# Fényképalbum9

szerző: PGY

## Stable isotope example

Only total and C-1 enrichment can be determined directly.

However, acceptable results can be obtained for each of the species in a mixture.

The overall method is much less sensitive than $^{14}C$ based approaches but does give positional information.

## Use of Tracers in Analytical Chemistry

**Tracer methods**

A radioactive nuclide is added to a system to study a given effect or property.

In this unit, we will consider some of the more popular approaches used in quantitative analysis.

Other units will consider radioreagents, large scale applications and other examples.

## Dilution Analysis

Several approaches based on the method of tracer addition / recovery and the interaction between the tracer ananalyte.

**Isotope dilution**
**Saturation analysis**
**Substiochiometric analysis**
**Reverse dilution**

## Isotope dilution

First used by Hevesy and Hobbie, Z. Analyt. Chem., **88**, 1 (1932)

Determination of lead in minerals.

### Overview of approach

A tracer is mixed with its inactive form in a sample. You can use it to follow a reaction or separation. This allows you to obtain quantitative analysis without quantitative recovery of you analyte.

## Isotope dilution

### Example

Determination of naphthalene in coal tar.

Add $^{14}C$ naphthalene to your sample.

Position of the label is not important in this case - no chemical reaction.

Tracer must be intimately mixed with the sample - via simple mixing, formation of a solution, ...

## Isotope dilution

Now, you conduct any sort of separation.

The only concern is that you obtain a 'pure' fraction.

Measure the amount of naphthalene

The mass of naphthalene obtained

The amount of $^{14}C$ label present.

$\%^{14}C_{recovered} = \%\ naphthalene_{recovered}$

## Isotope dilution

To use this method, you must know the specific activity of the tracer.

$S_0 = A_0 / W_0$

Where:

$S_0$ = specific activity

$A_0$ = total tracer activity

$W_0$ = tracer mass

## Isotope dilution

When you mix the tracer with the inactive form, you reduce the specific activity.

$$S_x = \frac{A_0}{W_0 + W_x}$$

$S_x$ is determined after recovering the pure fraction. $W_x$ is the total mass of analyte in your sample.

## Isotope dilution

The total mass of the analyte is $W_0 + W_x$

The mass recovered, $W_2$ while have the same proportions of labeled and unlabeled forms with an activity of $A_2$.

The total mass of analyte in your sample can be determined by:

$$W_x = W_0 \left( \frac{S_0}{S_x} - 1 \right)$$

## Isotope dilution

If $W_x >> W_0$ then

$$W_x = W_2 \frac{A_0}{A_2}$$

The only way you can know prior to doing the calculations is if $W_2$ is $>> W_0$.

Lets go back to the naphthalene example with some specific numbers to see how it works.

## Isotope dilution example

1.0 mg of labeled naphthalene with an activity of 100,000 cpm is added to a 10.00g sample of coal tar.

After an elaborate series of separations, 0.0300 g of pure naphthalene is recovered. It is found to have an activity of 4320 cpm.

What is the % naphthalene in the sample?

## Isotope dilution example

$S_0$ = 100,000 cpm / 1.0 mg
= 100,000 cpm/mg

$S_x$ = 4320 cpm / 30 mg
= 144 cpm / mg

$W_x$ = 1.0mg ( 100000/144 - 1) = 693.4 mg

%Naph = 100 x (0.6934 g / 10.00g) =6.9%

## Isotope dilution

The essential step in the method is the recovery of a pure fraction.

You may need to use a secondary method to insure that the collected fraction is pure - chromatography, NMR, melting point, ...

This approach can be applied to almost any method of analysis: gravimetry, colorimetry, chromatography, ...

## Another example of isotope dilution

One novel approach is the determination of total blood volume.

Using isotope dilution is much better than the alternative - collecting a total sample.

**Example**

5.0 ml of saline is injected into a person. It has an activity of 5.0 μCi (Na-24).

After 10 minutes, a 5 ml blood sample is taken - activity of 0.012 μCi.

## Another example of isotope dilution

Determination of total blood volume.

