Recording of thin layer chromatograms on Polacolor film under ultraviolet*,**

Thin layer chromatograms of many substances fluoresce under ultraviolet light or may be made to do so by appropriate post-development treatment. Several techniques for photographing fluorescence induced by trans-illumination of chromatograms have been reported. Black and white records of fluorescing spots were made on Agfa "copy rapid" negative paper from chromatograms on paper[1] and thin layer plates[2]. The construction of facilities to permit the color photography of ultraviolet fluorescence of thin layer chromatograms has been described[3–5]. Selection of color emulsions and filters for color recording of chromatograms has been discussed[5]. Thin layer chromatography of chlorophylls and pheophytins utilizing fluorescence to depict separation has been reported but description of the procedure employed for obtaining the black and white records presented was not indicated[6]. Since our study has been conducted, a report has been published in which reference was made to making permanent records of the fluorescence of thin layer chromatograms of free porphyrins on panchromatic film exposed through a red filter[9].

In a thin layer chromatographic study of the chlorophylls and a number of their derivatives, it was observed that most of the pigment spots of developed chromatograms, when viewed under ultraviolet of wavelength 3660Å, fluoresced characteristically (7). It was also observed that pigment spots of developed chromatograms exhibited marked differences in stability. For recording purposes, it became desirable to develop a procedure which would require a minimum of time and which would reduce to a minimum the destructive influences of chromatogram exposure to light and temperature associated with photographic procedures. It was found that color photographs could be rapidly made of the fluorescent spots of chromatograms of chlorophyll and derivatives and that such color photographs in combination with a description of the visual appearance of the chromatograms would provide an easily obtained, accurate permanent record.

Experimental

A fluorescence analysis cabinet*** was modified to provide a retractable platform on which the chromatoplate was mounted to permit the ready inspection of the chromatogram by illumination either with reflected or transmitted white light and inspection and photography under incident ultraviolet radiation. Essential details of the modification are shown in Fig. 1. Ultraviolet radiation was provided by two 80 W high intensity lamps (wavelength 3660 Å) mounted about $1^1/_2$ in. above and on either side of the chromatoplate, the lamp being tilted on edge at an angle of about 45°. Illumination of chromatograms by transmitted white light was provided by a fluorescent light tube mounted under the frosted glass which served as the retractable platform as shown in the diagram. A black matte-finished cardboard shield slotted for the chromatoplate was placed over the frosted glass. Suitably graduated plastic rules (which fluoresced white) were appropriately placed on either side of the slot in the

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Fig. 1. Fluorescence Analysis Cabinet modified to permit photography with the MP-3 camera as well as inspection of chromatograms under ultraviolet radiation and to permit inspection by means of transmitted and reflected white light.

cardboard shield to permit the ready location of chromatograms. The chromatoplates were 50 × 200 mm in dimension. The cabinet was placed on the base of a Polaroid MP-3 Industrial View Land camera stand thereby permitting either visual inspection or photography of the chromatogram without necessity of change of location. For maximum convenience, the camera-cabinet assembly was placed on a table of such height that the investigator might stand while making chromatogram inspection.

The mounting of the retractable platform at a level about 6 in. below the top of the fluorescence analysis cabinet permitted the easy and rapid focusing of the MP-3 camera without accessory lens tubes. All inspection and photography carried out under U.V. was conducted in a normally lighted laboratory.

The MP-3 camera was fitted with a 5 in. lens to which was attached a 2B Wratten filter to exclude stray U.V. Polaroid Polacolor Type 108 Land film was utilized in these studies, shutter setting was f5.6, and time of exposure was 18 sec. Development of the print was complete 1 min after exposure.

The photographic record of the fluorescing spots of the chromatogram was made at once following chromatogram development. This required about 2 min, following which chromatoplate evaluation could be made by visual inspection. In practice, this

activity consisted of description of the appearance of the spots of the chromatogram when illuminated either by transmitted or incident white light or by incident U.V. This evaluation may or may not include measurement of spot size and location on the plate. The photographic print, which is about 73 × 95 mm in size, provides a means of easily and exactly measuring the size and location of all fluorescing spots without damage to the plate. Therefore, these time-consuming operations might be conveniently carried out at the discretion of the investigator.

Results and discussion

The photography technique using Polacolor film was investigated in connection with a study of variations in procedures for the thin layer chromatography of chlorophylls and their derivatives[7]. Polacolor photos of a given chromatogram of these pigments and of certain carotenoids were found to be very similar to the visual appearance of the same chromatogram when viewed under U.V. This similarity prevailed with respect to sensitivity of spot detection and with respect to spot color, size and shape. For fluorescing spots there appeared to be a slight decrease in spot redness and in spot boundary sharpness and a slight increase in spot size in the Polacolor print when this record was compared with the chromatogram at the time of photographing.

Spots of certain substances absorb U.V. rather than fluoresce when irradiated with U.V. and accordingly appear black. The various carotenoids appear black immediately after chromatographing either when viewed or photographed under U.V. (3660 Å), but fade rapidly and then appear gray. Copper chelates are non-fluorescing and spots of these compounds on chromatograms appear black under U.V. irradiation (3660 Å). These spots are very stable.

