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Citation	北海道大學水産學部研究彙報, 4(1), 32-39
Issue Date	1953-05
Doc URL	http://hdl.handle.net/2115/22785
Type	bulletin (article)
File Information	4(1)_P32-39.pdf

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BEHAVIOR OF COLLOIDS IN EXTREMELY DILUTED SOLUTION OF POLYELECTROLYTE AS OBSERVED BY THE CHANGE IN SPECTRUM OF METACHROMATIC DYE *

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Certain dyes change color in the presence of various colloids. Familiar examples are the variation in color of a biological stain depending on the nature of the stainable substrate, which was called "metachromasy" by P. Erlich.

There are two generally accepted interpretations of the mechanism of metachromasy. E. G. Kelley[1] and L. Michaelis[2][3] have pointed out that all those dyestuffs which exhibit polymerization in aqueous solution show the effect of metachromasy. However, H. TERAYAMA[4] and R. C. Merril et al[5] have emphasized that the variation of color is induced by the electrostatical interaction between the colloidal polyions and the dye ions. Neither interpretation will be perfect for all experiments, but they are satisfactory for most practical experiments.

Hitherto many studies on the phenomenon have been carried out chiefly for the purpose of clarifying the causes of color change from the standpoint of molecular interpretation of the dyestuff. On the other hand, critical concentration of micell formation in colloidal solution, as in soap[6] or detergent,[7][8] has been determined by the change in color and the spectrum of metachromatic dye, such as pinacyanol. However, few studies on metachromatic dye as an indicator for a certain colloidal state of polymolecular solution have been carried out.

In this investigation, some spectrochemical observations were made on the change in absorption spectrum of metachromatic dye, such as toluidine blue or thionine, in the presence of algin, being a typical natural polyelectrolyte. The purpose was to clarify the colloidal states of algin in extremely diluted solution, where it could be hardly expected that ordinary methods, involving measurements of the viscosity or the osmotic pressure, would be entirely useful in respect to satisfactory accuracy.

Some physico-chemical properties, viz., viscosity, electroconductivity, pH and so on, of the diluted algin solution had been determined, and were compared with the results of the spectrochemical investigation.

It will become clear that the metachromasy method is excellent for investigation of colloid-chemical states in colloid solution, especially in extremely diluted condition.

Experimental

Commercial pruduct, MITSUISHI KOMBU, *Laminaria angustata* Kjellm., from HIDAKA, HOKKAIDO, was used as raw material.

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* This paper was presented at the annual meeting of the Japanese Society of Scientific Fisheries, Tokyo, April, 1952.

Na-alginate, so-called ALGIN, for the experiments was prepared by SUZUKI's method.[9] First, raw material was boiled in water, digested in $CaCl_2$ solution and further in NaCl solution, and the treated material was made soluble by adding Na_2CO_3. Filtering the gruel of algin, the filtrate containing crude Na-alginate was acidified with HCl, so that alginic acid was coagulated out. The coagulated alginic acid was washed with hot water (50-55°C), and converted to Na-alginate through neutralizing with NaOH solution.

The obtained Na-alginate was further purified repeatedly by the ordinary method. The purified algin was washed with ethanol, then with ether, and dried at 100°C for 2 hours.

The algin prepared by the above discribed method was snow-white and flocky, with humidity of 10.52%.

The aqueous algin solution was very viscous; average molecular weight calculated from intrinsic viscosity of the solution was about 29300 (degree of polymerization was 148). Certain physico-chemical properties of the aqueous algin solution are summarized in Fig. 1. (cf. the other reports[10] for details.)

On preparing the algin solution, the mixture of algin and distilled water was allowed to stand for 24 hours or over, making the dispersion of the colloid complete.

