The influence of certain metal ions on the visible spectra of food dyes

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SUMMARY

The visible absorption spectra of aqueous solutions of 22 food dyes were studied in the pure state and also in the presence of certain metallic ions (calcium, magnessium, aluminium, iron 11, iron 111, copper 11 and cobalt 11), at a variety of pH's (3.0, 6.4. 7.4. 12.5). Some mixtures showed a shift of colour from that of the pure dye to a sufficient extent to warrant furthur study by the methods of continuous variation and the straight line method in order to elucidate the mole ratio of a possible dye:metal complex present. The most marked colour change was found to be the addition of copper 11 ions to carmoisine at pH 6.4 and 7.4 when the addition of 0.0005M copper 11 ions caused a colour change in the solution of 0.0005M carmoisine from red to yellow-orange. Preliminary investigations were made for the possible use of carmoisine as a metallochromic indicator in the copper 11/E.D.T.A. titrations, but it was found that the reagent was not specific for copper 11 ions and other transition metals interferred with the end point. It was considered that carmoisine was inferior to the other excellent indicators now available for copper 11 ions. The compound formation between metal ions and dyes and their possible harmful effects upon metabolic and dietary processes are discussed, particular attention being paid to the uptake of iron in metabolic processes.

INTRODUCTION

Aspects of the chemistry and biochemistry of food additives have been a centre of much interest and experiment in recent years. The toxicity (4,5,7,8,9,43..), chromatographic properties and electro-phoresis of the food dyes have been some of the fields receiving attention. More information was urged by the Ministry of Agriculture, Fisheries and Food(1)It was noted that only comparatively meagre information was available concerning the possible interaction bewteen the food dyes and metal ions, and the spectrophotometric details of the dyes in aqueous solution and solutions containing metal ions were limited to a few isolated examples (23,24,25,36). This investigation has, therefore, been directed towards an examination of the influence, over a wide range of pH, of varying concentrations of calcium, magnesium, aluminium, cobalt ll, copper ll, iron ll and iron lll on the colours of most of the coal tar dyes permitted for use in food in the United Kingdom, as well as some dyes now removed from the permitted list.

The dyes used in this investigation were kindly donated by Pontings and were of the Hexacol range. They have been listed in Table 5 according to their structural similarity.

Minor (2) listed the requirements for a food dye and apart from the fact that the dye should not be injurious to health, he added that it should be fast to light, and should withstand relatively high temperatures and variable conditions of pH, and furthermore, it should not be affected by preservatives and other constituents of food. The bleaching effect of sunlight was well known and indigo carmine was particularly susceptible to spoilage. Some metallic ions caused discolouration and spoilage of the food dyes. Kitson and Strachan (3) showed that the colours of ponceau SX, tartrazine and amaranth at pH 3.5 were affected by the presence of copper and iron ions in solution at 54-68 ^{o}C, but no significant change was noted in the presence of aluminium ions. The dye / iron mixture yielded a precipitate in all three cases.

The following investigation is a compilation of spectrophotometric data of the aqueous solutions of the pure dyes and in the presence of some metal ions at various pH's. In some cases where colour changes occur when a metal ion is added to the dye solution, a closer study of the possible compound is made by the use of the methods of continuous variation and straight line methods. Particularly useful

aspects of this investigation was the monitoring of groups of food dyes as possible metallochromic indicators, and also the possible effects of the dyes upon metal uptake in the metabolism.

EXPERIMENTAL PROCEDURE FOR THE INVESTIGATION OF THE MIXTURES OF DYE SOLUTIONS WITH METAL ION SOLUTIONS.

The metal ions chosen for this study of possible food dye interaction, over a wide range of pH, are those normally encountered in food technology or human consumption, namely calcium, magnessium, aluminium, cobalt 11, copper 11, iron 11 and 111.

The metal ion solutions were prepared from the corresponding Analar sulphate except for iron 111 which was made from the ferric ammonium sulphate, and the calcium solution was prepared by dissolving the Analar carbonate in the minimum quantity of pure dilute hydrochloric acid. The concentrations of the metal ion solutions were exactly 0.0005M. Similarly the concentrations of the dye solutions were 0.0005M. The pH was maintained at the required values using glacial acetic acid for pH 3.0, Sörensen phosphate buffer mixture for pH 6.4 and 7.4, diethylamine for pH 12.5. All the solutions were made in high purity deionised water and the colours analysed with either a Unicam SP 700 or a Beckmann spectrophotometer. The region of the spectrum scanned was between 26,000 cm^{-1} to 10,000 cm^{-1} and the results were automatically graphed.

Varying volumes of the 0.0005M ion solution (from 0 to 40 ml) were mixed with 5 ml of 0.0005 M dye solution and 5 ml of the pH control solution added. The whole solution was made up to 50 ml with deionised water. The solutions were transferred to a 1 cm. glass cell and compared with 1 cm. of deionised water in a matched cell.

RESULTS

The results are given in Tables 1 . The food dyes in the table are conveniently classified according to their structure.

Those mixtures which showed a shift of their absorption spectra of 800 cm^{-1}, or more, from those observed for the free dye have been underlined. An * shows the presence of a point of inflection in the absoption spectra, and a crossed line / denotes the presence of a shoulder in the absorption spectra of the solution.

When a shift had taken place the value of the wave number of the solution containing the highest amount of metal ion solution was recorded.

A summary of the results of Table 1. is given in Tables 2. and 3.

The observed colour changes were not usually very marked, and in many cases amounted to only a change in hue. The copper 11,

carmoisine mixture at pH 6.4 and 7.4 gave the most outstanding visual colour change.

It must be noted that the food dye red FB gave an overpowering absorption over almost the whole range studied and consequently the solution was furthur diluted to 0.0001% in order that the solution was of use for the investigations.

STUDIES OF POSSIBLE COMPOUND FORMATIONS BETWEEN THE METAL IONS AND THE DYE SOLUTIONS.

Continuous variation method

From Table 1 it can be seen that variations in frequency of the absorption band maximum of the dye may be brought about by complex formation with the added metal ions. The possible compound formation is given in Table 2 and these are the combinations of dye plus metal which cause a shift of at least 800 cm^{-1} in the wave number.

An estimate of the structure and stability of the complex may be made by Job's method of continuous variation (53, 54) and also the amended methods of Vosburg and Cooper (55), Asmus(56), Klausen and Langmyher(57), Close and West (26,28).

The essential details of the Job's method is shown by considering the formation of the complex MA_n, M being the metallic ion and A the dye(both of the same molar concentration) the object of the experiment being to find 'n'.

$$M + nA = MA_n \qquad 1.$$

The metal and dye solutions are mixed in varying proportions but ensuring that the total concentration of the two are kept constant.

Suppose the mixtures are made up by adding V_m mls of

solution of the metal and V_A ml of the solution of the dye, then if no volume change occurs on mixing them, V (being the total constant volume) would be

$$V = V_A + V_m \qquad 2.$$

Initially the total concentration of each component of the mixture would be C_m^o of metal and C_A^o of the dye, then

$$C_m^o = (1 - x)m \qquad 3.$$

$$C_A^o = xm \qquad 4.$$

where $x = \frac{V_A}{V}$ and $1 - x = \frac{V_m}{V}$ 5.

If C is the concentration of the complex in the mixture, then from 1. the concenration at equilibrium of M is C_m and of A is C_A.

so at equilibrium $C_m^o - C_m = C$

$$C_A^o - C_A = nC$$

$$(1 - x)m = C_m + C \qquad 6.$$

$$xm = C_A + nC \qquad 7.$$

by applying law of mass action $K = \frac{C}{C_m C_A^n}$ 8.

K is called the stability constant of the complex for any given temperature.

From 6. and 7. then 8. is rewritten as

$$K = \frac{C}{C_m C_A^n} \qquad C = KC_m C_A^n$$

$$= K\left[(1-x)m - C\right]\left[xm - nC\right]^n \qquad 9.$$

The condition for C to have a maximum value then

$$C' = \frac{dC}{dx} = 0 \qquad 10.$$

Differentiating 9. with respect to x

$$C' = K\left\{n(m - C'n)\left[(1 - x)m - C\right](xm - nC)^{n-1} - (m + C')(xm - nC)^n\right\}$$

$$= K(xm - nC)^{n-1}\left\{n(m - C'n)\left[(1 - x)m - C\right] - (m + C')(xm - nC)\right\} \qquad 11.$$

For C to be a maximum then $C' = \frac{dc}{dx} = 0$

$$n(m - C'n)\left[(1 - x)m - C\right] - (m + C')(xm - nC) = 0$$

But $C' = 0$

so $nm\left[(1 - x)m - C\right] = m(xm - nC)$

$$n(1 - x) = x$$

$$n = \frac{x}{1-x} \qquad 12.$$

From this it can be seen that for C to be a maximum $n = \frac{x}{1-x}$

and so if C is plotted against the composition x of the mixture the curve will pass through a maximum value of x as given by 12. i.e. Maxima on the graph could be obtained when

$x = 0.5$ $n = 1$ MA

$x = 0.66$ $n = 2$ MA_2

$x = 0.33$ $n = 0.5$ M_2A

If E is the molar extinction coefficient of the complex and E_m and E_A are the extinction coefficient of the metal and dye respectively, then providing the Lambert-Beer Law is obeyed since $D = El$ (D is the optical density of the solution of the mixture and l is the thickness of the absorbing layer)

$$D = l(EC + E_m C_m + E_A C_A) \qquad 13.$$

Substituting for C_m and C_A from 6. and 7.

$$D = l\{EC + E_m[(1 - x)m - C] + E_A(xm - nC)\}$$

$$= l\{C(E - E_m - nE_A) + E_m(1 - x)m + E_A xm\}$$

$$= l\{aC + E_m(1 - x)m + E_A xm\} \qquad 14.$$

where $a = E - E_m - nE_A$

In the absence of compound formation i.e. concentration of complex $C = 0$, from 13. then $D_o = l(E_m C_m^o + E_A C_A^o)$ from 6. and 7. where $(1 - x)m = C_m + C$

$$C_m^o = C_m \quad \text{and} \quad C = 0$$

$$\text{then } (1 - x)m = C_m^o$$

$$\text{and } xm = C_A^o$$

$$D_o = l[E_m(1 - x)m + E_A xm]$$

But from 14. $D = l\{aC + E_m(1 - x)m + E_A xm\}$

so $D - D_o = l\,aC = \Delta D = y \qquad 15.$

l is the length of the absorbing layer and is a constant for any particular experimental run, and so it can be seen that ΔD is directly proportional to the concentration C of the complex; a being $E - E_m - nE_A$ is dependent upon wavelength and it can be concluded that ΔD is directly proportional to the concentration of the complex at any particular wavelength.

In cases where $E < E_m + nE_A$ 16.

then ΔD or y will be negative and so a minimum curve will be obtained instead of a maximum curve but the relationship $x = \frac{n}{n+1}$ still holds. The effect of changing the wavelength will be to change the value of ΔD but not its position with respect to x, provided that only one complex species is present. Efforts have been made to extend this method for polynuclear complexes.

If a single shaped curve is obtained for any two materials at various wavelengths at the same pH then it was concluded that only one coloured complex was present in the solution; however there might be colourless complexes present.

For the continuous variation curve which exhibited inflections for small values of x Asmus (56) concluded that for $m = 1$ $n > 1$. A continuous variation curve which was parabolic for values of x near zero and one and which had zero gradient at $x = 0$ and $x = 1$ indicated $m = n > 1$ in the formula of the complex M_mA_n and conversely the absence of inflections in parabolic portions indicated $m = n = 1$

The derivation of the above conclusions were based on the equilibrium $mM + nA = M_mA_n$

for which $$K = \frac{M_mA_n}{M^m A^n}$$ 1.

C_T is the total concentration of reactants $C_T = C_M + C_A$ and x the mole fraction $x = \frac{C_A}{C_A + C_M}$ then the equilibrium concentrations of A and M are

$$M = C_T(1-x) - m[M_mA_n] \qquad 2.$$

$$A = C_T(x) - n[M_mA_n] \qquad 3.$$

substituting the equilibrium concentrations in the law of mass action and rearranging terms

$$\frac{1}{K}[M_mA_n] = \left\{C_T(1-x) - m[M_mA_n]\right\}^m (C_Tx - n[M_mA_n])^n \qquad 4.$$

Let $y = \frac{[M_mA_n]}{C_T}$ then 4. becomes

$$\frac{yC_T}{K} = \left\{C_T(1-x) - m[yC_T]\right\}^m (C_Tx - n[yC_T])^n$$

$$f(x\ y) = (1-x-my)^m(x-ny)^n - \frac{1}{KC_T^{m+n-1}} \quad y = 0 \qquad 5.$$

$$\frac{dy}{dx} = \frac{\frac{df}{dx}}{\frac{df}{dy}}$$

$$-\frac{dy}{dx} = \frac{\frac{-df}{dx}}{\frac{df}{dy}}$$

$$= -\frac{\left[n(x-ny)^{n-1}(1-x-my)^m(1-n\frac{dy}{dx}) + (x-ny)^n(1-x-my)^{m-1}(-1-m\frac{dy}{dx}) - \frac{1}{KC_T^{m+n-1}}\frac{dy}{dx}\right]}{(1-x-my)^m\ n(x-ny)^{n-1}(\frac{dx}{dy}-n) + m(1-x-my)^{m-1}(-\frac{dx}{dy}-m)(x-ny)^n - \frac{1}{KC_T}\ m+n-1}$$

6.

When $\frac{dy}{dx} = 0$ or approaches 0 then the $\frac{dy}{dx}$'s and $\frac{dx}{dy}$'s are very

small and neglected in the expression for maximum value of x

So

$$-\frac{dy}{dx} = -\frac{\left[n(x-ny)^{n-1}(1-x-my)^{m}(1)+(x-ny)^{n}m(1-x-my)^{m-1}(-1)\right]}{(1-x-my)^{m}n(x-ny)^{n-1}(-n)+m(1-x-my)^{m-1}(-m)(x-ny)^{n}} - \frac{1}{KC_T^{m+n-1}}$$

$$-\frac{dy}{dx} = -\left\{\frac{(x-ny)^{n-1}(1-x-my)^{m-1}(n-nx-mx)}{(x-ny)^{n-1}(1-x-my)^{m-1}\left[-n^2-x(m^2-n^2)+nmy(m+n)\right]} - \frac{1}{KC_T^{m+n-1}}\right\} \quad \underline{7.}\ \underline{8.}$$

Changing the sign of bottom line

$$\frac{dy}{dx} = \left\{\frac{(x-ny)^{n-1}(1-x-my)^{m-1}(n-(m+n)x)}{(x-ny)^{n-1}(1-x-my)^{m-1}\left[n^2+x(m^2-n^2)+nmy(m+n)\right]} - \frac{1}{KC_T^{m+n-1}}\right\} \quad \underline{9.}$$

If $\frac{dy}{dx} = 0$ then for maximum values of x

$$(x-ny) = 0$$

$$\text{or} \quad (1-x-my) = 0$$

$$\text{or} \quad n-(m+n)x = 0 \quad \text{ie} \quad x = \frac{n}{m+n} \quad \underline{10.}$$

When $x = \frac{n}{m+n}$ there will be a maximum or minimum of the f(xy) curve.

When m=n=1 and then values of the gradient at the x=0, x=1

axis and when the concentration of the complex is negligible

ie $y = \frac{[Mm\ An]}{C_T} = 0$, then the gradient at these values abbreviates

to $\frac{dy}{dx} = \frac{1}{1+\frac{1}{KC_T}}$ but the value of x when m=n=2 is the same as

for m=n=1 but "practically a curve which exhibits inflections and is parabolic for values of x near 0 and 1 indicates the presence of a complex with m=n>1".(56)

It is unfortunate that the parabolic regions are those regions affected most by the highest degree of experimental inaccuracy (due to small values of one of the reactants added) care must be taken in being dogmatic in these regions but the conclusion can be best investigated by the Straight line method to elucidate any conclusions.

A furthur amendment to the Job method must be made when dealing with reaction in which one of the reactants absorbs in the same region as the complex. The Close and West procedure (10) and (11) makes an allowance for the excess dye stuff or colour reactant not complexed with the metal ion. The estimation is achieved by multiplying the optical density of pure dye alone, for any particular experiment, by the mole fraction of dye stuff present and then deducting this from the optical density of the same concentration of dye stuff and metal ions and this difference ΔD is plotted against x, the composition. The optical density chosen was usually the value where the chelate exhibited an absorption maximum and the dye stuff comparatively transparent and the metal ion transparent.

Thus this final $\Delta D/x$ plot showed an increase in colour of the complex due to addition of more complexing agent to an excess of metal ions followed by a decrease due to the scarcity of metal ions in the presence of excess dye or complexing agent.

EXPERIMENTAL PROCEDURE FOR THE CONTINUOUS VARIATION METHOD OF DETERMINING THE FORMULA OF A COMPOUND.

To find the value for n and m in the formuls Mm An then various volumes of metal ions were added to certain volumes of the dye solution in the ratio such that the total volume of metal plus dye is always a constant value.

Varying amounts of 0.0005M dye were mixed with varying amounts of 0.0005M metal ions according to the amounts stated below, and the solution was buffered (at 12.5 with diethylamine or at 7.4 with Sörensens mixture) and the solution made up to 50.00 ml. with deionised water.

x	mls. of 0.0005M dye	mls. of 0.0005M metal	Total vol. in mls.
0.1	0.5	4.5	5.0
0.2	1.0	4.0	5.0
0.3	1.5	3.5	5.0
0.4	2.0	3.0	5.0
0.5	2.5	2.5	5.0
0.6	3.0	2.0	5.0
0.7	3.5	1.5	5.0
0.8	4.0	1.0	5.0
0.9	4.5	0.5	5.0

The mixtures were studied spectrophotometrically between about 26,500 cm^{-1} and 10,000 cm^{-1} using a Unicam SP 700 and the absorption spectra was automatically graphed.

Solutions containing the stated amounts of dye alone (no metal ions) were prepared as for the mixtures and the

resultant spectra similarly recorded.

Results

A summary of the results is given in Table 4 . Because the solutions were very weak very little visual colour changes were observed, except in the case of the copper 11/carmoisine mixture at pH 7.4 which showed a gradation of colours from red at x = 0.9 to an orange at x = 0.7 and below. The very weak iron 11 and iron 111 solutions showed no noticeable precipitations at pH 12.5 and these solutions were considered, for the purpose of these experiments, to be true solutions.

When studying the graphed spectra the absorption maxima of the mixtures were calculated from the region of the spectra in which the dye solutions did not absorb, or which only absorbed to a small amount. These optical densities were calculated in the regions of the spectra in which the metals had no absorption.

For most continuous variation experiments with common substances, only the complex is coloured, and the reactants are virtually colourless so the consequent continuous variation graphs are easily calculated and drawn.

In the case of the food dyes and their corresponding

metal complexes, then both materials frequently absorb in the same region of the spectra and only the metal ion is colourless. It was found necessary to allow for the amount of the uncompounded dye when measuring the extinctions or optical densities of the complex. The technique adopted was that suggested by Close (65) in which the value of the optical density (D_1) of the pure dye was measured on a uniform scale and then multiplied by x, in order to find a value for the colour of the remaining uncompounded dye ($D_o = xD_1$). $x = \frac{\text{volume of dye}}{\text{volume of dye + volume of metal}}$

When x has a low value, then small amounts of dye would be present and large amounts of metal and so if any complex is formed then the amount of uncompounded dye would be proportional to xD_1.

The value of the optical density of the mixture D_2 would contain a contribution to the optical density value due to the uncompounded dye. Therefore the true value of the optical density of the complex is found by subtracting the estimated uncompounded dye colour (or optical density value xD_1) from the colour of the mixture D_2 ie. D_2-xD_1 or $D_2 - D_o$. When the various values of $D_2 - D_o$ are plotted against x, a maximum or minimum occurs at a point which would correspond to the formuls of the complex (see Graph 1-14).

It is noted that the majority of the graphs showed clear maximum or minimum values at the ratios quoted in Table 4. . There is possible evidence for the two compounds present in the copper II/carmoisine dye combination at pH 7.4 and at 16.000 cm^{-1} as shown by graph 2 which shows a weak maximum at metal:dye ratio 2:1 and a very strong minimum at metal:dye ratio 1:2.

Corrected graphs are compiled by plotting the optical density values/x graphs on a horizontal axis made by subtracting from the values of practical optical density the various values of optical density as indicated by joining the intersections of continuous variation graph with the x =0 ans x = 1 axis (see respective graphs).

The corrected continuous variation graphs can be used for the determination of the degree of dissociation of the complex present and also for calculating the value of the stability constant of the complex.

Stability Constants

The continuous variation graphs can be used to calculate approximate values of the degree of association of the complex and hence the dissociation constant or stability constants for the complex (65).

Stability, according to Cotton and Wilkinson (Adv. Inorg. Chem. p.539), can mean two things when considering a complex ion in aqueous solution; it can either refer to the thermodynamic stability of the complex at equilibrium with the reactants forming it and this is really a measure of the extent to which the complex can be transformed or decomposed into other species; or it could refer to the kinetic stability which is a guide to the speed with which transformations occur leading to the attainment of equilibrium. The thermodynamic stability constant is the relevant term to use in this context.

Consider the reaction

$$\underset{(1-d)C}{M_m A_n} \rightleftharpoons \underset{mdC}{mM} + \underset{ndC}{nA}$$

C is the concentration of the complex at the maximum optical density. d is the degree of dissociation of the complex.

$$K = \frac{M_m A_n}{M^m A^n} = \frac{(1-d)C}{(mdC)^m (ndC)^n}$$

When m=1 n=1 ie in MA

$$K = \frac{1-d}{Cd^2}$$

When m=1 n=2 ie MA_2

$$K = \frac{1-d}{4C^2d^3}$$

When m=2 n=1

$$K = \frac{1-d}{4C^2d^3}$$

The values of d can be obtained from the continuous variation graphs by extending the straight line portions of the side of the graph until they meet at a point (58). The distance of the tip of this point from the top of the maximum curve as compared with the total height of the point from the corrected axis is the value d the degree of dissociation.

It was assumed that if there was no dissociation the continuous variation curve would have been a sharp apex but due to dissociation of the metal/dye complex then the apex became blunt and curved. When the curves were very rounded at the maximum (or minimum) then this showed that a weakly stable complex was present (ie a high degree of dissociation). It has been suggested (52) that the complex could be stabilised by the addition of a water miscible solvent such as acetone or by adding an indifferent electolyte, which has the similar effect as salting out the complex.

Results

The degree of dissociation and stability constants (K) are summarised in Table 4 .

Sample calculation of the stability constants :-

2 : 1 iron 11/carmoisine at pH 12.5

$$K = \frac{1-d}{4C^2d^3}$$

d =0.13 C = concentration of complex at equilibrium at the maximum of the continuous variation curve

$= 1.65 \times 10^{-5}$ gm molecs/litre

$$K = \frac{1 - 0.13}{4(1.65 \times 10^{-5})^2(0.13)^3}$$

$$= 36.4 \times 10^{10}$$

1 : 1 iron 111/amaranth at pH 12.5

$$K = \frac{1-d}{Cd^2}$$

d = 0.10 $C = 2.50 \times 10^{-5}$ gm.molecs/litre

$$K = \frac{1 - 0.10}{2.50 \times 10^{-5}(0.10)^2}$$

$$= 36.0 \times 10^5$$

Straight line method for determination of the formulae of certain metal/dye complexes.

Theory:- $mM + nA = MmAn$

Let $K = \frac{[M]^m [A]^n}{[MmAn]}$ (1)

In this reaction Vo ml of solution M of concentration Co was added to a standard flask and the solution made up to V mls; similarily V mls of A of concentration ao were made up to V ml, the pH being maintained with a suitable buffer solution. The reactants were added in the desired proportions and the solution sampled and its colour measured spectrophotometrically at a suitable wavelength.

Initial concentration of metal M $c = \frac{CoVo}{V}$ (2)

" " " dye A $a = \frac{aov}{V}$ (3)

At equilibrium the concentration of reactants are

concentration of M = $[M] = c - m[MnAn]$ (4)

" " A = $[A] = a - n[MnAn] = a - \frac{n}{m}[c - [M]]$ (5)

$[Mm\ An] = \frac{E}{ed}$ (6)

where E = extinction of complex, e = molar extinction coefficient of complex, d = light path length in cms.

By combining equations (1),(4),(5),(6)

By combining equations (1),(4),(5),(6)

$$K = \frac{[M]^m[A]^n}{[MnAn]}$$

$$= \frac{\left[c - \frac{mE}{ed}\right]^m[A]^n}{\left[\frac{E}{ed}\right]}$$

$$K\frac{E}{ed} = \left[c - \frac{mE}{ed}\right]^m[A]^n \qquad (7)$$

Expanding $\left[c - \frac{mE}{ed}\right]^m$ using the binomial theorem

$$\left[c^m - \binom{m}{1} c^{m-1}\frac{mE}{ed} + \binom{m}{2}c^{m-2}\left(\frac{mE}{ed}\right)^2 \cdots (-1)^m\left(\frac{mE}{ed}\right)^m\right][A]^n = \frac{KE}{ed} \qquad (8)$$

Because $\binom{m}{1} = m$

then (8) becomes on rearrangemnt and taking $\frac{m}{1}$ term out of the brackets

$$\frac{1}{[A]^n} = \frac{c^m ed}{K}\left[\frac{1}{E} + \binom{m}{2}\left(\frac{m}{ced}\right)^2 E - \binom{m}{3}\left(\frac{m}{ced}\right)^3 E^2 + \cdots\right.$$

$$\left. \cdots (-1)^m\left(\frac{m}{ced}\right)^m E^{m-1}\right] - \frac{m^2 c^{m-1}}{K} \qquad (9)$$

Now by introducing (2),(3),(4),(5) and by assuming that

$[M_m A_n] \ll a.$

This assumption was made by both Azmus and Klausen and Langmyhr (57)

$$\text{Let } \frac{1}{v^n} = \frac{Co^m Vo^m a^n ed}{V^{m+n} K} X_m - \frac{m^2}{K}\left(\frac{CoVo}{V}\right)^{m-1}\left(\frac{ao}{V}\right)^n \qquad (10)$$

((10) is of the form $y = mx + c$)

Where $Xm = \frac{1}{E} + \binom{m}{2}\left(\frac{m}{aed}\right)^2 E - \binom{m}{3}\left(\frac{m}{aed}\right)^3 E^2 + \ldots\ldots$

$$\ldots\ldots + (-1)^m \left(\frac{m}{aed}\right)^m E^{m-1} \qquad (11)$$

Now by graphically plotting $\frac{1}{v^n}$ against Xm for different values of m and n then a straight line would be expected for the set of m corresponding to the actual m and n for the equilibrium

$$mM + nA = M_mA_n$$

Asmus developed a similar equation to (10) for and when m=1 then (10) becomes identical with the Asmus (59) equation. He considered only the equilibrium $M + nA = MA_n$.

Klausen and Langmyhr extended the Asmus method for polynuclear species and they rearranged equation (11) in the following way:-

To avoid the use of the molar extinction coefficient Xm was replaced by $\frac{Y_m}{ed}$. Also for convenience replace M_mA_n by Z and from (6) $M_mA_n = \frac{E}{ed}$ then equation (11) becomes

$$Y_m = \frac{1}{Z} + \binom{m}{2}\frac{m^2}{c^2} Z - \binom{m}{3}\frac{m^3Z^2}{c^3} \quad \ldots\ldots \quad (-1)^m \frac{m^mZ^{m-1}}{c^m} \qquad (12)$$

In equation (12) the contributions to the equation by the 2nd, 3rd etc. factors are small as compared with the first term $\frac{1}{Z}$, due to the small value of Z in relation to the value of c.

It can now be seen that the shape and position of the plotted curves are mainly determined by the values of n as $\frac{1}{\bar{\nu}^n}$ is a function of n and X_m or Y_m approximates to Z which is hardly effected by m. A straight line would be expected for correct values of n irrespective of values of m which shows that the method of Asmus for MA_n complexes was not able to distinquish between mono and polynuclear species.

Experimental Procedure for the Straight Line Method.

This method consists of adding varying amounts of dye to a constant concentration of metal and comparing the colour with the same concentration of uncompounded dye. As both the dye and the complex are coloured and absorb light of the same wave numbers, a slight adaption of the conventional method was necessary. The optical density of the estimated amount of uncompounded dye solution must be deducted from the optical density of the metal/dye mixture at each concentration ratio. Only amaranth/iron 111 at pH 12.5 combination was studied by this method as it was not necessary to confirm any other continuous variation graph ratios.

Each of a series of solutions of 2.00 ml. of 0.0005M iron 111 were treated with varying amounts of 0.0005M amaranth solution and the resulting mixture buffered at pH 12.5 with 5.00 ml. of diethylamine and the whole solution made up to 50.00 ml. with deionised water. Each solution was thoroughly mixed and samples and its colour graphed automatically using an Unicam SP 700 spectrophotometer. Similar procedure was repeated with the dye solutions alone (no iron 111 solution present) and both studied at the same wave numbers.

