CHAPTER II

THEORY

2.1 Isotope Dilution Technique

2.1.1 Background. Isotope Dilution Technique was introduced by Hevesy and Hofer in 1934 (64), but it was not until 1940 that the technique was revived by Rittenberg and Foster (65). Subsequent to 1940 the usefulness of isotope dilution methods has been reported frequently and is now accepted as an indispensable technique for performing various analyses which would otherwise be extremely tedious or impossible.

2.1.2 Basic Concept of Isotope Dilution. Determination of an inactive element by the isotope dilution method is based on adding its radioactive isotope to the test sample. Under such conditions the initial specific activity of the radioisotope changes as a function of the amount of the element present, and this permits determination of its content.

There are three general types of isotope dilution methods, that is, direct, inverse and double isotope dilution. These methods are based on the same fundamental principles but they differ both in technique and procedure and are applied under different circumstances.

2.1.2.1. Direct Isotope Dilution. This is used to determine an inactive compound by dilution with an active compound by adding a known amount of an active isotopically labelled compound to an unknown mixture containing the same compound.

2.1.2.2. Inverse Isotope Dilution. This is used to determine radioactive compound by dilution with inactive compound and the technique is reverse of direct isotope dilution.

2.1.2.3. Double Isotope Dilution. This is a procedure which does not require a knowledge of the specific activity. It is used to eliminate side reactions of the reagent by adding different quantity of carrier into equal quantity of radioisotope and sample. From the activity of equal quantity of working solution, the element can be determined.

Both direct and inverse isotope dilution are based on determination of the change in specific activity. This can be seen from the radioactivity mass balance relationship for 2.1.2.1.

$$yS = (x + y) S_x \qquad \text{.....(1)}$$

where S(A/m) and S_x (A_x/m_x) are the specific activities before and after isotopic dilution of the quantity, y, of a radiotracer with the quantity, x, of non-active form of the same element. A and A_x are the activities of the separated

amounts m and m_x of the element before and after mixing of active and non-active isotopes has taken place. Therefore

$$yA \frac{m_x}{m} = (x + y) A_x \qquad \text{.....(2)}$$

For analytical purposes, x should be measured through a change of A_x and the rest of the variables in (2) must be known within the working range of x. However, this is most difficult to achieve for the ratio m_x/m. Clearly a quantitative separation is useless as m = y and $m_x = x + y$.

2.1.3. Substoichiometric Isotope Dilution. The necessity of determining specific activity make it impossible to utilize isotope dilution analysis for determination of traces of elements because in all the above-mentioned alternatives it is essential to isolate at least sufficient substance so that the values of m and m_x can be determined either by weighing or by some physicochemical method.

However, if one separates from the solution of original specific activity ($S = A/m$) and from the solution formed by isotopic dilution ($S_x = A_x/m_x$) exactly equal amounts in weight of the element to be determined (substoichiometric separation $m = m_x$), its content (x) in the test sample can be computed directly from the isolated activities (66).

$$x = y \left(\frac{A}{A_x} - 1 \right) \qquad \text{.....(3)}$$

The substoichiometric principle permits direct isotope dilution technique to be used for determining traces of elements because the activity of minute quantities of radioisotopes can be easily measured.

For successive determination of the test element it is necessary to fulfil the following conditions:

a) To achieve isotopic equilibrium in the sample formed by mixing of radioactive and non-active species.

b) The amount of radioisotope (y) added to the test sample must be precisely known.

c) The activities A and A_x must be substoichiometrically isolated using exactly the same amount of reagents from both the standard radioisotope solution and its mixture with the test solution. Of course, these amounts must be smaller than that which stoichiometrically corresponds to the total amount of element in the less concentrated solution. The substoichiometric amount is choosen according to the known value y (66).

2.1.4. <u>The Advantages of Substoichiometric Isotope Dilution Technique.</u>

a) It is not neccessary to separate the test element from interfering elements quantitatively because it can be corrected from the added radioisotope.

b) Instrument and apparatus are common and inexpensive, also requires small quantity of radioisotope.

c) Sensitivity is very high.

d) It is very useful for the determination of elements for which other techniques are difficult.

e) It is rapid.

2.2 Sensitivity, Limitation, Accuracy and Precision.

2.2.1. Sensitivity of Substoichiometric Isotope Dilution Technique.

"Sensitivity" refers to the absolute detection limit or the relative detection limit of a method for determination. The absolute detection limit is defined here as the smallest amount of element to be determined, and the relative detection limit is defined as the smallest concentration of element that can reasonably be detected under the conditions of a method (2).

2.2.2. Limitation of Sensitivity of Substoichiometric Isotope Dilution Technique.

The sensitivity of this technique is limited by the following factors:-

a.) specific activity of the radioisotope

b.) counting efficiency and background of the counter.

c.) reproducibility of the substoichiometric isolation (e.g.fulfilling the condition $m = m_x$)

d.) reagent blank correction.

The higher the specific activity of a radioisotope used, the smaller is the amount of element which can be determined.

From the theory of substoichiometry it follows that the lower the amount of determined element, the more difficult it is to fulfil the condition $m = m_x$ which ensures the reproducibility of determination. Consequently, the choice of reagent suitable for isotope dilution analysis is limited.

2.2.3. Accuracy and Precision. Accuracy and precision are also important requirements for the quantitative analysis.

The term "accuracy" denotes the nearness of a determination to its accepted value and is expressed in terms of error.

