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SAMPLE PREPARATION TECHNIQUES IN TRACE ELEMENT ANALYSIS BY X-RAY EMISSION SPECTROSCOPY

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BY X-RAY EMISSION SPECTROSCOPY
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FOREWORD

This text on sample preparation technique in trace element analysis by x-ray emission spectroscopy has been written for I.A.E.A. under provisions of research contract 2947/RB and 3440/TC.

The text has been written with the aim of assisting laboratories introducing x-ray emission spectroscopy as an analytical tool with the most difficult problem in accomplishing this: mastering sample preparation techniques.

Many of the preparation steps have been used in the author's laboratory for over a decade. Many more have been used by researchers all over the world, and some of them have been described in detail, some only mentioned in the reference list. Many have been omitted, not because the author wanted that to do but because of being pressed into producing this manuscript (version 1.0). The author will be glad to accept any suggestions for addition, revisions or improvements of this text.

Let us help those laboratories who want to introduce x-ray emission spectroscopy and work with it.

Note: Mention of commercial products or company names does not constitute endorsement by the author or publisher.

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1. SAMPLING

1.1 Introduction

Sampling is often discussed because the greatest sources of error in many studies are usually in the sampling steps. As a general rule the analyst himself should be directly involved in the sampling procedure. In such a way it is easier to ensure that the samples are representative and that no significant changes in composition occur during sampling.

The major concern in sampling must be that the sample accurately reflects the variations in the material being sampled. Samples for laboratory assays may be selected on the bases of the capabilities of the analytical methods used: precision and accuracy, sensitivity, time considerations, costs, single versus multielement analyses - but special consideration must be given to specific media sampled. For example, greater homogeneity is often encountered in natural waters or in body fluids than in soils or tissues of organisms. Such characteristics always determine the sample sizes necessary to measure the variation accurately. The specific technique employed must be one capable of handling the analytical sensitivity required.

Great diversity in techniques and sampling procedures exist. For some cores general sampling designs have been developed. For example general sampling designs have been developed for environmental surveys and can serve as models for future programs (U.S. Geological Survey, 1972). Sampling plans involving large regions begin with a decision concerning the materials to be investigated from the lithosphere, hydrosphere, biosphere, or atmosphere. Increased detail can be ascertained from successive stages of sampling to a point determined by the demands of the problem or by the economics of the situation.

General and theoretical considerations of sampling are discussed in details by Sansoni and Iyengar (1980). In the process of chemical analysis only a small part of the total material is generally used to provide the analytical signal from which the concentrations of the components of interest are calculated. In general, a large sample is taken from the bulk material and transported to the laboratory (laboratory sample). Subsequently an aliquot is taken to provide a much smaller analytical sample. Of this, very often only a small fraction is actually used to produce the analytical signal. The sample and its subsamples must satisfy several requirements which can be listed as (Sansoni and Iyengar, 1980):

1. The mean composition of the laboratory and analytical samples should, in principle, be exactly the same as that of the bulk material to be evaluated (representative mean composition). However, this is an ideal condition which cannot usually be met in practice. Compromises are to increase the number of random aliquots to be analysed and to homogenize the bulk material before sampling.

2. The variance of the concentration levels within the laboratory and analytical samples should be the same as that of the bulk of material (representative variance).

3. The total error introduced during the entire sampling operations should be less than, or only of the same order of magnitude as, the error of the subsequent analytical procedure.

There are several sampling methods used. In random representative samples cannot be obtained by random sampling of inhomogeneous material unless the number of random samples taken is quite large. The overall error in this case largely depends on the degree of inhomogeneity.

For large samples that cannot be homogenized, it is necessary to use a sampling approach. This involves systematically sampling several constituent parts of a given material, e.g. by collecting samples from a population covering different age groups, sexes, geographical regions and nutritional habits.

In some cases the bulk material is made up of well-defined parts which are individually fairly homogeneous, e.g. different organs of the human body. The composition of the whole human body may be computed by analysing the individual homogeneous sections. This method is known as differential sampling. However, it is normally applied only to bulk materials that have clearly identifiable subdivisions and that cannot easily be homogenized in a single batch, as in the case of total body analysis (Sanson and Iyengar, 1980).

There are many papers written on this subject some of them are listed in the reference list. One of the important effects in sampling is caused by random particle distribution. This effect is based on the number of particles extracted per sample, i.e., the greater the number, the smaller the effect. Therefore, it can be generally stated that random particle distribution is of greatest influence when extracting small laboratory size samples having few particles. On the other hand, particle segregation exerts its greatest influence for bulk samples which cannot be conveniently mixed or processed through a device that eliminates particle segregation.

General sampling equation can be written as:

$$S^2 = A/W + B/N \quad (1.1)$$

where S^2 = the total variance for the system
A = sampling constant (random variance)
B = sampling constant (segregation variance)
W = size of the gross sample
N = number of samples collected

The random variance constant A is estimated by taking a series of small samples where the sampling variance is primarily due to random effects, and it is assumed that the segregation variance is negligibly small. Similarly, the segregation variance constant B is estimated by taking a series of large samples where the sampling variance is due primarily to segregation effects; and it is assumed that random effects are negligibly small.

Further, W and N in Equation (1.1) are in the nature of operating variables that can be manipulated within certain limits by the sampler. For instance after A and B have been determined and for a given S^2 and N, the sample weight, w, can be determined by substituting the equality $Nw = W$ into

Equation (1.1). This substitution gives--

$$w = A/(NS^2 - B) \quad (1.2)$$

Conversely, by specifying w , N can be determined. Therefore, the number and size of samples can be calculated for a specified sampling variance.

This approach is most applicable for the continuous analysis of similar lots of bulk material. This method is not, however, as helpful in estimating the expected sampling error for laboratory size samples.

Since segregation of particles in laboratory sampling can be largely eliminated, one needs to emphasize errors resulting from random particle distribution. Benedetti-Pichler considers this situation; and for a binary population Bernoulli Theorem yields the equation--

$$\tilde{\sigma}_p = \frac{d_A d_B}{d^2} (P_A - P_B) \sqrt{\frac{p(1-p)}{n}} \quad (1.3)$$

where $\tilde{\sigma}_p$ = the absolute standard deviation of the percent of component x in a mixture of particles A and B
 d_A = the density of the A particles
 d_B = the density of the B particles
 d = the weighted average density assuming all particles have the same volume
 P_A = the percent of the component x in the A particles
 P_B = the percent of the component x in the B particles
 p = the fractional number of the total particles which are type A
 $1-p$ = the fractional number of type B particles
 n = the total number of particles

This equation is idealized by assuming only two species of particles. However, this restriction can be overcome by considering that the components are either rich in x or poor in x . Equation (1.3) is also idealized by assuming that all particles are the same size.

Equation (1.3) can be modified to employ terms of particle size, sample weight, concentration, and density. For the simple case of two distinct particulate species of the same particle size, one obtains

$$S_E = (t_1 - t_2) \sqrt{W_1 W_2 \left(\frac{d_1 d_2}{d} \right) \left(\frac{V}{W} \right)} \quad (1.4)$$

where S_E = the absolute standard deviation of the concentration of element E
 t_1 = the weight fraction concentration of element E in species 1

t_2 = the weight fraction concentration of element E in species 2
 W_1 = the weight proportion of species 1
 W_2 = the weight proportion of species 2
 d_1 = the density of species 1
 d_2 = the density of species 2
 d = the weighted average density
 V = the volume of the individual particles
 w = the weight of sample taken

Let us consider the determination of trace elements in powder samples. If the element under consideration is a minor ingredient of one or more of the major constituents, its relative sampling error will be comparatively small because $(t_1 - t_2)$ will be very small. If on the other hand the trace ingredient is present as a major constituent of the species of minor abundance, its relative sampling error will be comparatively large. The latter situation would pertain to elements in certain mineral mixtures such as zirconium in beach sands where the zirconium is almost exclusively associated with individual zircon particles, gold in mineral sands, and metals in synthetic powder mixtures used to calibrate analytical systems for trace analysis.

For the case of more than two mineral species and with the simplifying assumption that all species have the same density, the following equation can be derived:

$$S_E = \left[(t_1 - t_2)^2 \frac{W_1 W_2}{n} + (t_2 - t_3)^2 \frac{W_2 W_3}{n} + (t_3 - t_1)^2 \frac{W_3 W_1}{n} \right]^{1/2} \quad (1.5)$$

Usually the interest is primarily to determine trace elements which are major components of species in minor abundance. In this case one can assume a binary system in which everything other than the species of interest is the second component.

For samples of wide particle size distribution the average volume, V , be replaced by a weighted average volume, \bar{V} , calculated from--

$$\bar{V} = \sum_{h=1}^k g_h v_h \quad (1.6)$$

where k = the number of groups of different particle size

v_h = the average volume of the individual particles of each group

g_h = the fraction by weight of the h group

One major drawback to the use of any estimate of particle volume is that, after grinding, different species are not likely to show the same particle size distribution due to differences in hardness and brittleness. For rock samples, minerals such as zircon, chromite, and magnetite which are harder than the bulk materials, may be concentrated in the larger fractions.

Since these coarse particles have the greatest influence on the standard deviation of sampling, such segregation could cause a serious discrepancy between expected and observed sample errors.

Another sampling equation sometimes used is:

$$s = \left[\frac{b}{p} \right]^{1/2} \quad (1.7)$$

where s = relative sampling standard deviation

b = weight of largest individual particle (assumed cubic)x100
weight of sample

c = weight percent of the analyte

1.1.1 Aerosol sampling

Aerosol sampling and analysis by the method of characteristic x-ray detection has received great attention in recent years. Sampling is usually done by collecting air particulates using a low volume total suspended particulate sampler.

Samples are usually collected on filters or by cascade impactors. As filter material Milipore, Nucleopore and other filters have been used. Some filter materials tend to be contaminated with elements of interest but are sometimes used when the sample is transferred to a solution for further analyses. Aerosol size fractionation is obtained by cascade impactors, where the aerosol to be sampled is drawn through jets of decreasing diameters.

An example of a commercially available impaction device is shown in Figure 1.1. This single impactor consists of five impaction stages in series plus an after filter. Air is taken in at the top and exhausted via a pump connected to the orifice at the bottom. The speed of the air flow is greater at each successive stage so that the largest particle ($\approx 4 \mu\text{m}$) impact on the surface below stage 1 and the smallest particles ($\approx 0.25 \mu\text{m}$) impact on the surface below stage 5. Very thin (50 to 100 $\mu\text{g}/\text{cm}^2$) polystyrene supported by glass impaction surfaces may be used to minimize the mass of the backing material of the sample. This device however does not produce a deposit of uniform areal density. Size fractionated samples contain information on aerosol generation mechanisms and are thus of interest in atmospheric chemistry and air pollution work.

Particulate matter can be filtered from the air and the filter examined directly by x-ray spectrometry. Detection limits of a few ng/cm^2 are now routinely achieved but require that background intensity be reduced to a minimum. To reduce the background care must be taken to remove sample supports or other material from the area which can be seen by the x-ray spectrometer; vacuum operation is necessary in order to eliminate air scatter. Usually the total sample is less than 1 mg/cm^2 and the filter is 1 to 10 mg/cm^2 which makes it the largest contributor to background intensity.

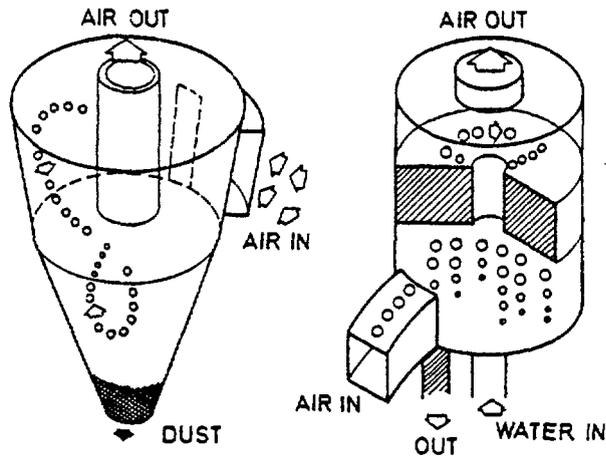


Fig. 1.1 The principle of a commercially available impaction device for aerosol sampling.

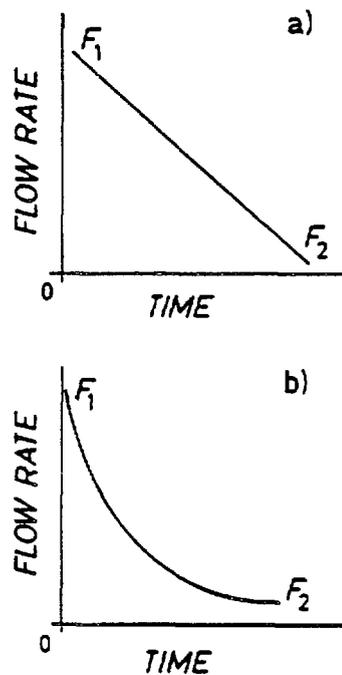


Fig. 1.2 Flow rate through a filter; (a) linear relationship caused by the loading of particulate matter on the filter device. (b) more realistic dependence of flow rate on time (after Morrison, 1967).

Each of components used in collection of air particulates needs to be considered individually since it may be a potential source of error. According to Morrison (1967) the components to be considered are: a filter holder, a filter media, a flow-measuring device or a volume totalizer such as a dry-gas meter, a timing device to record length of sampling time, and a pump.

A flow-rate device must be used in conjunction with a mechanism for recording the length of time of sample collection to allow for calculation of total quantity of air passed. This is a major source of error in the sampling process because a plot of flow rate versus time is not a linear relationship caused by the loading of particulate matter on the filter device. Figure 1.2 (a) (after Morrison, 1967) illustrates the assumed nature of flow versus time by this procedure. The determination of volume of air sampled is calculated by averaging the initial (F_1) and final (F_2) flow rates:

$$\frac{F_1 + F_2}{2} \times \text{length of time} = \text{volume of air sampled.} \quad (1.8)$$

Actually, the flow rate through a filter subjected to particulate buildup follows Figure 1.2 (b). The shape of the curve depends on the actual loading characteristics, which are the result of the total particulate burden of the air at any time t . It thus becomes clear that this is not a preferred method of estimating total volume of air sampled (Morrison, 1967).

A volumetric totalizer, such as a dry-gas meter, is not subject to errors of the extent described for the previous method and use of it probably represents the best method of measurement of air flow. However, this method will lead to incorrect estimates of the total volume unless certain precautions are taken. The major error lies in the change in temperature of the air being sampled and the resultant vacuum head produced between the filter head and the pump. If a vacuum recorder is placed in the airline to record continuously the pressure drop on the upwind side of the meter, appropriate mathematical corrections can be made. Another source of difficulty lies in the selection of a filter medium: because the particle-size distribution of suspended particulate matter in air ranges from submicron to very large, the experimental design must take this factor into account.

Any study designed to investigate the possible relationships between heavy metals in air and health or disease must employ the use of filter media that will have a high collection efficiency for submicron particles, because these sizes are respirable by man and animals. The foliar deposition and intake into the plants are also significant for these smaller-size particles (Morrison, 1967). The analysis of exposed filter paper poses another problem; it has been shown that metal particles are not uniformly distributed over the surface of the filter itself. This is presumably true of all airborne particles, making it necessary to ash the entire filter if reliable data are to be obtained. Kometani et al., (1972) have shown that particulate air pollutants collected on paper filters can be dry ashed at 500°C without serious loss of trace metals by volatilization. Conversion of metal salts to sulfates by the addition of H_2SO_4 prior to dry ashing ensures virtually complete recovery of the metals tested. Losses reported during dry ashing of particulate matter collected on glass fiber filters are not necessarily ascribable to volatilization as has commonly been supposed. Metals such as Pb, Zn, Cu, and

Cd react to varying extents with glass at high temperature to form insoluble metal silicates. Comparative studies of particulate matter collected on paper filters indicate that the results obtained by dry ashing compare favorably with those of accepted methods such as wet ashing and low-temperature ashing. Good recoveries of Pb, Cu, Zn, and Cd from samples were obtained by dry ashing at 500°C, for 1 hr even without the prior use of H₂SO₄.

Aerosol sampling is also discussed by Kemp and Møller (1981).

When determining the composition of an aerosol, two parameters are often of special interest: the time variations and the particle size distributions. Through the possibility of analysing very small amounts of material using x-ray emission spectroscopy, time sequential measurements can be achieved in a simple and handy way using a so-called "streaker", i.e. a sampler collecting a series of samples on a filter by moving the filter over a suction orifice (Nelson, 1977).

Valuable information about the normally "bimodal" size distribution of urban aerosols may be obtained by measuring only two size fractions, and discriminating the valley of the distribution, i.e., at a particle diameter of approximately 2 µm. Adding one more stage to the streaker may then increase the usefulness of the measurements considerably.

Compatibility with other samplers, e.g., standardized high volume samplers, is essential for intercomparing results from different projects. Due to the very different physical dimensions of the streaker from most other samplers, it is not obvious that this compatibility exists.

The aim in the construction of the streaker described in the following is to separate the two humps mentioned above and as far as possible to get compatibility with normally used high volume samplers. To get precise time resolution, streaking is performed in steps, thus creating discrete spots on the filters. The performance has been checked through simultaneous measurements in the field with a two-filter-high-volume sampler, fitted with a 12 µm pore size Nuclepore filter for collection of particles >2 µm (Heidam, 1979).

The main advantages of the streaker are the small size and the low power consumption. The air flow through the sampler, which is an important parameter determining its collection properties, is limited to approximately 1 l/min. In work by Kemp and Møller (1981) the air flow was chosen to be 0.58 l/min, which is well below the maximum capability of the pump used. The suction tube is open and points downwards (see Fig. 1.3). The diameter is 0.6 cm, giving an air velocity of 35 cm/s. The Reynolds number for the flow is approximately 150, which indicates that the flow is laminar. Compared to standard high volume samplers the velocity is approximately the same, but the very much smaller tube diameter may give rise to considerable differences in the collection efficiency for large particles.

An impaction stage is used for the size separation. The jet is formed in a linear slit having a length of 0.5 cm and a width of 0.025 cm. Using a channel length (i.e., the length of the tube having the slit cross section), which is long

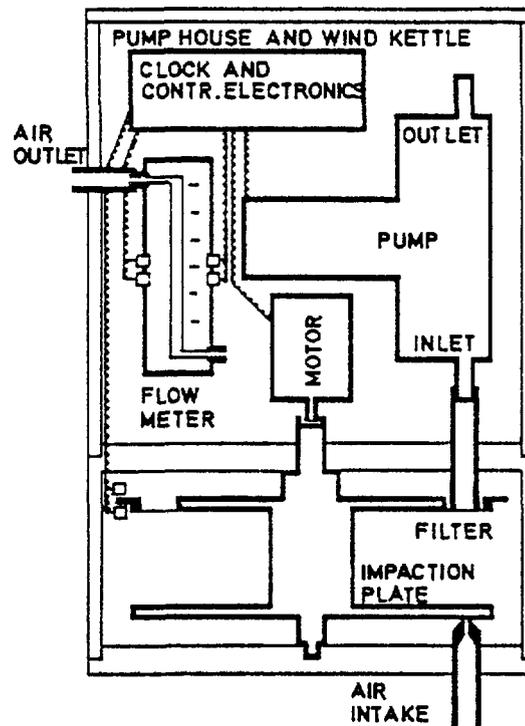


Fig. 1.3 Schematic of the device used by Kemp and Møller (1981) for aerosols sampling.

compared to the width, the air velocity in the centre of the slit is increased. In this way a cut at diameters of 2-2.5 μm is obtained, which would otherwise be difficult to reach with the low flow rate. However, since the boundary layers in the channel play an important role, the steepness of the cut-off might be somewhat smaller. Another factor which tends to diffuse the cut-off is that the distance between the slit and the impaction plate has to be rather high (≈ 0.1 cm) so as to reduce the effect of wobble in the plate position. The particles are impacted on a 12 μm thick mylar film coated with Apiezon L grease in order to avoid "bounce off".

Small particles are collected on a 0.4 μm pore size Nuclepore filter, covering a 0.25 cm x 0.75 cm oval suction orifice. The orifice has a smooth end, so the filter can pass over it without being damaged.

The filter and the impaction film are mounted on discs which can rotate around a common axis. The distance from the axis to the middle of the impaction jet and to the middle of the filter orifice is 3.75 cm. By rotating the discs it is possible to obtain 64 samples separated 5° from each other, on each film/filter. Exact positioning of the discs is obtained by pressing them towards a fixed stop during exposure. The exposure times are controlled by means of a quartz clock and the shifts are programmable in intervals between $\frac{1}{2}$ and 24 h in a 24 h cycle.

The pressure drop over the filter depends on its load, which makes flow stabilization necessary. Stabilization is obtained by regulating the supply voltage for the pump by means of a flow meter equipped with two sets of optocouplers "looking" at the flow meter ball. In this way, the flow is kept constant to within 5-10%.

