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INTERACTION OF DYES WITH SUBSTANCES OF HIGH MOLECULAR WEIGHT

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1. INTRODUCTION

In the technically important problem of the interaction of macromolecular substances (polymers) with complicated organic dye molecules, which underlies the dyeing of animal and vegetable fibres, we encounter combinations of various types of polymers and of dyes, both ionogenic and non-ionogenic; each individual case must be expected to have its own mechanism of interaction and its own type of bonding.

Increasing attention has recently been paid to a particular aspect of this problem — the interaction of polymer and dye *ions*, with an electrostatic type of bonding. We come up against this in practical work in various branches of chemistry, for example photographic chemistry in the optical sensitisation of photographic emulsions, histochemistry in the use of staining to differentiate tissues, and finally in the chemistry of surface-active agents in identification of the latter with dyes.

In all cases we are concerned with the same phenomenon — a change in the absorption spectrum (and also in the intensity of fluorescence) of the dye, which produces a change in colour of the material (in histochemistry this phenomenon has been termed "metachromasy" — for reviews see Refs. 1–4). This makes the application of the spectroscopic method of investigation especially fruitful.

In each of the above fields the use of this technique has led to definite advances, but nevertheless difficulties arise in practical work, indicating that insufficient study has been made of the problem. In several cases the results in different fields supplement one another; a detailed comparison of them should contribute to further progress. The purpose of the present review, which examines all the results obtained from a unified viewpoint, is to report the present position of the problem of the interaction of ionogenic high polymers and dyes in the above fields.

The fundamental condition for the formation of new absorption bands in the interaction of polymer and dye ions is that the ions should bear charges of opposite sign. Few data are available on the interaction of anionic dyes with cationic polymers; the combination of cationic dyes with anionic polymers has been studied to the greatest extent, and it is there that the main generalisations have been made.

Before passing to an account of work on the changes undergone by the spectra of dyes on interaction with polymers, we should briefly recall the properties of the most typical polymers (termed "chromotropes" in the literature on metachromasy), and also the spectral properties of the dyes employed.

2. MACROMOLECULAR SUBSTANCES PRODUCING A CHANGE IN THE COLOUR OF A DYE ("METACHROMASY")

Numerous investigations show[3] that, in addition to actual macromolecular substances of high molecular weight, substances of low molecular weight which can associate to form micelles of high molecular weight, are also effective.

Chromotropes of the first type include macromolecular electrolytes[5,6] from various sources; substances of anionic character investigated include polyelectrolytes of animal[7] (heparin†, chondroitin sulphate, hyaluronate, nucleic acids) and vegetable (agar-agar[8], alginates) origin, inorganic (silicates, polyphosphates), and finally synthetic (chitin sulphate, carboxymethylcellulose, polyacrylates). These polymeric substances contain hundreds of ionogenic groups

† Heparin is a substance, formed in the liver, which diminishes the coagulability of blood.

per molecule. The most effective are negatively charged groups: SO_4^- (agar-agar), SO_3^- (heparin), PO_3^- (nucleic acids), COO^- (chondroitin sulphate, hyaluronic acid), CH_3COO^- (carboxymethylcellulose). In most cases they have the structure of linear polyions with periodically repeating simple units. Proteins are amphoteric electrolytes[7,9,10]. Among anionic groups, carboxyls are of greatest value; the radicals of basic amino acids (arginine, lysine, and histidine[11]) act as cationic groups.

Chromotropes of the second type include anionic and cationic surface-active substances[12]: soaps and wetting agents (detergents), both natural[3] and, chiefly, synthetic, having long hydrocarbon chains (C_8–C_{20}) containing ionogenic groups at one end (in anionic substances SO_3^-, OSO_3^-, OPO_3^-, COO^-, with Na^+ ions for electrical neutrality)[13]. Surface-active substances include also the so-called colour couplers, which are necessary ingredients of each of the individual layers in multilayer materials for colour-photography[14].

3. SPECTRAL PROPERTIES OF SOME ORGANIC DYES IN THE DISSOLVED, ADSORBED, AND SOLID STATES

Dyes exhibiting metachromasy are limited to those which are water-soluble and saltlike, the dyeing ions being either positively charged (basic dyes) or negatively charged (acid dyes).

Basic dyes which have been examined in combination with substances of high molecular weight belong to various classes — thiazine, oxazine, azine, triphenylmethane, xanthen, acridine (see Table 2A), and finally polymethine, these last being used in the optical sensitisation of photographic films. Few acid dyes have been investigated (some azo dyes and xanthen dyes of the fluorescein series) (see Table 2B).

Many recent investigations have established that saltlike dyes can exist in different states in aqueous solution — as separate ions ("monomers") and in larger ionic aggregates. It is assumed to a first approximation that a solution contains a mechanical mixture of the various forms in a state of mobile equilibrium.

Each of these states of aggregation is characterised by its own absorption band in the visible region; disturbance of the equilibrium by some factor (the most important of which are temperature and concentration) produces a redistribution of the states with a corresponding change in shape of the curve[15-24] (Fig. 1). The intersection of all the curves in a single point (at 21 000 cm^{-1} in Fig. 1) indicates that only two states of the dye are present.

In some cases (Acridine Orange[21-25], polymethine dyes[26,27]) this situation is changed by an increase in concentration or a fall in temperature (Fig. 2 in Ref. 22, lower curves in Fig. 1), indicating the appearance of new states. Absorption bands corresponding to three states are shown particularly distinctly in the spectrum of several polymethine dyes (Fig. 2). In conformity with the terminology of Sheppard[28], these bands and the corresponding states of aggregation are denoted by the letters α, β, and γ; the β and γ bands are attributed to associated states of the dye.

The availability of a family of curves at different temperatures enables us to determine the heat of association[17,21,27,29-31]; from a series of curves for different dye concentrations we can determine the "order of association"

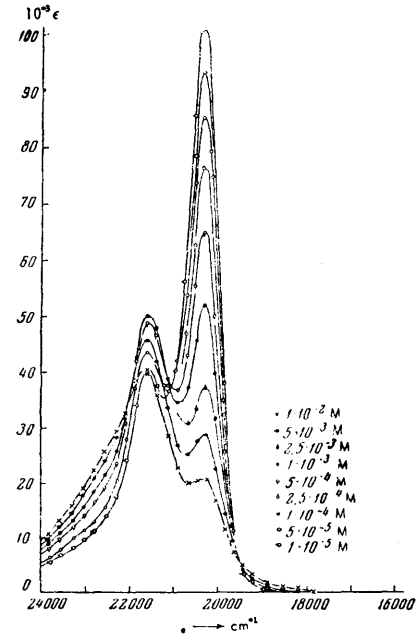


Fig. 1. Absorption curves for ether–alcohol solutions of Acridine Orange at -183° with different dye concentrations[22].

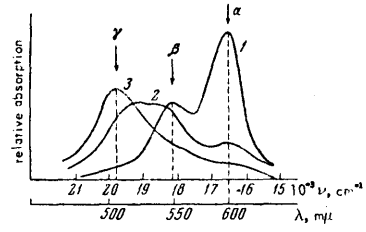


Fig. 2. Absorption curves for aqueous solutions of Pinacyanol chloride at different concentrations (M):
1) 3.2×10^{-6}; 2) 1.2×10^{-4}; 3) 1.8×10^{-3}.[26]

n by application of the law of mass action (Ostwald's dilution law). For the β centres we obtain $n \simeq 2$,[22,26,29] which indicates that they are dimeric in character; the α band corresponds to monomeric ions. The γ bands are produced by larger aggregates.

Sometimes a series of curves exhibit a second common point of intersection (at 22 000 cm^{-1} in Fig. 1), which indicates that centres of a third state are of the same kind. In conformity with Scheibe's view[26,33-40] of the dimer as the main "structural unit" in aggregates, the third state is regarded as tetrameric in these cases[21-23]. The absence of a second point of intersection (which is often observed) indicates the presence of still higher aggregates.

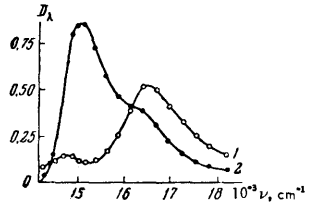


Fig. 3. Optical density curves (optical path 10 mm) for Methylene Blue in aqueous solution at 25°:
1) dimeric form; 2) monomeric form[17].

TABLE 1.

Dye	λ_{max}, mμ				Intensity ratio	Remarks
	β_1	α_2	α_1	β_2	β_1/β_2	
1. Methylene Blue[17,18]	605	610	665	678	1 : 0.3	
2. Thionine[41]	545	—	596	625	1 : 0.2	
3. Acridine Orange[22,25]	465	464	495	500	1 : 0.3	at (−183°C in alcohol solution[22]
4. Pyridocyanine dye[47]	457	457	495	510	1 : 0.2	at 77°K in a mixture of isopropanol and isopentane
5. Rhodamine B[43]	524	—	554	559	1 : 0.7	
6. Fluorescein						with dyes Nos. 6 and 7 dimerisation becomes appreciable only at concentrations of 0.01 M[43]
Ref. 43	472	—	496	505	1 : 1	
Ref. 42	472	—	492	512	1 : 0.	
7. Eosin[43]	495	—	517	522	1 : 0.9	
8. Chlorophyll a[49,50]	648	—	665	682	1 : 2.1	at room temperature in ethanol

From a knowledge of n we can obtain from the same set of curves values of the equilibrium constant (the weight fraction of the dye which is in the aggregated state) for each given case, and also absorption curves for the monomer, dimer, and higher aggregates in a pure form[17,25,41-43].

The absorption curves of the monomer and the dimer are each found to have two maxima (Fig. 3), while those for the tetramer have three.

The subsidiary maximum on the monomer curve, usually observed in the spectrum of a dye in organic solvents (with polymethine dyes two such maxima are observed[26]), is assigned[32] to vibrational transitions accompanying the main electronic transition $0 \rightarrow 0$ (α band). The intensification of these secondary bands ($0 \rightarrow 1$, $0 \rightarrow 2$) with dimers or higher aggregates was used to explain[21,22,25,32,33] the predominance of the corresponding maxima in the spectrum of aqueous solutions. Another view has been expressed recently, according to which[43-46] the excited state splits symmetrically into two, one of higher and one of lower energy than the original state, when two monomeric ions approach one another; the two maxima on the curve for the dimer correspond to these states.

Quantum-mechanical considerations based on crystal-field theory lead to the conclusion[47-49] that possible transitions between the ground and split excited levels are governed by the geometrical arrangement of the molecular planes with respect to the "growth axis" of the aggregate. According to this theory, the dimer will have two bands only when the molecular planes are arranged as in a "house of cards". If the planes are parallel, only one of the two possible electronic transitions is allowed in the two extreme cases (when they are perpendicular and parallel to the "growth axis"), giving a band of shorter wavelength in the first case and of longer wavelength in the second.

