*Influence of the Addition of High Molecular Electrolyte upon the Absorption Spectra and Fluorescence of Organic Dyestuffs. IV.**

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Introduction

In the earlier papers[1,2,3] of this series, the present authors have investigated the influence of the addition of high molecular electrolyte upon the absorption spectra and fluorescence of basic dyes. The conclusion then reached was that for the interpretation of metachromasy, the change of aggregation of dye ions caused by the addition of high molecular electrolyte is of primary importance rather than the direct interaction between dye and high molecular electrolyte.

Now, an investigation has been made with acidic dyes which, in contrast to basic dyes, have little tendency to associate in the aqueous solution and accordingly obey Beer's law quite well. The influence upon absorption spectra and fluorescence of such dyes caused by the addition of basic electrolytes of various molecular weights was examined in order to confirm our previous conclusion more definitely.

The acidic dyes examined were uranine, eosine and erythrosine, and the following basic electrolytes were used: a) chitosan hydrochloride b) glucosamine hydrochloride and c) several aliphatic amines of different chain length.

As the result of the investigation using various combinations of dyes and electrolytes, it was found that the data can be interpreted along the same reasoning as before, and the phenomenological interrelation has now become more comprehensive.

The present paper reports in a rather abbreviated form some experimental results and the discussions based upon them. The details will be published in J. Inst. Polytech. Osaka City Univ.

Experimental Results

Beckman spectrophotometer model D.U. was used for the measurement of absorption spectra and the relative intensities of the fluorescence.

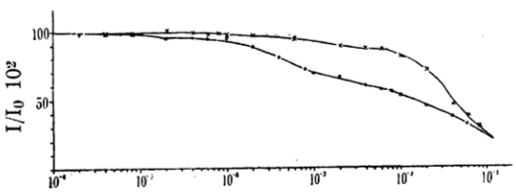
Sample.—Uranine (Merck) was purified by recrystallisation.

Glubler's eosine and erythrosine were used without further purification.

Chitosan hydrochloride and glucosamine hydrochloride were kindly supplied by Mr Kanji Okawa of the Institute of Cellulose Research of Osaka University.

Methylamine hydrochloride is of commercial supply, *n*-propylamine and *n*-dodecylamine hydrochloride were kindly supplied respectively by Mr. Shiro Morimoto and Shuji Saito of Osaka University.

1) Uranine *a) Chitosan hydrochloride.*— The absorption spectra and the relative intensity of fluorescence of the aqueous uranine solution containing various quantities of chitosan hydrochloride, are shown in Figs. 1 a and 2, respectively.



a. Chitosane hydrochloride (mol./*l.*) (log scale)
b. Glucosamine hydrochloride (mol./*l.*) (log scale)

Fig. 1. Change of relative fluorescence intensity of aqueous uranine solution by added chitosan hydrochloride, and glucosamine hydrochloride. Dye conc., 2×10^{-5}mol./*l*; temperature, 27°C.
○ a
× b

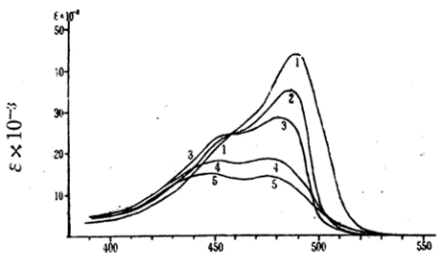


Fig. 2. Change of absorption spectrum of aqueous uranine solution by chitosane hydrochloride.
Dye conc. 2×10^{-5}mol./*l*. Temperature, 27°C. Quantity of added chitosane hydrochloride: (1) $0\text{–}4\times10^{-5}$mol./*l*. (2) 6×10^{-5} mol./*l* (3) 8×10^{-5}mol./*l*. (4) 6×10^{-4}mol./*l*. (5) 2×10^{-3}mol./*l*.

* Physicochemical Studies of Organic Dyestuffs in Relation to Photochemistry. VI.

