

26. *Fluorescence Quenching by Colloid Anions and Cations.*

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The fluorescence of solutions of certain basic and acidic dyes is quenched by the addition of small quantities of colloid anions and cations respectively. The magnitude of the effect, and its reversal by electrolytes are described. Possible explanations and applications of the effect are discussed.

AQUEOUS solutions of fluorescent dyes lose their fluorescence on concentration (Perrin, *J. Chim. physique*, 1928, **25**, 531) or on the addition of electrolytes, (Kortüm, *Z. physikal. Chem.*, 1938, *B*, **40**, 431). With particular groups of dyes, for example, the polymethine dyes (Scheibe, *Kolloid-Z.*, 1938, **82**, 1), the quenching of fluorescence has been shown to follow the aggregation of the dye molecules in solution. Bowen (*Quart. Reviews*, 1947, **1**, 1), in a review of work on fluorescence quenching, expresses the view that generally the monomeric dye is fluorescent whereas aggregates of two or more molecules are non-fluorescent.

Rabinowitch and Epstein (*J. Amer. Chem. Soc.*, 1941, **63**, 69) drew attention to the similarity between dyes and soaps; both contain large hydrocarbon portions to which strongly polar groups are attached, and many dyes show, in concentrated solution, the changes in viscosity and conductivity characteristic of the formation of colloidal aggregates in soap solutions.

A phenomenon similar to the quenching of fluorescence of dye solutions on concentration or the addition of salts is the metachromatic effect. Certain basic dyes, for example, toluidine-blue

and thionine, change in colour when treated in aqueous solution with a colloidal anion, for example, heparin or agar-agar. Michaelis and Granick (*ibid.*, 1945, **67**, 1212) have shown that this colour change also occurs when solutions of metachromatic dyes are concentrated or treated with electrolytes. They attributed the effect to the aggregation of the dye molecules by the colloidal anion, and demonstrated that the absorption spectrum of the monomeric dye differed from that of the aggregated dye. Similar to the metachromatic effect is the quenching of fluorescence of dye solutions by colloidal ions. The most familiar example of this phenomenon is the use of fluorescein-type dyes to indicate the charge on silver halide sols in the volumetric analysis of halides with silver nitrate (Fajans and Wolff, *Z. anorg. Chem.*, 1924, **137**, 221). There appears, however, to be no record of quantitative measurements of the quenching of fluorescence by colloid anions and cations.

During investigations of the properties of water-soluble, strongly acidic or basic colloids a convenient method for their analysis was required. The metachromatic colour change of basic dyes provided a basis for the analysis of colloid anions; the dyes were not, however, affected by colloid cations. Colour changes were noted on similar treatment of many acidic dyes, but no dye was particularly suitable for colorimetric estimation. It was noted, however, that the fluorescence of fluorescein and similar dyes was completely quenched by small concentrations of colloid cations. Similarly the fluorescence of rhodamine and other basic dyes was quenched by colloid anions.

It was found convenient to use the fluorescence-quenching properties for analysis of the colloid ions and for examination of their behaviour in solutions. A detailed description of these uses will be given later.

The quenching of fluorescence by colloid ions resembled closely the metachromatic effect, and like it appeared to be caused by aggregation of fluorescent monomeric dye ions by the colloid ion to form non-fluorescent aggregates. Unlike the metachromatic effect, however, the quenching of fluorescence was extremely sensitive to the presence of electrolytes. For example, the fluorescence of dichlorofluorescein quenched by a colloid cation could be restored by a concentration of a trivalent anion as low as 10^{-6} M.

The phenomenon of fluorescence quenching by colloid ions showed, therefore, features of general interest quite apart from its particular uses in the analytical methods mentioned above. A limited experimental examination of the quenching of fluorescence of dichlorofluorescein and Rhodamine 6G by colloid cations and anions respectively was therefore made. This paper describes these experiments and discusses some possible explanations of the phenomenon.