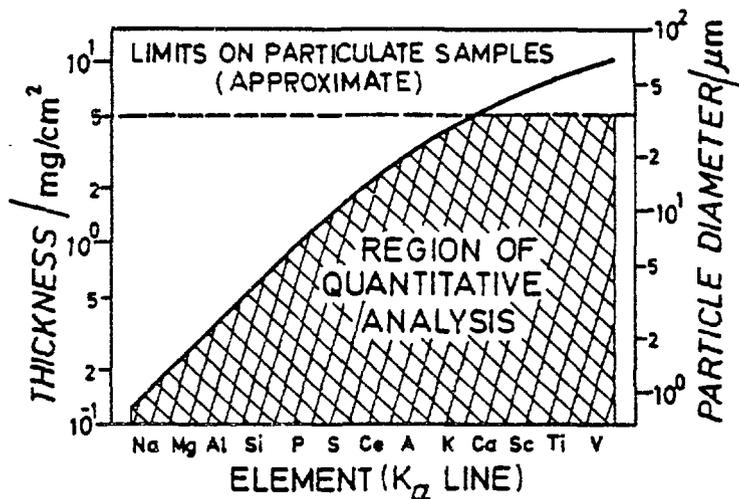


Fig. 1.4 Limits on total sample mass in aerosol sampling (Cahill, 1981).

The total power consumption of the streaker is approximately 3 W, with a 12 V supply voltage. More than 75% of the energy is used in the pump motor (Kemp and Møller, 1981).

Let us mention work by Adams and Grieken (1975) who have described a method for the evaluation of fluorescence radiation absorption in the analysis of air particulate material collected on depth filters and imperfect screen filters. The total absorption effect is divided into two components, one due to the particulate itself, and one due to the filter material. The first effect is calculated after evaluating the mass absorption coefficient by transmission measurements. The second correction is obtained by determining experimentally an hypothetical equivalent depth defined as the one hypothetical depth from which the particulate material would give rise to the same filter absorption effect as that obtained from the actual distribution. Measurements of the ratio of the fluorescent intensities obtained from the front side of the filter and from its back side allow this hypothetical depth to be calculated.

Cahill (1981) has discussed aerosol sampling for analysis by PIXE. Because of limiting penetrating power of charged particle beams used, only thin deposits of material, regardless of the penetrating power of the resultant characteristic x-rays, can be tolerated. Quantitative detection of these x-rays, however, causes even more severe restraints on total thickness. This is especially true for the elements sodium through calcium, elements extremely important for atmospheric physics and chemistry, comprising about 25% of the particulate mass. Fig. 1.4 illustrates the limits on total sample mass set by degradation of ion beam energy and concomitant changes in x-ray production cross-sections, and by x-ray attenuation corrections in mass layers and particles. One must also keep in mind the 65% or so of the mass in elements hydrogen through fluorine which PIXE cannot do quantitatively.

The only method certified in the United States for sampling of particulate matter is the high volume filter

sampler (Hi-Vol). Typical filters are about 500 cm² in area, so that ion beams sample no more than 0.5% of the collected matter and occasionally as little as two parts in 10⁵.

Large number of analyses are usually required together with both high time resolution and detailed size resolution. The characteristic temporal variations occur on several scales, from wind gusts (seconds) to diurnal patterns (hours) through synoptic behavior (days) to seasonal behavior scaled in months. The latter three are particularly informative, but to generate data at 2-hour resolution at one site with no size cuts for a year involves 4,380 analyses, entailing costs between about \$ 40,000 and \$ 100,00 (Cahill, 1981). However, diurnal patterns are often highly repetitive, and a limited number of such profiles, probably each season, might be adequate for many sites. Synoptic patterns are themselves characteristic of seasons, and seasons are among the most stable patterns since they average over many synoptic periods and hundreds of diurnal cycles.

These considerations also demand that one be careful to average over the shorter time cycles when interested in longer behavior. Thus, measuring for only 2/3 of a day may give misleading results, while sampling for two days might coincide with just one phase of a strong synoptic shift. Obviously, sampling in one season allows one to say little or nothing about annual cycles. One major time scale used for samplers involves the diurnal cycle, and such samplers generally use 2, 3, or 4 hour increments, with the Florida State streaker being a prime example of such a device matched to PIXE. Many devices use a 24 hour integration time to smooth out diurnal patterns, and in some circumstances occasionally allow 2 or 3 day periods in remote and/or stable conditions. The one-day-in-six 24 hour criterion used for many Hi-Vol networks defies statistical understanding even for seasonal averages. Ideally, one would like to take samples that would contain detailed time information that could be later used if desired. The rotating drum Multiday impactor used by the University of California, Davis, has such a capability for the $> 0.5 \mu\text{m}$ stages, as the ion beam is magnetically swept so as to average over 24 hours in a 2.5 cm strip. Resolution of 2 hours is easy for such samples, but since the filter switches every 24 hours, the size sample is incomplete.

Such considerations also hold for the streaker when a single time-averaged coarse stage filter is used to impose a 2.5 μm size cut on the device. Both the Multiday impactor and the streaker are being modified to give more complete time-size information, and these will allow detailed analyses of a single day if desired, while minimizing total numbers of analyses during routine monitoring. One might call such investigation "ex-post-facto experimentation", with the detailed analyses triggered by any unusual event that would benefit from detailed study in 2 hour increments, while normal analyses cover 24 hour durations (Cahill, 1981).

Compromises in size resolution are also aided by the systematic behavior illustrated by the bimodal distribution,

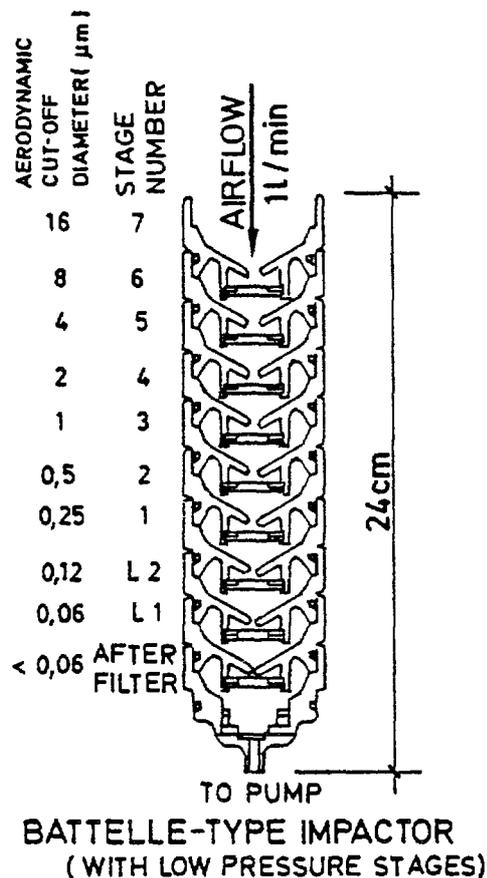


Fig. 1.5 Battelle type impactor with low pressure stages.

and by rejection of very large particles by the human nose. Thus, a natural first step would be to limit collection of very large particles that have minimal health effect, and such a limitation at 15 μm is proposed by the U.S. Environmental Protection Agency. This cut greatly aids PIXE (and XRF) as particle size corrections become very uncertain above 15 to 20 μm diameter. The next logical cut is at the minimum of the bimodal distribution, around 2 to 3 μm. Such a cut serves several purposes: it separates modes of different sources, making analysis both easier and more meaningful; it corresponds roughly to the partition between fine particles deeply penetrating the lung and the coarser particles lodged in nose, throat, and upper bronchial passages; it also puts minimal mass at the point of the cut, making results relatively insensitive to the shape of the separation function between coarse and fine. Four major devices operate at this cut, including a cyclone preseparator that eliminates the coarse particles, selective filtration through a nuclepore filter, physical impactors such as the Multiday, and virtual impaction. The latter depends on separation of air streams so that fine and coarse fractions largely end up on two filters.

The next cut that has proved useful lies at about the wavelength of light, $\approx 0.5 \mu\text{m}$. This cut bisects the accumulation mode into fractions that may scatter light differently, and a physical impactor is usually employed although other methods (spiral centrifuge), electrical methods, diffusion batteries) can be so utilized. There is a distinct difference between

particles above and below this cut in terms of both composition and impact upon visibility. The latter effect is predicted by Mie theory, as particles significantly smaller than the wavelength of light have minimal ability to scatter light.

Further size separations occur for the Lundgren impactor (5 fractions), the Batteile or Delron impactor (5 fractions), and the Anderson impactor (8 fractions), among others. Recently, Battelle impactors with low pressure stages have been designed and built, allowing 10 or so cuts down to 0.06 μm diameter, a diameter so small that mass values are falling rapidly with size (see Fig. 1.5). Even so, use of a filter is recommended, as in unusual circumstances, a significant mass mode at 0.03 μm , the condensation mode, can occur for short times in the presence of high temperature sources. The width of impactor separation functions set limits on the total number of cuts possible without serious overlap, and this number lies between 20 and 30 for the 0.05 to 15 μm range. This constraint of a fixed total number of analyses normally demands that best information is obtained when some size information is mixed with some time information, either through different devices (streaker and Battelle) or the same device (3 stage Multiday impactors), although exceptions can occur when size information is not required, or previously determined (lead from automobiles rarely varies much in size) (Cahill, 1981).

Table 1.1 lists the characteristics of a number of sampling devices heavily involved in PIXE analyses.

1.1.2 Water Sampling

The principal problems in water sampling consist of obtaining a representative sample, avoiding contamination and separating dissolved and particulate phases (Morrison et al., 1974). Many research groups have described sampling methods used. For example, often used sampling device consists of a Van Dorn bottle (sampling volume 3.51) made from PVC, that does not release any element in detectable amount to the water sample. Immediately after collecting, water should be transferred to polyethylene bottles previously cleaned with 10% nitric acid and rinsed with distilled water.

According to Morrison et al., (1974) very shallow streams, only a few meters wide, that are well mixed laterally as well as vertically may be sampled by dipping at mid-depth. Larger streams may require the composition of numerous samples according to some meaningful system. Each cross-section sample needs to be taken in such a way that it is velocity-integrated over the distance from the water surface to the water-bed interface. In larger streams the sampling is done from a bridge, cable, or boat.

The sample-bottle holders currently in use for sampling streams should have the brass intake nozzle replaced with one made of Teflon and the rubber gasket replaced with one made of silicone. It is important that the sampler be immersed in a flowing stream to wash off dissolved metals

Table 1.1
Particulate Samplers (after Cahill, 1981)

Unit (flow)	Size fractions	Volume/area ^{a)} (m ³ /cm ²)	Advantages	Problems	Improvements
High volume sampler (1500 l/min)	1	4	common, simple, cheap, legally acceptable	manual unit; no sizing; glass fiber filters	inlet control to 15 μm; teflon filters
Streaker (1 l/min)	1	2.2	simple, cheap, good time resolution, automatically, for 1 to 2 weeks	no sizing; clogging (?) inlet (?)	two stage sizing streaker
Virtual impactor (15 l/min)	2	2.7 (1.3)	sizing; two membrane filters	cost; manual; maintenance; particle loss in handling; clogging (?)	coated filters; automation
Stacked filter unit (10 l/min)	2	1.0 (0.5)	sizing; two membrane filters; simple and cheap	particle bounce/loss on coarse filter; manual	coated filters
Multiday impactor (30 l/min)	3	7.5/2.5F (2.5/0.8F)	extra cut at wave- length of light; automatic for 1 week	cost; maintenance	time variation for filter stage
Battelle impactor (1 l/min)	6	14 ^{b)} /0.9F (2.4/0.15F)	many size cuts	manual; heavy deposits may	low pressure stages

a) Volume of air (m³) per area of substrate (cm²) per 24 hour day, (values in parentheses are divided by the number of size fractions). F = filter stage for impactors.

b) assumes 0.1 cm² beam.

before inserting the sample bottle. With all plastic filters, filtration is best done in the field or as soon as practical. The membranes used for filtration should have a nominal pore size of no more than 0.1 μm . The actual particle size cutoff is unknown because, as sediment collects on the membrane, it generally filters more effectively than does the membrane itself (Morrison et al., 1974).

1.1.3 Soil Sampling

In soil sampling it is essential to recognize that soils are complex systems resulting from weathering processes. A soil profile reflects the magnitude of local weathering effects. Changes occur with depth in the distribution of organic matter in texture and structure, and clay contents (Morrison et al., 1974).

Some theoretical consideration of trace element sources, their redistribution by transport and deposition, and their behavior during weathering processes is essential in the evaluation of trace elements in soils. Models of soils need to be translated to real soils on landscapes that have properties important to a particular study. Soil survey maps are a means of locating potential sampling sites of specific soils. Natural soils rarely have sharply defined boundaries on a landscape and it is therefore necessary to check the actual soil to determine if it is suitable for study. Surface samples of soils are usually taken where information is needed about the role of soils on the trace element composition of shallow-rooted plants. Studies of trace element mobility and redistribution, whether from natural weathering or pollution, often require the collection of samples to greater depth. Only a few grams are usually needed for laboratory determinations. Samples are best collected from one face of a small hole. Because sampling tools are possible sources of trace metals, the use of a clean spade is preferable to the use of a soil auger, and-unless most samples are needed-the samples collected can be placed in a clean cloth bag.

For studies of most trace elements, it is necessary to break down soil aggregates. Riffle samplers may be used to reduce the amount of soil that might be fine-ground for trace element determinations. Sieves made of silk bolting cloth or nylon are free of trace metals and are useful for sieving ground-soil materials (Morrison et al., 1974).

When sampling rocks the methods must be designed to reduce the sampling error to a level commensurate with or less than the effects being studied. The sample must be ground to a sufficiently fine powder to yield an acceptable number of particles of each component of the heterogeneous material. This process introduces contamination, which must be minimized during the sample preparation.

1.1.4 Sampling of biological materials

Sampling of biological materials is discussed by many authors involved in trace element analysis. Processing and

storage of foods often result in drastic changes in trace element concentrations naturally present in plants. Some pertinent factors concerned with plant sampling are discussed below. It is stressed that in the plant sampling the number of individual plants needed to obtain a sample representative of the soil on which plants were grown. The species of plants collected and studied often differ as widely as do the kinds of soils on which they are grown. This problem was studied by Lazar and Beeson (1956). Six soil areas nearly chemically uniform and representing four soil series were used. Within each soil area, the individual plants were tagged and samples were collected from them over a 2-year period. At least five individual grass plants were needed to characterize the cobalt and copper status of soils when a grass was used, but the same information could be obtained by using leaves of two black-gum plants. Later, tests using leaves of black-gum (Kubota and Lazar, 1958; Kubota et al., 1960) showed that use of this plant can distinguish the cobalt concentrations of total, as well as extractable, forms of soil cobalt. Studies by Beeson and MacDonald (1951) of the effect of sampling dates on trace element concentration of alfalfa in New York showed that manganese, cobalt, and iron increase with the growing season. Marked initial decreases in trace element concentrations, however, were later observed by Loper and Smith (1961) in Wisconsin, also using alfalfa. When effects of high rates of fertilization used in the Wisconsin study (Loper and Smith, 1961) were reduced with cropping, the seasonal changes were essentially the same those of the New York study.

In terms of trace element concentration, plants appear to be species-distinct. Browse plants, herbaceous plants, sedges, and grasses grown in Alaska were all studied where they were growing in the surficial peaty mantle (Kubota et al., 1970). Marked species effects on the concentration of molybdenum were observed among species of common forage plants in Nevada, USA (Kubota et al., 1961).

The necessity for sampling specific parts of plants to identify trace element deficiencies or toxicities affecting growth of agricultural crops is well recognized. A comprehensive review was prepared, for example, by Tanaka and Yoshida (1970) to diagnose trace element deficiencies and toxicities using specific parts of the rice plant. Increases in trace element concentration with plant growth often result from increases in leaves of forage plants but not of stems or petioles (Beeson and MacDonald, 1951). Patterns of trace element changes evident in browse plants important for game animals (Kubota et al., 1970). Studies by Arkley et al., (1960) showed that trace elements carried in peat dust and deposited on plants are easily removed by washing, but those applied by sprays are not. Absorption of trace elements through leaf surfaces was noted as a factor. Surface absorption of trace elements by plants was also observed by Lagerwerff (1971) to be an important factor in increases caused by absorption of trace elements on leaf surfaces may also be enhanced with drying and changes in leaf-surface characteristics with plant maturity.

Sampling techniques and sample processing were examined some years ago in the study of vitamin levels in turnip greens (Southern Cooperative Group, 1951), but no systematic study has been made of sampling of fruits and vegetables for trace elements.

More attention has been paid to food processing and its effect on changes in trace element composition of fruits and vegetables. Losses of zinc and manganese from spinach, beans, and tomatoes in canning, as well as gains in zinc and manganese in canned beets, have been observed by Schroeder (1971). Peeling of some common fruits and vegetables has been found to result in losses of chromium, copper, and zinc, as well as lead (Cannon et al., 1972).

Sampling of animal and human tissues have been discussed in many papers.

Two types of sampling are used in experiments with animals and humans. In the first type the investigator uses experimental animals which can be sacrificed as necessary and dissected to produce whatever specific tissues are desired. Here replication of animals is common, total weight of samples is not limiting, and the analyst is generally present during the sampling. The major decisions involve selection of the specific tissue that will, on analysis, yield the most important data.

In the second type of experiment the animal or person being investigated must remain alive and experience minimum discomfort. Here the number of individuals is usually limited, and the analyst receives the samples from a nurse, technician, or other person who is generally not a part of the research team. In these instances, blood or serum, urine, hair, and perhaps needle biopsy materials are the tissues that are generally available. In this type of sampling, decisions include the kind of tissue or tissues most useful in characterizing the status of the subject with respect to the element in question and whether the data on environment, diet, health or disease, water supply, etc., are adequate for the purposes of the experiment. Very frequently the investigator is forced to use less than adequate samples rather than do nothing (Morrison et al., 1974).

Another decision in sampling under these conditions concerns contamination of samples during sampling. Blood samples drawn with stainless-steel needles may be unsatisfactory for chromium, and those drawn through rubber tubing may be unsatisfactory for zinc. Plastic urine containers may irreversibly absorb heavy metals. It would be useful for some central organization to sponsor the production and distribution of syringes, blood needles, and containers for blood and urine that would be suited to trace element studies on samples obtained from humans and domestic animals. A second desirable, but perhaps unattainable, feature would be the establishment of a uniform terminology suited to coding, for describing the environment, health and disease, diet, etc., of the subject sampled (Morrison et al., 1974).

Several authors have used following sampling method for fish and shellfish. Immediately after collection, samples of fish and shellfish should be frozen at -20 C. The dis-

section of the fish should be carried out with the organisms only partially thawed thereby obtaining muscle uncontaminated by drip and allowing easier cutting. As to shellfish shells should be allowed to open by gentle heating, taking care to avoid contamination of the soft tissues as much as possible.

In both cases dissection is to be carried out using plastic or quartz knives. The species should be chosen according to their taxonomic position and typical environment encountered in the region of interest.

Many procedures for tissue sampling have been described in the literature. Here we shall describe in some details procedure for human milk sampling as described by International Atomic Energy Agency (Byrne et al., 1979).

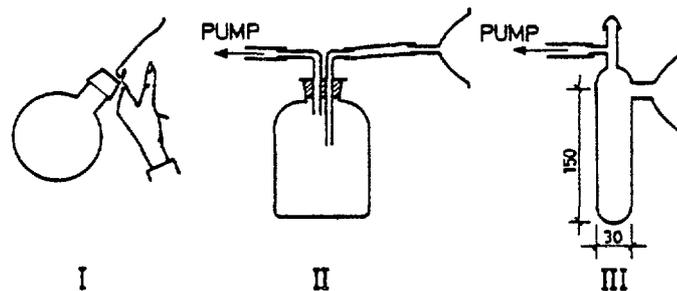


Fig. 1.6 IAEA/WHO sampling device for human milk.

Human milk should be sampled by direct expression into a 500 ml pyrex freeze drying flask (i), or via the IAEA/WHO device with a pump (iii) (see Fig. 1.6). Device (ii) is commonly used for withdrawing excess milk in maternity homes, but is not recommended, as the extra tubing and difficulties associated with cleaning the reservoir and its stopper increase the risk of contamination. This was confirmed in practise (Byrne et al., 1979).

All vessels and tubes were cleaned with a concentrated $\text{HNO}_3\text{-H}_2\text{SO}_4$ mixture and rinsed with doubly distilled water. After removal of excess water, drying is not necessary and only introduces further contamination risks. The nipple area of the breast was washed before sampling with soap and water, rinsed with distilled or de-ionized water and dried with a tissue. Ideally, both breasts should be emptied, and the sample mixed, or failing that, one breast. Protocol for this aspect have been established earlier (Byrne et al., 1979). However, some of the samples analysed in the study by Byrne et al. (1979), particularly of maturer milk, were not the whole contents of the breast, but represented excess milk from volunteers. In any case, for those elements of primary interest there, i.e. the "difficult" elements, magnitudes and methodological differences are of prime interest.

