(A) Introduction

One of the most important fields of analytical chemistry is the chemistry of chelate compounds. Many new analytical methods use chelating agents for titrimetric, gravimetric and colorimetric measurements. New types of chelates and chelating agents are constantly under investigations for possible analytical and industrial applications. The growing importance of the use of metal chelates in analytical chemistry may be realised by the ever increasing number of publications on this subject.

The term 'chelate' was proposed by Morgan(1) and Drew to designate the cyclic structures which arise from the combination of metallic ions with inorganic or organic molecules.

For the formation of metal chelate of an organic compound, the compound must have two or more atoms, usually oxygen or nitrogen, capable of coordination with a metal ion; that is, it must be a base having a pair of unshared electrons available for coordination. These coordinating atoms are so arranged that rings of five or six atoms, including the metal, will be formed in the process(2).
Coloured chelates are used for qualitative or quantitative determination of the elements. For qualitative detection, generally the spot test technique is followed whereas the colorimetric procedures based on the formation of coloured chelates, are utilised for the quantitative determination of elements.

(B) Colorimetric Analysis

1. Desirable Properties of Colour Systems

According to Moss(3), an ideal colour system for colorimetric determination would have the following characteristics:

(i) Stability for sufficient time to permit accurate measurement.
(ii) Intense colour.
(iii) Freedom from minor variations in pH and temperature.
(iv) Solubility of the coloured product in the solvent used.
(v) Agreement of the coloured system to Beer's law.

2. Desirable Properties of Colour Forming Reactions

Later, Mellon(4) suggests that an ideal colour forming reaction should meet the following requirements:
(i) Be free of interference due to other elements.
(ii) Use a colourless reagent.
(iii) Develop the colour rapidly.
(iv) React stoichiometrically.
(v) Be independent of any particular sequence if two or more reagents are used.
(vi) Yield a system having a maximum light absorption in the most desirable wavelength region of the spectrum.
(vii) Make use of a reaction of known mechanism.

Mellon, further adds that this list of requirements is not met by any reaction reported thus far and few reactants approach the ideal.

3. The Ideal Reagent

Moss(6) has suggested the ideal colorimetric reagent would have the following properties:

(i) Stability in solution.
(ii) Rapid colour development.
(iii) Stoichiometric reaction with the desired constituent.
(iv) Sufficient transparency in the spectral region involved in the measurement.
(v) Selectivity or specificity, so that the colour developed is a measure of the desired constituent only.
(vi) Freedom from interference by other constituents which might convert the reagent or desired constituent to an unreactive form, or complex formation leading to the incomplete colour development.

(vii) Capacity to function in most of the solvents.

In practice, very few reagents can fulfill all these exacting conditions.

4. Procedure for the Investigation of a New Reagent

Yoe(6) reports that organic compounds frequently react with inorganic ions to give intensely coloured substances. If only a comparatively few ions produce colour reactions with a given compound and the colour forms fairly rapidly, the compound gives promise of being a useful colorimetric reagent. It will then be necessary to carry an extensive critical investigation of the new reagent and its colour reactions. According to Yoe(7) the following steps should be followed for the investigation of a new organic colorimetric reagent:

(a) The Reagent:

(1) Suitable solvent.

(ii) Solubility in the solvent.

(iii) Stability in solution.
(b) The Colour Reaction:

(i) The effect of pH on the nature and the intensity of the colour.

(ii) The stability of the coloured complex to light.

(iii) The order of adding reagents.

(iv) The rate of colour formation.

(v) The nature of colour reaction (mole ratio of metallic ion to organic ligand).

(vi) Agreement with Beer's Law.

(vii) The sensitivity of colour reaction.

(viii) The optimum concentration range.

(ix) The effect of temperature on the colour reaction over the range 15°C to 35°C.

(x) The effect of foreign ions upon the coloured complex.

(xi) The determination of the optimum conditions for analytical use of the colour reaction.

(xii) The application of reagent to the analysis of a variety of standard samples.

Ye further remarked that much experimental work remains to be done to establish a better knowledge of the relation between the molecular structure of organic compounds and their colour reactions with inorganic ions.
5. Study of the Composition of the Complex

The methods mentioned below were used to find the composition of the complex:

(i) Job's method of continuous variation\(^{(8)}\).

(ii) Yoe and Jones mole ratio method\(^{(9)}\).

(iii) Harvey and Manning's slope ratio method\(^{(10)}\).

