## 3.0 *ANALYTICAL METHODS*

### 3.1 *Mass spectrometry*

#### 3.1.1 *The problem*

The low atomic weight elements of major biological or environmental interest, namely hydrogen, nitrogen, carbon, sulfur and oxygen all have naturally occurring stable isotopes. The most abundant isotopes correspond to the nominal atomic weight in each case, and the minor isotopes, which are one or two atomic mass units heavier, make up one percent or less of the isotopic mixture. The natural variation in the abundance of the heavier isotopes ranges from a factor of two for hydrogen to less than 10% for nitrogen, carbon and oxygen (Figure 3). Various processes of interest such as enzyme kinetic or equilibrium isotope effects or source mixing may result in changes of isotope abundance of only a small fraction of this range. The analytical problem boils down to measuring changes in the order of one part per thousand or less in a ratio of 1:100 (for carbon) at best and 1:10000 (for hydrogen) at worst.

#### 3.1.2 *The solution – Isotope ratio mass spectrometry (IRMS)*

A number of spectroscopic techniques such as nuclear magnetic resonance and infrared spectroscopy are able to detect and measure the abundance of stable isotopes, but **only** mass spectrometry is capable of solving the above problem in natural variations with a large sample throughput, at a modest cost. Mass spectrometry and isotopes have been intimately linked since Aston discovered the isotopes of many elements with the first mass spectrometer in 1919. Even with a mass spectrometer our problem is not easily solved and specially designed Isotope Ratio Mass Spectrometers (IRMS) have been developed for this purpose over the past forty years.

#### 3.1.3 *What is a mass spectrometer?*

Mass spectrometers come in many shapes and sizes designed for different purposes, but all contain three essential components: an ion source, a mass analyser and an ion detector (Figure 4). All three may be of very different designs but perform the following basic functions in any mass spectrometer. Firstly the sample under study is introduced to the **ion source** where the substance is converted into positive or negative ions and these ions are then focused into a beam which then enters the **mass analyser**. In the mass analyser ions of different mass/charge (m/z) ratio are separated either in time or space before entering the **ion detector** which produces a response proportional to the abundance of each mass/charge species separated by the mass analyser. This output is generally referred to as the mass spectrum. The ion source, mass analyser and detector are all contained within a high vacuum system to minimize dispersion of the ion beam by collisions with air molecules.

**What is special about an Isotope Ratio Mass Spectrometer?**

Measurement of ion beam intensities with the precision required to measure natural abundance isotope variations is not possible with most designs of mass spectrometer. All IRMS share

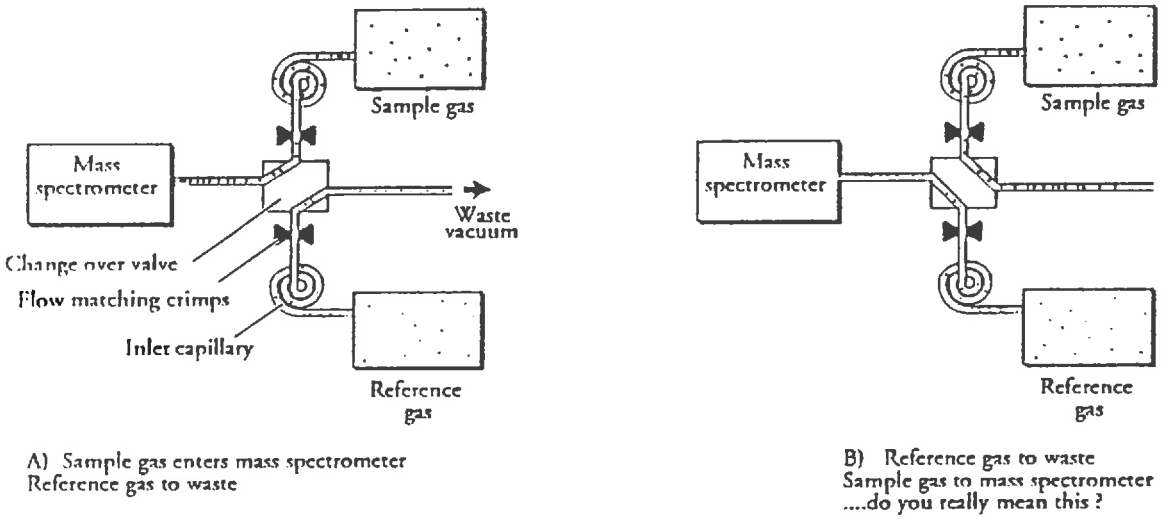


**Figure 5.** Diagram of Gas Dual Inlet for an IRMS. The 'cross over' flow of sample and reference gas is shown during the sequential measurement of the isotope ratio of each gas.

