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SPECTRAL FLUORESCENCE EFFICIENCIES OF CERTAIN SUBSTANCES WITH APPLICATIONS TO HETEROCHROMATIC PHOTOGRAPHIC PHOTOMETRY*

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Abstract

In extending the method of fluorescence photographic photometry recently described by the writers from homochromatic to heterochromatic usefulness, experiments have been carried out on the efficiencies of fluorescence of various substances in the visible and ultraviolet regions. A number of substances fluorescing at wavelengths between the red and the ultraviolet have been tested, and many of these have been found to exhibit the same variation of fluorescence efficiency with wave-length, when in a pure state and in solutions of proper concentration. The intensity of the fluorescence light emitted by them when illuminated with light of constant intensity at different wave-lengths was measured and found to be such that the number of quanta emitted was practically proportional to the number of incident quata over a wide range of wave-lengths. This is in contradiction to certain results previously announced by other workers who found the emitted energy proportional to the incident energy, but is confirmed by other results and by well-known photochemical laws. Small traces of impurities in certain of the fluorescing substances, particularly aesculin, produce large variations in the efficiency of response, which appears to explain the conflicting results previously obtained. The general relation found is applied to the development of a simplified method of heterochromatic photographic photometry useful in the range 3900–2000A, which it is believed can be extended to the Schumann region. The end desired is attained by photographing the fluorescence light emitted when the spectrum is allowed to fall on a plane surface of the substance, or by use of a dry plate with fluorescent coating, which shows not only constant contrast but almost uniform quantum sensitivity over a large spectral region. Small corrections need to be applied for variation in reflecting power of the coated emulsion, but these are all of the type which can be measured and controlled by homochromatic photometry.

I. Previous Data on Fluorescence Efficiencies

TWO of the most arduous requirements of photographic photometry are the calibration and standardization of the photographic plates used, i.e., the determination of the density—log-intensity curves of the emulsion for each wave-length desired, and the measurement of its variation in sensitivity from one spectral region to another. If a dry plate could be developed which showed uniform contrast at all wave-lengths, and which obeyed some general law of sensitivity variation both problems would be greatly simplified. The experiments described here were undertaken with a view to developing such a plate by making use of certain properties of fluorescent substances.

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* Much of the material discussed in this paper was presented at the Cleveland meeting of the American Physical Society, December 1930.

We have recently shown[1] that ordinary dry plates coated with certain fluorescent oils show constant contrast at all wave-lengths lying in the region of continuous absorption of the oil used, which in certain cases extended from 3300A to at least 900A. We have since[2] eliminated the variation in surface sensitivity occasioned by the flowing of the oils, together with the necessity of removing the oil from the surface of the emulsion prior to development, by using a fluorescent layer consisting of a coating of aesculin dissolved in gelatine which was allowed to dry on the original emulsion. In this way constant contrast was obtained at all wave-lengths below 3900A at least as far down as 2300A, and probably as far down as the plates are sensitive, the lower wave-length limit being presumably set by the absorption of the gelatine.

Anderson and Bird[3] found that they obtained exact agreement in the region 3350A to 2537A between spectral energy measurements made by means of a thermopile and by means of an optical fluorescence photometer employing aesculin, fluorescein, or uranium glass. Their results led us to expect that fluorescence methods of the type we had been studying would show uniform sensitivity at all wave-lengths for which they are suitable. In making the tests described below on this point, however, we found that curves which were expected to be horizontal straight lines were in fact hyperbolic, and such as to indicate that a uniform quantum efficiency rather than a uniform energy efficiency existed, a result much more in accord with the present picture of the quantum mechanism of fluorescence. From the known laws of photochemistry it appears more reasonable to suppose that the same number of fluorescence quanta will be emitted per 100 incident quanta no matter what the size (within limits) of the latter than that this fraction will vary in just the proper way to keep the amount of emitted energy proportional to the amount of incident energy. We accordingly investigated the literature further, and found that Nichols and Merritt[4] had noted a decrease in the energy efficiency of fluorescence of eosin and resorcufin at shorter wave-lengths, and that this decrease appeared about of the magnitude that one would expect to find if a uniform quantum efficiency existed. Wawilow[5] found that fluorescein showed a definite proportionality of the energy efficiency to the wave-length in the region 4047–2537A, indicating a uniform quantum efficiency, while aesculin solutions gave a fluorescence efficiency independent of wave-length in the same region, the former result being in contradiction and the latter in agreement with the results of Anderson and Bird. As the difference in the two results involves a discrepancy of about 40 percent some unknown variable factor appears to be operating. We accordingly determined to make a careful study of the efficiency of fluorescence of various substances in the desired range.