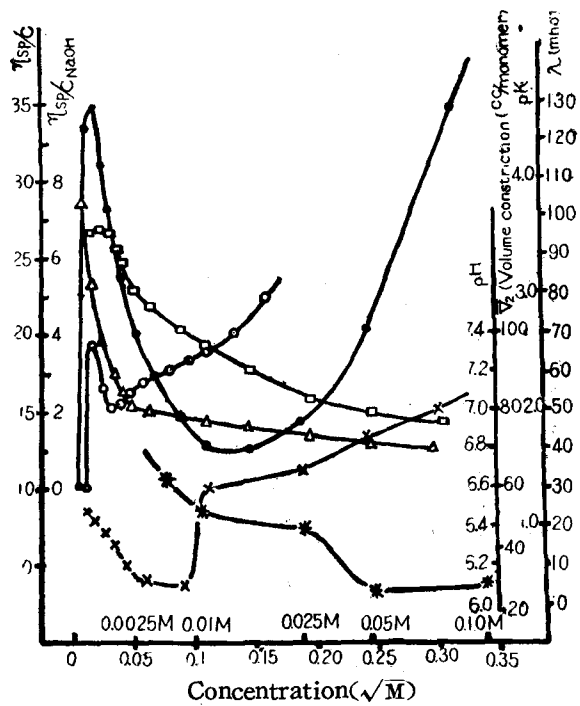

Fig. 1 Physical Properties of Diluted Algin Solution.[10]
●-●-● Reduced Viscosity, η_{sp}/C (C:g/dl)
○-○-○ Reduced Viscosity in 2N NaOH solution, η_{sp}/C_{NaOH}
△-△-△ Molar Electroconductivity, λ(mho/monomer)
□-□-□ Dissociation Constant, pk
×-×-× pH
※-※-※ Volume Constraction $\overline{V}_2$ (c.c./monomer)

The metachromatic dyes, viz. thionine or toluidine blue, were commercial products, being used without further purification. The thionine was purchased from Wakoh-Junyaku Co., and the toluidine blue was Grübler's Toluidine Blue 0. Each dye was dissolved in redistilled water to make 2.8mg M/l solution, pH 5-6. One ml of the dye solution was added into 10 ml of algin solution, and the variation in color of the mixed solution was measured spectometrically. However, the spectrum of the dye in 0.025 M/l or higher algin solution could not be measured owing to coagulation of the dye. The range of concentration of added algin solution was, consequently, $4.6\times10^{-5}-2.3\times10^{-2}$ M/l in the spectrochemical investigation.

