

CHAPTER II

PRINCIPLE AND THEORY

2.1 Visible Spectrometry

Methods based on the absorption of radiation are powerful and useful tools for analytical chemists. In the visible region, it becomes apparent that virtually every element and many ions and molecules are either colored, can react with a colored substance, or can react to form a colored substance. Each of these situations presents the possibility of a quantitative analytical determination. Spectrometric method is widely used for the quantitative determination of many trace substances, especially inorganic elements.

The basic principle of quantitative absorption spectroscopy lies in comparing the extent of absorption of a sample solution with that of a set of standards at a selected wavelength. When measurements of absorbances are carried out with spectrometers, the chosen wavelength should be appropriate to the spectral range concerned. In general, the maximum absorption-wavelength is chosen for maximum sensitivity. This chosen wavelength should not be in the region where the absorbance changes so rapidly with change in wavelength. The calibration curve obtained by the standard procedure usually becomes linear in a range of concentration of colored complex. The molar absorptivity can be calculated from the slope of the curve. Many effects of experimental variables involving in the formation of absorbing compound are:

a) pH. Since pH plays a very important role in complex formation, proper adjustment of pH or the use of a buffer often eliminates certain interfering reactions. The selectivity for certain metals is much improved in highly acidic media.

b) Reagent concentration. The amount of reagent required is dictated by the composition of the absorbing complex formed. An optimum concentration

of the reagents should be determined, since either not enough reagents or too much reagent can cause deviation from Beer's law.

c) Time. Formation of absorbing complex may be slow, in some cases requiring several minutes or a few hours for full color development after addition of the reagents.

d) Order of mixing reagents. Frequently it is important to add the reagents in a specified sequence, otherwise full color development will not be possible or interfering reactions may occur.

e) Temperature. The optimum temperature should be established in the procedure. Certain reactions require elevated temperature to decrease the time necessary for complete color development.

f) Stability. If the absorbing complex formed is not very stable, the absorbance measurement should be made as soon as possible. If the absorbing complex is photosensitive, precautions should be taken in order to avoid its photo-decomposition. Certain reagents may sometimes be added to help stabilize the absorbing complex.

g) Masking. The term masking refers to the addition of a complexing agent to form a metal complex of such stability that, in this case, color-forming reactions with another reagent do not occur to any appreciable extent.

h) Organic solvent. Many organic reagents or complexes are only slightly soluble in water. In such cases, it is necessary to add a water-miscible organic solvent to avoid precipitation or to aid color-development. In other cases, solvent extraction might be employed, for example, to separate the colored compound from excess reagent or from interfering substances.

i) Salt concentration. High concentrations of electrolyte may be form the ion-association complexes that cause a shift in the maximum absorption. This is a type of masking effect and usually causes a decrease in the absorption.

The spectrometric method is particularly valuable for studying complexes of low stability. Consider the formation of a complex M_mL_n , where M is a metal ion and L is a ligand:



The molar ratio of the two compounds of a complex is important. In a quantitative determination, an excess of ligand should be added in order to force the equilibrium toward completion.

The method of continuous variations (Job Method) is one of the most widely and numerous techniques for identifying complexes in solution by spectrometric measurements, first described by Job (22) and the later extended in applicability to include successive complex formation by Vosburgh and Cooper (23). The molar ratios may also be varied by changing the concentrations of both components while the total number of moles of both components are kept constant. The absorbance of each solution is measured at a suitable wavelength and corrected for any absorbance of the mixture if no reaction has occurred. The corrected absorbance is then plotted against the volume fraction (which is equal to the mole fraction) of one of the reactants (V_M/V_M+V_L). A maximum (or a minimum if the complex absorbs less than the reactants) occurs at a volume ratio $V_M:V_L$ corresponding to the combining ratio of cation and ligand in the complex. To determine whether more than one complex form between the reactants, the method is ordinarily repeated using different reactant concentrations and measurements at several wavelengths.

