

This Week's Citation Classic™

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Zscheile F P & Comar C L. Influence of preparative procedure on the purity of chlorophyll components as shown by absorption spectra.

Bot. Gaz. 102:463-81, 1941.

[Department of Agricultural Chemistry, Purdue Univ., Lafayette, IN]

Chlorophyll components *a* and *b* with reproducible spectroscopic properties were prepared by adsorption on sucrose but not dried. Analytical absorption values in ether solution make spectroscopic analysis more accurate than was possible before. [The SCI® indicates that this paper has been cited in over 160 publications since 1955, making it one of the most-cited papers ever published in this journal.]

Frederick P. Zscheile, Jr.
Department of Agronomy & Range Science
and
Agricultural Experiment Station
University of California
Davis, CA 95616

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"This paper is a culmination of a long search for better understanding of the photosynthetic process of the green plant. As a farm boy, I marveled at photosynthesis and was encouraged by very good high school teachers, J.A. Cocanouer and Mrs. Earl, in agriculture and chemistry at Chowchilla, California. At the University of California in Davis, C.S. Bisson and W.W. Robbins, in chemistry and botany, assisted me in many ways. I am thankful for the university scholarship during this and six additional years of study at Berkeley for my PhD degree in plant physiology.

"In the department of plant nutrition, C.B. Lipman and A.R. Davis were very helpful. D.R. Hoagland was my graduate adviser. For my graduate research, blue chlorophyll *a* and green *b* were prepared according to the directions of Willstätter and Stoll,¹ and separated by powder chromatography.² A second green fraction appeared on adsorption columns and was thought to contain a third component of chlorophyll. I took a course in photochemistry from T.R. Hogness, and I was convinced that methods for identification at that time were inadequate. Absorption spectra were measured by photographic spectroscopy and were not subject to quantitative applications. It was ap-

parent that some photoelectric method should be applied.

"Hogness moved to the University of Chicago and had a spectroscope that might serve my purpose. Under a National Research Council fellowship, I tackled the problem in the chemistry department of the University of Chicago, encouraged by E.J. Kraus in the botany department. Two years of research produced a photoelectric spectrophotometer³ for the ultraviolet and visible regions of the spectrum. This was probably the only such apparatus in the US at that time.

"A grant from the Rockefeller Foundation to Hogness provided expansion of this work to a larger room and included, in addition to the spectroscope (Zeiss), an early amplifying tube (FP 54-Pliotron, by General Electric), a long water-cooled hydrogen arc to provide ultraviolet, a long optical arm for the galvanometer, and later a small air-cooled hydrogen arc.

"After two years, I joined the faculty of agricultural chemistry at Purdue University at the invitation of H.R. Kraybill, who provided a larger and more elaborate double monochromator (Adam Hilger) for a third photoelectric spectrophotometer.⁴ Smaller spectrophotometers became economically available considerably later.

"The question of a third component of chlorophyll was caused by observation of pheophytin formation from component *a* during drying procedures.⁵ This paper with enthusiastic graduate student C.L. Comar has been cited because it provided very accurate spectra of chlorophylls *a* and *b*, with absorption constants (much higher than given by the earlier model⁶) that permit accurate analysis of plant extracts of pigments. The method was also applied to obtain similar constants for yellow carotenoid pigments of leaves.

"G. Mackinney, at Berkeley, soon published a study⁷ of the effects of various solvents on chlorophyll absorption spectra, using methods of measurement similar to ours. A recent review⁸ by Brown reports many spectroscopic studies on chlorophyll complexes extracted from plants and *in vivo*. Our studies on purified pigments prepared the way for much later work."

1. Willstätter R M & Stoll A. *Investigations on chlorophyll: methods and results*. Lancaster, PA: Science Press Printing, 1928. 385 p.
2. Trüvet M. Adsorptionsanalyse und chromatographische Methode Anwendung auf die Chemie des Chlorophylls. *Ber. Deut. Bot. Ges.* 24:384-93, 1906. (Cited 60 times since 1955.)
3. Zscheile F P, Jr., Hogness T R & Young T F. The precision and accuracy of a photoelectric method for comparison of the low light intensities involved in measurement of absorption and fluorescence spectra. *J. Phys. Chem.* 38:1-11, 1934.
4. Zscheile F P, Jr. Photoelectric spectrophotometry. The performance of a quartz double monochromator in an improved and more versatile photoelectric spectrophotometer. *J. Phys. Colloid Chem.* 51:903-26, 1947.
5. Number of chlorophyll components. *Bot. Gaz.* 103:401-3, 1941.
6. A quantitative spectro-photoelectric analytical method applied to solutions of chlorophylls *a* and *b*. *J. Phys. Chem.* 38:95-102, 1934.
7. Mackinney G. Absorption of light by chlorophyll solutions. *J. Biol. Chem.* 140:315-22, 1941. (Cited 990 times since 1955.)
8. Brown J S. Forms of chlorophyll *in vivo*. *Annu. Rev. Plant Physiol.* 23:73-86, 1972.