METHOD OF ELICITING ON ELECTROPHOREGRAMS AND CHROMATOGRAMS SPOTS OF SUBSTANCES ABSORBING ULTRAVIOLET RAYS IN THE 254-260 mμ ZONE (PHOTOGRAPHY IN ULTRAVIOLET LIGHT)

(UDC 543.544/.545 : 77.038)

Yu. N. Kasatkin and N. A. Fedorov

Department of Radiation Hygiene (Head-Active Member AMN SSSR Prof. F. G. Krotkov), Central Advanced Training Institute for Doctors, Moscow
(Presented by Active Member AMN SSSR V. V. Parin)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 59, No. 6, pp. 121-124, June, 1965
Original article submitted October 13, 1963

To elicit substances contained on chromatograms and electrophoregrams a physical method is used which is based on the selective absorption of ultraviolet rays with a specific wavelength by the investigated components.

The light filter and radiation source are chosen so that the maximum of radiation intensity is at the 250-260 mμ region of the spectrum. Limitation of the region of the radiation spectrum of the illuminator fosters a more distinct detection of the spots on the paper, and for individual substances the sensitivity of the method can reach fractions of a microgram.

Examining the strip obtained after electrophoretic or chromatographic separation of substances, we can note dark areas of more or less appreciable absorption of ultraviolet rays. The absorption spots can be elicited both on moist and on dried strips; no preliminary treatment of the strip is needed.

An investigation of strips in ultraviolet light is preferred over other methods (e.g., chemical) because the chemical structure of the substances does not change with a sufficiently quick inspection of the strip. The spots of UV-ray absorption can be easily removed from the strips and investigated further (chromatography, recording of absorption spectra, etc.).

Devices are described in the literature for obtaining ultraviolet radiation in a narrow zone with a sufficiently high intensity (254-260 mμ) [1, 3-8], which permit comparison of the travel of substances on chromatographic paper. But it frequently becomes necessary to obtain a more complete picture of the distribution of fractions on paper with all the characteristics of the movement of the substances, their mutual arrangement, etc., and also to get an idea about the density of the spots, etc. The means for expressing the schematic image of the results of chromatography or electrophoresis on paper are quite limited.

We worked out a method of photographing electrophoregrams and chromatograms in the passage of ultraviolet light. A quantitative study of substances elicited on prepared strips is possible on the basis of this method. It permits us to accurately determine on a paper strip the position of fractions of interest to the researcher based on curves recorded by means of a MF-4 microphotometer.

For photographing the electrophoregrams and chromatograms in transmitted ultraviolet light we propose a device (Fig. 1) which is mounted on a narrow stand (40 × 160 cm). On opposite sides of the stand are placed the camera (Fig. 1, 1) and illuminator (Fig. 1, 5). A wooden frame holder (Fig. 1, 3) with interchangeable diaphragms (Fig. 1, 4) is rigidly fastened to the stand along with the camera.

As an illuminator we used a UI-1 ultrachemoscope powered from a USN-350 ferroresonant stabilizer. During photographing it is necessary to rigorously maintain the heating conditions of the bulbs at the prescribed level. Changes of current strength supplying the illuminator lead to a change in the magnitude of the light flux from the

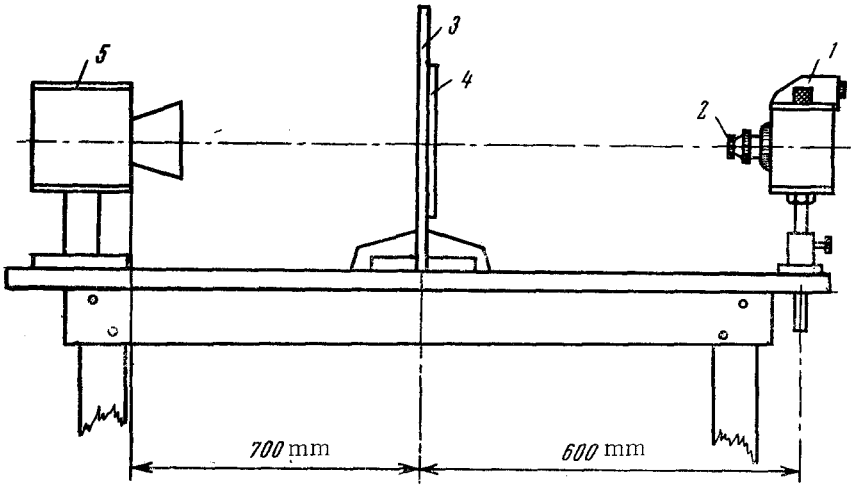

Fig. 1. Device for photographing electrophoregrams and chromatograms in transmitted ultraviolet light. Explanation in text.

