## INTRODUCTION

Amaranth, Ponceau 4R and Erythrosine are synthetic organic red colors using as color additives in food, drugs and cosmetics for many 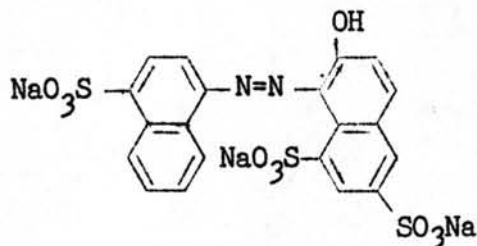years. Both Amaranth and Ponceau 4R are azo dyes but Erythrosine is an iodine substituted fluorescein. Preparations of Amaranth and Ponceau 4R are by diazotizing one mole of sodium salt of 1-amino-4-naphthionic acid and coupling the diazonium salt with one mole of sodium salts of 2-napthol-3, 6-disulfonic acid in case of Amaranth, and of 6,8-disulfonic acid in case of Ponceau 4R [1]. These two dyes are reddish brown powders which dissolve readily in water, glycol and glycerol, however, in 95% alcohol Ponceau 4R is better soluble than Amaranth [2]. Their structural formulae and their color indecies (C.I.) are shown below.
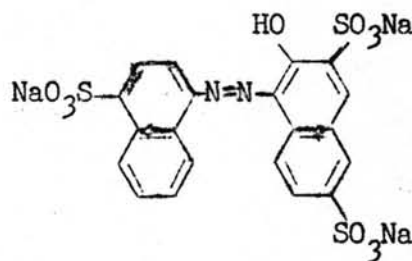

Amaranth

C.I. (1965) No. 16185

Ponceau 4R

C.I. (1956) No. 16255

Both Amaranth and Ponceau 4R are used as indicators in the oxidation titrations involving potassium bromate and potassium iodate, but they are destroyed by an excess of the oxidant[3,4]. By spectrophotometric titration, Amaranth was found to form an insoluble thorium-Amaranth compound with three moles of thorium to one mole of Amaranth; ionic bonding between Th(I) ion and the dye[5]. Amaranth reacted with Bi(III) ion providing a compound with the mole ratio of Amaranth to bismuth of 3:1 and the compound served as the basis of a sensitive colorimetric method for the determination of bismuth in alloys[6]. Amaranth formed one to one complex with Fe(III) ion in diethylamine buffer at pH 12.5 and its stability constant was found to be 3×10^6 [7]. By spectrophotometric method, complex formations between Cu(I) ions and Amaranth occurred at the mole ratios of 1:1 at pH 4.5 and 2:1 at pH higher than 4.5 and the structure of the later complex was purposed as a

salt forming Cu(II) ion and a coordinated Cu(I) ion per dye mole[8].
Ponceau 4R. formed a 2:1 complex with Fe(II) ion (metal:dye) at
pH 12.5 in diethylamine buffer and its stability constant was in
the order of 10^{11} [7]. In addition, paper chromatographic and
polarographic methods indicated that neither Amaranth nor Ponceau
4R. reacted with chromium or aluminium[9].

The structural formula and the color index (C.I.) of Erythrosine are shown below.
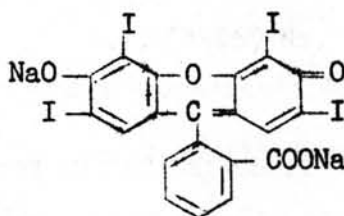
Erythrosine

C.I. (1956) No. 45430

Erythrosine is prepared by iodination of the fluorescein which is
synthesized by condensing phthalic anhydride with resorcinol in an
aquous or alcoholic solution[1]. It is a brown powder which is
soluble in water and 95% alcohol, giving solutions with slight
fluorescence[1]. It is readily soluble in glycerol and glycol and
the solutions have only fair resistance to light[1]. Erythrosine
has a fair resistance to oxidizing agent and is not reduced as
readily as the azo dye[1]. It was used under certain conditions
for colorimetric analysis of traces of most metal ions and amines

such as Li(I), Na(I), K(I), Ca(II), Sr(II), Mg (II), Ba (II), Zn (II), Pb (II), Cu (II), Mn (II), Cd (II), Co(II), Ni (II), Uo_2 (II), Fe (II), Ce (IV), Hg (II), methylamine, ethylaminediamine, piperazine, morpholine and quinine in the concentration of less than 1 or 0.1 ppm[10]. By spectrophotometric method, this dye formed 1:1 complex with paperine in chloroform solution[11]. The dye also formed a color complex with Cd(II) ion and 1, 10-phenanthroline in nitrate solution at pH 5-10, using 2 mole of 1, 10-phenanthroline and one mole of Erythrosine per atom of cadmium[12].