$S_0$ = 1mCi/ml

$S_x$ = 0.0024 mCi/ml

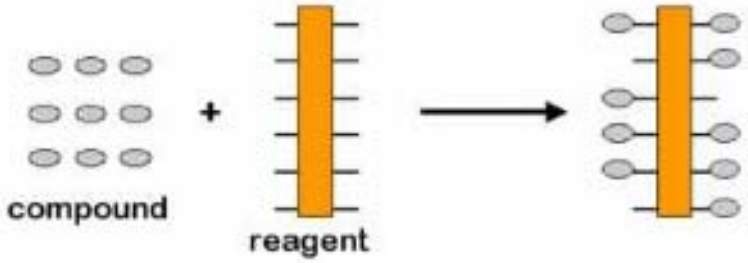
$V_{blood}$ = 5.0 ml( 1.0 / 0.024 - 1)

= 2100 ml

## Saturation analysis

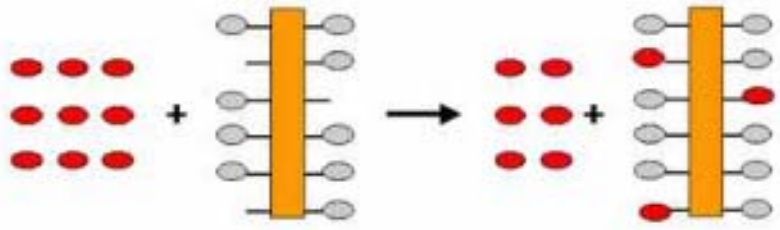
A type of isotope dilution where an irreversible reaction must be a part of the procedure.

**Step one** Excess reagent is added to an unknown amount of the analyte.




## Saturation analysis

**Step two** An excess of a radiolabeled form of the compound is now added.



## Saturation analysis

The bound and unbound forms are then separated and either fraction can be counted.

Amount of the compound is found by:

$$X_{sample} = X_t - X^*_{bound}$$

where

$X_{sample}$ = amount of the analyte

$X_t$ = total amount that can be bound

$X^*_{bound}$ = amount bound by active form.

## Saturation analysis

When this approach is applied to antibody - antigen systems, it is commonly called radioimmunoassay (RIA).

Prior to RIA, bioassays were the only option. It required a specific bacteria and that you observe how the sample affected it.

You could lose specificity, drugs would alter results. The source of the kit also could have an effect.

## RIA

First described by Berson and Yalow, 1960

Employed $^{131}I$ labeled insulin.

Studied two groups

1 - never took injected insulin

2 - had been given injected insulin

They found that the first group showed a rapid loss of $^{131}I$-insulin compared to the second group.

## RIA

The approach has tremendous selectivity and specificity since it is based on an antibody/antigen system.

Levels as low as $10^{-12}$ M can be determined for some systems.

The antibodies can be produced from rats, horses, ... In some cases, small 'tagged' molecules on polymers are used

Tests are typically available in 'kit' form.

## RIA

**Label to use.**

Any type of label can be employed although some are limited.

$^{14}C$ - Activity is usually too low

$^{3}H$ - Tends to exchange

$^{125}I$ and $^{131}I$ are both common.

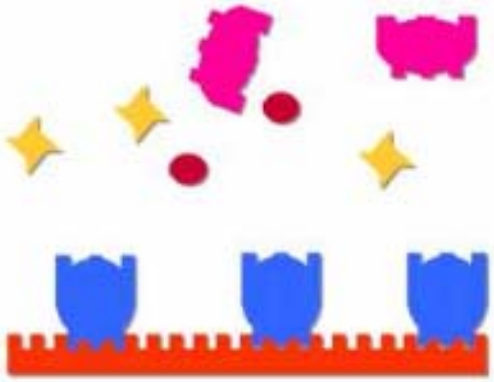
## Steps in an RIA

First the specific antibody must be collected.

An immunoadsorbent is added to a blood sample and allowed to incubate.

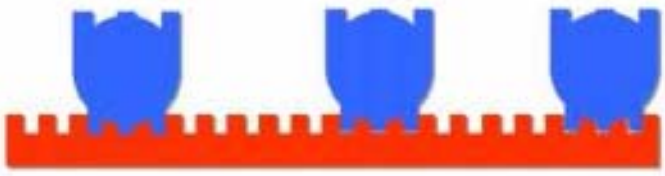
## Steps in an RIA

During incubation, the antibody if interest is collected on the immunoadsorbent.



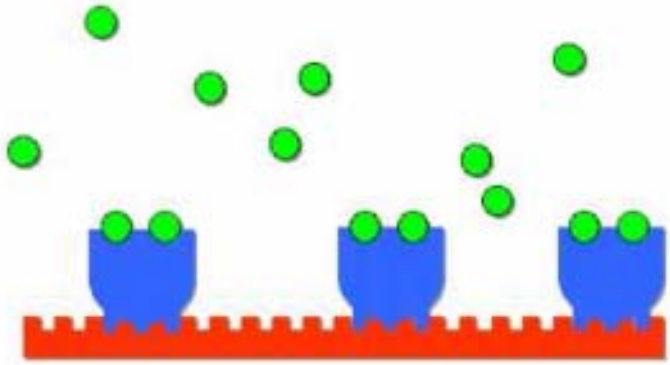
## Steps in an RIA

Now the sample can be washed to remove other immunoglobins and serum proteins.