Table 1 gives for several dyes the positions of the maxima due to the monomer (α_1 and α_2) and to the dimer (β_1 and β_2), and also the intensity ratios for the maxima β_1 and β_2; the fact that these last are different for dyes of different groups indicates, in conformity with what has been said above, that the dimers have different geometrical structures.

With several dyes the presence of ionic association can also be demonstrated by direct[51,52] physicochemical methods‡.

Thus with polymethine dyes of the pseudocyanine series[54] values of 3–6 have been obtained for n. In such cases the γ band predominates in the spectrum of aqueous solutions of the dye, even at low concentrations; the α band appears only when the solution is heated[320].

Binary association is possible also between ions of different dyes[55,56]; spectroscopic data show[55] that the ability of two dyes to combine with one another is greater the stronger the tendency of either of them to dimerise. Examination of the influence of solvent, temperature, and time of storage of the solutions indicates that the properties of "heterogeneous" complexes are analogous to those of ordinary "homogeneous" dimers.

In addition to selective light absorption, an important optical characteristic of solutions of several dyes is their luminescence. By no means all complicated organic molecules possess this property: of the above dye groups which exhibit metachromasy, only xanthen and acridine dyes luminesce (at room temperature).

A peculiarity of the fluorescence of complicated molecules is the existence of the so-called mirror-image symmetry of the absorption and luminescence spectra (Levshin's law)[15,16] with aqueous solutions of dyes capable of association (this applies to the monomers). In most cases dimers and higher aggregates (β and γ) do not luminesce[25,57], which is the main reason for the extinction of fluorescence with rise in concentration of the dye solution[15,57-61].

At high concentrations (0.01 M) the absorption spectra of aqueous solutions of some cationic polymethine dyes contain, at the longer wavelengths (relative to the α band), yet another absorption band (Fig. 4, curve *3*), which is very narrow (one-sixth of the width of the α band). In conformity with Sheppard's terminology[28], which is adopted also in the Soviet literature, we shall speak of the J band§ and hence of "J" structures.

A characteristic feature of these structures is their ability to luminesce, the position of maximum emission in

‡ With some azo dyes (Congo Red, Benzopurpurin), whose solutions form colloidal electrolytes[13], the degree of association determined by physicochemical methods may reach several hundreds[51,53].

§ This band was observed in an aqueous solution of pseudocyanine almost simultaneously by Jelley[62,63], after whom it was named, and by Scheibe, who, with his coworkers, investigated it in detail[26,33-40].

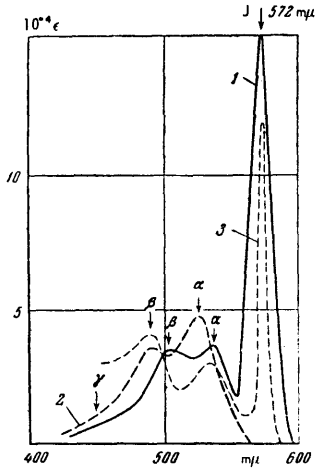


Fig. 4. Absorption curves for pseudocyanine (iodide):
1) in the presence of heparin, dye concentration $C_D = 5 \times 10^{-5}$ M, $C_{hep} = 10$ international units ml^{-1} in 0.16 M ≃ 0.95% ≃ isotonic NaCl solution[126]; 2) $C_D = 5 \times 10^{-4}$ M without heparin[126]; 3) $C_D = 1.05 \times 10^{-2}$ M, in relative units[27].

the spectrum almost coinciding with the absorption maximum¶. On the basis of the work of Scheibe and his collaborators[26,33-40] it is now accepted that these peculiar phenomena are caused by highly aggregated, polymeric threadlike structures formed by dye *ions*†, the presence of which produces a marked changed in the physicochemical properties of the solution (in particular an increase in viscosity leading to gel formation). According to Scheibe, individual ions in this aggregate are arranged in a regular order (like packs of cards); interaction between the π-electron clouds of separate ions arranged parallel to one another results in the formation of a single new cloud for the whole system. The minimum number of ions in such an "ordered aggregate" is of the order of tens and hundreds at room temperature, and about three at -183°.[40] As in the case of "heterogeneous dimerisation", here, too, the absorption resulting from the mixing of two dyes is not additive, a new J band being formed having properties intermediate between those of the bands due to the individual components of the mixture[26,34]. This shows that ions of the two dyes enter alternately into the polymeric structure, as is the case in mixed crystals.

The effect is shown best in the pseudocyanine group of dyes, in which the J bands are most narrow (4–7 mμ) and intense, their position varying from 574 to 604 mμ.[38] With polymethine dyes of other groups (carbocyanines and thiacyanines) the bands are wider (~ 15 mμ) and less intense. Among dyes of other types, only Rhodamine B in supersaturated aqueous solution has been shown to have such a band (of half-width 6 mμ)[43]. Differences in the positions of the maxima observed on passing from one dye to another are linked with the interplanar spacings along the axis of the polymer; the position of the maximum is unaffected by change in the anion of the dye[38], which supports the above view that it is the dye *ions* which polymerise. With rise in temperature the J bands are displaced towards longer wavelengths and become somewhat broader[34,40] (for the effect of low temperatures see Ref. 65). We shall return below to some other peculiarities of J bands which testify to the complexity of the J state, in connection with the properties of the adsorbed and solid states of polymethine dyes, where these characteristics are more strongly developed.

The association of dye ions to form dimers and other (including J) structures is considerably facilitated by the presence of electrolytes, as has been established by both physicochemical[51] and spectroscopic[15,21,54,57] methods. Thus the addition of salt (0.3 M NaCl) to an aqueous solution of Methylene Blue (10^{-4} g ml^{-1}) is equivalent[18] to increasing the dye concentration to 2.5 g ml^{-1}. With a still greater salt concentration (up to 1.5 M) and the same dye concentration the absorption maximum shifts to shorter wavelengths and the common point of intersection disappears, indicating formation of higher aggregates.

The introduction of organic compounds into an aqueous dye solution depresses the degree of association[18], pyridine and quinoline having the greatest effect.

The above association phenomena are most marked in aqueous solutions. It has been stated in several papers[28,38,39,65] that the presence of water is a necessary condition for association, but recent investigations have shown that the latter can be observed also in non-aqueous media[306], but at a different range of dye concentrations. Thus Rhodamine dimerises in water between about 10^{-4} M and 10^{-3} M, in glycerol at about 10^{-2} M, and in ethanol at about 10^{-1} M. At lower concentrations in alcoholic solutions this effect is observed on strong cooling (formation of J bands with pseudocyanine[40] and of dimers with Acridine Orange[22] and a pyridocyanine dye[47] at -183°).

The exceptional influence of water has been attributed to its high dielectric constant[21,22,27,40]. Recent work has shown, however, that the dielectric constant cannot be regarded as the dominant factor in the aggregation of dye ions. In fact, the ability of dyes to associate in single and mixed[19,66,67] solvents may differ greatly even when the physicochemical properties (in particular the dielectric constant) of the solvents are the same: the chemical nature of the solvent is of decisive importance. Furthermore, association, of the same type as in aqueous solutions, is observed both with similar and with different ions in some weakly polar media[41,61] (mixed solvents, for example dioxan + methanol), in spite of their low dielectric constant‡.

The above facts can be explained by intermolecular hydrogen bonding[52,67]. If OH and NH groups are present in the dye molecule, a hydrogen bond is formed[67] directly between its molecules; if such groups are absent, hydrogen bonding can occur *via* the intermediary of water molecules.

¶ Besides this "resonance" emission band, another, weaker luminescence band has been observed[27,35,57,64,65] at about 40 mμ from the first band on its long-wavelength side.

† Jelley[62,63], on the other hand, attributed them to threadlike individual undissociated molecules.

‡ A change in spectrum (redistribution of intensity at the maxima) with change in composition of the binary mixture occurs also with some non-saltlike dyes (for example with porphin colorants in aqueous acetone mixtures), and is also attributed[68] to association of their molecules.

If the structure of dye and solvent molecules is such that hydrogen bonding occurs predominantly between them, the association of dye molecules with one another is made more difficult.

From all that has been said (see also Ref.304) it is clear that the molecular structure of the dye plays an important part, both with respect to the possibility of hydrogen bonding and from the geometrical[69,70] point of view, in association phenomena of all types: indeed, it has been pointed out repeatedly[28,32,36,54,69,71-74] that a planar structure of the dye molecules is the fundamental condition for polymerisation. Quantitative support for the important role of the spatial arrangement of the parts of the molecule has been obtained[27] from a study of the tendency to association (assessed from the association constant and the binding energy of the aggregates) of a group of cyanine dyes.

When a dye capable of giving polymeric structures in aqueous solution undergoes physical adsorption[75-77], corresponding maxima appear in the spectrum of the adsorbate[78-81]. The proportions of the different structures are considerably influenced by the physicochemical properties of the adsorbent surface§ in addition to the solvent. Sometimes new maxima appear on adsorption (at 715 mμ with Methylene Blue)[83,84,305-307].

On adsorption the absorption maxima of dyes are displaced to shorter or longer wavelengths relative to their position in solution. The magnitude of the shift varies between 0 and 50 mμ. Appreciable differences in the shifts produced by adsorption on silver halides can be observed even within the same class of dyes[85,86]. (This holds also for the dyes formed when colour-photographic materials are developed[87].)

Much of the above applies also to J structures. When dyes of the polymethine type are adsorbed on mica, quartz, and other substances (silver halide microcrystals in a photographic emulsion) having negatively charged free groups on their surface, the J maxima usually appear at lower concentrations[37] than in water (10^{-4} M); they are displaced[38] compared with the position of the maxima in aqueous solution, and are also broader and weaker. Several structural details of J centres (the relative position of the ions, the slope of the planes of the ions with respect to the polymer axis, etc.) have been established by Scheibe and his coworkers[33-40] purely on the basis of adsorption experiments (and also on the orientation of the dye in a flowing concentrated aqueous solution). In particular, it has been established that the oscillations of the electric vector corresponding to the J band absorption and fluorescence take place parallel to the polymer axis. This conclusion was confirmed by other work[65], and also by more recent results[88] obtained from measurements of the polarisation spectra of stretched films of poly(vinyl alcohol) coloured with a pseudocyanine dye.

The ability of complex organic substances to luminesce is retained on adsorption. The spectra distribution of the fluorescence of dyes in the adsorbed state[89-91] indicates a shift to one side or the other relative to the luminescence spectrum in solution[89].