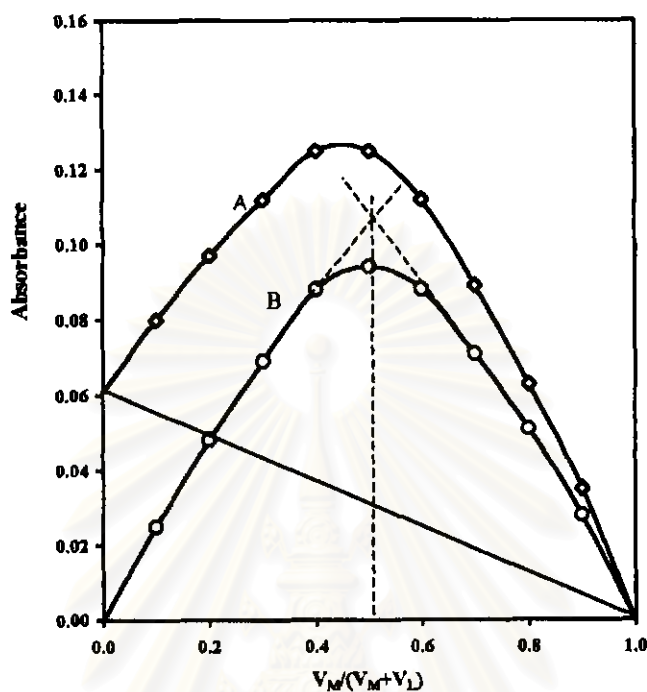


Figure 2.1 Continuous variation method

A : experimental absorbance value (not corrected)

B : absorbance after graphical subtraction

of the absorbance of the reagent (corrected)

Yoe and Jones (24) introduced the mole ratio method. They found that for a very stable complex, the concentration of one component is fixed and that of the other varied to give a series of concentration ratios $\left(\left[\frac{M}{L}\right] \text{ or } \left[\frac{L}{M}\right]\right)$. The absorbances of these solutions, measured at absorption maximum for the complex M_mL_n , increase linearly up to the molar ratio of the complex, at which virtually the whole amount of both components is complexed (assuming little dissociation). Further addition of component L cannot increase the absorbance, and the line becomes horizontal, or shows a break if component L absorbs at the same wavelength (Figure 2.2). In rare cases, an excess component L may cause a

decrease in absorbance owing to the stepwise formation of higher-order complexes that have smaller ϵ values at this wavelength.

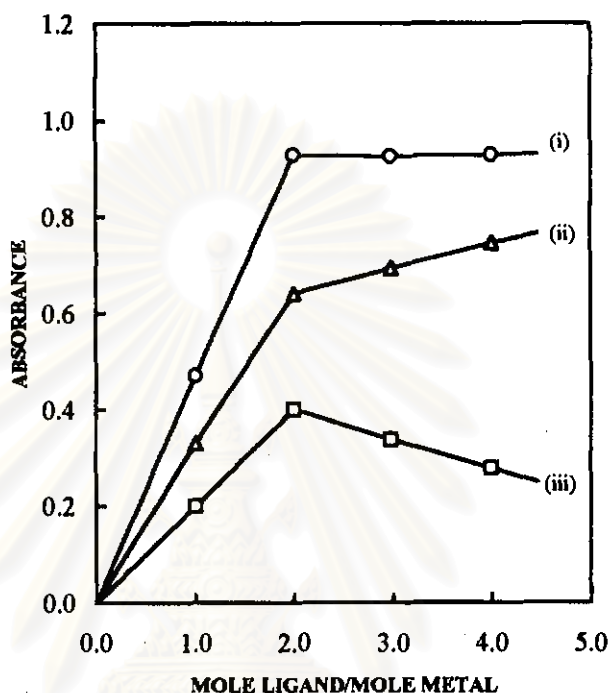


Figure 2.2 Molar-ratio method, showing different curves

- (i) Component L does not absorb at the wavelength of maximum absorption for the complex.
- (ii) Component L absorbs slightly at the wavelength of maximum absorption for the complex.
- (iii) An excess of component L causes a decrease in absorbance of the complex.

