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Note

Scanning of acrylamide gels by an inexpensive accessory apparatus for a spectrophotometer

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For electrophoretic studies, it is often necessary to measure the stained protein patterns quantitatively. However, the purchase of a commercial gel densitometer is not always the best solution and multiple use of an instrument is an important economic factor in a laboratory.

It is possible to construct a cheap additional device, if a photometer with sufficient room in the sample tray area is available, using the facilities and materials to be found in most small workshops. The principle behind this construction —an automatic advance which moves the gel along the slit— has been suggested previously[1–3].

THE ACCESSORY DEVICE

The device, shown in Figs. 1 and 2, was attached to a Perkin-Elmer 402 photometer for ultraviolet and visible adsorption spectra with a double optical null system. The available sample tray room in this special case is 270 × 120 × 150 mm. The distance from the measuring beam slit to the reference beam slit is 90 mm.

The accessory is mounted on a rectangular platform (270 × 120 × 10 mm) made of Perspex (a) and can be removed easily. On the vertically arranged plate of sheet brass (b), which was painted matt black at the front and back, two parallel-sided cell-guide rails of Perspex (c) were fixed. The reference cell (d) was short (20 × 15 × 4 mm) and stationary. The longer (85 mm) measuring cell (e) is moved along the slit (f) by an advance pin (g), which is fixed on a nut (h) and is driven by a threaded spindle, M 6 × 15 (i) equipped with a cog-wheel (j). Two pieces of brass tubing (k) constitute the bearings of the spindle. The movement is actuated by a 6 V Uniperm Super electromotor (l), adapted with a variable gear box (m) and a pinion (n).

The transmission ratio permits a cell speed of 0.25 cm/min, which, at a constant chart drive rate, doubled the abscissa. At the end of a run, a small 1.5-V servomotor (o) quickly returns the pin (g) to its starting position after the advance motor has been uncoupled from the cog-wheel by pulling it back.

The electrical equipment in this device consists of a transformer (p), a rectifier (q) to supply a low voltage to the advance motor, a battery (r) for the servomotor, and the connections to the power supply (s). The advance motor can be set in

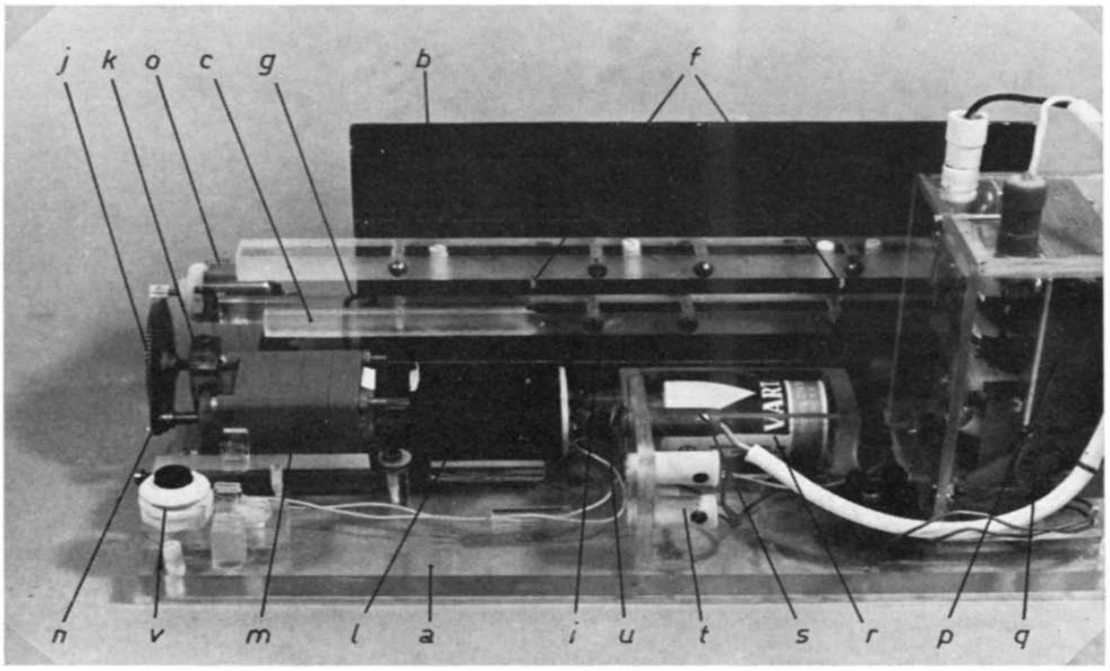

Fig. 1. Accessory device for a Perkin-Elmer 402 spectrophotometer for scanning acrylamide gels. The components are identified in the text.

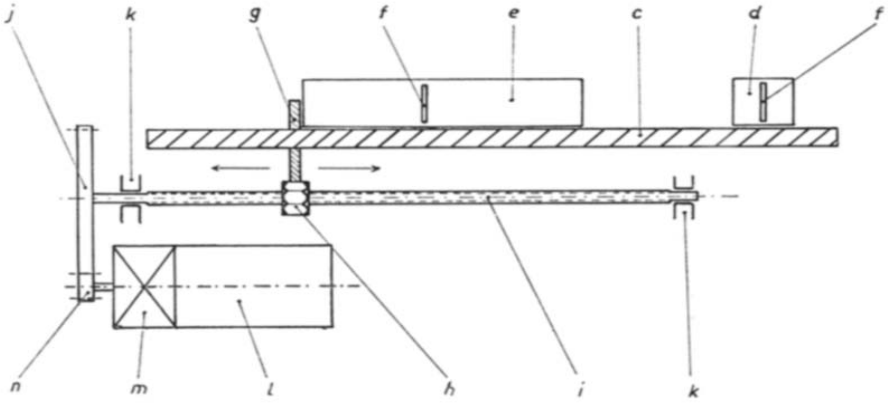

Fig. 2. Schematic diagram of the automatic advance. The components are identified in the text.

motion via circuit breaker (t) by the already closed sample room lid. A microswitch (u) operated by the nut (h) stops the movement automatically at the end of the scanning. A second press-button (v) starts the servomotor.