Very often trace element analysis is performed during autopsy studies. The following recommendations are useful to keep to a minimum the mineral contamination of, and the loss of, mineral elements from the samples.

1. Instruments used for the collection of specimens and their handling prior to the analysis must be acid-washed and cleaned with water of the highest purity. These instruments should be preferably of quartz, polyethylene, titanium and/or teflon; however, stainless steel instruments can also be used. (In case, the specimens cannot be collected following the recommended procedure, it should be the responsibility of the analytical laboratory to remove surface contamination before sampling the specimens for analysis).
2. Specimens should be collected as soon as possible after death (not more than 48h), suitably identified, and placed in sealed individual plastic bags. They must be kept frozen until analysis. (Hair samples need not to be kept frozen).
3. Clean metal-free plastic gloves should be worn during the handling of the samples which must be kept to a minimum.
4. No chemical fixatives should be used and samples should not be rinsed with water or any other medium, nor should they be pierced with a metal instrument.

Here are procedures for pre-analysis procedures for soft tissues, hard tissues and blood.

Soft tissues:

1. Allow the sample to thaw slowly in refrigerator for at least 24 hours. Remove plastic storage bags and allow the blood or any other fluid to drain. Obtain the wet weight. Carry out the entire procedure in a clean dust-free enclosure.
2. Using clean instruments and plastic gloves cut the samples into 2-cm cubes. The working surface should be a clean plastic sheet in a glove-box or laminar flow hood.
3. In cases where doubts exist as to the contamination of the specimen at collection the outer surfaces of the sample should be removed with the recommended instruments.
4. Homogenise sample cubes under liquid nitrogen using for instance the brittle fracture technique. Thoroughly mix the homogenate in clean tightly closed plastic containers. Store the homogenate at a temperature below -10°C .
5. Freeze-dry aliquots of homogenate prior to analysis and determine their dry weight; record the weight loss.

Hard tissues:

A. BONE

1. Allow the sample to thaw as outlined above for soft tissues. Obtain the wet weight.
2. Freeze the sample in liquid nitrogen and break up into small suitable pieces using instruments of

recommended material. Mix pieces and homogenise into powder using the brittle fracture technique, Mix the powder thoroughly and store at low temperature.

3. Aliquots are freeze-dried prior to analysis; care should be taken to record the weight loss.

B. HAIR

1. Wash hair (in a tied lock) successively once in acetone, thrice in water and once more in acetone. (Acetone should be of reagent grade and water of the highest purity.) Add sufficient amounts of the above solvents to cover the sample entirely. At each wash, allow the sample to stand at room temperature for 10 min in contact with the solvent with constant stirring. After each wash, decant the liquid and add fresh solvent. Carry out the washing in a dust-free enclosure (e.g. glove-box, laminar flow hood).
2. Allow the sample to air-dry over-night at room temperature between clean chromatography grade filter paper in a dust-free enclosure. Using plastic scissors or a titanium knife obtain the first centimeter (from the proximal end) sections of the lock.
3. Homogenise the proximal end sections obtained as described above into powder. One effective procedure is the brittle fracture technique. About 2g of hair are placed in a teflon container along with a teflon ball, and the lid is closed tightly. The container is cooled in liquid nitrogen for 3 min and then vibrated at 3,000 cycles per min for 2 min using a "micro-dismembrator" (B. Braun Melsungen AG, Melsungen, FRG). fine hair powder is obtained by repeating the procedure thrice. Mix the powder thoroughly and store at room temperature in a clean plastic container closed tightly.

BLOOD:

Elements of interest should be analysed in whole blood, plasma and blood cells. Blood should be collected by venipuncture using a heparinised evacuated blood collection tube (e.g. Venoject, Terumo Corp., Tokyo, Japan) to minimise metal contamination. Collected blood samples should be stored at a cold temperature to avoid microbial action. Blood cells and plasma should be separated by centrifugation.

1.1.5 Sampling of petroleum and its products

Sampling, which precedes analysis, should be carefully planned and executed because its quality determines the value of everything that follows. Generally, in the first step, a laboratory sample is removed from the bulk product to be analysed. The second step includes storage, transportation to the laboratory, with or without addition of some preservatives. In the third step this sample is then divided into the analytical subsamples.

The quantitative results generated from an analytical subsample are limited by how well the subsamples represents the bulk. Trace elements are generally not uniformly distributed in nonhomogeneous materials as most fuel oils. A trace element of interest in fuel oil may be associated with the particulate matter of sediment, with the solution, or both. Highly sensitive instrumental techniques often require a very small specimen, thereby increasing the danger that the specimen being analysed may not constitute a representative portion of the original sample.

Some of the recommended methods for the bulk sampling of petroleum and its products have been described in the following documents:

1. American Society for Testing Materials, Manual on Measurement and Sampling of Petroleum and Petroleum Products, 1957 Methods D270-57T, D923-56, D1145-53.
2. Institute of Petroleum (London), Standard Methods for Testing Petroleum and Its Products, Method 1P 60/61.
3. American Petroleum Institute, API Standard 2500, Measuring Sampling and Testing Crude Oil.

Although these provide sound guidelines, in practice the sampling of petroleum and related materials, as with other products, is largely a matter of sound judgment. In petroleum trace analysis, the problem of sampling often resolves itself to one of avoiding contamination (Milner, 1963). Let us describe the sampling method used by Witherspoon and Nagashima (1975):

Crude oil samples were collected in two-quart Mason jars by attaching a short length of small rubber hose through appropriate connections to a 2.5 cm (1 inch) gate valve normally present at the wellhead of any producing well. After the sample line was connected, the gate valve was opened so that the amount of oil and water being produced could be observed and the line purged. If the water-oil ratio was above approximately, 1.0, the produced fluids were first collected in a glass separator, the water drained off, and the oil poured directly into the sample bottle.

If the water-oil ratio was less than 1.0, the produced fluids were collected directly in the sample bottle. Each sample bottle was covered with aluminum sheet foil and the metal cap screwed on. Occasionally it was necessary, depending on atmospheric temperatures, to loosen the cap and to vent any natural gas that had been released. The crude oils were stored in these containers until investigated.

In the studies and in the monitoring of water pollution by oil, sample collection is also very important. A relatively large sample makes possible more accurate analysis and is likely to be more representative than a small sample. About one liter of the original petroleum sample should be collected along with similar amounts of oil from the water's surface and, if the oil reaches, from beaches and shores. Water collected with the oil should be kept at a minimum, to reduce bacterial activity.

Commonly available devices for collection of oil are described by Kawahara (1969). When the amount of spilled oil is appreciable, skimming oil into a container is recommended. Thin oil films can be collected using treated glass cloth. In all cases care must be taken to avoid contamination, and data on the collection location, conditions, date, time, and persons should be recorded.

Preservation procedure involves the containment of low boiling components and protection against oxidation and microbial attack. Procedure depends on the amount of water mixed with the oil. Crude oils and products containing less than 3% water can be preserved by sealing in glass bottles and storing at ambient temperature, upright, and in the dark (Kawahara, 1969). This will usually be the case with crude oils and products from tankers and pipelines. Petroleum from slicks and producing wells may contain a significant amount of water.

The bulk of the water should be removed and the sample container frozen and maintained at -20°C until the time of analysis to minimize bacterial alteration of the oil. An alternative procedure is to add about 100 ppm of mercuric chloride to the water phase to inhibit bacterial activity. The sample can then be stored at ambient temperatures. This alternative procedure may be preferred for oils that may become heterogeneous when held at -20°C .

Low-molecular-weight hydrocarbons can readily escape to the atmosphere. Rubber seals should be used on the sampling devices when the waters are to be analyzed for low-molecular-weight hydrocarbons. The addition of mercuric chloride or sodium azide is recommended before capping the bottle. The problems connected with the determination of high-molecular-weight hydrocarbons are more difficult to solve. Extreme care must be taken to use proper sampling equipment and carefully cleaned collection and storage vessels. Sampling devices should be constructed of glass, stainless steel, and/or Teflon. U.S. National Academy text on Petroleum in the Marine Environment (1975) prescribes the following procedure:

The retrieved samples should be transferred through clean Teflon tubing into sample bottles that have been cleaned previously with carbon tetrachloride. The bottles should be from 0.5 to 20 liters, depending on the sensitivity required, and an appropriate volume of hydrocarbon-free CCl_4 should be added to each sample. For certain analyses, it will also be appropriate to add hydrocarbon-free sodium chloride and hydrochloric acid.

The collection bottles should be capped immediately, using a clean Teflon liner, and the sample should be stored upright. Immediately after collection, the sample should be shaken in order to initially extract the hydrocarbons into the CCl_4 .

1.2 Sample storage

Storage of the sample obtained after the sampling procedure may have several purposes. First, the sample may have to be kept awaiting sample preparation and the following analytical steps if these cannot be carried out immediately after sampling. This is important in long-term investigations when it is desired to analyse all the samples in one series. Second, it may be necessary to prepare and store duplicate samples (identically prepared aliquots), one of which remains with the user of the analytical data for independent cross-checking at a later time, if necessary. Storage of duplicate samples for a definite interval is also necessary sometimes in forensic and clinical analysis for legal reasons. Analytical reference materials are also often produced in very large quantities and have to be stored for years. A last argument for long-term storage is the preservation of characteristic ecological, environmental or biological samples as specimen banks for use in future monitoring investigations (Sansoni and Iyengar, 1980).

For some samples the problems are not difficult. However, for biological samples there are number of difficulties. In many cases there is even a maximum acceptable storage time. It depends on the organ or tissue in question.

The storage temperature may be 2-4°C for short-term storage but should be $\leq -15^{\circ}\text{C}$ for longer preservation.

The total surface area of the container and the free space in it should be kept to a minimum. Containers with non-porous, smooth and non-wettable surfaces are generally preferable. As far as possible, whole organs or tissues should be stored without dividing them into smaller parts. The larger the amount of sample stored in the container, the less significant will be the influence of container surface.

Containers with non-wettable walls (teflon, polyethylene, etc.) are commonly used. Surface preconditioning, especially of quartz and glass containers, can be carried out by mineral acids such as HNO_3 , HCl and diluted HF , by chelating reagents (EDTA) and oxidants (H_2O_2), followed by thorough rinsing with demineralized distilled water (Sansoni and Iyengar, 1980).

These authors also list some common materials used for containers in decreasing order of importance in each group.

Polymers: polyfluorohydrocarbons (teflon, kel-F, tetzal, halar, etc.), polyethylene (high-pressure PE generally preferred to low-pressure PE), polypropylene (hostalen, hostafon, etc.), silicone rubber (one of the purest rubbers, but contamination risk by Zn is reported), polymethyl methacrylate (plexiglas, perspex; relatively low in trace element impurities). Glasses: ultrasilica (synthetic quartz), borosilicate glass. Metals: High-purity aluminium foil, platinum, high-purity titanium, etc.

The goal of any storage technique is the maintenance of sample integrity. Considerations of the container material are necessary regarding absorption from solution on the walls, leaching from the walls, loss through volatilization, degradation through photochemical or biological activity, and other factor. At present, such relatively inert materials

TABLE 1.2
TRACE ELEMENT IMPURITIES IN LABORATORY-WARE MATERIALS

Element	Glass	Polyethylene (high pressure)	Process unknown	Tygon	Plexiglas	Synthetic quartz	Teflon
	g/g	ng/g	ng/g	g/g	ng/g	ng/g	ng/g
Al	100000 ^a	80-3100	230-3000	55			
As						0.17	
Br			186			70	
Ca	1000 ^a	200-20000	200	5			
Cd						0.38	
Cl		1600	800-3000			3700	
Co	0.082	5	0.07-0.31		0.05	0.33	0.33-1.70
Cr		15-300	19-76	6	10	1.60	30
Cs	0.12		0.05		0.06	0.12	0.05
Cu		4	6.60-17	10	9.50	2.00	22
Fe	3000 ^a , 280 ^b	600-2100	10500	50	140	160	35
Hg						0.03	
K	3000 ^a					500	
Mg	600 ^a	80-1500		120			
Mn	1000 ^a	10	510	2	32		
Mo						0.40	
Na	300000 ^a	170-10000	44-25000				2500-5000
Ni				200		50	
Pb		200	200	200			
Rb						2.43	
Sb	2.92	5	0.18		0.01	0.40-3.80	
Se			43		0.002	0.70	
Si	400000 ^a	2000	2000	100			
Sr		800					
Th			3			0.46	
U			840				
Zn	0.73	90	28	5	10	34	8

^aPyrex.

^bBorosilicate.

TABLE 1.3
CONTAMINATION OF SOME MINERAL ACIDS BY LEACHING OF
CONTAINER WALLS DURING EVAPORATION

Element leached	HCl			HNO ₃			HF			
	Glass (ng/ml)	Poly- ethylene (ng/ml)	Poly- propylene (ng/ml)	Teflon (ng/ml)	Quartz (ng/ml)	Poly- ethylene (ng/ml)	Teflon (ng/g)	Quartz (ng/ml)	Poly- propylene (ng/ml)	Teflon (ng/g)
Al	10	3	0.1	0.3	10		2	20	0.54	3
Br						38				
Ca				5	60		4	60		1
Cd			0.03						0.0007	
Co				0.02		2.6	0.2			
Cr		0.02	0.03	0.9	0.6		7			0.4
Cu	3	4	0.01		11	160	0.01		0.2	0.4
Fe		0.7	0.6	4	10		14	20	0.3	3
Mg	7		0.02	3	10		7	20	0.12	3
Mn		0.005	0.001	0.5	0.4	50	0.2	0.6		0.1
Na	90	2			30	7.5				
Ni		0.03	0.01	0.3			1.0		0.02	0.4
Pb	2.30		0.06	440	0.5			1	0.03	0.1
Si	30	0.8	0.4	1			8			
Sn					0.4					
Ti		0.2	0.07	0.04	2					2
V							0.76			
Zn		7	0.02	0.04			0.04		0.1	

as quartz, teflon, polyethylene, hard glass, and so forth, have both attractive and undesirable attributes. An overriding consideration may be the duration of storage; short-term requirements clearly differ from those of longer periods.

Table 1.2 shows usual trace element impurities in some of often used laboratory-ware materials. Sometimes acids are used for sample preparation for storage. Contamination of some mineral acids by leaching of container walls during evaporation is shown in Table 1.3.

The systematics of storage have not as yet been adequately explored. The increasing need for baseline studies demands an evaluation of currently used storage methods. There are difficulties in predicting future disposition of stored samples, whether it be analysis of yet unconsidered species or the extension of present assays through enhanced capabilities of analyses.

During sample storage some changes in mean trace element composition of a sample may result from physical or chemical changes. The most often encountered change is the change in sample weight due to loss of water, if no special precautions are taken. This is a difficulty often experienced with biopsy samples of soft tissues. The necessary precautions are storage in closed systems, freezing at the sampling site, or repeated weighing of the sample as a function of time followed by extrapolation of the weight to the sampling time. In contrast, the residue from ashing or evaporation may absorb water from the surroundings.

Segregation of a heterogeneous mixture of solid particles of different size, shape and density can cause a considerable change in composition if aliquots are taken without prior re-mixing (Sansoni and Iyengar, 1980).

During reconstitution of frozen fluids by thawing, the protein part tends to form small lumps resulting in concentration gradients if the solution is not mixed thoroughly. Since many metals are bound to proteins or are components of specific enzymes, inhomogeneity in the fluid will affect the accuracy of the determination. Chemical processes that may result in changes in mean composition are hydrolysis, oxidation (e.g. decomposition), haemolysis of whole blood, denaturation of proteins by excess heating or chemical reagents, fermentation, photochemical reactions and microbial attack, e.g. fungus growth (Sansoni and Iyengar, 1980).

During sample storage loss of some elements may occur due to adsorption on container walls and tools particularly for low-level trace elements in body fluids.

With occasional exceptions quartz, teflon and high-purity polyethylene containers are generally suitable for storing dilute solutions, e.g. those used in the preparation of standards. Interactions between trace elements in dilute solution and various container materials have been reported for various elements (see list of references).

1.2.1 Losses from water by sorption on surface

Various studies have been published dealing with sorption phenomena in relation to matrix composition, concentration and chemical form of elements being determined, nature of container material, contact time, and addition of complexing agents and acids. Here, we shall describe the work by Masse et al (1981) on sorption behaviour of selected trace elements in distilled water and artificial seawater using polyethylene, poly tetrafluorethylene (PTFE; teflon) and borosilicate glass as typical container materials. The reagents and materials used in their work were described as: distilled water was prepared by distillation in quartz of demineralized water. For the preparation of artificial sea-water the following reagents were dissolved in 10 l of distilled water: 69,9 g of $\text{MgSO}_4 \times 7\text{H}_2\text{O}$, 50.5 g of $\text{MgCl}_2 \times 6\text{H}_2\text{O}$, 14.8 g of $\text{CaCl}_2 \times 2\text{H}_2\text{O}$, 1.00 g of KCl, 1.77 g of Na_2CO_3 , and 268 g of NaCl. Batches of distilled water and artificial sea-water were adjusted to pH 1, 2, 4 or 8.5 by suitable additions of nitric acid or sodium hydroxide.

The following radiotracers were used: ^{110}Ag (1.2 mCi mg^{-1}), ^{74}As (0.9 mCi mg^{-1}), ^{109}Cd (0.27 mCi mg^{-1}), ^{75}Se (3.8 mCi mg^{-1}), and ^{65}Zn (1.3 mCi mg^{-1}). From these radionuclides stock solutions were prepared at radioactive concentrations of 5-10 $\mu\text{Ci ml}^{-1}$ and a mass concentration of 10^{-4} M by adding amounts of the corresponding stable elements. The acidity of the stock solutions was adjusted to pH 2 by adding nitric acid.

The containers tested were 200-ml borosilicate glass bottles 100-ml high-pressure polyethylene bottles and 100-ml polytetrafluoroethylene bottles. New bottles were used exclusively. The differences in specific surface values were achieved by adding pieces of the material considered. To avoid the possibility of highly active sites for sorption arising from fresh fractures, the edges of the added pieces of borosilicate glass were sealed in a flame. Prior to the use of all materials, the surfaces were cleaned by shaking with 8 M nitric acid for at least 3 days and by washing five times with distilled water.

Specific surface is defined as the ratio of the inner container surface in contact with solution to the volume of the solution, denoted by $R(\text{cm}^{-1})$.

The following procedure was used by Masse et al., (1981): working solutions (1 l) which were 10^{-7} M in one of the elements to be studied were prepared by appropriate addition of the radioactive stock solutions to pH-adjusted distilled water and artificial sea-water. After the pH had been checked, 100-ml portions were transferred to the bottles to be tested. The filled bottles were shaken continuously and gently in an upright position, at room temperature and in the dark. At certain time intervals, ranging from 1 min to 28 days, 0.1-ml aliquots were taken. These aliquots were counted in a 3 x 3-in. NaI(Tl) well-type scintillation detector, coupled to a single-channel analyser with a window setting corresponding to the γ -rays to be measured. The counting times were chosen in such a way that at least 15 000 pulses were counted. The sorption losses were calculated from the activities of the aliquots and the activity of the aliquot taken at time zero.

Results obtained by Masee et al., (1981) are shown in Tables 1.4, 1.5 and 1.6. In these tables the percent loss of Ag, Cd and Zn from distilled water and artificial seawater stored in different containers is shown. Losses smaller than 3% are not indicated.

Here are the results for different elements as described by Masee et al., (1981).

Silver: At pH 1 and 2 sorption from either distilled water or artificial sea-water was not observed for any of the types of container materials. However, from distilled water at pH 4 silver was substantially sorbed on polyethylene, borosilicate glass, and PTFE. In polyethylene, silver was almost completely lost; in the case of larger R values the rate of loss increased. In borosilicate glass, inconsistent behaviour was observed which could not be explained, e.g. the percent sorption at pH 4 for $R = 1.0 \text{ cm}^{-1}$ and $R = 4.2 \text{ cm}^{-1}$ after 24 h. After 28 days the loss of silver appeared to be independent of the R value considered. In PTFE, silver was stable in solution up to 24 h in distilled water. At the end of a 28-day storage period the loss of silver in the PTFE vessel with $R = 5.5 \text{ cm}^{-1}$ was almost 4 times higher than in the vessel with $R = 1.0 \text{ cm}^{-1}$.

From artificial sea-water at pH 4, losses of silver were observed only in borosilicate glass containers. At pH 8.5, silver was sorbed from both distilled water and artificial sea-water regardless of the container material. The same anomaly as in distilled water was observed, though to a lesser extent.

