Chapter I

Introduction

The use of coloring matters in the preparation and the preservation of foodstuffs is now widespread. Substances used for food coloring can be grouped into natural colors and synthetic dyestuffs. Natural colors are obtained from mineral, vegetable and animal sources (1, 2). They may be available in different forms. Even from the same plant species, the nature and proportion of the colors and other components may vary widely because of the differences in soil, climatic conditions, age of plant and time of harvest (3). In considering such food colors, there are many problems owing to the lack of information relating to the adequate identification, chemical composition, instability, low tinctorial value and variation in strength (3, 4). Nowadays the synthetic products become practically available.

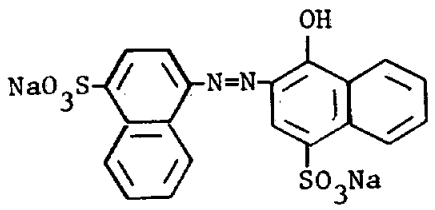
Synthetic dyes have been classified into a number of classes, according to their chemical structures, such as azo, pyrazolone, triphenylmethane, antraquinone, indigoid, xanthene, quinoline and polynuclear (1). Azo dyes are the most important class. The azo dyes are characterized by the presence of one or more azo (-N=N-) groups. They are prepared by diazotization of an aromatic primary amine to give a diazonium salt. The diazonium salt is then coupled with phenol or aromatic amine with a free o- and/or p-position, or with certain other components having reactive positions, such as β-ketonic acid arylamide (5).

Synthetic dyes have a wide range of colors. The compounds are colored depending on the chromophore functional groups, such as azo, azoxy, nitro, nitroso, carboxyl and ethylene groups. These dyes cannot be used indiscriminately for coloring food since many dyes are toxic, and food dyes have to be manufactured in a high state of purity and free from harmful constituents (5). All food dyes, the maximum limit for lead is 0.001%, for arsenic as As_2O_3 is 0.00014%, only a trace of other heavy metals (precipitated as sulfides) are permitted (6).

The permitted dyes; Azorubine, Sunset Yellow FCF, Tartrazine, and the nonpermitted dyes; Orange G, Orange RN which have similar structures were selected for this study. These dyes are of interest since they are hydroxy monoazo linkage which makes the complex formations simple. Green S was also selected for this study since it is an hydroxy substituted triphenylmethane compound and it is still used as food additive.

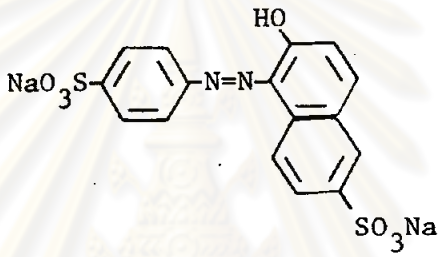
1.1 Preparations and properties of the dyes studied

Azorubine (Color Index Number 14720, Molecular weight 502.45) is the disodium salt of 2-(4-sulfo-1-naphthylazo)-1-naphthol-4-sulfonic acid. It belongs to the monoazo group of dyes. It is a water soluble food color (7, 8). Azorubine is prepared by coupling diazotized 1-naphthylamine-4-sulfonic acid with 1-naphthol-4-sulfonic acid (9). The structure is shown below.

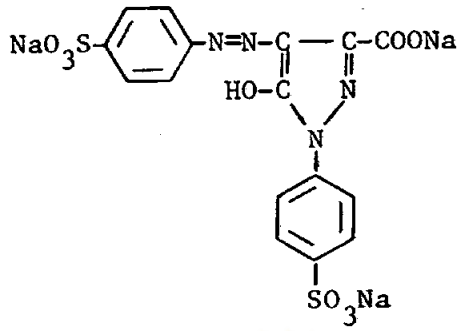


Sunset Yellow FCF (Color Index Number 15985, Molecular weight 452.40) is the disodium salt of 1-(4-sulfo-1-phenylazo)-2-naphthol-6-sulfonic acid. It is an acid dye of monoazo series, easily soluble in water, slightly soluble in 95% alcohol but is readily soluble in glycerol and glycols (7, 8, 10).

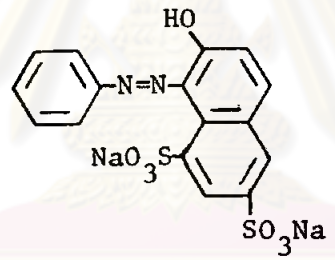
Sunset Yellow FCF is prepared by diazotizing sulfanilic acid and coupling the diazotized product with 2-naphthol 6-sulfonic acid (10, 11). The structure is shown below.



