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Note

Coupling of chemiluminescence thin-layer chromatographic detection to a Vidicon rapid scanning detector

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The preceding paper describes the use of peroxyoxalate chemiluminescence (CL) for detecting fluorescent compounds separated by thin-layer chromatography (TLC)[1]. One of the problems with this detection system is that intensity varies with time, which can lead to errors if there is a significant change during a complete scan of a plate. The intensity change with time can be minimized by reducing peroxide concentration; however, this also reduces sensitivity. In this paper we describe the coupling of CL detection with a Vidicon rapid scanning spectrometer.

The Vidicon detection head is a two-dimensional array of photodiodes in a total area 0.5 in. × 0.5 in. When photons strike a photodiode they cause a build up of charge. This charge is discharged and measured using an electron beam which completely scans the array once every 32 msec.

In the system used in this study the detector surface is divided into 500 separate channels in the horizontal direction. The intensity striking each channel is measured by a multichannel analyzer and displayed on a cathode ray tube (CRT).

Normally, the Vidicon detector is used to measure spectra in combination with a grating so that different wavelengths fall on different areas of the detector surface. The output of intensity *vs.* channel number is essentially a plot of intensity *vs.* wavelength. However, in this study optical dispersion is not used. Instead the emission from the TLC plate is focused onto the Vidicon detector surface. In this set-up, different detector channels correspond to different positions on the TLC plate. The CRT output of intensity *vs.* channel number is a record of emission intensity *vs.* plate position along one axis.

The Vidicon detector is generally used as an integrator. Successive scans of intensity *vs.* channel number are added together over some interval in time. This interval can be preset by the operator, *i.e.*, the Vidicon detector accumulates a fixed number of scans after a button has been pushed to start the integration.

Alternatively, the detector can be set up to accumulate scans until one channel has reached its maximum possible signal, at which point scanning stops and the integrated output is displayed on the CRT. For more information on Vidicon detectors the reader is referred elsewhere[2,3].

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There are several advantages to using a silicon Vidicon detector for a chemiluminescing TLC plate. Sensitivity is greater since the detector simultaneously measures emission from all areas of the plate along one dimension rather than looking at one particular spot at any given time. Also the rapid scan property of the detector eliminates error associated with CL intensity change with time and makes it possible to use higher hydrogen peroxide concentrations resulting in higher sensitivity.

EXPERIMENTAL

Instrumentation

The silicon Vidicon system used for TLC detection is diagrammed in Fig. 1. Light from the TLC plate is focused on the Vidicon detector surface by a f/2.0 lens system (Sankyo Kohki Japan Komura). The distances from the plate to the front lens and back lens to the detector surface are indicated on the figure. The effective focal length of the lens system is 5 cm. An SSR 1205 B detector head with a silicon intensified target was used in conjunction with an SSR 1205 A optical multichannel analyzer (OMA) console. Output as a function of channel number was displayed on a CRT and recorded on a Hewlett-Packard 7035 B X-Y recorder. The entire apparatus was in a dark room.

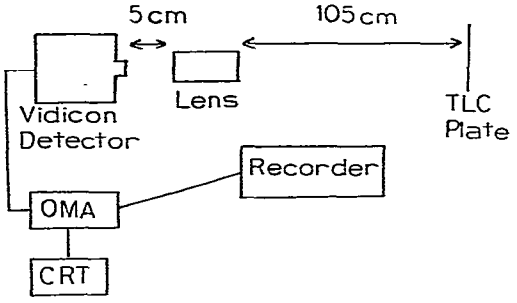

Fig. 1. Block diagram of Vidicon–TLC detection system.

The TLC plate was first sprayed with bis-2,4,6-trichlorophenyl oxalate (TCPO) solution and then positioned in front of the Vidicon detector and sprayed with hydrogen peroxide solution.

Integration was initiated as soon as the spots from the plate could be seen visually, which was a few seconds after spraying. The integration was performed in the full-scale-hold mode, *i.e.*, as soon as the channel recording the most intense signal had reached its maximum possible value, integration automatically stopped. The integrated data are stored in a memory register. The light from the TLC plate is then blocked off and the background signal is integrated for the same number of scans as required for the signal. The background is then subtracted from the signal. This procedure gives a significant improvement in signal-to-noise ratio.

