PART X

Tracer Methodology

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SECTION I

Basic Assumptions for Tracer Use

1. Radioisotopes are chemically identical with stable isotopes of the same element.

2. Radioactive nature of the isotope does not change the chemical or physical properties.

3. No observable radiolysis; i.e., no observable perturbation in the chemical behavior of a system containing radioactive atoms as a result of chemical products formed in the system by the absorption of the decay energy.

   NOTE: If tracer decays to radioactive daughter, must either:

   a. purify tracer before use and complete experiment and counting before significant amount of daughter activity grows in; or

   b. count tracer when parent and daughter are in equilibrium.

SECTION II

Self Decomposition of Labelled Compounds

Doses of 10^7 rads produce decomposition effects of the order of 1%.

For ^{14}C compounds if 1 millicuries per millimole in one year have ca. 10^7 rads

For ^{3}H compounds if 8 millicuries per millimole in one year have ca. 10^7 rads.

SECTION III

Radiometric Analysis

A. Precipitation: a slight excess of radioactive reagent added to a solution of the unknown to precipitate the unknown quantitatively.

Ex: Analysis of Zn(II)

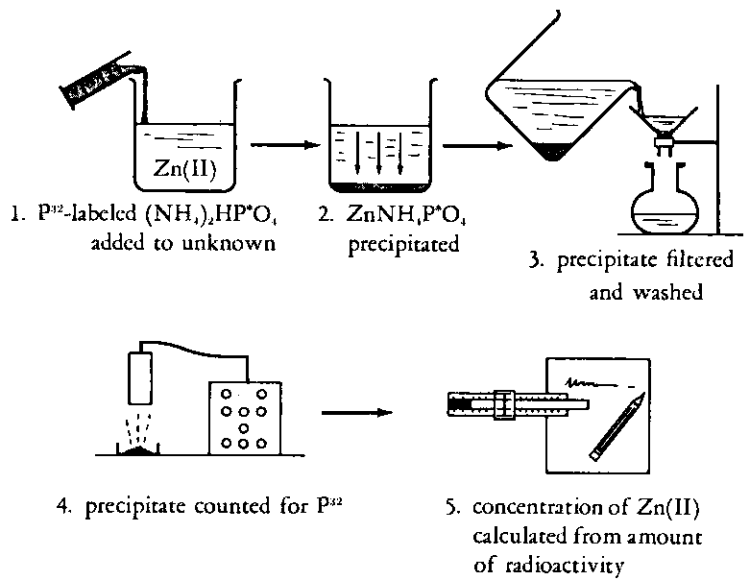


FIG. 1. Steps involved in the radiometric analysis of an unknown concentration of Zn(II) ions by use of a solution of ^{32}P-labelled $(NH_4)_2HPO_4$.

B. Titration: place identical aliquots of unknown solution in a series of test tubes; varying aliquots of precipitation (or extracting) reagent added. After precipitation, supernatant counted and "titration" curve plotted.

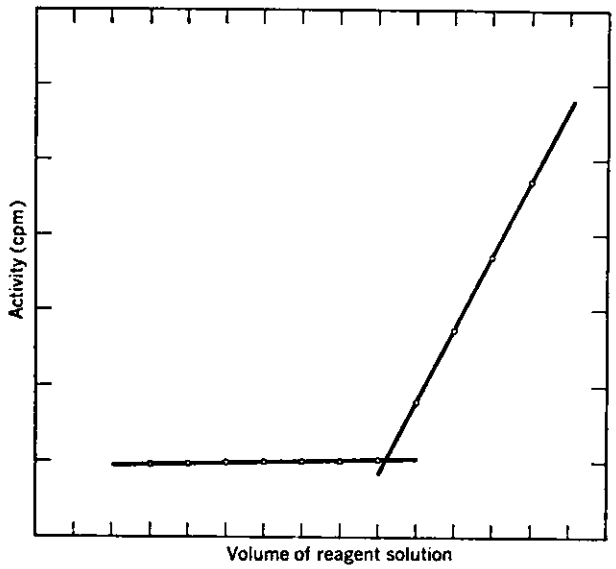


FIG. 2. Titration curve using radiometric analysis.

SECTION IV

Isotope Dilution

Valuable method for the analysis of complex mixtures of organic compounds.

A. Direct Isotope Dilution

Determination of the quantity of a particular compound in a mixture by the addition of a known amount of the same compound containing a radioactive label.