The absorption spectra of solid (semi-transparent) layers of dyes on different substrates depend[92-95] on the conditions of application. Their general features correspond to the spectra of concentrated solutions (this holds in particular for films which have been sublimed and examined under vacuum conditions). Sometimes the α and β bands can be traced quite clearly; in addition, bands characteristic of the crystalline state[96-98] appear, and also bands which are probably due to interaction with the substrate.

Thin films of polymethine dyes, in particular the pseudocyanines, also reveal the presence of J centres, in a series of modifications. As in the case of adsorption, the absorption bands of these centres are wider and weaker than in solution; resonance fluorescence is not observed. Depending on the experimental conditions (the presence of water), the separation of crystals of the dye can be observed in addition to the polymeric (ionic) state¶. Films obtained by the evaporation of concentrated solutions show[65,71] a new band of the J type, weaker and located near the α band; the oscillations of the electric vector are in a plane perpendicular to the polymer axis. This band is assigned[65] to fibres formed by parallel and interlinked J filaments. Another example of the complexity of the J state is the presence of a doublet structure in the absorption band observed with solid films of some pseudocyanine dyes[38] (in solution two bands, 10 mμ apart, have been observed only with one of these dyes)[38,39].

A still more complicated situation is found in the spectroscopic examination of a single film[38] of one of the above dyes: the position of the band maximum varies by 3 mμ at different points in the layer, and at the outset (as the film dries out) a band appears at shorter wavelengths, then suddenly changes its position and becomes wider. This phenomenon is influenced by the anion of the dye, indicating that the anion may be incorporated into the polymeric structure (anions must be involved in a solid film, from electrostatic considerations).

A doublet band (with the components 15 mμ apart) has been detected also[99] in the diffuse reflectance spectrum of polymethine (carbocyanine) dyes adsorbed on silver halide grains in a photographic emulsion.

Organic dyes in various states possess well defined photochemical properties[77,100-104].

The transformations of light energy occurring when adsorbed dyes are illuminated find wide application in the sensitisation and desensitisation of photographic emulsions (*e.g.* Refs.14, 77, 105-113).

The relative effectiveness of the various states of dyes in photographic films may vary within wide limits, owing to the above differences in the relative proportions of the different types of structure, depending on the physicochemical conditions at the surface of the silver halide.

A characteristic feature of solutions containing associated ions is their stability: precipitation does not occur even with a high degree of association . Such solutions

§ This gives a very delicate method for investigating the surface of absorbents; it has been used for a long time for the practical analysis and classification of clays[82-84]. On adsorbing such dyes as Methylene Blue, clays from different sources give appreciable differences in shade, caused by differences in intensity of the monomer and dimer bands in the spectrum.

¶ The appearance of a J band when an aqueous solution is "frozen out", as is observed[65] at temperatures up to -195°, should probably be attributed either to the separation of the dye in the solid state or to its adsorption on small crystals of ice.

differ in this respect from those containing aggregates of *neutral molecules*: in the latter case we are dealing with a colloidal solution of the dye, in which are suspended particles having a "phase interface"†; in time such particles separate out from the solution.

The optical properties of colloidal solutions of strongly absorbing substances (metals, dyes) are characterised by selective absorption and, when the particle size is sufficient, selective scattering. The spectral variation of the absorption and scattering can be calculated from the Rayleigh–Mee formulae for the optical properties of colloidal solutions [116-118]. Let us recall the main principles: (*a*) the intensity of both absorption and scattering depends on the particle size: in particular, with sufficiently fine particles scattering is absent, and the solution is optically clear; (*b*) the position of the absorption and scattering maxima, their half-widths, intensities, and also the type of dependence on particle size are entirely determined by the spectral variation and absolute magnitude of the optical constants‡ of the substance in the massive (solid) state. In particular, the positions of the maxima may vary to a greater or a lesser extent with the size of the particles§. When n increases on passing to longer wavelengths, which is the case with most metals, an increase in particle size is accompanied by a displacement of the maximum towards longer wavelengths. When the dispersion of the solid film changes in the opposite sense, an increase in particle size may cause the maximum to shift to shorter wavelengths.

The optical properties of dyes in the solid (massive) state are distinguished by the fact that the absolute values of the refractive index of the solid film are in the same sequence as the absorption coefficient κ_λ, and in conformity with this [116,118], according to the optical laws for colloidal solutions, the position of the bands roughly coincides with that of the maximum κ. In several cases the position of the latter approximately coincides with that of maximum absorption in a true solution; it can then be said that λ_{max} of a true (α) and a colloidal solution are close to one another. Such is the case with Fuchsine, for which the applicability of the Rayleigh–Mee formulae has been verified [118]. We can therefore speak of the similarity of the absorption maxima of colloidal (which we shall denote by the letter K) and true (α) solutions only to the extent that the latter corresponds to the maximum κ_λ. The same considerations apply also to the half-width of the absorption maximum. With polymethine dyes the maxima on the κ_λ curve and also in solution are very narrow; the maximum for the colloidal solution has a similar form.

The experimental preparation of colloidal solutions of readily soluble dyes involves several difficulties. One method [118] consists in adding a drop of a true solution of the dye (in an organic solvent) to a solvent in which this dye is insoluble; then (if the first solvent dissolves in the second) the dye separates in the form of collodial particles having a phase interface. Their size can be controlled by adjusting the proportions of dye and solvents.

There are indications that in some cases new absorption maxima (which are undoubtedly colloidal) have also been obtained directly at high (~0.1 M) dye concentrations.

Table 2, which gives the positions of the absorption maxima of dyes in the presence of macromolecular compounds, includes for some dyes the wavelengths λ_K in colloidal solutions prepared experimentally.

Colloidal solutions of Methylene Blue (Table 2A, II) have been obtained [121] by the procedure indicated above¶. With Toluidine Blue (Table 2A, V) the values of λ_K correspond to a saturated (20 M) solution of the dye in water; the data were obtained by means of a specially developed lenticular cell [122]. With Methyl Orange (Table 2B, I) the dye was added to an organic solvent in which it was almost insoluble [123].

4. SPECTRAL DATA ON THE INTERACTION OF DYES WITH MACROMOLECULAR SUBSTANCES HAVING OPPOSITELY CHARGED IONS

With cationic dyes the spectral changes produced by adding a macromolecular substance of anionic character to an aqueous solution of the dye consist mainly in the appearance of a new absorption maximum at shorter wavelengths† than the α–γ bands; when the concentration of macromolecular compounds is increased, a maximum appears on the long-wavelength side.

Fig. 5 shows a typical gradual change in spectrum of a dye (in this case a thiazine dye) produced by addition of deoxyribonucleic acid (DNA) [124]. A similar pattern is observed with other combinations of dye and high polymer [125-163].

The long-wavelength maximum is generally considerably more intense than the α maximum, and in several cases, but not always, is displaced to longer wavelengths (in the present case by ~10 mμ). Hence it must be different in nature [130-132,165,166] from the α maximum (see Section 5); we shall denote it by the symbol α' (see also Ref. 308).

Fig. 6 shows the variation in intensity of the α and short-wavelength maxima with the concentration of macromolecular substance (see also Refs. 11, 144, 151, 161, and 167–172). It is clearly seen that the range of concentrations of the latter at which the short-wavelength maximum is most strongly developed (the "optimum range") corresponds to the range of the greatest fall in the α maximum.

† Such solutions (which include also the esters of leuco vat dyes [114,115]) are quite different in nature from the above colloidal electrolytes (Congo Red, etc.) [5,13] having large charged micelles.

‡ At the present time values of the optical constants are known for only a very small number of dyes [119]. In particular, data are lacking on the oxazine and thiazine dyes, since in air solid films of these dyes are extremely rapidly converted into colloidal dispersions; the optical constants can be determined (by the method of Kravets *et al.* [120]) only for specular layers.

§ Thus with colloidal solutions of Na (in NaCl) the shift $\Delta\lambda_{max}$ is very large [116], ~20 mμ for a change in particle diameter 2ρ from "0" (the "finest" particles, when $2\rho/\lambda \to 0$) to 20 mμ; with Cu or Au, on the other hand, it is small, the adsorption curve remaining practically unchanged for a change in particle size 2ρ from the "finest" to ~60 mμ [117]. In this respect Fuchsine [118] occupies an intermediate position, λ_{max} remaining unchanged from the "finest" particles to those of ~20 mμ.

¶ When an electrolyte is added in high concentration to a dye solution, a precipitate is sometimes formed ("salting out"). In the case of Methylene Blue this deposit has a purple colour, as do colloidal solutions of this dye [121] ($\lambda_{max} \simeq 545$–560 mμ), which indicates that colloidal particles of the dye have separated out. A similar effect probably occurs in tablets of KBr + Methylene Blue [305].

† Sometimes it may arise on the long-wavelength side of the band (Rhodamine).

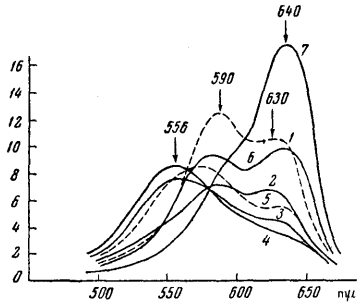


Fig. 5. Absorption curves for aqueous solutions (5×10^{-5} M) of Toluidine Blue in the presence of different concentrations (M) of deoxyribonucleic acid[124]:
1) 0; 2) 2.5×10^{-5}; 3) 4×10^{-5}; 4) 5×10^{-5}; 5) 2.5×10^{-4}; 6) 5×10^{-4}; 7) 2.5×10^{-3} M.

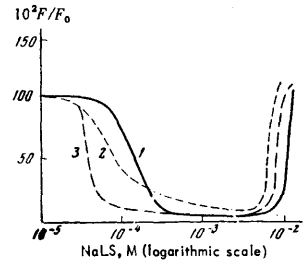


Fig. 7. Dependence of intensity of luminescence of dyes on concentration of NaLS for[150]:
1) Rhodamine 6G; 2) Trypaflavine; 3) Acridine Yellow.

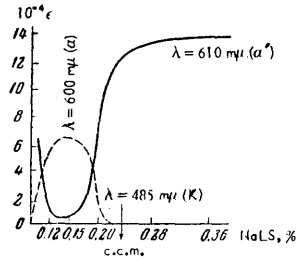


Fig. 6. Variation in intensity of α (α') and K maxima for 10^{-5} M Pinacyanol chloride with concentration of NaLS.[166]

A similar situation is found for bands arising on the short-wavelength side of the α–γ maxima in solutions of polymethine dyes with gradual change in the concentration of gelatin[173]. (In the scientific literature on photography these are termed "H bands"[14,73,173-176].)