The solutions were mixed in the following proportions:-

x	mls. of 0.0005M amaranth	mls. of 0.0005M iron 111
0.13	0.30	2.00
0.20	0.50	2.00
0.33	1.00	2.00
0.43	1.50	2.00
0.50	2.00	2.00
0.56	2.50	2.00
0.60	3.00	2.00
0.71	5.00	2.00

Results.

Only one combination was considered and this is shown on graph15 .

D_1 was the optical density of the pure dye solution and this was multiplied by x (the volume of the dye/total volume of dye plus volume of metal) to give the estimated optical density of the uncompounded dye in the mixture. The xD_1 value is subtracted from D_2 (the optical density of the mixture) to give the optical density (ΔD) of the complex. V is the volume of the dye solutions.

The eventual plotted graphs in this method are $\frac{1}{\Delta D} / \frac{1}{V^n}$

when n = 1,2,etc.... and to study the graphs to see which combination gives the straight line. It is clear that the straight line was formed when n = 1. It is already known from the continuous variation graphs that m = n for the formula of the iron lll/amaranth complex of $Fe_m(amaranth)_n$. Thus the formula is clearly Fe(amaranth) at pH 12.5.

THE STUDY OF CARMOISINE AS A POSSIBLE METALLOCHROMIC INDICATOR FOR COPPER 11.

The initial investigation of the effect of the various metals in solution with the listed food dyes showed that whereas in some cases the metal ion/dye mixture gave changes of hue, the copper/carmoisine mixture at pH 6.4 to 7.4 showed a distinct colour change which could be recognised with the naked eye, and because of this, investigations were made to study its possible use as a metallochromic indicator for copper 11 ions.

Experimental determination of the best working pH of the mixture for maximum observable colour change.

Procedure

5.00 ml of 0.0005M carmoisine solution was added to varying quantities of Sörensen buffer mixture and a constant amount of excess 0.01M copper 11 in solution was added in each case, then the whole solution was made up to 50.00mls. with deionised water. The pH of the solution was checked and recorded using a pH meter, and the colour of this solution was then spectrophotometrically determined.

Results

The optical densities of the solutions studied are graphically plotted against pH in graph 16 . Two values for wave number were chosen for spectrophotometric analysis 26,300 cm^{-1} and 18,900 cm^{-1}. At these two values the absorption of copper 11 ion solution was negligible. The graph clearly showed that the best working pH would be between 7.20 and 7.50 or even between the broader region 6.00 to 8.00 would still be permissable.

A series of experiments were performed involving the titration of copper 11 ions against EDTA at pH 7.4 using the carmoisine solution to act as an indicator of the end point, that is when there was a slight excess of copper 11 ions present.

Experimental procedure

20.00ml of 0.01M Analar EDTA(sodium salt) solution was mixed with a suitable quantity of Sörensen buffer solution to maintain the pH at 7.40, and to this mixture the 0.01M copper 11 ions were accurately added from a grade A burette until the 10.00 ml of 0.0005M carmoisine indicator present just changed from dull red to orange brown (visually determined). A blank titration was performed for the

indicator in the absence of any EDTA.

Results

The visual end point was from the dull red to the orange brown and was a sharp change, that is the colour change was easily seen for addition of a small volume of copper 11 solution, two or three drops of copper 11 being quite sufficient to bring about the complete colour transformation. A typical volumetric reading was

20.00ml of 0.01M EDTA 20.25ml of 0.01M copper 11 sol.

Blank 0.25ml.

True value of copper 11 solution needed to react with 20.00ml of 0.01M EDTA was therefore 20.00ml.

Similar titration experiments were performed in a series of standard flasks covering a range of colours either side of the visual end point and the solutions were made up to the same standard volume and their colours spectrophotometrically determined at the wave numbers 26,300 cm^{-1} and 18,900 cm^{-1}. The values of the optical densities were plotted graphically against the volume of copper 11 ion solution used, and are shown in graph 17.

Results

The graphs showed that the two possible end points can be determined spectrophotometrically which could not be easily discerned with the naked eye. Of the two end points on the spectrophotometric graphs only the latter could be considered as sharp visually. The changes in colour were from cerise to dark red followed by the change from dark red to orange.

In an endeavour to sharpen the end point of the copper 11/carmoisine end point with EDTA, a few drops of the highly blue coloured dye blue VRS was added to the buffered system. This had the effect of causing a sharp colour change at the end point from purple mauve to pale green (in the presence of excess copper 11 ions).

The procedure of the above experiments was repeated in the presence of blue VRS and are graphed on graph 18. The absorption maximum changedfrom 16,000 cm^{-1}(purple mauve) to 18,400 cm^{-1} (pale green). The absorption minimum changed from 24,400 cm^{-1} at the purple mauve to 23,400 at the pale green end point. There was a point of inflection in the spectrophotometric curve at 22,000 cm^{-1} at the start of the experiment and this had disappeared at the end point.

Blue VRS did not react with copper 11 ions but its presence was only used as a screening agent.

Simple preliminary metal ion interference experiments were performed with some other transition elements, namely nickel 11, cobalt 11, manganese 11, chromium 111, iron 11 ions.

Procedure

5.00ml of carmoisine were mixed with 10.00ml of EDTA and the solution buffered at 7.4 using Sörenen buffer solution and to this solution was added pure copper solution and this was then compared with the end point determined in the presence of 5.00ml of the seperate transition metal solutions.

Results

In all the cases an additive effect was observed when added to the EDTA and the end point changed at around the 10.00ml value. The cause of this colour change might be due to the transition metal ion/carmoisine complex and not the copper 11/carmoisine complex, or it could be caused by the copper 11 being displaced from the EDTA complex by the transition metal in sufficient quantities to cause the carmoisine to change colour. No furthur experiments were considered necessary to elucidate the problem. The gross

interference of the transition metals with the copper 11/ carmoisine end point makes this latter combination of little use as a metallochromic indicator unless masking reagents were used. Masking was not adopted due to the development of specific metallochromic indicators for copper 11 ions.

DISCUSSION

FEATURES OF THE SPECTRA OF THE DYES ALONE

A substance, when exposed to white light, will appear black if all the light is absorbed, or white if all the light is reflected. Some substances absorb a certain proportion of the light and reflect the remainder, in which case it would have the colour of reflected light. When only a certain single band of light is absorbed the substance would have the corresponding complementary colour of the absorbed band. A substance could appear blue either because it absorbed the yellow portion of the spectrum or because it absorbed all visible light except the blue which it reflected: the shades of blue would be different in the two cases. No dye gives totally pure shades but reflects a number of wavelengths to greater or lesser degree. Coloured substances owe their colour to the presence of one or more unsaturated linkages (49) and both the azo dyes and the triphenylmethanal anhydride dyes contain such unsaturated linkages. The linkages or groups giving colour to the substance are called chromophores. Some groups which, by themselves, do not confer colour on a substance, but deepen the colour of the chromophore, are called auxochromes.

Auxochromes are mainly acidic or basic groups e.g. OH, NH_2, SO_3^-, COO^-, NO_2. The basic groups were particularly auxochromic to dyes in which the benzene ring was part of the chromophore. Substitution in the ortho or para position gave the greatest intensification of colour whereas meta substitution had little or no effect. Radicals which bring about deepening of colour, i.e. shifting of an absorption band to a region of longer wavelengths, are often referred to as being bathochromic (the opposite of this effect is hypsochromic). Deepening of colour is the change from yellow - orange - red - purple - violet-blue - green - black.

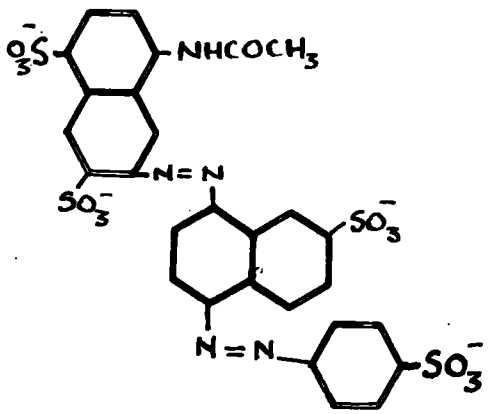
The principal characteristics of the absorption spectra of the food dyes followed the pattern mentioned. Thus a yellow dye showed absorption bands in the region of $23,000cm^{-1}$(435nm) to $25,000cm^{-1}$ (400nm); the orange dyes in the region of $21,000cm^{-1}$ (476) with a furthur band at $24,000cm^{-1}$ (417nm) to $25,000cm^{-1}$ (400nm); the red dyes had absorption bands at $19,000cm^{-1}$ (526nm) to $20,000cm^{-1}$ (500nm). The differences observed in the absorption band maxima for dyes of the same colour matched differences in hue, for example the red dyes tending towards an orange hue had absorption bands nearer to $20,000cm^{-1}$ (500nm), while those of a pronounced deep red had their absorption bands nearer $19,000cm^{-1}$ (526nm). The four

dyes that had absorption bands furthur towards the red end of the spectrum possessed, as expected, bluish characteristics in their colours. Thus violetBNP and black PN had absorption maxima at around 17,000cm^{-1} (588nm) while blue VRS and green S had their maxima nearer 16,000cm^{-1} (625nm). The structure of the dyes were really the underlying criteria for colour and these explained the absorption spectra values.(See Table 5).

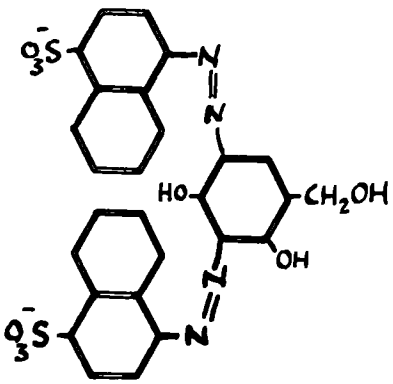
A yellow colouration in these food dyes was characteristic of the phenyl - azo - phenyl and phenyl - azo - pyrazole dyes i.e. yellow RY, yellow RFS and yellow 2G, and tartrazine respectively. The 1- and 2- naphthyl - azo dyes, with molecules of more enhanced bathochromic characteristics, were generally distinguished by their red colours. There were, however, several exceptions to the generality of the red character for the 1 and 2 naphthyl-azo dyes. The orange dyes, orange G, orange RN and sunset yellow FCF, phenyl-azo-naphthyl dyes, did not appear to carry substituents of potential bathochromic character as did the other dyes belonging to this group. (see structures table 5).

Another exception was black PN which demonstrates the bathochromic characteristics of the extended conjugation brought about by the favourable position of its extra azo grouping and absorbed at lower frequencies (longer wavelengths) than

the red dyes. Chocolate brown HT also had two azo groups but due to their unfavourable position an extended conjugation was not possible and so, with its two, apparently independent halves, absorbed at around 21,200cm^{-1} (472nm) close to the observed orange dyes.



Black PN



Chocolate Brown HT

The triphenylmethanol anhydride dyes, blue VRS, green S and violet BNP were characteristic of their class and all showed absorption of lower wave number than those of the red dyes. However, the absorption maximum had variable values for their extinctions and optical densities.

pH 3	Blue VRS	16.0(0.99)
	Green S	16.0(1.00)
	Violet BNP	17.0(0.98)

pH 3	Carmoisine	19.4(0.98)	Red Dyes
	Amaranth	19.0(0.92)	
	Red 10B	19.0(0.92)	

Except at pH 12.5 the effect of pH on the frequency of the absorption band was not great. At pH 12.5 however, there was a distinct tendency for the absorption maximum band to be shifted to a slightly different frequency.

Ponceau MX at pH 12.5 the 25.8 maximum is absent.

Amaranth at pH 12.5 there is a change of wave numbers from 19.0(0.92) to 20.2(0.79). hypsochromic shift

Fast Red E at pH 12.5 a change of wave numbers occurred from 19.6(0.86) to 21.0(0.72). hypsochromic shift

Carmoisine at pH 7.4 and 12.5 the ∮ 24.6 is absent. (∮ shoulder)

Black PN at pH 12.5 the absorption maximum changed from wave number 24.4(0.64) to 25.8(0.72).hypsochromic shift

Ponceau SX at pH 12.5 the absorption maximum moved from 20.0(0.92) to 21.0(0.84). hypsochromic shift

Red 2 G the split peaks converged at pH 12.5 from wave-numbers 20.0(0.95), 19.0(0.95) to 21.8(0.80).

Red 6B at pH 12.5 the absorption maximum changed from 19.0(0.93) to 21.6(0.83) hypsochromic shift

Yellow RY at pH 12.5 the absorption maximum changed from 23.0(0.89) to 22.0(0.83) bathochromic shift

Tartrazine at pH 12.5 the absorption maximum moved from 23.0(0.91) to 25.0(0.84). hypsochromic shift

Green S at pH 12.5 showed a slight variation the maximum of 25.0(0.54), 22.8(0.37) changed to a split peak at 25.0(0.64).

Sunset Yellow FCF progressed from wave numbers 24.2(0.61) to 22.2(0.74), 21.0(0.91) to 20.0(0.60). bathochromic shift

Another feature of the spectra was that the optical densities of the bands responsible for colours did not vary appreciably, only by a factor of two or three, in passing from one dye to the next. Red FB at the same concentration (0.0005m) was almost impervious to light and so the solution was considerably diluted (0.0001m) to make the solution comparatively transparent to light.

There was a tendency for the optical densities of solutions at pH 12.5 to be less than those of the solutions at other pH's. This was particularly true of violet BNP, a feature that was characteristic of triphenylmethanol anhydride dyes under alkaline solutions when they had basic (or positive) auxochromic groups.

The reason for red FB having appreciably greater optical densities was because of the presence of the benzthiazole grouping in this particular 2-naphthyl-azo-phenyl dye.

THE EFFECT OF METAL IONS ON THE SPECTRA OF DYE SOLUTIONS

For a visual recognition of a change of colour, a shift of at least 1,000cm^{-1} was apparently required in the position of a maximum of the absorption band of the dye solution in the presence of metal ions when compared with that dye in the absence of a metal ion. The maxima of absorption bands of the dye solutions in the presence of metal ions are underlined in Table 1 in cases where these differ by more than 800cm^{-1} from those of the free dye solution. This value is purely arbitrary but of significance in being near the change of value needed for a visual observation of colour change, and being sufficiently large not to be experimental error.

Table 2 summarises the pH values at which the added metal ion solutions appreciably effected the colour of the dye solutions. The majority of the changes of colour occurred at pH 12.5, almost 50% in fact (28 out of 55). At pH 7.4 there were only 3 recorded changes, at pH 6.4 there were 13 changes of colour and at pH 3 there were 11 changes of colour, (see also Table 3). Under none of the pH regions studied did either calcium or magnessium cause any change of colour from the colour of the pure dye. The majority, 5/6ths, of the colour changes caused by adding aluminium to pure dye solutions occurred in the acid region i.e. below pH 7. Similarly 3/9ths

of the colour changes caused by the addition of Cobalt ions occurred in the acid pH region, while copper 11 caused 8/9ths of its colour changes when added to the dye solutions in the alkaline region of pH. There seemed no systematic connection between the colour shifts of iron 11 and iron 111 at the various pH's, and in no case did iron 11 and iron 111 show identical colour change patterns at the pH's studied, although fed FB, yellow RY and yellow 2G did change colour at pH 12.5 when both iron 11 and iron 111 were added.

The majority of the colour changes were caused by the transition or 'd' block elements. 49 out of 55 of the changes were caused by these elements and of these 31 out of 49 were caused by addition of iron 11 or iron 111 solutions.

The largest changes in the absorption spectra of the dyes were brought about by the transition metal ions examined. The changes brought about by copper 11 and iron 11 tended to be hypsochromic, while those of iron 111 were more variable. In fact the most pronounced colour change observed visually was that brought about by copper 11 on carmoisine at pH 6.4, and especially pH 7.4 the change was from red to orange. This corresponded to a shift in the absorption band maximum of 1,200cm^{-1} (29nm) and 2,000cm^{-1} (47nm) at the respective pH values. Copper 11 ions were also responsible for a less

pronounced visual colour change over the normal colour of the free dye (orange red to orange) in ponceau MX at pH 12.5; this was characterised by a shift of 1,600cm^{-1} (37nm) in the maximum of the absorption band of the dye. A furthur example was the reddish tinge of colour exhibited by black PN in the presence of iron 111 ions at pH 12.5 with a shift of 3,000cm^{-1} (83nm) away from the bluish purple absorption at 17,600cm^{-1}(568nm). Red FB showed a visible change from red to red/purple when cobalt, iron 11 and iron 111 ions were added at 12.5.

It is interesting to note that the yellow dyes frequently showed changes of frequency in their absorption maximum with the metal ions, despite the fact that with the exception of yellow RY they did not possess suitably disposed groups for chelation. 9/19ths of the shifts of the yellow dyes (yellow RY, RFS, 2G and Tartrazine) occurred at pH 12.5, the remainder (10 out of 19)in the acid region of pH.

Phosphates play an active part in the forming of complexes with some metal ions. They are also a common constituent of foodstuffs and for this reason phosphate buffer solutions were selected for the neutral pH values of 6.4 and 7.4. Under these conditions, the dye competed with the phosphate for the metal ions, but despite this, shifts were observed in the frequencies

of the absorption maxima of a number of dyes in the presence of metal ions. With the excessive iron concentrations used to obtain the data of Table 1 , at pH 12.5 there was a tendency for the ultra-violet absorptions to spread into the visible region (due to slight cloudiness through slight precipitate formation), but nevertheless the frequencies of the colour causing absorption maximum were, in the majority of cases, easily distinquished. The cloudiness was very much less in evidence for the ion concentrations used to obtain the data for the calculating of the stability constants of Table 4 .

The most stable 1:2 metal:dye complex appeared to be that of iron 111/carmoisine which had a stability constant (K) value 2.6×10^{1} times that of the most unstable 1:2 complex that of cobalt 11/carmoisine, both at the same pH 12.5. The majority of the 1:2 complexes were of the same order in the $(15 - 80) \times 10^{10}$ region and all at pH 12.5.

The 2:1 complex of copper 11/carmoisine at pH 7.4 was also in the same range of values as for the 1:2 complexes.

The 1:1 complex formationof copper 11/red 10B, iron 11/ red 10B and iron 111/amaranth at pH 12.5 gave K values all closely resembling each other and differing by only a factor of 2 in the two extreme cases.

The 1:1 complex of iron 111/amaranth was also backed up

by the application of the alternative procedure of the straight line method.

All the complexes were shown to be in an equilibrium state with their reactants, but the degree of dissociation of all the complexes differed very little. The most stable system being iron 111/carmoisine, had a degree of dissociation (d) value of 0.06 whereas the most dissociated system iron 11/ ponceau 4R had a d value of 0.20.

It must be emphasised that the derivation of d and K have been based upon the approximations suggested by Close (65) but they are still of considerable use in work on the food dye/metal investigations.

The accuracy of the method for finding K depends upon the accuracy of the measurement of d from the continuous variation graphs. The method was originally derived by Diepe and Lindstrom (58). As 1% error in the estimation of d from the graph can lead to a maximum error in K, of 1:2 complexes, of a factor of 10 this is not so exagerated in the cases of the 1:1 complexes. It is therefore more desirable to study the d values of the complexes when a guide to their stability is required. The graphical measurement of d can be achieved with an accuracy of between 1 and $1\frac{1}{2}\%$. The individual errors in measurement of titration

volumes and their corresponding colour effects are somewhat eliminated by drawing the graph for continuous variation as this is made by considering the general shape of the curve and not necessarily joining each individual point.

The continuous variation curves show that for the limited number of dye/metal combinations studied the majority of mixtures formed a 2:1 metal:dye complex (see Table 4).

Both the red 10B/metal combinations ie. with copper 11 and iron 11, form a 1:1 complex of pH 12.5, whereas both the yellow RY/copper 11 and yellow RY/iron 11 form 1:2 complexes at pH 12.5. Carmoisine, cobalt 11, iron 11 and iron 111 at pH 12.5 all form 1:2 complexes. At pH 7.4 carmoisine formed a 2:1 dye/metal complex with copper 11. This latter combination was studied at three wavelengths. At 16,600 cm^{-1} the continuous variation graph shows a weak maximum at dye:metal ratio of 1:2 and a strong minimum at dye:metal ratio of 2:1. The studies at the other two wave numbers confirm the latter dye:metal 2:1 ratio. The study at 14,500 cm^{-1} showed parabolic portions on the continuous variation graphs which was shown earlier to indicate a second possible complex present other than the one recorded giving the major peak on the graph. Furthur experiments would have to be completed before the 1:2

dye/metal could be fully elucidated; it appeared that the complex was quite highly dissociated.

Iron 11 with ponceau 4R at 12.5 and chocolate brown HT at 12.5 both formed 2:1 metal:dye complexes. The only other continuous variation study was that of iron 111/amaranth at 12.5 which formed a 1:1 complex. This latter combination was confirmed to be a 1:1 complex by the straight line method. The continuous variation graph showed no parabolic portions near the x = 0 or x = 1 axis which confirms that no second species was present or that m = n>1.

Of all the continuous variation studies only the copper 11/carmoisine combination showed slight abnormality, which has already been discussed. It appeared that the only species of complexes present in any solution were those summarised in Table 4 , although the copper 11/carmoisine probably had a second species present particularly in solutions of low dye concentrations and fairly high copper concentrations. Furthur experiments would have to be performed to consider whether this 2:1 metal/dye complex breaks down before the formation of the 1:2 metal/dye complex or whether both are present in solutions in various proportions. The pH dependence of the 2;1 complex would be another avenue of furthur investigation.

METALLOCHROMIC INDICATORS

Dyes have to be individually monitored for their potential as indicators in complexometric titrations (26,27,28) and the present investigation could form such a service. The use of some of the dyes, under investigation, as indicators for oxidation titrations is well known. Whereas Lueck's (16) study with 0.06 per cent hydrogen peroxide showed no effect on the dyes, amaranth and ponceau 4R were destroyed by excess oxidant when they were used as indicators in oxidation titrations involving potassium bromate and potassium iodate (17,18). For the determination of hypobromite and bromite by titration against arsenius oxide, tartrazine can be used as the indicator (19). Similarly it was found that tartrazine was a good reversible indicator for the titration of arsenite with sodium hypochlorite (20) in a solution containing sodium bicarbonate and potassium bromide. Also tartrazine was found to be of use in the final titration with hypochlorite in the Kjeldahl's method for the determination of nitrogen in organic compounds (21). The amperometric determination of hafmium (IV) was achieved with tartrazine and gave a 1:1 ratio of Hf(IV) : tartrazine (22). Tartrazine had been developed as a selective reagent for zirconium (23) and was found to be adversely

masked by sulphate ions and tartaric acid but these did not prevent its use in the analysis of alloys and ores. The same dye had been used for the colorimetric determination of palladium ll in the presence of platinum(lV) (24). Both tartrazine and amaranth were recommended for an indicator in the determination of antimony lll using chloramine T (36). Amaranth formed an insoluble 3:1 bismuth:amaranth compound at pH 2.3 - 3.0 which found use in the determination of bismuth (25) in alloys using colorimetric analysis.

Azo dyes and triphenyl methanol dyes had been the centre of intense interest in the search for possible indicators suitable for use in complexometric titrations but the food dyes have not been exhaustively studied (3). A selective metallochromic indicator for the calcium and magnessium ions proved a centre of intense interest and this awkward problem was eventually resolved by calcichrome (26,27,28,29,30-35,37,40-45)(39)

Diehl and Ellingboe (1960)(37) predicted, after examination of a large number of monoazo compounds which combined with calcium and magnessium ions, that the criteria for compound formation was the presence of ortho and ortho'hydroxy groups with respect to the azo groups or ortho hydroxy and ortho' carboxyl groups.

Several of the dyestuffs used as metallochromic indicators in EDTA titrations belong to the O - O'- dihydroxy group of azo dyes (48) and under suitable conditions, gave well defined colour changes at the titration end points. These colour changes were due to the changes in electronic configuration brought about by the chelation arising from the favourable position of the O - O' hydroxy groups. However, only a limited number of ortho mono hydroxy azo dyes have applications as metallochromic indicators, and these, for example SPADNS, 3-(4-sulphophenyl azo)-4,5-dihydroxy naphthalene 2-7-disulphonic acid, normally have two hydroxy groups suitably disposed to form a ring system by chelation.

All, except four, of the azo dyes included in the present investigation have one hydroxy group in a position ortho to the azo linkage. Of the remainder, chocolate brown H.T. has two hydroxy groups - ortho to the same end of the azo linkage. Yellow 2G also has two hydroxy groups but neither are in a suitable position for chelation, as is the case of the mono hydroxy group of tartrazine. Yellow RFS is in the unique position of possessing not even a single hydroxy group.

From what has been said above it can be seen that the food dyes were not suitable dyes for chelation with calcium

and magnessium ions and this was confirmed by this present investigation. In fact acid alizarin black SN is of the type tris-hydroxy bis-azo dye, and calichrome, developed as a specific reagent for calcium (38) has the structure cyclo-tris-7-(1-azo-8 hydroxy naphthalene-3:6 disulphonic acid).

Other food dyes have been suggested as specific metallo-chromic indicators for various metal ions: orange G for example was selective for palladium ll in the presence of other elements, the maximum sensitivity being achieved between pH 6.5 and 6.8 (50).

Chelation of the food dyes with the particular metal ions chosen for this study, namely calcium, magnessium, aluminium, cobalt ll, copper ll, iron ll and iron lll, was possible only on a very restricted scale (see Table 5). The changes in the electronic configuration of the dyes were somewhat limited and these changes were almost exceptions to the rule. The carmoisine copper complex (1:2 metal:dye) at pH 6.4 or 7.4 was the outstanding example of the ability of some of the food dyes to be able to change its configuration sufficiently to cause a visible colour change. Possibly red FB/Coll combination could be useful, but this would need a more thorough investigation.

CARMOISINE AS A POSSIBLE METALLOCHROMIC INDICATOR.

The pH of 7.4 was found to be the most sensitive pH for the combination of copper II and carmoisine (see graph 16 + 19). T.S.West (51) has mentioned a few criteria when making a search for a possible metallochromic reagent, and suggested that a media of as low a pH as possible would be used for compounds containing weakly ionised hydroxy groups and sulphonic acid groups, in order to increase the solubility and stability. The food dyes, and carmoisine in particular, contain a hydroxy group and two ionised sulphonate groupings.

$$(M^+) \text{ metal ions} + \text{dye } (HA) \rightleftharpoons M_m A_n + H^+$$

$$mM^+ + nH^+A^- \rightleftharpoons M_m A_n + nH^+ \quad \text{(changes not balanced for simplicity)}$$

If the dye-metal complex was reasonably stable then the high stability constant may best be taken advantage of by using as acid a pH as possible in an endeavour to minimize the concentration of A^- ions from HA and so only the complexes of the highest stability will be formed at these very acid pH's. In alkaline pH the larger concentration of A^- would enable many metals to form stable complexes with A^- ions and so the reagent (the dye) would be less selective for any particular metal and at this pH masking reagents might have to be adopted.

The criteria of a good mettallochromic indicator is that it should provide a visually clear sharp colour change at the desired end point or equivalence point at the working pH. The necessity for a specific action with the desired metal is also very important because industrial analysis of metals usually deals with impure salts. The end point of copper 11/ EDTA when determined by carmoisine at pH 7.4 was somewhat hindered by the presence of nickel, chromium, manganese and iron ions in solution. The fairly high working pH of carmoisine made it less specific for copper 11 than other reagents. No masking experiments were considered necessary for this metal/dye combination as it was obviously not going to be of any extensive universal use as a metallochromic indicator for copper 11.

The stability constant for the copper 11/ carmoisine system at pH 7.4 was over the order 36.4×10^{10} which is rather a higher value than some metallochromic indicators but of the same magnitude as the 1:2 metal/dye complex of calcium/acid alizarin black SN. $K = 8.26 \times 10^{11}$. Also acid alizarin black SE/calcium 2:1 complex had a K value of 7.08×10^{11}. Some other saitisfactory calcium metallochromic indicators have K values of about 10^{5}.

It is of academic interest to notice that blue VRS in small quantities acted effectively as a screening agent in the titration of copper II/EDTA /carmoisine end point. The spectrophotometric graphs are given on graph 18. The end point was easily discerned to be from purple to pale green (in slight excess of copper II).

SOME BIOLOGICAL ASPECTS OF THE FOOD DYES.

The food dyes in this discussion were conveniently classified by the Ministry of Agriculture, Fisheries and Food (1). The Food Standards committee reviewed the toxicological evidence for the food dyes of 30 coloured dyes permitted in 1957 and classified them in groups A,B,C.

Class A contained colours which appeared to be innocuous when consummed in the amounts customarily used for colouring foods.

Class B contained colours for which evidence was scanty.

Class C contained colours which had been shown to have, or suspected to have harmful effects on health.

The committee commented, "It is with regret that we have to record that scientific literature reveals that for only comparatively few colours is there any information as to the chromic toxicity or carcogenicity. In the case of almost all the colours there is still lack of sufficiently comprehensive biological evidence to enable conclusive opinion to be formed."

The Pharmacology Panel, in the same report, considered the toxicity data for each colour and then furthur classified the food dyes. (Only the dyes relevant to this discussion are quoted.)

Group 1 contained colours for which available evidence suggested that they were acceptable for use in food:-

Amaranth and Green S.