Harvey and Manning proposed the slope ratio method in 1950 (25). The applicability of this method is suitable for systems in which a single complex is formed. When one component is kept constant in a sufficient excess amount for complete complex formation while the other one is varied in many amounts so that the concentrations of the complex are proportional to those varying amounts.

For the reaction: $mM + nL \longrightarrow M_mL_n$

and
$$[M_mL_n] = \frac{[M]}{m} = \frac{[L]}{n}$$

Absorbances of each solution were recorded under the Beer's law condition,

$$A = \varepsilon b [M_mL_n]$$

When the absorbances of the combined complex is plotted against the varied amount of the component (M or L), the slope of the straight line is:

$$\text{The slope (when varying M)} = \frac{\varepsilon b}{m}$$

$$\text{And the slope (when varying L)} = \frac{\varepsilon b}{n}$$

The ratio of n to m in the complex M_mL_n may be determined by taking the ratio of the two slopes.

$$\frac{\text{The slope (when varying M)}}{\text{The slope (when varying L)}} = \frac{n}{m}$$

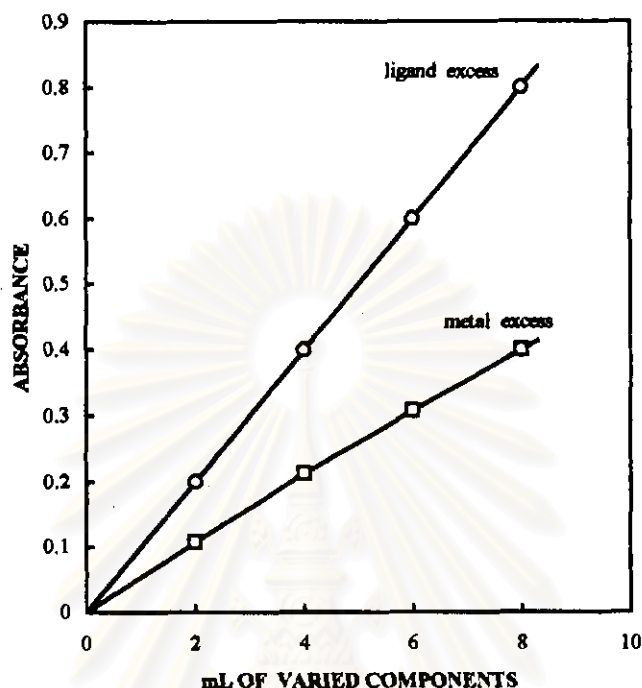


Figure 2.3 Slope-ratio method for the complex ML_2

2.2 Metallochromic Reagents

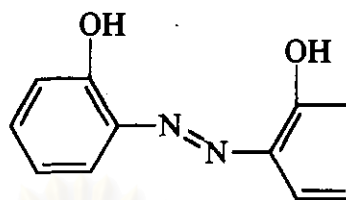
Metallochromic reagents are those that generally give reaction with metal ions which may be classified into various types as, metal indicators and redox indicators (26). Metal indicators are widely used in visible spectrometry.

Metal indicators are substances that undergo a marked color change when the concentration of free metal ion in a solution changes. These indicators usually have properties typical of acid-base indicators. The anion of the indicator is a base capable of donating one or more electron pairs and can hence function as a ligand. This base can react with a hydrogen ion or a metal ion (or with other electrophilic species). Often the color of the metal complex is about the same as that of the free indicator anion and the color change occurs when one or more hydrogen ions are replaced by a metal ion. It is possible to classify the metal

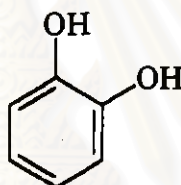
indicators broadly according to their mode or mechanism of reaction into four types: metallochromic indicators, diverse metal-sensitive and metal-specific indicators, fluorescence indicators, and turbidity indicators.