EXPERIMENTAL.

Measurement of Fluorescence.—Fluorescence and its quenching were measured in a simple fluorimeter which consisted of a 100-w. projection lamp, the light from which was passed *via* a lens through a boiling-tube containing the dye solution. Light scattered by fluorescence was measured by means of a barrier-layer-type photoelectric cell connected to a galvanometer. This instrument was calibrated against known concentrations of the dyes.

Materials.—The dyes used were dichlorofluorescein as the free acid, and Rhodamine 6G as chloride, of the grade normally used in adsorption titrations. Dichlorofluorescein was dissolved in 0.1% aqueous pyridine to avoid changes in fluorescence caused by contamination of the solution with traces of acid on the surfaces of the glassware. This precaution was not necessary with Rhodamine 6G, which was dissolved in distilled water.

Colloid Ions.—The colloid anion used in these experiments was sodium polyvinyl sulphate, prepared by treating polyvinyl alcohol with chlorosulphonic acid-pyridine (Lintot, *Arch. Biol.*, 1934, **46**, 509). The product was purified by repeated dissolution in water and precipitation with ethanol. The final material was a hygroscopic white powder, in which approx. 50% of the hydroxyl groups of the polyvinyl alcohol were esterified.

The colloid cation used was poly-(1-methyl-2-vinylpyridinium iodide). This was prepared by exposing redistilled 2-vinylpyridine to ultra-violet light in a quartz cell at room temperature for several days. The polymer produced was separated from unchanged monomer by gently warming the mixture *in vacuo* and was used in the next stage without further purification; the polyvinylpyridine was dissolved in 2 moles of methyl iodide at room temperature and the methiodide slowly precipitated during about 24 hours; unchanged methyl iodide was decanted and the quaternary salt purified by repeated dissolution in water and precipitation with acetone; during the later stages of the purification, the polymer tended to form a sol in the acetone and it was necessary to add a small quantity of potassium nitrate to assist precipitation; the final product was a hygroscopic yellow powder; titration with silver nitrate indicated that 70% of the polyvinylpyridine units of the molecule had been converted into quaternary salts.

RESULTS.

In view of the rather uncertain composition of the colloid ions the strengths of their solutions are expressed in terms of equivalence rather than as mass per unit volume. One equivalent of

sodium polyvinyl sulphate or of poly-(1-methyl-2-vinylpyridinium iodide) is defined as that quantity possessing 6.02×10^{23} ionogenic groupings.

Fig. 1 shows the quenching fluorescence of dichlorofluorescein by poly-(1-methyl-2-vinylpyridinium iodide), and of Rhodamine 6G by sodium polyvinyl sulphate; the concentration of the dyes is plotted logarithmically and is calculated on the assumption that the fluorescence remaining in the solution after successive additions of colloid anion or cation is due to free non-associated dye. It is realised that compounds formed by the colloid ion with the dye or with the associated dye may well have some fluorescence, but the assumption that they have none was thought justified since in both cases the fluorescence of the mixture was reduced almost to zero when sufficient colloid ion had been added. The diagrams show that the reaction occurs in two stages. Up to the addition of 2—3 equivalents of colloid ion fluorescence is quenched until less than 5% of the free dye remains in solution. Further additions cause a slow restoration of fluorescence.

It will be shown later that the addition of salts to the dye-colloid ion complex restores the fluorescence of the solution. It is likely, therefore, that the restoration of fluorescence following the addition of an excess of colloid ion is caused by a similar salt effect.

Effects of Salts on the Dye-Polyion Complex.—Fig. 2 shows the effect of adding various salts to a mixture of dichlorofluorescein and poly-(1-methyl-2-vinylpyridinium iodide). The salts used in this series of experiments all possessed univalent anions. All produced a restoration of the fluorescence, presumably by dissociating the dye-colloid ion complex. But three main groups of salts all restoring fluorescence in a different manner are discernible. First, low-

FIG. 1.
The quenching of fluorescence of Rhodamine 6G ($\times \times \times$) and dichlorofluorescein ($\circ \circ \circ$) by sodium polyvinyl sulphate and poly-(1-methyl-2-vinylpyridinium iodide) respectively.