(i) Job's Method of Continuous Variation:

This method is probably the oldest and most extensively studied. Even though the basic principle of the method has been independently described by several early workers\(^{(11)}\), it is usually ascribed to Job, as his presentation is of a greater generality, and particularly convenient for use with spectrophotometric measurements. When equimolar solutions of metal ion \(M\) and ligand \(A\) are mixed in different proportions, keeping the total volume constant, the concentration of the complex \(MA_n\), formed according to the reaction,

\[
M + nA \rightleftharpoons MA_n
\]

will be maximum when the reactants are present in the ratio of \(1:n\) in the solution. Usually the property of absorption of visible radiations is used as a measure of the concentration of a coloured complex. Other properties like molar refractivity or molar conductivity are also employed to estimate the amount of complex formed\(^{(12)}\).
A plot of absorbance against the concentration of A will show a maximum if $M_A^n$ is more strongly absorbing than $M$ or $A$, or will show a minimum if $M_A^n$ is less strongly absorbing than $M$ or $A$, at a value such that represents the mole ratio of $M$ and $A$ required to form the complex.

The original method suggested by Job is not applicable when two or more complexes are formed simultaneously in the solution. Vosburgh(13) and Cooper first mentioned the Job's treatment to suit some special cases in which two or three fairly strong complexes are formed between a metal ion and a ligand exhibiting spectra significantly different from one another. Selection of a suitable wavelength permits the determination of the composition of various complexes. Vosburgh and Cooper pointed out that when only a single compound is formed, the results are independent of the wavelength of the light used. After determining $n$ by the continuous variation method in equimolecular solutions, the value of $n$ can also be checked up by the use of non-equimolecular solutions.

The principal drawback of this method is the fact that when the value of $n$ is greater than 4, it cannot be determined with certainty from the plot of absorbance against mole fraction of the reactant. For complexes of mole ratios of 4:1, 5:1, and 6:1, the abscissa values in the plot for the method of continuous variation are
0.800, 0.833 and 0.857 respectively. As a result, an error of 2% in the preparation of the solutions or in the estimation of the maximum is sufficient to produce a unit change in the observed mole ratio (14). Rosasoti and Rosasoti (15) suggest that the method of continuous variation should be restricted to these systems in which there is no evidence for the formation of complexes higher than MA.

(ii) Yoe and Jones Mole Ratio Method:
The mole ratio method of deducing the composition of complexes in solution from spectrophotometric data was introduced by Yoe and Jones (loc. cit.). This method is also based upon reactions

\[ M + nA \rightleftharpoons MA_n \]

If the complex is very slightly dissociated, a plot of absorbance against the mole ratio of component A/M, with the concentration of M held constant, rises steeply from the origin as a straight line for the mole ratios below that corresponding to the complex formed and then breaks sharply to a constant absorbance at the molar ratio of A/M in the complex. Alternatively, the concentration of A may be held constant and that of M varied, the break in the curve then indicating the ratio M/A. If the complex is appreciably dissociated, the mole ratio plot shows no sharp break, but a gradual change of the slope, approaching asymptotically a constant absorbance at high mole ratios.
The breaks in the curves can often be sharpened by working at high ionic concentrations.

The mole ratio method is especially suitable for determining the composition of high ratio complexes. It provides more positive indication for the absence of any spectrophotometrically distinguishable complexes other than those proved to be present(16).

(iii) Harvey and Manning's Slope Ratio Method:
This method is a modification of the method of continuous variation, in which the ends of the curve are used, where there is a large excess of one or the other of the reactants. In the reaction

\[ mA + nB \rightleftharpoons A_mB^n \]

if the concentration of B is constant and in sufficient excess to make dissociation negligible, the equilibrium concentration of the complex \( A_mB^n \) will be essentially proportional to the analytical concentration of A added to the reaction; so

\[ (A_mB^n) = C \frac{C_A}{m} \]  \hspace{1cm} (1)

where the brackets refer to equilibrium concentration and C to the analytical or total concentration.
From Beer's Law there is the relation

$$E = \varepsilon \, d (A_m B_n)$$  \hspace{1cm} (ii)

where $E$ is the measured extinction, and the molecular extinction coefficient and $d$ the thickness of the cell in cm. Substituting the value of $(A_m B_n)$ from (i) into (ii)

$$E = \varepsilon \, d C_m / m$$  \hspace{1cm} (iii)