a number of special design features that enable sufficient precision to be achieved. The first IRMS was developed in 1950 (McKiney *et al.*, 1950), and many of their design principles are retained in modern instruments. An IRMS for low atomic weight elements operate using only low molecular weight 'fixed gases' as sample. Thus $H_2$ is used for $^{2/1}H$ ratio measurement, $N_2$ for $^{15/14}N$ and $CO_2$ for both $^{13/12}C$ and $^{18/16}O$. This sample gas is admitted to the mass spectrometer from a reservoir through a fine capillary to give a stable supply of gas to the ion source and to avoid diffusive fractionation of the isotopes in the inlet. The gas is ionized by an electron beam produced from a hot filament of rhenium or thoriated tungsten and accelerated through 3000 to 5000 volts before entering a magnetic sector mass analyser. In this type of mass analyser the ion beam passes through a magnetic field at right angles to its direction of travel from the source and the beam is bent by the magnetic field. Ions of different mass–to–charge ratio leave the source with equal velocity, but those of heavier mass, having a higher momentum, are less easily deflected, and ions of different mass/charge ratio are focused into different ion beams at the end of the mass analyser or 'flight tube'. The geometry of the source and flight tube in an IRMS is designed to give rather low resolution of the ion beams such that there is just about baseline separation between ion beams differing by one mass/charge unit, and each ion beam has a constant intensity over a significant portion of the peak width. These 'flat topped' ion beams are each detected by separate Faraday cup collectors. Separate collectors are required to cope with the large intensity range (up to 1:10000) between the most abundant and least abundant ion beams.

The ion beams are focused by adjusting the accelerating voltage and/or the magnetic field strength such that the middle of the flat top of each beam enters a Faraday cup. In this way small drifts in the focusing parameters do not alter the measured intensity ratio between the ion beams. The collectors are connected to ground through a large resistance, completing the circuit from the source. The ion current flowing through the resistor creates a voltage across it which is used as the output from the mass spectrometer, and this voltage is fed into a computer based data system via an impedance matching amplifier. The ion current through an IRMS is of the order of $10^{-8}$ amps for the most intense beam and $10^{-11}$ amps or less for the other beams. To produce a useful output voltage for the data system (a range of 1 – 10 volts) resistors of $10^8$ to $10^{12}$ ohms are required for these respective beams. By using a higher resistor for the less abundant ion beams the output fed to the data system can be brought in to the same voltage range for each beam. The respective ion beam intensities are then measured by integrating the output voltages over a time period using parallel voltage – frequency converters and counter circuits. A most important feature of the design is that the gain resistors and amplifiers must be very stable and produce a minimum of spontaneous noise.

Despite all the above adaptations to cope with large difference in ion currents and to achieve stability, it is not possible to make absolute measurements of isotope ratio of sufficient precision with such an instrument, and the method of differential measurement must be used to overcome the problem of instability during the measuring period. This requires the comparison of the isotope ratio of a reference gas with that of the sample, both measured under the same conditions and within a short time period of each other. The most effective way of arranging this is to use a **dual inlet** system, where the gas from separate storage volumes for the reference and sample are led through carefully matched capillaries to a system of crossover valves which allow the gases to enter the mass spectrometer or a waste vacuum line alternately (Figure 5). The cross over valves are designed to perturb the gas