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[1] Harrison and Leighton, J.O.S.A. **20,** 313 (1930).

[2] Harrison and Leighton, Phys. Rev. **34,** 779 (1930).

[3] Anderson and Bird, Phys. Rev. **32,** 293 (1928).

[4] Nichols and Merritt, Phys. Rev. **31,** 381 (1910).

[5] Wawilow, Phil. Mag. **43,** 307 (1922); Zeits. f. Physik **22,** 266 (1924); **42,** 311 (1927).

II. APPARATUS FOR MEASURING RELATIVE FLUORESCENCE EFFICIENCIES AT DIFFERENT WAVE-LENGTHS

A special monochromator was constructed, having quartz-fluorite achromatic lenses and a Cornu prism. The achromatic feature is very desirable so that a constant aperature may be kept for all wave-lengths, and if a sufficiently wide slit is used the spectral images are all of the same size. When this is the case a single thermopile reading gives the true intensity of a line; with the monochromators ordinarily used this is not true, and all spectral intensities should be integrated or special corrections applied. We have averaged a large number of runs through the mercury spectrum obtained with our instrument both by integrating and by merely reading maxima, and find no systematic deviations between the two averages. The prism was kept fixed, the thermopile being moved across the spectrum so that the line intensities were measured under exactly the same conditions as were used when they were all allowed to fall simultaneously on different portions of the fluorescent

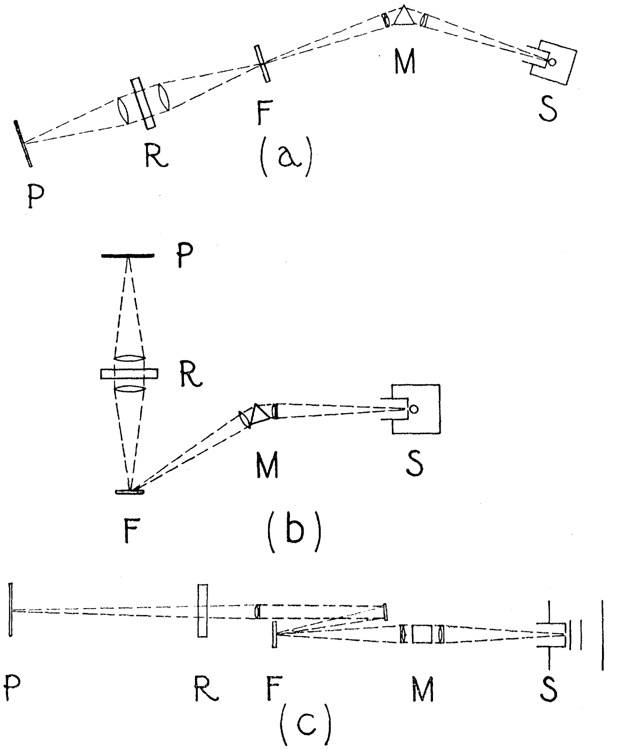

Fig. 1. (a) Quartz monochromator with achromatic lenses arranged to project a spectrum of constant magnification on a fluorescent surface, the resulting "homochromatic spectrum" being then photographed with a camera through the fluorescent material. A thermocouple or pile could be substituted in place of the fluorescent material, so that the energy distribution in the spectrum could be determined. (b) and (c) Alternative arrangements which allow the fluorescence light to be photographed from the front surface of the fluorescing material.

