A SIMPLE DENSITOMETER FOR PAPER ELECTROPHORESIS

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Many different methods have now been suggested for the quantitative analysis of electrophoregrams. The method of elution of the dye from the paper strip, followed by photometry by means of a spectrophotometer or photoelectric colorimeter [5] is laborious and unsuitable for work with a large quantity of material. A far more convenient method is direct photometry of the stained paper strip. Unfortunately most of the densitometers suggested for this purpose are unsatisfactory for some reason or other. Some [7, 8, 9] are complicated in their construction and cannot be made in every laboratory. In the descriptions of apparatuses for measuring the light flux during illumination of the photoelectric cell with light reflected from the surface of the electrophoregram [2, 3], no information is given to indicate the character of the relationship between the coefficient of reflection and the quantity of fixed dye. Other workers [6, 8] suggest constructing densitometers for measuring or recording the light flux when using transmitted light, although no account is taken of the need to convert the results into logarithms. The PDSh-1 densitometer developed in the A. N Bakh Institute of Biochemistry* suffers from the same disadvantage. Kreimer and co-workers [4] emphasize the necessity of taking logarithms of the magnitude of the light flux, but do not give the required formula. The logarithmic scale which they provide does not correspond to the known relationship between the coefficient of light transmission, which is proportional to the photoflux, and the optical density, which is proportional to the quantity of fixed dye.

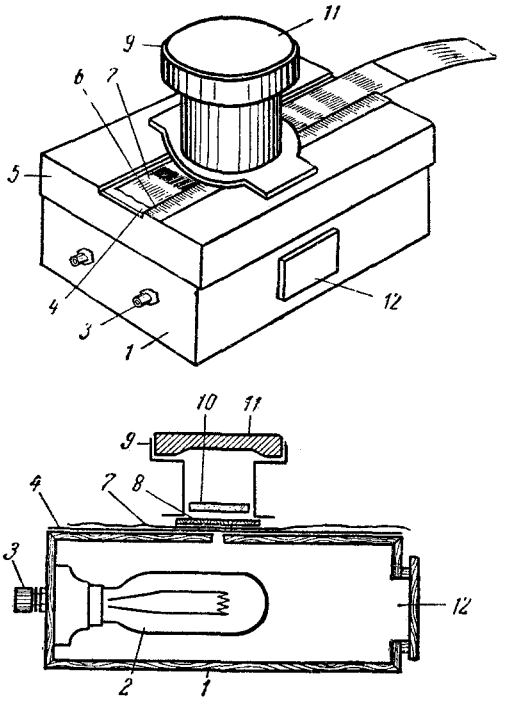

Fig. 1. Scheme of the light box of the apparatus.

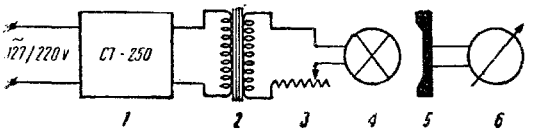

Fig. 2. Electrical circuit of the apparatus.

Some workers [1] have suggested a method of photometry of positive slides made from photographs of electrophoregrams, but this cannot be regarded as suitable. The fact is that the maximal and minimal degrees of blackening do not lie on the proportional part of the characteristic curve of the photographic material used, but in the flatter or steeper sections. This is more likely because the difference in the optical density of the stained parts (or fractions) with a high protein concentration and of the unstained zone is very great. In order to overcome this difficulty, a curve of blackening must be plotted for the particular emulsion used, but this is very complicated to do, and is impracticable in most laboratories on account of the lack of the special sensitometric apparatus [3]. Moreover, the preparation of positive slides in such cases is an additional laborious operation.

*Catalogue of the Exhibition of Economic Progress in the USSR, 1961.

We suggest a simple densitometer for analysis of electrophoregrams in transmitted light; the apparatus may be made in any laboratory. The parts from which it is built are not irreplaceable and similar components can be substituted without lowering the quality of the results.

