

A photographic method for recording chromatograms

Various methods have been used for recording thin-layer chromatograms of substances which are visible under ordinary light^{1,2}, and procedures involving photographic contact paper have been utilized for recording paper chromatograms of compounds which absorb in the ultraviolet region. A plastic film transfer technique for permanent preservation of the adsorbent layer of thin-layer chromatograms has recently been reported³, and reagents and equipment are commercially available⁴.

With compounds which do not yield visible spots on chromatograms without chemical development (*e.g.*, nucleotides and their derivatives), the spots can be outlined under ultraviolet illumination and, in the case of thin-layer chromatograms, their location can be transferred to tracing paper or cellophane sheets⁵ to form a type of permanent record. This procedure, requiring relatively long exposure to ultraviolet light, is tedious and also precarious because of the fragility of the layer.

A photographic method has been devised using Polaroid film. The procedure is simple and rapid, does not require elaborate equipment, gives a permanent record, decreases exposure of personnel to short wavelength ultraviolet radiation, and produces excellent results.

Method

A 4 × 5 view camera is attached to a vertical enlarger stand, the baseboard of which supports the chromatogram on a nonfluorescing background. The camera is fitted

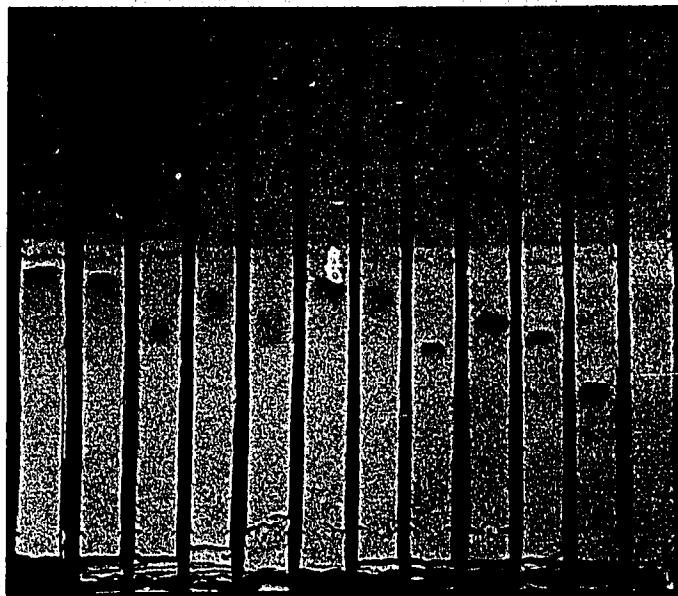


Fig. 1. Photograph from Polaroid negative of a thin-layer chromatogram (20 × 20 cm grooved-glass plate).

with a Polaroid Land Film Holder for film packets, and a Wratten G filter is used over the lens. The chromatograms are evenly illuminated by two ultraviolet lamps⁶ set at each side of the copyboard at a 45° angle, as in a standard photographic setup⁷. A photolamp or an incandescent lamp is used for illumination during critical focusing on the ground-glass screen. The room is then darkened, the ultraviolet lamps are

turned on, and an exposure is made on Polaroid film Type 55 P/N using a time of approximately 2 min at f 5.6 for a lens-to-subject distance of 18 in. The film yields a positive 4×5 -in. print in 20 sec and is usually satisfactory for determination of R_F values. In addition, a negative is obtained which can be processed without darkroom facilities. The negative can then be used for photographic enlargements to aid further in making desired determinations or filed for future reference. It was found that the Polaroid prints resulted in much better visualization, particularly of very weak spots, than those obtained by former methods.

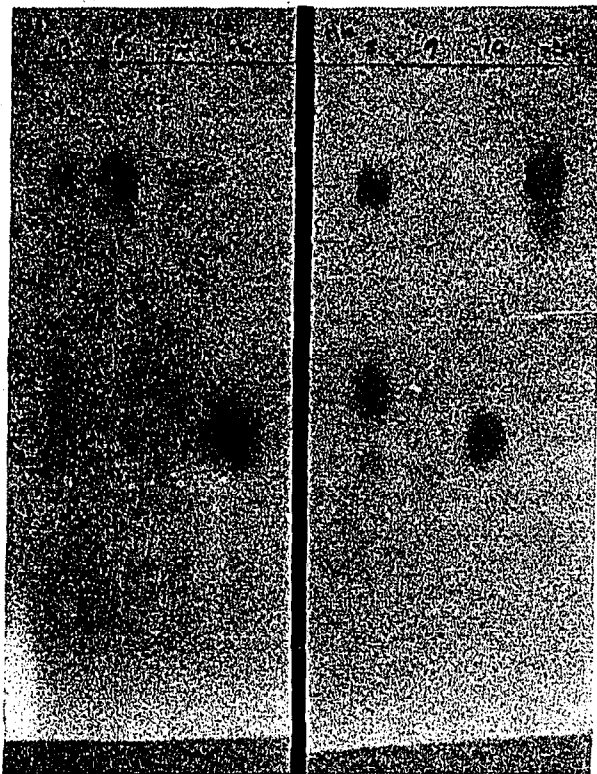


Fig. 2. Photograph from Polaroid negative of paper chromatograms (9 cm wide strips, Whatman No. 31).

Considerable modification could be made, depending on available equipment; for instance, satisfactory results were attained using only one ultraviolet lamp, although uniform illumination was more difficult.

Typical results obtained are illustrated in Fig. 1 for a 20×20 -cm grooved-glass TLC plate⁸ with cellulose as adsorbent and in Fig. 2 for 9-cm wide Whatman No. 31 paper strips.

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Separation and identification of methylol derivatives of cardanol

Various investigators have used different methods and techniques to separate and identify the reaction products of various phenols with aldehydes under alkaline conditions¹. With a substituted phenol, such as cardanol (the chief component of commercial cashewnut shell liquid), the identification of the intermediate products is even more difficult, and available methods do not enable easy separation for identification of these products.

Preliminary results indicate that it is possible to separate the intermediate products formed in the cardanol-formaldehyde reaction under alkaline conditions by using the technique of thin-layer chromatography².

Materials and method

(1) Cardanol (1 mol.) was treated with formaldehyde (1 mol.) in presence of 40% KOH solution (1 mol.) and the reaction mixture was kept at room temperature (25°) in a stoppered bottle. After a period of 24 hours, 1 g of the reaction mixture was removed from the bottle and diluted ten times with ethanol. The reaction mixture was kept for 11 days and at intervals of 24 hours, samples were taken out as indicated above.

(2) Finely divided silica gel (200 mesh) was mixed with twice its weight of water and a little gypsum. The paste was then uniformly spread on glass plates, 20 cm in length, and dried in an oven for 2 hours at 100°.

(3) The samples collected at intervals of 24 hours were taken for spotting. After spotting these solutions on the glass plates, the chromatogram was run for 55 minutes in ethyl acetate-toluene (60:40) mixture. Four spots were obtained from the samples taken on the fifth day and there was no change up to the eleventh day. The chromatograms were developed with diazotised sulphanic acid.

Three methylol derivatives with R_F values of 0.738, 0.586 and 0.448 respectively were detected. The fourth spot was identified and confirmed to be that of unreacted cardanol.

One of the derivatives was crystalline and melted sharply at 60°. It had an R_F value of 0.738. This derivative was separated and identified after hydrogenating the