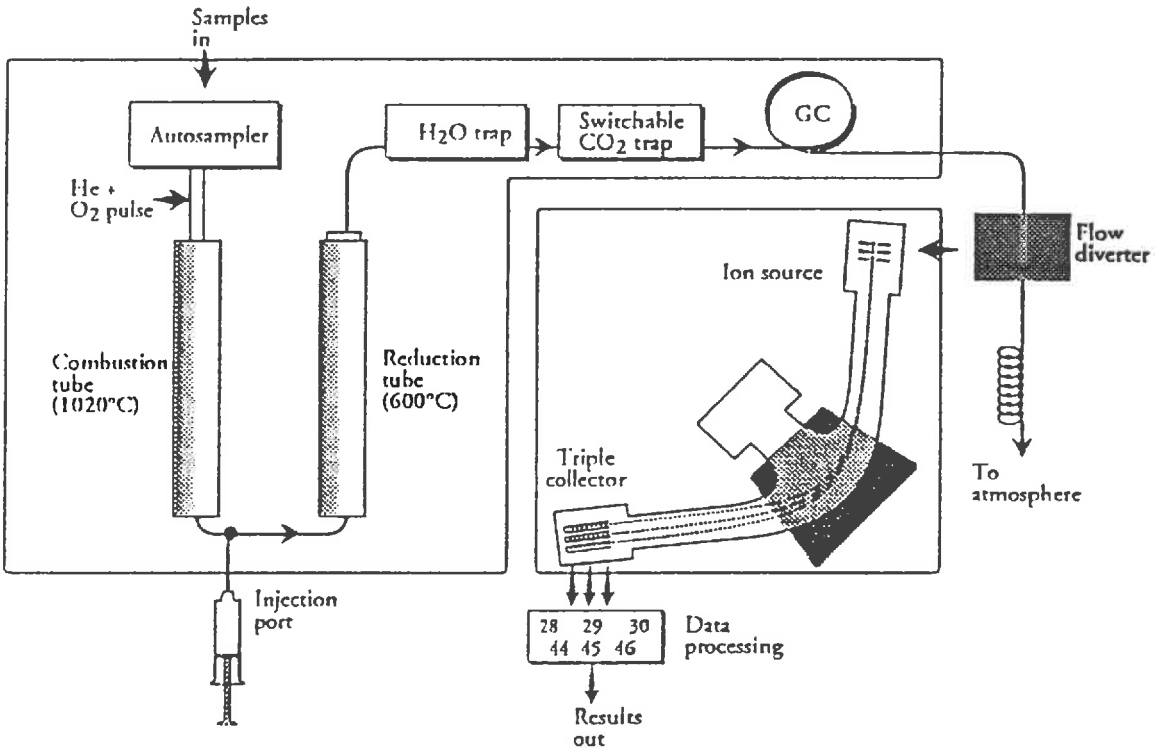


**Figure 6.** Diagram of a continuous flow IRMS (CF–IRMS). The sample preparation unit and mass spectrometer are integral parts of the system, linked by the continuous flow of helium carrier gas which carries the pulse of purified sample gas into the mass spectrometer.

flow as little as possible during the switch over, and to avoid cross mixing of the gases. The gas pressure in each storage volume can be adjusted and matched by altering the volume of the storage volumes with bellows ensuring the reference and sample gases are measured at the same ion current. Such a degree of controlled matching is only possible with gaseous samples.

With such an inlet, the reference and sample signals would each be integrated for 10 – 20 seconds after allowing a settling period of 5 – 15 seconds after each change over. This process is repeated for several (3 – 10) cycles and the data averaged over each cycle and over the set. In this way the drift in the detector system can be compensated for as far as possible and any aberrant measurements due to transient noise are rejected statistically. In contemporary instruments the whole of the measurement process, valve operation and data collection, is under the control of a computer based data system.

Instruments designed in this way are known as **dual inlet multi collector gas IRMS** and with low noise solid state electronics are capable of remarkable precision.

The development of low noise electronics has resulted in much greater stability than was possible when dual inlet IRMS were first developed, and reasonable stability is now possible over many minutes rather than a few seconds. This, and improved vacuum pumping, has led to the development of an alternative inlet system known as a **continuous flow** inlet where pulses of the gas under study are introduced to the source in a steady flow of helium carrier gas. Using such a system as many as ten different samples may be analysed between pulses of reference material which may be fed in as much as an hour apart. The continuous flow inlet is considerably simpler (and cheaper) than the dual inlet and suited to more rapid analyses, but is not capable of such great precision.

The continuous flow inlet is particularly suitable for use with sample preparation systems which produce a pulse of gas for analysis and a particularly important example of such a sample preparation device is an elemental analyser, which can oxidize samples of organic material to give a mixture of nitrogen and carbon dioxide. This gas mixture is carried by a constant flow of helium into a gas chromatograph where the gases are separated and emerge as two peaks which can be fed into the CF–IRMS. Within the IRMS the bulk of the helium is rapidly pumped away, allowing isotope ratio measurement of the $N_2$ or $CO_2$. An additional refinement, switching the IRMS tuning between the peaks, makes it possible to measure the isotope ratio (and amount) of both nitrogen and carbon dioxide from the same sample. Sample preparation is dealt with in more detail below, but with most CF–IRMS the sample preparation is an integral part of the system, and CF methods were first developed in conjunction with an elemental analyser to prepare nitrogen samples (Preston and Owens 1983).

**What does an IRMS tell us and is it what we want?**

The differential method of measurement – comparing sample with reference – and the range of isotopic variation studied has led to the use of the 'δ unit' notation.