material. This arrangement of apparatus is shown in Fig. 1 (a). *S* is a quartz mercury arc run from a storage battery under constant conditions at 73 volts and 3.2 amps. Light from this passes through a slit 0.5×3 mm, and the mercury spectrum is spread out by the monochromator *M* in the plane *F*, where a quartz cell with plane windows containing the fluorescent material being studied is placed. From the far side of the cell one sees the spectrum lines, but all below a certain wave-length appear of the same color, the fluorescent light in most of the substances studied comprising a comparatively narrow band of wave-lengths in some portion of the visible spectrum. An image of this "homochromatic spectrum" is now thrown on the photographic plate *P* by means of two quartz lenses whose distance apart can be varied to provide accurate focusing for light from materials which fluoresce with different colors, care being taken that none of the visible light not absorbed by the fluorescent material reaches the plate. *R* represents a filter whose transmission to the fluorescent wave-lengths is known; by photographing the homochromatic spectrum through filters of varying known transmissions data were obtained to calibrate the plate for the fluorescence wave-lengths. The filters were approximately neutral to that part of the spectrum covered by the fluorescent light, and were calibrated in terms of neutral screens in a separate apparatus by means of the fluorescence light itself, for each fluorescent substance.

In order to detect any errors that might be introduced through allowing the fluorescence light to pass through the fluorescent material, the arrangement shown in Fig. 1, (b) was also used. Here a chromatically uncorrected quartz lens is substituted for the camera lens of the monochromator so that the focal plane will not be normal to the optic axis, and the fluorescence light is taken off from the front face of the cell. With this arrangement the spectrum lines are unequally magnified, and a correction for this must be applied.

Still further to insure that unknown errors were not present, the third arrangement shown in Fig. 1, (c) was used to obtain supplementary data. The achromatic camera lens of the monochromator was replaced, and a mirror was introduced into the optical system so that the fluorescence light could still be taken from the front face of *F* while the uniform magnification of spectral lines was kept.

Eastman 36 plates were used for substances giving a blue or violet fluorescence, while Wratten panchromatic plates were found satisfactory when green and red fluorescing substances were being studied. Exposures of from 3 to 10 seconds sufficed for the first, while with the second 20 seconds or more were required.

In order to measure accurately the energy distribution in the spectrum in the focal plane at *F* a number of different thermoelectric devices were used, these being substituted for *C* when desired. One of the most probable causes of error in such work arises from the lack of true blackness of the absorber on the radiometer used. For example, the difference between results indicating an equal energy yield and an equal quantum yield could be entirely caused by a radiometer which had the absorption properties of copper rather than those of a true black body, and many commercial thermocouples are unsatisfactorily blackened. We accordingly obtained our results in terms of two Moll vacuum thermoelements in quartz, a similar instrument with a fluorite window, and three different Bi-Ag thirteen element thermopiles of our own construction. These all showed substantial agreement.

After a plate was obtained by taking a number of exposures to the homochromatic spectrum through various filters R it was measured on a projection lantern microphotometer and the data were plotted for the various spectrum lines as in Fig. 2, the log intensity values being known from the relative transmission factors of the filters. The curves for the various lines were found to be accurately parallel so long as the fluorescence light did not vary in quality with the color of the incident light, and so long as the number of quanta produced at a given wave-length remained proportional to the number of incident quanta. With the substances tested in the concentrations used these conditions were almost always fulfilled, and any departures from them could be readily detected.