Group 2 contained the dyes for which evidence suggested provisional acceptance for use in food, but about which furthur information was necessary:- carmoisine, fast red E, ponceau 4R, ponceau MX, sunset yellow FCF, tartrazine, black PN.

Group 3 classified the dyes for which the available evidence suggested possible toxicity and which ought not to be allowed in food:- ponceau SX.

Group 4 contains blue VRS for which, at the time, evidence suggested probable toxicity and which ought not to be allowed in food.

Group 5 Colours for which available evidence was inadequate were classified in this group and contains red 10B, red 2G, orange G, orange RN, yellow 2G, violet BMP, red 6B.

Group 6 Colours for which no information of toxicity was available included red FB, yellow RFS, yellow RY, chocolate brown HT.

Of the colous grouped above ponceau SX, blue VRS, yellow RFS and yellow RY were removed from the permitted food additives list in 1957. The Pharmacology Panel of 1957 emphasised that in no instance did the available evidence

fully comply with the Ministry of Health's "Guide to screening tests for cercinogenicity 19, 108 (1960)".

The Food Standards committee (1:) reported that little information was available on the chronic toxicity and carcinogenicity of food dyes and no limitation was made in the 1964 report on the quantity of colouring matter which could legally be added to food as they thought that with good commercial practice the colouring matter in food should be self limiting. the 1966 report of the same committee restricted the use of food dyes in certain foods but again no limit was imposed upon quantity in those cases where dyes were permitted. As from 27th June 1967 colouring matter was prohibited from being added to meat, game, poultry, fish, fruit, vegetables, tea, coffee, bread, milk, cheese and butter, although carotene or annatto was permitted for certain dairy products.

The study of "no effect" level and limiting quantities of food dyes have been investigated with a number of dyes. The short term study of chocolate brown HT in rats (4) showed that the maximum "no effect" level was regarded as 0.6% for the diet for 90 days and above this level male rats developed slight anaemia. The long term study of chocolate brown HT in rats (5) showed close agreement with the short term study

and concluded that the "no effect" level could be established at 0.5% in a 12 week diet of rats.

Ponceau MX had a low oral toxicity when fed to mice and rats, but growth retardation seen at the 2% dietary level for 90 days especially in females, was considered as a toxic manifestation. Also there was a possibility that the pigment present in the kidney tubules played a contributary part in evoking a urinary tract infection. Ikeda (60) reported a possible connection between liver tumour production by the 2, 4 xylylazo isomer of ponceau MX in rats and mice.

The metabolic fate of azo dyes such as the ones here considered was closely linked to their toxicity and azo reductive fissions were known to proceed readily in the body, (15) (61)(2,3). The azo reductive fissions of dyes which had a "sulphonated moiety" on one side of the azo link, and a heavily methylated phenyl moiety on the other, gave rise to an easily eliminated aminosulphonic acid derivative and to aromatic amines which do not undergo furthur metabolism to more easily excretable products (14).

Of the dyes considered, only ponceau MX would come into this category with perhaps ponceau SX as another possibility. Amines liberated after metabolic reduction is of considerable importance and much work has been done on this topic.

The effect of sunset yellow FCF upon cats and rats gave no abnormal behaviour (6) and in fact a 2% solution of the dye had a pseudo vitamin effect with growing rats when fed to rats with a diet containing no vitamin B_2 (7).

When rats were weekly injected with twenty milligrammes of blue VRS for a period of 45 weeks, eighteen of the twenty rats studied produced ulceration and abcess formation at the injection site. There were no marked effects when treated similarly with green S (8).

The effect of amaranth and tartrazine were compared with the effect of the carcinogen p-dimethyl-amino azo benzene on rats and the two food dyes showed no carcinogenic activity(9) Tartrazine containing carbon fourteen ^{14}C were injected into rats and rabbits and they were largely excreted unchanged in the urine within twenty four hours (10). Humans, when given tartrazine orally, excreted free and conjugated sulphanilic acid over a period of 48 hours. Clayson (46) studied the chemical carcinogenosis of many compounds including a group of materials phenyl azo-2-naphthol of which carmoisine and sunset yellow FCF are derivatives and showed that despite earlier observations these two materials were not carcinogenic.

L. Golberg (43) gave a review of artificial colouring materials and commented that the oral administration of

tartrazine gave no free dye in the urine or faeces but some sulphônilic acid. When the dye was given parenterally the animal was dyed bright yellow and free colouring appears in the urine but without sulphônilic acid. Many of the experiments with food dyes were performed by subcutaneous injections. Some ulceration occurred at the point of injection but extensive study of this effect gave the conclusion that the colouring materials themselves were not in any way connected with carcinogenicity but the fibro-sarcomas and ulcers were produced by the peculiar circumstances of the test procedure and in fact the production of these sarcoma provided no evidence for or against carcinogenicity.

It was reported that sulphonated colouring was not subjected to attack by the intestinal micro organisms and were sufficiently strong acids not to undergo appreciable absorption from the intestine. The triarylmethane colourings are extracted almost quantitatively in the faeces but the work of 1955 needed to be revised using modern apparatus and methods, but blus VRS was a non carcinogenic compound (47). The lipid soluble azo compounds did not undergo cleavage in the intestine but are absorbed intact and acted upon by liver azo reductase to form the corresponding primary amines. Another change which an azo compound

underwent involved protein-binding, hyroxylation and other effects such as N- and O- dealkylation which was brought about by microsomal processing enzymes.

It is believed that iron in the +2 oxidation state was the form most effectively utilised by the metabolisms of animals and towards this end iron lll is reduced to the +2 oxidation state before diffusion in the mucosal cell (62). Iron in the oxidation states +2 and +3 are available to the body and consideration should be given to the possible effect of food dyes upon the availibility of this iron.

The iron ll and iron lll solutions form compounds of varying stability and colour with all classes of food dyes. The majority 18/31 of the changes are at pH 12.5, these are the distinct visual colour changes. The metabolic pH's of 6.4 and 7.4 have respectively 8 and 1 possible compounds (Table 2). The iron/dye systems chosen for continuous variation study are given in Table 4 and the degree of dissociation are all in the region 0.06 to 0.20; the values of K vary between (18.8 to 399) x 10^{10} for the 1:2 metal/ dye complexes and the two 1:1 dye/metal combinations have stability constants of order of (29.4 to 36.0) x 10^{5}. All the continuous variation systems studied for iron ll and iron lll have been at pH 12.5 which is very much outside the region of the metabolic functions, but the K value shows that these

complexes are very stable. The colour changes with added iron ll and lll of the remaining dyes were not considered sufficient for continuous variation studies. Furthur investigations using other techniques would be of great use in giving information of the availability of iron ll and lll for metabolism in the presence of food dyes. The present investigation cannot be extended to compare the competition which possibly occurs between the iron/food dye complexes and the iron complexes with sugars or other polyhydroxy compounds which are claimed by Saltman (62) and Charley (63, 64) to be of great importance.

CONCLUSION

Small quantities of the metal ions used have only minimal effects upon the colour of the water soluble dyes studied. In acid solution the effect is almost non existent, but in the alkaline region of pH a few of the dyes are susceptible to the attack of certain metal ions, mainly the transition elements, and a colour change is often achieved, resulting in a metal:dye complex formation. The uptake of these metal ions by the dyes and the influence of this reaction upon metabolic processes would appear to be of importance and at present little has been published concerning these interactions. The nature of the iron/dye compounds and the possible loss of available iron for metabolic purposes needs a very close study. It would appear that the food dyes here studied are of no great use as metallochromic indicators for the more common metals although they might be of some importance for other elements, for example the other transition elements or the metals of the lanthanide series.

TABLE 1

ABSORPTION BAND MAXIMA (IN 10^{-3} cm^{-1}) AND OPTICAL DENSITIES (IN BRACKETS) AT VARIOUS CONDITIONS OF pH AND IN THE PRESENCE OF CERTAIN METAL IONS

(Data underlined relate to absorption band maxima in the presence of metal ions removed by 800 cm^{-1}, or more, from those observed for the free dyes.)

* point of inflection
/ shoulder

DYE	pH 3.0	pH 6.4	pH 7.4	pH 12.5
		Free Dye		
1-naphthyl-Azo-Phenyl Dyes				
Orange G	*25.0(0.51)	*24.5(0.50)	*25.0(0.50)	25.2(0.58)
	20.8(0.89)	20.8(0.88)	21.0(0.88)	20.2(0.49)
Orange RN	/24.2(0.56)	/23.8(0.56)	24.8(0.56)	23.0(0.62)
	20.8(0.87)	20.3(0.87)	21.0(0.87)	/20.0(0.52)
Sunset Yellow FCF	*24.2(0.61)	*23.8(0.60)	*24.4(0.61)	22.2(0.74)
	21.0(0.91)	20.4(0.89)	21.0(0.91)	*20.0(0.60)
Ponceau MX	25.8(0.43)	*25.6(0.45)	25.8(0.39)	-
	19.6(0.88)	19.7(0.88)	20.4(0.87)	20.0(0.69)
Chocolate Brown HT	21.6(0.86)	21.2(0.85)	21.6(0.86)	21.2(0.91)
1-Naphthyl-Azo-1-Naphthyl Dyes				
Ponceau 4R	20.0(0.99)	19.4(0.88)	20.0(1.00)	20.6(0.93)
Amaranth	19.0(0.92)	19.0(0.93)	19.6(0.91)	20.2(0.79)
Fast Red E	19.6(0.86)	19.7(0.85)-	20.2(0.84)	21.0(0.72)

TABLE 1 (cont.)

DYE	pH 3.0	pH 6.4	pH 7.4	pH 12.5
1-Naphthyl-Azo-2-Naphthyl Dyes				
Carmoisine	24.6(0.60)	24.4(0.40)	-	-
	19.4(0.98)	19.2(0.90)	19.6(0.88)	19.6(0.98)
Black PN	24.4(0.64)	24.3(0.66)	24.0(0.65)	25.8(0.72)
	17.6(0.98)	17.2(0.97)	17.2(0.96)	17.6(0.89)
2-Naphthyl-Azo-Phenyl Dyes				
Ponceau SX	20.0(0.92)	19.7(0.91)	20.0(0.89)	21.0(0.84)
Red 10B	19.0(0.92)	18.7(0.90)	19.0(0.91)	18.8(0.89)
Red 2G	20.0(0.95)	19.8(0.95)	20.0(0.95)	21.8(0.80)
	19.0(0.95)	18.7(0.95)	19.0(0.95)	
Red 6B	19.0(0.93)	18.9(0.94)	19.0(0.90)	21.6(0.83)
(a)Red F.B.	19.3(0.49)	19.6(0.49)	19.6(0.49)	18.8(0.48)
Phenyl-Azo-Phenyl Dyes				
Yellow RY	23.0(0.89)	22.7(0.88)	23.0(0.86)	22.0(0.83)
Yellow RFS	24.0(0.83)	23.3(0.84)	25.0(0.81)	24.0(0.86)
Phenyl-Azo-Pyrazole Dyes				
Yellow 2G	24.6(0.91)	24.7(0.91)	24.8(0.89)	25.0(0.90)
Tartrazine	23.0(0.91)	23.3(0.91)	23.8(0.91)	25.0(0.84)

TABLE 1 (cont.)

DYE	pH 3.0	pH 6.4	pH 7.4	pH 12.5
		Free dye		
Triphenyl-Methanol Anhydride Dyes				
Blue VRS	24.4(0.93)	24.0(0.74)	24.0(0.74)	24.4(0.43)
	16.0(0.99)	15.5(1.00)	16.0(1.00)	16.0(0.97)
Green S	25.0(0.50)	24.9(0.47)	25.0(0.54)	25.0(0.64) (split)
	22.8(0.48)	22.5(0.42)	22.8(0.37)	-
	16.0(1.00)	15.4(0.90)	16.0(1.00)	16.4(0.90)
Violet BNP	18.0(0.98)	18.0(0.96)	18.8(0.96)	18.4(0.78)
	17.0(0.98)	16.9(0.96)	17.8(0.95)	17.0(0.85)

TABLE 1. (cont)

DYE	pH 3.0	pH 6.4	pH 7.4	pH 12.5
	Dye in the presence of excess copper(11) ions			
1-Naphthyl-Azo-Phenyl Dyes				
Orange G	*24.8(0.51)	*24.4(0.50)	*25.0(0.49)	-
	20.8(0.88)	20.8(0.86)	21.0(0.87)	21.0(0.62)
Orange RN	/24.2(0.50)	/23.8(0.55)	26.4(0.58)	-
	20.7(0.87)	20.3(0.84)	21.2(0.84)	20.8(0.74) 17.6(0.66)
Sunset Yellow FCF	*24.4(0.57)	*23.8(0.63)	/24.8(0.57)	-
	21.0(0.88)	20.6(0.87)	20.8(0.86)	22.2(0.71)
Ponceau MX	25.6(0.44)	*25.0(0.55)	26.0(0.40)	-
	19.6(0.88)	19.8(0.85)	20.2(0.86)	21.6(0.67)
Chocolate Brown HT	21.6(0.86)	21.4(0.83)	21.6(0.80)	21.2(0.81)
1-Naphthyl-Azo-1-Naphthyl Dyes				
Ponceau 4R	19.6(1.00)	19.4(0.85)	19.6(1.00)	22.0(0.95)
Amaranth	19.0(0.92)	19.2(0.83)	19.6(0.85)	21.0(0.75)
Fast Red E	19.8(0.86)	19.70(0.79)	19.9(0.75)	21.4(0.69)
1-Naphthyl-Azo-2-Naphthyl Dyes				
Carmoisine	/24.8(0.61)	-	-	-
	19.4(0.98)	20.8(0.66)	21.6(0.63)	19.6(0.98)
Black PN	24.4(0.64)	24.3(0.66)	24.0(0.56)	25.6(0.73)
	17.6(0.96)	17.2(0.92)	17.6(0.95)	17.6(0.90)

TABLE 1. (cont.)

DYE	pH 3.0	pH 6.4	pH 7.4	pH 12.5
	dyes in excess of copper II ions			
2-Naphthyl-Azo-Phenyl Dyes				
Ponceau SX	20.0(0.91)	20.0(0.36)	20.6(0.70)	20.8(0.86)
Red 10B	19.0(0.92)	18.5(0.91)	19.4(0.91)	18.8(0.91)
Red 2G	20.0(0.95)	19.8(0.95)	20.0(0.92)	21.8(0.77)
	19.0(0.95)	18.7(0.95)	19.0(0.92)	
Red 6B	19.0(0.94)	18.9(0.94)	19.4(0.89)	21.6(0.83)
(a) Red F.B.	19.3(0.50)	19.6(0.45)	19.6(0.36)	18.5(0.49)
Phenyl-Azo-Phenyl Dyes				
Yellow RY	23.0(0.90)	22.7(0.86)	23.0(0.86)	23.0(0.78)
Yellow RFS	24.0(0.82)	23.2(0.85)	24.8(0.82)	24.0(0.93)
Phenyl-Azo-Pyrazole Dyes				
Yellow 2G	24.6(0.91)	24.8(0.90)	24.8(0.88)	25.2(0.88)
Tartrazine	23.2(0.91)	23.3(0.91)	23.6(0.92)	25.0(0.84)
Triphenyl-Methanol Anhydride dyes				
Blue VRS	24.4(0.90)	24.0(0.77)	24.4(0.79)	24.4(0.66)
	16.0(0.98)	15.2(1.00)	16.0(1.00)	16.0(1.00)
Green S	25.0(0.52)	24.8(0.52)	25.2(0.56)	26.6(0.55)
	22.8(0.47)	22.2(0.46)	22.8(0.40)	-
	16.0(0.98)	15.5(1.00)	16.4(1.00)	16.4(0.99)
Violet BNP	18.0(0.98)	18.0(0.93)	18.4(0.98)	-
	16.8(0.98)	16.8(0.97)	17.4(0.98)	16.8(0.10)

TABLE 1 (cont.)

DYE	pH 3.0	pH6.4	pH 7.4	pH 12.5
	Dye in presence of excess iron (11) ions			
1-Naphthyl-Azo-Phenyl Dyes				
Orange G	*25.0(0.51)	-	*24.4(0.49)	-
	20.8(0.89)	20.8(0.96)	20.8(0.85)	21.0(0.60)
Orange RN	/24.4(0.58)	24.8(0.60)	25.2(0.59)	/24.0(0.78)
	20.5(0.89)	20.4(0.87)	20.8(0.84)	-
Sunset Yellow FCF	*24.0(0.65)	/23.9(0.57)	*24.0(0.62)	*24.0(0.94)
	20.6(0.91)	20.6(0.83)	20.8(0.91)	-
Ponceau MX	25.6(0.43)	-	25.8(0.47)	-
	19.8(0.78)	19.8(0.95)	20.0(0.85)	20.2(0.74)
Chocolate Brown HT	21.4(0.85)	21.3(0.88)	22.0(0.86)	21.4(0.90)
1-Naphthyl-Azo-1-Naphthyl Dyes				
Ponceau 4R	19.6(0.99)	19.8(0.82)	20.0(1.00)	*23.0(0.96)
Amaranth	19.0(0.92)	19.0(0.92)	19.6(0.87)	20.4(0.81)
Fast Red E	20.0(0.84)	19.7(0.82)	19.9(0.80)	/25.0(0.84)
1-Naphthyl-Azo-2-Naphthyl Dyes				
Carmoisine	*24.4(0.60)	-	-	-
	19.3(0.98)	19.2(0.87)	20.0(0.86)	19.6(0.98)
Black PN	24.4(0.64)	-	24.0(0.60)	/24.4(0.84)
	17.4(0.98)	17.4(0.98)	17.6(0.96)	/20.6(0.83)

TABLE 1.(cont.)

Dye	pH 3.0	pH 6.4	pH 7.4	pH 12.5
	Dye in presence of excess iron (II) ions			
2-Naphthyl-Azo-Phenyl Dyes				
Ponceau SX	20.0(0.84)	19.6(0.90)	20.2(0.87)	21;4(0.93)
Red 10B	19.2(0.93)	18.9(0.89)	19.0(0.88)	19.0(0.91)
Red 2G	20.0(0.95) 19.0(0.95)	19.5(0.95) 18.7(0.95)	20.0(0.93) 19.0(0.93)	21.9(0.84)
Red 6B	19.0(0.94)	18.9(0.92)	19.4(0.93)	ǂ22.8(0.93)
Red FB	19.5(0.47)	ǂ20.6(0.69)	19.6(0.55)	*20.4(0.66)
Phenyl-Azo-Phenyl Dyes				
Yellow RY	22.8(0.89)	ǂ25.5(0.97)	22.8(0.82)	23.8(0.89)
Yellow RFS	23.6(0.77)	24.9(0.93)	24.8(0.81)	24.8(0.95)
Phenyl-Azo-Pyrazole Dyes				
Yellow 2G	24.4(0.91)	-	24.8(0.88)	25.8(0.96)
Tartrazine	23.6(0.90)	23.2(0.90)	23.6(0.87)	ǂ23.0(0.99)
Tri-Phenyl-Methanol Anhydride Dyes				
Blue VRS	24.4(0.93)	24.3(0.92)	24.6(0.68)	*26.6(0.96)
	15.6(0.95)	15.8(1.00)	16.0(1.00)	16.0(1.00)
Green S	24.0(0.45)	-	25.2(0.56)	-
	22.8(0.43)	-	*22.8(0.48)	-
	16.0(0.92)	15.5(0.99)	16.0(0.99)	16.4(0.97)
Violet BNP	ǂ18.0(0.98)	-	18.4(0.96)	ǂ18.4(0.62)
	17.0(0.98)	18.3(0.90)	ǂ17.6(0.96)	17.0(0.69)

TABLE 1 (cont.)

Dye	pH 3.0	pH 6.4	pH 7.4	pH 12.5
	Dye in presence of excess iron(III) ions			
1-Naphthyl-Azo-Phenyl Dyes				
Orange G	-	-	*23.8(0.57)	-
	20.8(0.97)	20.8(0.92)	20.6(0.89)	20.8(0.62)
Orange RN	24.4(0.60)	24.7(0.52)	24.7(0.53)	23.0(0.79)
	20.4(0.89)	20.6(0.94)	20.4(0.85)	*19.8(0.56)
Sunset Yellow FCF	*23.8(0.60)	*23.8(0.63)	*24.0(0.56)	22.5(0.78)
	20.6(0.89)	20.6(0.89)	20.4(0.26)	*20.0(0.66)
Ponceau MX	*25.3(0.96)	-	-	-
	19.6(0.88)	19.7(0.91)	19.7(0.89)	19.6(0.74)
Chocolate Brown HT	21.3(0.88)	21.4(0.85)	21.3(0.81)	21.3(0.92)
1-Naphthyl-Azo-1-Naphthyl Dyes				
Ponceau 4R	19.4(0.88)	19.4(0.89)	19.6(0.84)	20.6(0.65)
Amaranth	24.4(0.38) 18.9(0.95)	20.0(0.94)	19.3(0.93)	20.7(0.96)
Fast Red E	19.2(0.86)	19.6(0.81)	19.6(0.98)	20.6(0.79)
1-Naphthyl-Azo-2-Naphthyl Dyes				
Carmoisine	25.0(0.53)	-	-	-
	18.8(0.92)	19.2(0.94)	19.2(0.88)	20.2(0.98)
Black PN	24.4(0.67)	24.3(0.67)	24.3(0.60)	25.3(0.67)
	17.2(0.98)	17.2(0.97)	17.2(0.95)	17.2(0.91)

TABLE 1 (cont.)

Dye	pH 3.0	pH 6.4	pH 7.4	pH 12.5
	Dye in presence of excess iron (LLL) ioms			
2-Naphthyl-Azo-Phynyl dyes				
Ponceau SX	19.7(0.91)	19.7(0.89)	19.8(0.92)	20.6(0.87)
Red 10B	*24.0(0.48) 18.5(0.92)	18.7(0.90)	18.7(0.91)	19.0(0.95)
Red 2G	20.5(0.96) 18.5(0.96)	19.6(0.95)	19.5(0.92) 18.7(0.92)	20.0(0.87)
Red 6B	18.5(0.96)	18.9(0.94)	19.0(0.91)	21.0(0.85)
Red FB	19.7(0.53)	19.6(0.52)	19.6(0.52)	†20.4(0.66)
Phenyl-Azo-Phenyl Dyes				
Yellow RY	23.3(0.83) 19.6(0.80)	22.6(0.89)	22.5(0.89)	†26.0(0.95)
Yellow RFS	22.9(0.87)	24.4(0.89)	24.7(0.71)	-
Phenyl-Azo-Pyrazole Dyes				
Yellow 2G	†24.9(0.95)	25.5(0.92)	25.0(0.92)	†26.3(0.97)
Tartrazine	22.7(0.90)	23.8(0.98)	23.2(0.92)	†25.2(0.92)
Tri-Phenyl-Methanol Anhydride Dyes				
Blue VRS	24.3(0.95)	23.0(0.79)	24.2(0.75)	*24.0(0.91)
	15.4(0.97)	15.3(1.00)	15.3(1.00)	15.6(0.90)
Green S	*22.7(0.56)	-	*23.0(0.42)	-
	15.6(1.00)	15.4(1.00)	15.9(1.00)	16.0(0.99)
Violet BNP	†18.0(0.98)	18.0(0.92)	18.1(0.99)	*17.4(0.88)
	16.7(0.90)	16.7(0.98)	†16.7(0.98)	16.5(0.90)

TABLE 1 (cont.)

DYE	pH 3.0	pH 6.4	pH 7.4	pH 12.5
	Dye in the presence of excess cobalt(11) ions			
1-Naphthyl-Azo-Phenyl Dyes				
Orange G	*24.7(0.67)	*23.8(0.50)	*25.2(0.50)	25.0(0.66)
	20.8(0.87)	20.8(0.88)	21.0(0.89)	20.3(0.56)
Orange RN	24.4(0.38)	*23.8(0.56)	25.0(0.56)	22.8(0.72)
	20.4(0.88)	20.3(0.87)	21.2(0.87)	*19.8(0.56)
Sunset Yellow FCF	*23.8(0.58)	*23.8(0.60)	*24.6(0.59)	22.0(0.76)
	20.6(0.89)	20.4(0.89)	21.2(0.91)	20.0(0.66)
Ponceau MX	19.6(0.88)	19.7(0.88)	20.0(0.95)	23.7(0.40)
				19.3(0.59)
Chocolate Brown HT	21.3(0.85)	21.2(0.85)	21.6(0.86)	21.2(0.87)
1-Naphthyl-Azo-1-Napthyl Dyes				
Ponceau 4R	19.4(0.88)	19.4(0.88)	20.0(1.0)	20.0(0.62)
Amaranth	18.9(0.92)	19.0(0.93)	19.6(0.91)	20.0(0.67)
Fast Red E	19.2(0.87)	19.7(0.85)	20.2(0.84)	20.8(0.71)
1-Napthyl-Azo-2-Naphthyl Dyes				
Carmoisine	*24.0(0.40)	*24.4(0.40)	-	-
	18.8(0.92)	19.2(0.90)	19.8(0.84)	19.6(1.00)
Black PN	24.3(0.65)	24.3(0.66)	23.8(0.66)	25.3(0.78)
	17.2(0.98)	17.2(0.97)	17.4(0.95)	17.3(0.91)

TABLE 1 (cont.)

Dye	pH 3.0	pH 6.4	pH 7.4	pH 12.5
	Dye in the presence of excess cobalt (11) ions			
2-Naphthyl-Azo-Phenyl Dyes				
Ponceau SX	19.7(0.91)	19.7(0.91)	20.0(0.87)	20.6(0.86)
Red 10B	*23.6(0.34)	23.6(0.40)	19.4(0.89)	19.0(0.80)
	19.0(0.92)	18.5(0.91)		
Red 2G	20.0(0.96)	20.0(0.95)	20.6(0.95)	21.7(0.86)
	18.5(0.96)	19.0(0.95)	19.0(0.95)	
Red 6B	18.5(0.96)	18.9(0.94)	19.6(0.93)	20.8(0.82)
Red FB	19.3(0.49)	19.6(0.49)	19.6(0.45)	18.0(0.50)
Phenyl-Azo-Phenyl Dyes				
Yellow RY	24.4(0.75)	22.7(0.88)	23.0(0.86)	24.0(0.88)
Yellow RFS	24.4(0.70)	23.2(0.85)	25.0(0.84)	24.4(0.78)
	*19.6(0.62)			
Phenyl-Azo-Pyrazole Dyes				
Yellow 2G	24.4(0.90)	24.7(0.91)	25.0(0.97)	24.8(0.91)
Tartrazine	23.0(0.90)	23.3(0.91)	23.4(0.91)	24.8(0.90)
Tri-Phenyl-Methanol Anhydride Dyes				
Blue VRS	23.9(0.82)	24.0(0.75)	24.4(0.79)	24.2(0.80)
	15.4(0.98)	15.3(1.00)	16.0(1.00)	15.1(1.00)
Green S	24.9(0.50)	25.0(0.50)	25.2(0.55)	24.9(0.72)
	22.3(0.44)	22.3(0.44)	22.8(0.45)	-
	15.6(1.00)	15.4(0.99)	16.2(1.00)	16.0(0.99)
Violet BNP	*23.6(0.16)	18.0(0.96)	18.4(0.96)	16.7(0.15)
	16.8(0.98)	/16.4(0.96)	/17.2(0.95)	-

TABLE 1 (cont.)

DYE	pH 3.0	pH 6.4	pH7.4	pH 12.5
	dye in the presence of excess aluminium (111) ions			
1-Naphthyl-Azo-Phenyl Dyes				
Orange G	*24.7(0.67)	*24.0(0.53)	*25.0(0.51)	25.0(0.55)
	20.8(0.87)	20.8(0.88)	21.2(0.89)	20.0(0.51)
Orange RN	24.4(0.38)	/23.8(0.56)	24.6(0.57)	22.7(0.66)
	20.4(0.88)	20.3(0.87)	21.2(0.88)	/19.8(0.56)
Sunset Yellow FCF	*23.8(0.58)	*23.8(0.60)	*24.6(0.63)	22.1(0.70)
	20.6(0.89)	20.4(0.89)	21.4(0.91)	*20.0(0.60)
Ponceau MX	19.6(0.88)	19.8(0.88)	20.2(0.88)	19.5(0.68)
Chocolate Brown HT	21.3(0.85)	21.2(0.85)	21.8(0.84)	21.2(0.85)
1-Naphthyl-Azo-Naphthyl Dyes				
Ponceau 4R	19.4(0.87)	19.4(0.88)	20.0(1.00)	20.0(0.62)
Amaranth	18.9(0.92)	18.9(0.93)	19.6(0.91)	19.5(0.73)
Fast Red E	19.2(0.87)	19.9(0.85)	19.6(0.80)	20.8(0.71)
1-Naphthyl-Azo-2-Naphthyl Dyes				
Carmoisine	*24.0(0.40)	/24.4(0.40)	-	-
	18.8(0.92)	19.2(0.92)	19.6(0.98)	19.0(0.89)
Black PN	24.3(0.63)	24.3(0.66)	24.4(0.66)	25.4(0.74)
	17.2(0.98)	17.2(0.97)	17.8(0.96)	17.2(0.90)

TABLE 1 (cont.)