Method:

1. Addition of a known amount, W_a, of the radioactive compound with specific activity SA_a

$$SA_a = A_a/W_a \text{ (cpm/g)} \qquad (1)$$

where A_a is the activity of the added radioactive compound in counts/min

2. Original sample and added W_a well mixed

3. Isolation of the desired compound in high purity but not necessarily quantitatively

4. Determination of the weight, W_f, and specific activity, SA_f, of the isolated pure compound

$$SA_f = A_f/W_f \quad (cpm/gm) \tag{2}$$

5. Calculation of the weight of the desired compound, W_i, in the original mixture

$$W_i = W_f \left[\frac{SA_a}{SA_f} - 1\right] \tag{3}$$

Note: When the specific activity of the added radioactive compound, SA_a, is very high, $W_a << W_i$ and

$$W_i = W_f \left[\frac{SA_2}{SA_f}\right] \tag{4}$$

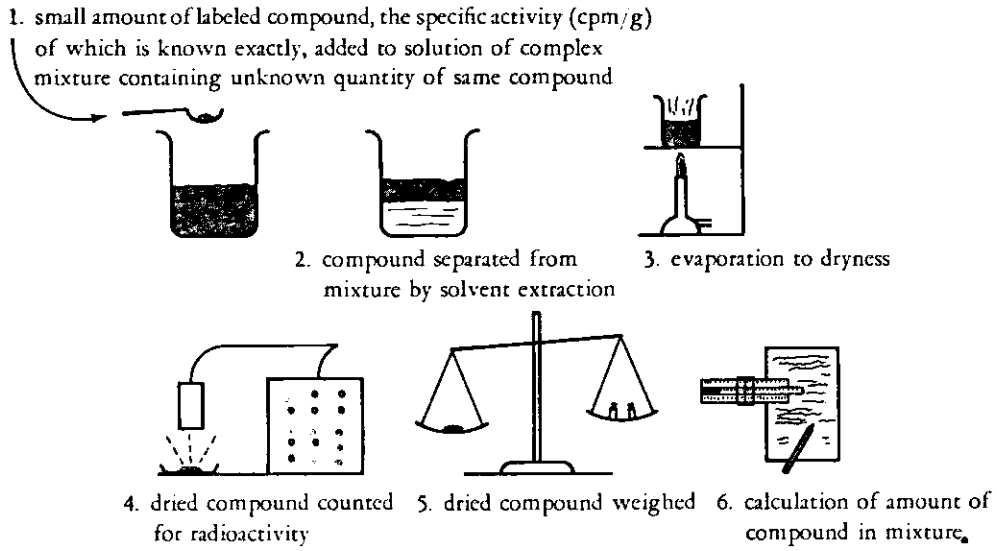


FIG. 3. Possible sequence of steps for determining the amount of one compound in a complex mixture by the technique of isotope dilution.

B. Reverse Isotope Dilution

Determination of a radioactive compound by the addition of the non-radioactive compound.

Method:

1. Determine the activity, A_1, of the radioactive compound in the mixture

2. Mix in known amount, W_2, of the stable compound

3. Again a high purity but not necessarily quantitative separation is performed yielding W_f of the pure compound

4. Measurement of the activity of the separated compound, A_2

5. Calculation of the amount, W_1, of radioactive compound present in original mixture

$$W_1 = W_f\left(\frac{SA_1}{SA_2}\right) - W_2 \qquad (5)$$

C. Derivative Isotope Dilution

A derivative of the unknown compound is made in high yield. A small sample of labelled derivative of known specific activity is added and the isotope dilution technique followed. Mathematically same as reverse isotope dilution.

D. Substoichiometric Dilution Analysis

Isolate equal but substoichiometric amounts of diluted and undiluted substances and compare activities. Can replace SA_1 and SA_2 in Eq. (5) by activities A_a and A_f.

E. Summary of Requirements and Advantages of Isotope Dilution

1. Requirements

Labelled compound must be chemically and radiochemically pure

The labelled compound and analogous unlabelled compound in the mixture must be in complete equilibrium

Separation must be complete, high purity, but not necessarily quantitative

2. Advantages

Quantitative isolation unnecessary

Simple counting instrumentation sufficient

SECTION V

Application of Radiotracers

A. Chemical Separations

Can determine yield in a separation by adding initially a known amount of radiotracer (either isotopic or sufficiently similar chemically). Measure amount of activity after separation process.

$$\text{Yield } (\%) = \frac{\text{activity recovered}}{\text{activity added}} \times 100\%$$

B. Solubility Studies

Can measure K_{SP} by adding tracer before precipitation of insoluble compound, then determining activity in filtrate.