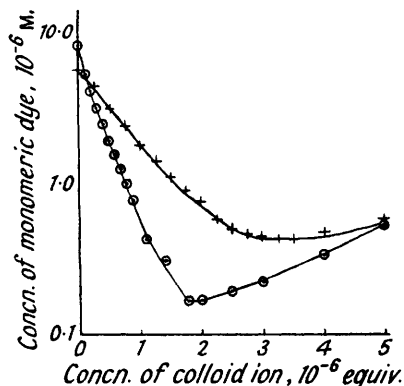
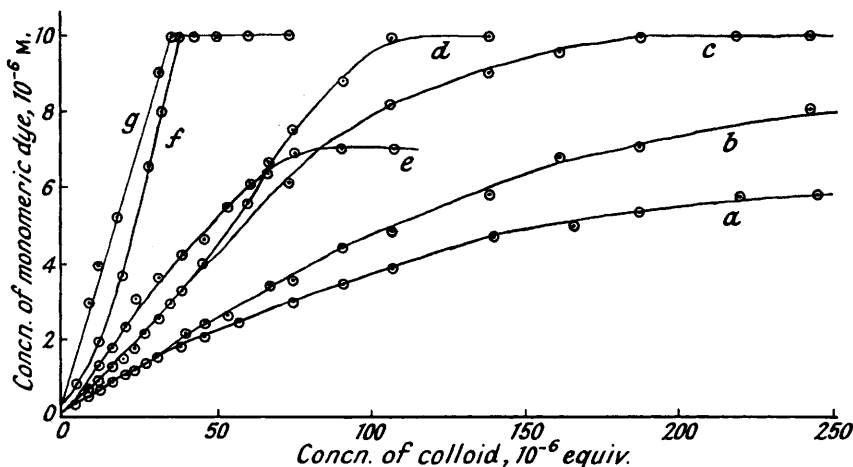


FIG. 2.

The restoration of fluorescence by the addition of univalent anions to a mixture of dichlorofluorescein and poly-(1-methyl-2-vinylpyridinium iodide). a, Sodium acetate; b, sodium chloride, c, potassium chloride; d, lithium chloride; e, sodium iodide; f, sodium oleate; g, sodium cetyl sulphate.



molecular-weight salts of strong acids and strong bases, *e.g.*, sodium chloride, potassium bromide, lithium chloride, with which the restoration of fluorescein anion from the complex proceeds until all, or nearly all, the dye has been liberated. Secondly, salts of strong bases with weak acids of low molecular weight, with which the liberation of dye from the complex is incomplete. Thirdly, salts of strong bases and high-molecular-weight acids, with which there is a stoicheio-

metric combination between the salt anion and the colloid cation leading to the liberation of all the dye when one molecular equivalent of salt has been added.

FIG. 3.

The restoration of fluorescence by the addition of bi-, ter-, and quadri-valent ions to a mixture of dichlorofluorescein and poly-(1-methyl-2-vinylpyridinium iodide). a, Magnesium sulphate, 10^{-4} M.; b, potassium oxalate, 10^{-4} M.; c, sodium sulphate, 10^{-4} M.; d, potassium ferricyanide, 10^{-5} M.; e, potassium ferrocyanide, 10^{-6} M.; f, sodium citrate, 10^{-4} M.