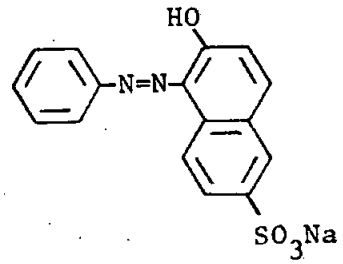
Tartrazine (Color Index Number 19140, Molecular weight 534.40) is the trisodium salt of 3-carboxy-5-hydroxy-1-(4-sulfo-1-phenyl)-(4-sulfo-1-phenylazo) pyrazole. It is a pyrazolone dye, easily soluble in water, slightly soluble in 95% alcohol but is readily soluble in glycerol and glycols (7, 8, 10). It has good resistance to light, acetic acid, hydrochloric acid and 10% sodium hydroxide solutions. However, toward 30% sodium hydroxide solution its resistance is only fair and it turns redder in hue (10). Tartrazine is prepared by treating phenylhydrazine-4-sulfonic acid with dioxytartaric acid. Alternatively it is prepared by coupling the diazotized sulfanilic acid with oxalacetic ether, condensing the product with phenylhydrazine-4-sulfonic acid and finally hydrolyzing the ester with sodium hydroxide solution (10). The structural formula of Tartrazine is



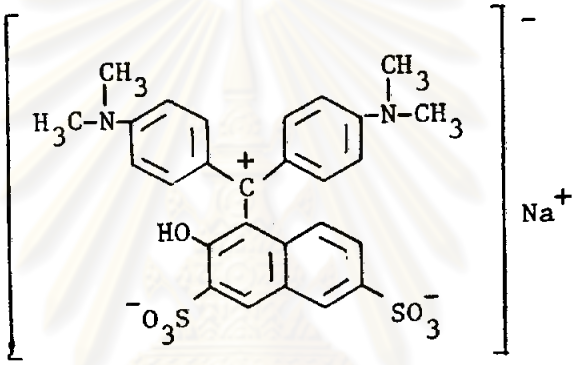
Orange G (Color Index Number 16230, Molecular weight 452.37) is the disodium salt of 1-phenylazo-2-naphthol-6, 8-disulfonic acid. It belongs to the monoazo group of dyes. It is a water soluble food color (7, 8). Orange G is prepared by coupling diazobenzene chloride with 2-naphthol-6, 8-disulfonic acid (9). Its structural formula is



Orange RN (Color Index Number 15970, Molecular weight 350.32), the sodium salt of 1-phenylazo-2-naphthol-6-sulfonic acid, is a water soluble food coloring. It is a monoazo dye. Orange RN is prepared by coupling diazobenzene chloride with 2-naphthol-6-sulfonic acid (9). The structure of Orange RN is shown below.



Green S (Color Index Number 44090, Molecular weight 576.64) is the monosodium salt of 4, 4′-bis (dimethylamino) diphenylmethylene-2-naphthol-3, 6-disulfonic acid. It belongs to the triarylmethane group of dye (8). It is a water soluble food color. Green S is prepared by condensing Michler's hydrol (4, 4′-dimethylaminodiphenyl methanol) with 3-hydroxy-2, 7-naphthalenedisulfuric acid in strong sulfuric acid and then oxidizing the leuco with lead peroxide or dichromate (11, 12). The structural formula of Green S is



1.2 Biological studies of the dyes

Biological studies of Sunset Yellow FCF, Orange RN and Orange G in rats showed that the major portions of the colors were reduced at the azo linkage by intestinal bacteria (3). The azo linkage of Orange RN was broken and the metabolites identified were 1-amino-2-naphthol-6-sulfonic acid, aniline, 4-aminophenol and 2-aminophenol. These metabolites are capable of producing Heinz bodies and associated haemolytic anemia (13).

It was suggested that Orange G and Orange RN have the same biological effect. Since their structure formulae were similar (13, 14).

Tartrazine and Sunset Yellow FCF were tested for mutagenic effect in a concentration of 0.5 g/100.0 cm^3 in cultures of Escherichia coli. No mutation was found (3). Biological studies of Sunset Yellow

FCF in mice showed no carcinogenic and no long term effect (15).

No carcinogenic property was found when Azorubine, Sunset Yellow FCF, Orange G, Tartrazine and Green S were tested in cultures of Escherichi coli (16).