## Steps in an RIA

Now we add our radiolabel - like $^{125}I$.



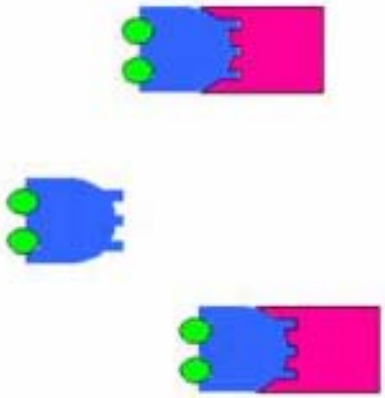
## Steps in an RIA

The labeled antibody is then removed from the adsorbent.

Finally, the reagent is ready to use.

In a kit, all of these steps are already done for you.

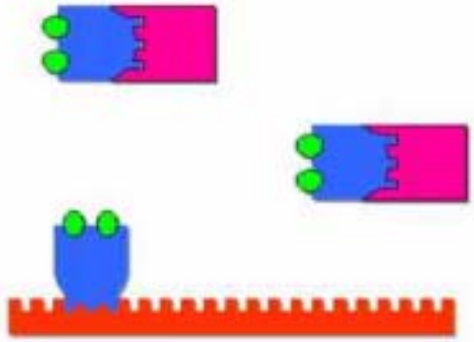
## Steps in an RIA



Our labeled antibody is now added in excess to our sample and allowed to incubate.

## Steps in an RIA

Our immunoadsorbant is then added to the sample resulting in all excess antibodies being 'precipitated.'

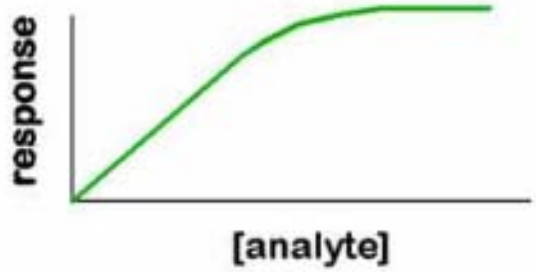


Either the supernate or the precipitated fraction can be assayed.

## Limit at high analyte concentration

With saturation analysis, you have a problems if there is too much analyte

All sites are used by the analyte so the curve reaches a maximum.




## Substiochiometric analysis

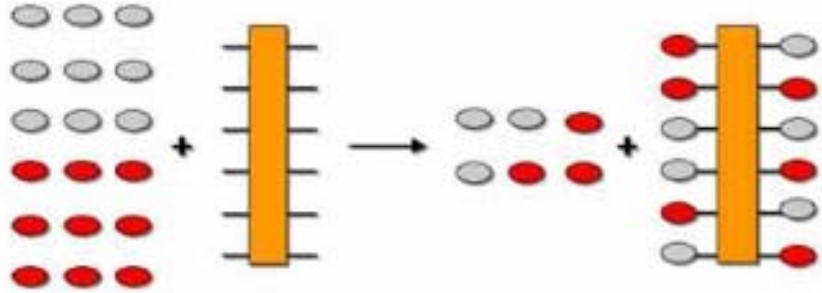
Another form of saturation analysis.

This approach is used when the analyte is bound irreversibly or has a slow equilibrium.

Less reagent is required and it gets around the limit at high analyte concentration.

labeled and unlabeled species are initially mixed and then allowed to compete for a limited amount of reagent.

## Substiochiometric analysis



## Substiochiometric analysis

The end result is the same isotope dilution so the amount of analyte can be calculated by:

$$W_x = W_0 \, ( A_0 / A_x - 1)$$

The supernate and the precipitate can be used and should give the same results.

Count both and compare the results.

## What if the reaction is reversible?

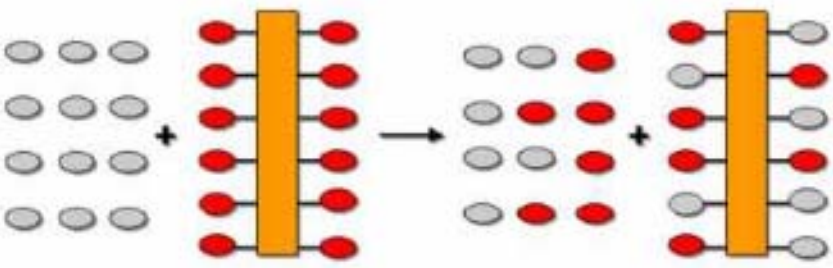
This is a relatively common occurrence.
Most RIA kits use reversible reactions.

When reversible, you can develop a kit that only needs strips already saturated with the labeled form of the antibody.

You can count the strip before and after incubation.

## Equilibrium based Substiochiometric analysis

The results are the same compared to irreversible type assays.