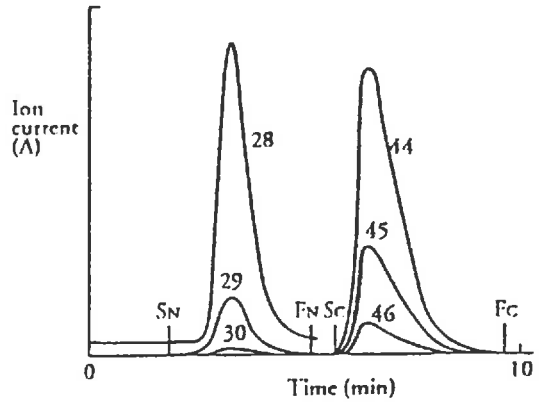


**Figure 7.** Continuous flow IRMS in operation. The real–time output shows a pulse of nitrogen followed by a pulse of carbon dioxide derived from combustion of one sample. Integration of the area under the mass 29 and 28 curves ($S_N$ to $F_N$) gives the $^{15/14}N$ isotope ratio. The mass spectrometer is then switched to measure the area under the mass 45 and 44 curves ($S_C$ to $F_C$) to give the$^{13/12}C$ ratio. These ratios are compared with those from reference material run before and after a set of up to ten samples. $\delta$ values are then calculated by comparing the sample and reference isotope ratios.

$$\delta = 1000 \times (R_{sample} - R_{standard})/R_{standard}$$

where $R_{sample}$ and $R_{standard}$ are the measured isotope ratios for the sample and standard gases respectively.

In other words, $\delta$ is the difference in parts per thousand between the isotope ratios of the sample and reference gases, expressed relative to the reference gas. Only if the reference gas is of known isotopic composition can the isotope ratio for the sample gas be measured. A number of precisely measured International Standards are available against which stocks of reference material can be calibrated for use as 'in house' standards enabling meaningful inter-laboratory comparison of results to be made.

However what is measured by an IRMS is the isotope ratio for a particular fixed gas $H_2$, $N_2$, $CO_2$ etc and the information required from an experiment is usually the isotope ratio of a particular element. Further processing of the information may be required to derive this information. For example, when analysing carbon dioxide we will measure the m/z 45/44 and 46/45 isotope ratios corresponding to the isotopomers of $CO_2^+$ while we would normally want to know either the $^{13/12}C$, $^{18/16}O$ or even $^{17/16}O$ ratio. More information is required to solve this (two measured isotope ratios and three derived isotope ratios) and only by assuming that $^{17}O$ abundance is co-variant with the $^{18}O$ abundance can $^{13/12}C$, $^{18/16}O$ abundances be estimated from the experimental measurement (Mook and Grootes 1973). These 'ion corrections' are normally carried out by the instrument software as part of the output report, but it should be remembered that an assumption is involved which may not be valid under non-equilibrium conditions and especially when any $^{18}O$ tracer has been used. Different corrections are required for $H_2$ analysis, where the measured m/z 3/2 isotope ratio is a combination of the required $^{2/1}H$ ratio and the $H_3^+/H_2^+$ ratio. $H_3^+$ is unavoidably formed in the ion source and has the same mass as dihydrogen containing $^2H$ and $^1H$. Careful source design can minimize the amount of $H_3^+$ formed but prior calibration of the instrument is required to correct for this interfering signal, and such correction is normally carried out under software control. With nitrogen neither type of ion correction is required at natural abundance, but correction for residual air in the mass spectrometer is often required.

The need to apply corrections to the measured isotope ratios is **not** a major drawback of the method compared with the significant advantages of using stable and readily prepared gases for the measurement. Providing the corrections are fully understood and carefully applied results of the highest precision and accuracy can be obtained by applying a standarised measurement method to a few gases derived from a wide variety of samples.

### 3.1.4 *Precision*

**What is possible?** All IRMS require a significant amount of sample material compared with say GC-MS or NMR analytical methods. Modern instruments require less than their older counter parts, mainly due to lower volume inlet systems, but the need for gas pressure balancing and the analysis period of several minutes does set a lower limit on the amount of sample needed. For maximum precision, 0.1 to 1 mg of gas is required depending on the instrument. With a dual inlet system and adequate sample, quite remarkable precision is possible, and typical manufactures specifications would be 0.01 $\delta$ ‰ for $CO_2$ and $N_2$ and

around 1 δ unit for $H_2$. 0.01 δ unit for carbon represents a difference of only one part in $10^7$ in the mole fraction of $^{13}C$ present. Continuous flow IRMS are of inherently lower precision, with 0.5 δ unit being attainable for nitrogen and 0.3 for carbon using around 100 μg of either. As little as 10 μg can be measured by continuous flow methods, or with low volume cold finger dual inlet systems, but with an inevitable loss of precision compared with when adequate sample is available.

**What is needed?** Meaningful information about natural processes may be obtained from a less than one δ unit range between samples for N, C or O. A recent publication (Charles & Fairbanks 1992) showed changes in oceanic water flows from the $^{13}C$ and $^{18}O$ variations in a deep sea core. The whole range of $\delta\ ^{13}C$ observed was less than 1.1 ‰ and that for $^{18}O$ less than 1.7 ‰. Similarly, significant differences between roots, root nodules and shoots in plants are detectable at the 0.3 $\delta^{13}C$ level. Clearly such work requires the maximum possible precision.