1.3 Literature survey of complex formations between the dyes and metal ions

The complex formations of some azo food dyes such as Amaranth, Ponceau 4R, Azorubine and Sunset Yellow FCF with Cr (III), Cd (II), Fe (II), Fe (III), Hg (II), Pb (II) and Cu (II) ions were studied (17, 18, 19, 20, 21).

Azorubine formed a 1:2 complex with Cu (II) ion (metal:dye) at pH 7.4 in phosphate buffer and its stability constant was found to be 2×10^{11} (18). By spectrophotometric method, complex formation between Cr (III) ion and Azorubine occurred at the molar ratio of 1:3 in 0.5 M sulfuric acid (19). In addition, Azorubine was suggested to be a suitable indicator for Cu (II) ion in complexometric titration (18, 20).

The complex formation between Cu (II) ion and Sunset Yellow FCF occurred at the molar ratios of 1:1 at pH 4.5 and 2:1 at pH 6.0 and structure of the later complex was proposed as a salt forming Cu (II) ion and a coordinated Cu (II) ion per dye molecule (21).

Tartrazine is used as an indicator for the determination of silver in dilute nitric acid solution by Volhard's method (22).

Since 1977, the Ministry of Public Health of Thailand has announced the following dyes as food additives: Azorubine (Carmoisine) Ponceau 4R, Erythrosine, Tartrazine, Sunset Yellow FCF, Riboflavin, Fast Green FCF, Indigotine and Brilliant blue FCF (23).

A search of the literature revealed that there were not many studies on the behavior of food dyes in the presence of metal ions. For this reason, the author decided to find out the suitable conditions for the complex formations between Azorubine, Sunset Yellow FCF, Orange G, Orange RN, Tartrazine and Green S with transition metal ion, such as Ti (IV), Cr (III), Mn (II), Co (II), Fe (II), Fe (III), Ni (II), Cu (II) and Zn (II) over a wide pH range of various buffer solutions as well as to determine the ratio of metal-dye in complexes and stability constants of complexes formed.

1.4 Techniques and Methods used

Numerous techniques have been used for studying complex formation. The spectrophotometric technique is the most useful one for elucidating the composition of complex ions in the solution and determining their stability constants. There are three methods: the method of continuous variation , the molar ratio method and the slope ratio method.

The method of continuous variations is one of the most generally and widely used, first described by Job (24). In this method (24, 25), it is convenient to start with identical concentrations of the cation and the ligand. A series of solutions in which the sum of the concentrations of the cation and the ligand in each solution is the same while their ratio varies, is prepared by adding y liter of the cation to (1-y) liter of the ligand (y<1). To determine the value of m and n in a complex system M_mX_n, the absorbance at which M_mX_n absorbed, but M and X do not, is plotted against y (mole fraction of M or X in the mixture). This will result in curve as shown in Figure 1. From the value of y at the maximum absorbance and the relationship $\frac{n}{m} = y/1-y$, the