The apparatus is shown schematically in Fig. 1. A wooden, metal, or plastic box 1 contains an illuminating lamp 2, the terminals 3 of which are located on the outside. Ventilation holes 12 are cut in the side walls of the box and covered with shields. In the top of the box is an aperture measuring 2 × 20 mm. If the box is made of wood, it is covered with a sheet of thin celluloid 4 (a piece of x-ray film from which the emulsion has been removed) to en-

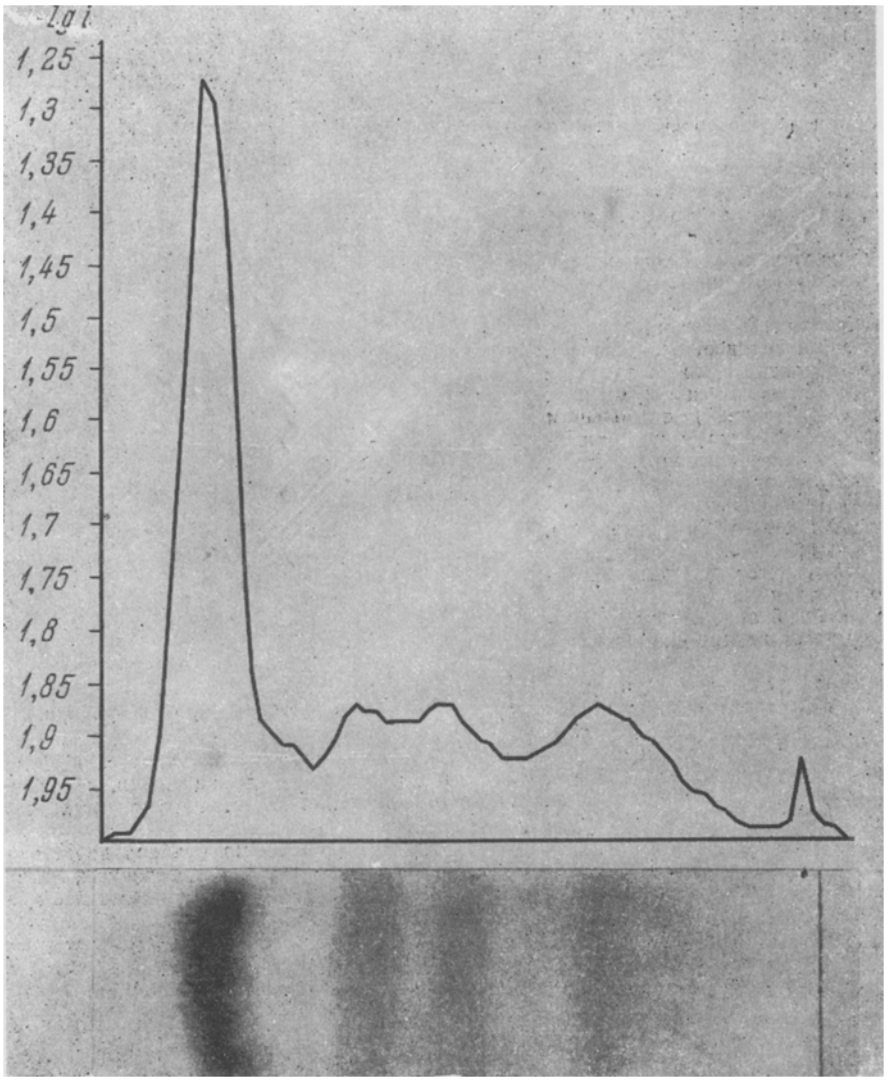

Fig. 3. Specimen densitogram.

sure sufficiently smooth movement of the oiled paper electrophoregram. Movement of the electrophoregram is controlled by the edges of a slit cut out of a cardboard lid 5, pressing down on the celluloid. A millimeter scale 6 is glued along the edge of the slit and is used for displacing the electrophoregram 7 for an equal distance (2 mm or any other desired distance) in relation to the aperture. The electrophoregram is pressed against the aperture (or the celluloid sheet 4) by a ground glass slide 8, glued to the cardboard hood 9 of the photoelectric cell 11. Inside the hood is a light filter 10; for protein fractions stained with bromphenol blue we obtained maximal color contrast (photoflux difference) by using an orange OS-12 photographic filter. To ensure complete illumination of the light-sensitive layer of the photoelectric cell 11, the hood 9 was about 25 mm high. This height was easily determined by placing a frosted glass over the aperture and observing the size of the surface illuminated by light passing through the oiled filter paper.