Cadmium: At pH 1 and 2, there was no significant sorption for any of the three container materials. At pH 4 sorption of cadmium was observed only from artificial sea-water stored in borosilicate glass. In distilled water at pH 8.5, cadmium was lost onto all three materials, whereas sorption of cadmium was not observed from artificial sea-water. In all cases, the amount of cadmium sorbed increased with increasing R value.

In general, the results of this study indicate losses of cadmium lower than those reported in the literature. However, unambiguous conclusions cannot be drawn because of the lack of information on various parameters, especially about the specific surface. This parameter seems to be critical in considering the sorption phenomena of cadmium, as may be seen from Table 1.5. Although the pH seems to be the dominant factor in preventing sorption of cadmium, it should be noted that at pH 8.5 and in the case of the highest R value cadmium was substantially sorbed from distilled water, whereas sorption was not observed from artificial sea-water. The absence of sorption of cadmium from sea-water may be explained either by the formation of chloride complexes, analogously to silver, or by competition between cadmium and other bivalent ions (Mg, Ca) in occupying active sorption sites.

In general, it may be concluded that for the determination of trace concentrations of cadmium, the sample has to be acidified to pH 4 and it is usually advantageous to use polyethylene or teflon containers.

Table 1.4

Sorption behaviour of silver (after Masee et al., 1981)

Matrix	Distilled water												Artificial sea-water											
Material	Polyethylene				Borosilicate glass				PTFE				Polyethylene				Borosilicate glass				PTFE			
pH	4		8.5		4		8.5		4		8.5		4		8.5		4		8.5		4		8.5	
R(cm ⁻¹)	1.4	3.4	1.4	3.4	1.0	4.2	1.0	4.2	1.0	5.5	1.0	5.5	1.4	3.4	1.4	3.4	1.0	4.2	1.0	4.2	1.0	5.5	1.0	5.5
Contact time	Sorption (%)												Sorption (%)											
1 min	-	8	-	12	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	-	-	-	-
30 min	7	12	19	33	-	3	6	19	-	-	-	10	-	-	7	8	-	-	-	-	-	-	-	3
1 h	10	15	25	36	-	4	9	21	-	-	-	10	-	-	6	5	-	-	3	3	-	-	-	4
2 h	12	20	36	44	3	5	15	27	-	-	3	13	-	-	10	9	-	-	3	5	-	-	-	6
4 h	14	28	45	49	4	8	18	35	-	-	3	14	-	-	14	13	-	-	3	4	-	-	-	7
8 h	17	42	51	49	9	12	23	42	-	-	4	18	-	-	16	18	3	4	5	10	-	-	-	8
24 h	25	66	72	49	32	18	26	48	4	6	5	25	-	-	24	28	4	4	6	9	-	-	6	12
2 d	30	83	76	60	72	31	28	58	3	5	7	24	-	-	35	36	6	7	10	31	-	-	13	17
3 d	37	91	75	74	83	56	65	56	5	18	9	26	-	-	44	45	6	11	31	80	-	-	13	23
7 d	51	88	66	78	84	79	70	60	6	32	11	27	-	-	64	64	74	60	27	73	-	-	14	29
14 d	83	95	61	98	85	70	70	57	7	55	19	28	-	-	66	72	81	76	39	84	-	-	20	30
21 d	95	100	59	100	84	75	72	64	10	54	20	27	-	-	58	77	80	73	39	64	-	-	26	37
28 d	96	100	59	100	82	80	72	63	15	55	22	28	-	-	46	78	82	71	40	67	-	-	27	37

^a Denotes a loss smaller than 3%.

Table 1.5
Sorption behaviour of cadmium (after Masee et al., 1981)

Matrix	Distilled water												Artificial sea-water											
Material	Polyethylene				Borosilicate glass				PTFE				Polyethylene				Borosilicate glass				PTFE			
pH	4		8.5		4		8.5		4		8.5		4		8.5		4		8.5		4		8.5	
R(cm ⁻¹)	1.4	3.4	1.4	3.4	1.0	4.2	1.0	4.2	1.0	5.5	1.0	5.5	1.4	3.4	1.4	3.4	1.0	4.2	1.0	4.2	1.0	5.5	1.0	5.5
Contact time	Sorption (%)												Sorption (%)											
1 min	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30 min	-	-	5	32	-	-	5	32	-	-	8	36	-	-	-	-	-	-	-	-	-	-	-	-
1 h	-	-	7	69	-	-	6	26	-	-	7	38	-	-	-	-	-	-	-	-	-	-	-	-
2 h	-	-	-	70	-	-	8	26	-	-	5	53	-	-	-	-	-	-	-	-	-	-	-	-
4 h	-	-	-	70	-	-	8	26	-	-	5	58	-	-	-	-	-	-	-	-	-	-	-	-
8 h	-	-	-	59	-	-	8	29	-	-	6	57	-	-	-	-	-	-	-	-	-	-	-	-
24 h	-	-	-	47	-	-	10	32	-	-	10	48	-	-	-	-	-	-	-	-	-	-	-	-
2 d	-	-	-	30	-	-	10	32	-	-	8	45	-	-	-	-	-	-	-	-	-	-	-	-
3 d	-	-	-	29	-	-	11	30	-	-	12	43	-	-	-	5	-	-	-	-	-	-	-	-
7 d	-	-	-	31	-	-	9	31	-	-	13	46	-	-	-	14	40	-	-	-	-	-	-	-
14 d	-	-	-	30	-	-	-	-	-	-	14	44	-	-	-	13	43	-	-	-	-	-	-	-
21 d	-	-	-	30	-	-	-	-	-	-	15	45	-	-	-	15	41	-	-	-	-	-	-	-
28 d	-	-	-	31	-	-	-	-	-	-	15	46	-	-	-	14	36	-	-	-	-	-	-	-

^aDenotes a loss smaller than 3%.

Table 1.6
Sorption behaviour of zinc (after Masee et al., 1981)

Matrix	Distilled water												Artificial sea-water											
Material	Polyethylene				Borosilicate glass				PTFE				Polyethylene				Borosilicate glass				PTFE			
pH	4		8.5		4		8.5		4		8.5		4		8.5		4		8.5		4		8.5	
R (cm ⁻¹)	1.4	3.4	1.4	3.4	1.0	4.2	1.0	4.2	1.0	5.5	1.0	5.5	1.4	3.4	1.4	3.4	1.0	4.2	1.0	5.5	1.0	5.5	1.0	5.5
Contact time	Sorption (%)												Sorption (%)											
1 min	-	-	-	-	-	-	21	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30 min	-	-	-	66	-	-	24	21	-	-	5	12	-	-	-	-	-	-	12	31	-	-	-	-
1 h	-	-	-	65	-	-	23	22	-	-	3	16	-	-	-	-	-	-	9	31	-	-	-	-
2 h	-	-	3	64	-	-	24	21	-	-	5	22	-	-	-	-	-	-	10	29	5	-	-	-
4 h	-	-	3	60	-	-	25	21	-	-	4	28	-	-	-	-	-	-	9	30	4	-	-	-
8 h	-	-	5	58	-	-	25	23	-	-	3	33	-	-	-	-	-	-	9	28	5	-	-	-
24 h	-	-	8	56	-	-	26	22	-	-	5	27	-	-	-	-	-	-	5	26	4	-	-	-
2 d	-	-	9	52	-	-	25	20	-	-	3	25	-	-	-	-	-	-	4	21	4	-	-	-
3 d	-	-	6	53	-	-	20	22	-	-	3	19	-	-	-	-	-	-	4	18	5	-	-	-
7 d	-	-	11	57	-	-	-	-	-	-	4	20	-	-	-	-	10	4	3	9	4	-	-	-
14 d	-	-	11	57	-	-	-	-	-	-	5	20	-	-	-	-	27	19	-	10	5	-	-	-
21 d	-	-	10	55	-	-	-	-	-	-	4	20	-	-	-	-	25	17	3	9	5	-	-	-
28 d	-	-	12	56	-	-	-	-	-	-	6	20	-	-	-	-	20	19	4	9	5	-	-	-

Zinc: At pH 4 losses of about 20% of zinc were observed from artificial sea-water stored in borosilicate glass. There was no apparent relation between the R value and the size of the losses. At pH 8.5 zinc was lost from distilled water in all container materials. The rate of loss in polyethylene and PTFE increased with increasing R value. In the case of borosilicate glass, an immediate loss of 20% was observed, whereas after 7 days all the initially sorbed ^{65}Zn activity was found to be in solution again. At pH 8.5 artificial sea-water showed some loss of zinc in polyethylene after 28 days. In borosilicate glass similar effects were observed for sea-water and distilled water, i.e., a decrease of loss with increasing storage time.

Arsenic and selenium: For arsenic (added as sodium arsenate) and selenium (added as sodium selenite), losses were insignificant in all the container materials considered, irrespective of matrix composition. For arsenic, literature data on sorption from aqueous matrices could not be found. For selenium, a some authors have mentioned small losses.

It was concluded that the sorption behaviour of trace elements depends on a variety of factors which, taken together, make sorption losses rather difficult to predict. However, the data from this study and from the literature indicate for which elements sorption losses may be expected as a function of a number of factors, such as trace element concentration, container material involved, pH, and salinity.

1.3 Sample fragmentation, powdering and homogenization

The preparation of samples for trace element analysis generally involves fragmentation of the bulk material, grinding to the desired particle size, and homogenization. Iyengar and Sansoni, (1980) have discussed the procedures in the case of biological samples.

Fragmentation and powdering of soft tissues can be accomplished by conventional means, provided that sufficient precautions are taken to avoid contamination. A particularly useful apparatus is a "microdismembrator" comprising a teflon vessel in which the sample is vibrated rapidly, together with a teflon-covered metal ball, at liquid-nitrogen temperature. Other suitable materials for grinders and homogenizers include ultrapure quartz, polymethylmethacrylate, high pressure polyethylene and high-purity titanium.

Fragmentation and powdering of hard tissues such as bone and teeth present formidable problems for the trace element analyst.

Homogenization involves not only fragmentation and powdering, but also mixing of different batches, and testing to confirm that the distribution of elements is adequately uniform. It is particularly necessary (a) for preparing biological standard reference materials, (b) before subdividing samples when comparing different analytical methods, and (c) to prevent the segregation of particles in samples containing a wide range of particle sizes.

Homogeneity tests may be carried out by determining certain elements in randomly selected subsamples, e.g. K, Zn, Se, Ag, which represent major, minor, trace and ultra trace levels, respectively. For examining different locations on the surface of pellets prepared from the powder, electron and ion microprobes can be used. It is also possible to measure the bulk density at different locations in the sample by examining the degree and rate of weight loss during drying, or by determining the constancy of the ash content at different locations. The uniformity of the particle sizes can be conveniently checked by sieving and observing the different sieved fractions.

1.4 Contamination of sample

Contamination of the sample by elements is often difficult to avoid. Contamination during sampling may occur from the environment of the sample, the sampling operation itself and the operating personnel. During the sampling operation, contamination may arise from dust and volatile contaminants in the air. In addition, the sampling tools may contribute contamination to a marked degree. Numerous possibilities of contamination of the samples by the operating personnel also exist. Exhaled air, spit, phlegm, sweat, cosmetics, tobacco ash, tobacco smoke or even clothing debris may contribute.

Sansoni and Iyengar (1980) have summarized types of contamination which may be derived from the laboratory atmosphere. This is shown in Table 1.7.

The basic requirement is to keep potential contamination hazards small in relation to the concentration levels in the sample. Iyengar and Sansoni (1980) have discussed the case of biological materials. For example, the elements Cu, Fe and Zn are present at mg/kg levels in most biological materials and a reasonably clean laboratory is usually adequate for the preparation of such samples. At the other extreme is the preparation of blood serum samples for the determination of Mn. The concentration of this element in blood serum is only ~0.6 µg/l and unusual precautions are prerequisites for the avoidance of external contamination since Mn is widely distributed in the environment, e.g. as air-borne dust. For determinations at the µg/kg level, clean air benches or glove boxes are usually indispensable for sample preparation.

Problems of contamination and loss of elements are present at every stage of analysis. They demand a minimum of sample manipulation and the use of appropriate equipment. Dust-free containment is a basic necessity at all stages of preparation. However, the magnitude of the contamination problem and its prevention are dependent upon the element involved. For example, Cr, Mn and Pb create more problems than Bi, Te, Th, Tl and U. Air-borne contamination is especially dangerous for Pb analysis at low concentrations (Iyengar and Sansoni, 1980).

The major source of contamination are usually chemical reagents used for the treatment of sample. Table 1.8 shows

Table 1.7

Trace element levels and laboratory atmosphere
(after Sansoni and Iyengar, 1980)

Element	Non-filtered	Filtered	Tobacco	Cosmetics	Sweat	Skin	Hair
	air	air	smoke				
	ug/g dust	ug/g dust	ug/g ^d	ug/g	ug/ml	ug/g wet	ug/g
Al	3000	6				1-2	4-29
As	55	<0.01	2.85			0.06-0.10	0.2-3.7
Br	23	<0.02	71.50	4000	0.2-0.5	4-10	<1-53
Ca	2690	<0.004		6x10	4-10*	250	200-3190
Cd	2.8	0.1				0.032	0.24-2.7
Cl	1.5	<0.005		630	1054-2041	2654	950-4805
Co	9	0.1	0.034	1.12	0.02	0.05	
Cr	39	<0.006	0.35			0.10	0.1-3.6
Cs	1.3	<0.01	0.01			0.03	0.4-1.1
Cu	213	<0.02			1.0-1.5	10-20	11-32
F	1.0	0.10			0.2-2.0		
Fe	3230	<0.006	7.30	1100	0.5-1.5	10	5-68
Hg						0.10	1.3-7.6
I	2.9	<0.01			0.01	0.10	<1-15
K	7920	<0.004		250	176-350	3067	150-863
Mg	2.4	<2390			1-48	58	19-163
Mn	116	<0.006			0.06	0.1	0.3-5.7
Mo						0.05	0.06-0.21
Na	2950	134			1017-3370	2000	18-1720
Ni	70	0.50			0.05-0.2	0.05	0.6-6.5
P	1150	1.50		1400	0.2-1.5	327	83-165
Pb	2150	<0.04			0.05-2.7	0.10	3-70
Rb	23	<0.01				2	0.2-0.50
S	20000	<0.003		400		1500	47700
Sb	14.8	<0.03	0.15			0.04	0.1-3
Se	0.6	<0.02	0.22			0.25	0.6-2.53
Si			9x10*			100-200	20-1950
Sn	9.6	<0.05					?
Sr	13.5	<0.01				0.10	0.05-0.92
Th							?
Ti	258	3		6300		0.50	0.05-14
Tl						0.03	0.012
U	0.1	<0.01					0.00013
V	259	0.005				0.15	0.005-0.53
Zn	1640	<0.02	10	3.5x10*	1	6-20	99-450

trace element impurities in some reagents used for sample preparation as summarized by Iyengar and Sansoni (1980).

Tap water shown in Table 1.8 is from UK, Belmont area, Surrey. It will be different composition in other localities. Distilled water in Table 1.8 was obtained by thermal distillation in quartz and then passed through a double stage mixed-bed ion exchanger followed by filtration through a complex teflon filter.

Table 1.8

Trace element impurities in some reagents used for sample preparation ($\mu\text{g/l}$)
(after Iyengar and Sansoni, 1980)

Element	H ₂ O		HCl		HF		HNO ₃		H ₂ SO ₄		HClO ₄		
	Tap	Demineralized	Single distillat.	p.a.	Ultra pure	p.a.	Ultra pure	p.a.	Ultra pure	p.a.	Ultra pure	p.a.	Ultra pure
Al	57	0.10	<0.002	8	0.80	4	0.5	7	1	8	-	-	-
As	-	-	-	-	-	-	-	-	0.005	-	-	-	-
Br	95	0.10	-	-	2.60	-	-	-	7				
Ca	55000	1	<0.0003	72	0.30	0.4	52	0.2	0.4	10	2	760	0.2
Cd	0.70	<0.10	<0.007	0.03	0.003	8	0.005	0.1	0.03	<1	<1	0.1	0.05
Cl	14100	1	<0.0004	-	-	-	-	-	-	-	-	-	-
Co	-	<0.10	0.02	0.09	0.01	<1	1	0.018	0.01	<1	<1	-	-
Cr	-	<0.10	0.0002	1.10	0.008	5	0.6	72	0.10	25	2	10	9
Cs	0.02	-	<0.00001	0.002	<0.002	-	-	<0.01	<0.1	-	-	-	-
Cu	-	0.20	<0.002	0.20	0.03	0.50	0.30	1.30	0.2	3	3	11	0.10
F	1.40	-	<0.0002	-	-	-	-	-	-	-	-	-	-
Fe	-	0.20	<0.0005	1	-	60	0.60	1300	0.80	8	-	330	2
Hg	-	<1	-	-	-	<10	<10	-	-	<10	-	-	-
I	9.40	-	<0.001	-	-	-	-	-	-	-	-	-	-
K	28000	0.04	<0.0001	200	0.10	0.40	1	<10	9	<10	4	200	0.6
Mg	10400	0.30	<0.0002	7	0.30	2	0.1	3	0.40	3.30	2	500	0.2
Mn	2.20	0.05	<0.0005	<2	0.001	0.60	0.03	9	2	8	0.8	-	-

Table 1.8 (cont.)

Element	H ₂ O		HCl		HF		HNO ₃		H ₂ SO ₄		HClO ₄		
	Tap	Demineralized	Single distillat.	p.a.	Ultra pure	p.a.	Ultra pure	p.a.	Ultra pure	p.a.	Ultra pure	p.a.	Ultra pure
Mo	-	0.02	-	0.02	-	-	-	-	-	-	-	-	-
Na	8100	0.03	< 0.0002	500	0.20	100	0.60	80	0.01	20	9	600	2
Ni	30	< 0.1	< 0.0002	0.20	0.005	0.50	0.05	0.07	0.03	< 1	0.20	8	0.50
P	43	0.004	< 0.0003	-	0.20	-	7	0.80	0.50	-	-	-	-
Pb	8.50	0.10	< 0.003	0.20	0.0015	2.20	0.002	0.20	0.01	1.2	1	2	0.20
Rb	10	-	< 0.001	-	-	-	-	-	-	-	-	-	-
S	14100	4	< 0.0003	-	3	-	-	0.60	15	-	-	-	-
Sb	0.60	< 0.50	< 0.002	0.20	0.38	-	3.0	0.03	0.04	-	-	-	-
Se	3.30	-	-	-	-	-	-	0.20	0.09	-	200	-	-
Si	4900	0.50	-	20	1	-	4	30	8	18	-	-	-
Sn	0.60	0.10	< 0.004	0.07	0.002	11	0.05	0.10	0.002	0.60	0.20	0.30	0.30
Sr	11000	0.06	< 0.007	2	0.06	0.50	0.10	0.20	0.01	0.40	0.30	14	0.02
Th	-	-	< 0.0002	-	-	-	-	-	-	-	-	-	-
Tl	-	< 0.1	-	-	0.006	-	0.50	0.50	0.80	-	-	-	-
Tl	-	-	< 0.0001	0.10	0.10	0.20	0.10	0.20	-	0.10	0.10	0.10	0.10
U	-	-	-	-	-	-	-	-	0.003	-	-	-	-
V	18.50	< 0.1	0.40	-	0.08	-	-	0.05	-	< 2.40	-	-	-
Zn	5.60	< 0.1	< 0.002	1	0.03	6	0.10	4	0.08	< 1	< 1	7	0.10

For the handling of biological samples tools should be made of materials that contain very low concentrations of the trace elements such as Cr, Mn and Ni are to be determined, it may even be acceptable to use steel knives for sample preparation, provided they are not freshly sharpened. Disposable plastic gloves, teflon tweezers, polyethylene and teflon foils, and wax paper (parafilm) are other handy aids.

If it is necessary to use tools made from materials that could be a potential source of contamination, appropriate monitoring should be carried out under realistic conditions. Problems of this kind arise particularly in the collection of biopsy samples (Iyengar and Sansoni, 1980).

2. SAMPLE PRETREATMENT

2.1 Preconcentration

Depending on the as-received state of the samples, the target preparation technique to be applied and the analytical results required, pretreatment of the samples may be necessary in order to obtain suitable targets. Pretreatment should in general be avoided as far as possible, as it is time consuming and may lead to contamination or loss of analytes. Pretreatment may be necessary for:

- homogenisation of the sample material;
- concentration of trace elements by removing matrix constituents;
- selective concentration of the elements of interest;
- selective removal of non-relevant interfering elements;
- selection of a certain chemical form of an element;
- selection of a specific constituent of the sample.

Whether required or not solutions have several general advantages over solid samples. In particular, it is much easier to prepare, mix, aliquot, and dilute samples and standards if they are in the form of solutions. Therefore we shall describe the ways in which various types of samples may be put into solution and discuss the errors that may be introduced by the solution procedure.