In many ecological studies there a high degree of natural variation of δ–values. Here the more pressing need is to process many samples. The requirement for rapid sample conversion and analysis makes combined combustion – continuous flow systems attractive for such studies.

### 3.1.5 *Availability and suitability IRMS instruments*

There are only three manufacturers of IRMS whose products are readily available in Europe. They are Finigan MAT (Bremen, Germany), VG Isogas (UK) and Europa Scientific (Crewe, UK). The first two specialize in dual inlet instruments, but also produce continuous flow variants and a number of automated sample preparation and inlet systems. Europa Scientific have set out to establish the continuous flow instrument with automated combustion sample preparation as their standard instrument, but they also produce other sample inlet systems and have recently manufactured a dual inlet variant of their MS. While the design of all these instruments is similar there are a number of factors which are different from manufacturer to manufacturer. For example the Europa and VG mass spectrometers use a permanent focusing magnet and continuously variable accelerating voltage to focus the ion beams in to a universal triple collector. Finigan use an electromagnet of variable field strength and a limited range of accelerating voltage to focus the ion beams into a particular set of collectors positioned to suit the gas under study. The gases to be used with such a design must be specified at the time of manufacture, whereas the VG and Europa instruments are inherently more flexible in this respect.

A laboratory which is expected to analyse a range of isotopes from a variety of samples requires a range of IRMS instruments and sample preparation systems. CF–IRMS instruments coupled to a combustion system can deal with many samples containing C and N. Where the highest precision is required, duel inlet systems are still required and a cryogenic trapping system of $CO_2$ preparation is required. Where the through put of different samples is not too high it is possible to connect several preparation systems to one mass spectrometer, but eventually a further instrument will be required if the number of samples processed rises. CF systems can cope with 100 plus samples per day, while cryogenic purification is much slower with 50 samples per day the upper limit. Where several isotopes are being studied it is often

necessary to dedicate an instrument primarily to one task, as resetting and stabilising can be time consuming. To date CF methods are well established for C and N from a large variety of samples and have also been used for $^{18}O$ in water. Deuterium has not been satisfactorily analysed by CF methods where interference from the helium carrier remains a problem. $SO_2$ has to date been studied by DI instruments although the development of CF methods can reasonably be expected in the future.

In summary a productive natural abundance isotope facility would require a minimum of one CF and one DI instrument with both on line and cryogenic sample preparation systems plus auxilliary kjeldahl, distillation, resin exchange and general wet chemistry facilities.

### 3.1.6 *Costs, reliability, development and backup*

The approximate costs are given in the table for a number of instruments and sample preparation systems.

**Table 1.** Availability and cost of IRMS and preparation systems

| Manufacturer | Instrument | DI/CF* | Preparation system | Cost (£K) |
|---|---|---|---|---|
| Finnegan MAT | Delta S CNOS | DI | | 130 |
| | Delta S CNOS-II | DI | | 136 |
| | | | Cryogenic traps, | 26 |
| | | | Elemental analyser and cryogenic trap | 52 |
| | Delta S | CF | | 97 |
| | | | Elemental analyser | 32 |
| VG Isogas | Sira CNOS | DI | | 103 |
| | | | Cryogenic traps, | 19 |
| | | | Elemental analyser and cryogenic trap | 45 |
| Europa Scientific | Tracermass | CF | | 70 |
| | Tracermass CNOS | DI | | 116 |
| | Tracermass CNOS-II | DI | | 123 |
| | | | Roboprep-CN | 26 |
| | | | Roboprep-G and autosampler | 19 |

Prices include VAT but do not include the provision of local services such as electrical supply, compressed air, cooling water etc.
*DI - Dual inlet, CF - Continuous flow

In addition to the capital cost, a number of other costs must be allowed for. IRMS instruments require a considerable amount of space, ideally at least 10 $m^2$ floor space per instrument and a dedicated power supply. Air conditioning is highly desirable, contributing to stable operating conditions, instrument reliability and operator comfort because the instruments produce large amounts of heat. Supplies of compressed gases, particularly compressed air for valve operation in dual inlet machines, are also needed. Many preparation systems use cryogenic traps and dedicated freezer units and a generous supply of liquid nitrogen may be required to maintain a high sample through put with such systems. The Europa instruments are designed as bench top rather than floor standing units and make slightly less demands on

space and operating conditions and do not generally require cryogenic supplies. When setting up an IRMS facility it is important to plan for breakdowns and maintenance. A 'dowry' to provide a stock of important replacements and consumables is advisable – for example an in–house supply of replacement turbomolecular pumps and power supplies can mean the difference between a half day down time and three to four weeks while a manufactures overhaul is carried out.