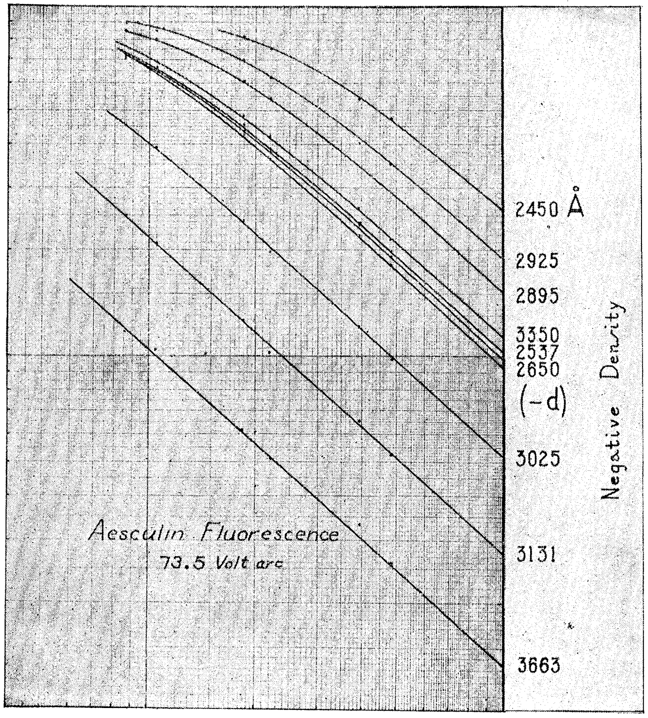

Fig. 2. Calibration curves for the photographic plate when exposed to the fluorescence light from aesculin produced by various incident wave-lengths.

By measuring the horizontal distances apart from the curves for the various lines, one obtains the relative intensities of fluorescence I_f of the lines. The ratio of this quantity to the actual intensity I_t of the incident light, as measured by the thermoelement, then gives a measure of the fluorescence efficiency for each wave-length.

III. Results

In Fig. 3 we show the types of curve obtained for various fluorescent substances when I_f/I_t was plotted against wave-length. Pure aesculin dissolved in water, a standard Wratten filter of aesculin in gelatin, a light machine oil (Cenco pump oil #11021), several standard automobile lubricating oils, chlorophyll dissolved in alcohol, and solid anthracene all showed similar variations in fluorescence efficiency between 3300 and 2400A, though they did not follow the relation found by Anderson and Bird. The strength of many of the solutions was found to have a great effect on the variation of fluorescence efficiency as is of course to be expected; the conditions depicted hold only when all of the incident light is absorbed in a very thin layer of fluorescing material. In chlorophyll this is particularly marked; in solutions of such dilution that a banded structure can be observed in the absorption spectrum, no regularity of response can be expected, of course. Various substances, such as a solution of quinine sulphate in water and calcium tungstate in powdered form, obey entirely different laws in the region tested.

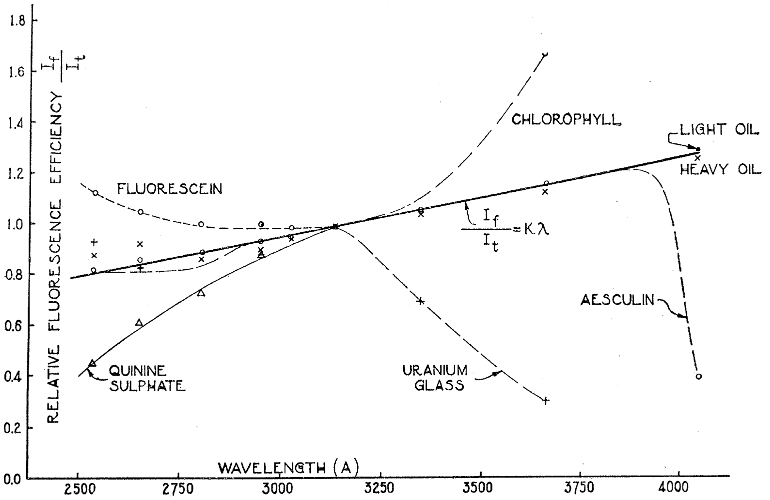

Fig. 3. Curves showing the variation of fluorescence efficiency with wave-length for various samples, the relative efficiency I_f/I_t being set at 1.0 for λ3131.