1) M. Koizumi and N. Mataga, This Bulletin, 26, 115 (1952).
2) M. Koizumi and N. Mataga, ibid., 27, 194 (1954).
3) N. Mataga and M. Koizumi, ibid., 27, 197 (1954).

Up to 10^{-5} mol./*l*. (referred to a unit group with one amino group) of the added chitosan hydrochloride, no change is observed.

In the range between -10^{-5} and -10^{-4} mol./*l*., the intensity of fluorescence slightly decreases, while the absorption spectra change conspicuously in such a manner as to suggest the existence of an association equilibrium of some kind between chitosan hydrochloride and uranine.

Beyond 10^{-4} mol./*l*., quenching of fluorescence gradually increases and at the same time the intensity of absorption wholly decreases.

There is observed no phenomenon of recovery either in fluorescence or absorption, however much chitosan hydrochloride is added.

b) *Glucosamine hydrochloride*.—The variations of the relative fluorescence intensity and the change of absorption spectra when various quantities of glucosamine hydrochloride (a structural unit of chitosan hydrochloride) are added to the uranine solution, are shown in Figs. 1 b and 3.

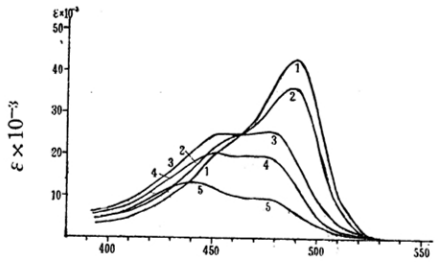


Fig. 3. Change of absorption spectrum of aqueous uranine solution by glucosamine hydrochloride.
Dye conc. 2×10^{-5}mol./*l*. Temperature 27°C. Quantity of added glucosamine hydrochloride (1) $0\sim10^{-4}$mol./*l*. (2) 6×10^{-4}mol./*l*. (3) 6×10^{-3}mol./*l*. (4) 2×10^{-2} mol./*l*. (5) 6×10^{-2}mol/l.

Up to 10^{-4} mol./*l*., there scarcely exists any change either in fluorescence intensity or absorption spectra.

Between ca. 2×10^{-4} mol./*l*. and ca. 10^{-2} mol./*l*., a slight decrease of fluorescence intensity is observed and quite similar changes of absorption spectra as those observed in the case of chitosan hydrochloride take place.

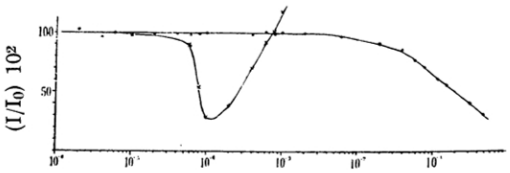
Beyond 10^{-2} mol./*l*., the intensity of fluorescence falls conspicuously and at the same time, the absorption spectra decline in the whole range of visible region. No phenomenon of recovery exists either in fluorescence or absorption.

c) *Aliphatic amino compounds*.—Only the result of methylamine will be described as a representative.

As small an addition as 2×10^{-5} mol./*l*. causes a rapid increase in the intensity of absorption and from this region up to 10^{-1} mol./*l*. There exists a change of absorption spectra suggestive of an association equilibrium of some kind. No change of fluorescence intensity is observed.

Above 10^{-1} mol./*l*. both the intensity of fluorescence and absorption decreases quite remarkably, and the feature of this decrease is somwhat similar to the case of glucosamine hydrochloride.

2) Eosine and Erythrosine *a*) *Chitosan hydrochloride*.—The relative intensities of fluorescence and the absorption spectra when various quantities of chitosan hydrochloride are added to the aqueous solution of eosine, are shown in Figs. 4 b and 5.



a. Glucosamine hydrochloride (mol./*l*.) (log scale).
b. Chitosan hydrochloride (mol./*l*.) (log scale).