A similar pattern is observed for the variation in intensity of the fluorescence of luminescent dyes on gradual addition of a macromolecular substance (Fig. 7)[150], this being parallel to the variation in intensity of the α band[144]. An appreciable increase in intensity of fluorescence (which is approximately doubled) compared with the region of the α band is observed also[131,132] in the region of the α' band. (For the quenching of luminescence at low polymer concentrations, see also Refs. 125 and 177–183).

Spectral data on luminescence are as yet limited to a few dye–polymer systems[184-187,309]. With increasing concentration of the latter, the luminescence band is displaced in the same direction as the absorption band: at first towards shorter, and then to longer, wavelengths compared with the luminescence band of the pure dye, in conformity with the law of mirror-image symmetry; the long-wavelength band should be attributed to luminescence of α' centres, and the short-wavelength band to that of the corresponding short-wavelength absorption centres. The intensity of emission in these bands varies: it is high for α and α' centres, and drops sharply at polymer concentrations corresponding to the formation of the short-wavelength absorption band, which makes it possible to assess the degree of interaction between polymer and dye[311]. With increase in polymer concentration, the degree of polarisation of the luminescence[184] remains low (as in an aqueous solution of the dye) up to a concentration corresponding to the minimum intensity of luminescence, after which it increases rapidly.

The extent of the "optimum" range, within which the short-wavelength maximum is most sharply defined, while the luminescence maximum is weakest, is different in the above groups of macromolecular substances. With surface-active agents combined with a polymethine dye (Pinacyanol) this range (assessed at 0.9 of the intensity of the short-wavelength maximum) is a fraction (0.3 in Fig. 6) of the polymer concentration at which the effect has its maximum development[135-139,166]; with other dyes it is rather wider (by factors of 1.5–2 in the case of a thiazine dye[146], and of 7 with Pyronine[148]). With polyelectrolytes combined with thiazine dyes and with Crystal Violet this region covers a factor of approximately one hundred in polymer concentrations[168,169]; with polymethacrylic acid and Rhodamine a fivefold difference is observed (according to luminescence data[184]). Nucleic acids, with which[124,170,171] the optimum range is very narrow, form an exception in the sequence of these substances. With gelatin and polymethine dyes[173] the width of the optimum concentration range exceeds the concentration at which the effect is a maximum by a factor of ~ 1.5.

Published values for the positions of the maxima with various combinations of dyes and polymers are collected together in Table 2. The most detailed data for most dyes (at different polymer concentrations) have been obtained for surface-active agents and also nucleic acids. With polymethine dyes results for polymers of the polyelectrolyte type, including gelatin, predominate; another group of polymers — the surface-active agents — is represented by the colour couplers used in colour development[212].

Table 2 includes the wavelengths of the maxima — $\alpha(\lambda_\alpha)$, $\beta(\lambda_\beta)$, $\gamma(\lambda_\gamma)$ — in aqueous (or aqueous–alcoholic) solutions of the dyes and, where available, values of λ for the J bands and of λ_K in colloidal solutions. Column 2

TABLE 2. Positions of absorption maxima of dyes in the presence of macromolecular substances*.

A. Cationic dyes + anionic polymers

Substance	λ_{max}, mμ		
	short wavelengths	long wavelengths	α′
1	2	3	4
Thiazine dyes			
I. Thionine, Lauth's Violet (C.I. 52000)**; λ_α = 596 mμ, λ_β = 545 mμ [42,188]			
Nucleic acid [158]	—	—	610
DNA [189,198]	—	—	605, 619
RNA[b] [190]	—	—	617
Mucin	535	—	—
NaLS[c] [146]	510	635–645	602
Na cellulose sulphate [193]	495	—	—
	510—535	635—645	α′—α=6–23
II. Methylene Blue (C.I. 52015); λ_α = 665 mμ, λ_β = 605 mμ [17,18], λ_K = 545 – 580 mμ [121]			
Nucleic acid [130]	—	—	670
$ChSO_4$[d] [162,251]	570	—	—
Agar-agar, Ref. 191	562±3	—	—
Ref. 160	560	—	—
Ref. 1	~565	—	—
Polyacrylate [192]	585	—	—
Polyacrylic acid [192]	590	—	—
NaVin[e] [192]	573	—	—
NaSPVA[f] [192]	567	—	—
KPVS[g]	550—560	—	—
NaLS [146]	(540?) 560	~750	665
Gelatin 2221 [191]	~565	—	—
Gelatin 12350 [191]	~570	—	—
Na cellulose sulphate [193]	565	—	—
	550 590	~750	α′—α=0–5
III. Azure A (C.I. 52010); λ_α = 620 mμ, λ_β = 590 mμ			
RNA [162]	—	—	640
Heparin [129,142,162]	525, 525, 530	—	—
$ChSO_4$ [129,162]	540, 550	—	—
Cartilaginous tissue[h] [127-129]	545, 550	—	—
Hyaluronic acid [129]	(510), 550	—	—
Rice starch [129]	(500)	—	—
Mucoid film[i] [127]	550	—	—
Na pectinate [162]	545	—	—
Metaphosphate [162]	530	—	—
TPBS[j] [162]	(510)	—	—
KSCN [162]	520	—	—
Na cellulose sulphate [193]	513	—	—
	510—550	—	α′—α=20
IV. Methylene Green (C.I. 52020); λ_α = 655 mμ, λ_β = 605 mμ			
KPVS [146]	~550	—	658
NaLS [146]	~530	~720	655
	530, 550	~720	α′—α=0–3

Also: thiazine and oxazine dyes + biological colloids [131,132,193] + inorganic polymers [196,198]; Azure A + KI [162].

V. Toluidine Blue (C.I. 52040); λ_α = 630 mμ, λ_β = 600 mμ [191], λ_K = 540 – 560 mμ [122]

Nucleic acid [158,163]	580	—	640, 648
DNA [124,164]	558, 560	—	640
RNA, Refs. 124, 196	—	—	640
Ref. 196	589	—	650
Heparin [197,198]	530, 520	—	—
$ChSO_4$ [196,199]	545, 550	—	—
Agar-agar, Ref. 191	537± 2	—	—
Ref. 1	540	—	—
Ref. 100	540	—	—
Carragheenin[k] [196]	540	—	—
Cartilaginous tissue [199]	555	—	—
Na pectinate [189]	555	—	—
Na arabate [189]	565	—	—
Na alginate [189]	555	—	—
Amyloid [199]	560	—	—
Silicate [200]	540	—	—
Na_2O, $3.3SiO_2$ [156,157]	535, 560	—	—

TABLE 2 (contd.)

1	2	3	4
Na_2O, Si_2O_5 [191]	558± 1	—	—
Na_2SiO_3 [191]	556± 1	—	—
Hexametaphosphate [130]	520	—	—
Carboxymethylcellulose, Ref. 191	550± 1	—	—
Ref. 158	550	—	—
Polyacrylate [192]	555	—	—
Polyacrylic acid [192]	570	—	—
NaVin [192]	555	—	—
SPVA [192]	540	—	—
KPVS [192]	(490) 540	660	—
Gum arabic [196]	560	—	630
Aerosil [22,188]	(500)	700	635
NaLS, Ref. 146	(490)	700	635
Ref. 163	578	670	638
Na oleate [158,163]	570	—	630
Na arginate[l] [141]	540	—	—
Gelatin 2221 [90]	560	—	—
Gelatin 12350 [90]	575	—	—
NaLS + alcohols [312]	(486—500)	676	635—640
	520—575	—	α′—α=20
Oxazine dyes			
VI. Capri Blue (C.I. 51015); λ_α = 660 mμ, λ_β = 610 mμ [160]			
Nucleic acid [160]	—	—	657
	—	—	α′—α=15
VII. Nile Blue (C.I. 51180); λ_α = 650 mμ, λ_β = 600 mμ [157]			
Na_2O, $3.33SiO_2$ [157]	570	—	—
Na cellulose sulphate [193]	560	—	—
	560—570	—	—
Azine dyes			
VIII. Phenosafranine (C.I. 50200); λ_α = 523 mμ [190]			
Nucleic acid [160]	500	—	540
DNA [190]	?	—	544
RNA [190]	?	—	542
Depolymerised DNA [190]	—	—	(523)
	—	—	α′—α=17–21
IX. Safranine (C.I. 50240); λ_α = 520 mμ [190]			
DNA [190]	?	—	541
RNA [190]	?	—	543
Na_2O, $3.3SiO_2$ [156]	510	—	—
	~510	—	α′—α=21–23
Triphenylmethane dyes			
X. Fuchsine base (C.I. 42510); λ_α = 543 mμ, λ_β = 480 mμ [160], $\lambda_K \simeq$ 500 mμ [118]			
Nucleic acid [160]	—	—	560
Agar-agar [160]	485	—	—
	—	—	α′—α=17
XI. Rosaniline hydrochloride (C.I. 42500, 42510); λ_α = 545 mμ, λ_β = 475 mμ [152]			
DNA [182]	470	590	560
	470	—	α′—α=15
XII. Crystal Violet (C.I. 42555); λ_α = 590 mμ, λ_β = 540 mμ [160]			
Nucleic acid [160]	—	—	600
Heparin [169]	510	—	—
$ChSO_4$ [201,251]	510	—	—
Agar-agar [160]	510	—	—
Cartaginous tissue [199]	512	—	—
Bovine serum albumin [134]	—	—	610
Denatured	—	—	600
Natural			
Na_2O, $3.33SiO_2$ [157]	510	—	—
	510—512	—	α′—α=10–20
Xanthen dyes			
XIII. Pyronine G (C.I. 45005); λ_α = 545 mμ, λ_β = 510 mμ			
KPVS [130,143,181]	465	600	545
NaLS [146]	450	—	550
NaLS	—	—	560
	450—465	600	α′—α=0–15

Also: triphenylmethane dyes + surface-active agents [202] + nucleic acids [203]
pyronine + nucleic acids [204,205].

TABLE 2 (contd.)