Presented as an example of recording of chromatograms photographed under incident U.V. are the Polacolor prints in Fig. 2. Pigments were chromatographed on an oil-impregnated Kieselguhr G-coated plate according to a variation of the method of EGGER[8] developed in this laboratory[7]. In this study, it was observed that when chromatograms of chlorophylls and derivatives are prepared using Kieselguhr or certain other coating media there is a noticeably rapid alteration of the appearance of the spots of certain of the pigments. This alteration is seen as fading or change in color when viewed under white light and as a change in fluorescence color varying progressively from red to pink to white or gray when viewed under U.V. Polacolor photos of chromatograms which had undergone the alteration mentioned above were found to provide faithful reproduction of the change which was seen to have taken place upon visual evaluation of the chromatogram under U.V. An example of the ability of Polacolor film to portray pigment change is presented in Fig. 2 which contains a Polacolor print of a chromatogram photographed immediately after development and a second print of the same chromatogram photographed upon removal from storage in the dark, 3 h after development. Ektachome and black and white transparencies of the prints provide excellent projector slives of the records of the chromatograms.

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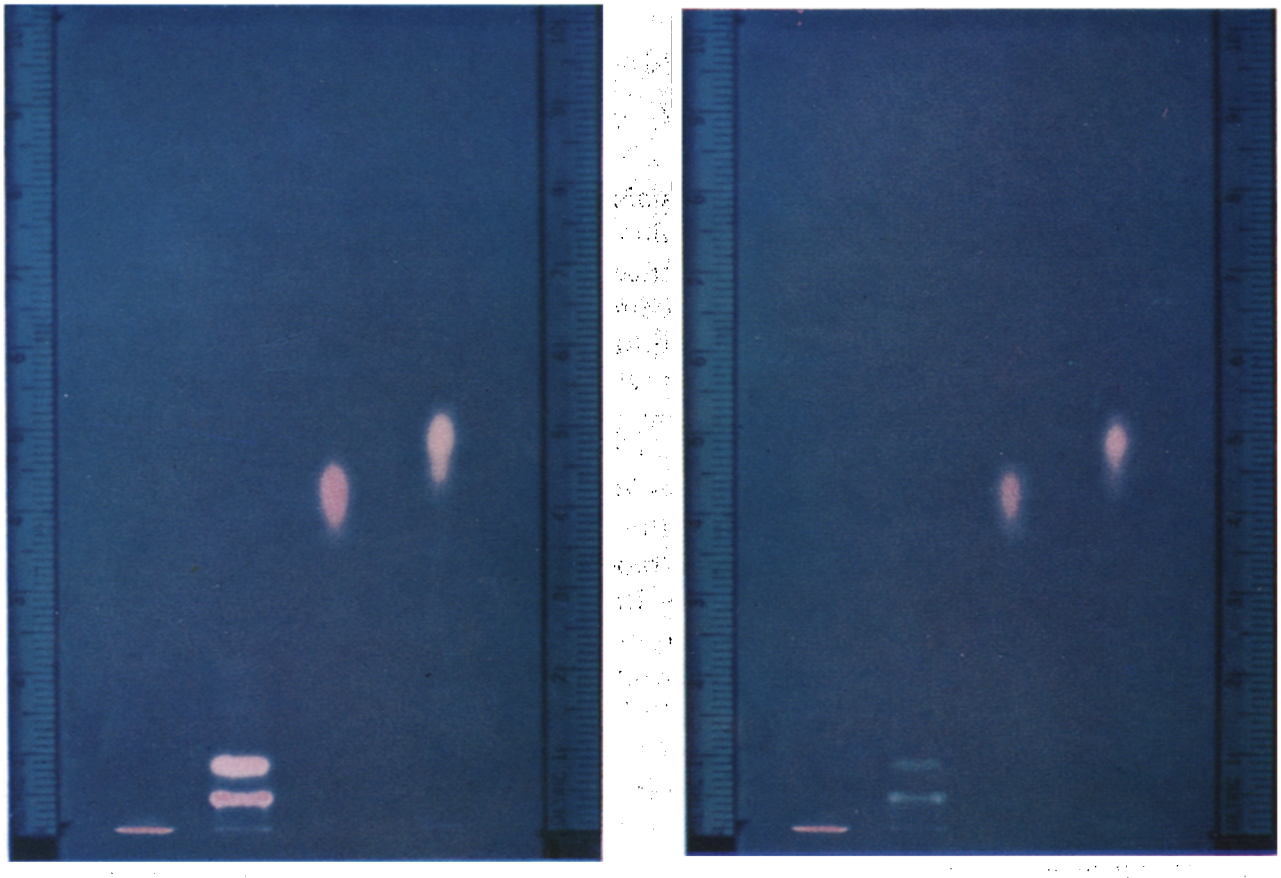

Photographed immediately. Photographed after 3 h.

Fig. 2. Polacolor print of oil-impregnated Kieselguhr G-coated chromatoplate photographed at intervals indicated after chromatogram development. Sample identity, from left to right: (1) Pheophytins a and b (single band). (2) Mixture of purified chlorophyll a and chlorophyll b with trace of pheophytin; lowest band = trace pheophytin, middle band = chlorophyll a, upper band = chlorophyll b. (3) Pheophorbide a. (4) Pheophorbide b.

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Department of Food Science,
Department of Agricultural Information,
Department of Chemistry,
North Caroline State University, Raleigh, N.C. (U.S.A.)

I. D. JONES
L. S. BENNETT
R. C. WHITE

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