IRMS instruments are inherently reliable as a result of their design requirements for stability and the use of only pure gases in the mass spectrometer and experience of all three manufacturers has shown this to be the case. In terms of 'customer support' both for fault correction and methods development there are considerable differences between the attitudes of the different manufactures. Europa Scientific have proved satisfactory for fault correction and are positively involved in method development. This method development is carried out both by their own staff (23% of sales is directed to development) and in collaboration with customers, for which they have provided some financial support. Europa are also willing to tackle method development under contract from customers. Finigan also provide satisfactory fault support and build highly reliable instruments, but have a rather narrower view of method development, carrying out in–house development work in response to well established demands for preparation systems. VG are widely considered to provide poor fault support, even under warranty, appear to have an under resourced development section which again responds to well established demand, and are rather negative about method development with customers.

**Staff requirements.** IRMS are sophisticated high capital cost instruments and though reliable and relatively simple to operate on a day to day basis, require a significant investment in staffing to achieve satisfactory results and efficient use of machine time. The operator must understand the principles and complexities of both the sample preparation and IRMS systems and be able to relate to the applications for which the results are used and the particular problems of sample analysis posed. It is not satisfactory to regard the IRMS system as a Black Box which produces numbers. Post–doctoral level staffing with a substantial experience of analytical methods and isotope analysis is required for the effective running of an isotope analysis facility, and present experience has shown that the day to day operation, maintenance and dedicated method development with 2 to 3 IRMS instrument is a full time task for at least one person. Technical assistance is invaluable both for the supervised operation of IRMS instruments and sample preparation. Grinding and weighing material for combustion/IRMS instruments is unavoidably time consuming and setting up samples for other automated systems is also a considerable consumer of technical help.

### 3.2 *Sample preparation*

The restriction of IRMS to a few fixed gases means that much work must often go into converting the material of interest – animal, vegetable or mineral – into a suitable gas for isotope analysis. This gas must be pure to enable sample and reference matching during the measurement and prevent reactions in the ion source from interfering. For example, a small trace of carbon dioxide in nitrogen will give rise to some $CO^+$ in the ion source and this has the same signal (mass/charge) as $N_2$. It is equally important that the isotope ratio of the element of interest in the prepared gas truly reflects that present in the original starting