1	2	3	4
XIV. Rhodamine 6G (C.I. 45170*); λ_α = 527 mμ, λ_β = 500 mμ [87]**			
DNA [190]	—	—	533
RNA [190]	—	—	536
Na alginate [161]	485	—	—
KPVS [150]	490	560	527
NaLS [149]	~504	565	535
K octyl sulphate [149]	—	570	532
Polymethacrylic acid [144]	510	—	527
	495—510	560—570	α′—α=0—10
Acridine dyes			
XV. Acridine Yellow (C.I. 46025); λ_α = 440 – 445 mμ [188]			
NaLS [149]	<350	490	455
DNA [188]	325—330	—	455—460
	350	490	α′—α=20
XVI. Acridine Orange (C.I. 46005); λ_α = 495 mμ, λ_β = 465 mμ [125], $\lambda_\gamma \simeq$ 445 mμ [26]			
DNA, Refs. 166, 205, 206, 309	—	—	507
Refs. 185, 207	465	—	502
Ref. 310	—	—	504
Refs. 308, 313	464	—	504
Ref. 314	464	—	—
RNA, Ref. 318	464	—	504
Refs. 166, 207	—	—	506
Heparin, Refs. 166, 207	450	—	502
Ref. 125	452	—	—
Poly-C^{m} [133]	435	—	—
Poly-U^{n} [166]	440, 450	—	498
Poly-P^{o} [166]	455	—	492
Poly-A [113]***	464	—	—
	435—465	—	α′—α=3—11
XVII. Trypaflavine (C.I. 46000); λ_α = 450 mμ, λ_β = 432 mμ			
KPVS [146,147]	432?	~500	—
NaLS [144,146]	440	~500	460
Silica gel [186]	—	—	460
	440	500	α′—α=10
XVIII. 3,6-Diamino-10-methylacridine Cl; λ_α = 450 mμ [185]			
DNA, Ref. 190	—	—	470
Ref. 185	430	—	465—470
RNA [190]	—	—	468
	430	—	α′—α=15—20
XIX. 3-Amino-7-dimethylaminoacridine; λ_α = 500 mμ			
DNA [190]	—	—	550
RNA [190]	—	—	550
	—	—	α′—α=50
XX. 2,8-Diaminoacridine sulphate (Proflavine); λ_α = 445 mμ [188]			
DNA, Ref. 208	—	—	462
Ref. 185	425	—	460
RNA [208]	—	—	462
	425	—	α′—α=15—77

1	2	3	4	5	6
Polymethine dyes					
XXI. 3,1′-Diethyl-5,6-dimethyl-8′-chlorothia-4′-quinocarbocyanine iodide; λ_α = 645 mμ, λ_β = 580 mμ, λ_γ = 550 – 560 mμ [191] (alcoholic – aqueous solution)					
Agar-agar [191]	540	—	—	—	
Gelatin 2221 [191]	542	~700	—	—	—
XXII. 1,1′-Diethyl-2,2′-quinocyanine chloride (iodide); λ_α = 524 mμ, λ_β = 490 mμ, λ_γ = 454 mμ, λ_J = 572 [126] (574 [26]) mμ, λ_K = 400 mμ [27]					
Heparin [126]	400	566—572	538	508	—
$ChSO_4$ [126]	?	572	524	488	—
Hyaluronic acid	400?	575	524	490	454
NaPES^{p}	—	572	538	508	—
	400?	566—572	α′—α 0—14	β′—β 0—18	γ′—γ 0

TABLE 2 (contd.)

1	2	3	4	5	6
XXIII. 3-H-3′-Ethyl-4-oxo-5-[(3′-ethylbenzothiazolin-2′-ylidene)isopropylidene]-thiazolinothiacyanine iodide; λ_α = 600 mμ [173], λ_β = 550 mμ [173], J = 650 mμ [173]					
Gelatin 2221 [173]	470?	~650	600	~560	—
Gelatin 12350 [173]	500	~650	?	~550	—
	470—500	650	α′—α 0	β′—β 0—10	
XXIV. Pinacyanol chloride (1,1′-diethyl-2,2′-carbocyanine chloride); λ_α = 600 [26] (610 [136]) mμ, λ_β = 545 [26] (550 [209], 552 [138]) mμ, λ_γ = 510 [26] (522 [209], ≃530) mμ, $\lambda_K \simeq$ 480 mμ [29], λ_J = 635 mμ [27]					
Nucleic acid [189]	—	—	610	—	—
Heparin [160]	~490	640	—	—	—
Agar-agar [189]	480	~640	—	—	—
Silicates [154,155]	478—498	~640—640	—	—	—
TPBS [162]	~490	~650	—	—	~519
NaLS [154,158,167]	480, 185	—	615	570	—
			610	562	
KLS^{c} [136]	480	—	615	570	—
K oleate [136]	467	—	620	575	~537
K myristate [136]	480	—	613	570	—
K octanoate [210]	489	—	607	563	—
K nonanoate [210]	490	—	604	561	—
Gelatin, Ref. 209	~480	~630	(590)	(545)	—
Refs. 40, 211	~490	—	?	~575	—
	467—498	630—650	α′—α 4—15	β′—β 15—30	γ′—γ 9
XXV. 3,3′-Diethyl-4,5:4′5′-dibenzoxacarbocyanine p-toluenesulphonate; λ_α = 545 mμ [191], λ_β = 500 mμ [191], $\lambda_\gamma \simeq$ 480 mμ (aqueous – acetonitrile solution)					
Agar-agar [191]	455	—	—	—	—
Carboxymethylcellulose [191]	450	—	—	—	—
Gelatin 2221 [191]	455	—	~550	~510	—
Gelatin 12350 [191]	460	—	545	500	—
	450—460	—	0—5	0—10	—
XXVI. Pinachrome Blue (6,6′-diethoxy-1,1′-diethyl-2,2′-carbocyanine bromide); λ_α = 615 mμ [191], λ_β = 560 mμ [191], λ_γ = 525 mμ [191] (aqueous – alcoholic solution)					
Agar-agar [191]	503±0.5	—	—	—	—
$Na_2Si_2O_5$ [191]	508±0.5	~650	—	—	—
Na_2SiO_3 [191]	513±0.5	~650	—	—	—
Carboxymethylcellulose [191]	498±0.5	660—670 700	~610—615	—	—
Gelatin 2221 [191]	513±0.5	670—680	~635	~560	~540
Gelatin 12350 [191]	507±0.5	670—680	~635—640	~560	~535—540
	498—513	650—680	α′—α −5±25	β′—β=0	γ′—γ= =10—15
XXVII. 3,3′-Diethylthiacarbocyanine chloride; λ_α = 550 [209] (548 [212]) mμ, $\lambda\beta$ = 505 [209] (515 [212]) mμ, λ_γ = 475 mμ [211] (?)					
Gelatin [209]	?	~580	~560	505	?
Agar-agar [209]	?	~580	?	?	?
Colour coupler^{q} [212,246]	?	?	570	?	?
	58	580	α′—α 10—20		

Also: azo dye + mucopolysaccharides [171].

(Absorption curves for other polymethine dyes in the presence of coloured colour couplers are given in Ref. 212.)

E. Anionic dyes + cationic polymers

Substance	Short wavelength λ_{max}, mμ	
Azo dyes		
I. Methyl Orange (C.I. 13025); λ_α = 507 mμ (acid form), $\lambda\beta$ = 463 mμ (alkaline form) [213], λ_K = 415 – 420 mμ		
β-Lactoglobulin [123]	412	
Human blood serum albumin (cryst.) [123]	433	
Octadecyltrimethylammonium chloride [213]	426	
	412—433	
II. Azo dye^{r}; λ_α = 472 mμ		
Bovine blood serum albumin (cryst.) [214]	440	
Xanthen dyes		
III. Fluorescein (Uranine) (C.I. 45350); λ_α = 490 mμ		
Chitosan chloride [151]	445	—
Glucosamine chloride [151]	~436	—
	435—445	—

TABLE 2 (contd.)

Substance	Short wavelength λ_{max}, mμ	
IV. Resorcinol–benzoin; λ_α = 488 mμ		
N-Methylquinolinium perchlorate [215]	495	
V. Eosin (C.I. 45380); λ_α = 515 mμ		
Chitosan chloride [151]	~480	~533
Glucosamine chloride [151]	~480	~520
N-Methylquinolinium perchlorate [215]	—	~530
Serum albumin	—	530, 539
	~480	displaced by 5–25 mμ

Also: xanthen (phthalein) dyes + proteins; azo dyes + proteins [216-220], + surface-active agents [136,221], + inorganic colloids [222]; xanthen dyes + surface-active agents [136,177,178,223-226], + polyelectrolytes [194,196-200,227-232]. Indigoid dyes + polyelectrolyte [233]; anionic dyes + cationic inorganic complexes and biological materials [234].

* The superscript letters refer to notes below.

** The "Farbstofftabellen" numbers cited in the Russian paper have been replaced by "Colour Index" numbers (Ed. of Translation).

*** This C.I. number and the corresponding Schultz number given in the Russian paper actually represent Rhodamine B; Rhodamine 6G is C.I. 45160 (Ed. of Translation).

**** Ref.113 seems to provide no clue to the nature of "Poly-A" (Ed. of Translation).

Key to superscript letters in Table 2.

a DNA = deoxyribonucleic acid.
b RNA = ribonucleic acid.
c NaLS (KLS) = Na (K) dodecyl (lauryl) sulphate (surface-active agents).
d $ChSO_4$ = chondrotin sulphate (polyelectrolyte).
e NaVin = Na salt of vinyl acetate–maleic acid copolymer.
f NaSPVA = Na salt of sulphonated poly(vinyl alcohol).
g KPVS = K poly(vinyl sulphate).
h Cartilaginous tissue contains mainly $ChSO_4$ [f] and hexauronic acid.
i Mucoid is an animal tissue containing up to 95% of hyaluronic acid.
j TPBS = triisopropylbenzenesulphonate.
k Carragheenin is a substance resembling agar-agar [8].
l Sodium arginate (sodium 2-amino-5-guanidinovalerate).
m Poly-C = polyribocytidylic acid.
n Poly-U = polyuridylic acid.
o Poly-P = sodium polyphosphate glass
p NaPES = sodium polyethylsulphonate.
q Sodium 1-hydroxy-2-(*N*-1-naphthyl-*N*-octadecylcarbamoyl)naphthalene-4-sulphonate (surface-active agent).
r *p*-(*p*-Phenylazo)phenylazophenyldimethylammonium acetate.

contains values of λ_{max}‡ for the short-wavelength maximum, and columns 4–6 for the α', β', and γ' maxima. Nucleic acids are no exception in the sense of yielding a short-wavelength maximum; with Acridine Orange the coincidence of its position with that of the dimeric maximum may give rise to doubt as to its nature§, but its presence in other cases, for example with Toluidine Blue in solution and also on paper chromatograms [235], leaves no doubt as to the theoretical possibility of its existence.

Values obtained by different workers for λ_{max} are identical within the limits of accuracy of the determination (2–3 mμ). More detailed data [191], where each value is based on a series of up to ten independent experiments, show a reproducibility of 1–3 mμ for Methylene Blue and Toluidine Blue, and of 0.5 mμ for Pinacyanol, which possesses a narrower maximum. These results suggest that the different positions of the maxima for a given dye in the presence of different polymers are to a considerable extent real.

It is significant that gelatin is no exception to the sequence of polymers; with solutions of dyes in the presence of gelatin, values of λ_{max} for the short-wavelength band (H band) lie within the limits of scatter of these values for other polymers. This applies not only to polymethine dyes but also to dyes of other classes. With the similarity mentioned above between the concentration dependence for the two systems — polymethine dyes + gelatin (H band) and other dye–polymer combinations — it compels us to acknowledge their identity and to speak of their common nature.