Körbl and Pribil(26) introduced the term metallochromic indicator to designate a metal indicator that has acid-base activity and that functions in a complexometric titration by reacting with the metal ion to form a chelate substantially soluble in the titration medium. Metallochromic indicators are substances which react with metal ions to form colored products having the properties necessary in visual indicators. Among these indicators, metallochromic indicators are the most significant and relevant to visible spectrometry. The color-species is usually due to the formation of a chelate complex, but there are a few instances where inorganic anions such as iodide or thiocyanate form simple coordination complexes of sufficient absorption in the visible range of the spectrum. Metallochromic indicators can be classified on the basis of their structures to be: azo dyes, phthaleins, sulfophthaleins, triphenylmethane dyes, anthraquinone dyes, and phenolic substances. In the structure of metallochromic indicators one or more ligand groups must be joined directly to a resonance system (such indicators are usually so-called mordant or chrome dyes). The change in the spacing of low-lying π electron energy levels resulting from the chelation causes a shift in the wavelength of the absorption bands and hence a change in color. The extent of the shift may depend to some extent on the metal ion involved. For this reason, metallochromic indicators function in this way as acid-base indicators, pH has a profound effect on their mode of action and range of application. Sharper end points are usually obtained when an extremely small concentration of a metallochromic indicator is employed in a complexometric titration. For this reason the indicator in either its metallized or its free form need only be added in concentration of 10^{-6} - 10^{-3} M (27) to give a color change that is still clearly detectable. The indicators are arranged principally according to their structures. Naphthylazo compounds (28) constitute a large group, most of which have hydroxyl groups substituted adjacent to the azo bond, while a few also depend on

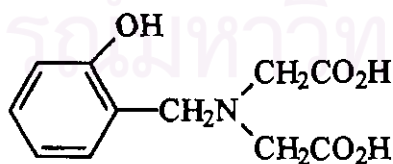
carboxyl or arsonic acid groups. These azometallochromes depend on reaction with the azo bond as well as with the hydroxy group.



The second group comprises phenolic compounds ranging in complexity from catechol to hydroxy-substituted triphenylmethane compounds. All these molecules depend solely on the presence of vicinal hydroxyl groups for their activity. This differentiates them from the hydroxylazo indicators of the first group and those described below.



The third group also depends on the presence of a hydroxy group for its metallochromic response, but the principal reactive center in the molecule is an "ortho" substituted aminomethyldicarboxymethyl group. Attachment of a metal ion at this center in the molecule results in reaction with the adjacent phenolic group with consequent changes in the chromophore of the organic reagent.



The fourth group deals with indicator substances not catered for in the above sub-classification of direct metallochromic indicators.

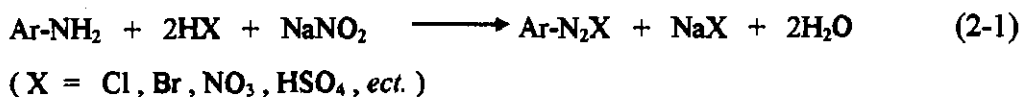
2.3 General Method for Preparation of Azo Dyes

The azo dyes form the largest group of all the synthetic colorants and play a prominent part in almost every type of application. The chromophoric system consists essentially of the azo group, $-N=N-$, in association with one or more aromatic systems. There may be one or more than one azo group present in the dye molecule giving monoazo, disazo, trisazo, tetrakisazo, and polyazo dyes according to whether there are one, two, three, four or more azo groups present in the dye molecule. The azo compounds of simpler structure are usually yellow to red in color; those of more complex constitution, violet to blue and black. Amongst analogously constituted compounds with the same number of azo groups, those containing naphthalene nuclei have a darker color than those containing benzene nuclei (29).