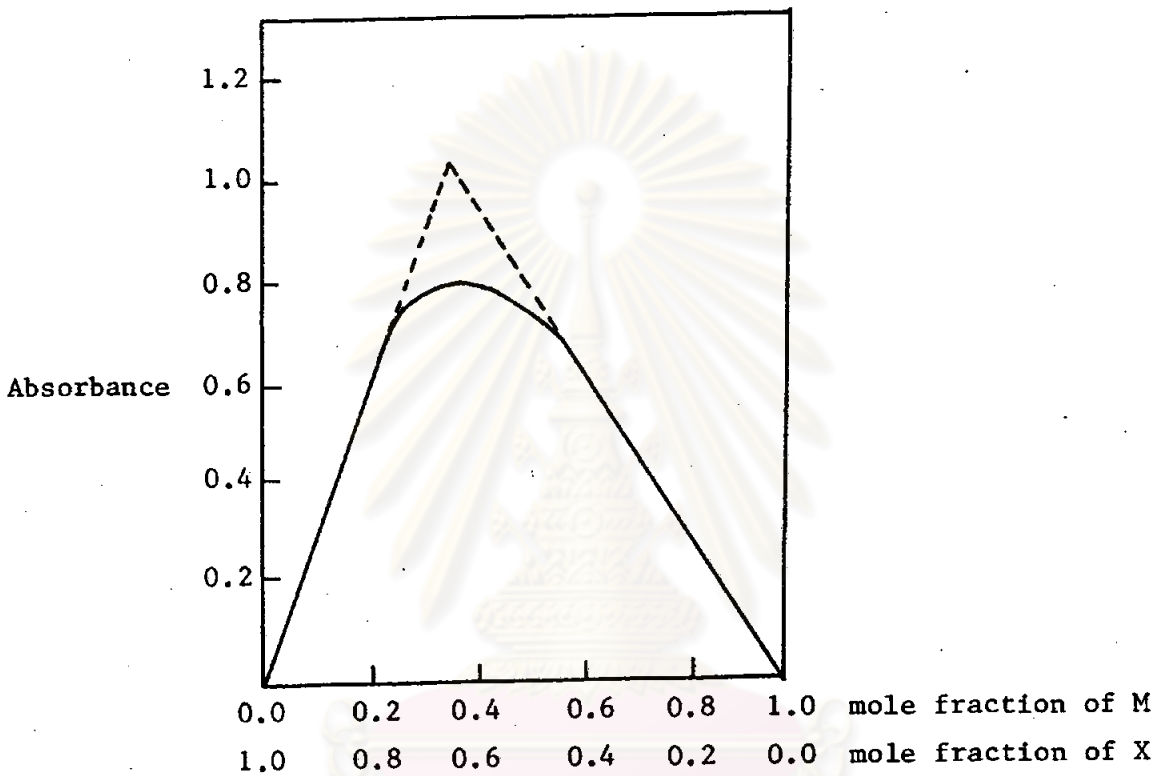


Figure 1 Continuous variation plot for the 1:2 Complex, MX_2

value of n/m is obtained. The curvature of the experimental lines shown in Figure 1 is resulted from the incompletness of the complex formation reaction because of the dissociation of the complex. The composition of the complex can be found by extrapolating the straight-line portions of the graph to intersect each other. From the dissociation of complex one can determine, under certain conditions, the stability constant of the complex.

The molar ratio method (26, 27) is similar to the method of continuous variations. The difference lies in the fact that the concentration of metal (or ligand) is held constant rather than the sum of the ligand and metal concentration. In this method a series of solutions is prepared in which the concentration of the metal ion (or ligand) is kept fixed while that of the other is varied to give a series of [ligand]/[metal] or [metal]/[ligand] ratios. The absorbance of each solution, is then measured at a wavelength where the complex ion absorbs, and a plot of absorbance against molar ratio of the reactants resembles Figure 2. The complex composition can be found by extrapolating the straight-line portions of the graph, which is to say that the point at which the lines intersect together corresponds directly to the molar ratio of the ligand to the metal ion in the complex. The curvature in Figure 2 depends on the degree of dissociation of the complex. If the dissociation of the complex is too large the molar ratio plot will become a smooth continuous curve with no straight line portions that can be extrapolated to give the combining ratio. In such cases, better results can often be secured by the slope-ratio or the continuous variation method. However, the curvature around the end point of a molar ratio plot can be turned to good advantage and used for the calculation of the dissociation constant of the complex.

