Institute of Experimental Physics

Connection Between Absorption and Emission of the Alcoholic Solution of Acridine Orange NO

By P. Szőr

Introduction.

P. Ramart, M. Gromez and M. Martynoff investigated the ab-sorption of the acridine dyestuffs. (1). Particularly the absorption of the solid gelatine solution of acridine orange NO was studied by H. Mischung, (2) while P. Fröhlich and H. Mischung studied its emission (3). In this paper the emission and absorption of the alcoholic solution of acridine orange base was established.

The dyestuff acridine orange consists of two tautomer forms; which are in equilibrium, and are influenced by alkalis and acids. Under the influence of the former II., under that of the latter I. is predominant.

The object of the following investigations was to determine to which extent these tautometer forms participate in the establish-ment of the phenomena of fluorescence. For this reason acid or alkali, respectively, were added to influence the tautomer forms in the one or other direction.
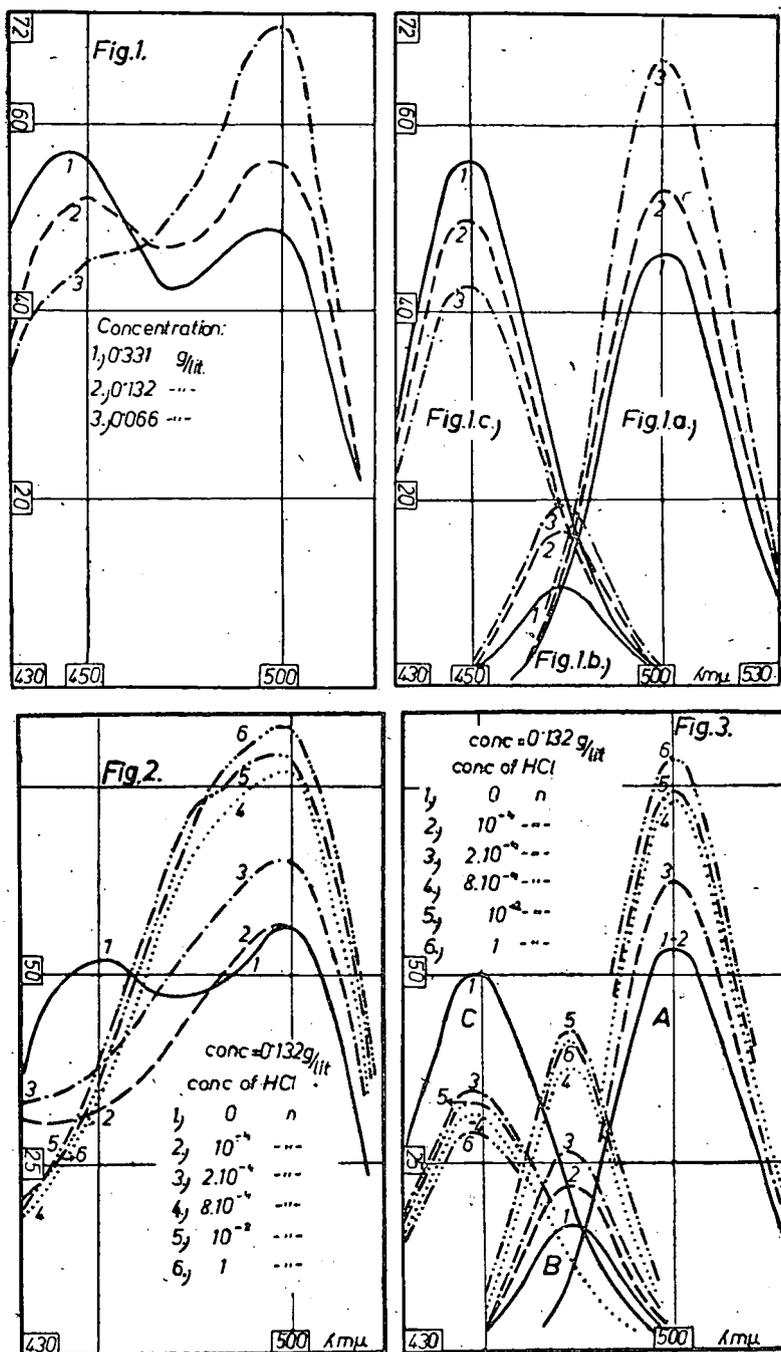
Measurements.