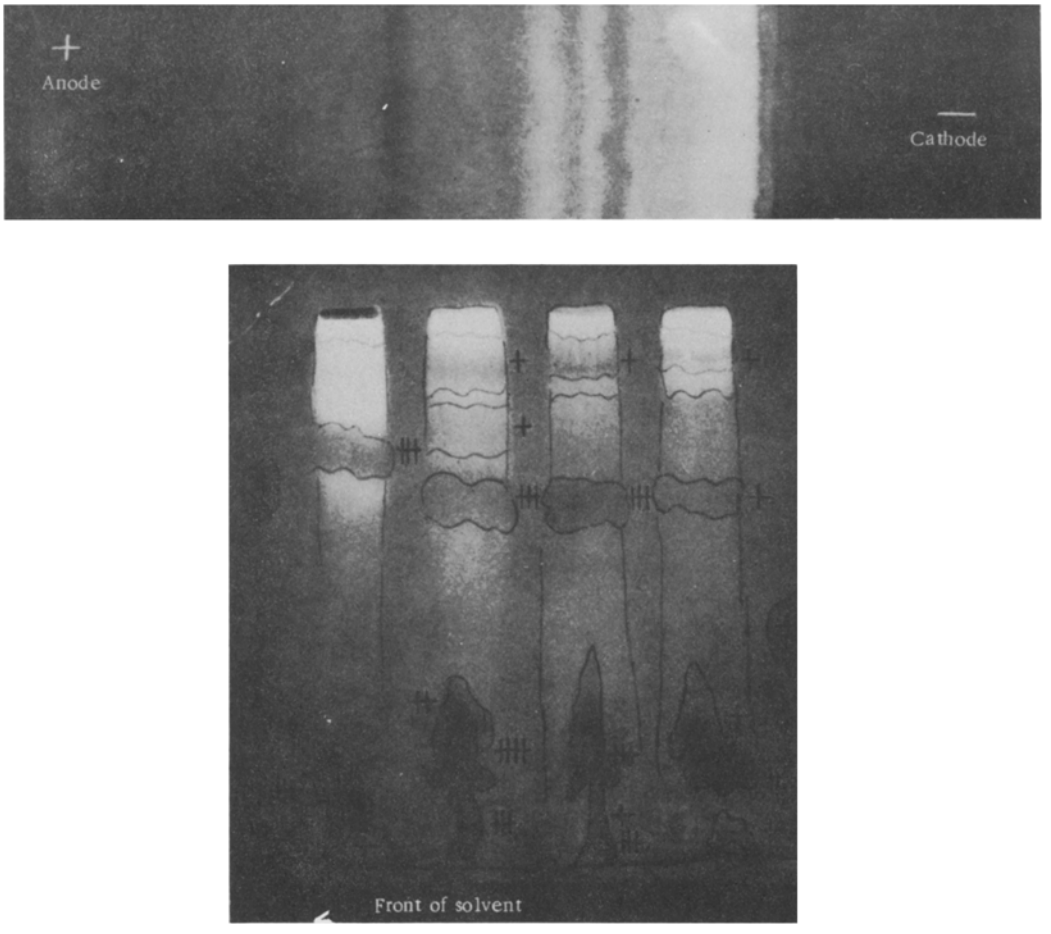

Fig. 2. Photograph of electrophoregram and chromatogram of eluate of rat urine.

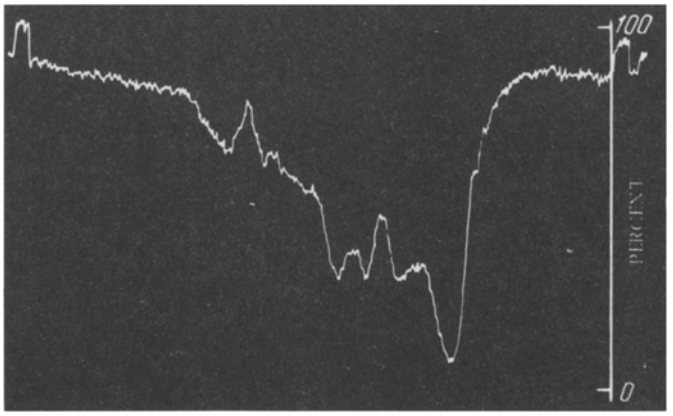

Fig. 3. Recording of the transmission coefficients from the negative of an electrophoregram.

bulbs, which makes it completely impossible to compare the values of the transmission coefficients obtained on individual negatives. If there is no ferroresonant stabilizer in the laboratory, the illuminator should be supplied from a LATP laboratory autotransformer with proper control of the voltage and current strength. In our laboratory we use BUV-30-p bactericidal lamps in the illuminator.