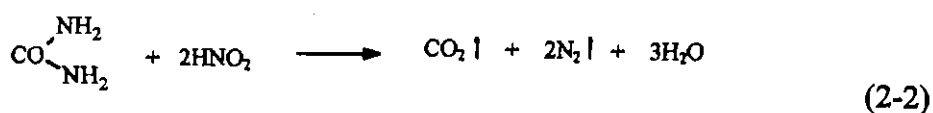
Azo dyes are made almost exclusively by the diazotization of a primary aromatic amine to give a diazo or diazonium salt, a reaction of fundamental importance to the dye discovered by Griess in 1862 (30). The diazo compound is then coupled with a second substance, usually a phenol, an enolizable ketone or an aromatic amine, an azo compound being formed. The participating molecules are known as diazo component and coupling component, respectively.

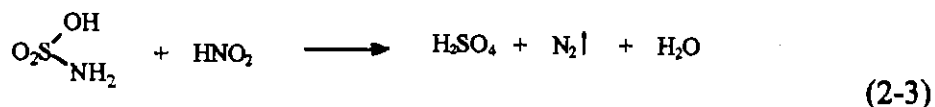
2.3.1 The Diazotization (31)

The diazotization of an aromatic primary amine is the first of the two reaction steps by which practically all azo dyes are produced. Normally an aqueous solution of the amine is converted into the diazonium ion at a temperature of about $0-5^{\circ}\text{C}$ by the action of sodium nitrite in the presence of mineral acid. The use of at least two (usually 2.5-3.0) equivalents of mineral acid in accordance with Equation (2.1) is essential for smooth reaction, the overall equation for this process is shown as follows.



A higher proportion of hydrogen ions than that indicated in Equation (2-1) is used in diazotizing weakly basic amines, since this results in the equilibria in the nitrous acid solution shifting towards the production of the more electrophilic particles. Thus *p*-nitroaniline is dissolved in hot 5-10 mol/L HCl and the solution is either cooled rapidly or poured on ice. Nitroanilium chloride precipitates before hydrolysis to the base occurs. Smooth diazotization results on the immediate addition of nitrite. Fierz and Blangey described the detail and provided a useful summary of most of the essential variations possible in diazotization methods. With extremely weakly basic amines diazotization can be performed in sulfuric acid (90-96%). The diazotizing agent is nitrosyl sulfuric acid (HSO₄NO), which can be readily produced. Nitrosyl sulfuric acid is astonishingly stable in highly concentrated sulfuric acid (>85% up to 50°C). Diazotizations of 2,4-dinitro-6-halogenoanilines are, however, dangerous in higher concentrations of nitrosyl sulfuric acid and may explode. When nitrite is added to the sulfuric acid solution of the amine an excess must be avoided. Excess nitrite would exert an unfavorable influence on the stability of diazonium ions, apart from which it could form nitroso compounds with naphthols, secondary and tertiary amines, and diazo compounds in the subsequent coupling reaction with primary amines. Any excess of nitrite can be readily detected with starch iodide paper (instantaneous bluing) and can be destroyed with urea. The reaction yields gaseous products, as shown by Equation (2-2), but as already mentioned by Zollinger this reaction is slow. The recommendation has to replace urea by sulfamic acid, (32) which reacts according to Equation (2-3).

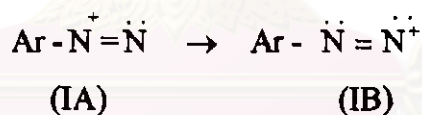




2.3.2 The Coupling Reaction

Diazo coupling can be regarded as an electrophilic substitution by a diazonium cation. It follows therefore that the position where coupling will occur are those at which there is increased electron density, generally at carbon atom in aromatic systems or heterocyclic systems aromatic in character, and, in some cases, at activated carbon atoms occurring in an aliphatic chain as in acetoacetanilide.