Most metals are easy to dissolve in mineral acids. HNO_3 and HCl , either singly or in combination, are commonly used.³ If large quantities of tungsten are present, the use of H_3PO_4 helps keep the tungsten in solution. HF may be added to destroy any residue of silica. Zirconium and its alloys can be dissolved in HF and HNO_3 . Some alloys may contain carbide nitride, oxide, or intermetallic inclusions which are resistant to simple acid treatment. The use of fusion or Teflon bomb techniques may be required in such cases (O'Haver, 1976).

Fusion, often in Na_2O_2 or Na_2CO_3 , is the classic way of solubilizing geochemical materials, especially if the analysis of silicon is desired. A more recent development is the lithium metaborate (LiBO_2) fusion. In this procedure, 0.2 g samples are fused with 1 g LiBO_2 and poured while still molten into 100 ml 3% HNO_3 .

Fusion techniques retain silicon in solution and are thus useful if silicon is to be determined. The major disadvantages are the time and special equipment required (platinum ware, muffle furnace), and the large excess of flux added to the sample. Obviously, the major cation in the flux cannot be determined and, furthermore, trace impurities in the flux material may lead to excessively high blanks.

HF, in combination with other mineral acids, is often used to dissolve silicateous material. The use of HF drives off silicon as the tetrafluoride and thus is suitable only if silicon is not to be measured. Teflon containers are recommended. If large amounts of calcium are present, CaF_2 may precipitate. Also, HF does not dissolve zircon or carbon residue.

A dissolution technique based on a pressure decomposition vessel, commonly called a Teflon bomb is often used. The sample is placed in a specially designed Teflon container, treated with aqua regia and HF, tightly sealed in a stainless steel bomb, and heated at 110°C for 30-40 min. This technique has been found useful with glasses, nitrides, and other refractory material that is difficult to dissolve by other methods. The advantages are that excess alkali salts are not added and that silicon is retained.

Cement samples can be treated with 4 N HCl. Silica is not dissolved. Glass can be dissolved in HF in a Teflon beaker, evaporated to dryness, and the residue taken up in HCl. H_3BO_3 may be added to aid in the elimination of F^- . Coal ash is basically a silicate material; dissolution in HF, sometimes with HClO_4 , has been found useful if silica is not determined (O'Haver, 1976).

Samples containing large amounts of organic matter represent a large and important class of materials for which the sample preparation steps are often long and involved and sometimes a major source of analytical error. This includes solid biological samples such as plant and animal tissue, man-made organic materials such as plastics, and liquid samples such as blood, urine, oils, and liquid fuels, when analyzed for elements at concentration levels too low to allow the use of dilution methods.

The two most widely used methods for the destruction of organic matter are dry ashing and wet digestion. This is described in paragraph 2.2 and 2.3.

A very often used step in sample preparation is evaporation. This is a simple, but slow, preconcentration method for solutions. Its main advantage is that it does not involve the use of large amounts of reagents or of complicated glassware thus contamination is minimized. The main disadvantage is that the total dissolved solids content of the solution is increased. Also, the procedure is very slow, requiring many hours even for modest concentration factors. It is necessary to use a dust cover over the evaporation dish to prevent contamination. Clean, dry, filtered air should be passed over the dish to carry away the vapor. Polyethylene or Teflon evaporation dishes are best. The dust cover can also be fabricated from a large plastic beaker fitted with a side arm on the flushing air. The sample is usually acidified (e.g. with 1 or 2% concentrated HCl by

volume) to prevent hydrolysis. Even so precipitation losses from CaSO_4 , BaSO_4 , silica, and so on, may be expected in some cases. Volatility losses also occur, especially for mercury and to a lesser extent for arsenic and antimony. The lowest possible temperature should be used consistent with reasonable evaporation times. Boiling should be avoided. Either a heat lamp or a hot plate may be used, or both.

2.2 Dry ashing

The purpose of the ashing is to destruct the organic matrix, thereby concentrating the trace elements in the sample.

In dry ashing, the organic matter is decomposed at high temperature in the presence of atmospheric oxygen. In a typical procedure, the sample is weighed into a clean silica or platinum dish, covered with a crystallizing dish, and dried under a heat lamp until the water has evaporated and the sample has a brittle, charred appearance. The sample dish is then placed in a muffle furnace, usually at 500°C , until ashing is complete. After cooling the ash is taken up in dilute mineral acid (O'Haver, 1976).

Dry ashing is relatively simple, can accommodate large samples, and does not require the addition of large amounts of potentially contaminating reagents. However, its principal disadvantage is the serious losses of trace elements that can occur because of (a) volatilization, (b) retention on the walls of the ashing dish, and (c) retention in the acid-insoluble fraction of the ash. Volatility losses are especially serious for mercury and selenium, for which dry ashing procedures are not recommended. Under certain conditions, arsenic, boron, cadmium, chromium, iron, lead, phosphorus, vanadium, and zinc have also been reported to be lost. Elements that occur as volatile organic complexes, such as copper, iron, nickel, and vanadium porphyrins in petroleum, can be lost through volatilization even at comparatively low temperatures. Nonmetals form many volatile compounds which are easily lost.

Wall retention has been found to be a problem, especially with cobalt, copper, iron, silver, aluminium, and manganese, when using silica dishes. Some elements for example, silicon, aluminium, calcium, copper, tin, beryllium, iron, niobium, and tantalum, may react to form acid-insoluble compounds in the ash, especially at ashing temperatures above 500°C .

Kometani et al., (1972) have described procedure for dry ashing of samples in this way. Samples and metal salts in open Pt crucibles were heated in air in a carefully regulated muffle furnace. Filter paper samples were placed in a vented furnace at 300°C for 1/2 hour. At this temperature the paper was charred slowly and did not ignite to a flame. The preliminary heating ensures that sample loss will not occur by turbulence and high temperatures associated with flaming. The temperature was raised to and held at 500°C for 1 hour. The residue in the crucible was dissolved with $\text{HF} + \text{HNO}_3$ (1+3), evaporated to dryness and diluted for analysis by AAS. Unused filters were similarly treated for determination of metal blank corrections.

Dry ashing is often used for the preparation of crude oils for trace element analysis.

Wet samples to be analyzed usually contain materials other than oil, such as brine and sand, which have to be removed before the oil is analyzed. The raw crude oil should first be washed several times with distilled water in order to remove the brine, each time draining off and discarding the water layer. To remove the last traces of water and any suspended sand or clay particles, the oil should be filtered through a dry medium-fine filter paper.

A dry ashing method is described by Horr et al., (1961); first a convenient volume, usually a liter, should be weighed. Small volumes (25 ml) of the weighed sample are then transferred to a 250-ml tarred platinum dish, and each increment ignited and allowed to burn freely in the open dish until reduced to dry char before the next increment is added. The sample is added in small portions to minimize possible losses caused by too rapid combustion, spattering, or mechanical entrainment. This is a slightly modified ASTM D482-46 procedure. The charred mass is then heated in an electric muffle furnace at 500°C until all carbon is removed.

Morgan and Turner (1951) have shown by radioactive tracer technique that no significant losses in inorganic ash occur if the ashing is carried out below 550°C. The losses of metals in the dry ash method have been found not appreciable for crude oils and residual stocks; however, in charge stocks and overhead fractions obtained by vacuum distillation, losses of metals may be considerable (Gamble and Jones, 1955); Karchmer and Gunn, 1952). Loss of some metals during the ignition has been recognized (Milner et al., 1952; Davis and Höck, 1955). This affects nickel and vanadium primarily because they are present in petroleum in the form of volatile porphyrins.

2.3 Wet ashing

In wet digestion, the sample is treated with concentrated mineral acids and/or strong oxidizing agents in solution. Oxidizing conditions are maintained throughout the procedure. Most often, the mixture is heated to 100-200°C to aid the digestion process. Wet digestion is fast and is much less troubled with volatilization losses, because of the lower temperature. The major disadvantage is the possibility of contamination from the large excess of reagents employed.

The most widely used acids for wet digestion are HNO₃, H₂SO₄, and HClO₄. A 3:1:1 mixture, respectively, dissolves its weight of most organic materials. H₂SO₄ is eliminated if the sample contains large amounts of calcium, because of the danger of the coprecipitation of trace elements on CaSO₄. The insolubility of certain other sulfates (e.g., of Pb²⁺, Ag⁺, Ba²⁺) and chlorides (e.g., of Ag⁺, Pb²⁺) limits the choice of suitable digestion acids in certain cases. Other oxidizing agents, such as H₂O₂ and permanganate are occasionally employed in wet digestions. Salts of molybdenum (VI) are sometimes used as catalysts to speed up the oxidation reactions.

Although volatility losses are less severe in wet digestion than in dry ashing, they are not entirely absent.

Mercury is lost when wet digestion are performed in open beakers: an enclosed reflux system is recommended. Elements that form volatile oxides, such as ruthenium and osmium, are lost. If the organic material is allowed to that during the digestion, reducing conditions are temporarily induced and losses of selenium, arsenic, and antimony, which form volatile hydrides, may occur. The presence of organically bound chlorine has been shown to lead to losses of germanium and arsenic which form volatile chlorides.

The use of concentrated HClO_4 in wet digestion procedures requires several precautions. Its use is desirable, because the hot, concentrated acid is an exceedingly strong oxidizing agent, capable of destroying the most resistant organic materials. However, this high reactivity can lead to violent explosions if the acid is mishandled. First, it must be realized that the acid is an oxidizing agent only if both hot and concentrated. The cold, concentrated (70%) acid, although a strong acid, is not an oxidizing agent and will not even oxidize iodide to iodine or iron (II) to iron (III). Basically, the rules of safe handling are (O'Haver, 1976):

1. Never bring undigested organic matter directly into contact with hot, concentrated HClO_4 ; a fire or explosion may result.
2. Always predigest organic matter with HNO_3 first to destroy the easily oxidized compounds.
3. If hot, concentrated HClO_4 is spilled, dilute immediately with quantities of water. This dilutes and cools the acid and effectively "turns off" its oxidizing power.
4. Never boil HClO_4 to dryness. If the digestion cannot be watched.

Wet ashing is also applied to crude oil sample preparation. Horeczy et al., (1951) have shown that wet ashing (ashing with sulfuric acid) recovers more metal than dry ashing. They added synthesized porphyrin complexes of nickel, copper, iron and vanadium to crude oil, and after ashing by the two processes, analyzed for the metals. Their results indicated essentially complete recovery for samples that were wet ashed, but significant losses from the dry ashed samples.

To eliminate such losses a number of investigators have utilized methods of fixing the metals by pre-sulfating the oil before igniting it.

Wet oxidation of oil is similar to the wet ashing procedure of biological and other organic material. A technique frequently used is to char the sample by heating it with sulfuric acid and then adding concentrated nitric acid in 1 or 2 ml increments; alternatively or additionally, strong hydrogen peroxide is added dropwise directly into the charred digestion mixture.

In general, the characteristics of the wet-oxidation techniques are that relatively large acid-to-sample ratios are needed and limited amounts of sample can be decomposed in a reasonable length of time. The two methods of ashing show generally close agreement for the metals contained in oil.

Table 2.1

Comparison of Results for Samples Prepared
by Dry Ashing and Wet Oxidation

(After Horr et al., 1961)

Method of ashing	Ash (%)	Concentration in crude oil (ppm)												
		Cu	Ni	Co	V	Cr	Pb	Mo	Y	As	U	Fe	Zr	Sr
dry	8.6	75	690	115	17	18	150	230	100	7.70	37.00	2.60	70	10
wet	9.7	61	720	115	17	22	160	220	116	8.30	33.00	2.40	55	9.2

Table 2.1 shows the comparison of data for metals in crude-oil samples as obtained by dry ashing and wet oxidation of the oil (Horr et al., 1961).

In another report Agrawal and Fish (1972) compared three methods of ashing for the determination of V, Fe, Ni, Cu, Mg, Na, K, and Ca. Wet ashing gave the most reliable results.

Barney (1955) and Barney and Haight (1955) compared results when a variety of concentration procedures were used. These included (a) dry ashing, (b) total sulfated ashing, (c) partial sulfated ashing, (d) extraction with iodine solution, (e) extraction with a mixture of acetic acid and hydrobromic acid, (f) extraction with a mixture of acetic acid and hydriodic acid, and (g) extraction with sulfuric and hydrochloric acid. Their results indicated quantitative recovery of copper, iron, lead, nickel, and vanadium by procedures (b), (e), and (g).

Gleim et al., (1975) describe the sample preparation technique for crude oils. In their measurement a sulfuric and nitric acid wet digestion process was selected for sample preparation. This process differs from the wet ashing procedure in that the former is carried out in a liquid acid medium from start to finish at a relatively low temperature (280°C), whereas in the wet ashing procedure after acid charring the oil, the car is subjected to a 600°C muffling step. The procedure used by Gleim et al., (1975) was as follows:

Two portions of oil, 55 g \pm 0.1 g, were wet-digested separately with sulfuric and nitric acids in a glass laboratory digestion apparatus. Prior to wet digestion, 1,000 μ g of a Conostan molybdenum sulfonate was added to one of the portions of the oil. After digestion, these two portions of the digested oil would now represent a modification of the classical AAS method of standard additions with one added desirable feature: the added molybdenum standard would be subjected to the same harsh acidic treatment as any of the molybdenum present in the original oil. The authors refer to this procedure as the "cooked" method of standard additions, and find it to be the ultimate in matching atomic absorption response between the sample, and the sample plus the added standard. Smith et al., (1975) have summarized the sample preparation techniques for trace element analysis of crude oils by X-ray fluorescence.

2.4 Low-temperature ashing

Feasibility of low temperature ashing as a sample preparation technique for X-ray fluorescence analysis of biological samples has been investigated in details by Pallon and Malmqvist (1981).

The purpose of the ashing is to destruct the organic matrix, thereby concentrating the trace elements in the sample. The traditional method of dry ashing organic material is to use an oven in which the sample can be heated to temperatures of several hundred degrees centigrade. Due to the high temperature, volatile compounds or elements can be lost (Gorsuch, 1959). In the low temperature ashing technique (Gleit, 1963, Mangelson et al., 1979), oxygen molecules in a gas stream are transformed into highly active oxygen atoms by using a high frequency electromagnetic field. The excited atoms react with the sample, converting primarily hydrogen, carbon and nitrogen into volatile oxides. Thus the matrix can be ashed without the use of a high temperature.

The probability of losing volatile elements during low temperature ashing is expected to be much smaller than in traditional ashing in an oven, but it cannot be neglected. The behavior of elements such as Hg, Br and Se is particularly difficult to predict. Another problem is the risk of sample contamination, which increases with the number of sample preparation steps. To check if such effects occur, well-known standard materials can be analysed. NBS reference materials have well-known compositions, which have been certified by the U.S. National Bureau of Standards (NBS Special Publication 260), and determined by many independent analytical techniques. In the study reported by Pallon and Malmqvist (1981), two common NBS standard materials, bovine liver and orchard leaves, were chosen. The NBS reference materials, originally freeze-dried and homogenized, were put into a glass crucible for ashing. The sample masses were determined before and after ashing by weighing. The blood plasma was first freeze-dried at liquid nitrogen temperature thus reducing its mass by a factor of 11. Next, 450 mg of dried blood plasma was low temperature ashed for 12 h yielding a mass reduction factor of 7.

Targets were prepared from both the freeze-dried matter and the ashed residue. Thick pellets were pressed from the freeze-dried parts of all three materials. From the ashed residues, thick targets were made from blood plasma and bovine liver but not from orchard leaves, due to lack of material. The thick pellets had diameters of 6 mm.

Targets were also prepared by dissolving the rest of the ashed residue in 0.5 ml of 7-molar supra-pure nitric acid and pipetting between 5 and 20 μ l on to Nuclepore filters (N 040, pore size 0.4 μ m, diameter 25 mm). In the study by Pallon and Malmqvist (1981) targets were irradiated by 2.55 MeV protons from the 3 MV Pelletron accelerator in Lund. In order to decrease the count rate from dominant elements such as S, K and Ca, an external pin-hole absorber was used. It consisted of a 340 μ m thick Mylar film with a small hole for the low energy X-rays to pass through. This absorber was chosen from our standard set of different absorbers as being the most suitable one. It would, however, have been better to use one with a ten times smaller hole.

Table 2.2

Ratios of element abundances in NBS bovine liver
(SRM 1577) as measured by PIXE (Pallon and
Malmqvist, 1981) to the stated values (in percent)

Element	Dried, thick (pellets)	Ashed, dissolved (pilletted on Nuclepore)	Ashed, thick (pellet)	NBS
P	92.7 \pm 2.7	63.6 \pm 3.6	116 \pm 9	(100)
Cl	122 \pm 4	0.8	0.9	100
K	105 \pm 6	88.7 \pm 4.1	127 \pm 9	100 \pm 6.2
Ca	145 \pm 8	145 \pm 12	169 \pm 16	100
Mn	115 \pm 4	99.0 \pm 2.9	90.3 \pm 7.8	100 \pm 9.7
Fe	93.3 \pm 5.6	89.6 \pm 3.7	101 \pm 8	100 \pm 7.5
Cu	98.4 \pm 5.2	88.1 \pm 2.6	95.9 \pm 7.8	100 \pm 5.2
Zn	96.2 \pm 4.6	92.3 \pm 7.7	100 \pm 8	100 \pm 7.7
Se	100 \pm 27	82	27	100 \pm 9.1
Rb	98.4 \pm 5.5	95.6 \pm 5.5	90	100 \pm 5.5

Table 2.3

Ratios of element abundances in NBS orchard leaves (SRM 1571) as measured by PIXE (Pallon and Malmqvist, 1981) to the stated values (in percent)

Element	Dried, thick ^{a)}	Ashed, dissolved ^{b)}	NBS
S	84.0 ± 10	65.8 ± 2.6	(100)
Cl	82.6 ± 14	58.0 ± 4.3	(100)
K	109 ± 3	100 ± 3	100 ± 2.0
Ca	100 ± 3	95.7 ± 4	100 ± 1.4
Mn	114 ± 5	100 ± 3	100 ± 4.4
Fe	110 ± 17	73.3 ± 3	100 ± 6.7
Cu	117 ± 42	100 ± 8	100 ± 8.3
Zn	96.0 ± 8	324 ± 16	100 ± 12
As	150 ± 40	160 ± 20	100 ± 20
Br	100 ± 10	50 ± 10	(100)
Rb	100 ± 8	100 ± 17	100 ± 8.3
Sr	83.8 ± 5	97.3 ± 11	(100)
Pb	133 ± 22	122 ± 18	100 ± 6.7

a) cellulose matrix has been assumed.

b) samples pipetted on to Nuclepore filters.

Normalized to Mn.

The thick pellets were analysed for 300-500 s using a beam diameter of 4 mm giving a total accumulated charge of 3 μC . The pipetted targets were analysed for 300-700 s using a beam diameter of 8 mm.

The total accumulated charge was 10 μC . All irradiations were performed in a vacuum chamber and for some hot carbon filament was used to avoid charge build-up on the samples.

The obtained results are summarized in Table 2.2 and 2.3 which show the contents of the NBS standards bovine liver and orchard leaves, as found in the PIXE-analysis, as percentages of the values stated by NBS. The left-hand column in Table 2.2, named dried thick, gives the values for the original freeze-dried matter. Except for Ca these agree quite well with the certified values. In the case of orchard leaves the measured values, shown in Table 2.3, show good agreement with the certified values.

During ashing of bovine liver, more than 90% of the chlorine and part of the selenium present are lost. When calculating the contents of the pellet, thick target corrections due to proton slowing-down and X-ray attenuation must be used. The low-Z element values are especially sensitive to the matrix composition, which is not completely known in the ashed specimen.

From the results in Tables 2.2 and 2.3, one can conclude that volatile elements are lost to some degree, but that most of elements are well preserved after low temperature ashing. If enough ashed material is available and it is possible to make a good estimate of the major constituents of the ash matrix, thick pellets are to be preferred. This approach has several advantages:

- (a) Easier target handling, including fewer preparation steps and minimum requirements of chemical treatment, decreasing the risk of contamination.
- (b) The sensitivity (pulses/(ppm x μC)) is often higher for thick targets, but the uncertainties when calculating the corrections are larger especially for lighter elements.
- (c) Corrections made to thick target data can be based on the fact that the matrix is infinitely thick for protons and that absorption occurs for all X-ray energies. Pipetted samples can in general not be regarded as thin targets with negligible X-ray absorption. When the pipetted ash solution dries on the thin filter, crystalline structures may result. These crystals can be too thick, thus making the self-absorption of low energy X-rays and slowing-down of protons severe problems. To avoid this, pipetted solutions have to be diluted, or a tedious absorption control of each target made. The first alternative decreases the sensitivity, the second is time consuming: neither is very attractive (Pallon and Malmqvist, 1981).