An announcement of January 19, 1976 from the Food and Drug Administration (F.D.A.) in U.S.A. pointed the carcinogenic property of Amaranth in Osborne-Mendel mice and the certificate of this dye for using in food, drugs and cosmetics was terminated since January 28, 1976[13]. In Thailand, the announcement of the Ministry of Public Health number 37(1977)[14] for red food dyes was to cancel the announcement of the Ministry of Public Health number 11(1972) which was the permission of Amaranth and to permit the following dyes as food additives: Pomceau 4R, Carmoisine or Azorubine and Erythrosine.

In the light of the above and a search of the literature revealed that there was a paucity of the behavior of red food dye additives in the present of metal ions. The present investigation has, therefore, been directed to study of the suitable conditions for the formations of compounds or complexes between Amaranth, Ponceau 4R or Erythrosine with metal ions such as Cd(II), Hg(II), Pb(II), Fe(II) and Fe(III) in various buffer solutions at many pH values as well as to determine the ligand numbers and stability constants of the compounds or complexes formed.

Numerous spectrophotometric techniques have been devised for identifying complexes in the solution. The method of continuous variations[15] is simple and widely used, however, it is mainly useful for solutions where only one complex is formed. In this method, the sum of the total analytical concentrations of complexing agent and metal ion is held constant and only their ratio is varied. To determine the value of n in a complex system AB_n, the absorbance at a given wavelength, e.g., at which the complex absorbed, is plotted versus the mole fraction of A or B in the mixture. A triangle curve is obtained and the point where the maximum absorbance of the curve located indicates the composition of A or B in the complex. In a weak complex system, a curvature of the graph is obtained. It is customary in such a case to extrapolate the two straight-line portions on each side to intersect each other in order to use this point of intersection as a more precise estimation for the position of maximum absorbance
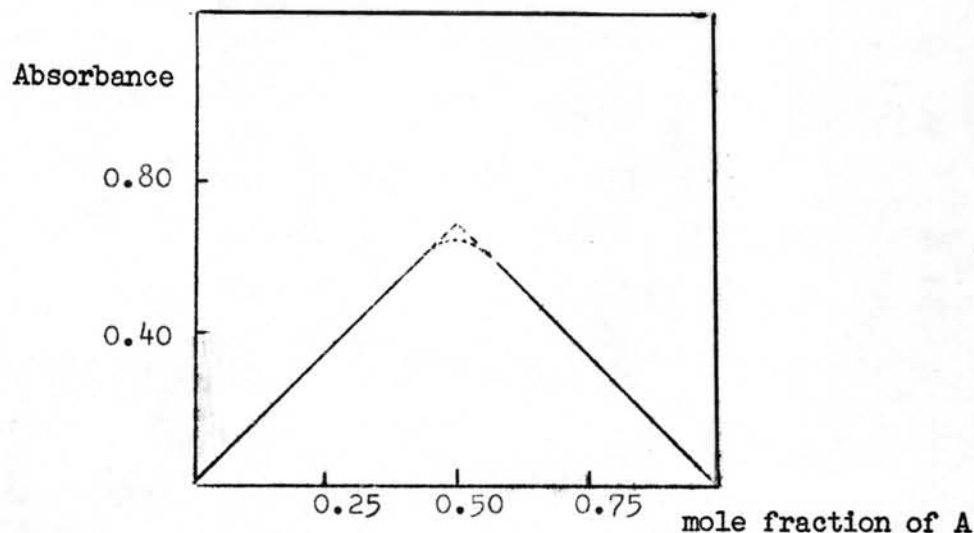

Figure 1. Continuous variation plot for the 1:1 complex, AB

(15)

