Simple Densitometer for Microbiology Laboratories

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A densitometer composed of a microscope and a colorimeter is described. Results obtained with the microscope densitometer and with a standard densitometer compare favorably.

Densitometer tracings of chromatographic separations are a permanent record of the chromatographic results and can be used to quantitate the results. However, in spite of its value a densitometer may be used too infrequently or sporadically in microbiological research to justify its purchase. Faced with such a situation, we developed a densitometer composed of a microscope and a small colorimeter, instruments found in virtually all microbiology laboratories.

The densitometer consists of a Leitz Ortholux microscope (E. Leitz, Inc., Rockleigh, N.J.) and a Bausch and Lomb 340 colorimeter modified with an external phototube. The phototube (Bausch and Lomb 33-29-71, Rochester, N.Y.) is held in a light-impermeable box by an eight-prong vacuum tube socket; the light-sensitive portion of the tube is positioned behind a window (2.5 cm in diameter) in the box. The box is mounted on the camera tube of the microscope so that the phototube receives the maximum amount of transmitted light. Shielded electrical wire connects the mounted phototube to an eight-prong plug inserted into the phototube socket of the colorimeter. The light from the microscope lamp passes through the condensor, objective, body tube, and camera tube to the phototube. The amount of light reaching the phototube is read from the colorimeter.

In a typical application of the microscope densitometer (MD), a destained polyacrylamide electrophoresis gel of skim milk caseins was photographed in natural light against a white background with a single-lens (55 mm f/1.8) reflex camera and Kodachrome II film. The focusing distance was approximately 50 cm. The photograph of the gel, a 2- by 2-inch (5.08- by 5.08-cm) slide transparency, was placed on the graduated mechanical microscope stage, and the microscope with a x25 objective was focused on an area of the slide corresponding to a blank portion of the gel. With the light path open to the phototube, the microscope lamp was adjusted to give a 100% transmittance (%T) spectrophotometer reading. The spectrophotometer had previously been adjusted to 0% T with the light path closed. The image of the gel sample slot was centered in the microscope field, and the %T was recorded. The slide was repeatedly advanced by 0.25 mm using the stage vernier scale, and each time the %T was recorded. So that the MD could be compared to a standard densitometer, the destained gel itself was scanned with a Photovolt 530 densitometer-recorder system (Photovolt Corp., N.Y.) using a red filter and a 1- by 15-mm slit.

The results obtained with the MD were very similar to those obtained with the standard densitometer. The major bands were readily detected by the MD, and the resolution of these bands was as good as with the Photovolt (Fig. 1). Resolution of the minor bands was poorer, but the standard densitometer appeared to be

Fig. 1. Comparison of microscope and Photovolt densitometer scans and corresponding electrophoretic gel pattern.
only slightly better for detecting these bands. The MD also was satisfactory for quantitative estimates of the total casein and of the major casein fractions (Table 1). The percentages of \( \kappa \), \( \beta \), and \( \alpha \)-casein calculated from MD tracings agreed well with those obtained with the Photovolt densitometer. Likewise, the total casein content of the diluted samples estimated by the MD method agreed well with the Photovolt results and with the expected dilutions of 66.7 and 80%.

The MD appears to be a relatively simple, convenient, and reliable method of densitometry. Since a photographic transparency of the chromatogram is scanned rather than the chromatogram itself, the MD method should be applicable to a variety of chromatographic methods, and we have used the MD method successfully with thin-layer chromatograms.

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**Table 1. Comparison of the microscope and standard densitometers for quantitative estimation of skim milk casein fractions**

| Sample                  | Microscope densitometer | Photovolt densitometer |
|-------------------------|-------------------------|------------------------|
|                         | Calculated\( a \) dilution (%) | Casein fractions\( a \) (%) | Calculated\( a \) dilution (%) | Casein fractions\( a \) (%) |
| Undiluted Skim Milk     | 0.0 | 6.0 | 45.3 | 48.9 | 0.0 | 5.0 | 45.7 | 49.4 |
| 1:3 Dilution            | 64.3 | 8.2 | 46.2 | 45.9 | 63.7 | 6.4 | 47.5 | 46.0 |
| 1:5 Dilution            | 78.9 | 6.9 | 47.3 | 46.2 | 83.0 | 1.8 | 48.9 | 49.2 |

\( a \) Based on total casein measured for each dilution.
\( b \) The amount of each fraction is expressed as a percentage of the sum of all three fractions. Total casein and casein fractions were quantitated by planimetry of densitometer tracings (optical density versus distance).