## RIA summary

RIA assays offer significant improvements in sensitivity and selectivity compared to bioassays.

Simple test kits can be developed

- Over 30 tests on the market at the present time.
- Tests typically come with calibration curves and simple procedures.
- Reduced training time, fewer screw-ups.

## Reverse dilution

The opposite of isotope dilution.

A radioactive species is already present and want to know how much there is.

This is useful for confirming the specific activity of your tracer prior to use.

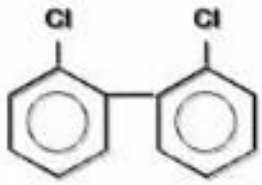
A known amount of carrier is added to a known activity of tracer.

An assay is then conducted and a 'pure' fraction recovered.

## Reverse dilution

**Example**

You just purchased a $^{36}Cl$ labeled PCB sample and you want to know how much of the following form is present in labeled form.




PCBs actually exist as a mixture of over 200 forms.

## Reverse dilution example

You initially purchased 5 mg of tracer with an activity of 1.0 mCi (Cl-36)

A 0.1 mg tracer sample is added to 1.0 g of the specific PCB you are interested.

The mixture is separated and a portion of the peak associated with the specific PCB is collected and counted.

## Reverse dilution example

You collect a total of 0.30 grams of the species with an activity of 4μCi.

Now calculate the amount of the material in the sample using:

$$W_T = \frac{W_x}{\left(\frac{A_0}{A_x} - 1\right)}$$

## Reverse dilution example

$W_T$ = (0.3g) / [ (0.2mCi / 0.004mCi) - 1]

= 0.061 mg

% species = 100 x 0.061 mg / 0.10 mg

= 61%

## Stable isotope methods

Most radio-tracer methods can also be conducted using stable isotopes.

$^{13}C$ vs $^{14}C$, $^{2}H$ vs $^{3}H$, ....

There are not radioanalytical methods but are identical in their approach. All you need is a way to detect and quantify the amount of the stable isotope.

MS offers this method of detection.

## Stable isotope methods

Relative advantages

Many stable isotope labeled materials are commercially available.

No need to deal with the regulations associated with radioactive materials.

The potential exists to obtain 'positional' information - where the label is.

Since stable labels are often used in NMR, the methods provide complementary information.

## Stable isotope methods

**Relative disadvantages.**

Materials are about as expensive as radiolabeledform but you typically need more of them.

Methods are not as sensitive as with radioisotopic techniques.

The mass spectral information can be more difficult to interpret.

## Stable isotope example

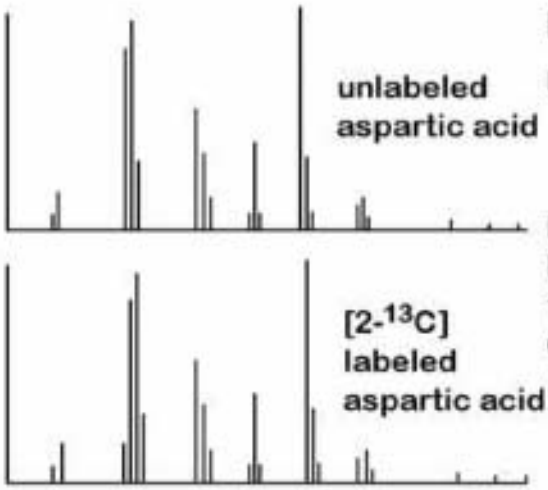
A series of $^{13}C$ labeled forms of aspartic acid were evaluated by GC/MS.

Labeled forms

C-1, C-2, C-3, and C-4

Standards were prepared of each form and various mixtures as N-trifluoroacetyl n-propyl esters.

## Stable isotope example




Example mass spectra.

Note that many lines shift by 1 amu but others do not.

## Stable isotope example

Not all lines will show a clean shift if an isotope is present.

Lines may arise from more than a single source.

First step is to identify lines that show a relative clean shift of 1 amu when labeled.

Even for 'clean' lines

You still must account for the naturally occurring $^{13}C$ that is already present.

## Stable isotope example

### Determination of enrichment

$$E = \frac{I_{(p+1)} - (I_p \times f)}{I_{(p+1)} - (I_p \times f) + I_p}$$

| | |
|---|---|
| E | = enrichment, amount of label present |
| $I_p$ | = intensity of unenriched line |
| $I_{(p+1)}$ | = intensity of enriched line |
| f | = correction factor for normal 13C intensity |

## Stable isotope example

For the aspartic acid study, the following line sets were fund to be suitable:

139/140 - C-2 and C-3 enrichment
142/143 - C-2 enrichment
212/213 - base enrichment of species
226/227 - total enrichment less C-1