STUDIES IN LUMINESCENCE.

BY EDWARD L. NICHOLS AND ERNEST MERRITT.

XIII. THE SPECIFIC EXCITING POWER OF THE DIFFERENT WAVE-LENGTHS OF THE VISIBLE SPECTRUM IN THE CASE OF THE FLUORESCENCE OF EOSIN AND RESORUFIN.

IN the case of either a solid, like anthracene, or of a liquid such as one of the fluorescent dyes, fluorescence may usually be excited by light of a great variety of different wave-lengths. In fluorescent solutions there is usually a well-marked absorption band lying close to the fluorescence band on the side toward the violet, and light of any wave-length within the limits of this absorption band will excite fluorescence. In fact fluorescence may usually be excited by light of much shorter wave-length, so that the solution will be lighted up when exposed to an ultraviolet spectrum. Simple inspection, however, is sufficient to show that the intensity of the fluorescence excited by different portions of a given exciting spectrum is widely different. It is not clear whether this variation is due to the fact that certain wave-lengths are particularly effective in exciting fluorescence, or whether it results merely from the fact that the absorbing power of the material is different for different wave-lengths. It is clear that light cannot produce excitation unless it is absorbed by the fluorescent solution. Differences in absorbing power might therefore produce wide variations in the apparent effectiveness of different spectral regions in producing fluorescence, even if the specific exciting power, *i. e.*, the fluorescence excited per unit of *absorbed* energy, were in reality constant for all wave-lengths.

The determination of the relation between the specific exciting power and the wave-length of the exciting light is a problem of some interest, whose results possess also considerable significance on account of their bearing upon the theory of fluorescence. The present paper deals with the determination of this relation for eosin and resorufin.

The exciting light was furnished by a Nernst glower, which took the place of the slit of a large spectrometer. A narrow region in the spectrum thus formed was used in exciting the solution studied, and the intensity of fluorescence produced was measured by a spectrophotometer as the wave-length of the exciting band was varied.

The arrangement for exciting and observing fluorescence will be made more clear by inspection of Fig. 90. The light of the Nernst

Fig. 90.

glower, after passing through the large spectrometer before mentioned, was reflected directly upward by a total reflecting prism as shown in the figure, and passed through a horizontal slit S into the cubical glass vessel which contained the solution to be studied. The spectrometer was adjusted so as to bring the spectrum in focus at the slit S . One vertical face of the cubical cell was covered with a sheet of metal containing a slit S' as shown in the figure. The exciting light passing through the slit S excited a narrow, vertical strip in the liquid to fluorescence, and this was observed through the slit S' , in front of which the collimator slit of the spectrophotometer was placed. The comparison source for the spectrophotometer was an acetylene flame sliding upon a photometer track so that it could be set at different distances on the ground glass in front of the slit. This procedure makes it possible to keep

the slit width constant, and therefore eliminates the errors which might be introduced in measurements where the variation of intensity is great. The collimator slit of the spectrophotometer was broad and the instrument was set so as to measure the central part of the fluorescence band.

The Nernst glower which furnished the exciting light was attached permanently to the collimator tube of the large spectrometer, and in fact took the place of the slit of this instrument. The wave-length of the light used for excitation was varied by swinging the arm carrying the collimator and glower, the other portions of the spectrometer remaining fixed in position. At frequent intervals, corresponding to wave-lengths of the exciting light which differed only slightly from one another, the intensity of the fluorescence excited was measured by the spectrophotometer. The cell containing the fluorescent solution was then emptied and carefully cleaned and a piece of magnesium carbonate was mounted obliquely in the cell so as to reflect light from the slit *S* into the spectrophotometer. The intensity and range of wave-lengths of the light thus reflected were measured for each of the spectrometer settings previously used. Finally the absorption of a layer of the solution of known thickness was measured for some definite wave-length in the spectrum. By a separate series of measurements the relative reflecting power of the magnesium carbonate was determined throughout the region used.

The procedure in computing results was as follows: To correct for the unequal reflecting power of magnesium carbonate at different wave-lengths, the observed intensity, I_0 , of the exciting light was divided by the reflecting power of magnesium carbonate for that particular wave-length. This correction reached approximately ten per cent. as its highest value. Using the values previously determined by us for the distribution of energy in the spectrum of the acetylene flame, we next determined the relative energy intensity of the light used for excitation as a function of the wave-length. It will be noticed that this procedure not only takes account of the difference in quality and energy distribution between the Nernst glower and the acetylene flame, but also eliminates any errors which might arise on account of selective transmission or other causes anywhere in the apparatus.

The center of the slit S' through which the fluorescence was absorbed was 8.2 mm. above the bottom of the cell containing the solution. On the average therefore the exciting light had passed through 8.2 mm. of solution before reaching the region whose fluorescence was measured by the spectrophotometer. The light available at this point to produce excitation was therefore less than that falling upon the face of the cell in the ratio of one to $e^{-0.82\alpha}$. It was therefore necessary to compute this factor $e^{-0.82\alpha}$ for each wave-length. In the case of eosin the solution was used the same that had been employed in determining the absorption curve given in our last paper for the more concentrated of the two solutions there studied. The value of α for each wave-length could therefore be read off at once from this curve. In the case of resorufin a different solution was used. Since, however, it has been shown that the form of the absorption curve is the same for all concentrations, it was sufficient to determine the absorption for one particular wave-length and then to reduce the values read from the curve of our last paper in a constant ratio.