I. Measurements of absorption.

1. Absorption of neutral solutions.

The alcoholic solution of the acridine orange base was in-vestigated in three different concentrations, i. e. a concentration of $c = 331$ mg/lit, 132 mg/lit, and 66 mg/lit respectively. The absorp-tion values were converted into a concentration of 3160 mg/lit. These absorption values are shown in Fig. 1. The figure displays two maxima. Thus it could be concluded that there are two partial bands, however, the analysis made by the method of Kiss and Sándorffy exhibited three partial bands (4). In this analysis the established maximum (λ_m) and the k distribution constants were considered to be as permanent within the same band, and concer-ning the other curves, the construction of the original curve with values λ_m and k; in alkaline and in acidified solutions was attemp-ted. A similar method of analysis was employed by Gilbert N. Lewis, Theodor T. Magel, and David Lipkin, (5). On investigating the absorption curves they used the absorption curve of a highly diluted solution as base and a_m as unit, this basic curve was sub-stracted from the other curves thus a new band was obtained.

Fig. 1. shows the analysis of the alcoholic solution of the acridine orange base. The maxima of each band are 500, 473, and 448 $m\mu$. The k-values are 0,91, 0,75, and 1,2, respectively. This fi-gure demonstrates that the maximum of band III diminishes with the decrease of the concentration, while that of bands II and I

below); with the aid of these λ_m and k-values the λ_m and k-values, of band III were calculated from neutral solutions.

Fig.1.

Concentration:
1, 0·331 g/lit.
2, 0·132 ---
3, 0·066 ---

Fig.1.c.)   Fig.1.a.)

Fig.1.b.) $\lambda m\mu$

Fig.2.

conc = 0·132 g/lit
conc of HCl
1,   0   n
2, 10^{-4} ---
3, 2.10^{-4} ----
4, 8.10^{-4} ---
5, 10^{-2} ---
6,   1   ---

$\lambda m\mu$

conc = 0·132 g/lit
conc of HCl   Fig.3.
1,   0   n
2, 10^{-4} ---
3, 2.10^{-4} ---
4, 8.10^{-4} ---
5, 10^{-2} ---
6,   1   ---

C   A

B

$\lambda m\mu$

increases. The λ_m and k-values of bands I and II were established from the acidic solutions (as regards their absorption curves see-

On adding the area of each partial band at each concentration, we find that the total of these areas agrees in all three cases within the limit of error. The area of a band is expressed by the formula $\alpha_m k \sqrt{\pi}$ (4). Thus for a solution of concentration of c—331 mg/lit, $T = 117.6 \sqrt{\pi}$ for that of $c = 132$ mg/lit, $T = 121.5 \sqrt{\pi}$ and finally for that of $c = 66$ mg/lit, $T = 128.9 \sqrt{\pi}$. Thus it is evident that changes in the concentration do not effect the relative number of the absorbing centres; but that they only are converted.

2. *Absorption of acidified solutions.*

The measurements were made in an acidified medium with a solution of $c = 132$ mg/lit Fig. 2. shows the absorption bands of these solutions and Fig. 3. the analysis. It can be seen that by increasing the concentration of HCL bands I and II also increase, while band III diminishes. When the concentration of the acid reaches 1,6 molecules HCL per dyestuff molecule, in spite of the very high increase in the concentration of HCL the absorption values scarcely change. This phenomenon may lead to the conclusion that if there are 1-2 molecules of HCL per 1 molecule of dyestuff, form I dominates, thus further molecules of HCL can have but a slight affect on the absorption, or equilibrium of dyestuffs.
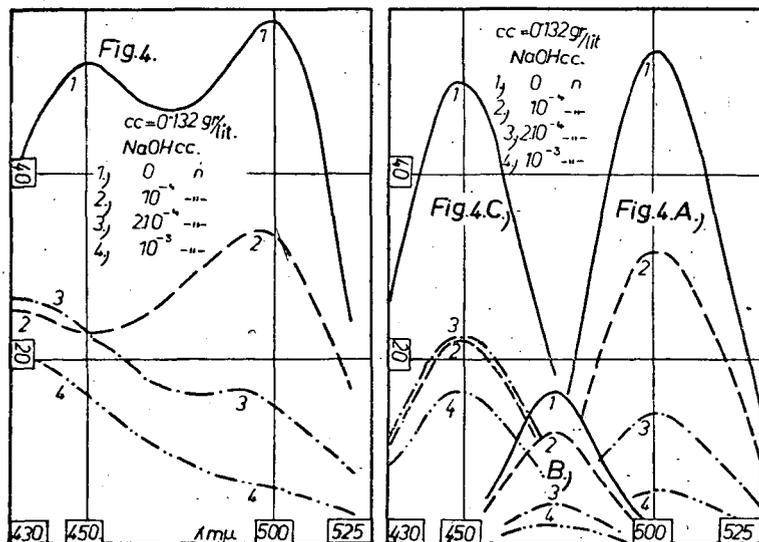
3. *Absorption of alkaline solutions.*

Fig. 4. demonstrates the absorption bands — as measured in NaOH alcoholic solution of acridine orange base — and also the partial bands. Similarly to the acidified medium the three bands are also present, but in the solutions of greater concentrations band III is predominant. However, in acidified solutions band III is not enchanced on adding alkali, but diminishes, nevertheless as bands II and I decrease more than band III, the latter remains predominant.