The diazonium cation can be represented by two resonance structures (IA) and (IB), of which (IA) makes the main contribution on account of its stability, but it may be the unstable structure (IB)

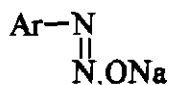


having a nitrogen atom with a sextet of electron, which enables the attachment to the anionoid carbon atom in the phenoxide ion to take place (33) with the formation of the azo linkage $\text{Ar} - \text{N} = \text{N} - \text{Ar}'$.

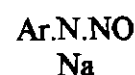
Iso-diazotates or nitrosamines are produced when diazo solution are poured into warm dilute caustic soda, a stable sodium compound being precipitate. The compounds produced in transforming the diazo compound to stable sodium salt are considered to be :



Syn- or normal diazotate
(unstable, couples easily)



Anti or iso-diazotate
(unstable, couples with
difficulty)



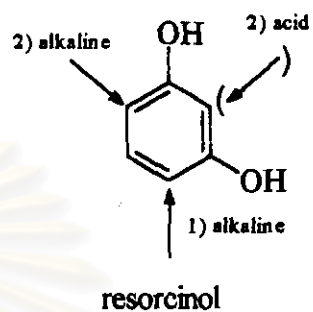
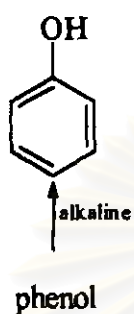
Nitrosamine
(stable)

The anti-diazotate or nitrosamines are converted into the reactive syn-diazotate or diazonium compound by treatment with acids.

The coupling position (attachment of an azo group to a carbon atom) in a coupling reaction is governed by a general rule, which follows from the mechanism of the reaction. The diazonium group attacks a position which has been activated as a site of high electron density. Coupling therefore take place in the *o*- or *p*- position to the directing hydroxyl of amino group in the second component. If both of these positions are occupied, no coupling will take place, or one of the substituents will be displaced. Coupling never takes place in the *m*-position to the directing group. A few well-known coupling components are shown in Figure 2.4 together with the favored position of coupling under the condition normally used (30).

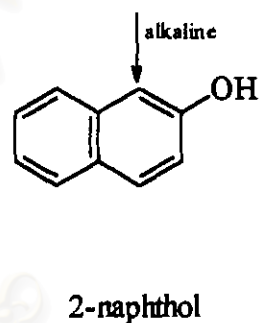
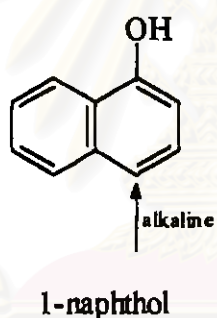
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Hydroxy derivatives



Naphthalene derivatives

1. Naphthols



2. Naphthylamines

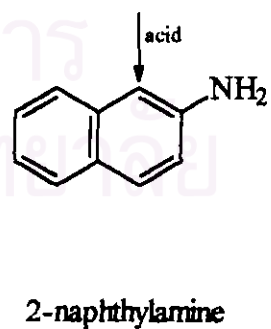
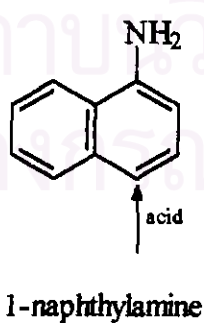
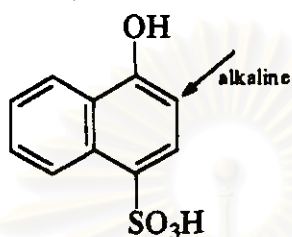


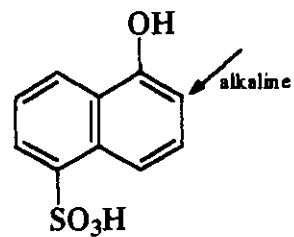
Figure 2.4 The well known coupling components