DYE	pH 3.0	pH 6.4	pH 7.4	pH 12.5
	dye in the presence of excess aluminium (111) ions			
2-Naphthyl-Azo-Phenyl Dyes				
Ponceau SX	19.7(0.91)	19.7(0.91)	20.2(0.91)	20.6(0.86)
Red 10B	23.6(0.84) 19.0(0.92)	18.5(0.91)	19.0(0.91)	18.5(0.89)
Red 2G	20.0(0.96)	18.5(0.95)	20.0(0.95)	21.7(0.76)
	18.5(0.96)	20.0(0.95)	19.0(0.95)	
Red 6B	18.5(0.96)	18.9(0.94)	19.6(0.94)	20.8(0.78)
Red FB	19.3(0.49)	19.6(0.49)	19.6(0.49)	18.8(0.49)
Phenyl-Azo-Phenyl Dyes				
Yellow RY	24.0(0.79)	22.5(0.89)	22.8(0.86)	24.3(0.85)
Yellow RFS	24.4(0.70)	24.3(0.88)	24.8(0.84)	24.4(0.78)
Phenyl-Azo-Pyrazole Dyes				
Yellow 2G	24.4(0.90)	24.7(0.91)	25.0(0.89)	25.0(0.88)
Tartrazine	22.7(0.90)	23.3(0.92)	23.6(0.91)	24.9(0.87)
Tri-Phenyl-Methanol Anhydride Dyes				
Blue VRS	24.4(0.91)	24.1(0.74)	24.6(0.79)	24.0(0.75)
	15.8(0.98)	15.3(1.00)	16.4(1.00)	15.1(0.97)
Green S	24.8(0.54)	24.9(0.50)	25.0(0.52)	24.9(0.64)
	*23.0(0.45)	22.4(0.45)	-	-
	15.4(1.00)	15.6(0.99)	16.0(1.00)	15.9(0.99)
Violet BNP	*23.5(0.15)	18.4(0.96)	18.8(0.97)	16.4(0.82)
	17.0(0.77)	16.8(0.96)	18.0(0.95)	-

TABLE 1 (cont.)

DYE	pH 3.0	pH 6.4	pH 7.4	pH 12.5
	dye in the presence of excess calcium ions			
1-Naphthyl-Azo-Phenyl Dyes				
Orange G	*25.0(0.50)	*23.9(0.50)	*24.8(0.51)	25.4(0.59)
	20.8(0.90)	20.7(0.88)	21.0(0.88)	20.2(0.52)
Orange RN	/24.2(0.56)	/23.8(0.56)	/24.6(0.56)	23.3(0.60)
	20.5(0.88)	20.3(0.87)	21.4(0.88)	/20.5(0.51)
Sunset Yellow FCF	*24.4(0.63)	*23.8(0.60)	*24.0(0.61)	-
	21.0(0.91)	20.4(0.89)	20.8(0.91)	22.2(0.76)
Ponceau MX	20.0(0.88)	19.7(0.87)	20.0(0.87)	20.0(0.64)
Chocolate Brown HT	21.6(0.86)	21.2(0.85)	22.0(0.85)	21.2(0.87)
1-Naphthyl-Azo-1-Naphthyl Dyes				
Ponceau 4R	19.4(1.00)	19.4(0.88)	20.0(1.00)	20.6(0.95)
Amaranth	19.0(0.92)	19.0(0.93)	19.8(0.91)	20.4(0.76)
Fast Red E	19.6(0.87)	19.7(0.83)	20.4(0.84)	21.0(0.73)
1-Naphthyl-Azo-2-Naphthyl Dyes				
Carmoisine	/24.6(0.60)	/24.4(0.40)	-	-
	19.4(0.98)	19.2(0.90)	19.8(0.87)	19.8(0.97)
Black PN	24.4(0.63)	24.3(0.66)	24.0(0.66)	-
	17.6(0.98)	17.2(0.97)	17.6(0.96)	17.6(0.91)
2-Naphthyl-Azo-Phenyl Dyes				
Ponceau SX	19.8(0.91)	19.7(0.91)	20.4(0.91)	20.8(0.85)
Red 10B	19.2(0.92)	18.4(0.91)	19.4(0.91)	19.0(0.90)
Red 2G	20.0(0.95)	20.0(0.95)	20.0(0.95)	21.6(0.78)
	19.0(0.95)	19.0(0.95)	19.0(0.95)	

TABLE 1 (cont.)

DYE	pH 3.0	pH 6.4	pH 7.4	pH 12.5
	dye in the presence of excess calcium ions			
2-Naphthyl-Azo-Phenyl Dyes cont.				
Red 6B	19.0(0.94)	18.9(0.94)	19.4(0.94)	21.6(0.75)
Red FB	19.6(0.49)	19.6(0.49)	19.6(0.49)	18.8(0.48)
Phenyl-Azo-Phenyl Dyes				
Yellow RY	22.6(0.90)	22.5(0.89)	23.2(0.86)	22.4(0.81)
Yellow RFS	24.0(0.78)	23.0(0.84)	25.0(0.81)	24.0(0.87)
Phenyl-Azo-Pyrazole Dyes				
Yellow 2G	24.8(0.91)	24.7(0.91)	24.6(0.90)	25.0(0.88)
Tartrazine	22.4(0.90)	23.2(0.91)	23.6(0.91)	25.0(0.86)
Tri-Phenyl-Methanol-Anhydride Dyes				
Blue VRS	24.6(0.91)	24.0(0.74)	24.4(0.68)	24.2(0.73)
	16.0(0.98)	15.8(1.00)	16.0(1.00)	15.8(1.00)
Green S	25.0(0.54)	24.9(0.50)	25.0(0.52)	25.6(0.57) (split)
	23.0(0.49)	22.2(0.44)	22.7(0.38)	-
	16.0(1.00)	15.2(0.99)	16.1(0.98)	16.2(0.97)
Violet BNP	18.0(0.98)	18.0(0.96)	18.4(0.96)	-
	17.0(0.98)	16.7(0.96)	17.2(0.95)	17.0(0.18)

TABLE 1 (cont.)

Dye	pH 3.0	pH 6.4	pH 7.4	pH 12.5
	Dye in the presence of excess magnesium ions			
1-Naphthyl-Azo-Phenyl Dyes				
Orange G	*25.0(0.52)	*23.9(0.50)	*24.0(0.50)	25.4(0.59)
	20.7(0.90)	20.6(0.88)	21.0(0.88)	20.4(0.52)
Orange RN	/24.4(0.57)	/23.8(0.56)	/24.6(0.56)	23.0(0.61)
	20.8(0.87)	20.3(0.87)	21.6(0.88)	/20.4(0.54)
Sunset Yellow FCF	*24.4(0.63)	*23.8(0.60)	*24.2(0.61)	-
	20.8(0.91)	20.4(0.89)	20.8(0.91)	20.2(0.74)
Ponceau MX	19.8(0.89)	19.6(0.87)	20.2(0.95)	19.9(0.70)
Chocolate Brown HT	21.3(0.85)	21.2(0.85)	21.6(0.85)	21.2(0.85)
1-Naphthyl-Azo-1-Naphthyl Dyes				
Ponceau 4R	19.6(0.98)	19.4(0.88)	20.0(1.00)	20.8(0.94)
Amaranth	19.4(0.91)	18.7(0.93)	19.6(0.91)	20.0(0.77)
Fast Red E	19.8(0.85)	19.7(0.85)	20.0(0.84)	21.2(0.71)
1-Naphthyl-Azo-2-Naphthyl Dyes				
Carmoisine	/24.4(0.59)	/24.4(0.40)	-	-
	19.2(0.98)	19.2(0.90)	19.6(0.90)	19.6(0.96)
Black PN	24.5(0.63)	24.3(0.66)	24.0(0.63)	25.6(0.80)
	17.9(0.98)	17.2(0.97)	17.4(0.96)	17.6(0.95)

DYE	pH 3.0	pH 6.4	pH 7.4	pH 12.5
	dye in the presence of excess magnesium ions			
2-Naphthyl-Azo-Phenyl Dyes				
Ponceau SX	20.0(0.91)	19.7(0.91)	20.0(0.91)	20.8(0.85)
Red 10B	19.0(0.91)	24.8(0.39)	19.6(0.91)	19.0(0.90)
		18.8(0.91)		
Red 2G	20.0(0.95)	20.0(0.95)	20.0(0.95)	21.8(0.77)
	19.0(0.95)	19.0(0.95)	19.0(0.95)	
Red 6B	18.8(0.94)	18.9(0.94)	19.4(0.94)	21.0(0.76)
Red FB	19.6(0.49)	19.6(0.49)	19.6(0.49)	18.8(0.48)
Phenyl-Azo-Phenyl Dyes				
Yellow RY	22.6(0.90)	22.6(0.89)	23.2(0.86)	22.0(0.80)
Yellow RFS	23.6(0.78)	23.3(0.85)	24.8(0.81)	23.8(0.95)
Phenyl-Azo-Pyrazole Dyes				
Yellow 2G	24.6(0.91)	24.7(0.91)	24.8(0.90)	25.2(0.96)
Tartrazine	22.9(0.91)	23.8(0.92)	23.8(0.91)	25.0(0.91)
Tri-Phenyl-Methanol Dyes				
Blue VRS	24.4(0.90)	24.4(0.68)	24.4(0.68)	24.4(0.73)
	15.9(0.98)	16.0(1.00)	16.0(1.00)	16.0(0.98)
Green S	25.0(0.44)	25.0(0.52)	25.0(0.53)	25.0(0.53)
		/22.7(0.38)		
	22.6(0.49)	-	/22.8(0.35)	-
	16.0(0.93)	16.1(0.98)	16.1(1.00)	16.4(1.00)
Violet BNP	/18.0(0.98)	18.0(0.96)	18.4(0.96)	-
	16.4(0.97)	/16.7(0.96)	/17.2(0.95)	17.0(0.17)

TABLE 2.

Summary of the pH values at which the mixtures of dye and metal ions solutions gave a shift of 800 cm^{-1} or more in the absorption maximum

DYE	Ca	Mg	Al	Co(11)	Cu(11)	Fe(11)	Fe(111)	
Orange G					12.5	12.5	7.4	
Orange RN					7.4 12.5	6.4 12.5		
Sunset Yellow FCF						12.5		
Ponceau MX				12.5	12.5			
Chocolate Brown								
Ponceau 4R						12.5		
Amaranth					12.5		3.0 6.4	
Fast Red E						12.5		
Carmoisine					6.4 7.4			
Black PN						12.5		
Ponceau SX								
Red 10B			3.0	3.0 6.4			3.0	
Red 2G							12.5	
Red 6B						12.5		
Red FB				12.5		6.4 12.5	12.5	
Yellow RY			3.0 12.5	3.0 12.5	12.5	6.4 12.5	3.0 12.5	
Yellow RFS			6.4	3.0		6.4 12.5	3.0 6.4	

TABLE 2. (cont)

DYE	Ca	Mg	Al	Co(11)	Cu(11)	Fe(11)	Fe(111)	
Yellow 2G						12.5	6.4 12.5	
Tartrazine						12.5		
Blue VRS						12.5	6.4	
Green S					12.5			
Violet BNP			3.0 6.4	3.0 6.4			12.5	

TABLE 3.

Summary of Table 2 showing the number of absorption maximum shifts with the addition of metal ions.

	pH 3.0	6.4	7.4	12.5	TOTAL
No. of colour changes					
when CALCIUM ions added	0	0	0	0	0
" Magnesium " "	0	0	0	0	0
" Aluminium " "	3	2	0	1	6
" Cobalt(II)" "	4	2	0	3	9
" Copper(II)" "	0	1	2	6	9
" Iron(II) " "	0	4	0	13	17
" Iron(III) " "	4	4	1	5	14
TOTAL	11	13	3	28	55

TABLE 4. Metal/dye complexes.

Ratio dye/metal	Metal	dye	pH	Wave No. $x10^{-3}cm^{-1}$	d	K
1:2	cobalt 11	carmoisine	12.5	20.0	0.17	$15.5x10^{10}$
2:1	copper 11	carmoisine	7.4	16.6	0.087	
				24.0	0.15	
				14.5	0.14	$36.4x10^{10}$
					Av. 0.13	
1:2	copper 11	yellow RY	12.5	22.0	0.10	$82.6x10^{10}$
1:1	copper 11	red 10B	12.5	20.0	0.08	$57.5x10^{5}$
1:2	iron 11	carmoisine	12.5	19.6	0.13	$36.4x10^{10}$
1:2	iron 11	choc.br.HT	12.5		0.13	$36.4x10^{10}$
1:2	iron 11	yellow RY	12.5	22.4	0.15	$25.8x10^{10}$
1:2	iron 11	ponceau 4R	12.5	20.0	0.11	
			12.5		0.20	
					Av. 0.16	$18.8x10^{10}$
1:1	iron 11	red 10B	12.5	23.4	0.11	$29.4x10^{5}$
1:1	iron 111	amaranth	12.5	20.0	0.10	$36.0x10^{5}$
1:2	iron 111	carmoisine	12.5	20.0	0.06	$399x10^{10}$

TABLE 5.
Classification Group 1.

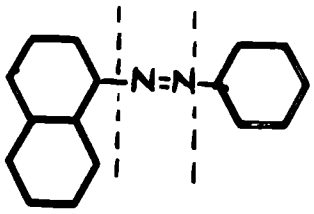
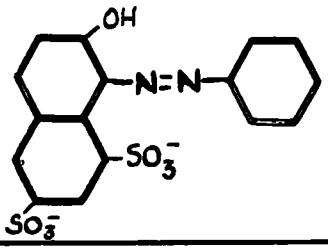
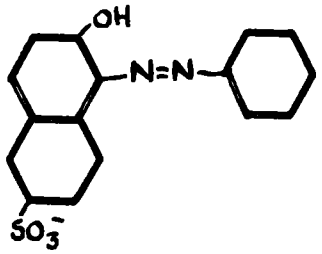
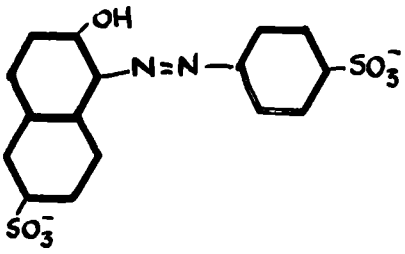
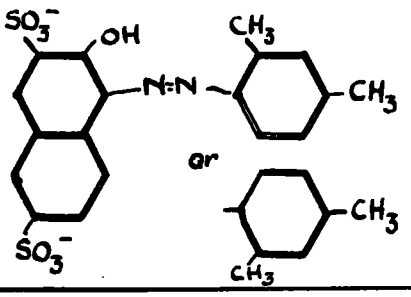
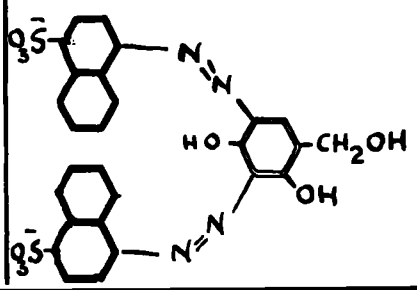
Name of dye	Chemical Name	Molec. wt.	Colour index 1956	Structure
	1-naphthyl-azo-phenyl			
Orange G	di sodium salt of 1-phenyl azo-2 naphthol-6:8 di sulphonic acid	452	16230	
Orange RN	sodium salt of 1-phenyl azo -2 naphthol 6 sulphonic acid	350	15970	
Sunset Yellow FCF	di sodium salt of 1-p-sulpho-phenyl azo - 2 - naphthol-6 sulphonic acid	452	15985	
Ponceau MX	di sodium salt of 1(2:4 or mixed xylazo)- 2 - naphthol-3:6-di sulphonic acid	480	16150	
Chocolate Brown HT	di sodium salt of 2:4-di hydroxy-3:5-di(4-sulpho-1-naphthylazo)benzyl alcohol	652	20285	

TABLE 5.

Classification Group 11.

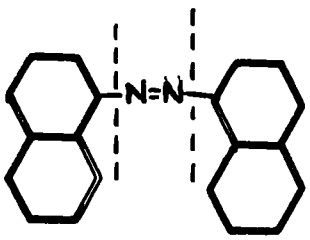
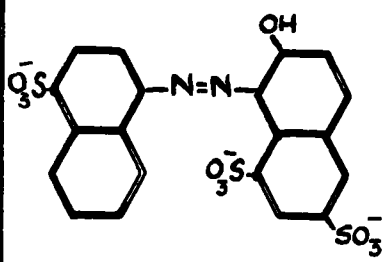
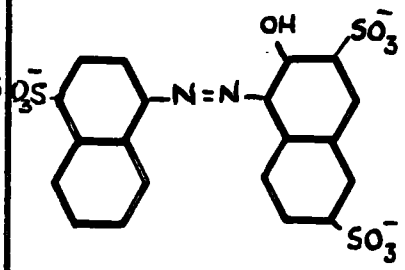
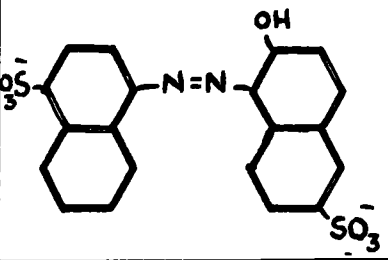
	1-naphthyl-azo-1 naphthyl			
Ponceau 4R	tri sodium salt of 1-(4 - sulpho-1-naphthyl azo)-2 - naphthol-6:8 di sulphonic acid	604	16255	
Amaranth	tri sodium salt of 1-(4sulpho -1-naphthyl azo) 2-naphthol- 3:6-di sulphonic acid	604	16185	
Fast Red E	di sodium salt of 1-(4-sulpho -1-naphthyl azo)-2 hydroxy naphthalene-6-sulphonic acid	502	16045	

TABLE 5.

Classification Group 111.

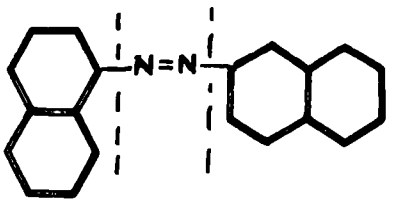
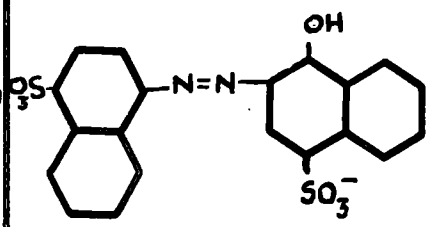
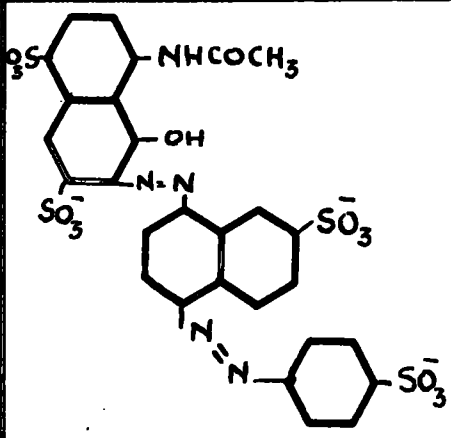
	1-naphthyl-azo-2-naphthyl			
Carmoisine	di sodium salt of 2-(4-sulpho -1-naphthyl azo) 1-naphthol- 4-sulphonic acid	502	14720	
Black PN	tetra sodium salt of 8 acetamido -2-(7-sulpho-4-p-sulpho phenyl azo-1-naphthyl azo) 1-naphthol 3:5 di sulphonic acid	866	28440	

TABLE 5.

Classification Group IV.

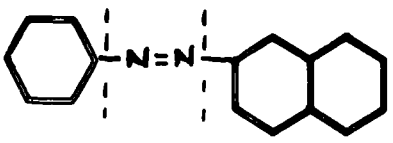
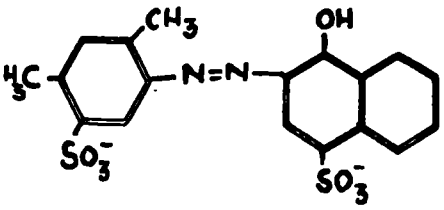
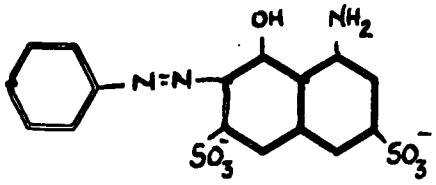
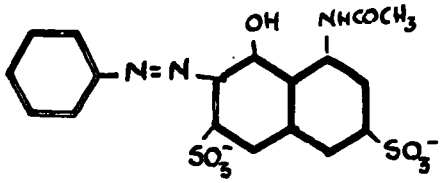
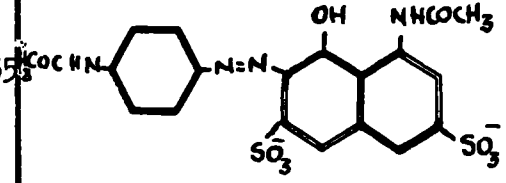
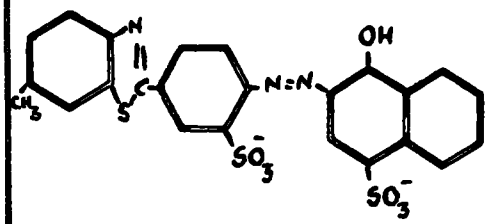
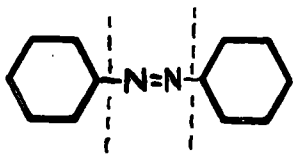
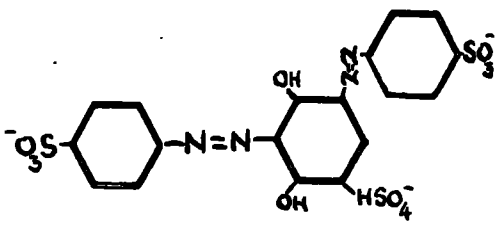
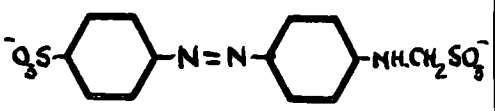
	2-naphthyl azo phenyl			
Ponceau SX	di sodium salt of 2-(5-sulpho 2:4 xylyl azo) 1-naphthol 4 sulphonic acid	480	14700	
Red 10 B	di sodium salt of 8-amino -2-phenyl azo-1-naphthol-3:6-di sulphonic acid	467	17200	
Red 2 G	di sodium salt of 8-acetamido-2-phenyl azo-1-naphthol 3:6 di sulphonic acid	509	18050	
Red 6B	di sodium salt of 8-acetoamido-2-p-acetoamido - phenyl azo-1-naphthol 3:6 di sulphonic acid	566	18055	
Red FB	di sodium salt of 2- 4(1-hydroxy-4-sulpho-2-naphthyl azo)-3-sulpho phenyl 6-methyl benzothiazole	583	14780	

TABLE 5.

Classification Group V.

	phenyl - azo - phenyl			
Yellow RY	tri sodium salt of 2:6-di (4-sulpho phenyl azo)1:3 di hydroxy benzene 4 sulphuric acid *	418	14330	
Yellow RFS	di sodium salt of 4 sulpho -4(sulpho methyl ammo)azo benzene	432	13011	

* not as once thought to be di sodium salt of 6-p sulpho phenyl azo resorcinol-4 sulphonic acid.

TABLE 5.

Classification Group VI.

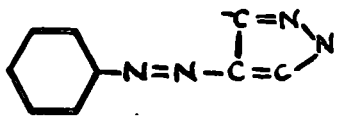
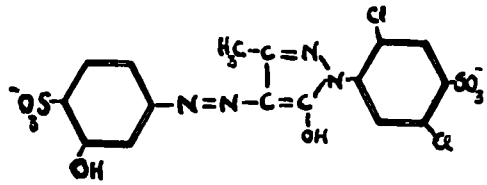
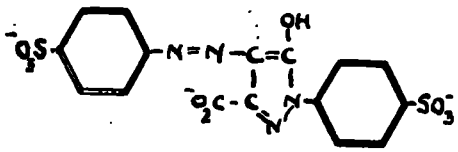
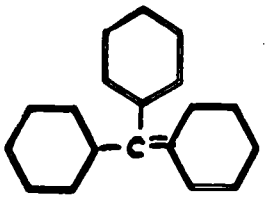
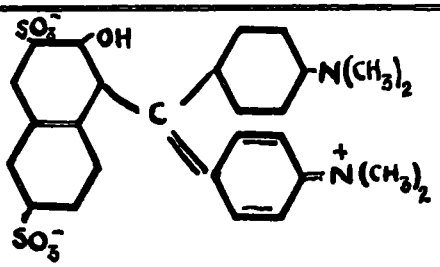
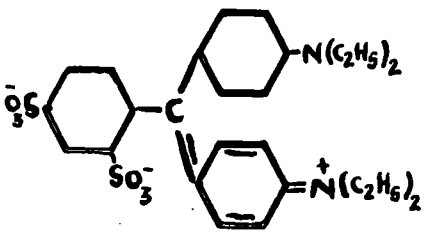
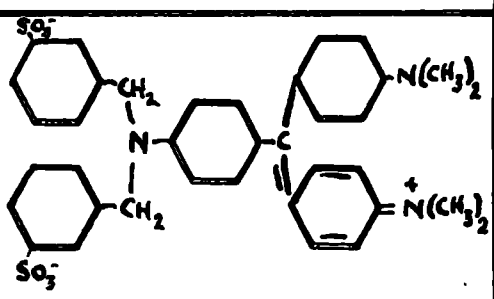
	phenyl-azo-pyrazole			
Yellow 2G	di sodium salt of 1-(2:5-di chloro 4-sulpho phenyl) -5-hydroxy-3-methyl-4-p-sulphophenyl azo pyrazole	568	18965	
Tartrazine	tri sodium salt of 5-hydroxy -1-p sulpho phenyl-4-p-sulpho phenyl azo pyrazole -3-carboxylic acid	534	19140	

TABLE 5.

Classification Group V11.

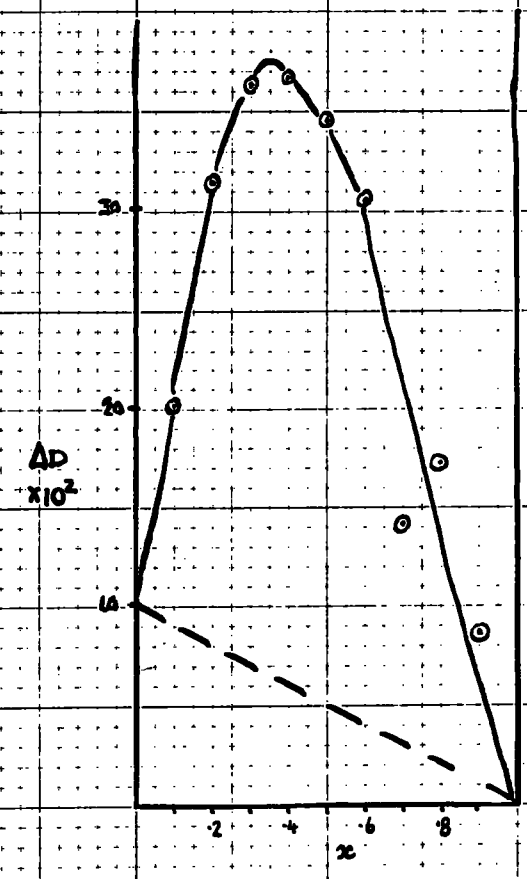
	tri phenyl methanol anhydride			
Green S	sodium salt of di-(p-di methyl amino phenyl)-2-hydroxy-3:6-di sulpho-naphthyl methanol anhydride	576	44090	
Blue VRS	sodium salt of 4:4'-di(di ethylamino)-4":6" di sulpho tri phenyl methanol anhydride	576	42045	
Violet BNP	sodium salt of 4:4'-di(di methyl amino)-4"-di-(p-sulphobenzylamino)tri phenyl methanol anhydride	720	-	

GRAPH 1.