The absorption spectrum was

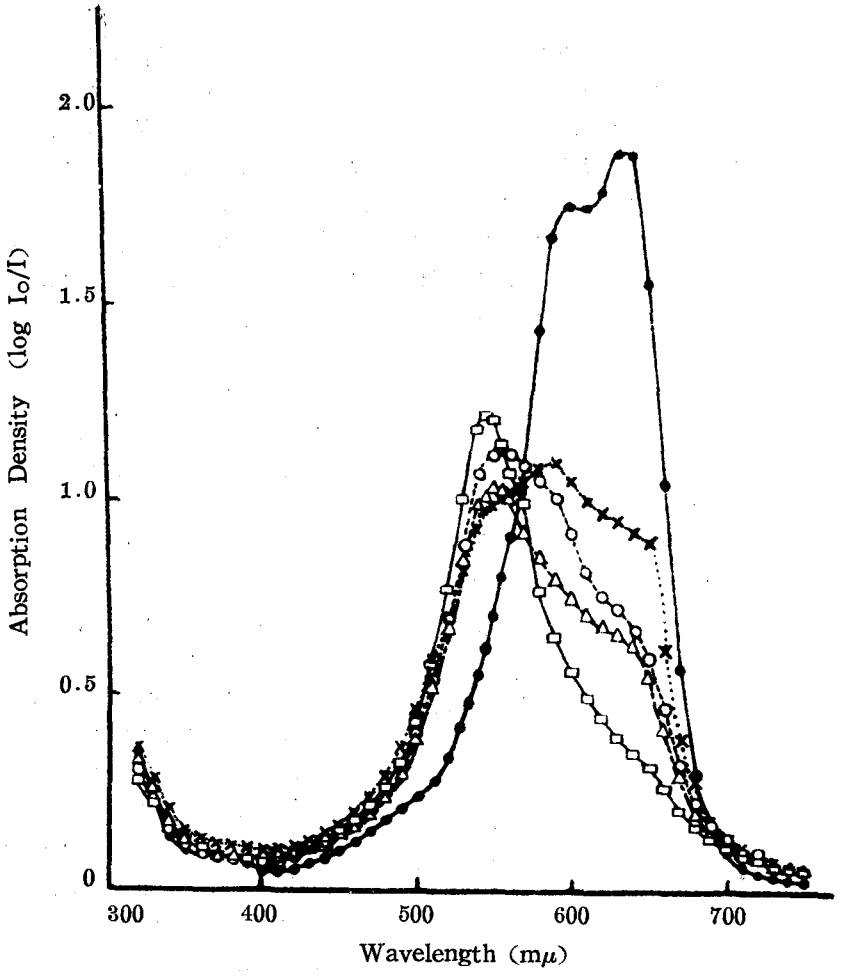

Fig. 2 Absorption Spectra of Toluidine Blue in Water and in Algin Solutions.

●—●—● in H_2O
△---△---△ in 4.6×10^{-5} M/l Na-alginate
□—□—□ in 4.63×10^{-4} M/l Na-alginate
○--○--○ in 4.63×10^{-3} M/l Na-alginate
×···×···× in 2.31×10^{-2} M/l Na-alginate

Table 1. The Intensities of Band Maxima in 2.8 mg/l Solution of Toluidine Blue.

Solvent	α-Band		β-Band		μ-Band	
	mμ	log Io/I	mμ	log Io/I	mμ	log Io/I
H_2O	632	1.810	601	1.753	—	—
4.6×10^{-5}M/l Na-alginate	640	0.625	590	0.954	550	1.122
4.63×10^{-4}M/l Na-alginate	(640)	(0.361)	(590)	(0.651)	544	1.223
4.63×10^{-3}M/l Na-alginate	640	0.620	590	0.802	550	1.020
2.31×10^{-2}M/l Na-alginate	640	0.925	590	1.085	554	0.993

Table 2. The Intensities of Band Maxima in 2.8 mg/dl Solution of Thionine

Solvent	α-Band		β-Band		μ-Band	
	mμ	log Io/I	mμ	log Io/I	mμ	log Io/I
H_2O	595	2.752	560	2.400	—	—
4.6×10^{-5}M/l Na-alginate	600	2.391	564	1.773	505	0.751
4.63×10^{-4}M/l Na-alginate	584	1.012	525	1.604	515	1.583
4.63×10^{-3}M/l Na-alginate	640	0.925	590	1.085	544	0.993