On observing the absorption bands ranging between 440 and 430 $m\mu$ (see fig. 4.), in alkaline solutions a divergence between the analysis and the actually measured values can always be found. The same difference can also be observed in the neutral solutions of higher concentration. The acidified solutions on the other hand, do not show such differences. This seems to infer that by adding alkali a new maximum develops at the border of the ultraviolet and the visible spectrum. This subject was not investigated in detail.

On the addition of NaOH the size of the maxima of the partial bands decreased during the measurements. Therefore the solution were kept 1-2 days in daylight. By this time every band developed its definite form, which remained unchanged even under further excitation. All the measurements were made with such solutions. HCL and neutral solutions were exposed to the sun-light for as long as two months but no changes could be observed.

This phenomenon suggests the assumption that changes of the structure of acridine orange base in an alkaline medium cannot be ascribed solely to the effect of alkali but that the energy of light is also required.

II. Measurements of emission.

The emission of acridine orange base was also measured. The measurements were made with the spectral photometer of König—Martens. The intensity of the emission was compared with a Tungsten filament lamp of 12 Volt, 36 Watt, the spectral distribution of which was established with a photocell. The intensity of the emission in the figures is given in arbitrary units.

1. Emission of neutral solutions.

The fluorescence emission of three solutions of different concentration were measured. The concentrations of the neutral solutions were identical to that of the alkaline solutions. Fig. 5. shows
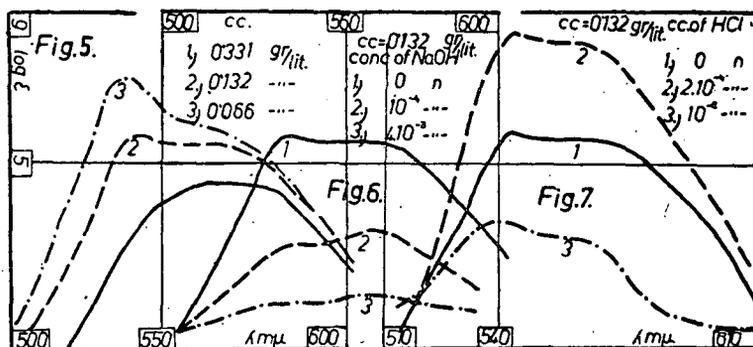
the spectral distribution of the emissions. It demonstrates the increasing of the emission at diminishing concentration although the emission values are not converted into concentration units. The figure demonstrates furthermore that with decreasing concentration the absorption at wave length 540 $m\mu$ increases considerably and shows a maximum, whereas the emission value remains almost unchanged at 565 $m\mu$.

2. *Emission of acidified solutions.*

Figure 6. displays the result of the emissions of acidified solutions. As compared with neutral solutions, we cannot find any difference in the form of the emission curves, only the emission values differ. A small amount of HCL increases the emission, while a greater concentration of HCl diminishes it. On the other hand, HCL enhances the absorption, in spite of the fact of the absorption, value changing only to an insignificant extent above a certain HCL concentration. Till a certain HCL concentration is reached the emission increases, but on exceeding it diminishes. Thus there is a HCL concentration above which the absorbed energy is transformed in a slighter degree into emitted energy. The detailed investigation of this problem is in progress.

3. *Emission of alkaline solutions.*

On addition of alkali to alkaline solutions the emission and absorption diminish. It was established in connection with emission measurements that light exerts an effect on alkaline solutions. This is similar to the effect found for absorption, i. e. the emission decreases during excitation. Consequently the measurements were made in solutions which were exposed 1-2 days to sun-light after which the emission bands took up their final forms.

4. *Connection between absorption and emission bands.*

On observing the slight variations of the emission under the influence of HCL and alkali it can be stated that HCL enhances the band in the emission near 540 $m\mu$ (Fig. 6.), while in alkaline solutions the band near 565 $m\mu$ predominantes (Fig. 7.). Comparing these with the absorption bands we find that for acidified solutions in which the partial bands of absorption I and II predominate (Fig. 2.), the partial band of the emission near 540 $m\mu$ dominates. For alkaline solutions, where partial band III predominates (Fig. 4.), the partial band at 565 $m\mu$ is in prominence. It may be inferred therefore that the emission band at 540 $m\mu$ is caused by the absorption bands I and II and the emission band at 565 $m\mu$ by absorption band III. Consequently the emission of tautomer form I shows the band at about 540 $m\mu$ and that of tautomer form II the band near 565 $m\mu$.
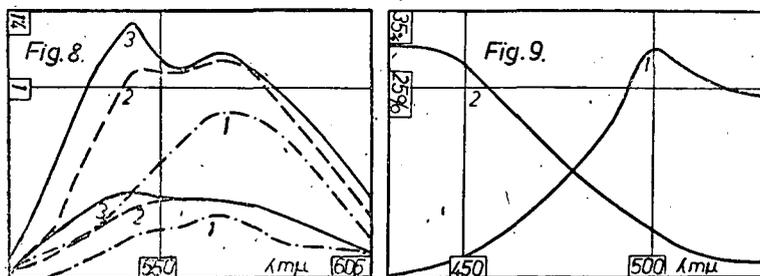
Fig. 8.