The molar-ratio method is very similar to the method of
continuous variations. The difference lies in the fact that the
total analytical concentration of metal (or ligand) is held constant
rather than the sum of the ligand and metal concentrations. At a
selected wavelength, e.g. at which the complex absorbed, the curve
of the absorbance-molar ratio of ligand to metal is used for
determining the complex composition. The point where the straight
line portions are extrapolated to cross each other determines the
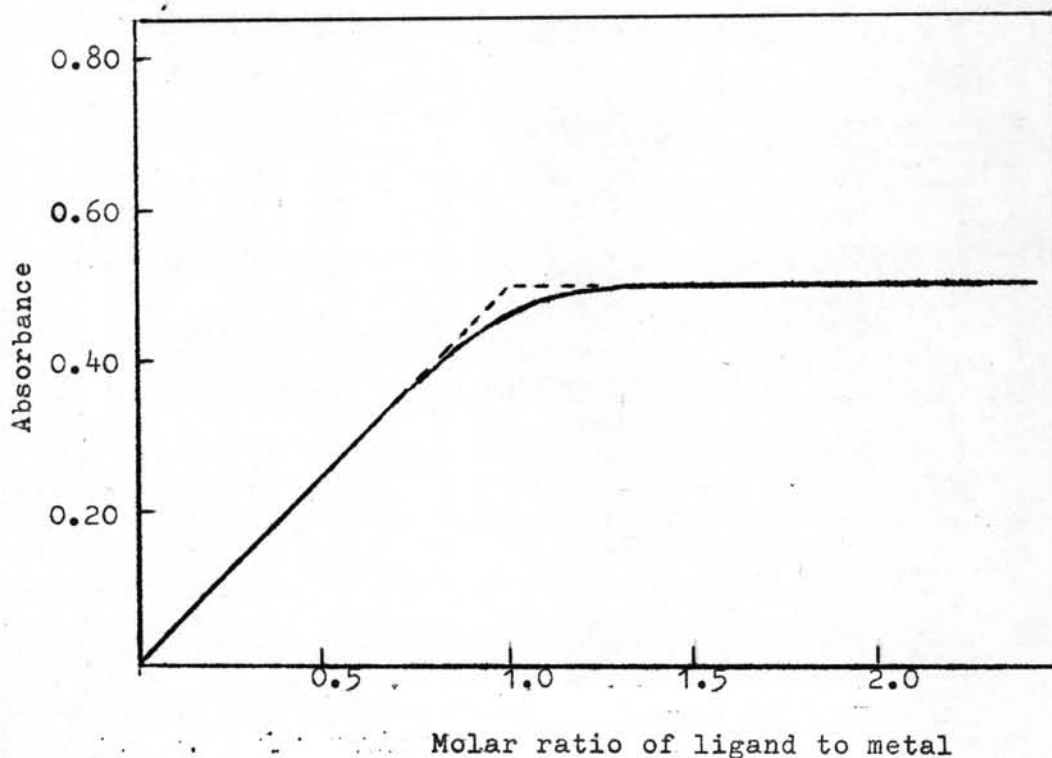molar ratio of ligand to metal (See Fig 2)

Figure 2. Molar ratio plot for the 1:1 complex, AB

The last method which is most valuable for weak complexes is the slope-ratio method. This method is [15] applicable only when one complex species is formed and Beer's law is followed. The absorbance measurements involved both the solutions containing a large excess of the metal ion and the solutions containing a large excess of the ligand. The plot of the absorbance of the complex from the solution containing a large excess of metal ion versus the total ligand concentration gives a straight line whose slope is S_x, which is equal to $\varepsilon_c \frac{b}{n}$, where ε_c is the molar absorptivity of complex, b is the cell length and n is the number of moles of ligand in the complex. The other plot of the absorbance of the complex from the solution containing a large excess of ligand vs the metal ion concentration also gives a straight line with a slope of S_m which is equal to $\varepsilon_c \frac{b}{m}$ where m is the number of moles of metal ion in the complex. The formula is then determined by $\frac{S_m}{S_x} = \frac{n}{m}$ for the complex $A_m B_n$.

Owing to the precipitation of the compounds formed between dyes and metal ions studied in the solutions and no absorption of compounds in the visible and ultraviolet regions was detected, the molar ratio method was selected for determining compound compositions as well as the stability constants throughout this thesis. The absorbances of dyes were measured at the wavelengths where the dyes absorbed in the visible and ultraviolet regions by spectrophotometric method and the absorbances of metal ions were measured at the wavelengths where the metal ions absorbed by

atomic absorption spectrophotometric method.