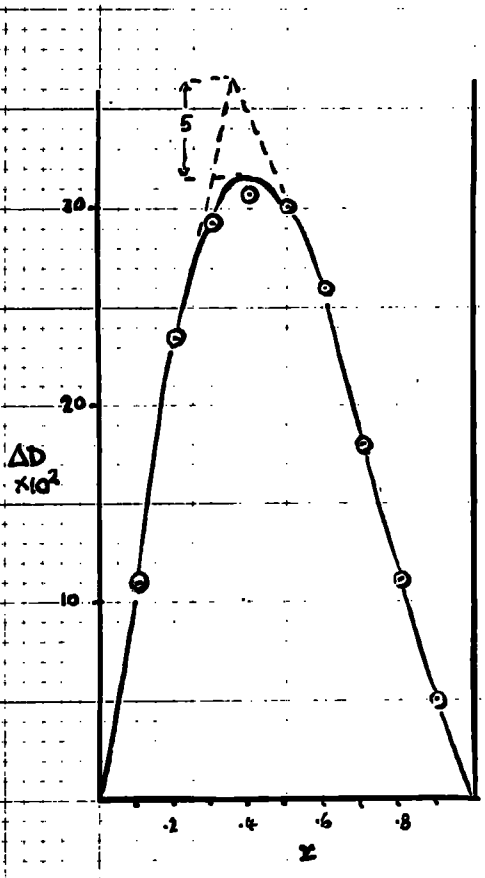
Cobalt II / Carmoisine

pH 12.5 wave number 20,000 cm^{-1}

Dye	Metal	x	D_1	$D_0 = xD_1$	D_2	$\Delta D = D_2 - D_0$	ΔD^1
0·5	4·5	0·1	0·19	0·019	0·229	·201	0·110
1·0	4·0	0·2	0·33	0·066	0·380	·314	·234
1·5	3·5	0·3	0·49	0·147	0·510	·363	·293
2·0	3·0	0·4	0·58	0·232	0·590	·368	·308
2·5	2·5	0·5	0·67	0·335	0·680	·345	·300
3·0	2·0	0·6	0·74	0·444	0·750	·306	·260
3·5	1·5	0·7	0·80	0·560	0·790	·140	·180
4·0	1·0	0·8	0·88	0·656	0·830	·174	·110
4·5	0·5	0·9	0·87	0·783	0·870	·087	·050



Original graph



Corrected graph

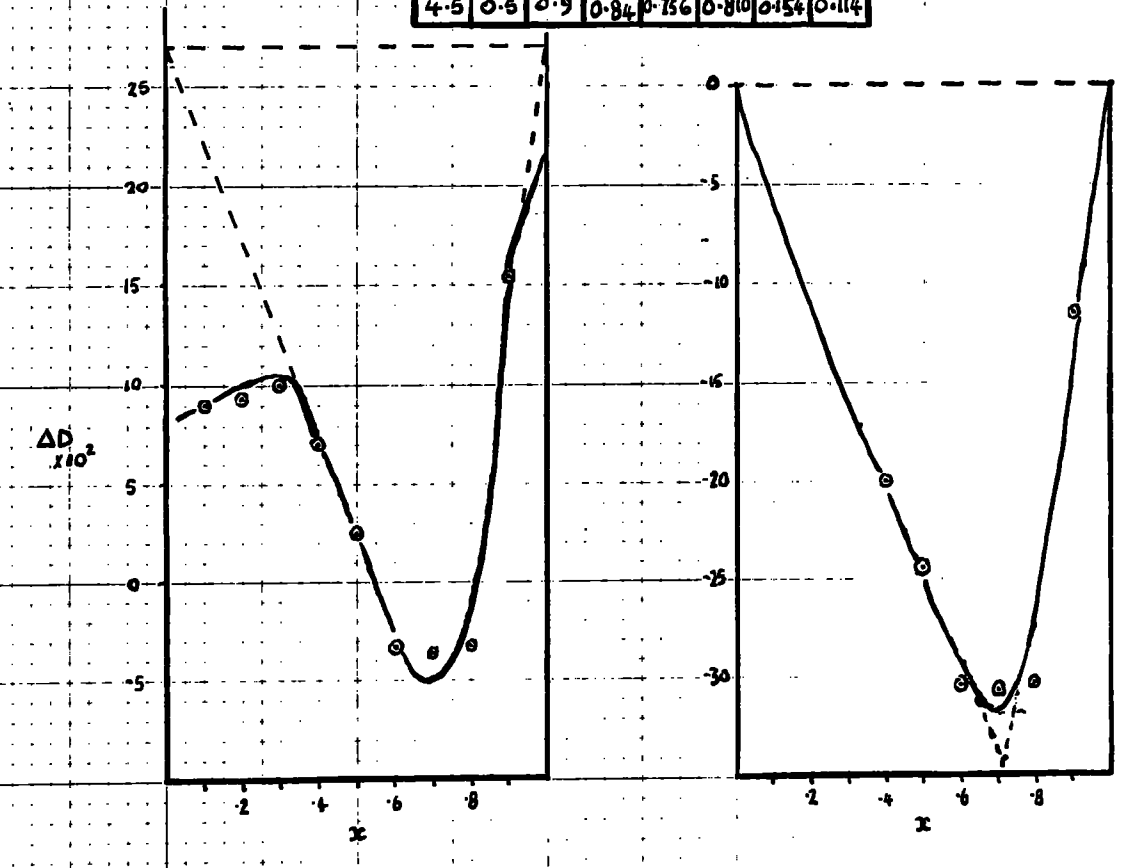
$d = \frac{5}{36} = 0.17$

GRAPH 2.

Copper II / Carmoisine

pH 7.4 wave number 16,600 cm^{-1}

Dye	metal	x	D_1	$D_0 = xD_1$	D_2	$\Delta D: D_2 - D_0$	ΔD^1 Diff.
0·5	4·5	0·1	0·20	0·020	0·110	0·090	
1·0	4·0	0·2	0·38	0·076	0·170	0·094	
1·5	3·5	0·3	0·50	0·150	0·250	0·100	
2·0	3·0	0·4	0·55	0·220	0·290	0·070	$\bar{0}$·20
2·5	2·5	0·5	0·65	0·325	0·350	0·025	$\bar{0}$·245
3·0	2·0	0·6	0·72	0·432	0·400	$\bar{0}$·032	$\bar{0}$·382
3·5	1·5	0·7	0·78	0·546	0·510	$\bar{0}$·036	$\bar{0}$·306
4·0	1·0	0·8	0·84	0·672	0·640	$\bar{0}$·032	$\bar{0}$·302
4·5	0·5	0·9	0·84	0·756	0·810	$\pm\bar{0}$·154	$\bar{0}$·114



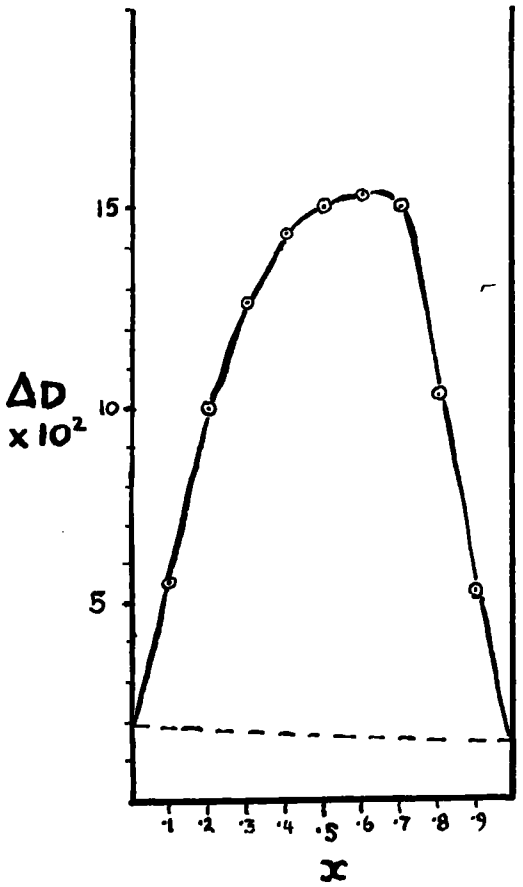
$d = \frac{3}{34.5} = 0.087$

Original graph Corrected graph

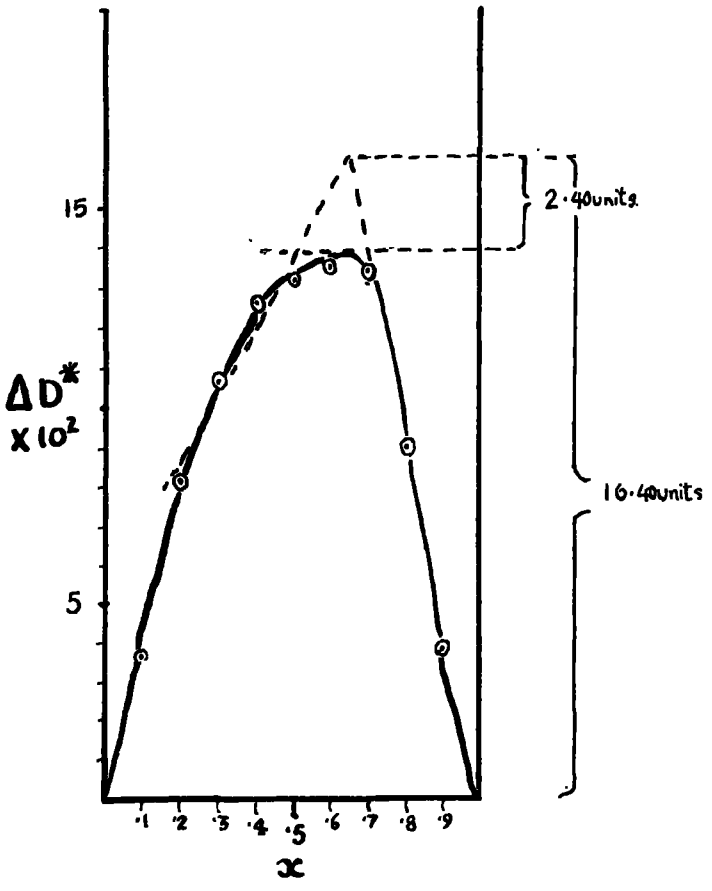
GRAPH 3.

Copper / Carmoisine
pH 7.4 wave number 24,000 cm^{-1}

Dye	Metal	x	D_1	$D_0 = xD_1$	D_2	ΔD D_2-D_0	ΔD^*
0·5	4·5	0·1	·045	·0045	0·060	·0555	·0360
1·0	4·0	0·2	·100	·0200	0·1200	·1000	·0820
1·5	3·5	0·3	·145	·0435	0·1700	·1265	·1070
2·0	3·0	0·4	·190	·0760	0·2200	·1440	·1270
2·5	2·5	0·5	·230	·1150	0·2650	·1500	·1320
3·0	2·0	0·6	·260	·1560	0·3100	·1540	·1360
3·5	1·5	0·7	·300	·2100	0·3600	·1500	·1350
4·0	1·0	0·8	·340	·2720	0·3750	·1030	·0900
4·5	0·5	0·9	·370	·3330	0·3850	·0520	·0390



Original graph



$$d = \frac{2{\cdot}40}{16{\cdot}40} = 0{\cdot}15$$

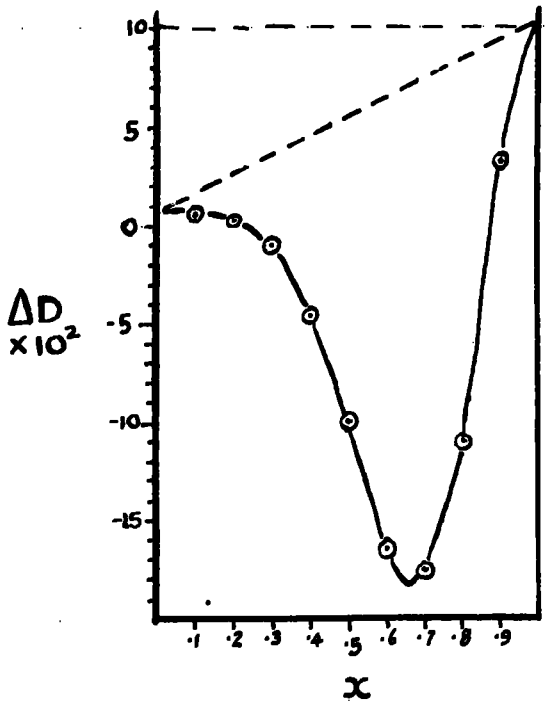
Corrected graph

GRAPH 4.

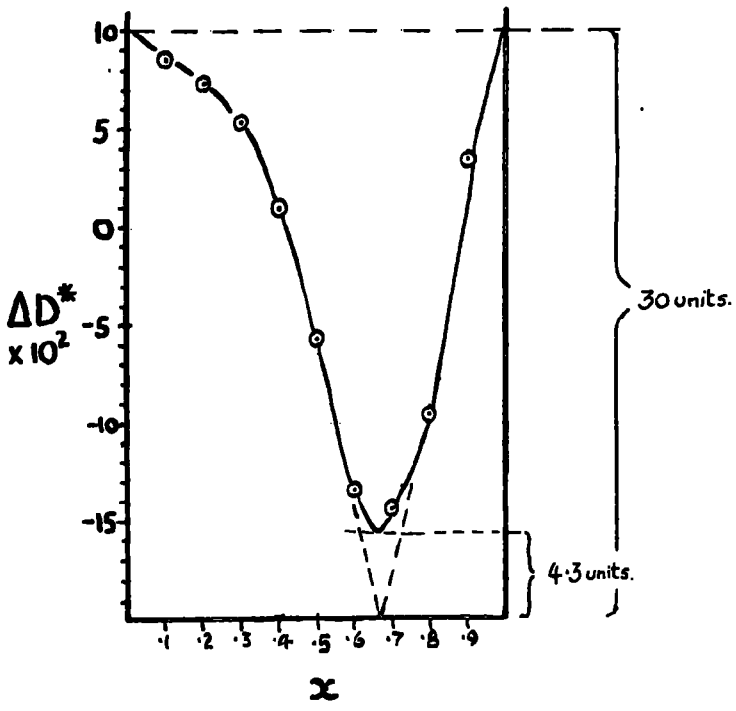
Copper / Carmoisine

pH 7.4 wave number 14,500 cm^{-1}

Dye	Metal Cu^{II}	x	D_1	$D_o = xD_1$	D_2	$\Delta D = D_2 - D_o$	ΔD^*
0·5	4·5	0·1	0·13	0·013	0·020	·007	·086
1·0	4·0	0·2	0·29	0·058	0·060	·002	·072
1·5	3·5	0·3	0·40	0·120	0·110	-·010	·052
2·0	3·0	0·4	0·45	0·180	0·134	-·046	·010
2·5	2·5	0·5	0·54	0·270	0·170	-·100	-·056
3·0	2·0	0·6	0·63	0·375	0·210	-·165	-·132
3·5	1·5	0·7	0·69	0·485	0·310	-·175	-·141
4·0	1·0	0·8	0·75	0·600	0·490	-·110	-·096
4·5	0·5	0·9	0·77	0·688	0·720	·032	·036



Original graph



Corrected graph

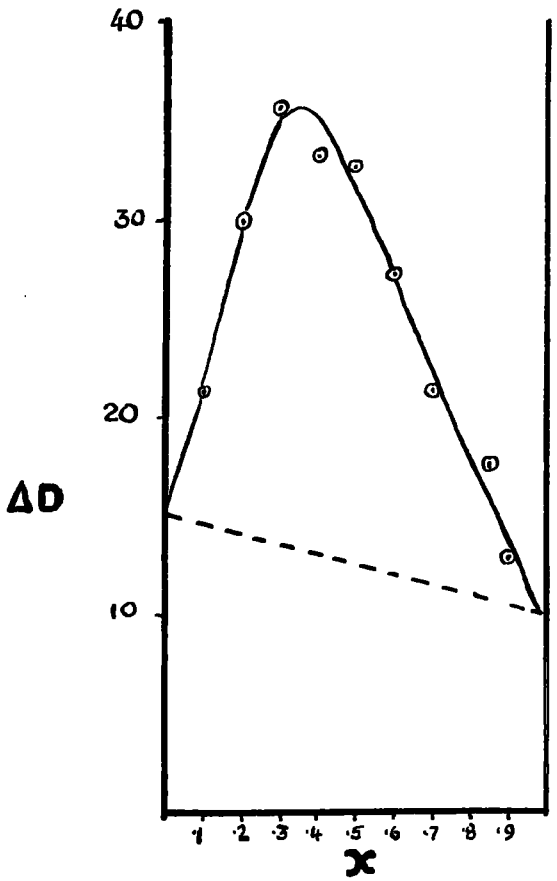
$d = \frac{4.3}{30} = 0.14$

GRAPH 5.

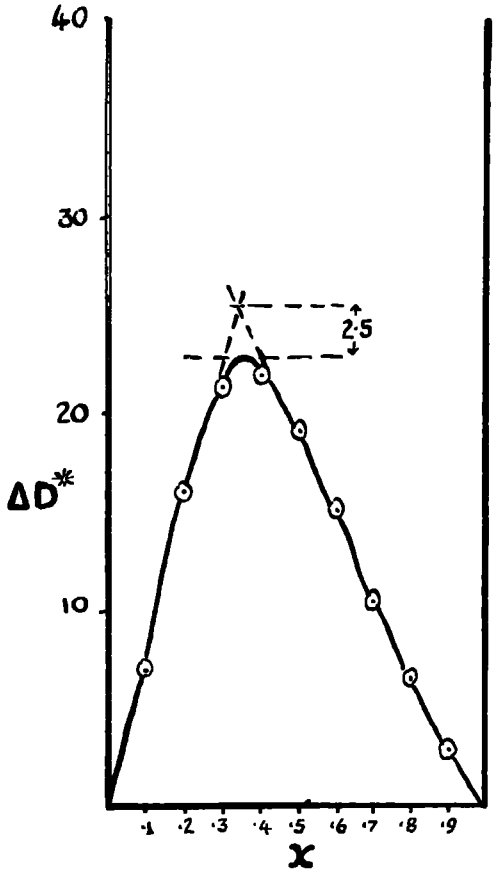
Copper II / Yellow RY

pH 12.5 wave number 22,000 cm^{-1}

Dye	Metal	x	D_1	$D_0 = xD_1$	D_2	$\Delta D = D_2 - D_0$	ΔD^*
0·5	4·5	0·1	·170	·017	·230	·213	·070
1·0	4·0	0·2	·300	·060	·360	·300	·160
1·5	3·5	0·3	·410	·123	·480	·357	·215
2·0	3·0	0·4	·510	·204	·535	·331	·220
2·5	2·5	0·5	·582	·291	·620	·329	·190
3·0	2·0	0·6	·660	·396	·670	·274	·150
3·5	1·5	0·7	·710	·497	·710	·213	·105
4·0	1·0	0·8	·752	·600	·775	·175	·066
4·5	0·5	0·9	·790	·711	·840	·129	·030



Original graph

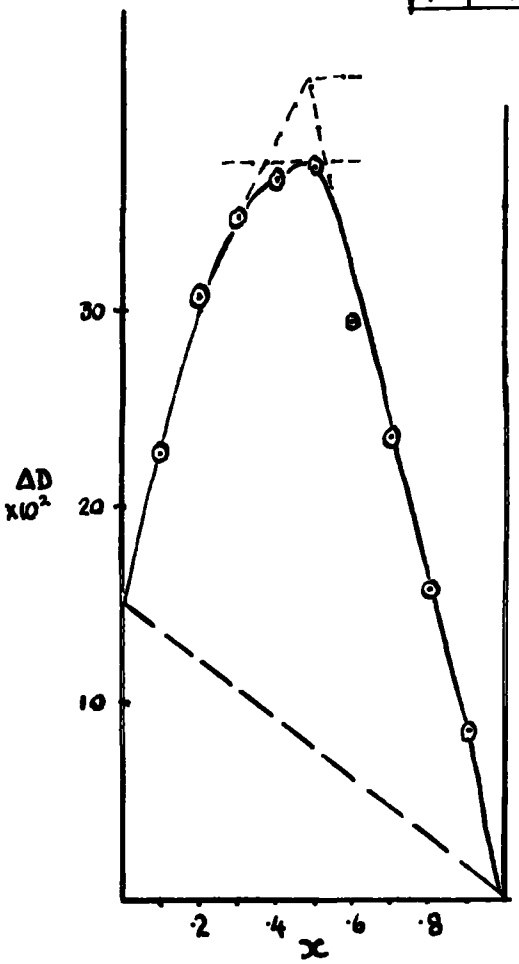


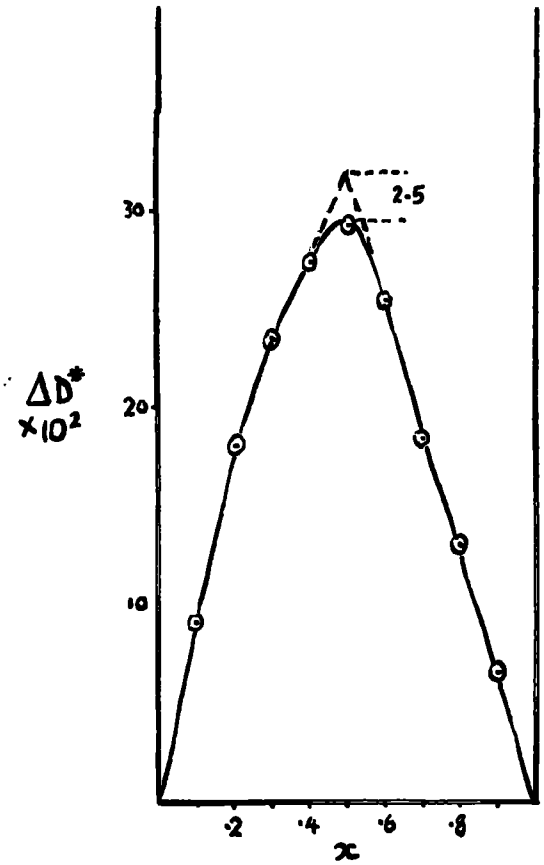
Corrected graph $d = \frac{2·5}{25·5} = 0.098 \simeq 0.1$

Copper II / Red 10B

pH 12.5 wave number 20,000 cm^{-1}

Dye	Metal Cu II	x	D_1	$D_o = xD_1$	D_2	$\Delta D = D_2 - D_o$	ΔD^*
0·5	4·5	0·1	·210	·021	·250	·229	·090
1·0	4·0	0·2	·355	·071	·370	·309	·180
1·5	3·5	0·3	·495	·149	·495	·347	·235
2·0	3·0	0·4	·540	·216	·580	·364	·273
2·5	2·5	0·5	·580	·290	·660	·370	·292
3·0	2·0	0·6	·730	·438	·730	·292	·254
3·5	1·5	0·7	·780	·546	·780	·234	·182
4·0	1·0	0·8	·792	·634	·792	·158	·130
4·5	0·5	0·9	·848	·756	·840	·084	·064





$d = \frac{2 \cdot 5}{32} = 0 \cdot 08$

Original graph

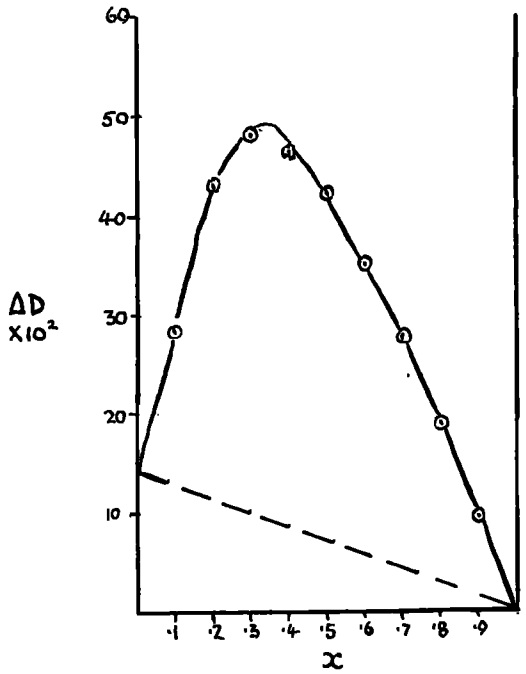
Corrected graph

GRAPH 7.

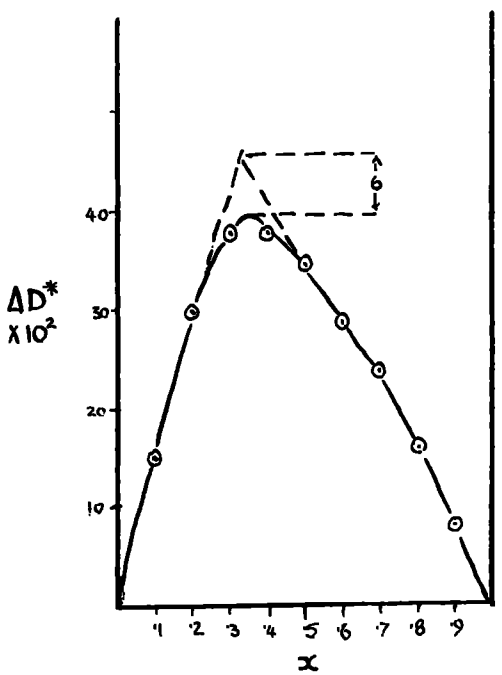
Iron II / Carmoisine

pH 12.5 wave number 19,600 cm^{-1}

Dye	Metal	x	D_1	$D_o = xD_1$	D_2	$\Delta D = D_2 - D_o$	ΔD^*
0·5	4·5	0·1	·304	·0304	·3120	·2916	·1500
1·0	4·0	0·2	·545	·1090	·5450	·4360	·3000
1·5	3·5	0·3	·680	·1940	·6750	·4810	·3800
2·0	3·0	0·4	·774	·3096	·7740	·4644	·3800
2·5	2·5	0·5	·853	·4265	·8500	·4245	·3500
3·0	2·0	0·6	·898	·5388	·8910	·3522	·2900
3·5	1·5	0·7	·930	·6510	·9300	·2790	·2400
4·0	1·0	0·8	·952	·7616	·9520	·1904	·1600
4·5	0·5	0·9	·970	·8730	·970	·0970	·0800



Original graph



$d = \frac{6}{46} = 0.13$

Corrected graph

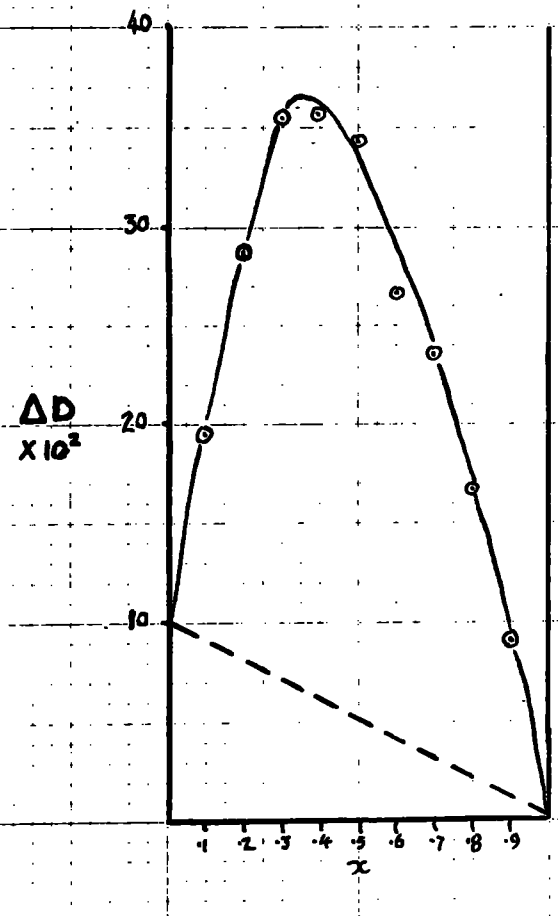
GRAPH 8.

Iron II / Chocolate Brown HT

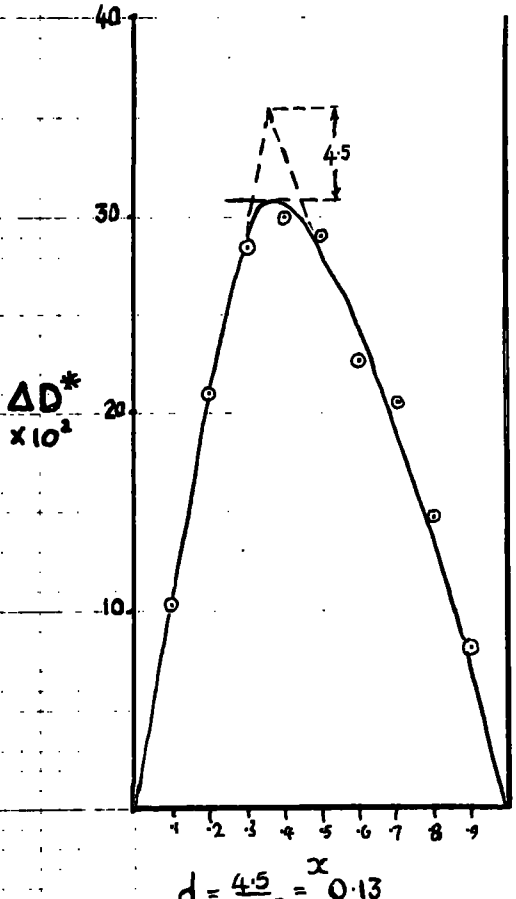
pH 12.5 wave number 21,000 cm^{-1}

Dye	Metal	x	D_1	$D_0 = xD_1$	D_2	$\Delta D = D_2 - D_0$	ΔD^*
0·5	4·5	0·1	·235	·0235	·2200	·1960	·1020
1·0	4·0	0·2	·355	·0710	·3600	·2890	·2100
1·5	3·5	0·3	·450	·1350	·4900	·3550	·2850
2·0	3·0	0·4	·570	·2280	·5850	·3570	·3000
2·5	2·5	0·5	·655	·3270	·6700	·3420	·2900
3·0	2·0	0·6	·770	·4620	·7300	·2680	·2280
3·5	1·5	0·7	·775	·5460	·7800	·2340	·2050
4·0	1·0	0·8	·820	·6560	·8250	·1690	·1480
4·5	0·5	0·9	·850	·7650	·8550	·0900	·0800

Original graph



Corrected graph



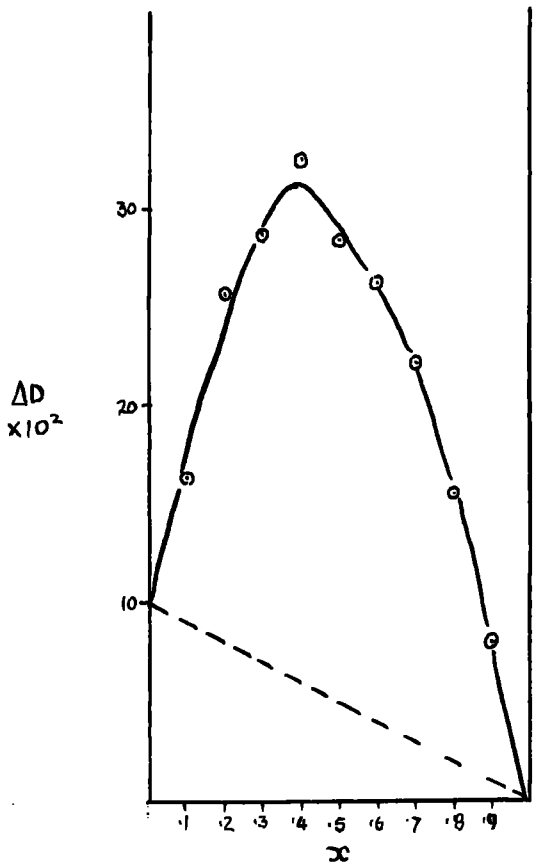
$d = \frac{4.5}{35.5} = {}^{x}0.13$

GRAPH 9.