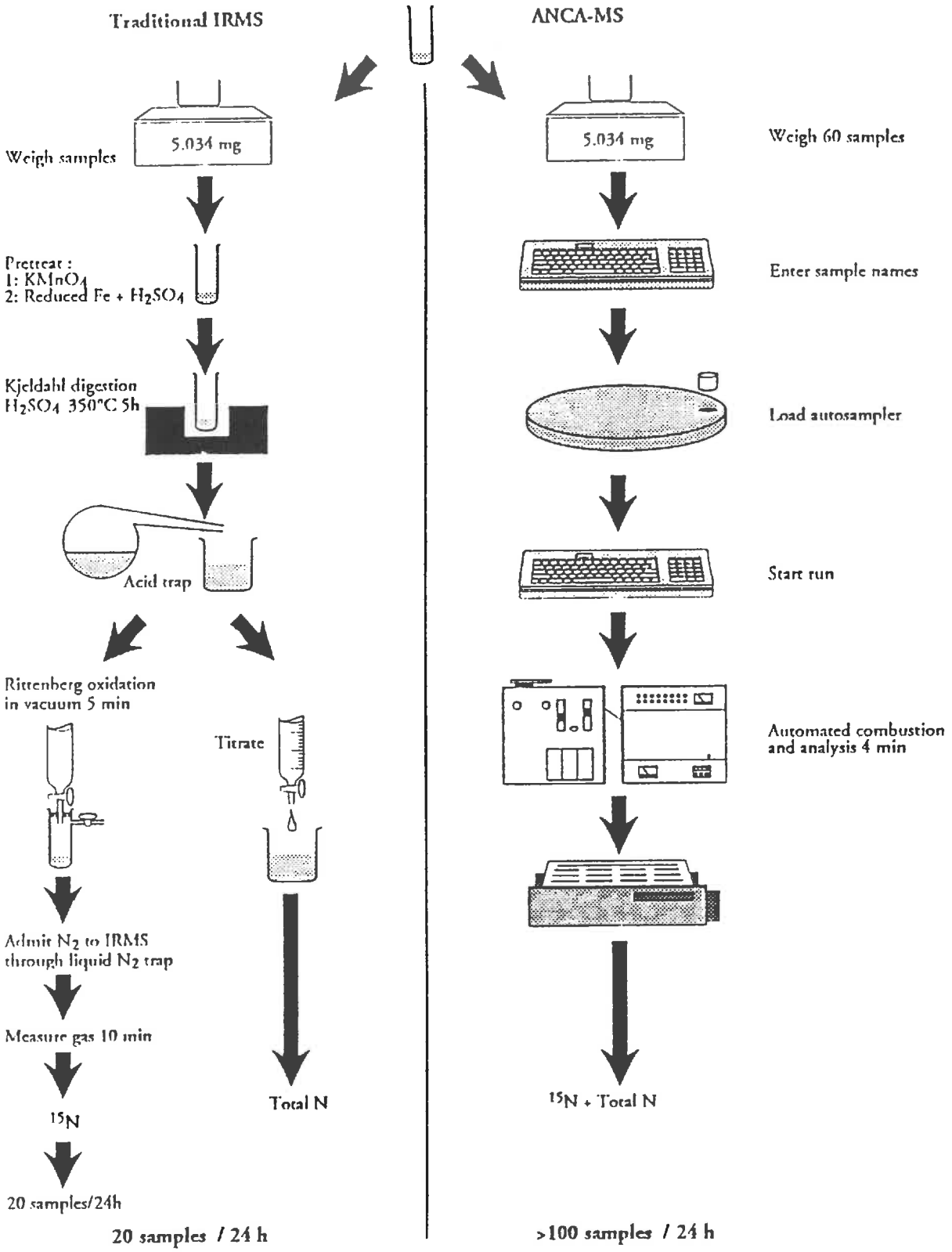


**Figure 8.** Comparison of traditional 'wet chemistry' sample preparation of nitrogen for IRMS measurement with on-line continuous flow sample preparation and IRMS measurement.

material. This means that the conversion step or steps must be **complete** to avoid fractionation or that an equilibrium is set up under identical conditions for all the samples. Clearly, sample preparation is a non-trivial part of the isotope analysis and may require as much time and care as the final IRMS measurement.

It is often possible to integrate and automate sample preparation systems with an IRMS and this has great advantages. Automated systems can operate unattended overnight, making efficient use of instrument time, and can often produce greater reproducibility from sample to sample and batch to batch than is possible by the most patient and careful operator. An important example of such an integrated system is the elemental analyser, CF-IRMS described above, and the figure indicates the saving in labour and potential errors made possible by the integrated combustion system.

Figure 8 shows two alternative methods for $N_2$ preparation from organic or inorganic material, the Kjeldahl-Rittenberg method being used to prepare a nitrogen sample for a duel inlet instrument, while the on-line combustion is designed to feed a CF-IRMS. Other methods which have been used for nitrogen preparation are sealed tube combustion followed by manual cryogenic purification (Handley *et al.*, 1991), or on-line combustion followed by automated cryogenic purification (available for Finigan and VG instruments). Apart from the obvious labour saving over the manual methods, the on-line combustion and CF-IRMS is inherently more satisfactory than the other methods since the nitrogen sample is measured above a constant and controlled background from the helium carrier. With all the other methods uncontrolled leakage of nitrogen from air or concentration of nitrogen from the helium carrier will result in a variable degree of contamination of the sample derived nitrogen. At best this can be corrected for but always remains a problem particularly with small or variable sample sizes.

**Table 2.** Preparation methods for IRMS

| Isotope | Source | Preparation method | Available automated | DI or CF |
|---|---|---|---|---|
| $^{13}C$ | Air | Cryogenic trapping | Yes | DI |
| | Organic matter | Combustion† & cryogenic trapping | Yes | DI |
| | | Combustion & GC separation | Yes | CF |
| $^{15}N$ | Organic matter | Combustion & cryogenic trapping | Yes | DI |
| | Nitrate | Combustion & GC separation | Yes | CF |
| | Ammonia | Kjeldahl digestion/Rittenberg oxidation | No | DI |
| $^{18}O$ | Water | Equilibration with $CO_2$, | | |
| | | Cryogenic trapping | Yes | DI |
| | | Equilibration with $CO_2$, GC separation | Yes | CF |
| | | Reaction with guanidine hydrochloride | No | DI |
| $^{2}H$ | Water | Uranium reduction | Yes* | DI |
| | | Zinc reduction | No | DI |
| | | Platinum catalyst equilibration | No | DI |

†Automated combustion methods generally use an elemental analyser for oxidation/reduction and gas separation
*This system has perennial difficulties with memory effects

On the other hand, cryogenic trapping of $CO_2$ prior to admission to a dual inlet instrument provides the method with the highest possible precision for $CO_2$ analysis. However cryogenic trapping is about 3 to 4 times slower and requires a considerable supply liquid nitrogen.

Even with automated systems a considerable labour may be needed to feed the system with sample material. With most preparation systems there is a need for the same amount of material to be analysed each time and this demands the careful aliquoting of liquid samples or weighing of solids. Solid samples must also be finely ground before use to ensure that a representative sample is taken. The amounts of say plant material required for an elemental analyser system is of the order of 1 mg which must be weighed into a tin foil sample pot. The time consuming steps of grinding and weighing have been a characteristic of all elemental analyser use for many years, and are largely unavoidable.