The electrical circuit of the apparatus also is very simple (Fig. 2). The voltage of the illuminating circuit is stabilized by means of a type ST-250 stabilizer 1, and then reduced by a transformer 2 (for example, a T-3 transformer from a type FM photometer). A variable rheostat 3 (15-20 Ω, 2-3 A) is used to regulate the brightness of the light source 4 (a 30 watt lamp 12 from type MF-2 microphotometer, or similar) when the arrow of the measuring apparatus is set before carrying out photometry. The current produced in the circuit of the photoelectric cell 5 is measured by the apparatus 6. We used a selenium cell with a sensitivity of 410 μA/lu and a multiscale microammeter M-95. Maximal sensitivity of the circuit in terms of photoflux difference was ensured by an additional shunt included in the microammeter; under these circumstances the value of one scale division was 5×10^{-5} A. It should be noted that during short-term operation of the selenium cell it works on the linear part of its light characteristic curve [7], thus ensuring that the photoflux is proportional to the coefficient of light transmission.

The stained and dried electrophoregram is soaked in vaseline oil. For this purpose the rolled up strips are immersed in a jar of oil and placed in a vacuum exsiccator. Air is withdrawn from the exsiccator by an initial vacuum pump (or other suitable means) until foam reaches the top of the jar. Air is then readmitted into the system to break up the foam, and the aspiration is repeated. The strip is removed from the oil and dried with filter paper, and then placed in the slit of the lid 5 (see Fig. 1) of the apparatus so that the unstained part of the electrophoregram lies opposite the aperture. The illuminating lamp and rheostat 3 (see Fig. 2) are switched on, and the arrow of the apparatus is set at the division corresponding to maximal current (100 on the M-95 microammeter), thereby taking the coefficient of light transmission of the unstained zone as 100%. If a small pencil mark is made on the strip, it can be moved through the width of the aperture (2 mm or other distance) and the reading of the apparatus obtained. After the complete electrophoregram has been subjected to photometry, the results are analyzed.

Since in this scheme the photoelectric cell is working on short-term operation, the value of the photoelectric current i will be directly proportional to the coefficient of light transmission.

$$i = k\tau; \tag{1}$$

The optical density D bears a logarithmic relationship to the coefficient of light transmission:

$$D = \log 1/\tau; \tag{2}$$

Transforming Eq. (2), we obtain $D = -\log \tau$.

Since $\log \tau = \log i - \log k$ [from Eq. (1)], then:

$$D = \log k - \log i. \tag{3}$$

This equation is also used for plotting the curve of optical density, which is directly proportional to the quantity of fixed dye. In practice, this amounts to plotting a curve for which the length of the electrophoregram is plotted along the axis of abscissas, and the values obtained by taking the logarithm of the photoelectric current, because the value of $\log k$ is constant. Next, the ratio (in percent) between the areas of the parts of the curve (peaks) corresponding to the individual fractions of the protein mixture is determined. These areas are proportional to the quantity of dye combined with the proteins and, with a slight approximation, to the quantity of protein in the fractions. The areas may be determined by planimetry or by weighing the cut out peaks on torsion scales, and the result is expressed as a percentage of the weight of the paper (the weight of the whole curve is taken as 100%).

A specimen curve is given in Fig. 3. Electrophoresis of the blood serum of a healthy human subject was carried out for 22 h in a veronal buffer at pH 8.6 and ionic strength 0.09, at 100 V and 7 mA, in a vessel built in accordance with the scheme of Flynn and de Mayo [9].

SUMMARY

A description is presented on a nonrecording densitometer for photometry of electrophoregrams in passing light. The instrument may be easily made in any biochemical laboratory with the aid of available parts; the parts may be replaced by analogous ones without impairing the quality of the device. A method is presented for tracing the curve of the optic density and for calculation of the ratio of the fractions of the mixture studied.

LITERATURE CITED

1. V. M. Vadimov, Labor. delo, 5, 54 (1957).
2. V. Z. Gorkin and V. F. Levitin, Byull. éksper. biol., 7, 76 (1955).

3. B. R. Kirichinskii and B. A. Roitrub, Labor. delo, 5, 51 (1957).
4. A. Ya. Kreimer, O. M. Preger, and I. G. Burnashov, Labor delo, 7, 58 (1961).
5. N. M. Rudoi, Labor delo, 6, 9 (1955).
6. P. S. Khomulo and E. A. Levin, Fiziol. zh. SSSR, 8, 1024 (1960).
7. L. P. Shuvatov, Labor. delo, 6, 39 (1957).
8. F. Flynn and P. de Mayo, Lancet, 1951, v. 2, p. 235.
9. M. Lederer, Introduction to Paper Electrophoresis and Related Methods [Russian translation](Moscow, 1956).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