In several cases interaction of basic dyes with high polymers is accompanied by the appearance of a new absorption band in the long-wavelength part of the spectrum beyond the α band¶. As an example, Fig. 8 shows a series of absorption curves for Thionine in the presence of the

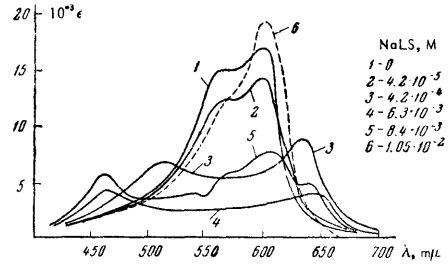


Fig. 8. Absorption curves for aqueous solutions (5×10^{-5} M) of Thionine in the presence of different concentrations (M) of sodium dodecyl sulphate at 17° [146]:
1) 0; 2) 4.2×10^{-6}; 3) 4.2×10^{-4}; 4) 6.3×10^{-3}; 5) 8.4×10^{-3}; 6) 1.05×10^{-2} M.

‡ Values are given for λ_{max} corresponding to the optimum polymer concentration, *i.e.* the shortest wavelengths. As can be seen from Fig. 7, at other concentrations an apparent shift in the maximum to longer wavelengths is sometimes observed owing to superposition of the dimer maximum; if this phenomenon has, nevertheless, occurred, the values of λ_{max} are indicated by the symbol ~.

§ Indeed it has been assumed [314], on the basis of adsorption and luminescent data, to be dimeric in nature.

¶ The colour caused by the long-wavelength bands (greenish in the case of dark blue dyes) is attributed in the literature to "negative" metachromasy" [1,189], in contrast to "positive" metachromasy, which is associated with the short-wavelength maximum. The spots on paper chromatograms of DNA stained with Toluidine Blue and Azure A [235] have a reddish violet tinge at the periphery, and are greenish in the centre, which indicates a spatial separation of the substances responsible for the corresponding absorption bands in the case of DNA (with RNA only a greenish tinge is observed).

surface-active agent sodium dodecyl sulphate. Here the long-wavelength maximum ($\lambda \simeq 640$–650 mμ) arises together with the short-wavelength maximum (curves *3* and *4*) at some intermediate ("optimum") concentration; at higher concentrations both maxima give way to the α′ maximum (curve *6*). Positions of the long-wavelength band for several other cases are given in column 3 of Table 2A; with thiazine, xanthen, and acridine dyes it is observed only in the presence of surface-active agents and nucleic acids. The maximum is not usually sharp, appearing in several cases merely as a shoulder on the absorption curve. With polymethine dyes the long-wavelength maximum is observed also in the presence of polymers of other types, including gelatin. In some cases (the pseudocyanine XXII in Table 2A) a narrow band is present, coinciding in position and character with the J band (Fig. 5, curve *2*). (We may compare also the long-wavelength bands for carbocyanine dyes arising in the presence of surface-active colour couplers[212].) With other polymethine dyes (Table 2A), especially in the presence of gelatin, this band, coinciding in position with the J band in aqueous solution, has the same diffuse character that other dyes show in the presence of surface-active agents and nucleic acids.

The complexity of the J state, exhibited in the variety of spectral curves, has been mentioned above; there is every reason to suppose that the same state is involved in all the cases of the appearance of a long-wavelength band which have been examined†. In conformity with this, the long-wavelength bands occurring in the presence of polymers will be denoted by the symbol J′, in contrast to such J bands in solution and on adsorption on a silver halide. The J′ bands are narrow and intense only with dyes of one type, the isocyanines and carbocyanines; with all other dyes they are more or less diffuse.

Extending the analogy with the absorption bands observed in photographic emulsions, we must identify the α′ bands with the B bands[209].

5. NATURE OF ABSORBING CENTRES FORMED IN THE INTERACTION OF DYES WITH MACROMOLECULAR SUBSTANCES HAVING OPPOSITELY CHARGED IONS

All investigators (*e.g.* Refs. 2, 3, 162, 173–175) agree that the short-wavelength maximum is due to aggregated dye structures produced in combinations of a cationic dye with an anionic polymer as a result[125,126,152,162,164,166,195,239,308] of adsorption of positively charged dye ions on negatively charged terminal groups of the polymer. The distance between neighbouring groups is usually of the order of 3–5 Å, and since no electrostatic repulsion is present, owing to the mutual neutralisation of charges, the electron levels of dye molecules situated side by side and held together by the polymer substrate coalesce. Compact neutral dye particles are obtained, which are the same as the colloidal particles (with a "phase interface") discussed above.

Comparison of the short-wavelength absorption maxima of Methylene Blue and Crystal Violet in the presence of polymers with the spectra of colloidal solutions of these dyes has shown their similarity[121]. The coincidence of λ_{max} for the short-wavelength absorption band with λ_K is found also with some other dyes (Table 2A, dyes V, X, and XXII)‡.

All these results support the above attribution to the colloidal nature[240] of these absorption bands, which we shall therefore term K bands. This is also supported by direct observations. Thus a strong yellowish green luminescence is observed[200] when dye solutions containing polymers are examined in the ultramicroscope; this luminescence may change shade in various ways, as with colloidal solutions of gold, for example.

Some spectral phenomena accompanying the interaction of polymers and metal ions must also be discussed in terms of the formation of colloidal particles. Thus the spectrum of the complex formed by copper ions with imidazole[241] (λ_{max} 278 and 580 mμ) agrees with that of colloidal solutions of metallic copper[117] and differs sharply from the spectrum of copper ions (λ_{max} 700 mμ); all these phenomena are characteristic of poly-*N*-vinylimidazole[242] ($\lambda_{max} \sim 630$ mμ) and of bovine serum albumin[243].

A similar phenomenon to the possibility of aggregation of ions of different kinds, both with dimers and with J centres (Section 3), is observed also in the interaction of dyes with polymers: a mixture of two dyes in the presence of a polymer gives not two but one K maximum, with properties intermediate between those of this maximum for each dye separately[196]. This fact completely fits the above hypothesis of the formation of K particles.

Complete coverage of all the end-groups of the polymer molecules takes place at a definite ratio of polymer and dye concentrations. Several investigations show[124–129,132,152,164,168,169,176,221,235,244] that the maximum effect occurs at the stoichiometric (1 : 1) ratio of numbers of charged groups in dye and polymer; thus for one molecule of heparin[125,126], containing about 50 SO_3H end-groups, there are, under optimum conditions, on the average 50 (between 35 and 75) dye molecules (Acridine Orange, diethylpseudocyanine). Taking the distance between the end-groups as 4 Å, we obtain a value of ~20 mμ for the size of the colloidal dye particle.

The polymer concentration corresponding to the above stoichiometric ratio is in fact the above-mentioned optimum concentration for the formation of K particles; its value should obviously depend on the dye concentration[167]. This is shown very clearly by results[184] obtained on the quenching of the luminescence of a dye at different concentrations: the values of the above optimum concentration for a polymethacrylate are 10^{-2}, 10^{-3}, and 5×10^{-4} M at Rhodamine 6G concentrations of 10^{-4}, 10^{-5}, and 10^{-6} M respectively.

At a given dye concentration the values of the above optimum concentration (in practice we shall always be dealing with a more or less wide range of optimum concentrations) are different for different polymers. This forms the basis of a method for differentiating histological tissues. A

† In the scientific literature on photography, the long-wavelength bands observed with polymethine dyes in the presence of gelatin have long been assigned to the J state[73,74,176,209,236].

‡ Conversely, in cases where the aggregates causing a given band in the dye spectrum are unknown, their colloidal nature can be assessed from the coincidence of λ_K with λ_{max} for the short-wavelength band in the presence of polymers. Such is the case with the weak band at ~480 mμ in an aqueous solution of dye XXIV[103], which is revealed when the solution is illuminated (these structures being significantly more stable to light than the structures α–γ).

necessary condition for the application of this method, which is not always observed, is to vary the dye concentration [245] between wide limits, so that it is possible to cover both the optimum and adjacent ranges§.

Further increase in the polymer concentration diminishes the probability of covering all possible adsorption centres, and polymer molecules may be present on which are adsorbed dye particles containing smaller numbers of molecules, right down to three, two, and finally one. The spectrum of these dye structures adsorbed on polymers should differ from the spectrum of the dye ions in the absence of polymer (see above, Section 4). In fact, we have seen above (Table 2A, columns 4–6) that at high polymer concentrations the α' bands differ from the α bands¶. By analogy we shall speak also of β' and γ' bands. They can readily be identified for polymethine dyes (see Fig. 4). Thus the α', β', and γ' centres should be attributed to monomeric, dimeric, and polymeric structures linked with the macromolecular substance (the type of bonding in the β' and γ' centres is still not clear).

We should take the same view of the nature of the absorption bands in the case of the interaction of anionic dyes with cationic polymers. Here, too, the short-wavelength maximum coincides with the colloidal maximum (Table 2B, dye I); the long-wavelength maximum should probably be attributed to an α' band†.

Interaction of thiazine dyes and anionic polymers results in the appearance of further bands — in addition to K and α' (β', γ') bands — having $\lambda_{max} < \lambda_K$ and $\lambda_{max} > \lambda_{\alpha'}$. The nature of the former must be linked with that of the so-called "red form" of Methylene Blue ($\lambda_{max} \sim 506–523$ mμ), which has been detected in several systems [78,79,237,238,315,316]. It has been ascribed [315] to an isomer of the dye. The latter bands, which we have denoted by the symbol J', should be assigned to ordered aggregates of *ions* of the dye combined with the macromolecule. This is an important difference between these bands and the K bands, which are caused by the aggregation of neutral dye molecules. The difference in character is illustrated by a difference in the photochemical activity of these structures: in the sensitisation spectra of photographic emulsions, H(K) bands are often hardly in evidence [86,111–113,305]‡, whereas J(J') structures are most effective.

In all the cases examined above we are dealing with polymer–dye complexes. The mutual saturation of the hydrophilic groups of the two components of the complex markedly reduces the solubility, with separation of a precipitate§ in the case of both K and J' structures. With K structures the deposit comes down in the form of films (agar-agar [121], gelatin [191], chondroitin sulphate [251]) [317], filaments (carboxymethylcellulose [191], carragheenin [252], nucleic acids [217,253]), and granules [253], which are more or less deeply stained the colour of the K structures.