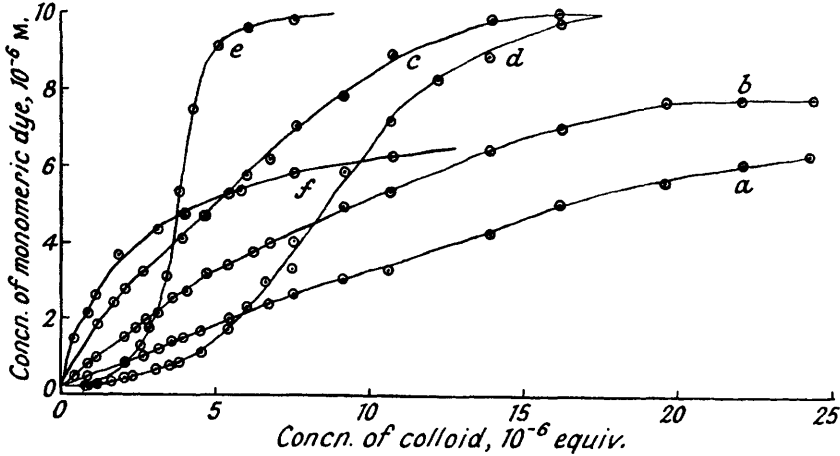


Fig. 3 shows a comparison of the effects of adding uni-, bi-, ter-, and quadri-valent anions to the dye-colloid ion complex. The salts used in this series were all of strong bases and strong acids of relatively low molecular weight. The restoration of free dichlorofluorescein from the

FIG. 4.

The use of dichlorofluorescein as an indicator of the state of aggregation of benzylhexadecyldimethylammonium chloride.

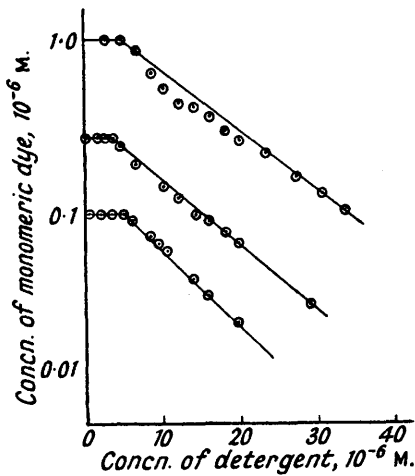
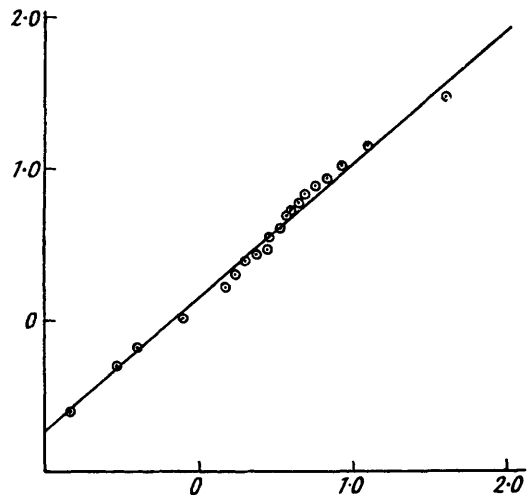


FIG. 5.

The relation between the proportion of fluorescence quenched by unit quantity of poly-(1-methyl-2-vinylpyridinium iodide) and the concentration of dye remaining in solution in a fluorescent state (ordinate). The data for this relation are derived from Fig. 1 and are plotted in logarithmic units.



complex occurs with increasing ease as the valency of the anion is raised. The ratios of the concentration of anions of various valency necessary to restore fluorescence to the same extent falls much in the same order as the concentrations of the same salts necessary to precipitate negatively charged colloids. The restoration of the fluorescence of Rhodamine, from its complex with

polyvinyl sulphate, by cations was closely similar. The relationship between cation concentration and the extent of fluorescence restoration depended on the valency and size of the cation used.

