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Autoradiography of thin-layer radiochromatograms using Polaroid film

Autoradiography of thin-layer radiochromatograms has commonly been performed by exposure of the radiochromatogram to X-ray film in a photographic darkroom for various periods followed by development of the film with a suitable developer. This procedure requires the space for the photographic darkroom and the tedious use of wet-process developers. Radiochromatographic scanners and cameras have recently been employed as an alternate method for the detection of labeled compounds on TLC plates, but price restrictions discourage many investigators from using this technique. It was, therefore, desirable to devise an autoradiographic procedure which would be both inexpensive and uncomplicated and would not require a darkroom or a wet-process development. Incorporation of Polaroid film, as noted in a previous report[1], and a suitable exposure cassette into the autoradiographic process proved to be an excellent technique which may be used by investigators who desire an inexpensive, uncomplicated autoradiographic procedure. The procurement of a positive

print in black and white or in color, as ^{14}C and ^{3}H were found to produce different colors upon exposure, also adds value to this technique.

Experimental

Construction of the cassette. The light-tight cassette is pictured in Fig. 1 (A). It is made of 1/4 in. black plexiglas and has dimensions of $5\frac{1}{4} \times 7 \times 3\frac{1}{4}$ in. and a slot for the insertion of a Polaroid 545 Land Film Holder (B). A hinged $\frac{3}{4}$-in. plexiglas lid (C) is lined with $\frac{1}{8}$-in. black foam-rubber strips which fit around the rectangular frame of the film holder. Attached to the lower side of the lid is a movable plexiglas pressure plate (D) measuring $3\frac{11}{16} \times 4\frac{11}{16} \times \frac{1}{4}$ in. which can be lowered or raised with the aid of four guide pins (E) and a knurled screw (F). A thumb screw (G) on the side of the cassette is screwed into the lid to hold it tightly in place against the film holder.

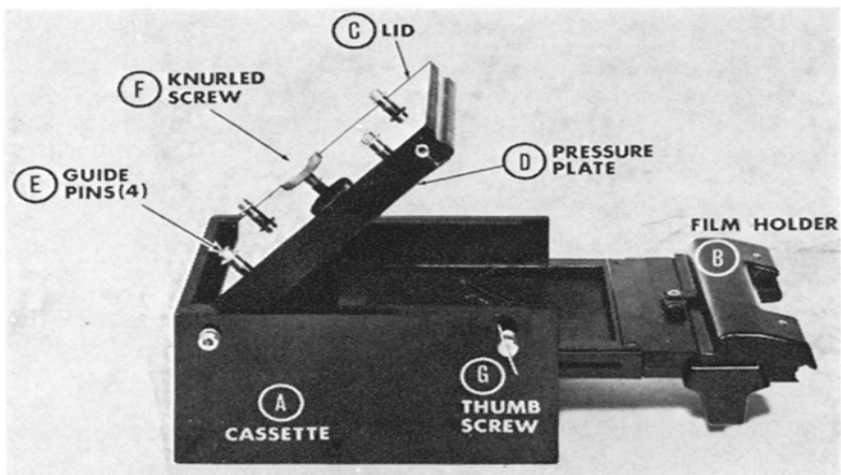

Fig. 1. Autoradiographic cassette.

Autoradiographic procedure. A radiochromatogram with a maximum dimension of 9 × 12 cm is attached to the movable pressure plate (D) with doubly adhesive tape. The hinged lid is set against the film holder and screwed tightly in place with the thumb screw (G). The 4 × 5 in. film packet (Polaroid Type 57 or 58) is inserted into the film holder with the front side of the packet (the side normally facing the lens) facing the radiochromatogram, and the protective envelope is then withdrawn. The movable pressure plate containing the radiochromatogram is lowered with the aid of the guide (E) and knurled screw (F) onto the film. Exposure times depend upon the activity of the material. After exposure, the movable pressure plate is lifted from the film and the protective envelope containing the positive is inserted back into the film holder. The film is then processed by pulling the film from the holder which breaks the pod containing the developer.

Results

A radiochromatogram containing 10,000 d.p.m. of [^{14}C]glycine and 1,000,000 d.p.m. of [^{3}H]valine was exposed to Polaroid Type 57 film (black and white) for 96 h. The positive print is pictured in Fig. 2. The same radiochromatogram, when exposed to Polaroid Type 58 film, gave a positive print after 240 h, which exhibited a very

faint blue area due to the tritiated material and a greenish white area due to ^{14}C. Greater activities of tritium or longer exposure times produced brighter areas. The whiteness of the ^{14}C area also increased on longer exposure times. For Type 57 film, the exposure times were similar to those necessary for autoradiographs on X-ray film using a wet-process development. This new technique eliminates a darkroom and the

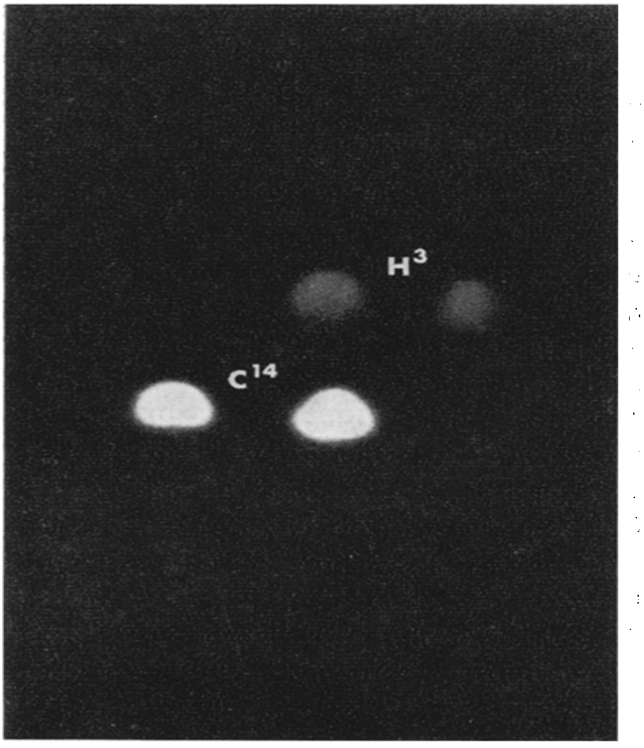

Fig. 2. A Polaroid print of a radiochromatogram containing [^{14}C]glycine and [^{3}H]valine.

use of wet-process developers. Its timesaving ability to differentiate ^{14}C and ^{3}H makes it useful in studies involving the two isotopes such as those dealing with metabolic transformations, organic and biochemical mechanisms, and in any other studies where doubly labeled substances must be chromatographed.

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