C. Solids

1. Surface area: add tracer to slurry of solid, allowing exchange or sorption by surface; e.g. use ^{212}Pb to measure surface area of $PbSO_4$:

$$\frac{^{212}Pb_{surface}}{^{212}Pb_{solution}} = \frac{Pb_{surface}}{Pb_{solution}}$$

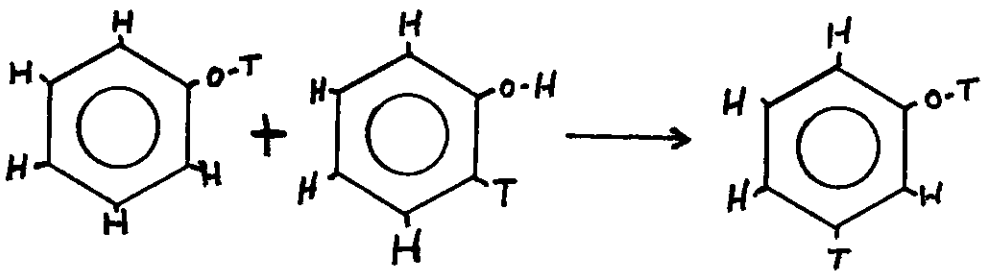
Found 5 x $10^3 cm^2/g$ $PbSO_4$ as surface area

2. Diffusion: add tracer to surface, after period of time, solid divided into sections of known thickness, X, and their specific activity (SA) measured. Diffusion coefficient, D, given by

$$\frac{d(SA)}{dt} = \frac{Dd^2(SA)}{dX^2}$$

D. Kinetics and Mechanisms

1. Hydrogen Migration

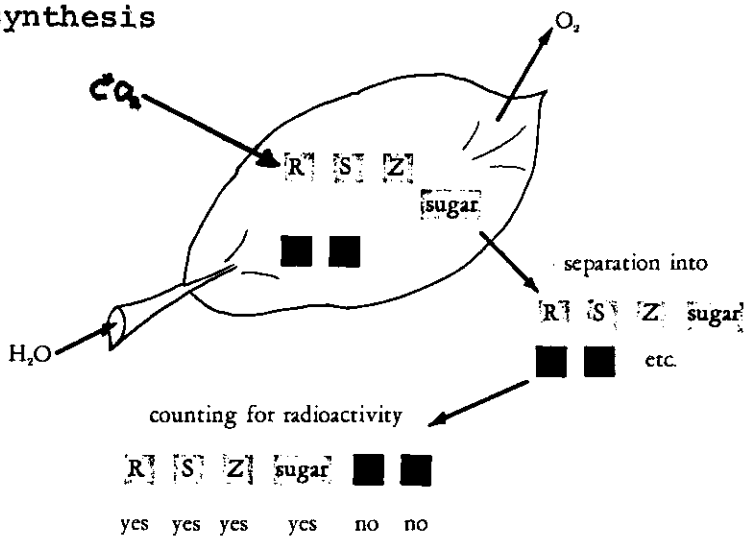


(T=tritium)

2. Isotopic Exchange

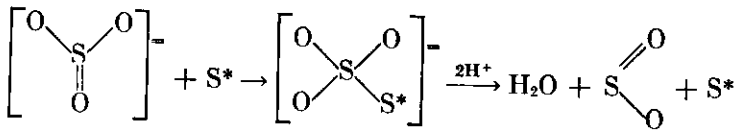
$$Cr^*(III) + Cl^- + Cr(II) \longrightarrow [Cr^{*}\cdots Cl\cdots Cr]^{+4} \rightarrow Cr^*(II) + Cl^- + Cr(III) \quad (6)$$

3. Photosynthesis



$$6C\overset{*}{O}_2 + 12H_2O \xrightarrow[\text{chlorophyll}]{\text{light}} {}^{*}C_6H_{12}O_6 + 6O_2 + 6H_2O$$

E. Structural Studies



F. Geochronometry, Isotope Dating

The decay of naturally occurring radioisotopes can be used for the measurement of age.