Table 2 illustrates a number of common preparation methods for samples of biological interest. Those which can be readily automated are indicated and these combined preparation system – IRMS are often capable of a high sample throughput. Many can be adapted to other sources than they are originally designed for.

Most studies of natural abundance variations in carbon and nitrogen have used bulk samples, without much or any chemical separation of the components of the sample. While much interesting information can be obtained in this way, the detailed understanding of many of the mechanisms which control the eventual isotopic composition of the material will increasingly require chemical separation of sample material. The separation methods used will vary somewhat with the compounds being studied, but the fundamental requirement is for a method which is quick and efficient. Complete separation of all of a compound from the rest of the matrix ensures that no fractionation will occur (although the risks of fractionation decrease as the molecular weight increases). High pressure liquid chromatography (HPLC) has considerable potential as the most general and flexible method for compound separation. HPLC can be applied to a large range of neutral and ionic compounds and provides rapid and efficient separations. HPLC can be used both as an analytical and preparative method, and in the later case the compound of interest is finally recovered from a relatively small volume of solvent. For IRMS analysis 0.1 to 0.5 mg of compound would be required which would require columns of more than the minimum analytical capacity, but still of modest capacity and solvent consumption. HPLC has been used successfully to isolate amino acids for low level tracer studies of protein metabolism (Preston & McMillan, 1988) and should be readily extended to other compounds.

Automated HPLC systems can include autosampler injection and fraction collection of the eluate, and can be programmed for multiple analyses of the same sample with pooling of the eluate fractions. This allows the accumulation of a sufficiently large sample for IRMS analysis with little operator intervention. Such HPLC systems cost from £K20 to 30 depending on the configuration and detector system required. Modest technical support is required for solvent preparation and sample loading for established methods. A larger time input is required to develop isolation methods, and even with a well developed method some considerable technician time may be required to prepare samples in a form suitable for HPLC analysis. We shall acquire an HPLC–quadrapole mass spectrometer.

An ideal goal is the linking of an HPLC to an IRMS. HPLC–thermospray–MS systems are

available which are useful for enrichment studies, but their operation at natural abundance levels will require a great deal of development. The availability of tested HPLC methods for natural abundance studies is however a necessary preliminary to developing an integrated HPLC–IRMS.

A further refinement in sample preparation is the isolation of particular atoms in a molecule for isotopic analysis. In many compounds this may not be practicable, and in compounds containing only one nitrogen this would not be necessary for nitrogen analysis. However organic molecules with many carbon atoms can sometimes be degraded by chemical or enzymic methods to reveal the isotopic signature of one or more of the constituent atoms. The ninhydrin reaction is used routinely to liberate the carboxyl carbon of amino acids in low level tracer studies (Scrimgeour *et al.*, 1988 B), and enzymic treatment has been used to degrade sugars to determine natural abundance variations (Scrimgeour *et al.*, 1988 A). Other examples are the pyrolytic decomposition of aromatic carboxylic acids to liberate the carboxyl carbon (Stantrock & Hayes, 1985) and the stepwise degradation of fatty acids (von Unruh & Hayes, 1975). Of relevance to the understanding of cellulose metabolism are the methods for the isolation of carbon, hydrogen and oxygen for isotope analysis from cellulose. For the first two elements this involves the preparation and purification of nitro cellulose and for oxygen the preparation of the pure carbohydrate before degradation to water or carbon dioxide for isotope analysis (Sternberg, 1989). The application of such methods to natural abundance studies clearly requires the dedication of a considerable effort in method development and routine sample processing.

In some instances, such as environmental research or large scale agricultural trials, chemical degradation, followed by IRMS will be necessary. In other cases, NMR will be more appropriate for identifying isotopic markers on chemical groups.

### 3.3 *Sulfur*

The discussion thus far has ignored the isotopic analysis of sulfur, which nevertheless fits into the general principles described for H, C, N and O. $^{34}S$ with an abundance of about 4.22 atom% is somewhat more abundant than the other isotopes described and the range of natural variation is somewhat smaller as a proportion of the fractional abundance being below 10% of the mean. $SO_2$ is the only form in which S can be measured with conventional gas IRMS, being near the upper end of the mass range of most instruments. The major problem with $SO_2$ is not the IRMS measurement, but the difficulty in preparing and handling pure $SO_2$. Serious problems due to adsorption on the surfaces of dual inlet systems can be minimized by heating the inlet and using only very pure dry sample gas, but this ideal is not easily achieved. CF–IRMS would seem to offer answers to these problems, as $SO_2$ can be prepared using conventional elemental analyser techniques, and the presence of a large excess of helium should flush the $SO_2$ through the preparation and inlet systems with the minimum opportunity for adsorption. These methods are under development, but have not yet been fully tested.