The term "precision" is used to describe the reproducibility of results. It can be defined as the agreement between the numerical values of two or more determinations that have been made in an identical fashion, and is expressed in terms of deviation.

The fundamental difference between accuracy and precision is that "accuracy" involves a comparison with respect to a true, or accepted value; in contrast, "precision" compares a result with the best value of several determinations made in the same way (67).

The most important factor that influence accuracy and precision is fulfilling the condition $m = m_x$ (substoichiometric isolation).

2.3. Solvent Extraction with Dithizone.

2.3.1. Solvent Extraction.

If to a system consisting of two immiscible solvents is added a third component which is soluble in both, then the third component will distribute itself between the two solvents in a definite manner. The law was clearly formulated by Nerst in 1891(68). If C_1 and C_2 represent the concentrations of the third component, respectively, in the two solvents, then

$$\frac{C_1}{C_2} = k(\text{a constant}) \qquad \text{.....(4)}$$

This is known as the partition law or distribution law and the constant K is the partition coefficient or the distribution coefficient.

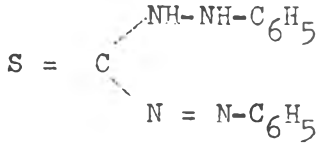
In determination of trace amount of metals by isotope dilution analysis, the amounts of metal ions isolated are of the order 10^{-6} to 10^{-9}g., which corresponds approximately to a 10^{-5} to 10^{-8}M solution of the reagent in an organic phase. Therefore one usually chooses the volume of the organic solvent ten times as small as the volume of the aqueous phase. In addition, the choice of the organic reagent is limited by three factors (66).

a.) The reagent used must form an extractable chelate with a sufficiently high value of extraction constant K, so that the determination need not to be carried out in such a basic medium that hydrolysis and sorption of the test metal ion onto the wall of vessels used would interfere with the determination.

b.) The organic reagent is a weak acid and at higher pH it passes into the aqueous phase because of dissociation.

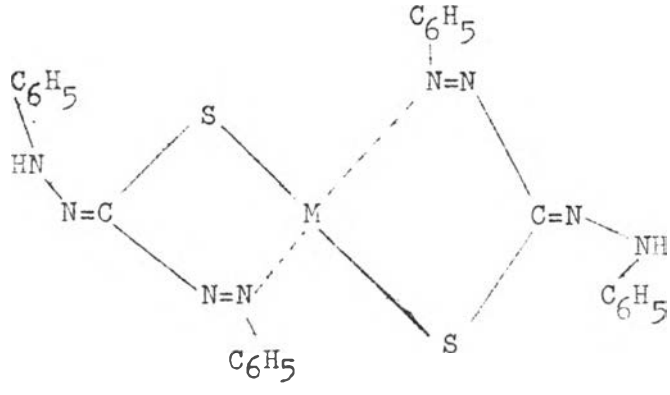
c.) The reagents used must be comparatively stable against decomposition by light, oxidising agent, etc. even in extreme dilution. Therefore it will probable be necessary to improve their stability against decomposition in very dilute solutions by using their zinc salt.

2.3.2. Dithizone. Since Fisher's discovery of dithizone in 1925 (69), this reagent became, and still is, the most successful one for extraction and spectrophotometry of mercury and other heavy metals. The chemical formula of dithizone or diphenylthiocarbazone is



. It is a well known chelating agent in solvent extraction (70). Carbon tetrachloride and chloroform are two solvents mostly used in the preparation of dithizone solution for analytical purposes. When a solution of dithizone in organic solvent is shaken with an

aqueous solution of a reacting heavy metal, an internal complex dithizonate is formed which generally is soluble in the organic solvent



Because the extractibility of metal dithizonates decreases in the order Pd (II) > Au (III) > Hg (II) > Ag (I) > Cu (II) > Bi (III); Pt (II) > In (III) > Zn (II) > Cd (II) > Co (II) > Pb (II) > Ni (II) > Sn (II) > Tl (I), (4), the separation of mercury by dithizone is very selective. Furthermore the extraction constant of mercury dithizonate has a very high value (log K=26.8 in CCl_4). This solution, however, must not contain any oxidants which would decompose dithizone. Hydroxylamine hydrochloride is normally added before extraction. From Table 2.1 as shown below, it can be easily seen that mercury can be extracted very selective from hydrochloric acid solution containing EDTA and hydroxylamine hydrochloride. Regarding this condition, Au and Pd are reduced to metals, Cu is masked by EDTA and Ag by chloride.

Table 2.1 Masking agents in Dithizone Reactions (71).

Conditions	Metals Reacting
Basic solution containing cyanide	Pb (II); Sn (II); Tl (I); Bi (III)
Slightly acid solution containing cyanide	Pd (II); Hg (II); Ag (I); Cu (II)
Dilute acid solution containing thiocyanate	Hg (II); Au (II); Cu (II)
Dilute acid solution containing thiocyanate plus cyanide	Hg (II); Cu (II)
Dilute acid solution containing bromide or iodide	Pd (II); Au (II); Cu (II)
Dilute acid solution containing EDTA	Ag (I); Hg (II)
Slightly acid solution (pH 5) containing thiosulfate	Pd (II); Sn (II); Zn (II)
Slightly acid solution (pH 4-5) containing thiosufate plus cyanide	Sn (II); Zn (II)
Citrate and tartrate in basic medium	Usually do not interfere with extraction of reacting metals

In substoichiometric isotope dilution technique zinc dithizonate is more advantagesthan dithizone because its stability towards oxidising agent is much higher.