The complex will not pass through a regenerated cellulose membrane [167]; it has been isolated by centrifuging, dissolving the precipitate in alcohol, and recrystallising. With Pinacyanol + sodium dodecyl sulphate, large needle-shaped monoclinic crystals were obtained; chemical analysis showed that, as with other dye–polymer systems, their composition did not entirely agree with calculation. With K particles separation of a coloured precipitate is observed at some intermediate relative concentrations of polymer and dye [1,3,125,169,173,198,200,239,251] (there are indications [2,11,127,171,183] that this takes place at the stoichiometric ratio of charged groups in dye and polymer mentioned above). The formation of a precipitate (sometimes immediately the solutions of polymer and dye are mixed, sometimes over a period of time [318–320]) occurs as a result of the molecules, deprived of their charge, combining with the dye adsorbed on them (perhaps by hydrogen bonding [254]); individual coloured polymer molecules may remain suspended in the solution for a long time, revealing themselves optically by an absorption spectrum with a K maximum and also by a Tyndall cone [200,239,318].

6. CONDITIONS FOR COMPLEX FORMATION BETWEEN MACROMOLECULAR SUBSTANCES AND DYES HAVING OPPOSITELY CHARGED IONS

The primary condition for the formation of polymer–dye complexes with aggregated dye particles (K or J' structures) is the presence of polymer molecules having a sufficiently large number of charged groups.

At low concentrations surface-active agents exist as individual ions [12,255]. With rise in concentration the physicochemical properties of the solution (electrical conductivity, surface tension, viscosity, specific volume) undergo sudden changes [255] within definite intervals of concentration, outside which these properties remain constant. Thus with sodium dodecyl sulphate changes have been noted [255] at the concentrations 0.005–0.007, 0.019–0.021, 0.028–0.030, and 0.05–0.07 M. A similar pattern is observed with other wetting agents which have been investigated [255,256]. Among these intervals, one (in the case of sodium dodecyl sulphate 0.028–0.030 M) is outstanding, the changes in physical properties being most marked in this range. The existence of such a concentration range was noted long ago as a characteristic feature of solutions of surface-active agents; it is attributed to the formation of micelles [255,257], which are in thermodynamic equilibrium with the molecules or ions forming them. The comparative narrowness of this

§ Actually, differences in the shade or colour of the luminescence between different regions of a preparation can be expected only if the relative dye and polymer concentrations correspond to the optimum concentration in one region and to either the α or the α' range [205,206,245] in the other region; if conditions correspond to the same condition in both regions, the change in colour is insignificant, which may lead to incorrect conclusions.

¶ With several polymethine dyes, interaction with colour couplers is accompanied [212,246] by the appearance (sometimes together with a long-wavelength band of the J type) of a band which has been displaced by 10–15 mμ to longer wavelengths compared with the α band (for example, dye XXVII in Table 2A). In the original papers this band is denoted by the letter *k* and is attributed to the adsorption of dye on "colloidal particles" (*i.e.* micelles) of the component, which is fully consistent with the above views. On this basis we shall include these bands in the α' type.

† The coincidence of its position with that of the long-wavelength maximum of the dimer (Table 1) is probably fortuitous.

‡ The spectral contiguity of the H (K) and γ (γ') bands may lead to their being confused. This is probably the case in Refs. 72, 85, and 113: feebly active states denoted as H II are obviously K states, while the more active are γ states.

§ The fact that a water-insoluble precipitate comes down when oppositely charged ions interact is widely known; in particular, it occurs when solutions of anionic and cationic dyes are mixed [247], and also with macromolecular substances having oppositely charged groups (heparin + albumin at pH < 4.5 [248], polyelectrolyte + surface-active agent [249], see also Ref. 250).

range enabled it to be regarded as practically a single concentration, the so-called critical micelle concentration (c.c.m.), the values of which for a series of surface-active agents[13] lie within the limits 10^{-4}–10^{-1} N. Recent investigations indicate[255], however, that the process of association in solutions of surface-active agents begins at considerably lower concentrations. The subsequent ranges are linked either with further development of this process (stepwise association) or with a change in the character of the micelles resulting from their rearrangement.

It follows from the above that the picture of micelle formation in such a solution is still far from clear; this also makes it difficult to examine conditions for the formation of a complex between surface-active agent and dye. It was at first suggested that, since the change in colour of a solution or the extinction of its fluorescence (caused by separation of K complexes) takes place in the region of the critical micelle concentration, these optical phenomena were caused by micelle formation.

This view forms the basis of a spectroscopic method for determining c.c.m. which has found widespread use[135-139,210,258]. The concentration of surface-active agent which corresponds to the maximum change in the optical characteristics of the dye is determined. As noted above, this corresponds to the formation of polymer–dye complexes; thus in essence the spectroscopic method records[167] the separation not of pure micelles but of their complexes with an aggregated dye.

To what extent these two processes coincide is still obscure. Values of c.c.m. determined by physicochemical methods prove[225] to be smaller in the presence of a dye: *i.e.* a dye facilitates micelle formation. Values of c.c.m. obtained by the spectroscopic method are usually also somewhat smaller[139,167,259,260] than when physicochemical methods are used. Probably, however, the separation of surfactant–dye complexes, if it does precede the separation of pure micelles, is not very intense¶, so that, for a rough estimate of the concentration of micelle formation, the optical method, which is experimentally considerably simpler than most physicochemical methods, is nevertheless worthy of attention.

The separation of dye–surface-active agent complexes in other regions of stepwise association is also not understood (results[167] show that this can occur: the optimum region for the separation of K complexes corresponds to the second of the critical concentration ranges for sodium dodecyl sulphate).

A detailed study of the absorption spectra of surface-active agent plus dye solutions in all the regions of stepwise association should provide important help in clarifying these matters; it might give information on the nature and the structure of the aggregates in each concentration range.

In solutions of polymers of the polyelectrolyte type and at low concentrations, macromolecules having a large number of charged groups are formed; it is to be expected that polymer–dye complexes will be precipitated also on great dilution of the polymer solutions. Since these are masked spectroscopically by the predominant free dye molecules, the lower limit of the optimum concentration range should be indefinite, as is in fact observed. The structure of the macromolecules (in the sense of the type of link between the monomeric units) should be of considerable importance. This structure may change in the presence of a dye, since the electrostatically neutralised macromolecules will be coagulated, which is a general characteristic of neutral polymer molecules[192,231,232]. Structural rearrangements may well be possible with this type of polymer too (this is indicated by the presence of a second region of separation of K particles[168]). Thus stretched molecules of polymethacrylic acid[184] are able to react with one another to form characteristic aggregates comprising "packs" of molecules. Further increase in the concentration results in the production of "globules".

The degree of homogeneity of the polymer solution should play an important part. Actually, we are always concerned in practice with a mixture of macromolecules having different degrees of polymerisation[6], and there are indications that the active centres in a polymer molecule are heterogeneous: nucleic acids[208] and serum albumin[262] each show two groups of such centres, each of which is characterised by its own bonding constant.

Examination of the picture sketched above for the formation of polymer–dye complexes shows that the structure of the monomeric units in both polymer[6] and dye is very important. In fact, if dye ions are to combine into a single whole, they must be arranged sufficiently close together. (For the J structure this minimum distance has been determined as 3–5 Å.[63,73,263].) The three-dimensional structure of the dye molecule therefore has a similarly important role in the separation of aggregates in the presence of a polymer[171,190,193,208,264] to that in polymerisation in solution, which has been discussed above.

Replacement of methyl by ethyl groups at nitrogen atoms in a dye is known to prevent the formation of H(K) centres[173]; not only the nature of the substituents but also their position is of substantial importance[86].

In the polymers it is necessary that the distance between neighbouring charged groups should correspond to the minimum† mentioned above (in other words, there should be a minimum density of charge on the surface of the polymer molecules)[2,73,162]. The flexibility of the polymer molecules would have some influence, by allowing the distance between the charged groups to fit the required distance between the planes of the dye molecules[165,166]. A quantitative indication of aggregating power is given by the intensity ratio of the α' and K bands[165,166,304,313]; this "packing factor" depends appreciably on the molecular structure of the polyanion.

The intensity of the effect (with the same dye at a constant concentration) is proportional to the total quantity of complexes which separate in unit volume[171]: *i.e.* it is determined by the total number of charged groups per unit volume[2,162,194,214] of the polymer solution. This number

¶ A further cause of uncertainty in the optical determination of c.c.m. is that, as mentioned above, the separation of K centres takes place at stoichiometric proportions of dye and polymer, and consequently depends on the dye concentration[167,184]. (More definite results are to be expected from a direct optical method — from the ultraviolet absorption spectra of solutions of surface-active agents[261].)

† Metachromasy, *i.e.* the separation of K particles, is absent in dilute solutions of hyaluronic acid, in which the distance between the COO groups is 10.3 Å; it appears only in gels or films of this substance[162]. The effect is less marked in synthetic polynucleotides than in the natural derivatives, where the distance between the phosphate groups is less[265].

depends on the degree of dissociation of the ionised groups in the polymer, which is determined mainly by the pH of the solution, being low at low pH and high at high values. This explains why metachromasy is not usually observed at pH < 3–4 [1,2,164,192,194,208,266] (it becomes more intense with increase in pH [261]). In solutions of polymers containing strongly ionised groups (sulpho, quaternary nitrogen), on the other hand, pH has practically no effect [227] (with heparin the effect is observed also at pH ~2 [198])‡.

The total number of groups is affected by the configuration of the polymer molecules; thus the separation of H(K) centres is more intense and starts at lower concentrations in a more hydrolysed gelatin [173].

The degree of polymerisation of the polymer has no influence on the intensity of the effect [164,194,268] above some minimum degree of polymerisation (in the case of polymetaphosphates, eight PO_3 groups [206] or $M > 3000$ [268]). The effect remains unchanged also during the aging of polymers [268] (polymetaphosphate), when some of the side chains split off, and under the action of ultrasonic waves [268]. It is not entirely clear how the intensity of this effect depends on the nature of the groups.

The number of charged groups (charge density) in the polymer molecule (or group of molecules forming a single common substrate for the dye particle) determines the size of the particle and hence (see Section 3) the position of the maximum λ_K. We have seen above (Table 2) that values of λ_K for the same dye in the presence of different polymers may vary by 15–30 mμ. Calculation (largely tentative) shows that such differences correspond to changes in diameter of the dye particle from 20 to ~80–120 mμ, and hence to changes in the number of charged groups from ≃50 to 200–300. The charge density for several polymers of the polyelectrolyte type changes [230] within similar limits (by factors of 3–5). However, in order to assess the possibility of such differences in any actual case, a more detailed idea of the structure and shape of the micelles of the given substance is needed (some information is given in Refs. 184, 269–271, and 321).

Examination of the degree of polarisation of luminescent dyes in the presence of polymers should have an important role in this connection. Study of the interaction of dyes with native and denatured polymers is promising [308,309,313,314]. In the case of nucleic acids there is some suggestion of the formation of complexes (intercalation [322]) of inclusion compounds. The different cases of the interaction of dyes with proteins have indicated [272–276] that denatured proteins, whose molecules are uncoiled, are far more active [277] than the native proteins, which have coiled molecules (see also Refs. 180–182), while fibrillar proteins are more active than globular proteins.