Fig. 4. Change of relative fluorescence intensity of aqueous eosine solution by added chitosane hydrochloride and glucosamine hydrochloride. Dye conc. 10^{-5}mol./*l*. Temperature, 27°C.
○ a
× b

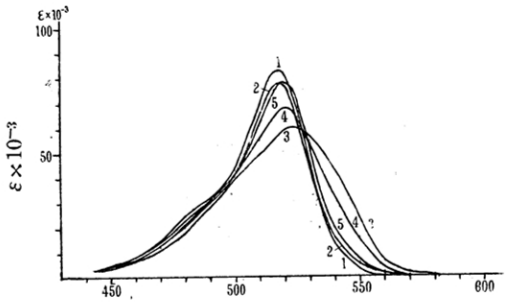


Fig. 5. Change of absorption spectrum of aqueous eosine solution by chitosane hydrochloride.
Dye conc. 10^{-5}mol./*l*. Temperature, 27°C. Quantity of added chitosane hydrochloride. (1) $0\sim10^{-5}$mol./*l*. (2) 6×10^{-5}mol./*l*. (3) 10^{-4}mol./*l*. (4) 4×10^{-4}mol./*l*. (5) 10^{-3} mol./*l*.

There occurs no change either in absorption or fluorescence untill the concentration of chitosan hydrochloride reaches about 10^{-5} mol./*l*.

Beyond this concentration, the fluorescence is quenched gradually and the absorption spectrum shifts toward red and somewhat broadens accompanied with the decrease of extinction.

Such a change becomes most prominent near 10^{-4} mol./*l*., but over this point a marked phenomenon of recovery comes to be observed both in absorption and fluorescence and when the concentration reaches 10^{-3} mol./*l*., the shape of the absorption curve becomes quite similar to that in the pure aqueous solution and the intensity of fluorescence exceeds to some extent, that of the pure aqueous solution.

In the case of erythrosine, the main features

of change of absorption spectra caused by the addition of chitosan hydrochloride, are quite similar as in the case of eosine.

b) Glucosamine hydrochloride.—The effects of glucosamine hydrochloride upon the relative intensity of fluorescence and absorption spectra of the aqueous eosine solution are shown in Figs. 4 a and 6.

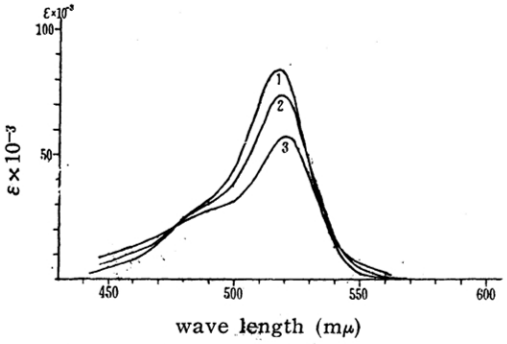


Fig. 6. Change of absorption spectrum of aqueous eosine solution by glucosamine hydrochloride.
Dye conc. 10^{-5}mol./*l.*, Temp, 27°C. Quantity of added glucosaminehydrochloride.
(1) $0\text{–}6\times10^{-3}$mol./*l.*, (2) 8×10^{-2}mol./*l.*, (3) 3.36×10^{-1}mol./*l.*

No marked change is observed either in fluorescence or absorption below 10^{-2} mol./*l.*, but over this point the intensity of fluorescence gradually falls and the absorption also declines in the whole range.

Quite similar results were obtained for the absorption spectra of erythrosine.

c) Aliphatic amino compounds.—When methylamino hydrochloride is added to the aqueous eosine solution, there is no marked change either in fluorescence or absorption until the concentration reaches as much as ca. 4×10^{-1} mol./*l.*, beyond which the intensity of fluorescence gradually falls accompanied with the decline of extinction in the entire region. The state of affairs for erythrosine is quite similar to eosine.

But in the case of dodecylamine hydrochloride, which is capable of forming micelle[4], quite different phenomena were observed, the changes both in fluorescence intensity and absorption curve are analogous to the case in which alkyl sulfate of long chain is added to basic dyes.