Our results as depicted in Fig. 3 show then, that the conclusion of Anderson and Bird, that in general, fluorescent substances will be found to have a constant fluorescence efficiency at wave-lengths shorter than a certain limit cannot hold. In order to obtain a still further check on the results we set up an optical method of measurement somewhat similar to that used by Anderson and Bird; the arrangement is shown in Fig. 4. Light from the monochromator *M* passing through the micrometer slit *S* is made parallel by the lens *L*, and after passing through the diaphragm *D* which maintains a constant aperture for all wave-lengths falls on the quartz cell *C* containing the fluorescent substance. The fluorescent light is taken off at an angle different from that of the reflected light and passes into the Lummer-Brodhun photometer *P* where it is compared visually in intensity with light from an incandescent lamp shining through a filter and a solution of the fluorescent substance being studied, which gives a constant comparison source of the proper color.

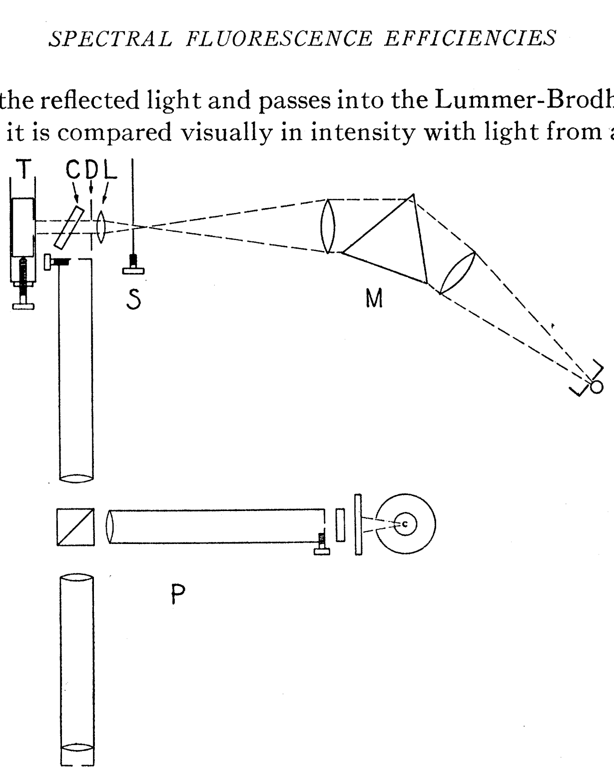

Fig. 4. Lummer-Brodhun photometer arranged to measure fluorescence efficiencies, giving an optical check on the results obtained photographically with the apparatus depicted in Fig. 1.

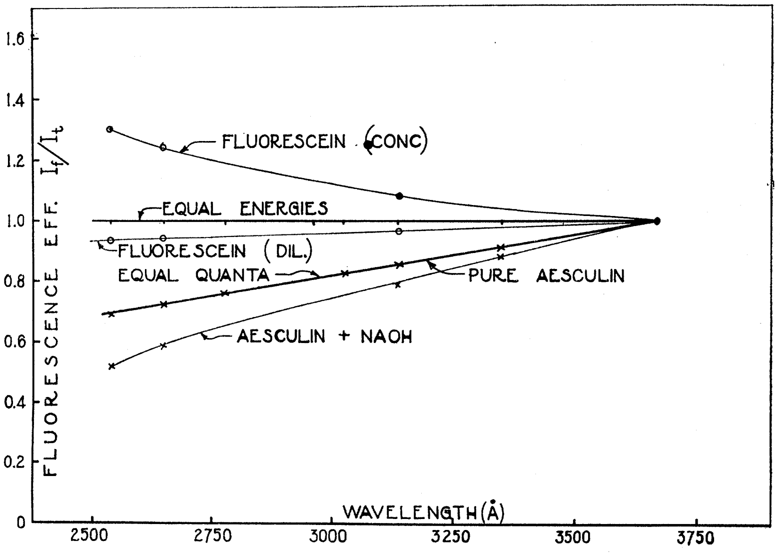

Fig. 5. Variation in fluorescence efficiency for fluorescein and aesculin with different dilutions and degrees of contamination, I_f/I_t being set arbitrarily at 1.0 for λ3663.