The structure of polymers should influence also the shape of the colloidal particles.

7. PHYSICOCHEMICAL PROPERTIES OF COMPLEXES OF MACROMOLECULAR SUBSTANCES AND DYES HAVING OPPOSITELY CHARGED IONS

The stability of a polymer–dye complex can be influenced by all the factors which weaken or strengthen the bonds in the complex.

Numerous experiments show that, when a polymer solution containing K complexes is heated to 60°–70°, [2,132,149,152,159,164,171,192,198,199,230,266,278,279,319] and also when alcohols and other organic substances are added to it, [2,3,125,126,162,164,199,230,240,278,280] the K maximum disappears from the spectrum of the solution, the α and β maxima becoming stronger. Corresponding to this, the fluorescence also changes. The effect of heating is reversible: on cooling, the absorption in the region of the K maximum is more or less reestablished [319]. This effect indicates rupture of the bonds holding ions of the dye and the polymer, a colloidal dye particle being being split off. The thermal stability of the complexes depends on the nature of the polymer [319].

The mechanism by which organic compounds of low molecular weight act on the polymer–dye complex is not yet clear; it is obvious that, as with the action of heat, the primary effect consists in fission of the polymer–dye bond. Different polymers require different quantities of alcohol to break down the colour centres [280]; the effect is stronger in alcohol–water mixtures [278].

Compared with the solutions, the precipitates are very stable towards both heat and organic solvents. The solubility of the precipitated complexes§ is of importance in the study of the metachromasy of biological materials, but the literature contains only isolated data of a qualitative character. Thus the water-insoluble complexes of cationic dyes and anionic polymers are soluble in alcohol–pyridine [121] and alcohol–water mixtures [73], chloroform [3,142], ether [142], alcohols [167], and benzene [225]; a good solvent for a cationic surface-active agent is also a good solvent for the complexes which it forms with anionic dyes [220].

The polymer–dye complexes are considerably affected by salts¶. In the interaction of a cationic dye with an anionic polymer, small concentrations (up to 0.1 M) of salts [168] break down existing complexes [2,125–127,137,154,155,162,177,192,195,208,230,231,279,283]; the effect increases with increase in valency [154,195] of the inorganic cation, but the anion is of no significance [230]. The stability of the complex is governed to a large extent by the above-mentioned surface charge density of the polymer; it falls in the sequence of groups: sulphate, phosphate, carboxyl [230]. A similar phenomenon has been noted for complexes of an anionic dye with a cationic polymer when organic acids are added to a protein–dye mixture.

‡ Ionisation also governs [184] the shape of the macromolecules in solution: at low pH they are coiled up, and with increasing degree of ionisation become extremely unsymmetrical. A decrease in pH in breakdown of the packs with the formation of globules. The possibility of the structure of a protein changing with pH has also been pointed out [10,217].

§ A method for the analytical determination both of the combined dye and of the polymer is based on precipitation of the complexes. When a solution of the polymer and the dye is shaken with petroleum ether, the complex separates at the interface between the solution and the solvent; absorptiometric measurements on the solution before and after this operation give the quantity of combined dye [281]. The polymer concentration can be obtained by extracting the complex, dissolving it in a suitable solvent, and making absorptiometric measurements on the solution [220,282].

¶ Simple ionic salts are implied; inorganic and organic polymers behave similarly in the presence of dyes (see Table 2, A and B).

In both cases we are dealing with competition, which is very general in physical chemistry[284], between ions of the dye and of the salt (or aromatic acid)[216]. A similar phenomenon is observed when to a polymer–dye solution is added a second polymer having similar ions to the first (silicate + + surface-active agent[155], polyelectrolyte + surface-active agent[177], nucleic acid + inorganic colloid[285]), where the ions of the two polymers compete for the dye ions. This effect occurs also when proteins are added to solutions of anionic polymers[2,3,126,197,228,286], especially if the protein is strongly basic (protamine). The polymer–protein complex is obviously less stable than the polymer–dye complex, and therefore certain agents (ascorbic acid, heating to 90°)[286] reestablish the K band†

The dispersing action of proteins on salt solutions of several dyes[276] should also be regarded as competition (between ions of the dye and of the protein) for the other ion of the dye.

At low pH the formation of K complexes is difficult (Section 6); but once precipitated, the complexes prove to be very stable[220] with respect to pH, since owing to loss of water they are unable to interact with hydrogen ions in the environment.

Complex formation has a considerable influence on the reactivity of dyes. Thus with indicators of the phthalein series, addition of a protein to an acid solution of the dye restores the primary form of the latter[183]. The time required for reduction of dyes by various reducing systems is appreciably increased in the presence of polymers[233]; this is possible when the ions of dye and polymer are of different kinds, but is not observed when they are similar.

Dyes are considerably more stable to light in the presence of polymers than in pure solution[163,171,182,214]; the photochemical stability of the complexes depends on the nature of the polymer[319].

The depressant action of colour couplers on the degree of optical sensitisation[212,246,324] with some dye–coupler combinations should probably[14,287] also be explained by bonding of the dye.

Great resistance is observed also towards "alkaline" fading; the bleaching of triphenylmethane dyes at pH ~ ~10–12 is markedly diminished in the presence of anionic surface-active agents, and is intensified on addition of cationic surface-active agents[202].

8. OTHER CASES OF INTERACTION BETWEEN MACROMOLECULAR SUBSTANCES AND ORGANIC MOLECULES

(*a*) Interaction between Ionogenic Polymers and Organic Substances Having Oppositely Charged Ions

This covers the interaction of cationic surface-active agents (pyridinium salts) with the dye Congo Red[288,289], an aqueous solution of which contains large micelles and is a colloidal electrolyte. In neutral solution the dye has an absorption band at ~500 mμ; on addition of a surface-active agent a new band appears at 460–470 mμ, and at high concentrations of the agent (0.25 M) a maximum becomes visible at 530 mμ. A water-insoluble precipitate separates over a definite range of concentrations of the surface-active agent (0.5–5 M depending on the dye concentration). The same final effect is observed also in an acid solution of the dye: addition of the surface-active agent changes the colour of the solution from blue to orange red (λ_{max} = 470 mμ); at a definite (optimum) concentration a precipitate is formed. The general picture of the phenomenon fully corresponds to that described above; it is assumed that the ions of the surface-active agent interact with a sulpho-group in the dye. However, the nature of the complex formed when both components are micellar in character is obscure[290].

Another example from this field is the interaction of serum albumin[291] with dinitrobenzene derivatives (of anionic type). Combination of the albumin with a dinitroaniline derivative displaces the absorption maximum of the latter (350–370 mμ).

In the manufacture of photographic colour films we encounter[87] the interaction of gelatin with indoaniline and azomethine dyes formed in the colour development of these materials. The absorption maxima of these dyes are displaced in the presence of gelatin (compared with an alcoholic solution) by up to 30 mμ on either side; these shifts cannot be explained as resulting from a change in polarity of the medium and in the polarisability of the dye molecules. In some cases the addition of gelatin produces a new maximum on the short-wavelength side, which can reasonably be assigned to an aggregated state of the dye. All these facts indicate that these dyes interact with the gelatin; a knowledge of the nature of the resulting complexes is of fundamental importance for a rational development of colour-sensitive photographic materials.

The same type of interaction occurs also between molecules of two polymers having oppositely charged ions. An example is the formation of mucoproteins, which are complexes of the positive groups of a protein and, for example, heparin or chondroitin sulphate[248]; the physicochemical properties of these complexes (in particular the stoichiometric proportions 1 : 1) are the same as in the above cases of the interaction of polymers and dyes. The absence of spectroscopic data prevents us, however, from assessing the nature of these complexes (isolated α′ or aggregated K).

(*b*) Interaction between Non-Ionic Polymers and Dyes

This question is of practical importance in connection with surface-active agents, in view of the possible application of the spectroscopic method to the determination of critical micelle concentrations.

Experiments with several dyes in the presence of polyethylene glycol and laurylethyl alcohol[292,293] show the absence of precipitation and, in the case of fluorescent dyes, extinction of the luminescence; no new maximum can be observed on the absorption curves. With Pinacyanol the spectral changes produced by increasing the concentration of surface-active agent consist in an increase in intensity of the monomeric maximum (α) at the expense of the dimeric (β), with a shift in these maxima to longer wavelengths. With Erythrosine (C.I. 45430) there is only a displacement of the maximum.

It can be concluded from all these results that, in contrast to the above cases of interaction between dyes and

† There may be other explanations of the action of proteins (steric masking of anionic groups, formation of a covalent bond between hydroxyl groups of the protein and anionic groups of the polymer)[3].

oppositely charged polymers, no polymer–dye complexes are formed in the present case; the shift in the maxima is to be attributed to a " solvent effect".

Other workers[289,294], who have investigated the interaction of polyethylene glycol and of several other neutral substances (polyvinyl alcohol, several surface-active agents) with an azo dye, speak of the formation of complexes: $mD + nS = D_mS_n$,[294] where D is a dye molecule, S a polymer molecule, and D_mS_n a molecule of the complex; with polymers n/m tends to $\frac{1}{2}$.

In several cases polyvinylpyrrolidone can also be regarded[182,215,263,295,296] as a non-ionic polymer, its solutions being used as an artificial substitute for blood plasma; other data[7,250], however, indicate that this substance possesses feebly basic properties, and hence should interact with anionic dyes. This has received experimental confirmation: physicochemical[263,295,296] and optical (luminescence[215]) methods have established the formation of complexes between this polymer and these dyes.

(*c*) Interaction between Dyes and Complexes of Two Macromolecular Substances

Ternary systems of this type are met in investigating the properties of complexes of surface-active agents with polymers[297-300], in particular the solubilising power of surface-active agents[12], which is changed by this interaction. For this purpose fat-soluble dyes are employed; quantitative data on their solubilisation are obtained spectroscopically. Changes have been noted in the absorption spectrum of the dye, which indicates that its molecules are incorporated into the complex.

(*d*) Interaction of Dyes with Macromolecular Substances by Formation of a Covalent Bond

Dyes of a new type (the so-called reactive dyes[301]) form covalent bonds with polymers; their use is continually extending for various kinds of colouring, in particular in histochemistry[302].

A covalent bond has also been detected between proteins and triphenylmethane dyes[275,276,303]; here the dye adds on to thiol or amino-groups in the proteins, with the result that a new absorption band appears in the ultraviolet region of the spectrum, close to the band due to the carbinol base. Proteins prove to be more reactive when denatured than in the native state.

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