It was advantageous to mount an additional slit device (not shown in the photograph) independent of the photometer. This device consisted of razor blades, at the front of the vertical plate (b), which could be moved towards each other. The slits can be adjusted with the aid of a sheet down to 30 μm. The compromise between

slit-width and sufficient energy conditions for the detector —indicated by the control ampere meter— provided the best resolution. However, exact synchronization of the slit position with the optical path must be observed. This can be achieved by placing a small mirror behind the front plate in the dark.

EXPERIMENTAL, RESULTS AND DISCUSSION

A reference gel eliminates optical faults in cells and gels and compensates for different gel background stainings. The reference should be a gel which has been treated in the same way as the sample. It is advantageous to use the destained piece of the end of a sample. Cylindrical gels are put into tubes while in water so as to prevent air bubbles from forming. Strips are held in a parallel-sided glass container. In such an arrangement, the gels can be moved back and forth. In order to scan the maximum length of the gels, it is essential to position the tubes or slides so that one end just reaches the lid of the cell compartment when the advance pin is at the appropriate extreme of its travel.

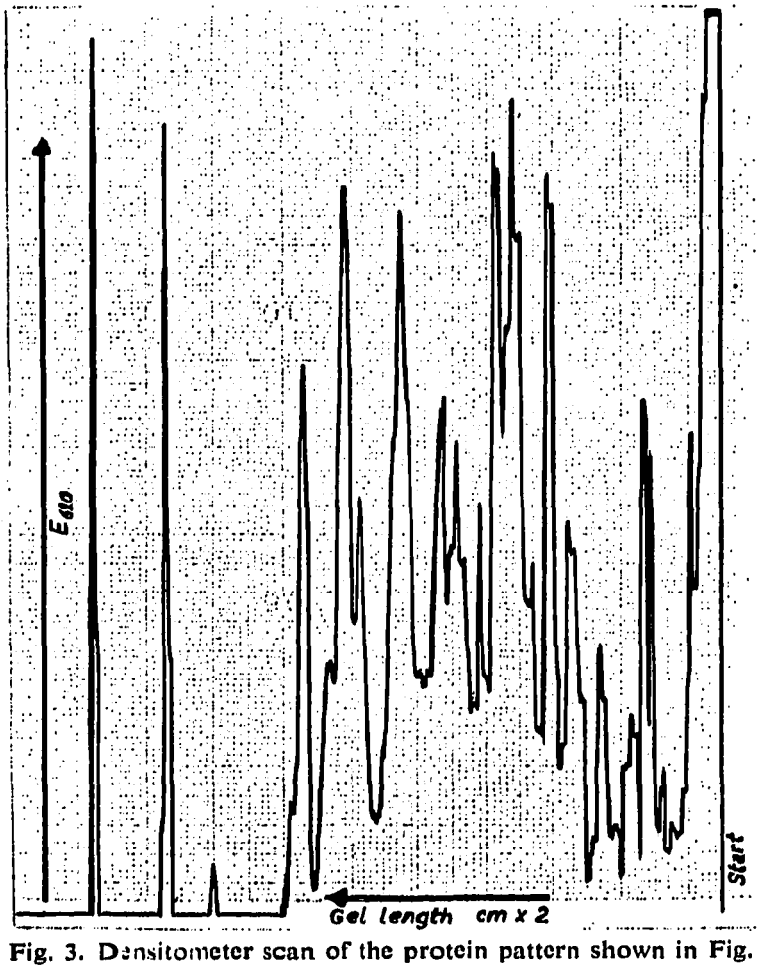

Fig. 3. Densitometer scan of the protein pattern shown in Fig. 4.

An example of the scanning ability, of a complex protein pattern of a bacterial cell extract, is shown in Fig. 3 in comparison with a photograph (Fig. 4). It shows the superior resolution even of those bands located in narrow ranges.

A device of this type will fit only if dimensions of about $200 \times 80 \times 80$ mm in the sample tray area are available (in this case, the transformer will be placed outside) and the distance between the measure and the reference beam is large enough to cover a sufficient length of the gel.

The use of silica instead of glass slides should permit the scanning of unstained gels at 280 nm.

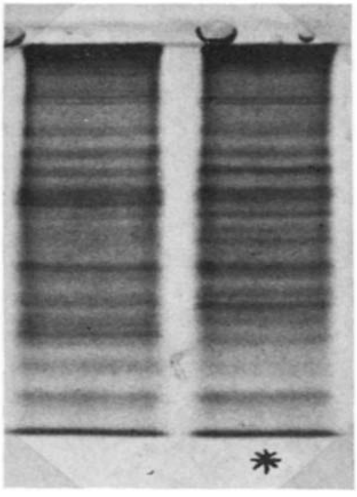

Fig. 4. Bacterial protein pattern.

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