Discussion

It can safely be concluded from the above results, that in the case of uranine there exists no action specific to high molecular electrolyte and the effects of the added electrolyte upon fluorescence and absorption are mainly due to the direct interaction between amino group and dye ion, most probably the ionic association.

Indeed, there is some difference between the effect of aliphatic amine and glucosamine or chitosan hydrochloride, but this is quite natural in view of the fact that aliphatic and ring compounds generally differ much in the property.

It may be worth adding here that in the case of uranine, the fluorescing power is not affected much by its association with amino group, since there is no marked change of intensity in the region where absorption spectra conspicuously change.

Now the state of affairs is quite different in the case of eosine and erythrosine; when the amino compounds of low molecular weight such as methylamine and glucosamine hydrochloride are added to the solution, no change suggestive of strong interaction between amino group and dye ion is perceived at all. Therefore, only a weak interaction due to ionic interaction or van der Waals' force will exist in this case. The conspicuous change of absorption and fluorescence intensity observed in the case of chitosan hydrochloride must accordingly be attributed to the specificity of high molecular substance. The intense quenching of fluorescence by dodecylamine and the phenomenon of recovery occurring prior to micelle formation must also be due to the same reason.

For the interpretation of these phenomena, it is quite natural to adopt the same reasoning as that used in the case of basic dyes; thus they are ascribed to the accumulation of dye ions around high molecular ions by virtue of the weak interaction between them. For example, quenching is produced by the increase of concentration of dye ions around high molecular electrolyte, while the recovery takes place because the accumulation of dye ions around high molecular electrolyte becomes less marked as the latter is increased. In fact, the concentration quenching of the pure aqueous solution of eosine was examined and found to be quite remarkable. The above explanation as it runs, is completely analogous to that applied to the similar phenomena observed when high molecular acidic electrolytes are added to basic dyes. It is an interesting problem to be settled why the interaction of amino group and dye ion is so strong in case of uranine while it is rather weak in eosine and erythrosine; perhaps steric hindrance due to Br and I would be an important factor for it, though the question must of course await further investigations.

Another important result obtained, which shows a sharp contrast to basic dyes, is that no such band as deserves to be called a metachromasy band appears in eosine-chitosan

4) M.L. Corrin and W.D. Harkins, *J. Chem. Phys.*, **14**, 641 (1946).

hydrochloride, in spite of the marked accumulation of dye ions around high molecular electrolyte.

One cannot but attribute this difference to the fact that acidic dyes have scarcely any tendency to associate while basic dyes easily dimerise or polymerise. Thus our previous conclusion[1,2,3] that the phenomenon of metachromasy is mainly due to the change of aggregation of dye, conforms quite well with the present results. One may rather say that metachromasy is the conspicuous change of absorption spectra produced by the change of aggregation of dye ions accumulated around high molecular electrolyte when the interaction between dye and functional group of electrolyte is moderate.

Summary

The relative intensity of fluorescence and the change of absorption spectra were examined when chitosan hydrochloride and other amine hydrochloride were added to the aqueous solution of uranine, eosine and erythrosine, which are all acidic and have no tendency to associate.

It was found that in the case of uranine, the interaction between amino group and the dye ion is so strong that it directly influences the absorption band remarkably, and thence the interaction is commonly observed irrespective of whether the molecular weight of electrolyte is high or low. In the case of eosine and erythrosine there exists no such strong interaction between amino group and dye ion because the amino compound of low molecular weight produces no marked change in either fluorescence or absorption; a quite notable change is observed in the case of the amino compounds of high molecular weight; thus for example, the fluorescence at first drops and then recovers as the concentration is increased. The phenomena were interpreted as due to the concentration effect of dye ions gathered around the electrolyte ion by weak attraction. This interpretation is quite analogous to that already applied for basic dyes.

There appeared, however, no metachromasy band and this was ascribed to the fact that the acidic dyes have scarcely any tendency to associate; thus our opinion put forth for the case of basic dyes, that the change of aggregation of dye ions is mainly responsible for metachromasy, was confirmed once more in the case of acidic dyes.

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