$E$ is plotted against different analytical concentrations of $A$, keeping the concentration of $B$ constant and in excess. Over the straight line portion of the curve, equation (iii), is valid and this straight line will have a slope given by

$$\text{Slope}_1 = \varepsilon \, d / m$$  \hspace{1cm} (iv)

Similarly, if $A$ is the component in constant excess and the concentration of $B$ is varied

$$(A_m B_n) = C_B / n$$  \hspace{1cm} (v)

and if $E$ is plotted against $C_B$, the slope of the straight line portion of the curve will be

$$\text{Slope}_2 = \varepsilon \, d / n$$  \hspace{1cm} (vi)

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

$$\text{Slope}_1 / \text{Slope}_2 = n / m$$  \hspace{1cm} (vii)
This method, in common with other spectrophotometric methods, serves only to establish the ratio of colour forming radicals to the metal ion.

6. Evaluation of Stability Constants:

The stability constant of the complexes formed were calculated from the results obtained by

(i) the mole ratio method.

(ii) the method of continuous variation.

(i) The Mole Ratio Method:

If the dissociation of the complex be represented by

\[ M^n_{m} = M^m + nF^0 \]

\[ c \quad o \quad o \quad \text{initial concentration} \]

\[ c(1-\alpha) - m\alpha c \quad n\alpha c \quad \text{equilibrium concentrations} \]

the dissociation constant \( k \) may be written as

\[ k = \frac{(n(1-c)(n\alpha c))}{c(1-\alpha)} \quad (1) \]

where, \( c \) = concentration

\( \alpha \) = the degree of dissociation of the complex, and

\( m \) and \( n \) are integral numbers.

The values of \( m \) and \( n \) for the complex having been determined, \( \alpha \) may be calculated by the relation

\[ \alpha = \frac{E_m - E_s}{E_m} \quad (2) \]
where $\alpha$ is the degree of dissociation, $E_m$ is the maximum absorbance and $E_s$ is the absorbance at the stoichiometric ratio.

The stability constant is the reciprocal of the dissociation constant and is obtained by the relation,

$$K = \frac{c(1-\alpha)}{(m \alpha c)(n \alpha c)}$$

(ii) The Method of Continuous Variations:

From the results obtained in this method, the stability constant $k$ can be determined with the help of relation (1). The degree of dissociation $\alpha$ is calculated by relation (2), where $E_m$ is the theoretical absorbance calculated from the molecular extinction coefficient and $E_s$ is the observed absorbance.

7. Sensitivity of the Colour Reaction and the Optimum Concentration Range

The sensitivity of the coloured complex can be observed by preparing a series of solutions in which the concentrations of nickel ions is varied. A known excess of the reagent is added and the pH values adjusted to the optimum.

The sensitivity was always determined on Sandell scale (17), i.e., the lowest concentration of nickel ions, which gave a difference of 0.2% in transmittances in the blank and sample solutions. However, as the spectrophotometric error is at a minimum in the absorbance range of 0.2 to 0.7, the range of concentrations of nickel ions which could give
this range of absorbance with the help of a particular reagent, was taken as the optimum concentration range.
REFERENCES


5. As per reference 3, page 21.


17. idem, ibid., page 108.
ANALYTICAL APPLICATION OF INVESTIGATED THIOSEMICARBAZONES

Hovorka(1,3) used salicyldenethiosemicarbazone as a reagent for the gravimetric determination of cadmium, chloride, fluoride, tartarate and citrate interfere in the determination.

Guha Sircar(2) carried out preliminary investigation on thiosemicarbazones of salicylaldehyde and vanillin as reagents for silver, mercury and copper. Valacil(4) studied the extraction of nickel-salicyldenethiosemicarbazone with a mixture of butylecetate and butylphosphate. The extraction depends on the pH of aqueous media.

Valacil(5,7) studied the extraction of manganese-salicyldenethiosemicarbazone complex in benzene-butylphosphate mixture and applied the process to the photometric determination of manganese. Beer's Law is obeyed in the range 0.16 to 6 μg/ml and Sandell sensitivity is 0.014 μg/cm², the absorbance is not affected by common masking agents such as potassium thiocyanate, sodiumpotassiumtartrate and sodiumcitrate.

Isagi(6) developed a process for the spectrophotometric determination of copper with salicyldenethiosemicarbazone. The optimum pH is 2.5-3.5 and maximum absorbance is obtained at 630 μm, ten-fold excess of cobalt interfere.
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