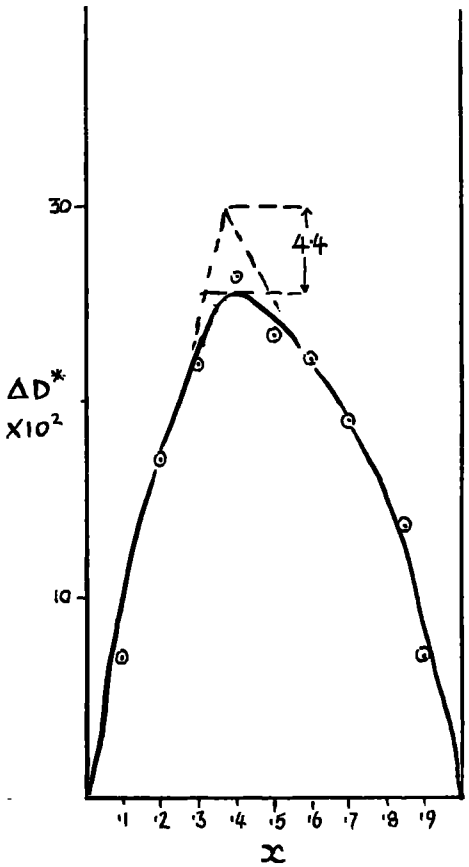
Iron II / Yellow RY

pH 12.5 wave number 22,400 cm^{-1}

Dye	Metal	x	D_1	$D_0 = xD_1$	D_2	$\Delta D = D_2 - D_0$	ΔD^*
0·5	4·5	0·1	·170	·017	·180	·163	·070
1·0	4·0	0·2	·305	·061	·320	·259	·170
1·5	3·5	0·3	·436	·131	·420	·289	·220
2·0	3·0	0·4	·530	·212	·540	·328	·265
2·5	2·5	0·5	·594	·297	·582	·285	·235
3·0	2·0	0·6	·670	·402	·665	·263	·222
3·5	1·5	0·7	·715	·501	·722	·221	·190
4·0	1·0	0·8	·770	·616	·770	·154	·135
4·5	0·5	0·9	·805	·725	·805	·080	·070



Original graph



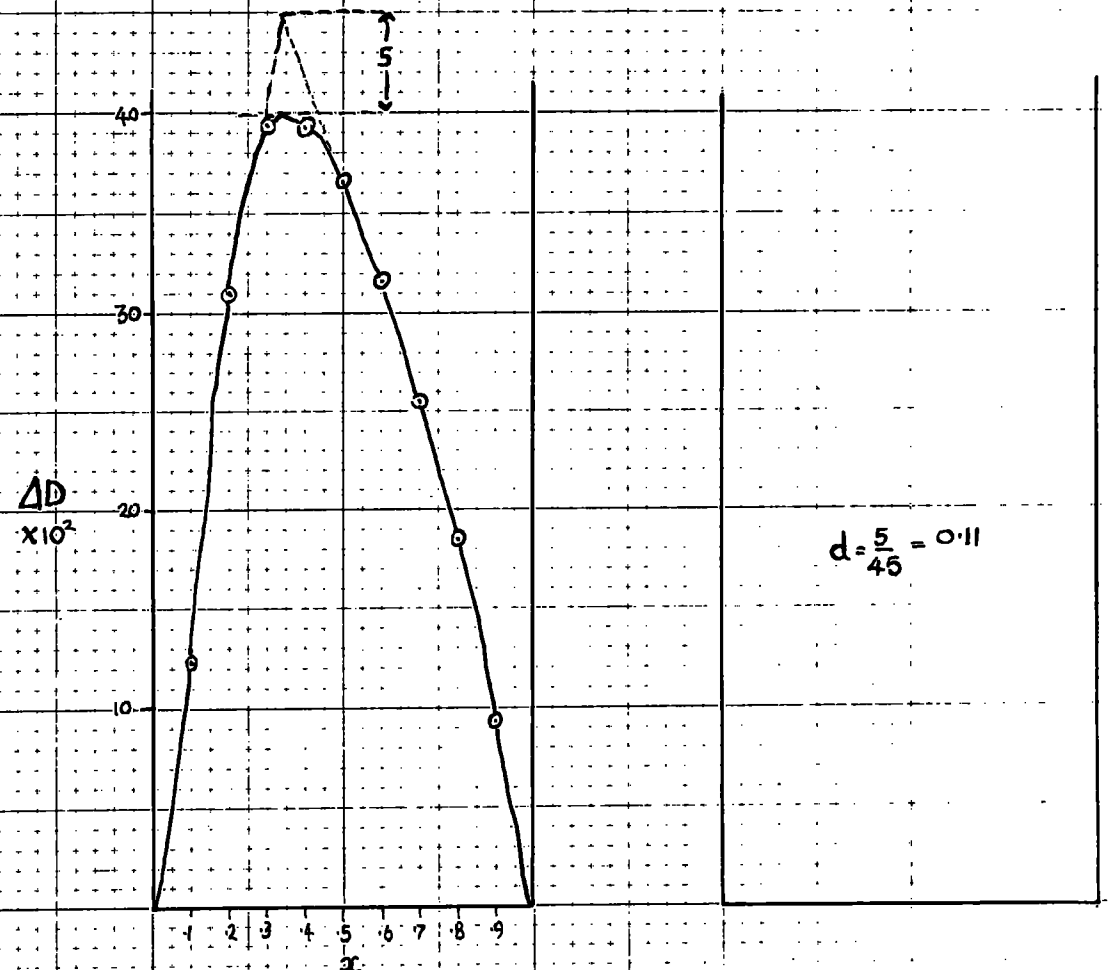
Corrected graph $\frac{4.4}{30} = 0.15$

GRAPH 10.

Iron II / Ponceau 4R

pH 12.5 wave number 20,000 cm^{-1}

Dye	Metal	x	D_1	$D_o = xD_1$	D_2	$\Delta D = D_2 - D_o$	ΔD^*
0·5	4·5	0·1	·160	·016	·140	·124	
1·0	4·0	0·2	·350	·070	·380	·310	
1·5	3·5	0·3	·518	·156	·540	·394	
2·0	3·0	0·4	·645	·260	·652	·392	
2·5	2·5	0·5	·730	·365	·730	·365	
3·0	2·0	0·6	·790	·474	·790	·316	
3·5	1·5	0·7	·852	·595	·848	·253	
4·0	1·0	0·8	·880	·704	·888	·184	
4·5	0·5	0·9	·910	·819	·910	·091	



$d = \frac{5}{45} = 0.11$

Original graph

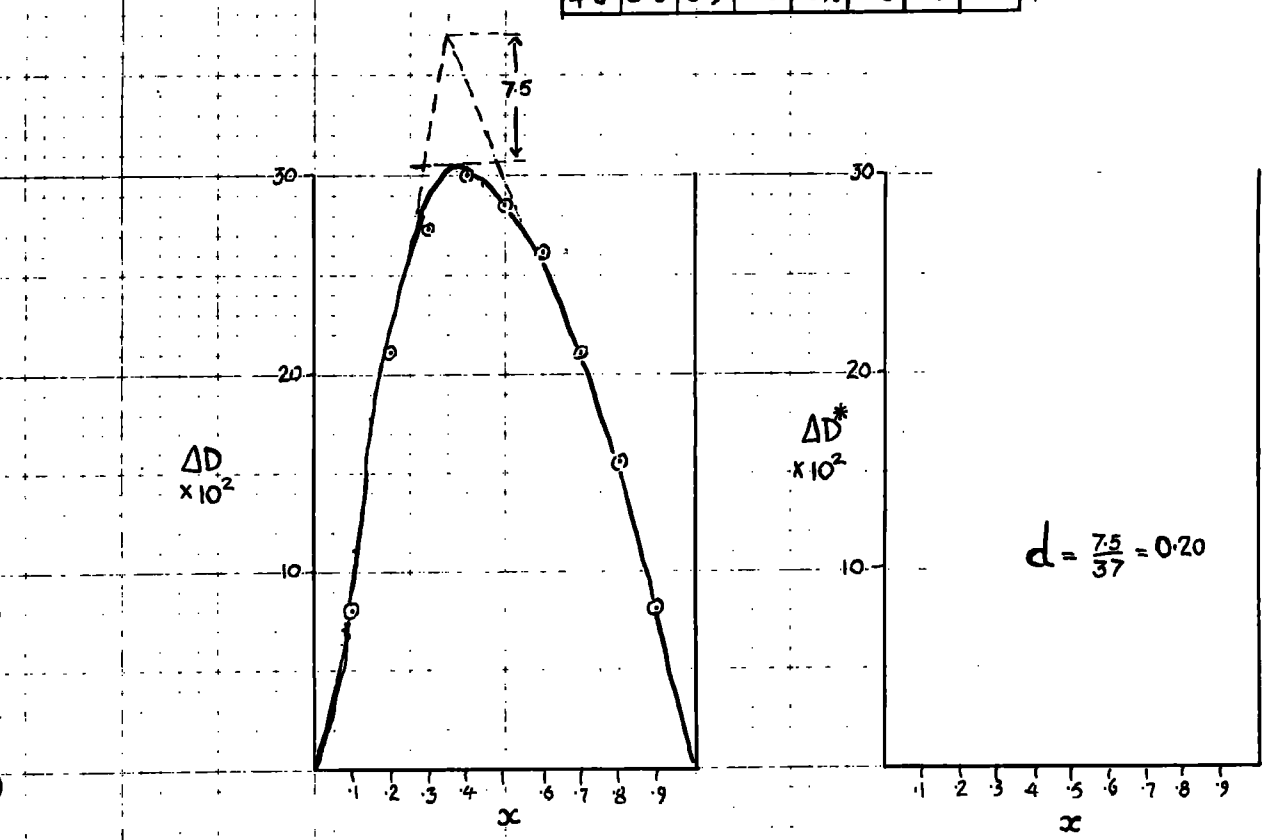
Same as original

GRAPH 11.

Iron II / Ponceau 4R

pH 12.5 wave number 26,000 cm^{-1}

Dye	Metal	x	D_1	$D_0 = x D_1$	D_2	$\Delta D = D_2 - D_0$	ΔD^*
0·5	4·5	0·1	·002	·0002	·0800	·080	
1·0	4·0	0·2	·146	·0292	·2400	·211	
1·5	3·5	0·3	·288	·086	·3600	·274	
2·0	3·0	0·4	·410	·164	·460	·300	
2·5	2·5	0·5	·510	·255	·540	·285	
3·0	2·0	0·6	·583	·350	·611	·260	
3·5	1·5	0·7	·687	·480	·693	·212	
4·0	1·0	0·8	·728	·582	·738	·156	
4·5	0·5	0·9	·770	·693	·773	·080	



Original graph

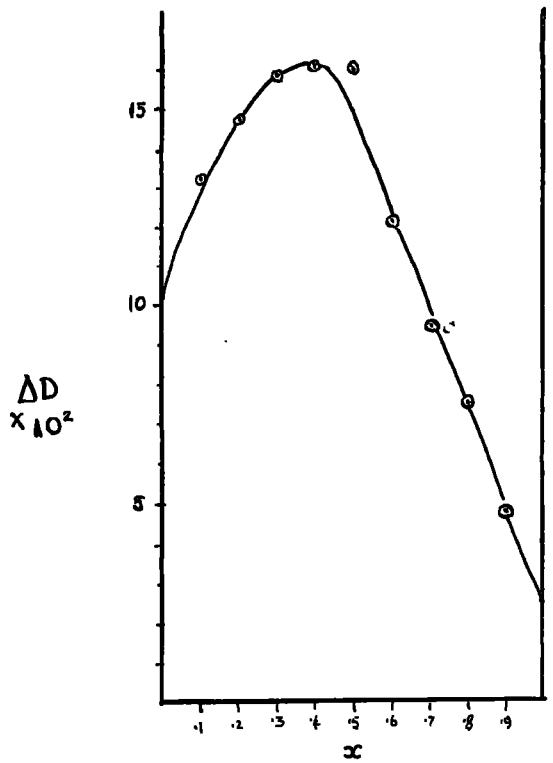
Same as original

GRAPH 1?.

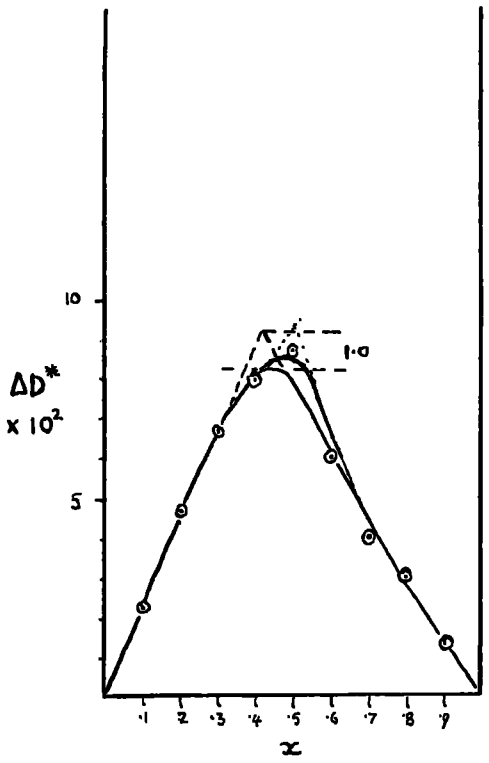
Red 10B / Iron 11

pH 12.5 wave number 23,400 cm^{-1}

Dye	Metal	x	D_1	$D_b = xD_1$	D_2	$\Delta D = D_2 - D_b$	ΔD^*
0·5	4·5	0·1	·070	·007	·140	·133	·023
1·0	4·0	0·2	·110	·022	·160	·148	·047
1·5	3·5	0·3	·170	·051	·210	·159	·068
2·0	3·0	0·4	·210	·084	·245	·161	·080
2·5	2·5	0·5	·240	·120	·280	·160	·088
3·0	2·0	0·6	·280	·168	·290	·122	·060
3·5	1·5	0·7	·312	·218	·312	·094	·040
4·0	1·0	0·8	·345	·270	·345	·075	·030
4·5	0·5	0·9	·380	·342	·390	·048	·013



Original graph



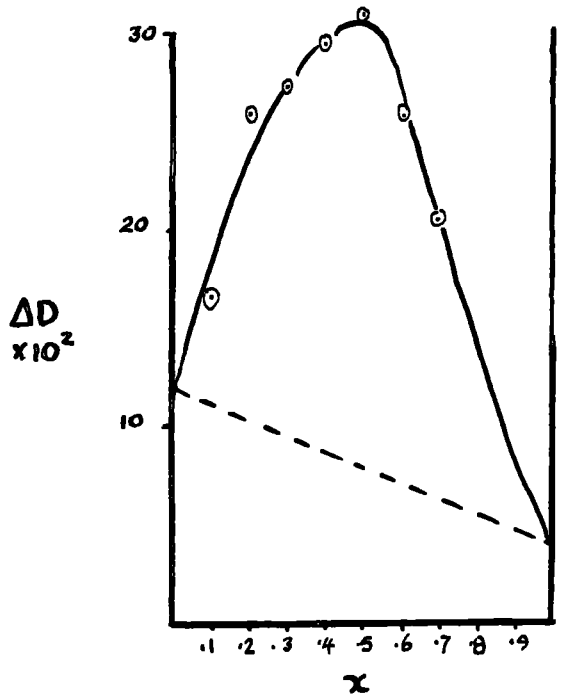
Corrected graph $d = \frac{1.0}{9.25} = 0.11$

GRAPH 13.

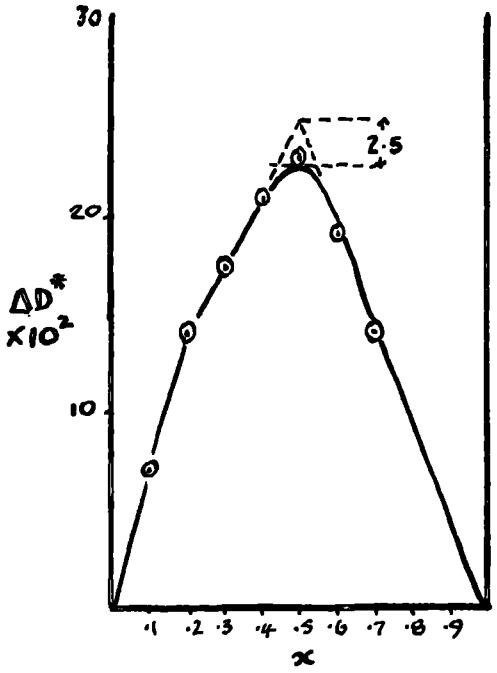
Iron III / Amaranth

pH 12.5 wave number 20,000 cm^{-1}

Dye	Metal	x	D_1	$D_o - \frac{1}{2}D_1$	D_2	$\Delta D - D_2 - D_o$	ΔD^*
0·5	4·5	0·1	·120	·012	·180	·168	·070
1·0	4·0	0·2	·250	·050	·310	·260	·140
1·5	3·5	0·3	·360	·108	·380	·272	·175
2·0	3·0	0·4	·460	·184	·480	·296	·210
2·5	2·5	0·5	·480	·240	·550	·310	·230
3·0	2·0	0·6	·595	·357	·615	·258	·190
3·5	1·5	0·7	·650	·455	·660	·205	·140
4·0	1·0	0·8			·710		
4·5	0·5	0·9			·745		



Original graph



$d = \frac{2.5}{25} = 0.1$

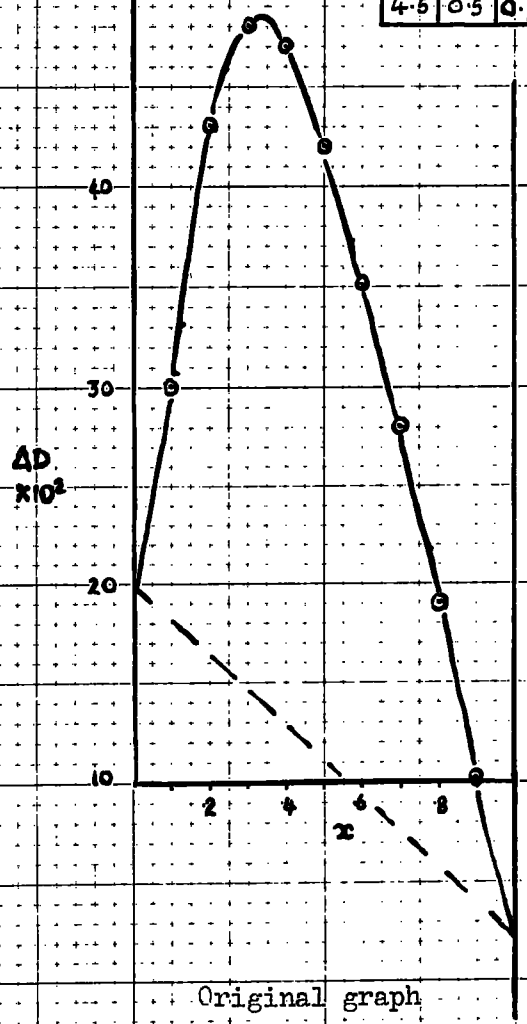
Corrected graph

GRAPH 14.

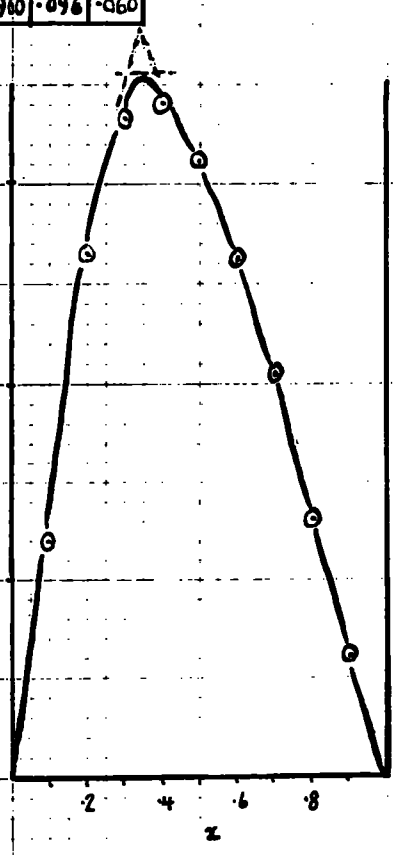
Iron III / Carmoisine

pH 12.5 wave number 20,000 cm^{-1}

Dye	Metal	x	D_1	$D_0 = x D_1$	D_2	$\Delta D = D_2 - D_0$	ΔD^*
0·5	4·5	0·1	0·320	0·032	0·330	·298	·120
1·0	4·0	0·2	0·560	0·110	0·540	·430	·265
1·5	3·5	0·3	0·680	0·204	0·690	·486	·332
2·0	3·0	0·4	0·780	0·312	0·780	·468	·340
2·5	2·5	0·5	0·860	0·430	0·850	·420	·310
3·0	2·0	0·6	0·890	0·544	0·890	·346	·260
3·5	1·5	0·7	0·930	0·651	0·930	·279	·205
4·0	1·0	0·8	0·950	0·760	0·950	·190	·130
4·5	0·5	0·9	0·960	0·864	0·960	·096	·060



Original graph



Corrected graph

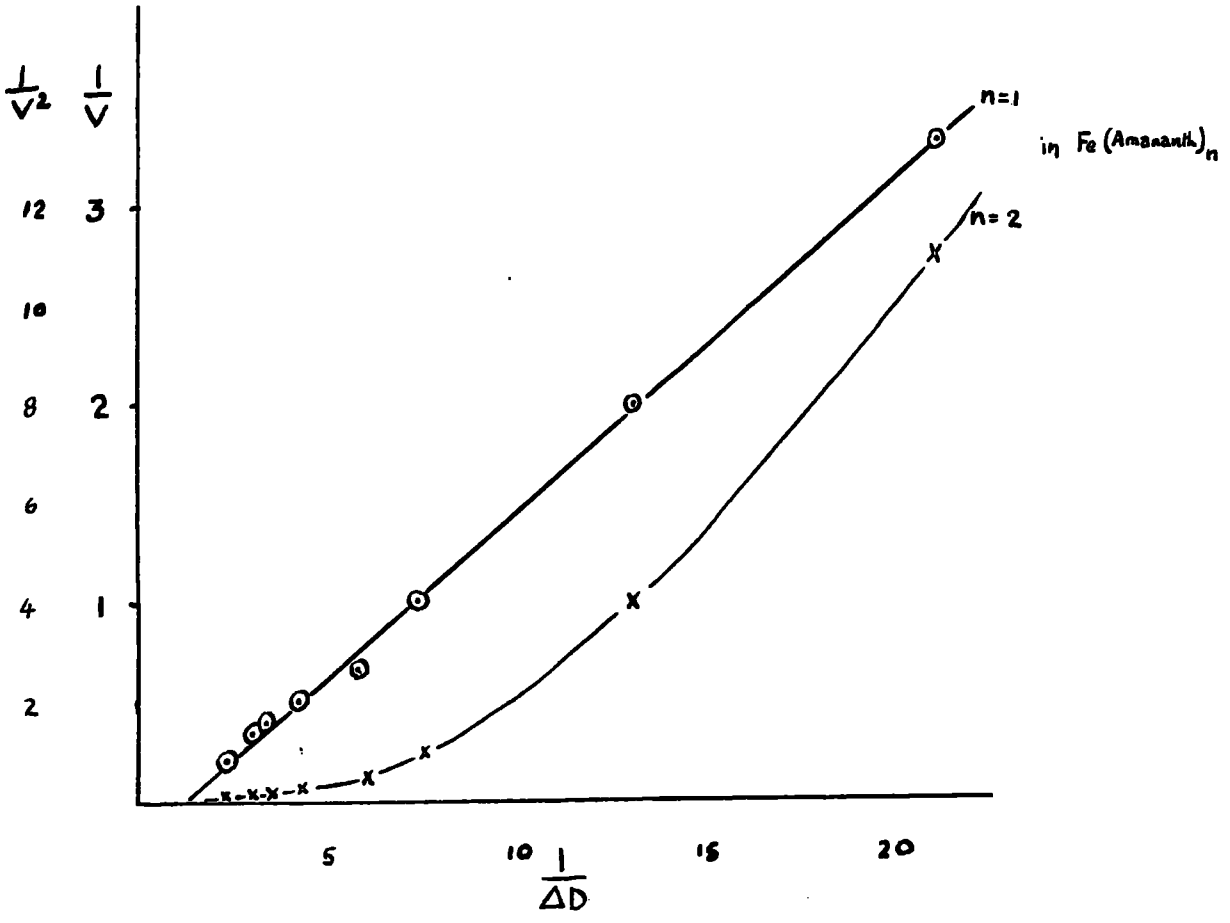
$$d = \frac{2.25}{38.00} = 0.06$$

GRAPH 15.