2.5 Loss of elements during sample pretreatment

During the process of sample pretreatment element losses can occur as well as possible contamination. The most usual steps in sample pretreatment are drying and ashing. These two steps are almost always done when analysing biological material.

Iyengar and Sansoni (1980) have discussed the losses of elements in biological material which was subject to dry ashing at temperatures around 500°C . Their results are shown in Table 2.4. Losses are observed for elements such as Ag, As, Co, Cr, Hg, I, K, Na, Pb, Se, Sn and Te.

Some losses are observed even during oven drying of different biological matrices. Data presented by Iyengar and Sansoni (1980) on the loss of elements during oven drying are shown in Table 2.5. The losses range from not detectable up to more than 50%.

Table 2.6 shows the recovery of various elements in different matrices following freeze drying as reported by the same authors. Basically freeze drying is well suited for drying biological samples, yet care should be taken to prevent contamination from the metallic housing of the freeze drier for elements such as Cr, which may volatilize and be trapped by the sample. Use of non-metallic components such as perspex, or preferably quartz, is desirable for the construc-

Table 2.4

Loss of elements during dry ashing of biological samples
(After Iyengar and Sansoni, 1980)

Element	Matrix	Procedure or mode of incorporation	Temperature (°C)	Time (h)	Loss observed (%)
Ag	Animal, liver kidney	Chemical analysis	450	?	5
			450	?	20
Al	Animal, liver kidney	Chemical analysis Chemical analysis	450	?	16
			450	?	12
As	Ox, blood (dry)	Radioisotope, spiking	850	16	35
			550	16	29
			450	16	28
	Rat, bone blood kidney	Radioisotope, intravenous	450	16	44
			450	16	86
			450	16	82
Ba	Animal, liver kidney	Chemical analysis	450	?	
			450	?	4
Ca	Human, rib	Radioisotope, spiking	420	16	1
			600	16	1
			710	16	1
Cd	Animal, liver kidney	Chemical analysis	450	?	0.7
			450	?	6
	Rat, liver liver kidney	Chemical analysis platinum dish	600	16	1.6
			500	16	2
		500	16	4.4	
Co	Animal, liver	Chemical analysis	450	?	14
	Mollusc	Radioisotope, metabolized	450	?	26
			800	?	22
Cr	Sugar, refined brown	Graphite furnace	450	?	0
			450	?	13
	unrefined	Chemical analysis	450	?	47
	Mollasses		450	?	52
	Sugar, refined brown	Chemical analysis muffle furnace	450	?	63
			450	?	62
	unrefined		450	?	86
	Mollasses		450	?	89
	Animal, kidney liver	Chemical analysis	450	?	25
			450	?	7
	Rat, liver	Radioisotope, platinum dish	700	16	2.2
500			16	6.1	
Rat, blood		700	16	51.3	
		500	16	4	
Cu	Animal, kidney liver	Chemical analysis	450	?	0.4
			450	?	0.2
Fe	Animal, kidney liver	Chemical analysis	450	?	0.1
			450	?	0.3
	Rat, liver blood	Chemical analysis platinum dish	500	16	No loss
		500	16	0.4	

Table 2.4 (cont.)

Element	Matrix	Procedure or mode of incorporation	Temperature (°C)	Time (h)	Loss observed (%)
Hg	Fish (whole)		110	24	81.4
K	Human, rib		420	16	1
			600	16	55
			710	16	90
Mn	Mollusc metabolized	Radioisotope	450	?	15
			800	?	21
	Animal, kidney liver	Chemical analysis	450	?	0.4
			450	?	0.3
Mo	Animal, kidney liver	Chemical analysis	450	?	1.5
			450	?	0.4
Na	Human, rib	Radioisotope, spiking	420	16	3
			600	16	10
			710	16	20
	Ox, blood		450	16	Slight
Ni	Animal, kidney liver	Chemical analysis	450	?	15
			450	?	3
Pb	Animal, kidney liver	Chemical analysis	450	?	12
			450	?	2.4
	Human, rib		600	16	5
			710	16	40
Sn	Animal, kidney liver	Chemical analysis	450	?	0.3
			450	?	11
Sr	Animal, kidney liver	Chemical analysis	450	?	0.5
			450	?	2.5
	Ox, blood	Radioisotope, spiking	450	16	9
	Rat, bone blood kidney	Radioisotope, intravenous	450	16	Slight
			450	16	16
450			16	5	
Zn	Mollusc	Radioisotope, metabolized	450	?	33
			800	?	44
	Seaweed	Chemical analysis	500	16	No loss
			1000	16	No loss
	Mussels	Chemical analysis	500	16	No loss
			1000	16	No loss
	Ox, blood	Radioisotope, spiking	450	16	No loss
			550	16	No loss
			850	16	No loss
	Rat, blood	Chemical analysis, new porcelain	700	16	1
	Rat, blood	Chemical analysis	500	16	No loss
	Animal, kidney liver	Chemical analysis	450	?	1
			450	?	1
	Rat, liver	Chemical analysis, etched porcelain	700	16	1.1
500			16	1.3	

Table 2.5

Loss of elements during oven drying of biological samples (After Iyengar and Sansoni, 1980)

Element	Matrix	Procedure or mode of incorporation	Temperature (°C)	Time (h)	Loss observed (%)
Cd	Oyster	Radioisotope, metabolized	120	48	No loss
			90	48	No loss
			50	48	No loss
	Rat liver kidney	Radioisotope, intravenous	110	16	1
			110	16	1
Co	Oyster	Radioisotope, metabolized	120	48	No loss
	Mollusc	Radioisotope, metabolized	110	?	14
	Rat, many tissues	Radioisotope, intravenous	80	72	No loss
			110	24	No loss
			120	24	No loss
Cr	Rat liver blood	Radioisotope, intravenous	120	48	No loss
			110	16	3
Fe	Oyster	Radioisotope,	110	16	5
	Rat blood	intravenous	105	48	No loss
Hg	Human urine	²⁰³ Hg-organic, intravenous	80	72	3
			105	24	15
			120	24	25
	Plankton	Chemical analysis	60	50	51-60
	Rat liver	Radioisotope, metabolized	80	72	5
			105	24	3-10
			120	24	7-15
	Rat brain muscle	Radioisotope, metabolized	120	24	5-16
			120	24	5-21
	I	Human urine	Radioisotope, metabolized	80	72
105				24	4
120				24	7
Rat muscle blood serum erythrocytes brain kidney lung		Radioisotope, metabolized	120	24	5
			120	24	7
			120	24	7
			120	24	8
			120	24	10
			120	24	15
			120	24	7
Mn	Oyster	Radioisotope,	50-120	48	No loss
	Mollusc	metabolized	110	?	14
Pb	Oyster	Radioisotope, metabolized	60	48	10
			100	48	17
			120	48	20
Sb	Rat blood	Radioisotope, metabolized	105	24	5
			120	24	5
	Rat brain kidney lung spleen		120	24	8
			120	24	9
			120	24	6
			120	24	7

Table 2.5 (cont.)

Element	Matrix	Procedure or mode of incorporation	Temperature (°C)	Time (h)	Loss observed (%)
Se	Herbage	Chemical analysis	30	12	No loss
			60	12	No loss
			100	12	No loss
	Rat blood brain lung muscle	Radioisotope, metabolized	120	24	5
			120	24	5
			120	24	5
			120	24	5
	Human urine	⁷⁵ Se organic, intravenous	80	72	12-30
			105	24	30-50
			120	24	50-65
	Oyster	Radioisotope, metabolized	60	48	5
			100	48	5
			120	48	20
Zn	Rat blood liver	Radioisotope, intravenous	110	16	No loss
			110	16	No loss
	Rat, many tissues	Radioisotope, metabolized	80	72	No loss
			110	24	No loss
			120	24	No loss
	Mollusc	Radioisotope, metabolized	110	?	9

tion of the sample housing compartment of the freeze drier. Generally freeze drying of biological samples has been reported to be satisfactory for most elements in different matrices.

2.6 Chelation and Solvent Extraction

Solvent extraction can be conveniently used for preconcentration and for separation of transition metals from large amounts of alkali metals. Most commonly, metals in an aqueous solution are extracted into an immiscible organic solvent, usually with the use of a chelating agent, and the organic phase is analyzed directly without back-extracting into the aqueous phase.

The most common used chelating agents for the solvent extraction of metals are listed in Table 2.7. The recommended pH ranges for the extraction of metals by dithizone, cupferron, oxine, sodium diethyldithiocarbamate (Na DDC), and ammonium pyrrolidine dithiocarbamate (APDC) are listed in Table 2.8. Note the APDC is the most comprehensive, that is the least selective reagent. It can conveniently be used because several trace metals can be extracted simultaneously from an aqueous solution and determined in a single extract (O'Haver, 1976).

The most commonly used extracting solvents are CHCl_3 , CCl_4 , methyl isobutyl ketone (MIBK), esters such as ethyl³

Table 2.6

Loss of elements during freeze drying of biological samples (After Iyengar and Sansoni, 1980)

Element	Matrix	Procedure or mode of incorporation	Pressure (Torr)	Time (h)	Loss observed (%)
Co	Oyster	Radioisotope, metabolized	?	24	No loss
Cr	Oyster		?	24	No loss
Fe	Oyster		?	24	No loss
Hg	Fish	Chemical analysis	?	?	20
	Fish homogenate	Chemical analysis	?	?	16-39
		Radioisotope, spiking	?	?	No loss
	Butterfish	Chemical analysis	?	?	70
	Human brain (pons)		?	?	18-57
	Plankton		?	?	50-64
	Guinea-pig, rat:	Methylmercury (^{203}Hg)	?	?	3
	muscle		0.05	24	3.3
	liver				1.7
	kidney				No loss
	heart				1.5
	blood				2.8
	faeces				No loss
	muscle	Phenylmercury (^{203}Hg)	0.05	24	No loss
	liver				2
	kidney				No loss
blood				No loss	
faeces				9.3	
	Sea cucumber	Chemical analysis	?	?	59
	Water		0.01-0.05	24-72	39
	Human urine	^{203}Hg -organic, intravenous	0.05	48	2
I	Water	Chemical analysis	0.01-0.05	48-72	32
	Human urine	Radioisotope, metabolized	0.05	48	2
Mn	Oyster	Radioisotope, metabolized	?	24	No less
Pb			?	24	No less
Se	Human urine	^{75}Se -organic, intravenous	0.05	48	3

acetate or propionate, and ethers such as ethyl ether. The solubility of MIBK in water (20 ml liter^{-1} at 25°C) is too great to allow very large concentration factors. Methylamyl ketone (MAK) is much less soluble in water and is a suitable alternative. However, MIBK is less expensive and more widely available.

O'Haver (1976) has described an example of a typical extraction procedure suitable for many metals:

Table 2.7

Chelating agents commonly used in preconcentration
(After O'Haver, 1976)

Common Name	Chemical Name	Commonly Used for
APDC	Ammonium pyrrolidone dithiocarbamate	Cu, Pb, Cd, Co, Mn, Fe, Ni, Bi, Zn, As, Ir, Pd, Pt, Se, Te, Tl, Mo, V, Cr
Cupferron	Ammonium salt of N-introsophenylhydroxylamine	V, Ti, Cu, Mn, Fe, Ni
Oxine	8-Hydroxyquinoline	Al, alkaline earths, others
Dithizone	Diphenylthiocarbazon	Ag, Pb, Cd, Zn, Cr
ACAC	Acetylacetone; 2,4-pentanedione	Transition metals
NADDC	Sodium diethyldithiocarbamate	Pb, Cu, Fe, Mn, Te

Adjust 100 ml of the aqueous solution to the appropriate pH range (usually between 3 and 4) by the addition HCl or NH₃. Transfer to a separatory funnel. Add 5 ml of a freshly prepared 1% solution of APDC in water and shake to mix. Add 10 ml MIBK, shake 2 minutes, and allow the phases to separate. The organic (upper) phase may be used for the analysis.

It is sometimes convenient to use a narrow-necked volumetric flask or similar vessel instead of a separatory funnel. After the phases have separated, additional water can be poured in to raise the level of the organic phase into the neck, if needed.

In solvent extraction work, it is always necessary to carry the blank and the standards through the same extraction procedure (O'Haver, 1976). The purpose of this is to (1) allow for less than 100% extraction, (2) correct for the concentration of reagent impurities, (3) eliminate calculation of the exact concentration ratio, and (4) provide standard solutions in the appropriate chemical form and matrix.

Cronin and Leyden (1979) have described a method of uranium concentration on to a solid substrate in a single step. The method utilized a silica-cellulose filter chemically-modified by bonding a silanedithiocarbamate to the silica surface. The aqueous sample is continuously cycled through the filter in a closed-loop flow system. In their work the extraction and subsequent enrichment of uranium onto the filter is accomplished by ion-pairing of the tris-carbonato complex of uranium (UO₂(CO₃)₃⁴⁻) with an immobilized derivative of ethylenediamine. The silica-cellulose filter is chemically modified by silylation with a silane-ethylenediamine. This method combines the advantages of a one-step filter pretreatment,

Table 2.8

Recommended pH ranges for solvent extraction
of metal chelates (After O'Haver, 1976)

Element	Dithizone	Cupferron	Oxine	NADDC	APDC
Ag	0-7	-	3-6	8-11	1-10
Al	-	3.5-9.0	5-6	-	-
As	-	-	-	6	2-6
Be	-	-	6	-	-
Bi	2	-	-	8-11	1-10
Cd	6-14	-	6	8-11	1-6; 1-10
Co	6-8	-	5-6	3.6; 8-11	2-4; 1-10
Cr	0.5	-	-	3.6	3-9
Cu	2-5	7; 1	2-6	3.6; 8-11	0.1-8; 1-10
Ga	-	-	-	-	3-7
In	-	-	-	-	1-10
Fe	-	7	6	3.6	2-5; 5 [±] 0.3
Hg	0-4	-	-	8-11	2-4; 1-10
Pb	7-10	-	6	3.6; 8-11	0.1-6; 1-10
Mn	-	7	-	3.6	2-4; 5 [±] 0.3
Mo	-	-	1.8-2.6	-	3-4; 3-6
Ni	6-8	7	6	3.6; 8-11	2-4; 1-10
Se	-	-	-	6	3-6
Sb	-	-	-	-	1; 3,7
Sn	-	-	2.5-6	-	3-6
Tl	-	-	-	8-11	3-10; 3-10
V	-	1	-	6	1-2; 4; 3-6
W	-	-	-	-	1-3
Zn	6-9	-	6	3.6; 8-11	2-6; 1-10

a single step UO_2^{2+} extraction and enrichment onto a solid substrate and the simplicity of an x-ray fluorescence determination. The details of experimental procedure are as follows:

N- β -ethyl- γ -aminopropyl trimethoxysilane (Dow-Corning Z-6020) was used as a 10% v/v solution in toluene. The filters (22 or 25 mm diameter) were punched from sheets of SG-81 (Whatman, Inc.), 20% w/w silica gel in cellulose after silylation. Stock solutions were prepared from reagent grade chemicals, copper from the chloride salt as a 1000 mg l⁻¹ aqueous solution and uranium from uranyl acetate as a 5000 mg l⁻¹ solution. Working standards were subsequently prepared from these solutions as needed. Samples from various stages in the uranium solution mining process were obtained from Wyoming Minerals, Inc. These samples represented a range of uranium concentrations in a varying matrix, although all samples were aqueous solutions.

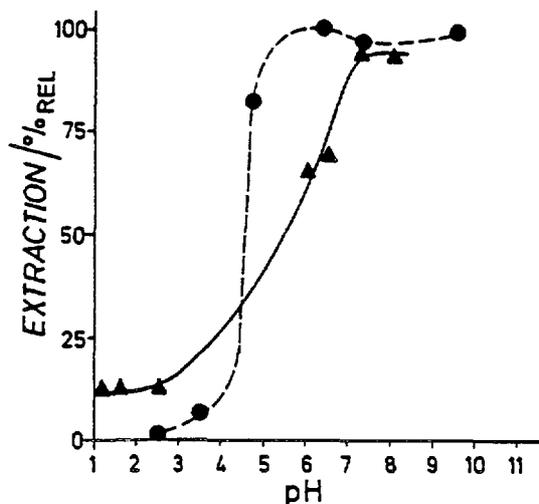


Fig. 2.1 The relative amount of Cu^{2+} extracted by the silylated filters at various pH values (Cronin and Leyden, 1979).

The relative amount of Cu^{2+} extracted by the silylated filters at various pH values was determined using 50 ml of 2 ppm Cu^{2+} solution, adjusted to constant ionic strength ($\mu = 0.5$) with NaClO_4 . The pH of the solutions was adjusted using either 0.1 N HCl or 0.1 N NaOH . The final pH was determined using a pH meter with a combination pH electrode. The relative concentration of Cu^{2+} on the filters at the various pH values was determined as in the chemical capacity study.

Uranium enrichment on the silylated filter involves the ion-pairing of $\text{UO}_2(\text{CO}_3)_3^{4-}$ with the immobilized ethylenediamine between pH 6 and 8. Thus the uranium working standards and the process samples were prepared as follows: the required aliquot of either the stock solution or process sample was pipetted into a volumetric flask and brought to volume so that the sample used for enrichment would be 0.1 M in $(\text{NH}_4)_2\text{CO}_3$ and at a $\text{pH} = 6.5 \pm 0.2$. In the case of the process samples, the aliquot was first pretreated by acidifying to pH 2 and heating to just boiling followed by cooling and neutralization. The pretreatment is required to remove any carbonate that might be present.

The procedure for uranium extraction involves a 25 ml sample and a cycling time of 60 min. This is followed by washing the filter with three 5 ml portions of 0.1 M $(\text{NH}_4)_2\text{CO}_3$ solution ($\text{pH} = 6.5$). The filter is then prepared for XRF analysis by air drying and placing it in a 25 mm diameter sample holder (Chemplex) between two sheets of 6.3 μm thick Mylar film (Cronin and Leyden, 1979).

The results showing the extraction of Cu^{2+} on SG-81 are presented in Fig. 2.1 and it has the same pH dependence as on the bulk silica gel. It was concluded that the immobilized ethylenediamine on the SG-81 filters was similar to the ethylenediamine immobilized on bulk silica gel. In addition, even though the capacity of each filter is low, their convenient physical form makes the filters attractive as preconcentration tools in XRF analysis.

Two experiments were done to determine the suitability of the filters for XRF analysis. The first study dealt with determining the necessity of counting both sides of the filter in the XRF spectrometer. A series of solutions of various uranium concentrations was preconcentrated by this method followed by x-ray counting of the $U_{L\alpha}$ line on both sides of the filters. A plot of cps of the contact side of the filters (the side facing the sample solution) versus cps of the average of the front and back counts of the filter was constructed and a straight line was obtained with a slope of 0.96 ± 0.03 and a correlation coefficient of 0.9991. This indicates that counting of only one side of the filter is sufficient for the uranium determination. In the second experiment pressing of the filters to improve the precision of the counting statistics was investigated. A series of filters containing varying amount of uranium was first measured by XRF followed by pressing at 10.000 psi for 20s in a one inch die and remeasured. Using two-group statistics, no significant difference at the 95% confidence level was observed between the XRF signals from the unpressed and pressed filters.

Two methods were used to determine the percent recovery of uranium of the filters. In the first method, two filters were used in succession; a sample was preconcentrated onto one filter, the filter was removed and a second filter was used to concentrate the uranium remaining in the sample. The second method involved a comparison of the x-ray counts of a sample filter with another filter spiked with a known amount of uranium. Mean recovery ranges from 70 to 95% for 10 ppm (Cronin and Leyden, 1979).

2.7 Ion exchange

Ion exchange is becoming an increasingly popular method of separation and preconcentration in trace element analysis, especially for water analysis at ultratrace levels. Basically three different types of ion exchange resins are used for these purposes: cation exchange, anion exchange, and chelating-type resins. Cation (acid) exchange resins are those that exchange cations with the solution, replacing all the cations in solution with H^+ or Na^+ . Anion exchange resins replace the anions in solution with OH^- or Cl^- . Chelating resins contain functional groups similar to those in conventional chelating agents. These resins remove from solution any ions with which the functional groups can form a chelate bond. They are somewhat more selective than cation or anion exchange resins. All three types are widely used for trace element concentration. The large ion exchange capacity of many resins means that large volume of dilute solutions can be passed through the exchange column with nearly complete retention of the ionic species. The retained ions are then eluted with a relatively small volume of a strong acid (e.g. 2 M HNO_3) or for anion exchange resins, an alkali (e.g. 4 M NH_4OH). Nearly 100% recovery can be obtained for many metals. If the sample contains large amounts of major cation (e.g. Na^+ , K^+ , Mg^{2+} , Ca^{2+}), a cation exchange column may not be practical because it may be saturated by the major cations, thus preventing complete retention of trace cations. In such

cases, a chelating resin that does not retain alkali or alkaline earth elements is preferred (O'Haver, 1976).