By varying slit *S* the intensity of the light falling on *C* is varied to give a match in the photometer; the cell is then removed and the incident light allowed to fall directly on the surface thermopile *T*, where its total intensity is integrated. Results obtained in this way again contradicted those of Anderson and Bird, and showed a linear variation of fluorescence efficiency with wave-length for most substances.

We noted during the course of this part of the work that often the curves for one cell filled with aesculin differed from those of another, and soon found that minute traces of alkaline impurities had a large effect on the result. With fluorescein, also, concentration appeared to play a large part. Fig. 5 shows results obtained with these substances under various conditions, and appears to explain the results of Anderson and Bird for fluorescein, at least.

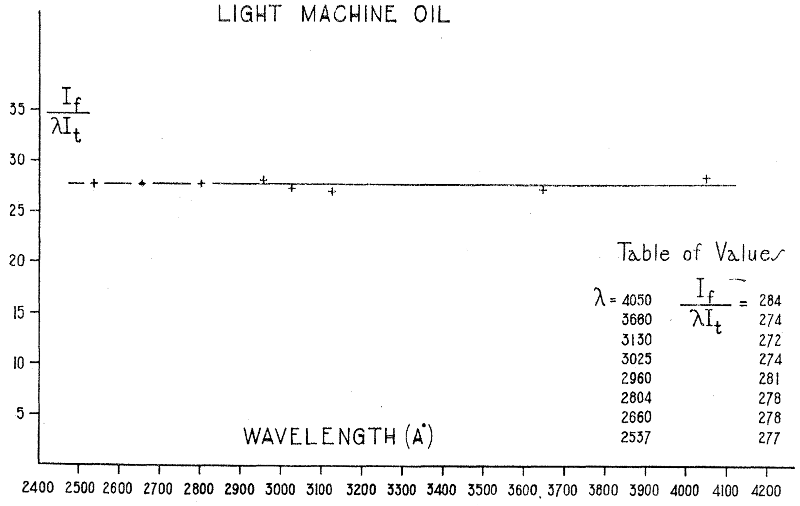

Table of Values

$\lambda =$	$\frac{I_f}{\lambda I_t} =$
4050	284
3660	274
3130	272
3025	274
2960	281
2804	278
2660	278
2537	277

Fig. 6. Curve showing constancy of quantum yield obtained at various wave-lengths with an oil sample of the type used for coating plates.

IV. CONCLUSIONS AND APPLICATION TO PHOTOMETRY

Turning now from the exceptions to the rule to the gratifying number of substances which were found to obey a simple law within a certain spectral range, we see at once that while the uniform fluorescence efficiency does not ordinarily exist, a uniform quantum yield often does. In Fig. 6 we show results (obtained from a single plate) for the type of lubricating oil which we have found most useful for coating plates to produce uniform contrast. Here we have plotted $I_f/\lambda I_t$ against wave-length; obviously, within the range plotted a constant number of fluorescent quanta are emitted per 100 incident quanta.

The greater part of this work was done by the writers at Stanford University during 1929–30; on account of the contradiction between many of the results and those of previous workers a new set-up of the apparatus has been made at the Massachusetts Institute of Technology by one of us and the work has been repeated by Mr. A. L. M. Dingee, who is continuing and extending it. We are indebted to Mr. Dingee for the data plotted in Fig. 6, which agree entirely with our previous results, and give points lying somewhat closer to the theoretical curve than ours had done, due to the fact that in obtaining them the incident light was varied by means of filters of platinum on quartz, in place of the variation of the fluorescence light by means of the filters *R*. This reduces small errors arising from the non-neutrality of the filters, the platinum filters being calibrated with monochromatic light at each wave-length used.

