY

Masaru SHIRAKI, Masahiko YOSHIURA, and Keiji IRIYAMA

Research Institute for Polymers and Textiles, Sawatari 4-1, Kanagawa-ku, Yokohama 221

A thin-layer chromatographic method for the analysis of leaf pigments and their derivatives by commercial silica gel layers has been developed. The method is rapid and easy and is suitable for checking the purity of chlorophyll samples for various investigation.

In *in vitro* studies of chlorophylls, prepared or purchased chlorophylls should be checked for possible contamination of yellow pigments and chemical degradation products of chlorophylls. Commercial chlorophyll a was reported[1] to contain xanthophylls although it was free from chlorophyll b and it has been generally recognized that chlorophylls are decomposed by acids, alkalies, oxidizing agents, oxygen, heat, and intense light. Therefore, we should check the purity of chlorophylls also after experiments as the existence of the degradation products in chlorophylls may have induced erroneous experimental results. Thin-layer chromatography (TLC) has been widely used for the separation of chloroplast pigments.[2),3)] In this letter, we propose a rapid and easy method for checking the purity of chlorophylls by silica gel TLC during their preparation and physicochemical studies.

All the experiments were carried out at room temperature in total darkness or under green dim light. Spinach leaves (100 g fresh weight) were homogenized for 3 min in Warling blendor with 500 ml of acetone. The green juice obtained were filtrated through a pad of cotton to remove coarse debris and the filtrate was centrifuged at 10,000xg for 5 min to remove insoluble materials. The deep green supernatant solution (acetone-extract) was employed in the preparation of chlorophylls a and b according to the method of Iriyama et al.[4),5)] Acetone-extract, chlorophylls a and b were thus prepared. Chlorophylls a′ and b′ were obtained by re-fractionation of chlorophylls a and b on a sugar column according to the method of Sievers and Hynninen.[6)] Pheophytin a was prepared according to the method of Hynninen.[7)] 2.5x10 cm of commercial silica gel sheets (silica gel "Spotfilm" without fluorescence indicator, Tokyo Kasei LTD, Tokyo, Japan) were used. Solutions of test materials in diethyl ether were spotted with 2 μl micropipette 1.5 cm from the lower edge. The chromatogram were developed in thin-layer chambers. The atmosphere in the chamber was pre-equilibrated with the developing solvents for at least 15 min before the sheets were inserted. After the solvent front had ascended 7 cm (about 10 min), the sheets were dried. No chemical degradations were observed for chlorophylls a and b during the separation by the silica gel sheets.

Fig. 1 shows the thin-layer chromatogram of acetone-extract developed in 10 %

pentane solution of *tert*-butyl alcohol. The solvent system provided complete separation of the leaf pigments on the silica gel layer. Solvent system (*tert*-butyl alcohol : benzene = 1 : 9) had also provided good separation of the pigments in acetone-extract in the sequence carotenes-chlorophyll a-chlorophyll b-lutein-violaxanthin-neoxanthin.

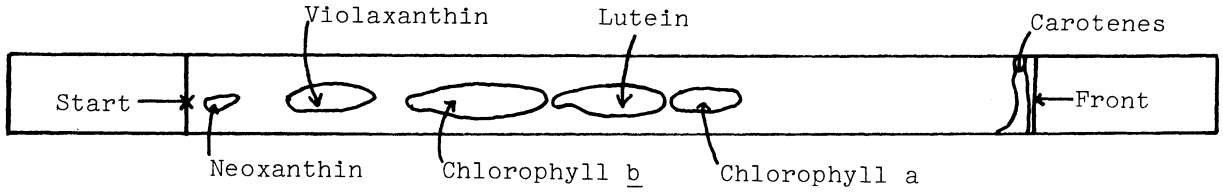

Fig. 1. Thin-layer chromatogram of acetone-extract on silica gel layer developed in solvent system (*tert*-Butyl alcohol : pentane = 1 : 9).

Fig. 2 shows the thin-layer chromatogram of mixed pigments (chlorophyll a, chlorophyll a', chlorophyll b, chlorophyll b', and pheophytin a) developed in solvent system (*tert*-butyl alcohol : pentane : acetone = 0.5 : 9 : 0.5). The solvent system provided complete separation of five pigments on the silica gel sheet.

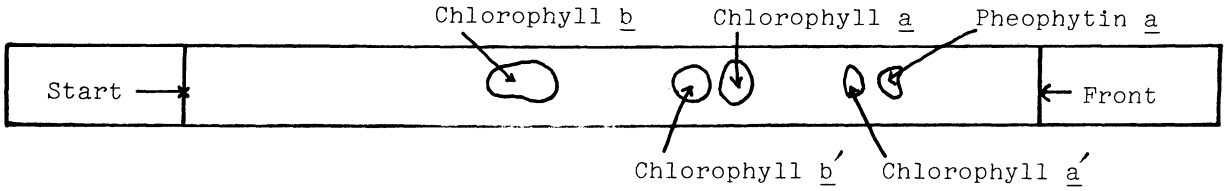

Fig. 2. Thin-layer chromatogram of mixed pigments (chlorophyll a, chlorophyll a', chlorophyll b, chlorophyll b', and pheophytin a) developed in solvent system (*tert*-butyl alcohol : pentane : acetone = 0.5 : 9 : 0.5).

The results in this study show that TLC on commercial silica gel sheets in microscale with the solvent systems containing *tert*-butyl alcohol is a rapid and easy method for checking the purity of chlorophylls a and b in chlorophyll studies

References

1) M. Mangel, D.S. Berns, and S. Ilani, J. Membrane Biol., 20, 171 (1975).
2) Z. Sestak, Photosynthetica, 1, 269 (1967).
3) H.H. Strain, and W.A. Svec, Advan. Chromatogr., 8, 119 (1969).
4) K. Iriyama, N. Ogura, and A. Takamiya, J. Biochem., 76, 901 (1974).
5) K. Iriyama, M. Shiraki, and M. Yoshiura, Chem. Letts., 787 (1977).
6) G. Sievers, and P.H. Hynninen, J. Chromatogr., 134, 359 (1977).
7) P.H. Hynninen, Acta Chem. Scand., 27, 1771 (1973).

(Received November 17, 1977)