Beamish (1967) has reported methods of separating both microgram and milligram amounts of the six platinum metals from large proportions of iron, copper and nickel by cation-exchange columns. The isolation of rhodium, iridium, platinum and palladium by anion-exchange columns has also been subject of number of papers. In the work by Taylor and Beamish (1968) quantitative separations of microgram quantities of osmium and ruthenium from large proportions of copper, iron and nickel were accomplished by the use of anion-exchange paper. Accurate determinations of osmium and ruthenium were made by adaptation of X-ray fluorescence.

Use of chelating ion exchange resin in the determination of uranium in ground water by x-ray fluorescence is described in details by Hathaway and James (1975). In their work all test solutions were prepared from a common ground-water sample. Spiked solutions were prepared using a solution of uranyl nitrate. Solutions used in the studies on extraction time, pH dependence, and recovery in multiple extractions were spiked to 50 ppb (ug/l) above the natural uranium level of the ground water. Preacidification of the water samples consisted of treatment with 3 ml concentrated HCl per liter of water 12 hours prior to addition of resin.

Samples used for the standard additions-calibration curve were triplicate sets of unspiked ground water, and ground water spiked to 20, 60, and 100 ppb above the natural uranium level.

The procedures used were as follows: a 0.3-ml glass scoop was used to add two level scoopfuls (0.6 ml equals 60 mg dry weight) of prepared Chelex-100 resin to 1-liter samples of water contained in 1-liter Erlenmeyer flasks. The pH of the solutions was then adjusted to the desired value, with the aid of a pH meter, using a dilute NaOH solution.

All samples, except those in the extraction time study, were stirred for 3 hours on a magnetic stirring table. All flasks were sealed during the extraction period in order to minimize contamination and CO₂ uptake in the higher pH solutions. After stirring the solutions for the proper length of time, the resin samples were collected on 0.45- μ filter and then dried for 1 hour at 45°C.

Filtrates from solutions used in the multiple extraction studies were treated as new samples and run through the above procedure two more times.

The dried resin samples were each mixed about 500 mg of Somar Mix (a binder), and then spread uniformly over the surface of 1.25-inch planchets which were half-filled with boric acid as a backing agent. The samples were then pressed to 10,000 psi. The resulting pellets were quite stable when stored in a Chamber with silica gel.

Pellets were counted for 100-second intervals at both the analyte and background lines.

The use of ion-exchange resin-loaded filter paper for automatic analysis of dissolved metal pollutants in water is described in an article by Ho and Lin (1982). The system consists of a sample tank, a roll of 3.75 cm wide ion-exchange

resin-loaded filter paper on a supply reel, a filter-paper transport mechanism, a collection tank, and a spent-water tank. A water sample as large as 500 ml can be processed from the sample tank through the ion-exchange filter paper and into the collection tank. The water in the collection tank will either recycle back to the sample tank or go to the spent-water tank. The water transport lines and tanks are made of plexiglass and Teflon in order to reduce the possibility of sample contamination. The filter-paper transport mechanism includes a precision motor that moves the filter paper forward and backward in precise increments, a photoelectric sensor that detects seams in the paper, a cutter, and a transporter that moves the cut filter paper to the x-ray chamber for analysis.

The use of ion-exchange resin-loaded filter paper for water sample preparation is also described by Campbell et al., (1966) and Law and Campbell et al., (1974). In their works chemical and x-ray characteristics of Reeve-Angel cation and anion exchange resin-loaded paper disks were investigated. Chemical characteristics include exchange capacity, effects of pH, salt concentration, and competing ions, and distribution of collected ions. X-ray characteristics include x-ray transmission coefficient as a function of wavelength, relationship of x-ray intensity to quantity atomic number, and distribution of collected ions, reproducibility of intensity measurements, and limits of detection.

2.8 Electrodeposition

The use of constant current electrodeposition of reducible metal ions upon a pyrolytic graphite roll to prepare samples for wavelength dispersive x-ray fluorescence analysis has been described by Vassos et al., (1973). In the work of Boslett et al., (1977) a technique by which trace amounts of the aqueous metal ions nickel (II), copper (II) and zinc (II) are preconcentrated on the end face of an ordinary spectrographic graphite roll by potentiostatic electrodeposition is described. The thin metal film that results from the electrodeposition is analyzed by XRF. Controlled potential electrodeposition has the capability to selectively separate trace concentration metal ions from a solution that may contain interfering metal ions. In the work by Boslett et al., (1977) stock solutions of zinc (II) acetate, copper (II) perchlorate, and nickel (II) chloride were prepared by dissolving reagent grade salts in distilled-deionized water. The stock solutions were standardized against dried primary standard disodium dihydrogen ethylenediaminetetraacetate dihydrate ($\text{Na}_2\text{H}_2\text{EDTA}\cdot 2\text{H}_2\text{O}$) and determined to be 0.0996 F $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2$, 0.1039 F $\text{Cu}(\text{ClO}_4)_2$ and 0.0991 F NiCl_2 . Solutions containing trace-levels of the metal ions were prepared by diluting microliter amounts of the stock solution in distilled-deionized water.

Supporting electrolyte was prepared in concentrated form by dissolving reagent grade sodium acetate in distilled-deionized water and adjusting to pH 6.0 with glacial acetic acid. The total analytical concentration of acetate was 2.60 F. This solution was cleaned of metal contaminants by electrolysis for 18h over a cathodic mercury pool at -1.700 V US. saturated

calomel electrode (SCE). The details of the procedure used are as follows:

A solution of distilled-deionized water and sufficient amounts of stock supporting electrolyte to make the solution 0.10 F in total acetate at pH 6.0 was prepared and degassed by purging with nitrogen for 10 min. The appropriate microliter amount of previously standardized stock metal solution was added. The final volume in all cases was 120.0 ml. Electrodeposition proceeded at -1.300 V vs. SCE, with vigorous stirring. All electrode positions were run at ambient temperature. On completion of the deposition, the graphite rod was removed from the cell, with the voltage applied, and rinsed quickly in distilled-deionized water. After air drying, the working surface was sprayed lightly with the acrylic lacquer to fix the deposit, and a 1/4- to 1/2-inch length of the carbon rod was cut off for analysis.

Counting times were generally 400 s (live time), except for the analysis of solutions of very low concentrations (<20 ppb), for which counting times as long as 1200 s were used and normalized to an equivalent 400-s count. Data for the calibration curves were obtained from digitized spectra by a computer-assisted integration of the analytical peaks.

The concentration of a metal ion in solution that is being potentiostatically electrodeposited on an electrode surface decreases with time according to equation:

$$C = C_0 e^{-kt} \quad (2.1)$$

where C_0 is the initial concentration, and k is a constant characteristic of the electrodeposition system, the metal ion, and its environment. The amount of material Y deposited on the electrode at time t also depends upon the initial concentration, as shown in equation:

$$Y = C_0 VM(1 - e^{-kt}) \quad (2.2)$$

in which V is the solution volume and M is the gram-atomic weight of the metal ion.

The electrode upon which the thin metal film was deposited may then be inserted directly into the modified x-ray spectrometer sample holder for quantitation. For a given deposition time, the intensity of characteristic fluorescence emitted from the sample is proportional to the amount of metal deposited, up to the critical thickness, beyond which additional metal deposited results in no incremental fluorescence emitted. Thus, Equation (2.2) may be rewritten as:

$$I_t = I_\infty (1 - e^{-kt}) \quad (2.3)$$

in which I_t is the intensity of characteristic fluorescence emitted from the electrode surface after electrodeposition for some time t , and I_∞ is that intensity which would be emitted after all of the metal ion has been deposited on the electrode ($t = \infty$).

Bosiett et al., (1977) have concluded that MDL for Cu^{2+} using a 6-h. deposition is 0.9 ppb. Similar results were also obtained for Zn.

In spite of encouraging results, a more widespread analytical application of the technique is limited to the elements which can be recovered from aqueous solution in sufficient yield.

An improvement of this method has been reported by Wundt et al., (1975). They have developed an analytical procedure for transition elements based on the electrodeposition of their anionic cyano complexes from mixed organic aqueous media in a high potential electric field was developed.

For optimizing the electrodeposition procedure, solutions of the individual elements (10^{-3}M) containing cyanide of different concentrations ($5 \times 10^{-3}\text{M}$ - $7.5 \times 10^{-2}\text{M}$, pH 11) were studied. For most mono- and bivalent transition metals, they were obtained from weighed amounts of the respective chlorides (Co, Ni, Cu, Zn, Cd, Pt, Au, Hg) or nitrates (Ag). Because of their redox and hydrolytic behavior, Fe(II), Fe(III), and Co(III) were used in the form of their cyano complexes. Those of V(III), Cr(III), and Mn(II) were prepared by standard methods. Because of the limited stability of these cyano metalates in aqueous solution, the oxo compounds NH_4VO_3 , K_2CrO_4 , and KMnO_4 were also examined. All reagents were Suprapur or of analytical grade, the water was deionized and bidistilled. For deposition, aliquots of these solutions were diluted with methanol, ethanol, and 2-propanol to give alcohol mole fractions between 0.4 and 0.9.

To favor the elution of the cyano complexes, which is possible only from basic medium, the cation-exchange resin (Dowex 50W-X8, 200 400 mesh) was preconditioned with a mixed solution of KOH (1 M) containing KCN (1 M) for eliminating contaminants, repeatedly washed with bidistilled water, and dried at 70°C .

The effectiveness of deposition is most sensitive to the nature of the organic component and its ratio to water. Because of their solvating properties and their miscibility with water, polar organic solvents like alcohol proved to be most suitable for the electrodeposition technique. Preliminary experiments showed, however, that deposition was incomplete in aqueous mixtures of methanol and ethanol, irrespective of their composition and the variation of the other parameters. Also, the cyano metalate films tended to become less adhesive. This seems to be due to the relative high dielectric constants of the media causing high current densities (10 mA cm^{-2} at 400 V) and consequently the corrosion of the aluminum anode.

Quite different results were found with 2-propanol as the organic component. With all other parameters optimized quantitative deposition as well as thin, uniform, and firmly adhering films were obtained, when aqueous cyanide solutions (pH 11) with Fe(III), Co(III), Ni(II), Cu(I), Zn(II), and Cd(II) were mixed with appropriate amounts of 2-propanol to give mole fractions of the deposition solution between 0.50 and 0.78. For Fe(II) quantitative deposition is possible only in a small region of 2-propanol mole fractions, whereas Co(II) is deposited with an incomplete but constant yield (70%). These

conditions were realized after 30-min deposition time at high₂ voltages above 1000 V and current densities of about 10 mA cm². Extending the range of mole fractions beyond 0.50 and 0.75 leads to a steep decrease of the deposition yield and to badly adhering deposits.

The deposition yield is sensitive also to the ionic composition of the aqueous solution, namely the pH and the degree of complex formation (Wundt et al., 1979).

3. SAMPLE PREPARATION FOR PIXE

A major problem in quantitative PIXE analysis is the target preparation; most of the positive characteristics of PIXE may be spoiled by inadequate target preparation. In this context it should be noted that PIXE is a surface analysis technique. For instance, the range of 3-MeV protons in organic material is less than 20 mg/cm².

If targets thicker than the proton range ("infinitely" thick) are analysed for their "bulk" composition, the surface under irradiation should be representative for the whole target. This means that such targets should be homogeneous, implying constant target composition throughout the target volume to be irradiated. Because of the homogeneity, matrix corrections can then be applied when a quantitative analysis is required.

For thin targets ($\lesssim 1$ mg/cm²) PIXE may be considered as a quantitative trace element analysis method, since matrix corrections are negligible. Thick targets ($\gtrsim 1$ mg/cm²) should meet the requirement of uniformity, implying a constant area density over the target area to be analysed. For thin targets uniformity is not a strict requirement, when the beam spot is bigger than the area of sample.

Thick targets have the following disadvantages (Kivits, 1980):

- data analysis is complicated and uncertainties are relatively large due to the varying cross sections for the x-ray production by protons which are slowed down in the target as well as due to absorption and enhancement effects;
- charge build-up in the target may cause an increased Bremsstrahlungs continuum, especially if the protons are stopped in the target;
- target damage may occur by heating, even at low beam intensities (e.g. 10 nA/cm²).

For homogeneous thick targets, the disadvantage of inaccuracy can be overcome, to a certain extent, by calculation of the matrix corrections. The effect of charge build-up may be reduced by using targets with thicknesses less than the proton range. Special additions may allow targets to withstand heat generation, at the expense of loss of sensitivity and the risk of contamination, however.

On the other hand the use of thick targets also has advantages. Thick targets are generally less complicated and

faster to prepare than thin targets, and involve less risk of contamination and loss of elements to be analysed. With respect to a bulk analysis, the preparation of thick targets is less dependent on sample inhomogeneity.

3.1 Backing materials

With charged particles the simplest is the direct bombardment of a specimen. This method is often used for such materials as certain biological tissues, e.g., teeth, or metallurgical samples, where it is difficult or impossible to obtain thin targets. The drawbacks are mainly the lower sensitivity and the need for various corrections in calculating the results.

When thin targets are prepared the material to be analyzed should be deposited on some suitable backing. There exists a great number of methods for bringing a sample into a form suitable for analysis and for depositing it on the backing. The method chosen depends on the type of material to be analyzed.

When charged particles are used for the excitation of characteristic x-rays then a sample to be analyzed has to be maintained under vacuum during ion bombardment. This means that volatile compounds or gas contained in the sample will be removed to a large extent during pumpdown. For example water will disappear from tissue samples so that one is analyzing mainly dry matter, and the sensitivity to Br and Hg (for instance) will depend on the chemical form they have in the original sample.

A backing material is necessary for many types of samples. Mechanical strength and good electric and thermal conductivity is desired and it has to be of high purity material, able to withstand high beam intensities. The continuous background radiation produced by the backing ought to be as small as possible, and this favors thin backings consisting of low Z elements.

Many different kinds of backing materials are used in different laboratories. Some investigators favor thin carbon foils, some others are using different plastic foils (Formvar^R, Kapton, Mylar^R, polysterene and Hostaphane). Often Millipore^R, Whatman^R, Nucleopore^R and other filters are used.

Here we shall present procedures for the floating of formvar: A solution of 50 ug/ml formvar in spectroanalyzed 1,2-dichloroethane should be prepared and stored in a glass container, (the container must be glass since 1,2-dichloroethane, a strong organic solvent, will dissolve polyethylene). Target frames should be cleaned by soaking overnight in acetone. The frames should be stored in acetone, since they oxidize in water and air, and should be rinsed with doubly distilled water (DDW) just prior to use. All work should be done in a clean box to reduce dust contamination.

Details are described in a paper by Valković (1974). A plexiglass trough 2.5 cm wide by 17.7 cm long and 5 cm deep was cleaned with ethanol and rinsed with DDW. The trough was then filled with DDW and a drop of the formvar solution

was placed at one end of the trough. The formvar was drawn across the water as a thin film with the edge of the frame held by an alligator clip. The formvar film was then draped over the hole in the target frame by dipping the frame in and out of the coated water and folding the formvar back on itself. Four layers of formvar film proved to give the support necessary for the backing. The wet targets were then stored in a special dust free holder until dry. Preparation of Formvar films is also discussed by Bearse et al., (1973).

X-ray spectrum from Formvar obtained by Valković et al., (1974) exhibits no characteristic x-ray peaks. The thinness and low effective atomic number of Formvar^R foils confer on them the advantage of extremely low bremsstrahlung background: if trace metal impurities can be eliminated, Formvar^R will afford better detectability limits than its competitors. The disadvantage is that Formvar^R is very fragile and is a bad heat conductor; breakage rates are likely to be high and beam currents must be kept low, increasing running time. Valković et al., (1974) have circumvented the heat dissipation problem by evaporating a 100 ug/cm² aluminum layer onto 100 to 150 ug/cm² Formvar^R. This allowed them to use 300 nA beams with normal films. Another disadvantage of Formvar^R relative to carbon is that it is destroyed by acidic solutions. Figure 3.1 shows the spectrum of x-ray radiation resulting from 3 MeV bombardment of Al-Formvar^R backing. It is essentially free of any lines and it can be easily approximated by a polynomial for background subtraction.

When higher concentrations have to be measured in powdered samples different adhesive plastic tapes can be used. Figure 3.2 shows the spectrum from the Scotch tape (3M Company, Minneapolis) with the significant contaminant being bromine only.

The use of thin films to support liquid, powdered and slurry samples in x-ray spectroscopic sample cups is a state-of-the-art. Polyester film supports are the most commonly used and preferred because of their unique properties. The chemical composition of polyester attenuates absorption of the primary x-rays and characteristic radiation emitted by the sample. The degree of attenuation is further controlled by the gauge of the film used; the thinner the gauge, the less the absorption of x-rays. The inherent high strength of polyester film also permits safe sample handling and retention concurrent with maintaining taut film surfaces to define statistically reproducible target-to-sample distance. For example, Chemplex "X-Ray Mylar Films" are polyesters (polyethyleneterephthalate) and have all of the unique combination of properties required for x-ray spectroscopy.

Chemplex "X-Ray Mylar Film" is available in three gauges covering the entire x-ray spectral range:

One has a gauge thickness of 2.5 μm . It is used for applications requiring reduced absorption of the primary x-rays and characteristic long wavelengths, including the "L" spectral line series.

Another has a gauge thickness of 3.6 μm . This is a general purpose film gauge for both short and long wavelength investigations. It is particularly well suited for analyzing samples containing mixtures of both heavy and light elements.

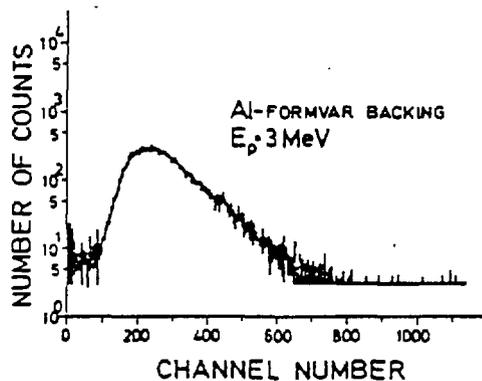


Fig. 3.1 X-ray spectrum resulting from 3 MeV bombardment of Al-Formvar backing.

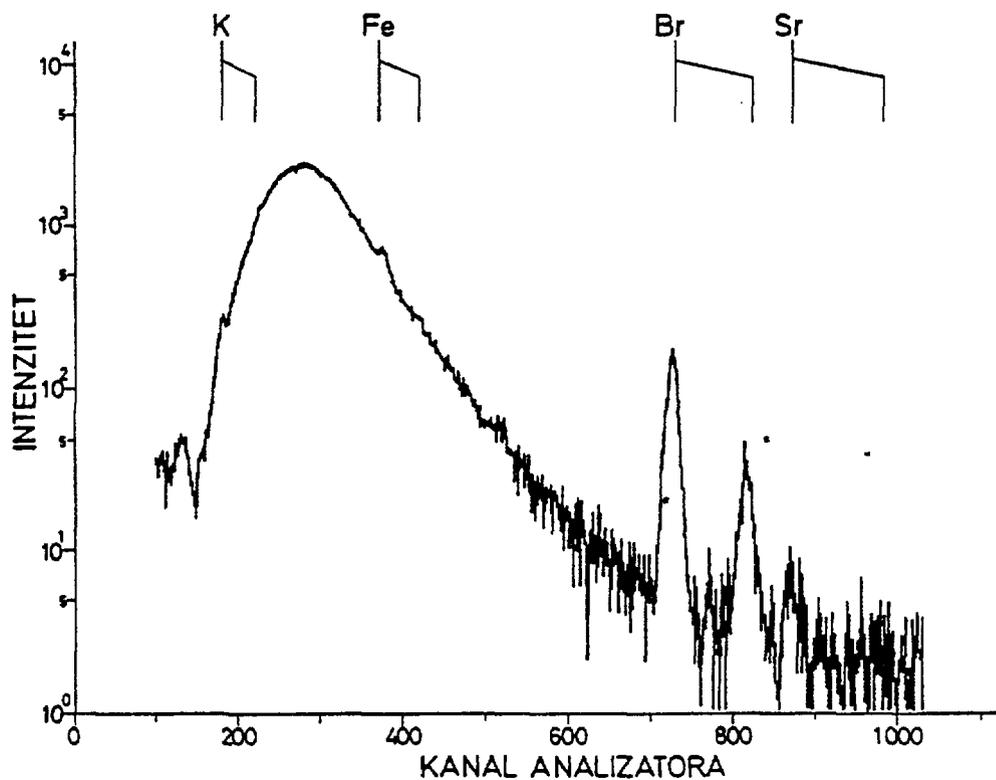


Fig. 3.2 X-ray spectrum resulting from 3 MeV proton bombardment of Scotch tape.

Third is a 6.3 μm gauge film used primarily for short wavelength (heavy element) determinations. Its applications may be extended to include moderately high concentrations of elements having long wavelengths.