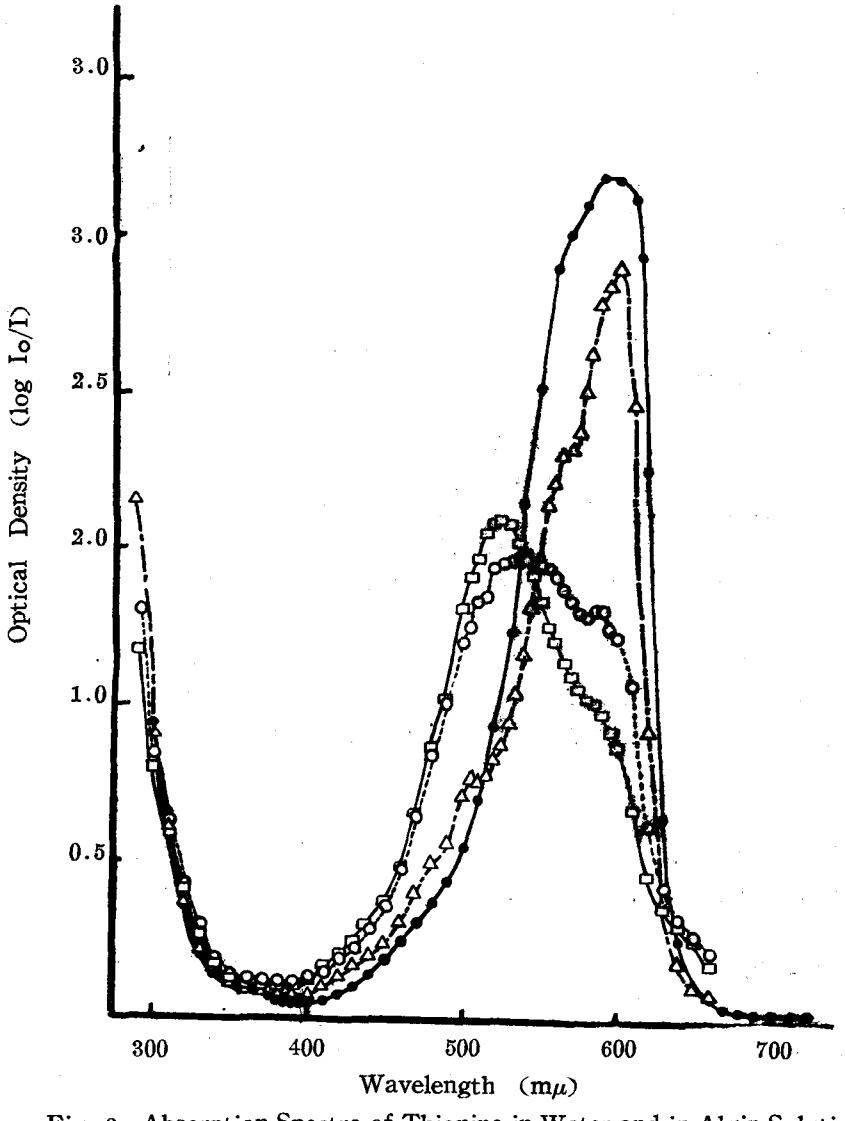

Fig. 3 Absorption Spectra of Thionine in Water and in Algin Solutions.

●—●—● in H_2O □—□—□ in 4.63×10^{-4}M/l Na-alginate
△---△---△ in 4.6×10^{-5}M/l Na-alginate ○--○--○ in 4.63×10^{-3}M/l Na-alginate

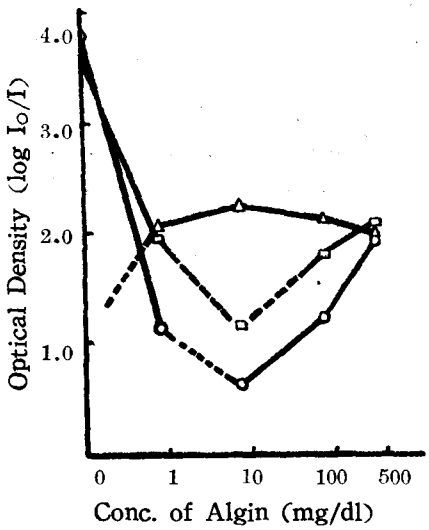

Fig. 4 The Variations in Intensities of Absorption Maxima of Toluidine Blue in Water and in Algin Solution.

○-○-○ α-Band (632~646mμ)
□-□-□ β-Band (601~590mμ)
△-△-△ μ-Band (544~560mμ)

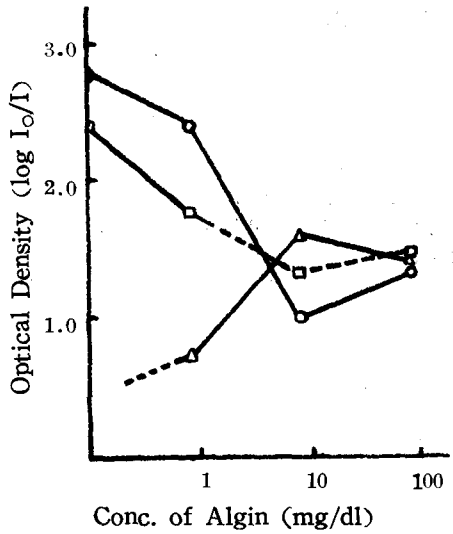

Fig. 5 The Variations in Intensities of Absorption Maxima of Thionine in Water and in Algin Solution.