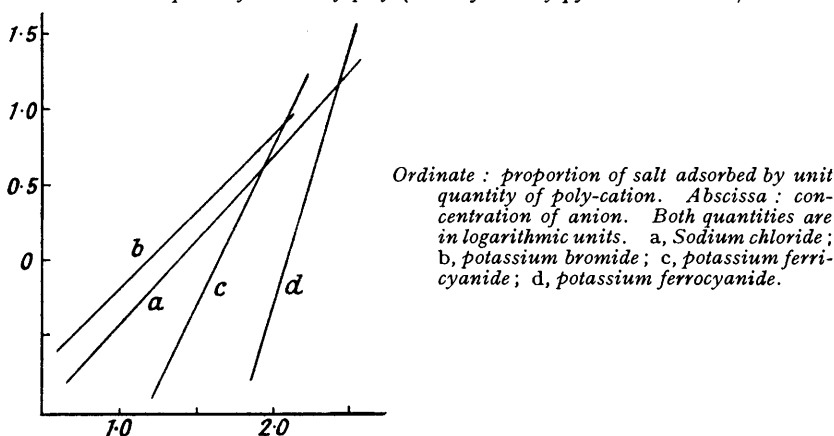
Suppression of Fluorescence by Detergents.—Fig. 4 shows the suppression of the fluorescence of dichlorofluorescein by benzylhexadecyldimethylammonium chloride: after a certain limiting quantity of the cationic detergent has been added, an effect occurs closely similar to that given by the colloid cation. It is well known that detergents above a certain limiting concentration exist in solution as colloidal aggregates. Such aggregates are obviously similar in structure to the colloid ions. The concentration of detergent at which fluorescence quenching commences is the same as that at which an abrupt change in conductivity occurs (Hartley and Runnicles, *Proc. Roy. Soc., A*, 1938, **168**, 420). This observation, and the indication from Fig. 2 that the limiting concentration of detergent at which quenching occurs is independent of the dye concentration, suggest that measurements of this type might prove useful in studying the behaviour of detergent solutions. Michaelis (*J. Phys. Coll. Chem.*, 1950, **54**, 1) has shown a metachromatic effect to occur with anionic soaps and basic dyes and similarly suggests the use of the latter as indicators of the colloidal state of anionic detergent solutions. The fluorescence of Rhodamine 6G is, as might be expected, quenched by anionic detergents.

DISCUSSION.

The changes in absorption spectra of the metachromatic basic dyes on reaction with colloid anions have been attributed by Michaelis and Granick (*loc. cit.*) to the aggregation of the dye molecules by the colloid ions. The fluorescence-quenching phenomena described in this paper resemble the metachromatic effect and could similarly be explained by the aggregation of fluorescent dye molecules by the colloid ions. The ready reversal of fluorescence quenching by ions of the same sign as the dye ion suggests, however, that adsorption of the dye by the colloid ion, rather than aggregation, is responsible for the fluorescence quenching. Fig. 5 shows the quenching of fluorescence of dichlorofluorescein by poly-(1-methyl-2-vinylpyridinium iodide) plotted to illustrate the relation between the proportion of dye "adsorbed" by a unit quantity of colloid cation and the dye concentration remaining in solution. The linear relation over a wide range of values is in accord with the Freundlich adsorption isotherm.

FIG. 6.

The adsorption of anions by poly-(1-methyl-2-vinylpyridinium iodide).



Regardless of the exact mechanism of fluorescence quenching, it is probable that the restoration of fluorescence by salts is caused by adsorption of the added ions by the colloid, leading either to its inactivation as a quencher of fluorescence, or to the displacement of adsorbed dye. Fig. 6 shows the relation between the proportion of various ions adsorbed by poly-(1-methyl-2-vinylpyridinium iodide) and the concentration of the ions. The proportion of the ions adsorbed by the colloid was calculated on the assumption that the quantity of ion adsorbed was equivalent to the quantity of dye restored to a fluorescent condition. The linear nature of the relation appears to confirm this assumption.

This interaction between the colloid ions and the fluorescent dyes appears, therefore, to be

sufficiently slight to enable the latter to be used as internal indicators of the charge on colloid ions. Michaelis's suggestion that metachromatic dyes would be of value as internal indicators of the colloidal state of detergents is thus confirmed and extended to other colloid types.

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