Iron III/amaranth Straight line Method wave no. 19,230cm^{-1}

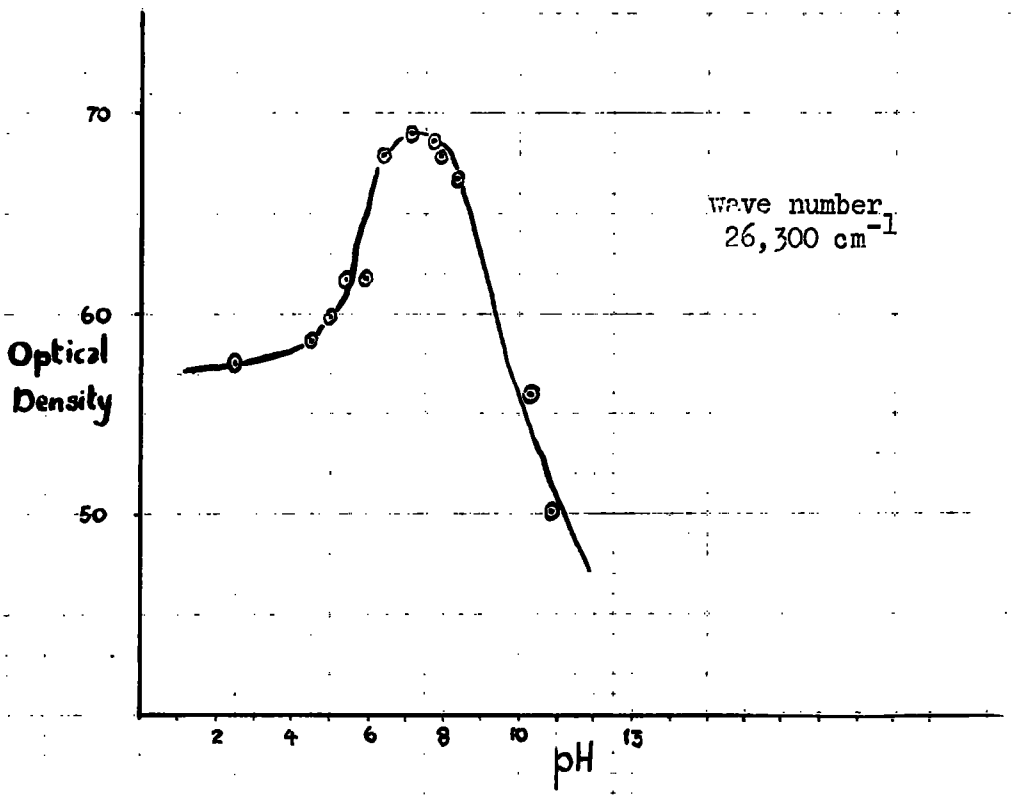
pH 12.5

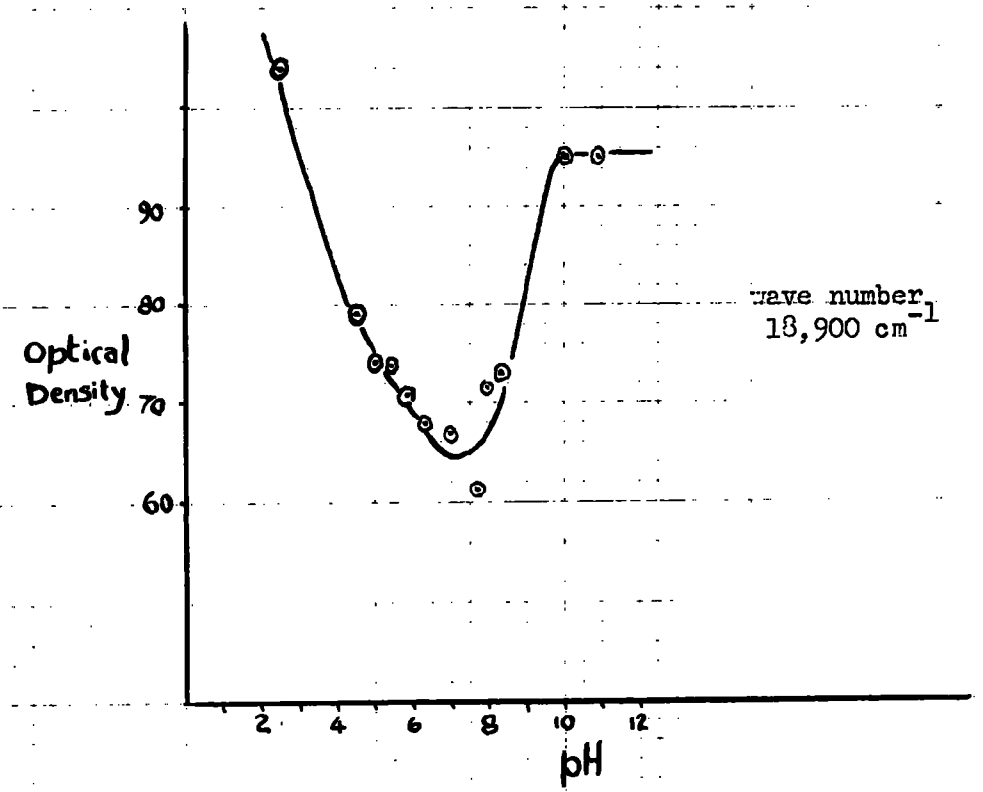
$\frac{V}{mk\ of\ Amaranth}$	$\frac{1}{V}$	$\frac{1}{V^2}$	$x = \frac{mls.\ dye}{Total\ volume}$	D_1 dye alone	$x D_1$	D_2 mixture	$\Delta D = D_2 - xD_1$	$\frac{1}{\Delta D}$
0·30	3·30	11	0·130	0·480	·0625	·1100	·0475	21
0·50	2·00	4·0	0·200	0·465	·0930	·1700	·0770	13
1·00	1·00	1·0	0·330	0·490	·1630	·3000	·1370	7·3
1·50	0·67	0·45	0·430	0·500	·2150	·3900	·1750	5·7
2·00	0·50	0·25	0·500	0·490	·2450	·4900	·2450	4·1
2·50	0·40	0·16	0·560	0·495	·2770	·5700	·2930	3·4
3·00	0·33	0·11	0·600	0·490	·2940	·6250	·3310	3·0
5·00	0·20	0·04	0·710	0·515	·3650	·8100	·4450	2·3



GRAPH 16.

pH studies of Copper II / Carmoisine

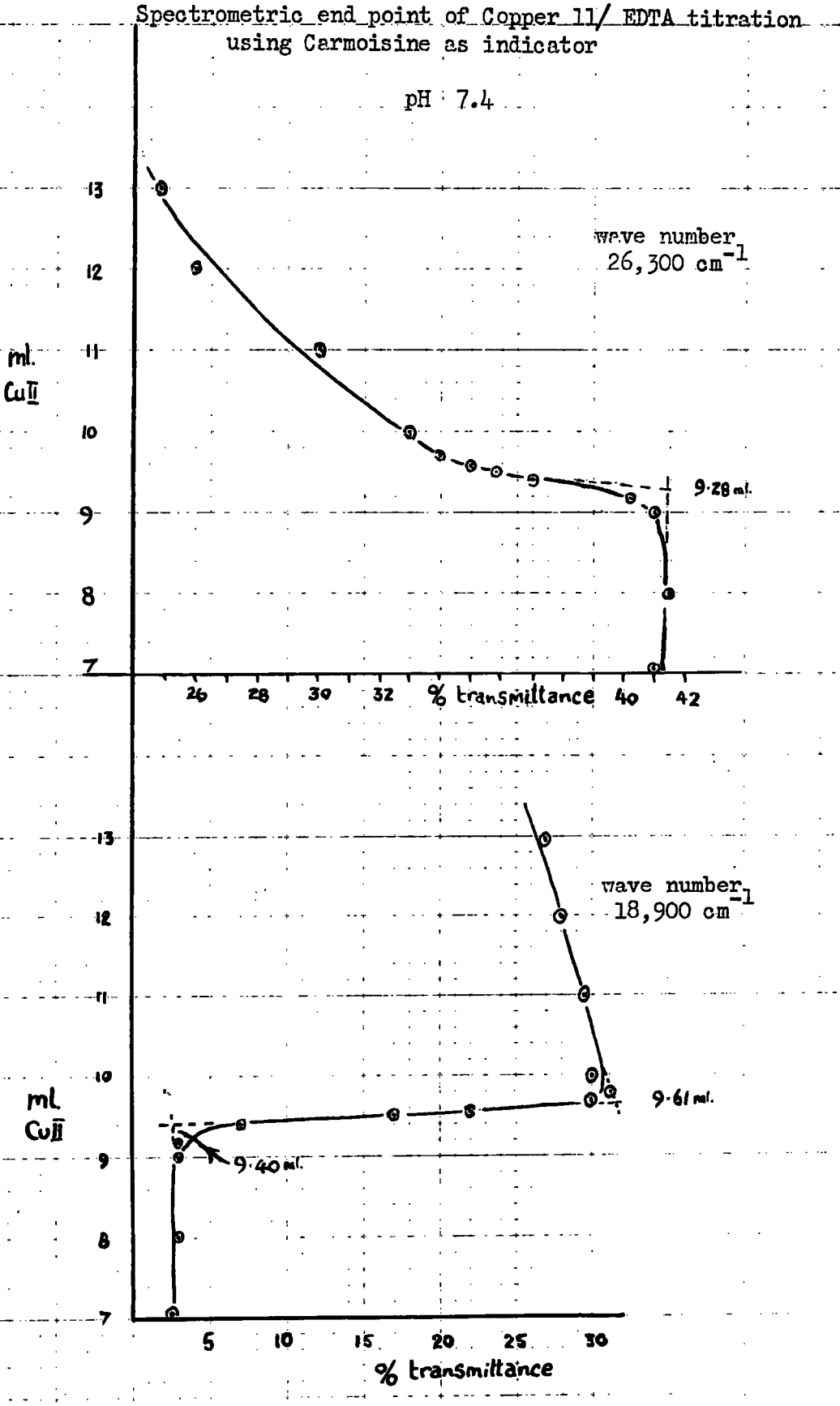




GRAPH 17.

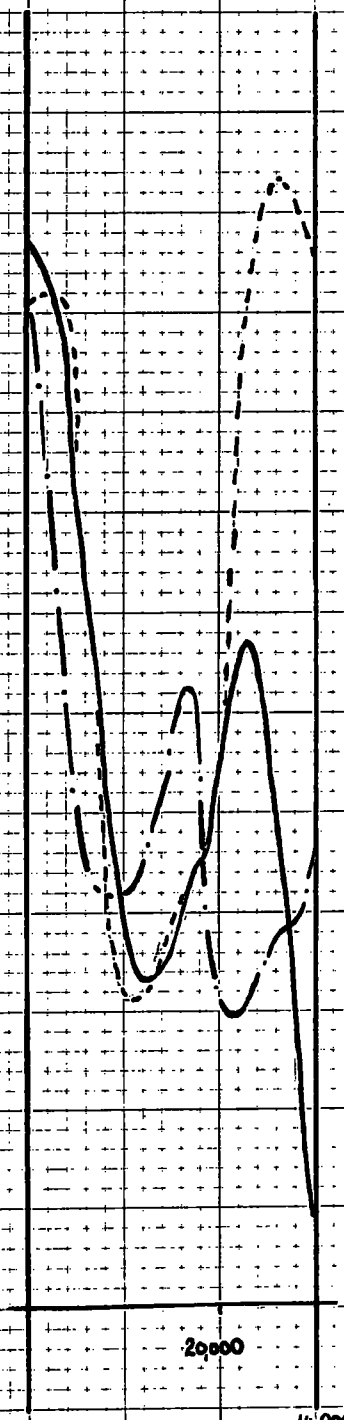
Spectrometric end point of Copper II/ EDTA titration using Carmoisine as indicator

pH 7.4



GRAPH 18.

Colour changes at the end point of copper 11/carmoisine and blue VRS at pH 7.4



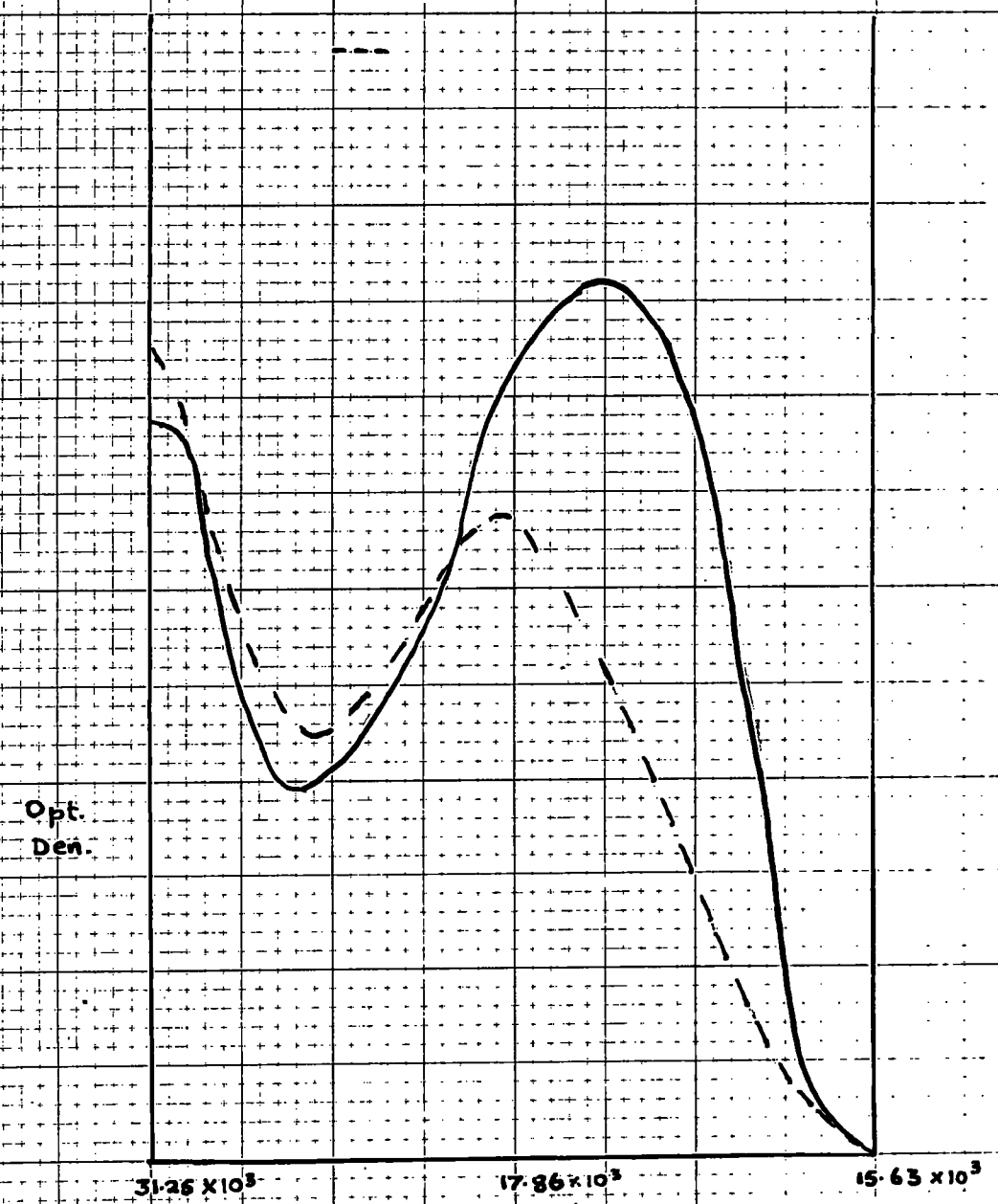
carmoisine and blue VRS and copper 11

carmoisine and blue VRS

blue VRS and copper 11

GRAPH 19.

Copper II/carnoisine pH 7.4



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The influence of certain metal ions on the visible spectra of food dyes

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Summary. The visible absorption spectra of twenty-two food dyes have been plotted both in the free state and in the presence of added calcium, magnesium, aluminium, iron (II), iron (III), copper (II) and cobalt (II) ions at the pH values 3·0, 6·4, 7·4 and 12·5.

The frequencies of the absorption band maxima of the free dyes are briefly discussed in relation to colour and structure. While added metal ions do not, in general, have an appreciable effect on the spectrum of the dye, the transition metal ions studied frequently bring about a shift in the characteristic frequencies of the free-dye absorption maxima. Examples of where this effect is greatest are carmoisine in the presence of copper (II) at pH 6·4 and 7·4, and black PN in the presence of iron (II) at pH 12·5.

The possibility of dye complexes affecting iron metabolism is also briefly discussed.

Introduction

The essential requirements of a food dye have been listed by Minor (1962). Apart from the fact that the dye should not be injurious to health, it should also be fast to light and should withstand relatively high temperatures and variable conditions of acidity. Furthermore, it should not be affected by preservatives and other constituents of food.

The bleaching effect of sunlight on dyes is well known, and of the food dyes permitted in the United Kingdom (Her Majesty's Stationery Office, 1966), indigo carmine is particularly susceptible to such spoilage. On the other hand, a search of the literature reveals that relatively little attention has been given to a systematic study of the behaviour of food dyes under various conditions although, of course, a great deal is known, and can be predicted about the breakdown characteristics of individual dyes. A number of investigations on the metabolic fate of several food dyes have been carried out over recent years (Radomski & Diechmann, 1956; Koether, 1960; Daniel, 1962; Manchon, 1965), and with regard to their possible denaturing prior to ingestion, Lueck (1965) has studied the effect of heat, reducing agents and

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oxidizing agents on a selected range. Thus, black PN, cochineal red A, ponceau 6R and yellow 27175N were found to be unstable to boiling and sterilization under certain conditions of pH. Most of the eighteen dyes studied were affected by ascorbic acid and sodium sulphite.

While in Lueck's (1965) study 0·06% hydrogen peroxide was found to be without effect, it is well known that certain oxidizing agents denature food dyes, for example, amaranth and ponceau 4R are destroyed by excess of oxidant when they are used as indicators in oxidation titrations involving potassium bromate and potassium iodate (Belcher & Nutten, 1955; Vogel, 1962).

Azo dyes have been the centre of intense interest in the search for possible indicators suitable for use in complexometric titrations (Close & West, 1960a, b), but despite a similarity between many of the compounds investigated and the food dyes containing azo groupings, the investigations have not included the food dyes. The same is also true of investigations in the search for indicators among the triphenylmethane dyes (Brazier & Stephen, 1965).

In the light of the above there is a paucity of systematic information on the behaviour of food dyes in the presence of metal ions normally encountered in the practice of food technology and of food preparation. The present investigation has, therefore, been directed to an examination of the influence over a wide pH range of varying concentrations of calcium, magnesium, aluminium, cobalt (II), iron (II), iron (III) and copper (II) ions on the colours of most of the coal tar food dyes permitted in the United Kingdom, as well as some of those that have recently been deleted from the permitted list (Her Majesty's Stationery Office, 1957, 1966), that is, ponceau SX, yellow RY, yellow RFS and blue VRS.

Experimental

Materials

The dyes studied were of the 'Hexacol' range and were kindly donated by L. J. Pointing and Son Ltd, Hexham, Northumberland.

The metal ion-containing solutions were prepared from the appropriate AnalaR sulphate except for iron (III), when the alum was used, and calcium, where the solution was prepared by dissolving AnalaR calcium carbonate in the minimum quantity of hydrochloric acid.

Procedure

A range of solutions was prepared for each metal ion and each dye by mixing varying volumes of aqueous 0·0005 M metal ion-containing solution, 5 ml aqueous 0·0005 M dye solution, the appropriate volume of a suitable reagent to ensure the required conditions of pH, followed by de-ionized water to give a total volume of 50 ml. The absorption spectra of these solutions were recorded with a recording spectrophoto-

meter. Spectra of solutions containing 40 ml of the metal ion-containing solution but no dye were also recorded, but the absorption was minimal and, therefore, ignored.

Solutions of pH 3·0 were obtained by adjustment with about 5 ml glacial acetic acid; those of pH 12·5 by adjustment with about 5 ml diethylamine; those of pH 7·4 by adjustment with about 10 ml of a Sörensen type buffer solution prepared from 80·0 ml of 0·200 M disodium hydrogen phosphate and 20·0 ml of 0·0667 M potassium dihydrogen phosphate and, finally, solutions of pH 6·4 were obtained by adjustment with about 10 ml of a similar buffer solution prepared from 30·0 ml 0·200 M disodium hydrogen phosphate and 70·0 ml 0·0667 M potassium dihydrogen phosphate.

Results

Table 1 summarizes the frequencies (cm^{-1}) of the absorption band maxima observed between about 25,000 cm^{-1} (400 nm) and 12,500 cm^{-1} (800 nm) together with the optical densities for the free dyes (5 ml aqueous 0·0005 M dye solution made up to 50 ml as described above) and for the dyes in the presence of excess metal ions (5 ml aqueous 0·0005 M dye solution + 40 ml 0·0005 M metal ion-containing solution (35 ml at pH 6·4 and 7·4) made up to 50 ml as described above). Where the absorption band maxima of the free dye and of the dye in the presence of excess metal ions differ by a frequency of greater than 800 cm^{-1} (that is, a wavelength of about 20 nm at 500 nm), the figures recorded in the latter case are shown in italics in Table 1.

Variations in frequency of the absorption band maxima of the dye may be brought about by added metal ions due to complex formation. An estimate of the structure and stability of the resulting complex may be made by Job's (1928) method of continuous variation as used by Close & West (1960a, b). This kind of calculation involving a plot of the differences in optical densities between the complex and the estimated amounts of non-complexed dye against solution composition has been made in a limited number of cases and the results are summarized in Table 2.

Discussion

Features of the spectra of dye solutions

The principal characteristics of the absorption spectra of solutions of the dyes naturally follow a pattern according to the colour imparted by the solution. Thus, the yellow dyes show absorption bands in the region of 23,000 cm^{-1} (435 nm) to 25,000 cm^{-1} (400 nm), the orange dyes in the region of 21,000 cm^{-1} (476 nm) with a further band at 24,000 cm^{-1} (417 nm) to 25,000 cm^{-1} (400 nm), while the red dyes have absorption bands at 19,000 cm^{-1} (526 nm) to 20,000 cm^{-1} (500 nm). The differences observed in the absorption band maxima for dyes of the same colour match differences in hue, for example, the red dyes tending towards an orange hue have absorption bands nearer to 20,000 cm^{-1} (500 nm) while those of a pronounced deep red have their absorption bands nearer 19,000 cm^{-1} (526 nm).

TABLE 1. Absorption band maxima (in 10^{-3} cm^{-1}) and optical densities (in parentheses) at various conditions of pH and in the presence of certain metal ions

Dye	pH 3·0	pH 6·4	pH 7·4	pH 12·5	pH 3·0	pH 6·4	pH 7·4	pH 12·5
	← Free dye →				← Dye in the presence of excess copper (II) ions →			
1-Napthyl-azo-phenyl dyes								
Orange G	*25·0 (0·51)	*24·5 (0·50)	*25·0 (0·50)	25·2 (0·58)	*24·8 (0·51)	*24·4 (0·50)	*25·0 (0·49)	—
	20·8 (0·89)	20·8 (0·88)	21·0 (0·88)	20·2 (0·49)	20·8 (0·88)	20·8 (0·86)	21·0 (0·87)	*21·0 (0·62)*
Orange RN	†24·2 (0·56)	†23·8 (0·56)	24·8 (0·56)	23·0 (0·62)	†24·2 (0·50)	†23·8 (0·55)	*26·4 (0·58)*	—
	20·8 (0·87)	20·3 (0·87)	21·0 (0·87)	†20·0 (0·52)	20·7 (0·87)	20·3 (0·84)	21·2 (0·84)	†*20·8 (0·74)*
								17·6 (0·66)
Sunset Yellow FCF	*24·2 (0·61)	*23·8 (0·60)	*24·4 (0·61)	22·2 (0·74)	*24·4 (0·57)	*23·8 (0·63)	†24·8 (0·57)	—
	21·0 (0·91)	20·4 (0·89)	21·0 (0·91)	*20·0 (0·60)	21·0 (0·88)	20·6 (0·87)	20·8 (0·86)	22·2 (0·71)
Ponceau MX	25·8 (0·43)	*25·6 (0·45)	25·8 (0·39)	—	25·6 (0·44)	*25·0 (0·55)	26·0 (0·40)	—
	19·6 (0·88)	19·7 (0·88)	20·4 (0·87)	20·0 (0·69)	19·6 (0·88)	19·8 (0·85)	20·2 (0·86)	*21·6 (0·67)*
Chocolate Brown HT	21·6 (0·86)	21·2 (0·85)	21·6 (0·86)	21·2 (0·91)	21·6 (0·86)	21·4 (0·83)	21·6 (0·80)	21·2 (0·81)
1-Naphthyl-azo-1-naphthyl dyes								
Ponceau 4R	20·0 (0·99)	19·4 (0·88)	20·0 (1·00)	20·6 (0·93)	19·6 (1·00)	19·4 (0·85)	19·6 (1·00)	22·0 (0·95)
Amaranth	19·0 (0·92)	19·0 (0·93)	19·6 (0·91)	20·2 (0·79)	19·0 (0·92)	19·2 (0·83)	19·6 (0·85)	*21·0 (0·75)*
Fast Red E	19·6 (0·86)	19·7 (0·85)–	20·2 (0·84)	21·0 (0·72)	19·8 (0·86)	19·7 (0·79)	19·9 (0·75)	21·4 (0·69)
1-Naphthyl-azo-2-naphthyl dyes								
Carmoisine	†24·6 (0·60)	†24·4 (0·40)	—	—	†24·8 (0·61)	—	—	—
	19·4 (0·98)	19·2 (0·90)	19·6 (0·88)	19·6 (0·98)	19·4 (0·98)	*20·8 (0·66)*	*21·6 (0·63)*	19·6 (0·98)
Black PN	24·4 (0·64)	24·3 (0·66)	24·0 (0·65)	25·8 (0·72)	24·4 (0·64)	24·3 (0·66)	24·0 (0·56)	25·6 (0·73)
	17·6 (0·98)	17·2 (0·97)	17·2 (0·96)	17·6 (0·89)	17·6 (0·96)	17·2 (0·92)	17·6 (0·95)	17·6 (0·90)
2-Naphthyl-azo-phenyl dyes								
Ponceau SX	20·0 (0·92)	19·7 (0·91)	20·0 (0·89)	21·0 (0·84)	20·0 (0·91)	20·0 (0·36)	20·6 (0·70)	20·8 (0·86)
Red 10B	19·0 (0·92)	18·7 (0·90)	19·0 (0·91)	18·8 (0·89)	19·0 (0·92)	18·5 (0·91)	19·4 (0·91)	18·8 (0·91)
Red 2G	20·0 (0·95)	19·8 (0·95)	20·0 (0·95)	21·8 (0·80)	20·0 (0·95)	19·8 (0·95)	20·0 (0·92)	21·8 (0·77)
	19·0 (0·95)	18·7 (0·95)	19·0 (0·95)		19·0 (0·95)	18·7 (0·95)	19·0 (0·92)	
Red 6B	19·0 (0·93)	18·9 (0·94)	19·0 (0·90)	21·6 (0·83)	19·0 (0·94)	18·9 (0·94)	19·4 (0·89)	21·6 (0·83)
(a) Red FB	19·3 (0·49)	19·6 (0·49)	19·6 (0·49)	18·8 (0·48)	19·3 (0·50)	19·6 (0·45)	19·6 (0·36)	18·5 (0·49)
Phenyl-azo-phenyl dyes								
Yellow RY	23·0 (0·89)	22·7 (0·88)	23·0 (0·86)	22·0 (0·83)	23·0 (0·90)	22·7 (0·86)	23·0 (0·86)	*23·0 (0·78)*
Yellow RFS	24·0 (0·83)	23·3 (0·84)	25·0 (0·81)	24·0 (0·86)	24·0 (0·82)	23·2 (0·85)	24·8 (0·82)	24·0 (0·93)

Phenyl-azo-pyrazole dyes								
Yellow 2G	24·6 (0·91)	24·7 (0·91)	24·8 (0·89)	25·0 (0·90)	24·6 (0·91)	24·8 (0·90)	24·8 (0·88)	25·2 (0·88)
Tartrazine	23·0 (0·91)	23·3 (0·91)	23·8 (0·91)	25·0 (0·84)	23·2 (0·91)	23·3 (0·91)	23·6 (0·92)	25·0 (0·84)
Triphenyl-methanol anhydride dyes								
Blue VRS	24·4 (0·93)	24·0 (0·74)	24·0 (0·74)	24·4 (0·43)	24·4 (0·90)	24·0 (0·77)	24·4 (0·79)	24·4 (0·66)
	16·0 (0·99)	15·5 (1·00)	16·0 (1·00)	16·0 (0·97)	16·0 (0·98)	15·2 (1·00)	16·0 (1·00)	16·0 (1·00)
Green S	25·0 (0·50)	24·9 (0·47)	25·0 (0·54)	25·0 (0·64)	25·0 (0·52)	24·8 (0·52)	25·2 (0·56)	*26·6 (0·55)*
	22·8 (0·48)	22·5 (0·42)	22·8 (0·37)	—	22·8 (0·47)	22·2 (0·46)	22·8 (0·40)	—
	16·0 (1·00)	15·4 (0·90)	16·0 (1·00)	16·4 (0·99)	16·0 (0·98)	15·5 (1·00)	16·4 (1·00)	16·4 (0·99)
Violet BNP	†18·0 (0·98)	18·0 (0·96)	18·8 (0·96)	†18·4 (0·78)	†18·0 (0·98)	†18·0 (0·93)	18·4 (0·98)	—
	17·0 (0·98)	†16·9 (0·96)	17·8 (0·95)	17·0 (0·85)	16·8 (0·98)	16·8 (0·97)	†17·4 (0·98)	16·8 (0·10)
	← Dye in presence of excess iron (II) ions →				← Dye in presence of excess iron (III) ions →			
1-Naphthyl-azo-phenyl dyes								
Orange G	*25·0 (0·51)	—	*24·4 (0·49)	—	—	—	*23·8 (0·57)*	—
	20·8 (0·89)	20·8 (0·96)	20·8 (0·85)	*21·0 (0·60)*	20·8 (0·97)	20·8 (0·92)	20·6 (0·89)	20·8 (0·62)
Orange RN	†24·4 (0·58)	*24·8 (0·60)*	25·2 (0·59)	†*24·0 (0·78)*	24·4 (0·60)	24·7 (0·52)	24·7 (0·53)	23·0 (0·79)
	20·5 (0·89)	20·4 (0·87)	20·8 (0·84)	—	20·4 (0·89)	20·6 (0·94)	20·4 (0·85)	*19·8 (0·56)
Sunset Yellow FCF	*24·0 (0·65)	†23·9 (0·57)	*24·0 (0·62)	**24·0 (0·94)*	*23·8 (0·60)	*23·8 (0·63)	*24·0 (0·56)	22·5 (0·78)
	20·6 (0·91)	20·6 (0·83)	20·8 (0·91)	—	20·6 (0·89)	20·6 (0·89)	20·4 (0·26)	*20·0 (0·66)
Ponceau MX	25·6 (0·43)	—	25·8 (0·47)	—	*25·3 (0·96)	—	—	—
	19·8 (0·78)	19·8 (0·95)	20·0 (0·85)	20·2 (0·74)	19·6 (0·88)	19·7 (0·91)	19·7 (0·89)	19·6 (0·74)
Chocolate Brown HT	21·4 (0·85)	21·3 (0·88)	22·0 (0·86)	21·4 (0·90)	21·3 (0·88)	21·4 (0·85)	21·3 (0·81)	21·3 (0·92)
1-Naphthyl-azo-1-naphthyl dyes								
Ponceau 4R	19·6 (0·99)	19·8 (0·82)	20·0 (1·00)	**23·0 (0·96)*	19·4 (0·88)	19·4 (0·89)	19·6 (0·84)	20·6 (0·65)
Amaranth	19·0 (0·92)	19·0 (0·92)	19·6 (0·87)	20·4 (0·81)	*24·4 (0·38)* 18·9 (0·95)	*20·0 (0·94)*	19·3 (0·93)	20·7 (0·96)
Fast Red E	20·0 (0·84)	19·7 (0·82)	19·9 (0·80)	†*25·0 (0·84)*	19·2 (0·86)	19·6 (0·81)	19·6 (0·98)	20·6 (0·79)
1-Naphthyl-azo-2-naphthyl dyes								
Carmoisine	*24·4 (0·60)	—	—	—	25·0 (0·53)	—	—	—
	19·3 (0·98)	19·2 (0·87)	20·0 (0·86)	19·6 (0·98)	18·8 (0·92)	19·2 (0·94)	19·2 (0·88)	20·2 (0·98)
Black PN	24·4 (0·64)	—	24·0 (0·60)	†*24·4 (0·84)*	24·4 (0·67)	24·3 (0·67)	24·3 (0·60)	25·3 (0·67)
	17·4 (0·98)	17·4 (0·98)	17·6 (0·96)	†*20·6 (0·83)*	17·2 (0·98)	17·2 (0·97)	17·2 (0·95)	17·2 (0·91)

