The source, purification and analysis of methylene blue have already been described³.

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Metachromasy

MANY dyes when used to colour certain tissue constituents (chromotropes) absorb light of wave-length shorter than that absorbed by the dyes in solution¹. Similar metachromatic behaviour is exhibited in solution when the dyes interact with soluble chromotropes, for example nucleic acids², polyphosphates³ and gelatine⁴. Metachromasy may also be induced in solution in the absence of chromotropic substances in three different ways⁵: by increasing the dye concentration (dye-induced), by adding salt (salt-induced) and by lowering the dielectric constant of the solvent from medium (about 20) to low (about 3) values (solvent-induced).

The phenomenon is exhibited only by dyes in which the characteristic ionic charge is an integral part of the chromophoric system and is distributed throughout the system by resonance, that is, most common basic dyes and a few acid dyes. The following discussion is therefore restricted to basic dyes, but the same arguments apply *mutatis mutandis* to dyes, such as fluorescein, with a distributed negative charge.

Metachromasy in solution has recently been attributed primarily to interaction of the dye ions and counter-ions⁵, although in the past interaction of dye ions themselves was generally considered to be responsible. Usually the colour change is small and the hypsochromic (*H* -) band in the visible spectrum is removed by only about 50 mμ from the normal (*M* -) band; the interacting dye ion and counter-ion are considered to be separated by solvent molecules. In solvents of low dielectric constant⁶ (and certain strongly basic solvents⁶) strong interaction occurs to form an undissociated species in which the ions are in contact and not separated by solvent molecules. In this case the colour change is large, for example, solutions of methylene blue become red and, indeed, a red form exists in crystals of the dye⁷.

In organic solvents of moderate dielectric constant, for example simple alcohols, dye-induced metachromasy (at room temperature) only occurs at extremely high dye concentrations, but occurs at increasingly low concentrations as the dielectric constant is lowered (see ref. 5 and other references quoted therein). This is in keeping with electrolytic dissociation theory. In solvents of high dielectric constant, long-range electrostatic forces are smaller and solvation effects are relatively more important⁸. The marked metachromatic effects observed in water are attributed to the inability of water to solvate the large organic residues in the dye ions. The dye ions therefore tend to aggregate and the aggregation of organic ions is known to be associated with the uptake of counter-ions^{9,10}.

The foregoing concepts can also explain metachromasy induced by interaction with chromotropic substances either in tissues or in solution. Basic dyes exhibit metachromasy when they interact with negatively-charged substrates; the colour change is more marked the more strongly acidic the active groups (sulphate > phosphate > carboxylate)¹. In solution, metachromasy of cyanine dyes induced by interaction with gelatine is only observed at a pH above the iso-ionic point of gelatine⁴. Ionic interaction of the dye ion with an ionic group in the substrate must clearly alter the charge distribution in the dye ion and, as described previously⁵, this produces a hypsochromic spectral shift. We therefore believe that metachromasy induced by chromotropes should be attributed primarily to ionic interaction with the substrates, even though there is evidence¹¹ that basic dyes tend to aggregate when they interact with biological substrates. The aggregation requires uptake of counter-ions to reduce electrostatic repulsion between the dye ions and this will have an additional effect.

The interaction of a metachromatic dye and a chromotrope in solution often exhibits a curious phenomenon. As increasing amounts of the chromotrope, for example polyphosphate³ and gelatine⁴, are added to a solution of dye, the *M*-band of the dye is gradually replaced by the *H*-band. However, if still more chromotrope is added, until it is well in excess of the dye, the *H*-band gradually disappears and is replaced by a band at the same or slightly longer wave-length than the original *M*-band. This phenomenon is probably related to the behaviour of 1,1'-diethyl-2,2'-carbocyanine chloride in the presence of soaps, for example potassium laurate or oleate¹². At the dye concentration used the dye absorbed light mainly in the *H*-band ($\lambda = 553$ mμ) and partly in the *M*-band ($\lambda = 610$ mμ). Both these bands diminished as soap was added and were replaced by a new band at much shorter wave-lengths ($\lambda = 480$ mμ), comparable in fact with those associated with absorption by the undissociated species of the dye insolvents of low dielectric constant⁵. When more soap was added, however, this band disappeared sharply and was replaced by a band at 610 mμ, in fact the normal *M*-band; the sharp spectral change occurred at the critical micelle concentration of the soap¹². It is well known that the solubility of dyes is increased in the presence of soap micelles¹³ and indeed there is evidence from diffusion data that sulphonated anthraquinone dyes form mixed micelles with surface-active agents¹⁴. We suggest that the basic dye ions are incorporated in the micelles of the soap and the aggregates of the chromotrope in such a way that they are effectively in a Faraday cage and are therefore subjected to a uniform electrical field. The charge distribution and thus the electronic configuration of the dye ion is therefore the same as that of the unperturbed dye ion, which absorbs in the *M*-band.

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