We turn now to the direct application of these results to heterochromatic photographic photometry. An Eastman 36 plate was coated with the light machine oil and was found to give a response which agreed quite accurately with that expected from the uniform quantum yield law. However, a correction is required here, even when the necessary conditions of layer thickness, etc. have been fulfilled. For there is a variation of the amount of reflection from the air-oil interface with wave-length which may not be negligible. In the cases recorded above, where the oil or other material was enclosed in quartz, the thermopile was also enclosed in quartz, so the variation in loss of light at the air-quartz surface could be neglected. The loss at the quartz-oil surface was found to be exceedingly small, so that its variation was entirely negligible. The magnitude of the correction required for the coated plate at the air-oil face can be readily determined, however, by the methods of homochromatic photometry.

Apparently then, this method can be used in place of a thermopile, provided proper precautions are taken to see that the simple law assumed holds. Otherwise errors similar to or greater than those often made by the gratuitous assumption of the reciprocity law in photographic photometry are liable to occur. The sensitivity of such a coated plate is several hundred times as great as that of a thermopile, and we believe that its greatest application will be, not in supplanting this, but in supplementing it for use with weak spectra and in regions difficult of access, such as the Schumann region. A further advantage is that the coated plate bridges the gap in sensitivity between the thermopile and the photographic plate as ordinarily used; we have found that in order to satisfactorily record directly on a photographic plate a spectrum which was about as weak as could conveniently be measured with the average thermopile-galvanometer combination, and exposure time of about 1/500 second was sufficient. This inconveniently small time of exposure gives inaccurate results with the mercury arc or a similar source due to the flickering which occurs at high intensities. The fluorescence method, on the other hand, bridges the gap, and in addition transforms all energies into a single set of wave-lengths, so that various spectral lines can be reflected or absorbed equally without introducing relative errors. Much higher resolving power can be used than with the thermopile, and very weak lines can be accurately measured.

For many kinds of work a still further simplification would result if the fluorescent dry plates discussed above[2] should follow the same law. Accordingly photographs of the spectrum were taken on plates prepared with the pure aesculin coating in gelatin as described, and a uniform quantum yield was found within the same limits as for the oil coated plates. We accordingly possess a dry plate which shows uniform contrast and almost uniform quantum sensitivity, which provides a known energy sensitivity distribution if one knows the wave-lengths photographed. We are now investigating further methods of improving the uniformity of this type of plate, and especially of extending the method to wave-lengths shorter than 2000A.

Much of the apparatus used in this investigation was purchased with a grant from the Rumford Committee of the American Academy of Arts and Sciences, which is hereby gratefully acknowledged.

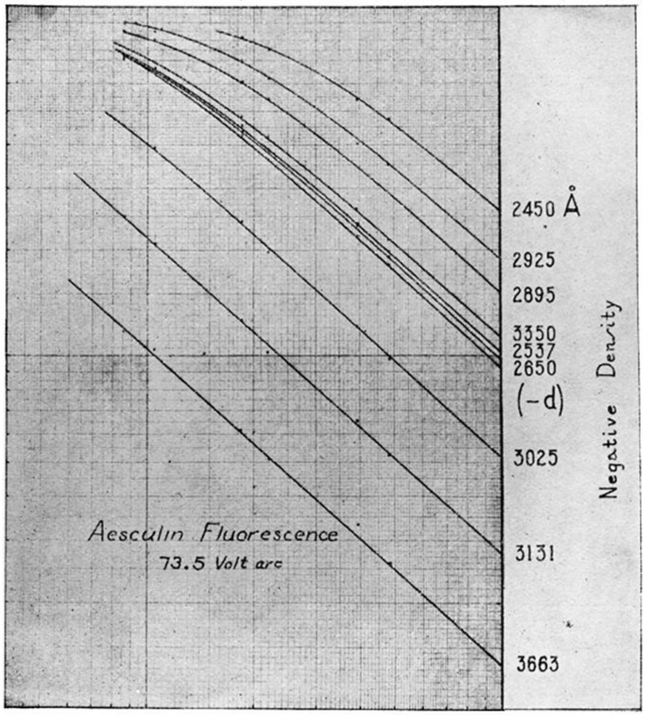

Fig. 2. Calibration curves for the photographic plate when exposed to the fluorescence light from aesculin produced by various incident wave-lengths.