During photographing the electrophoregrams and chromatograms are fastened to the interchangeable diaphragms with the appropriate aperture, and then the diaphragm is placed in the frame holder.

In our laboratory small, not more than 2 mm wide, reflex cameras ("Practiflex," "Exacta," "Zenith") with adapter rings for objectives and a ZhS-18 photographic light filter are used for ultraviolet photography.

When selecting the light filter it is necessary to pay attention to the quality of its glass—it should not fluorese in the rays of the ultrachemoscope. If the needed light filter is not available in the laboratory, we recommend that such a filter be made: a sheet of double-sided x-ray film on a colorless base is treated in a freshly prepared solution of hyposulfite until completely clear. The film must be carefully washed in tap water and then rinsed several times in distilled water, after which the film is placed for 15-20 min in a saturated solution of picric acid and then washed several times in distilled water and dried at room temperature. From the colored film cut out 2 disks equal to the diameter of the mounting camera lens and accurately attach it by adhesive tape to a cardboard cylinder previously prepared to fit the lens mounting. After these manipulations the light filter is ready for use.

Before beginning to photograph serial paper strips it is necessary to select the exposure for a given assortment of photographic material. The exposure of the film is always determined by constructing the characteristic curve [5] for a given assortment of material, illumination, and conditions of treatment.

Figure 2 shows the electrophoregram and chromatogram of the eluate of rat urine.

Figure 3 shows the recording curve of the transmission coefficients obtained on the MF-4 microphotometer from the negatives of the electrophoregram shown in Fig. 2, a.

In this case, for photographing the electrophoregrams and chromatograms we used RF-3 film which was treated according to the instructions for this photographic material. Photographing was done with a 13-min exposure under illumination by three BUV-30-p lamps with UFS-1 light filters at a voltage of 120 V and current strength of 0.8 A, ZhS-18 light filter, 2-mm adapter ring, and f/5.6.

The negatives proved to be completely suitable for making quantitative measurements on the MF-4 microphotometer. To record the transmission coefficients it is necessary to use optics with the least magnification. The slit width of the microphotmeter should be not more than $^1/_5$ of the width of the area of the negative being photometered (in practice the narrowest band obtained on the negative is selected on the screen of the microphotometer and the $^1/_5$ width of the photometer slit is established relative to it).

Recording of the transmission coefficients is done opposite the fog of the treated film, for which purpose metal plates 6 mm wide are attached along the edges of the electrophoregram or at the upper and lower edges of the chromatogram during photographing (see Fig. 3).

The graininess can play an important role in photometry, and its effect can be reduced to a minimum if photographing is done on large negative materials by means of appropriate cameras. The obtained negatives can be deciphered by using densitometers, which are widely applied in electrophoresis and personnel dosimetric monitoring. Although this method is more accurate it has a number of faults which in total yield an error within the limits of that obtained when working on small material.

It is necessary to point out another important fact: large positives obtained in projection printing from micro-sized negatives are completely unsuitable for a quantitative assay by the densitometric method, since in the photo-printing process there is an appreciable repeated correction of the light contrasts on the negative. If for photometry the use of large objects is desirable, then cameras measuring 6 × 9 cm to 18 × 24 cm can be used for these purposes. Photographing paper strips in transmitted UV light does not differ from the method described in this article. In these cases high-quality emulsions on a solid base should be used.

LITERATURE CITED

1. E. M. Brumberg, Dokl. AN SSSR, 72, 5 (1950), p. 885.
2. Yu. N. Gorokhovskii, Spectral Investigations of the Photographic Process [in Russian], Moscow (1960).
3. N. A. Fedorov and Yu. N. Kasatkin, Vopr. med. khim., 2 (1961), p. 211.
4. J. E. Edström, Nature, 168 (1951), p. 876.
5. R. Markham and J. L. Smith, Biochem. J., 45 (1949), p. 294.
6. Idem, Nature, 163 (1949), p. 250.
7. E. Sempinska, Postepy Biochem., 2, (1956), p. 131. X.
8. T. Wiéland and L. Bauer, Angew. Chem., (1951), Bd. 63, S. 511.

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.**