Fig. 9.

In order to determine whether this supposition is correct the neutral solutions were excited through a filter. The results of the respective measuremnts are shown in Fig. 8. — Fig. 9. shows the transmissivity of the filters. Fiter *a)* transmits the part corresponding to absorption band I, and filter *b)* the part corresponding

to band III. The curves under 𝑎) demonstrate in Fig. 8 the emission, measured at the excitation with filter 𝑎), and the curves under 𝑏) show the measurements with filter 𝑏). Although apparently the transmissivity of both filters in bands I and III is almost identical the intensity of the emission curves differs greatly. The emission diminishes using filter 𝑏) to the same extent to which the transmissivity of the filter diminishes in absorption band I. Namely the area of the emission curve of solution 2. ($c = 132$ mg/lit) yields 1.74 units for excitation with filter 𝑎) and 0.56 units for filter 𝑏). On the other hand, the transmissivity of filter 𝑎) amounts to 30 percent at 500 $m\mu$ and 7.5 percent for filter 𝑏). The diminution of the transmissivity is fourfold, that of the emission 3.1 fold. On the base of this approximative calculation it can be postulated that the energy absorbed by absorption band I is transformed into emitted energy, i. e. tautomer form I emits.

In order to establish the emitting, tautomer form and the emssion band which corresponds to a certain absorption band the emission bands must be analysed.

Such an analysis had been already employed by P. Borissov (6). His method is in principle identical with the analysis of Kiss and Sándorffy (4), but for the fact of P. Borissov establishing the distribution of the emission value according to λ, while Kiss and Sándorffy employed ν for the establishment of the distribution of the absorption. In contrast to B. Borissov the analysis was attampted — similarly to the absorption according to ν.

This analysis may be carried out beyond 550 $m\mu$ i. e. the emission curve may be given by the formula

$$J = J_o \, e^{-\left(\frac{\Delta\nu}{h}\right)^2}$$

The curve between 500 and 550 $m\mu$, particularly the ascending part of the curve, is so abrupt that the distribution function may not be employed. The divergence between the emission curve and the distribution function is explained by the fact that the absorption and emission bands cross each other, consequently the solution absorbs its emitted light, i. e. self-absorption occurs. The nearer the wave-length of the emitted light is to 500 $m\mu$, the greater is the self-absorption. Consequently, the smaller the measured emission relatively is, the greater is the self-absorption at the corresponding wave-length. Thus the measured emission decreases, by degrees towards the 500 $m\mu$, i. e. the steepness of the curve does not diminish according to the requirements of the distribution function but increases. Hence an attempt to analyse fails and the absorption and emission cannot be compared.

Summary.

The absorption and demission of the alcoholic solution of acridine orange NO was measured.

The absorption bands are composed of three partial bands. The changes of the partial bands in acidified and alkaline mediums and the comparison of the respective emission bands seem to point

to the fact that the fluorescence emission is caused by one of the tautomer forms of the dyestuff.

Owing to self-absorption an analysis of the emission bands could not be accomplished.

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About an Effect of Oriented Molecules on Polarized Light

By L. Szalay

Introduction.

According to the investigations of P. Fröhlich and L. Szalay the dyestuff molecules are oriented in rigid gelatineous solution of acridine orange under illumination(1). If a part of the solution is preexcited by polarized light and after the preexcitation the phosphorescence emission is measured by weak polarized light, at the same time illuminating also the unexcited part of the preparation, the intensity of emission of the preexcited surface is greater or smaller than the intensity of emission of the unexcited surface. When the directions of polarization of preexciting and exciting lights are parellel, the preexcited surface has a more intensive phosphorescence emission than that of its surroundings; when they are perpendicular, the phosphorescence emission of the preexcited surface is smaller than that of its surroundings.

The effect is similar to that found by G. N. Lewis and his co-workers (2), according to which the absorption of dyestuffs, exposed to and measured by polarized light, depends on the direction of polarization of the measuring light. The absorption is great when the directions of polarization of exciting and measuring lights are parellel, and small if they are perpendicular.