ISOTOPE DILUTION TECHNIQUES

OBJECTIVES:

To illustrate the principles of isotope dilution methods and their application to analytical procedures.

To analyze an "unknown" solution by means of an isotope dilution technique.

THEORY:

Isotope dilution analysis was introduced by Hevesy and Hofer in 1934, but it was not until 1940 that the technique was revived by Rittenberg and Foster. 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. Volumetric analysis depends upon the use of a titrant which is specific for the substance sought, and gravimetric analysis depends upon the ability to separate the substance sought in pure form for subsequent weighing. Often it is impossible to accomplish either of these aims, and one must resort to isotope dilution methods for analysis.

There are three general types of isotope dilution methods. These are (a) direct, (b) inverse, and (c) 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.

Direct Isotope Dilution - (Determination of an Inactive Compound by Dilution with an Active Compound). The following technique is used to determine the quantity of a nonradioactive or untagged constituent in a mixture of closely related compounds which are difficult to separate quantitatively by conventional methods. It is applicable to <u>both</u> inorganic <u>and</u> organic mixtures.

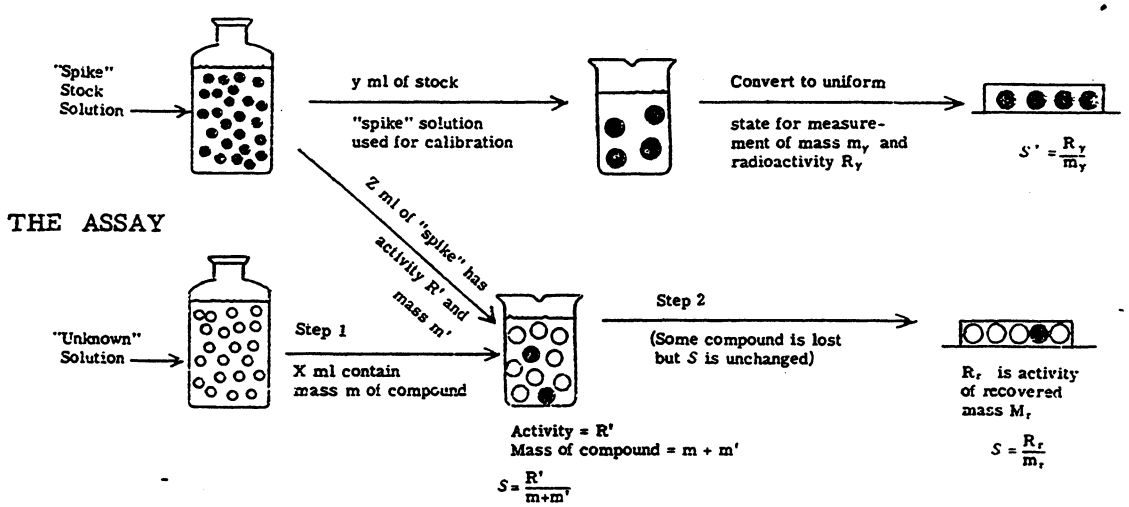
The technique consists of:

Step 1. Addition of a "spike" consisting of a known amount of isotopically labeled compound with a known specific activity S' to the unknown mixture containing the same compound made up of stable isotopes. The two are thoroughly mixed to obtain a uniform distribution.

Step 2. Suitable treatment of the mixture to isolate the same compound in pure form. It is essential that the isolated compound be pure, but it is not necessary that all of the compound be recovered from the mixture. Thus, it is possible to avoid tedious processes required for quantitative separations and frequently to carry out analyses otherwise impractical.

Step 3. Determination of the isotope content of the isolated portion by measurement of its specific activity S. The ratio of active and inactive molecules depends on the relative masses of (active) substance added m' and (inactive) substance originally present m.

CALIBRATION OF THE "SPIKE" (i.e., determination of S')



If

R' = activity (cps) in spike used for assay

m' = mass of spike used for assay

m = mass of inactive substance in unknown used in assay

then the specific activity of the spike S' is given by

$$A' = R'/m' \qquad (1)$$

and the specific activity of the substance isolated in pure form from the mixture is

$$S = R'/(m' + m) = R_r/m_r \qquad (2)$$

It is desired to know the mass m of substance in the unknown in terms of easily measurable quantities. This may be accomplished by dividing equation (1) by equation (2) and solving for m. Thus,

$$\frac{S'}{S} = \frac{R'/m'}{R'/(m' + m)} = \frac{(m' + m)}{m'} = 1 + \frac{m}{m'} \qquad (3)$$

$$\frac{m}{m'} = \frac{S'}{S} - 1 \qquad (4)$$

$$m = m' [(S'/S) - 1] \qquad (5)$$

Example: A sample of mass m was spiked with 0.05 grams of sodium phosphate having an activity of 1950 cps.

$$S' = R'/m' = 1950/0.05 = 39{,}000 \text{ cps/g}$$

0.1 grams (i.e., m_r) of pure sodium phosphate was recovered having an activity R_r of 60 cps.

$$S = 60/0.1 = 600 \text{ cps/g}$$

$$m = m' [S'/S) - 1]$$

$$m = 0.05 [39{,}000/600) - 1] = 3.20 \text{ grams}$$

MATERIALS REQUIRED:

Ferric chloride solution containing about 10 mg Fe per ml and a small amount of ^{59}Fe; ammonium hydroxide; "unknown" iron solution.

PROCEDURE:

To calibrate the tracer (spike) solution, place into one 5-ml centrifuge tube (Tube A):

1.0 ml of radioactive iron solution

3.0 ml of distilled water

To assay the unknown solution, place into a second 5-ml centrifuge tube (Tube B):

1.0 ml of "unknown" iron solution

0.20 ml of radioactive iron solution

3.0 ml of distilled water

Precipitate ferric hydroxide in each tube by adding, slowly with stirring, 6N NH_4 OH. Centrifuge. Decant the supernatant liquids and redisperse each precipitate in about 3 ml of water plus 1 or 2 drops of 6N NH_4OH. Centrifuge and decant the supernatant liquids. Transfer most of each precipitate to a tared planchet using acetone. Completely dry the ferric oxide residues cautiously under the heat lamp.

Determine the mass (nearest tenth mg) of the ferric oxide in each case.

Count the samples using an aluminum absorber (approximately 200 mg/cm^2) between the sample and the counter. The absorber permits only the gammas to be counted and minimizes errors caused by self absorption of the betas.

DATA:

	(m) Mass Fe_2O_3 mg	(R) Activity cpm	Specific Activity S = R/m
Tube A (known	_____(m_y)	_____(R_y)	_____(S')
Tube B (unknown)	_____(m_r)	_____(R_r)	_____(S)
Mass of spike (i.e., mass of iron in 0.200 ml)			_____(m')

CALCULATIONS:

Assuming the radioactive iron solution to contain 10 mg Fe per ml, calculate the mg of Fe per ml in the "unknown" solution.

The mass of iron (m) in 1 ml of unknown solution is given by the formula

$$m = [(S'/S) - 1]\, m'$$