○-○-○ α-Band (584~600mμ)
□-□-□ β-Band (546~564mμ)
△-△-△ μ-Band (522~530mμ)

observed with a Beckman's quartz spectrophotometer Model DU. Optical density, log I_o/I, where I_o and I are the intensities of the incident and transmitted light, was obtained at 10 mμ intervals of the range from 300 mμ to 900 mμ at wavelength. All the absorption curves were obtained at room temperature (25-27°C).

Previous to observing the spectrum of the dye solution, the absorption spectrum of 0.05M algin solution had been observed, and then it was hardly possible to detect any absorption, but only a slight absorption was observed in the ultraviolet, i.e. 0.02 in optical density at 300 mμ. Consequently, it may be stated that measurement on absorption spectrum of the dye in algin solution is not influenced by the absorption spectrum of the added algin.

Results

The absorption spectra of 2.8 mg/dl toluidine blue in water and in diluted solutions of Na-alginate, are shown in Fig. 2. Table 1 gives the intensities of the band maxima for these solutions.

The toluidine blue in water has two band maxima in the visible, occurring at 632 mμ (α-band) and at 601 mμ (β-band). Addition of algin to the dye reduces the intensities of these bands, while, a new band depending on metachromatic properties develops, and then the color of the dye changes into purple in algin solution in spite of its appearing blue in water.

On absorption spectrum of the dye in 4.6×10^{-5}M/l algin solution, the intensity of α-band at 640 mμ declines to about one-third and β-band is almost annihilated, but a new

one, the μ-band, appears at 550 mμ.

The absorption spectrum of toluidine blue in 4.63×10^{-4}M/l algin solution is very characteriestic. That is, the peak of μ-band at 544 mμ develops progressively, while, both α- and β-band have quite disappeared. With further increasing the concentration of added algin, the intensity of μ-band begins to decrease.

It is remarkable that, in 4.63×10^{-3}M/l algin, the height of μ-band decreases and, on the contrary, that of the α- and β-bands apparently increases.

The absorption spectrum of the dye in higher concentration of algin, 2.31×10^{-2}M/l, where the gel structure develops gradually, shows three peaks of band maxima, viz., α-(at 646 mμ), β-(at 590mμ) and μ-band (at 554mμ).

Fig. 3 shows the absorption spectra of thionine in water and in diluted algin solution. The variations in intensities are summarized in Table 2.

The aqueous thionine solution shows two band maxima, α-band at 595 mμ and β-band at 560 mμ. Addition of algin weakens the intensities of the above bands, but a new band at 530-550 mμ develops in certain concentration of algin as well as in the case of toluidine blue. On the absorption of thionine in 4.6×10^{-5}M/l algin solution, intensities of α-band at 600 mμ and of β-band at 564 mμ decrease slightly, while a new band appears at 530 mμ. The absorption band at 530 mμ has been called μ-band or γ-band, which is characteristic of metachromatic effect.

In the presence of 4.63×10^{-4}M/l algin, the absorption spectrum changes remarkably. That is, the intensity of μ-band increases distinctly at 525 mμ, although both α- and β-band fade and almost vanish. However, when the concentration of added algin is 4.63×10^{-3}M/l the intensity of α-band at 590 mμ becomes higher again, and especially β-band at 546 mμ shows its highest peak of absorption, although the height of μ-band at 530 mμ decreases.

With further increase of the concentration of algin, the dye coagulates, and consequently the spectrochemical observation of absorption spectrum becomes almost impossible.

Discussion

From the above mentioned results, it is plain enough that the presence of about 5×10^{-4} M/l algin in these dye solutions exerts most remarkable influences upon metachromatic change in color of these dyes.

The absorption spectrum of the metachromatically adsorbed dye shows very characteristic properties: The α-band and β-band are almost or even entirely annihilated, and a new band called μ-band appears; in toluidine blue where the α-and β-band are respectively at 632-646 mμ and 590-601 mμ, the new band has its peak at 544-550 mμ, varying slightly according to conditions.