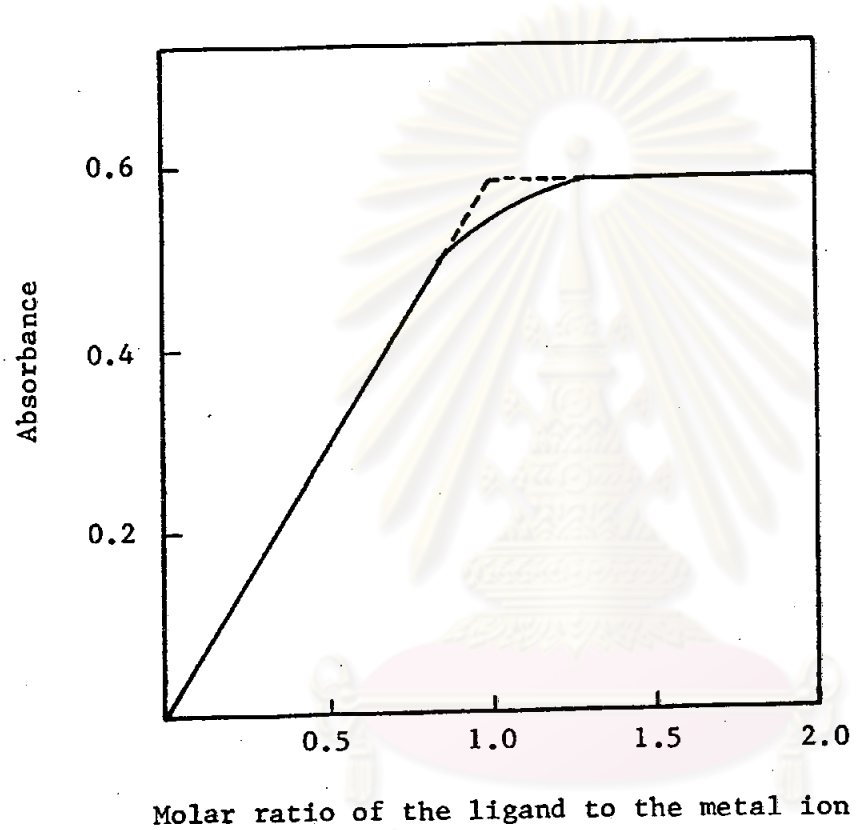


Figure 2 Molar ratio plot for the 1:1 complex, MX

Frequently, two compounds or two species of a compound existing in equilibrium exhibit overlapping absorption bands. Their absorption curves intersect at the fixed wavelength. This point of intersection will not be affected by changing the pH of the medium. Any other changes affecting the equilibrium will not affect this point either. Such point is called the isobestic point (iso-absorptive point). The existence of one or more isobestic points in a system is a good indication of chemical equilibrium between two compounds (28, 29). The complex formations between the metal ion and the dye for this case can be written as the following equilibrium (21):

$$nM^{m+} + H_xD \rightleftharpoons [M_nD]^{(mn-x)} + xH^+ \qquad (I)$$

Let; H_xD = formular of dye

C_D^o = analytical concentration of the dye, mole/liter

C_D = equilibrium concentration of the dye, mole/liter

C_M = equilibrium concentration of metal ion, mole/liter

C_x = equilibrium concentration of the metal-dye complex, mole/liter

ε_D = molar absorptivity of the dye at any given wavelength

ε_x = molar absorptivity of the complex at any given wavelength

ε = the apparent molar absorptivity of the metal-dye solution

K = equilibrium constant

From Beer's law:- $A = \varepsilon C_D^o = \varepsilon_D \cdot C_D + \varepsilon_x \cdot C_x$ (1)

$$C_D^o = C_D + C_x \qquad (2)$$

combining (1) and (2) $\frac{C_x}{C_D} = \frac{\varepsilon - \varepsilon_D}{\varepsilon_x - \varepsilon}$ (3)

$$K = \frac{C_x \cdot C_{H^+}^x}{C_M^n \cdot C_D} \qquad (4)$$

at a fix pH; C_{H^+} is constant; $K' = \frac{K}{C_{H^+}^x}$

$$\frac{C_x}{C_D} = K' \cdot C_M^n \qquad (5)$$

combining (3) and (5) $\frac{\varepsilon - \varepsilon_D}{\varepsilon_x - \varepsilon} = K' \cdot C_M^n$

$$\varepsilon = \varepsilon_x + \frac{1}{K'} \cdot \frac{\varepsilon_D - \varepsilon}{C_M^n}$$

If the metal concentration, C_M^o is kept large compared to the dye concentration, then $C_M \sim C_M^o$, so that

$$\varepsilon = \varepsilon_x + \frac{1}{K'} \cdot \frac{\varepsilon_D - \varepsilon}{(C_M^o)^n}$$

Thus, a plot of ε vs. $(\varepsilon_D - \varepsilon)/(C_M^o)^n$ should give a straight line for any system obeying equilibrium of the reaction (I). This procedure is also the molar ratio method since the dye concentration is fixed while the metal ion is varied.

The last method is the slope ratio method (26, 27). This procedure is particularly useful for weak complexes, it is applicable only when a single complex is formed, the complex must exhibit characteristic absorption difference from its progenitor, and it must obey Beer's law. The method is based on the absorbance measurement made with a solution containing the large excess of either the metal ion or the ligand to repress the dissociation of the complex. If the complex formed in the reaction

$$mM + nX \rightleftarrows M_m X_n$$

is to be identified, small amount of M is added to a solution containing a very large excess of X. The slope S_m, of the plot of absorbance VS formal concentration of M is determined which is equal to ε_c. b/m where ε_c. is the molar absorptivity of the complex, b is the cell length and m is the number of moles of metal ion in the complex. Similarly, if a small concentration of X is added to a solution containing a very large excess of M, the solpe S_x, of the plot of the absorbance against the formal concentration of X is determined which is equal to $\varepsilon_c b/n$ where n is the number of mole of ligand in the complex. The formular of complex $M_m X_n$ is then determined by the relationship:

$$\frac{S_m}{S_x} = \frac{n}{m}$$