1. Requirements

a. Decay rate of nuclide must be known

b. Radioactive equilibrium must be established

c. Sampling must be representative of material to be dated

d. Analytical methods must be accurate

e. The isotopic distribution must not have been altered by external processes

The range of applicability of a given method is approximately 10 $T_{1/2}$ of the parent

2. The Lead Method

Based on the decay of uranium and thorium

	Establishment of equilibrium (years)
$^{238}U \rightarrow {}^{206}Pb + 8\ {}^{4}He$	10^6
$^{235}U \rightarrow {}^{207}Pb + 7\ {}^{4}He$	10^5
$^{232}Th \rightarrow {}^{202}Pb + 6\ {}^{4}He$	10^2

Example of calculation of age by Pb method

Assume a mineral which originally contained uranium but no thorium or lead. After a time t long enough for equilibrium (10^6 y for ^{238}U-^{206}Pb), the ratio of ^{206}Pb to ^{238}U atoms is:

$$\frac{N_{206}}{N_{238}} = e^{\lambda 238 t} - 1$$

λ_{238} = decay constant for ^{238}U

$= 0.154 \times 10^{-10}$

For $t < 1.2 \times 10^9$ y

$t = (N_{206}/N_{238})/\lambda_{238}(y)$

3. Rb and K Methods

Similar equations for use of

$$^{87}Rb \xrightarrow{\beta^-} {}^{87}Sr(\text{stable})$$

and

$$^{40}K \begin{cases} \xrightarrow{\beta^-} {}^{40}Ca(\text{stable}) \\ \xrightarrow{\text{E.C.}} {}^{40}Ar(\text{stable}) \end{cases}$$

4. Radiocarbon Dating

Used for dating relatively young samples 1000 to 30,000 years

$$^{14}C \xrightarrow{\beta^-} {}^{14}N(\text{stable})$$

$$\lambda(^{14}C) = 1.245 \times 10^{-4}\ y^{-1}$$

Given the biological specific activity of carbon as 15.3 dpm/g, the age of a sample, t, can be calculated after the determination of its specific activity, SA, by:

$$t = \frac{1}{\lambda} \ln \frac{15.3}{SA}\ (y)$$

SECTION VI

Problems

1. One millimole of a compound with the empirical formula MCl_3 is dissolved in an aqueous solution containing 1 milliequivalent of radioactive Cl^-. At intervals, samples of the compound are isolated from aliquots of the solution. The activity of the compound increases slowly until it reaches 6/7 of the total activity. What is the simplest molecular formula consistent with these data? If the original specific activity of the Cl^- was 1000 cpm per mg of AgCl, what will be its final specific activity?

2. A 2.0 ml sample of an aqueous solution containing 0.1 microcurie per ml of tritium is injected into the blood stream of an animal. After allowing sufficient time for complete circulatory mixing, a 1.0 ml aliquot of blood is removed and found to have an activity of 1480 dpm of tritium. From this, calculate the blood volume of the animal.

3. A Grignard reaction was run beginning with 1.55 gm of bromobenzene. After completion of reaction, 2.5×10^4 dpm of ^{14}C labeled benzoic acid was added to the reaction mixture. The benzoic acid was extracted once with petroleum ether and the extract was found to have 55 mg of benzoic acid with a specific activity of 190 dpm per mg. What was

the acid yield in the Grignard reaction?

4. A 0.1 gm sample of animal tissue was taken to be analyzed for histamine. After extraction of the histamine, it is reacted with ^{131}I labeled pipsyl chloride reagent with a specific activity of 2×10^6 cpm per micromole. After removal of unreacted reagent, 3000 micromoles of unlabeled pipsylhistamine are added to the mixture. After purification by recrystallization a 3000 micromole (33 mg) sample was found to contain 400 cpm. What was the amount of histamine in the sample?

5. A reaction mixture containing butyl alcohol was reacted with excess ^{14}C labeled acetic anhydride which had an activity of 5000 cpm per mg. The butyl acetate was isolated and found to have a total activity of 3.4×10^4 cpm. Calculate the amount of butyl alcohol in the reaction mixture.

6. A 1 ml sample of a solution containing trace amounts of lead and bismuth was subjected to paper chromatography in alcoholic hydrochloric acid solution. After development of the chromatogram, the wet strip was exposed to ^{35}S - labeled hydrogen sulfide gas whose activity was 2×10^5 cpm per microgram of H_2S. Upon drying and counting the strip, a spot corresponding to the position of PbS was found to have an activity of 1900 cpm while

a second at the correct position for Bi_2S_3 was found to have 475 cpm. What are the concentrations of lead and bismuth per liter in the solution?

7. A 10 ml sample of an unknown Ba(II) solution was titrated by addition of a 0.010 M sulfate solution containing 100,000 cpm per ml; 100 microliter additions were made and a filtered aliquot of the supernatant solution counted after each addition. No significant counts were observed until the 6th addition when 235 cpm were obtained; subsequent 100 µl additions gave the following count rates in the solution aliquot; 1170, 2080, 2980, and 3860 cpm. What was the concentration of the barium solution?