L. Michaelis[3] has given an interesting explanation of the variation of these three bands: The α-band is characteristic of the monomeric dye-cation and corresponds to an electronic oscillator along the x-axis of the dye molecule (the long axis). The β-band is character-

istic of the dimer, and has been interpreted as due to an oscillator in the y-direction (the shorter axis in the plane of the molecule). The μ-band is due to the effect of high reversible polymerization of the dye. However, in the case of metachromasy in the presence of diluted algin solution, it is unlikely that the dye should have been adsorbed in the form of polymolecular dye micells, rather that there might be a monomolecular distribution of the dye over the negatively charged sites of the algin polyion. The absorption spectra of the metachromatic dyes are very sensitive to external fields.

According to the electronic interpretation, metachromasy is due to a deviation in the absorption band to shorter wavelength.

The deviation of the absorption band is due to an increase of energy difference between the stable and the excited state, or, in other words, to restriction of quantum resonance in the dye molecule, and the restriction may depend upon electrostatical interaction between the dye ion and the colloidal ion.

In the experiments, the absorption maximum of μ-band deviated slightly with increase in concentration of added algin, and the wavelength was the shortest in about 5×10^{-4}M/l algin, where resonance of in the dye molecule was restricted firmly, on the other hand, in the above mentioned concentration of algin, the wavelengths of these absorption maxima were rather longer, where the restriction was merely loose.

The above considerations may suggest that the deviation with concentration of added algin influences not only the appearance of the characteristic three bands, viz., α-, β- and μ-band corresponding to reversible aggregation of the dye, but also to slight deviation in absorption maximum of the μ-band, in turn, corresponding to the degree of resonance restriction by algin ion.

In other words, metachromatic effect occurs in sol solution of algin where algin ions would be able to move apart, and the actual number of dissociated radicals in algin will have influence upon both the polymolecular aggregation and the resonance of the dye molecule.

But ionic dissociation of algin is complicated, being due to the colloidal state in aqueous soluton. In an extremely diluted solution of algin, the positive charged Na-ions can diffuse away from the negative polyion, but the individual negative charges move apart only until the chain of the polyion has reached its maximum extension under the influence of their mutual repulsion. As the concentration increases and Na-ions are drawn into the sphere of the polyion, internal repulsions will be compensated, and the normal tendency of the chain to curl up will appear, possibly enhanced by interionic attraction of the sort which stabilizes the salt crystal.

Consequenty, the effect of metachromasy will be distinguished in extremely diluted algin solution, where the actual number of negative charges on polyion will be most abundant.

The relation between the change in color of the metachromatic dye and the colloidal behavior of added algin will be explained by the colloid-chemical interpretation concerning

the dye and algin.

It may be suggested that metachromatic change is very useful as an index for indicating the critical concentration in extremely diluted solutions of polyelectrolyte where the chain of polyion is on the maximum extension under electrostatical repulsion.

Summary

The variation in color of a metachromatic dye, such as toluidine blue or thionine, in algin solution was observed with Beckman's spectrophotometer.

Algin is a typical natural polyelectrolyte, being in various states in its aqueous solution from the viewpoint of colloid chemistry.

According to the results of the experiment, observation of absorption spectrum of the dye solution in the presence of polyelectrolyte, as algin, will be very useful for investigations on colloid-chemical states in the polyelectrolyte solution, especially in extremely diluted condition. That change in color of the dye will be an index for indicating the critical concentration in the polyelectrolyte solution, where the chain of polyion will be on the maximum extension under electrostatical repulsion.

Acknowledgement

The author wishes to thank Prof. N. SUZUKI, Hokkaido University, and Prof. H. TOMINAGA, Tohoku University, for their constant encouragement during the course of this work. He is indebted also to Mr. K. TANII and Mr. E. EBIKO, Tohoku Regional Fisheries Research Laboratory, for the use of a Beckman's spectrophotometer.

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(水産科学研究所業績 第158号)