Having computed in this way the intensity of the rays which actually reach the region in the solution that is under observation, and which are therefore available for producing excitation we must next compute the amount of energy actually absorbed in each case by multiplying $I_0e^{-0.82\alpha}$ by α . Finally the absorbed fluorescence divided by the absorbed energy which produced it gave the quantity sought, that is, the intensity of fluorescence excited by unit quantity of absorbed energy of the particular wave-length in question.

In the case of eosin the observed data as well as the derived quantities corresponding to several steps in the computation are given in Table I. In the case of resorufin the same thing is shown graphically (Fig. 91). The observed points in each case are connected by full lines, while in the case of computed values the points are connected by broken lines. In curve *I* we have the intensity of the exciting light after reflection from magnesium carbonate. Curve *II* gives the energy intensity of the light actually reaching that part of the solution opposite the slit S' . The ordinates of curve *III* are obtained by multiplying each of the ordinates of

TABLE I.

Eosin.

1 λ	2 Intensity of Fluores- cence.	3 Intensity of Exciting Light.	4 Reflecting Power of MgCO_3 .	5 I_0 Corrected for Reflection.	6 Energy in C_2H_2 Spectrum.	7 I_0 (Energy.)	8 $I_0 e^{-ax}$	9 $a/I_0 e^{-ax}$	10 Specific Exciting P_0
.479 μ	2.2	8.25	102	8.08	1.15	9.3	7.0	2.45	0.9
.485	2.9	8.4	103	8.15	1.38	11.2	7.6	3.53	0.82
.491	4.1	8.3	104	7.98	1.59	12.7	8.4	4.24	0.97
.497	5.3	8.4	105	8.00	1.77	14.2	8.65	5.20	1.02
.501	6.3	10.1	108	9.37	1.90	17.8	10.1	6.98	.90
.509	8.6	8.9	111	8.00	2.13	17.1	8.12	7.37	1.17
.514	10.4	11.2	111	10.1	2.26	22.8	8.13	10.3	1.01
.521	11.0	11.0	111	9.9	2.40	23.8	5.60	9.9	1.11
.526	13.0	12.2	111	11.0	2.58	28.4	5.98	11.4	1.14
.534	17.0	12.0	110	10.8	2.90	31.3	9.76	13.8	1.23
.540	18.3	12.5	109	11.5	3.14	36.2	19.6	14.7	1.25
.547	11.0	12.2	108	11.3	3.4	38.4	31.0	8.07	1.37
.556	4.8	12.7	107	11.9	3.84	84.5	42.7	3.85	1.25

Fig. 91.

Resorufin.

curve *II* by α . Curve *III* therefore gives the energy actually observed from the exciting light for each wave-length. When the ordinates of curve *IV*, which shows the observed fluorescence, are

Fig. 92.

divided by the ordinates of curve *III* we have the quantity desired, namely, the fluorescence excited per unit of observed energy (curve *V*).

The results for eosin are shown in Fig. 92 and for resorufin in Fig. 93. In the case of the latter figure the values corresponding

Fig. 93.

Resorufin. Intensity of fluorescence excited by different wave-lengths per unit of absorbed energy. The circles and crosses give the results of two independent sets of observations. Curve *A* gives the coefficient of absorption and curve *F* the energy distribution in the fluorescence spectrum.

to those shown in Fig. 91 are marked by circles. The crosses show the values obtained by another set of observations which are not here recorded in detail. The individual points on these curves show considerable erratic variation. In many cases these can be traced back to irregularities in the original data, doubtless due to some experimental errors. In making computations we have made no effort to eliminate these errors by smoothing the curves, but have taken each observation as it stood and carried through the work to the final result. Smooth curves have, however, been drawn among the computed points in Figs. 92 and 93. The data and results recorded here represent the best of a number of series of observations. While those not recorded gave more irregular results than here shown they all agreed in indicating the same general trend in the final curve.

In each of these two figures also we have plotted in the curves for the coefficient of absorption (A) and for the energy distribution in the fluorescence spectrum (F). It would seem reasonable to expect that the curve of specific exciting power might show some peculiarities either in the region of maximum absorption or in that of most intense fluorescence. It appears, however, that nothing of this kind occurs.

Before beginning these experiments we were of the opinion that either the specific exciting power would prove to be constant, so that the same quantity of absorbed energy would produce the same fluorescence regardless of its wave-length, or else that the effectiveness of the exciting light would prove to be greater for the shorter wave-lengths. The readiness with which fluorescence is excited in the ultra-violet spectrum made the latter view seem plausible. As it turns out neither of these views is in accord with the facts. We were unfortunately unable to extend the observations to the ultra-violet spectrum. It seems clear, however, that if we confine our attention to wave-lengths falling within the range of one absorption band, the light lying near the red side of the band is more effective in producing fluorescence than that lying on the violet side, and the change in the specific exciting power as we pass through the band is continuous, without any indication of anything selective in the neighborhood of the region of maximum absorption.