TABLE 1 (*Continued*)

Dye	pH 3·0	pH 6·4	pH 7·4	pH 12·5	pH 3·0	pH 6·4	pH 7·4	pH 12·5
2-Naphthyl-azo-phenyl dyes								
Ponceau SX	20·0 (0·84)	19·6 (0·90)	20·2 (0·87)	21·4 (0·93)	19·7 (0·91)	19·7 (0·89)	19·8 (0·92)	20·6 (0·87)
Red 10B	19·2 (0·93)	18·9 (0·89)	19·0 (0·88)	19·0 (0·91)	*24·0 (0·48) 18·5 (0·92)	18·7 (0·90)	18·7 (0·91)	19·0 (0·95)
Red 2G	20·0 (0·95) 19·0 (0·95)	19·5 (0·95) 18·7 (0·95)	20·0 (0·93) 19·0 (0·93)	21·9 (0·84)	20·5 (0·96) 18·5 (0·96)	19·6 (0·95)	19·5 (0·92) 18·7 (0·92)	*20·0 (0·87)*
Red 6B	19·0 (0·94)	18·9 (0·92)	19·4 (0·93)	†*22·8 (0·93)*	18·5 (0·96)–	18·9 (0·94)	19·0 (0·91)	21·0 (0·85)
(a) Red FB	19·5 (0·47)	†*20·6 (0·69)*	19·6 (0·55)	**20·4 (0·66)*	19·7 (0·53)	19·6 (0·52)	19·6 (0·52)	†*20·4 (0·66)*
Phenyl-azo-phenyl dyes								
Yellow RY	22·8 (0·89)	†*25·5 (0·97)*	22·8 (0·82)	*23·8 (0·89)*	23·3 (0·83) *19·6 (0·80)*	22·6 (0·89)	22·5 (0·89)	†*26·0 (0·95)*
Yellow RFS	23·6 (0·77)	*24·9 (0·93)*	24·8 (0·81)	*24·8 (0·95)*	*22·9 (0·87)*	*24·4 (0·89)*	24·7 (0·71)	—
Phenyl-azo-pyrazole dyes								
Yellow 2G	24·4 (0·91)	—	24·8 (0·88)	*25·8 (0·96)*	†24·9 (0·95)	*25·5 (0·92)*	25·0 (0·92)	†*26·3 (0·97)*
Tartrazine	23·6 (0·90)	23·2 (0·90)	23·6 (0·87)	†*23·0 (0·99)*	22·7 (0·90)	23·8 (0·98)	23·2 (0·92)	†25·2 (0·92)
Tri-phenyl-methanol anhydride dyes								
Blue VRS	24·4 (0·93)	24·3 (0·92)	24·6 (0·68)	**26·6 (0·96)*	24·3 (0·95)	*23·0 (0·79)*	24·2 (0·75)	*24·0 (0·91)
	15·6 (0·95)	15·8 (1·00)	16·0 (1·00)	16·0 (1·00)	15·4 (0·97)	15·3 (1·00)	15·3 (1·00)	15·6 (0·90)
Green S	24·0 (0·45)	—	25·2 (0·56)	—	—	—	24·9 (0·57)	—
	22·8 (0·43)	—	*22·8 (0·48)	—	*22·7 (0·56)	—	*23·0 (0·42)	—
	16·0 (0·92)	15·5 (0·99)	16·0 (0·99)	16·4 (0·97)	15·6 (1·00)	15·4 (1·00)	15·9 (1·00)	16·0 (0·99)
Violet BNP	†18·0 (0·98)	—	18·4 (0·96)	†18·4 (0·62)	†18·0 (0·98)	18·0 (0·92)	18·1 (0·99)	**17·4 (0·88)*
	17·0 (0·98)	18·3(0·90)	†17·6 (0·96)	17·0 (0·69)	16·7 (0·90)	16·7 (0·98)	†16·7 (0·98)	16·5 (0·90)
	← Dye in the presence of excess cobalt (II) ions →				← Dye in the presence of excess aluminium (III) ions →			
1-Naphthyl-azo-phenyl dyes								
Orange G	*24·7 (0·67)	*23·8 (0·50)	*25·2 (0·50)	25·0 (0·66)	*24·7 (0·67)	*24·0 (0·53)	*25·0 (0·51)	25·0 (0·55)
	20·8 (0·87)	20·8 (0·88)	21·0 (0·89)	20·3 (0·56)	20·8 (0·87)	20·8 (0·88)	21·2 (0·89)	20·0 (0·51)
Orange RN	24·4 (0·38)	†23·8 (0·56)	25·0 (0·56)	22·8 (0·72)	24·4 (0·38)	†23·8 (0·56)	24·6 (0·57)	22·7 (0·66)
	20·4 (0·88)	†20·3 (0·87)	21·2 (0·87)	*19·8 (0·56)	20·4 (0·88)	20·3 (0·87)	21·2 (0·88)	†19·8 (0·56)

Sunset Yellow FCF	*23·8 (0·58) 20·62 (0·89)	*23·8 (0·60) 20·4 (0·89)	*24·6 (0·59) 21·2 (0·91)	22·0 (0·76) *20·0 (0·66)	*23·8 (0·58) 20·6 (0·89)	*23·8 (0·60) 20·4 (0·89)	*24·6 (0·63) 21·4 (0·91)	22·1 (0·70) *20·0 (0·60)
Ponceau MX	19·6 (0·88)	19·7 (0·88)	20·0 (0·95)	*23·7 (0·40)* 19·3 (0·59)	19·6 (0·88)	19·8 (0·88)	20·2 (0·88)	19·5 (0·68)
Chocolate Brown HT	21·3 (0·85)	21·2 (0·85)	21·6 (0·86)	21·2 (0·87)	21·3 (0·85)	21·2 (0·85)	21·8 (0·84)	21·2 (0·85)
1-Naphthyl-azo-1-naphthyl dyes								
Ponceau 4R	19·4 (0·88)	19·4 (0·88)	20·0 (1·00)	20·0 (0·62)	19·4 (0·87)	19·4 (0·88)	20·0 (1·00)	20·0 (0·62)
Amaranth	18·9 (0·92)	19·0 (0·93)	19·6 (0·91)	20·0 (0·67)	18·9 (0·92)	18·9 (0·93)	19·6 (0·91)	19·5 (0·73)
Fast Red E	19·2 (0·87)	19·7 (0·85)	20·2 (0·84)	20·8 (0·71)	19·2 (0·87)	19·9 (0·85)	19·6 (0·80)	20·8 (0·71)
1-Naphthyl-azo-2-naphthyl dyes								
Carmoisine	*24·0 (0·40) 18·8 (0·92)	†24·4 (0·40) 19·2 (0·90)	— 19·8 (0·84)	— 19·6 (1·00)	*24·0 (0·40) 18·8 (0·92)	†24·4 (0·40) 19·2 (0·92)	— 19·6 (0·98)	— 19·0 (0·89)
Black PN	24·3 (0·65) 17·2 (0·98)	24·3 (0·66) 17·2 (0·97)	23·8 (0·66) 17·4 (0·95)	25·3 (0·78) 17·3 (0·91)	24·3 (0·63) 17·2 (0·98)	24·3 (0·66) 17·2 (0·97)	24·4 (0·66) 17·8 (0·96)	25·4 (0·74) 17·2 (0·90)
2-Naphthyl-azo-phenyl dyes								
Ponceau SX	19·7 (0·91)	19·7 (0·91)	20·0 (0·87)	20·6 (0·86)	19·7 (0·91)	19·7 (0·91)	20·2 (0·91)	20·6 (0·86)
Red 10B	*23·6 (0·34)* 19·0 (0·92)	*23·6 (0·40)* 18·5 (0·91)	19·4 (0·89)	19·0 (0·80)	*23·6 (0·34)* 19·0 (0·92)	18·5 (0·91)	19·0 (0·91)	18·5 (0·89)
Red 2G	20·0 (0·96) 18·5 (0·96)	20·0 (0·95) 19·0 (0·95)	20·6 (0·95) 19·0 (0·95)	21·7 (0·86)	20·0 (0·96) 18·5 (0·96)	18·5 (0·95) 20·0 (0·95)	20·0 (0·95) 19·0 (0·95)	21·7 (0·76)
Red 6B	18·5 (0·96)	18·9 (0·94)	19·6 (0·93)	20·8 (0·82)	18·5 (0·96)	18·9 (0·94)	19·6 (0·94)	20·8 (0·78)
(a) Red FB	19·3 (0·49)	19·6 (0·49)	19·6 (0·45)	*18·0 (0·50)*	19·3 (0·49)	19·6 (0·49)	19·6 (0·49)	18·8 (0·49)
Phenyl-azo-phenyl dyes								
Yellow RY	*24·4 (0·75)*	22·7 (0·88)	23·0 (0·86)	*24·0 (0·88)*	*24·0 (0·79)*	22·5 (0·89)	22·8 (0·86)	*24·3 (0·85)*
Yellow RFS	24·4 (0·70) *19·6 (0·62)*	23·2 (0·85)	25·0 (0·84)	24·4 (0·78)	24·4 (0·70)	*24·3 (0·88)*	24·8 (0·84)	24·4 (0·78)
Phenyl-azo-pyrazole dyes								
Yellow 2G	24·4 (0·90)	24·7 (0·91)	25·0 (0·97)	24·8 (0·91)	24·4 (0·90)	24·7 (0·91)	25·0 (0·89)	25·0 (0·88)
Tartrazine	23·0 (0·90)	23·3 (0·91)	23·4 (0·91)	24·8 (0·90)	22·7 (0·90)	23·3 (0·92)	23·6 (0·91)	24·9 (0·87)
Tri-phenyl-methanol anhydride dyes								
Blue VRS	23·9 (0·82) 15·4 (0·98)	24·0 (0·75) 15·3 (1·00)	24·4 (0·79) 16·0 (1·00)	24·2 (0·80) 15·1 (1·00)	24·4 (0·91) 15·8 (0·98)	24·1 (0·74) 15·3 (1·00)	24·6 (0·79) 16·4 (1·00)	24·0 (0·75) 15·1 (0·97)

TABLE 1 (*Continued*)

Dye	pH 3·0	pH 6·4	pH 7·4	pH 12·5	pH 3·0	pH 6·4	pH 7·4	pH 12·5
Green S	24·9 (0·50) 22·3 (0·44) 15·6 (1·00)	25·0 (0·50) 22·3 (0·44) 15·4 (0·99)	25·2 (0·55) 22·8 (0·45) 16·2 (1·00)	24·9 (0·72) — 16·0 (0·99)	24·8 (0·54) *23·0 (0·45) 15·4 (1·00)	24·9 (0·50) 22·4 (0·45) 15·6 (0·99)	25·0 (0·52) — 16·0 (1·00)	24·9 (0·64) — 15·9 (0·99)
Violet BNP	*23·6 (0·16) 16·8 (0·98)	18·0 (0·96) †16·4 (0·96)	18·4 (0·96) †17·2 (0·95)	16·7 (0·15) —	*23·5 (0·15) 17·0 (0·77)	18·4 (0·96) †16·8 (0·96)	18·8 (0·97) †18·0 (0·95)	16·4 (0·82) —
	← Dye in the presence of excess calcium ions →				← Dye in the presence of excess magnesium ions →			
1-Naphthyl-azo-phenyl dyes								
Orange G	*25·0 (0·50) 20·8 (0·90)	*23·9 (0·50) 20·7 (0·88)	*24·8 (0·51) 21·0 (0·88)	25·4 (0·59) 20·2 (0·52)	*25·0 (0·52) 20·7 (0·90)	*23·9 (0·50) 20·6 (0·88)	*24·8 (0·50) 21·0 (0·88)	25·4 (0·59) 20·4 (0·52)
Orange RN	†24·2 (0·56) 20·5 (0·88)	†23·8 (0·56) 20·3 (0·87)	†24·6 (0·56) 21·4 (0·88)	23·3 (0·60) †20·5 (0·51)	†24·4 (0·57) 20·8 (0·87)	†23·8 (0·56) 20·3 (0·87)	†24·6 (0·56) 21·6 (0·88)	23·0 (0·61) †20·4 (0·54)
Sunset Yellow FCF	*24·4 (0·63) 21·0 (0·91)	*23·8 (0·60) 20·4 (0·89)	*24·0 (0·61) 20·8 (0·91)	— 22·2 (0·76)	*24·4 (0·63) 20·8 (0·91)	*23·8 (0·60) 20·4 (0·89)	*24·2 (0·61) 20·8 (0·91)	— 20·2 (0·74)
Ponceau MX	20·0 (0·88)	19·7 (0·87)	20·0 (0·87)	20·0 (0·64)	19·8 (0·89)	19·6 (0·87)	20·2 (0·95)	19·9 (0·70)
Chocolate Brown HT	21·6 (0·86)	21·2 (0·85)	22·0 (0·85)	21·2 (0·87)	21·3 (0·85)	21·2 (0·85)	21·6 (0·85)	21·2 (0·85)
1-Naphthyl-azo-1-naphthyl dyes								
Ponceau 4R	19·4 (1·00)	19·4 (0·88)	20·0 (1·00)	20·6 (0·95)	19·6 (0·98)	19·4 (0·88)	20·0 (1·00)	20·8 (0·94)
Amaranth	19·0 (0·92)	19·0 (0·93)	19·8 (0·91)	20·4 (0·76)	19·4 (0·91)	18·7 (0·93)	19·6 (0·91)	20·0 (0·77)
Fast Red E	19·6 (0·87)	19·7 (0·83)	20·4 (0·84)	21·0 (0·73)	19·8 (0·85)	19·7 (0·85)	20·0 (0·84)	21·2 (0·71)
1-Naphthyl-azo-2-naphthyl dyes								
Carmoisine	†24·6 (0·60) 19·4 (0·98)	†24·4 (0·40) 19·2 (0·90)	— 19·8 (0·87)	— 19·8 (0·97)	†24·4 (0·59) 19·2 (0·98)	†24·4 (0·40) 19·2 (0·90)	— 19·6 (0·90)	— 19·6 (0·96)
Black PN	24·4 (0·63) 17·6 (0·98)	24·3 (0·66) 17·2 (0·97)	24·0 (0·66) 17·6 (0·96)	— 17·6 (0·91)	24·5 (0·63) 17·9 (0·98)	24·3 (0·66) 17·2 (0·97)	24·0 (0·63) 17·4 (0·96)	25·6 (0·80) 17·6 (0·95)
2-Naphthyl-azo-phenyl dyes								
Ponceau SX	19·8 (0·91)	19·7 (0·91)	20·4 (0·91)	20·8 (0·85)	20·0 (0·91)	19·7 (0·91)	20·0 (0·91)	20·8 (0·85)
Red 10B	19·2 (0·92)	18·4 (0·91)	19·4 (0·91)	19·0 (0·90)	19·0 (0·91)	24·8 (0·39) 18·8 (0·91)	19·6 (0·91)	19·0 (0·90)
Red 2G	20·0 (0·95) 19·0 (0·95)	20·0 (0·95) 19·0 (0·95)	20·0 (0·95) 19·0 (0·95)	21·6 (0·78)	20·0 (0·95) 19·0 (0·95)	20·0 (0·95) 19·0 (0·95)	20·0 (0·95) 19·0 (0·95)	21·8 (0·77)

Red 6B	19·0 (0·94)	18·9 (0·94)	19·4 (0·94)	21·6 (0·75)	18·8 (0·94)	18·9 (0·94)	19·4 (0·94)	21·0 (0·76)
(a) Red FB	19·6 (0·49)	19·6 (0·49)	19·6 (0·49)	18·8 (0·48)	19·6 (0·49)	19·6 (0·49)	19·6 (0·49)	18·8 (0·48)
Phenyl-azo-phenyl dyes								
Yellow RY	22·6 (0·90)	22·5 (0·89)	23·2 (0·86)	22·4 (0·81)	22·6 (0·90)	22·6 (0·89)	23·2 (0·86)	22·0 (0·80)
Yellow RFS	24·0 (0·78)	23·0 (0·84)	25·0 (0·81)	24·0 (0·87)	23·6 (0·78)	23·3 (0·85)	24·8 (0·81)	23·8 (0·95)
Phenyl-azo-pyrazole dyes								
Yellow 2G	24·8 (0·91)	24·7 (0·91)	24·6 (0·90)	25·0 (0·88)	24·6 (0·91)	24·7 (0·91)	24·8 (0·90)	25·2 (0·96)
Tartrazine	22·4 (0·90)	23·3 (0·91)	23·6 (0·91)	25·0 (0·86)	22·9 (0·91)	23·8 (0·92)	23·8 (0·91)	25·0 (0·91)
Tri-phenyl-methanol-anhydride dyes								
Blue VRS	24·6 (0·91)	24·0 (0·74)	24·4 (0·68)	24·2 (0·73)	24·4 (0·90)	24·4 (0·68)	24·4 (0·68)	24·4 (0·73)
	16·0 (0·98)	15·8 (1·00)	16·0 (1·00)	15·8 (1·00)	15·9 (0·98)	16·0 (1·00)	16·0 (1·00)	16·0 (0·98)
Green S	25·0 (0·54)	24·9 (0·50)	25·0 (0·52)	25·6 (0·57)	25·0 (0·44)	25·0 (0·52) †22·7 (0·38)	25·0 (0·53)	25·0 (0·53)
	23·0 (0·49)	22·2 (0·44)	†22·7 (0·38)	—	22·6 (0·49)	—	†22·8 (0·35)	—
	16·0 (1·00)	15·2 (0·99)	16·1 (0·98)	16·2 (0·97)	16·0 (0·93)	16·1 (0·98)	16·1 (1·00)	16·4 (1·00)
Violet BNP	†18·0 (0·98)	18·0 (0·96)	18·4 (0·96)	—	†18·0 (0·98)	18·0 (0·96)	18·4 (0·96)	—
	17·0 (0·98)	†16·7 (0·96)	†17·2 (0·95)	17·0 (0·18)	16·4 (0·97)	†16·7 (0·96)	†17·2 (0·95)	17·0 (0·17)

(a) The data for Red FB were obtained with solutions diluted, in all cases, by a factor of five.

Data in italics relate to absorption band maxima in the presence of metal ions removed by 800 cm^{-1}, or more, from those observed for the free dyes.

* Inflexion. † Shoulder.

TABLE 2. Stability constants of selected metal–dye complexes

pH	Metal	Dye	Metal–dye ratio in complex	Stability constant of complex
12·5	Iron(II)	Ponceau 4R	2 : 1	6×10^{11}
12·5	Iron(III)	Amaranth	1 : 1	3×10^{6}
7·4	Copper(II)	Carmoisine	1 : 2	2×10^{11}
12·5	Copper(II)	Yellow RY	2 : 1	8×10^{11}

The four dyes that have absorption bands further towards the red end of the spectrum possess, as expected, bluish characteristics in their colours. Thus, violet BNP and black PN have absorption maxima at around 17,000 cm^{-1} (588 nm) while blue VRS and green S have their maxima near 16,000 cm^{-1} (625 nm).

The colours and, hence, absorption spectra of the dyes studied, fall into a pattern according to structure of the dye molecule. Thus, a yellow coloration is characteristic of the phenyl-azo-phenyl and the phenyl-azo-pyrazole dyes. On the other hand, 1- and 2-naphthyl-azo dyes, with molecules of more enhanced bathochromic characteristics, may generally be distinguished by their red colours.

There are, however, several exceptions to the generality of the red character for the naphthyl-azo dyes. For example, the orange dyes, namely orange G, orange RN and sunset yellow FCF, which belong to the phenyl-azo-naphthyl group, do not appear to carry substituents of potential bathochromic character as do the other dyes belonging to this group. Another exception is black PN which brings out the bathochromic characteristics of the extended conjugation brought about by the favourable position of its extra azo grouping and absorbs at lower frequencies than the red dyes. Chocolate brown HT also has two azo linkages, but due to their unfavourable position, an extended conjugation is not possible and hence, with its two apparently independent phenyl-azo-naphthyl halves, its absorption maximum at around 21,200 cm^{-1} (472 nm) is close to that observed for the orange dyes.

The triphenylmethanol anhydride dyes, namely, blue VRS, green S and violet BNP are characteristic of their class and all show absorptions well on the low frequency side of those of the red dyes.

Except at pH 12·5, the effect of pH on the frequency of the absorption band is not great. At pH 12·5, however, there is a distinct tendency for the absorption band to be shifted to a slightly different frequency. For example, this is evidenced by the orange colour of red 6B and red 2G at this pH.

The only other feature of the spectra of the dye solutions that calls for comment is that with the exception of red FB, the optical densities of the bands responsible for the colours do not vary appreciably (only by a factor of two or three) in passing from

one dye to the next. However, there is a tendency for the optical densities of solutions at pH 12·5 to be less than those for solutions at other pH values. This is particularly true of violet BNP, a feature that is characteristic of triphenylmethanol anhydride dyes under alkaline conditions when they have basic (or positive) auxochromic groups. The optical densities of the red FB solutions are appreciably greater, due possibly to the presence of the benzothiazole grouping in this 2-naphthyl-azo-phenyl dye.

The effect of metal ions on the spectra of dye solutions

Table 1, and the trends noted above, reveal that there are differences of 1000 cm^{-1} (25 nm at 500 nm) or more, in the absorption band maxima of the main colour bands. For a pronounced change of colour to be observed visually, a shift of at least this magnitude is apparently required in the position of a maximum of an absorption band of a dye solution in the presence of metal ions when compared with that in the absence of metal ions. Towards this end, the maxima of absorption bands of the dye solutions in the presence of metal ions are italicized in Table 1 in cases where these differ by more than 800 cm^{-1} (20 nm at 500 nm) from those of the free dye solution.

Several of the dyestuffs used as metallochromic indicators in EDTA titrations belong to the *o-o'*-dihydroxy group of azo dyes (Barnard, Broad & Flashka, 1956), and under suitable conditions, give well-defined colour changes at the titration end-points. These colour changes are due to changes in the electronic configuration brought about by chelation arising from the favourable position of the *o-o'*-hydroxy groups. However, only a limited number of *ortho*-monohydroxy azo dyes have applications as metallochromic indicators and these, for example, the sodium salt of 3-(4-sulphophenylazo)-4,5-dihydroxynaphthalene-2,7-disulphonic acid (SPADNS), normally have two hydroxy groups suitably disposed to form a ring by chelation (Barnard, *et al.*, 1956). All, except four, of the azo dyes included in the present investigation have one hydroxy group in a position *ortho* to the azo linkage. Of the remainder, chocolate brown HT has two hydroxy groups—*ortho* to the same end of the azo linkage; yellow 2G also has two hydroxy groups, but neither are in a suitable position for chelation, as is the case of the monohydroxy group of tartrazine. Yellow RFS is in the unique position of possessing not even a single hydroxy group.

At best, chelation of the food dyes with metal ions is possible on a more limited scale than that indicated above for the metallochromic indicators with the result that the consequent changes in electronic configuration are on a more restricted scale. In confirmation of this, it may be seen from Table 1 that appreciable changes in the absorption spectra [shifts of greater than 800 cm^{-1} (20 nm at 500 nm) in the position of the maximum of the absorption band], and hence of colour of the dyes brought about by metal ions are not, by any means, the rule. Lesser changes are, as might be expected, more frequent and certain dyes, for example, ponceau SX, give only small changes. Again, with added calcium or magnesium ions, there is a negligible effect.

This is to be expected since these ions do not usually have a strong affinity towards complex formation. Aluminium ions, on the other hand, do cause a few changes in the absorption spectra, more especially for red 10B, red 2G, yellow RFS, yellow RY and violet BNP.

The larger changes in the absorption spectra of the dyes are brought about by the transition metal ions examined. Changes brought about by copper (II) and iron (II) tend to be hypsochromic while those of iron (III) are more variable. In fact, the most pronounced colour change observed visually is that brought about by copper (II) on carmoisine (normally red) at pH 6·4, and especially at pH 7·4 when the colour is orange. This corresponds to a shift in the absorption band maximum of 1200 cm^{-1} (29 nm) and 2000 cm^{-1} (47 nm) at the respective pH values. Copper (II) ions are also responsible for a less pronounced visual colour change over the normal colour of the free dye (orange red → orange) in ponceau MX at pH 12·5. This is characterized by a shift of 1600 cm^{-1} (37 nm) in the maximum of the absorption band of the dye. A further example is the red colour exhibited by black PN in the presence of iron (II) ions at pH 12·5, a shift of 3000 cm^{-1} (83 nm) away from the bluish purple absorption at 17,600 cm^{-1} (568 nm).

It is interesting to note that the yellow dyes frequently show changes of frequency in their absorption maxima with the metal ions, despite the fact that with the exception of yellow RY, they do not possess suitably disposed groups for chelation.

Dyes have had to be monitored individually for their potential as indicators in complexometric titrations (Close & West, 1960a, b; Brazier & Stephen, 1965). The present investigation might form such a monitoring and suggests that carmoisine might be a suitable indicator for copper (II). Indeed, its 1 : 2 (metal–dye) complex stability constant of the order of 10^{11} (Table 2) would serve to confirm this. However, while the dye functioned at the predicted end-point in the titration of copper (II) with EDTA, it was considered to be inferior to the other excellent indicators now available.

Even though phosphates play an active part in forming complexes with metal ions, they are also a common constituent of foodstuffs and for this reason phosphate buffer solutions were selected for the neutral pH values of 6·4 and 7·4. Under these conditions, the dye competed with the phosphate for the metal ions, but despite this, shifts were observed in the frequencies of the absorption maxima of a number of dyes in the presence of metal ions (Table 1). With the excessive iron used to obtain the data of Table 1 at these pH values, and also at pH 12·5, there is a tendency for the ultraviolet absorptions to spread into the visible region (due to a slight cloudiness through slight precipitate formation) but nevertheless, the frequencies of the colour causing absorption maxima can, in the majority of cases, easily be distinguished. The cloudiness is very much less in evidence for the iron concentrations used to obtain the data for the calculation of the stability constants shown in Table 2.

An interesting facet of this work is the possible effect of food dyes on iron metabolism.

It is believed that iron in the +2 oxidation state is the form more effectively utilized by the body and that towards this end, iron (III) is reduced to the +2 oxidation state before diffusion in the mucosal cell (Saltman, 1965). Since, it appears that iron in both the +2 and +3 oxidation states are available to the body, the question arises of whether the food dyes affect iron metabolism. Some of the dyes clearly form complexes with iron and since the formation of biological iron chelates is claimed to be important in iron metabolism (Charley *et al.*, 1963a, b), it is interesting to have some indication of the stability of the iron–dye complexes. As can be seen from Table 2, the stability constant for the 1 : 1 complex with amaranth at pH 12·5 is of the order of 10^6, while the 2 : 1 (metal–dye) complex with ponceau 4R, at the same pH has a stability constant of around 10^{11}. These figures relate to the more alkaline pHs. Conditions in the human body are more acid with the pH of human saliva at around 7·4, the stomach being distinctly acid, and, finally, a pH of 6·5–7 being characteristic of the lumen of the intestine which is the region normally associated with iron absorption. However, the present investigation cannot throw any light on how far these iron–dye complexes compete with complexes of iron with materials, such as sugars and other polyhydroxy compounds, which are claimed to be highly significant in iron metabolism (Charley *et al.*, 1963a, b; Saltman, 1965).

The triphenylmethanol anhydride dyes also do not show an appreciable change of frequency in absorption band maxima in the presence of metal ions, although changes in hue are frequently apparent. Here again, this time in the presence of metal ions, a pH of 12·5 is sufficiently alkaline for the basic (or positive) auxochromic groups of violet BNP to have an influence, thus causing fading and, of course, the extreme fall in optical density noted above for the free dye.

Conclusion

Traces of metal ions do not, in general, have an appreciable effect on the colour of coal-tar food dyes; indeed extreme alkaline conditions have the more pronounced effect. There is, however, the question of the possible role of the dyes in influencing iron metabolism and it is suggested that further enquiry on this point is desirable.

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