Naphthalene derivatives

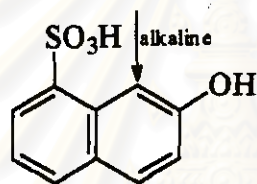
3. Naphtholmonosulfonic acids



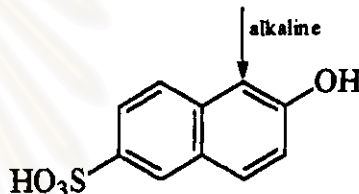
Nevil and Winther's acid



1-naphthol-5-sulfonic acid

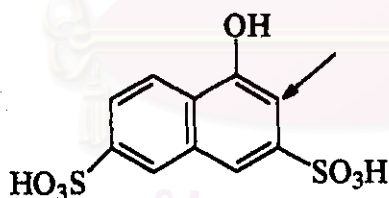


Crocein acid

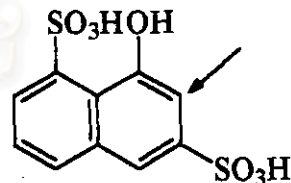


Schaffer's acid

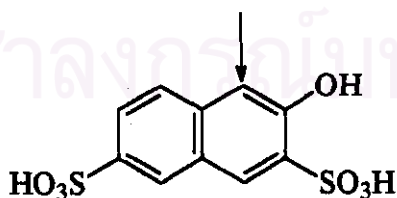
4. Naphtholdisulfonic acids



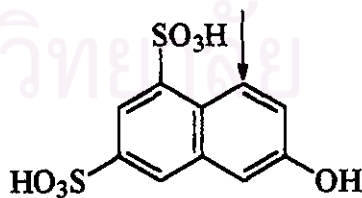
1-naphthol-3,6-disulfonic acid



Epsilon acid



R acid

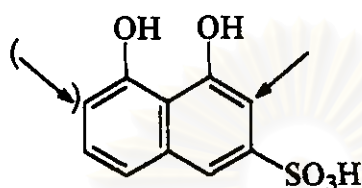


G acid

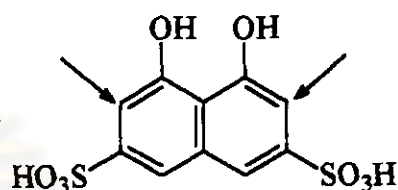
Figure 2.4 (cont.)

Naphthalene derivatives

5. Dihydroxynaphthalenesulfonic acids

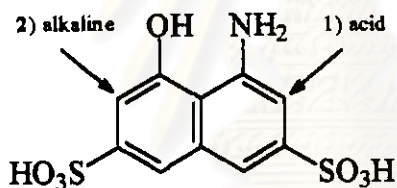


4,5-dihydroxynaphthalene-
1-sulfonic acid

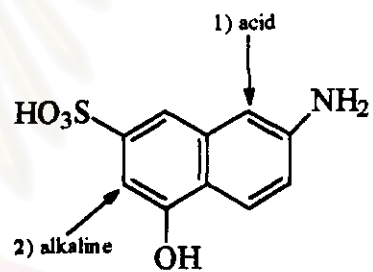


Chromotropic acid

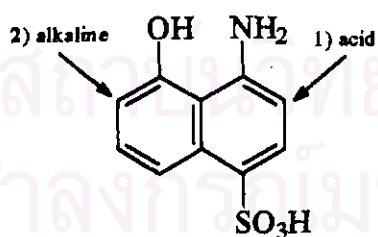
6. Naphthylaminesulfonic acids



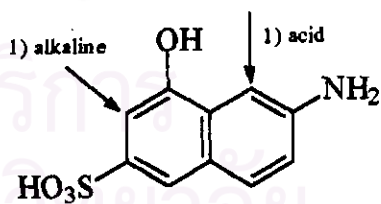
H acid



J acid



S acid



Gamma acid

Figure 2.4 (cont.)