Chemicals, materials, and procedures were identical to those reported in the preceding paper[1].

RESULTS

Fig. 2 shows the signal observed for 0.5 μg of dansyl-arginine, dansyl-glycine, and dansyl-leucine applied directly to silica gel. The areas of the peaks are comparable but the peak heights differ significantly. This differs from the results using the scanning fiber optic detection system of the previous paper[1].

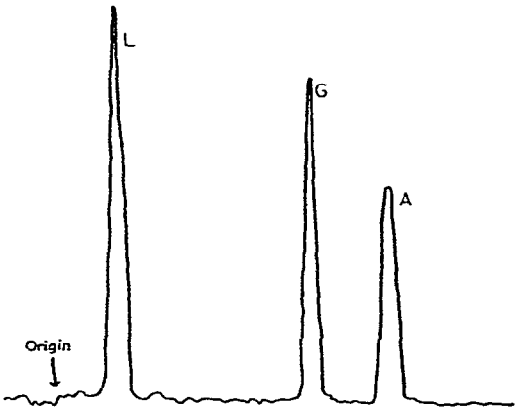

Fig. 2. CL intensity *vs.* plate position as measured by the Vidicon detection system for dansyl compounds spotted directly on a TLC plate. 0.5 μg dansyl compound per spot, 8×10^{-3} *M* TCPO, and 1.2 *M* H_2O_2. The data shown are the accumulation of 116 scans requiring 3.7 sec. The aperture of the lens system was set at 4. A = Dansyl-arginine; G = dansyl-glycine; L = dansyl-leucine.

DISCUSSION

This study demonstrates the possibility of using a Vidicon detector for measuring fluorescent compounds separated by TLC. It should be noted that chemically exciting the luminescence is ideally suited for coupling to the Vidicon. With conventional fluorescence, it would be necessary to design to design a system for evenly illuminating the plate as well as for keeping source radiation from reaching the detector.

The sensitivity advantage of the Vidicon is clearly demonstrated by the fact that only a few seconds were required to obtain the data of Fig. 2. Sensitivity could be further increased by increasing the diaphragm opening and by using a lens system with a shorter focal length so that the TLC plate could be positioned closer to the detector. This would also be advantageous in that the entire system would be more compact. The sensitivity advantage reduces the time required for measuring an entire plate. The detection limits will be essentially the same as with conventional scanning[1] because they are imposed by background luminescence.

The detector system used in this study was designed to resolve intensity *vs.* position in one dimension only. However, other Vidicon detectors have the ability to resolve in two dimensions. This creates the possibility of measuring emission from a complete plate simultaneously including both standards and samples for several compounds. With appropriate data analysis, this could be a tremendously powerful technique for analyzing TLC plates.

The Vidicon detector has inherently higher resolution than the fiber optic detection system described in the previous paper[1]. The Vidicon as set up in this study has 500 resolution elements corresponding to 5 in. along the TLC plate or 0.01 in./resolution element. For the fiber optic, the smallest possible resolution element is the optic diameter or 0.125 in. It is felt that this difference is primarily responsible for the variation in peak height for the three zones. With higher resolution the peak value will be more sensitive to zone geometry. Another source of variation may be uneven spraying with hydrogen peroxide since this had to be done in the dark. At the higher hydrogen peroxide concentrations used in this study, CL intensity changes rapidly with time (see Fig. 4, preceding paper[1]). Therefore the measured intensity will be more sensitive to variations in the amount of peroxide reaching different areas of the TLC plate.

For quantitative analysis, it would be best to measure both standards and samples for the same compound on the same detection run. If this is not done, it would be necessary to control excitation conditions, *i.e.*, H_2O_2 spraying, time between spraying and measuring, etc., to achieve quantitation.

REFERENCES

1 T. G. Curtis and W. R. Seitz, *J. Chromatgr.*, 134 (1977) 343.
2 Y. Talmi, *Anal. Chem.*, 47 (1975) 658A.
3 K. W. Bush and G. H. Morrison, *Anal. Chem.*, 45 (1973) 719A.