Chemplex "X-Ray Mylar Films are supplied in 7.6 cm x 91.4 m rolls in dispensers with cutting edges. Each roll adequately prepares over 1200 samples at two inches (5 cm) per sample, is convenient to use, clean and eliminates waste.

Fig. 3.3 can serve as a guide in helping to select the appropriate gauge of Mylar film for any given x-ray spectral wavelength investigation.

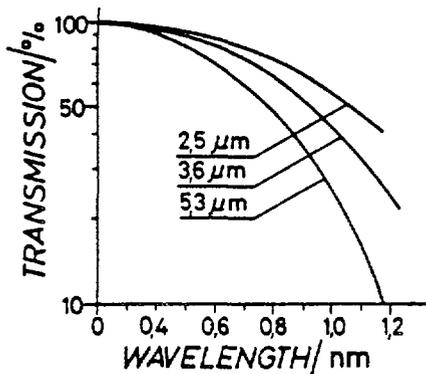


Fig. 3.3

X-ray transmission through Mylar films of different thickness.

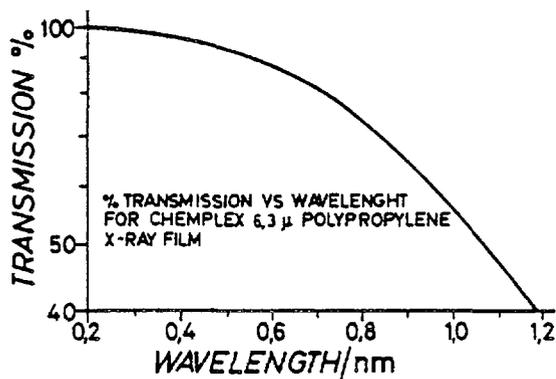


Fig. 3.4

X-ray transmission through 6.3 μ m polypropylene x-ray film.

Percent transmission for each of the three different gauges is related to wavelength. Simply determine the wavelength of the element to be investigated and select the gauge of Mylar film displaying the greatest transmission. For multi-element determinations the transmission values for the shortest and longest wavelengths should be considered.

Elements having wavelengths of 0.2 nm or less exhibit virtually 100 percent transmission through each gauge.

The possibility of pinholes, pores and variations in gauge thickness existing in all thin film sample supports regardless of form and packaging may present leakage of a sample with subsequent potential contamination and damage to the analytical instrumentation and its components, variations in quantitative data and impose bodily injury to the user. It is strongly recommended that each product and section to be used be subjected to judicious testing, use, applications and evaluation prior to actual use by the user.

Polypropylene x-ray films are unique by combining a low mass absorption coefficient value with a gauge thickness of 6.3 μ m and a density of 0.9 gm/cm³. These two properties contribute to the transmission of primary x-rays and characteristic radiation emitted by a specimen as shown in Fig. 3.4. To further enhance low level elemental determinations, Chemplex polypropylene x-ray film is processed without additives, stabilizers or lubricants, which frequently cause limitations on detectability.

The high sample retention strength of polypropylene x-ray film permits safe sample handling in XRF Sample Cups. The sample planes are maintained taut for uniform target-to-sample distances and statistically replicate intensity

measurements. Chemical resistance to acids, alkalies and oils is excellent, and good for organic solvents.

In analyzing liquid and powdered samples in vacuum containment of the sample and maintaining a flat reproducible sample plane, as defined by the thin-film sample support are important considerations. Samples encased in closed sample cups without provision for venting present the possibility of internal pressure build-up and distension or rupture of the thin-film sample support. Samples introduced in open sample cups may abruptly and vigorously outgas and spatter.

Microporous film permits permeation of entrapped air and sample vapors in a closed sample cup through tortuous micrometer-size channels. An equalization of pressure within the closed sample cup and the vacuum environment is established and avoids distension of the thin-film sample support. Intensity variations are minimized by maintaining a flat uniform sample plane and a reproducible target-to-sample distance. The $0.1 \mu\text{m}$ micro pore channels prohibit the penetration and escape of sample material.

The opacity of microporous film is an indicator for gaseous permeation. The disappearance of opacity indicates "wetting" by low surface tension liquids which may cause diminished gas permeability with continued use. High surface tension liquids "bead" and resist penetration. Generally, gentle heating restores microporous film to original porosity for reuse.

Microporous film may be used with Chemplex "XRF Sample Cups". An "XRF Sample Cup" is equipped with a Chemplex sample support the sample is introduced, and microporous film is affixed to the top opening with a snap-on ring. Care should be exercised in avoiding contact of the microporous film with a liquid sample to prevent unnecessary permeation of the micro pore channels.

Thin carbon foils (20 to $60 \mu\text{g}/\text{cm}^2$) are very convenient backings. They are mechanically strong, and the breakage rate is usually less than 5%. Their high heat conductivity enables them to withstand $\approx 1 \mu\text{A}$ proton currents for over 60 min, but this advantage over Formvar^R becomes less significant in view of the need to restrict currents to $\leq 0.5 \mu\text{A}$ to prevent loss of volatiles from the specimen. For these thicknesses bremsstrahlung background is negligible in comparison with background generated in the specimen material.

The effects of self-absorption are very important and should be considered when the sample thickness exceeds some value. An expression can be obtained for the magnitude of the emitted radiation as a function of sample thickness if one idealizes the geometry and makes several simplifying assumptions. It is felt that although the problem is greatly idealized, the answers so obtained are useful in estimating the errors one can expect due to thickness or absorption effects in the sample (Zeitz, 1969).

The necessary condition for a quantitative analytical method is that the magnitude of the detected line must be independent of sample thickness, or stated in another way, that the magnitude of the detected line must be a function

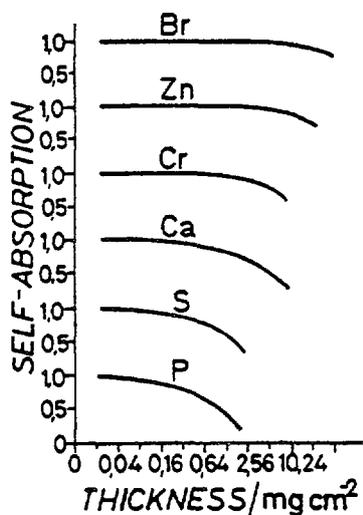


Fig. 3.5

Self-absorption for K_{α} lines of different elements in biological tissue (Zeitz, 1969).

of the amount of element present in the sample and not its thickness. One can develop an expression which will indicate the error in accuracy (precision) to be expected due to the absorption or thickness effect. The results of the calculation by Zeitz (1969) are shown in Figure 3.5. The ordinate is essentially the ratio of the power of K radiation of element A actually emitted from the sample into a relatively small, well-defined solid angle to the power which would be emitted into this same solid angle if there were no absorption within the sample.

The actual numbers will depend on the tissue composition and for different tissue composition will be slightly different. It can be shown that similar curves can be constructed for the case of "fluorescence in reflection", or the detection of fluorescent radiation emitted from the same side of the sample as that of the entering exciting radiation. Curves for this case vary little from those given in Figure 3.5.

Careful choice of backing materials is very important in trace element analysis since the background highly affects the accuracy of the measurement and the detection limit. The ideal backing is a thin film composed of elements of low atomic number and possessing the properties of high mechanical and chemical strength and of high electric and thermal conductivities. Further-more, it must be of very high purity so that the characteristic x-rays from impurity elements can be ignored. In the study by Kaji et al., (1977) the advantages and disadvantages of carbon, Formvar and Mylar as backing materials have been examined from the practical viewpoint.

Several authors have compared background spectra from thin plastic films such as Mylar, Kapton and Teflon. According to some reports carbon films of $40 \mu\text{g}/\text{cm}^2$ thickness can withstand up to 1 h irradiation by $2.5 \mu\text{A}$ of 1.5 MeV protons. The use of thin films as backing will reduce the bremsstrahlung background significantly compared to a thick backing. Heat dissipation by radiation is no doubt important at high temperature for carbon films, but for thin aluminium films of good conductivity the temperature rises only a few degrees because of heat dissipation. According to some reports Mylar can withstand proton irradiation by $150 \mu\text{A}$ at 3.3 MeV, with some evidences of deformation, while 0.3 mil. of Kapton

can withstand a 30 min irradiation by 200 uA protons at 3.5 MeV, but will burn away after a few minutes irradiation with 350 uA.

In the study by Kaji et al., (1977), carbon films were prepared by evaporating onto a clean glass plate coated previously with a thin layer of glucose. The high solubility of glucose facilitated the removal of the carbon film from the glass plate on the surface of water by dipping it in water and then the carbon film was mounted on a brass-plate target holder. The x-ray spectrum from the carbon foil thus prepared contained characteristic peaks of impurities such as Fe, Cu and Zn superimposed on the rather low continuous background. These suggest that carbon films are inadequate as backings unless ultra-pure carbon is employed.

Background spectra of commercial Mylar films bombarded by 3.5 MeV protons show small amounts of Ca, Fe and Zn superimposed on the fairly high background were found in 10 μm Mylar film. However, the background level of 4 μm Mylar is sufficiently low for the analysis of trace elements. Thus, Kaji et al., (1977) have adopted Mylar as the backing, taking into account its mechanical and chemical strength and the relatively low background.

In their work Johansson et al., (1970) have used thin foils of polystyrene as a backing materials.

Samples to be analysed are applied on thin foils ($\approx 40 \mu\text{g}/\text{cm}^2$) or polystyrene, either by being directly collected on these foils, as in the aerosol analysis program, or transferred to them. Solutions, e.g. can be spotted with a pipet and, after evaporation, the residue can be analysed directly provided care is taken to avoid absorption problems. Polystyrene is easy to handle and has considerable strength. It also withstands sufficient beam intensities to allow count-rate limited analysis.

The carrier foils are prepared from a 4% solution of polystyrene in benzene. Microscope slides previously coated with NaOH are inserted and then withdrawn at a constant rate of several cm/s. After evaporation of the benzene, the polystyrene coating can be cut to suitable pieces (e.g. 1 cm^2), then floated off by obliquely lowering the glass slide into distilled water. The foils are then easily picked up on an aluminium frame with an ellipsoidal hole. The whole target preparation procedure is performed in a dust free laminar flow work station to reduce contamination. Eight frames with samples are mounted together on a rod, held in a barrel that, when closed, shields the targets from airborne contaminants during the transportation to the irradiation site. The foils generally have low blank values. In 33 blank foils, Ca was detected in 28 foils with an average of 3.4 ng Ca per foil in the beam, Cl in 20 foils (4.8 ng), S in 16 (4.6 ng), Mn and Fe in 11 (2 ng) as aerosol deposits from a single-orifice impactor, are completely enveloped by the beam. In the case of large homogeneous samples, the positioning facilities are only manipulated to make sure that no beam hits the frame on which the sample is suspended.

In the case of selfsupporting samples there is no need for backing material. However, even in this case the sample material (usually solid) has to be mounted on the grame in order to be exposed to proton beam. Since in many cases rather thin samples ($\approx 5-20 \mu\text{m}$) are required cutting by microtone is often used. In order to efficiently use microtone the material to be investigated must be first embedded into the araldite.

Very often used araldite is AY103 with hardener HY956. The only contaminants in araldite interfering with trace element analysis are Cl and Br. For nonconducting samples, for example biological material, it is necessary to evaporate an aluminium layer (5-10 nm) to prevent charging of the target and to reduce heating of the sample.

3.2 Target uniformity and homogeneity

Target uniformity and homogeneity should be checked for any adapted procedure of target preparations. This is needed especially when the beam spot size is smaller than the target area.

Let us describe uniformity test and homogeneity test as performed by Kivits (1980) for their sample preparation procedure.

They tested the uniformity and homogeneity of targets prepared using a solution consisting of Mn, Cu and Y (as nitrates) each 5 g/l. For testing they used a 2-mm diameter 3.05-MeV proton beam, scanning along a diameter of the target. Since rotational symmetry may be assumed, this scanning gives the radial distribution of the elements over the target. Results are given in Table 3.1. The normalised x-ray peak intensities of the three elements show standard deviations of about 6%. In calculation of the mean value, the position at $r = -13.4$ mm is not taken into account because this spot is just at the edge of the wetted area, as was observed after irradiation on the basis of the colour change. The areas normally analysed with PIXE have diameters ranging from 5 to 15 mm; for such large areas the authors claim a non-uniformity of lower considerably than 6%. Inhomogeneity, i.e. deviation from a constant composition of elements, is clearly demonstrated in the last two columns of Table 3.1. The standard deviations for the ratios Cu/Mn and Y/Cu are 2.3% and 3.3% respectively, including the spot at -13.14 mm.

3.3 Reproducibility

For any adapted method of target preparation the reproducibility should be tested. This is usually done by dividing a larger sample into a number of subsamples and then preparing the number of targets following identical steps in target preparation. Results obtained by Wheeler et al., (1974) from the measurements of some 50 samples of human blood serum are presented in Figure 3.6.

The distribution of concentration values obtained for Ca, Fe, Cu, Zn, Br, and Rb shown, together with their

Table 3.1

Results of scanning the target across a diameter
(27 mm) with a proton beam of 2 mm diameter,
as reported by Kivits (1980)

r_i (mm)	Uniformity test			Homogeneity test	
	$\frac{C_{Mn K}}{\bar{C}_{Mn K}}$	$\frac{C_{Cu K}}{\bar{C}_{Cu K}}$	$\frac{C_{Y K}}{\bar{C}_{Y K}}$	$\frac{C_{Cu K}}{\bar{C}_{Mn K}}$	$\frac{C_{Y K}}{C_{Cu K}}$
11.8	1.10	1.05	1.02	0.335	0.352
7.6	0.92	0.93	0.89	0.349	0.349
3.8	0.98	1.01	1.04	0.358	0.373
0.0	0.98	0.97	1.02	0.347	0.380
-8.4	1.02	1.04	1.03	0.356	0.359
-13.4	0.84	0.83	0.84	0.345	0.370
	1.00	1.00	1.00	0.348	0.364
	± 0.07	± 0.05	± 0.06	± 0.008	± 0.012

standard deviations obtained by least squares fitting to a normal distribution. The results show that trace elements can be measured at the 1 ppm level (as seen in Cu) with an accuracy of $\pm 15\%$. The greater uncertainty in determining Br concentrations is consistent with the findings that Br volatilizes even at low beam intensities.

Kivits (1980) has also tested the reproducibility of his method of target preparation from homogenized fish meal. For this purpose 1 gram was introduced into a 10 ml Formvar-dioxana solution. The average number of x-rays per second and per nA, with attendant relative standard deviation, was determined for some elements (P, S, Cl, K, Ca, Fe): for P K $26.8 \pm 5.3\%$; for S K $25.8 \pm 9.2\%$; Cl K $29.2 \pm 5.1\%$; K K $28.6 \pm 9.7\%$; Ca K $59.5 \pm 4.7\%$; Fe K $2.1 \pm 11.3\%$. Statistical errors are negligible. This means that the irreproducibility over the ten targets in the amounts of material irradiated is at most about 5%, assuming that the elements phosphorus, chlorine and calcium are homogeneously distributed over the sample. The high relative standard deviation for the other elements is largely due to the inhomogeneity of the original sample. The contribution of sample inhomogeneity to the P, Cl and Ca concentration is shown by the relative standard deviation of the intensity ratio between the K x-rays of an element and those of calcium in each target: P K/Ca K $= 0.45 \pm 5.4\%$ and Cl K/Ca K $= 0.49 \pm 5.5\%$. The relative standard deviation observed indicates for these elements a certain sample inhomogeneity, too. This implies that the irreproducibility of the amount of material irradiated is substantially less than 5 per cent.

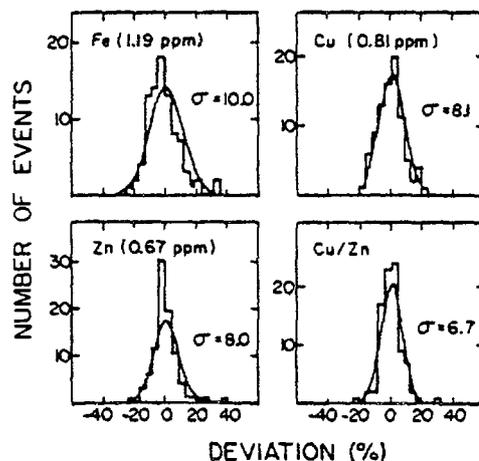


Fig. 3.6 Distribution of concentration values measured for Fe, Cu and Zn in human blood serum by PIXE (after Wheeler et al., 1974).

3.4 Effect of irradiation

Several investigators have studied the effect of irradiation on the sample. Probably the most detailed study has done by De Rooij et al., (1981) and we shall report some of their findings.

Important parameters for losses of elements during irradiation are the volatility, and the thermal and radiation stability of the analytes. Other parameters are the volatility of the radiolytic and thermolytic products of the analytes. With respect to the thermal and radiation stability, the beam intensity and irradiation time are important parameters.

In the study by De Rooij et al., (1981) the effect of the several parameters was investigated by repeated bombardment with 3.05 MeV protons of the same target. The elemental concentrations were determined for each of these measurements. To obtain an idea about the effect of volatilisation losses in inorganic compounds, they investigated the behaviour of inorganic chlorides and bromides. For these experiments the beam intensity was kept constant at 50 nA/cm². The initial concentrations of the halogenides were taken as 7.5 ug/cm². Table 3.2 shows the losses after 15 minutes irradiation for various chlorides and bromides. There are considerable differences in percentage loss between the halogenides; these losses occur usually in the first 8 min of the bombardment. Two typical curves showing the time-dependent behaviour are shown in fig. 3.7. In these cases, the amount of copper and rubidium remained constant ($\pm 2\%$) during irradiation. The losses of the halogenides observed in our experiments are of the same order as those observed by Ishii et al., (1975) under comparable conditions.

The second parameter investigated by De Rooij et al., (1981) was the concentration of the volatile element. The experiments, were repeated for CuBr₂, with concentrations ranging from 5 to 20 ug/cm² bromine. All curves had a similar shape, showing a plateau after about 10 min. The plateau corresponds to a bromine decrease of 13% \pm 1%, independent of

Table 3.2

Percentage loss of Cl and Br for several compounds. Beam intensity 50 nA/cm², halogenide concentration 7.5 ug/cm², proton energy 3.05 MeV and irradiation time 15 min.

(after De Rooij et al., 1981)

Compound	Binding energy (eV/atom)	Percentage loss
CuBr ₂	3.4	14
NaBr	3.7	6
RbBr	3.9	6-9
CsBr	4.2	20
ZnCl ₂	2.4	5
CuCl ₂	3.9	20
KCl	4.4	5
BaCl ₂	5.0	10-12

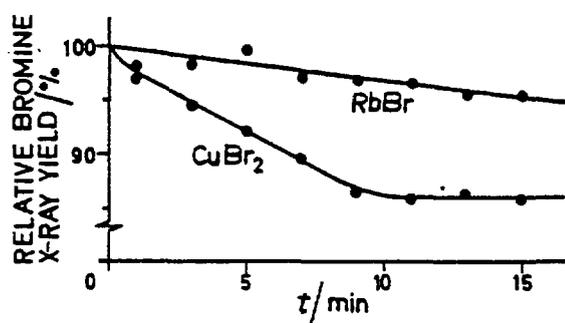


Fig. 3.7 Percentage loss of bromine for rubidium bromide and copper bromide on Selectron filters, as a function of the irradiation time. The bromine concentration was 7.5 $\mu\text{g}/\text{cm}^2$; the beam energy 3.05 MeV and the beam intensity 50 nA/cm². (After de Rooij et al., 1981).

the initial concentration. The effect of beam intensity on losses was checked for CuCl₂ (7.5 $\mu\text{g}/\text{cm}^2$ chlorine), as is shown in fig. 3.8 for 50 and 100 nA/cm² beam intensities.

As it is seen from the Figure 3.8 substantial volatilization may occur during the irradiation. Similar effects have been reported by Alexander et al., (1974), see figure 3.9.

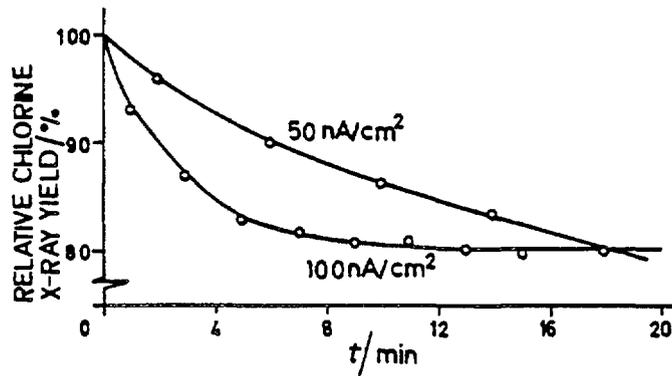


Fig. 3.8 Percentage loss of chlorine for copper chloride on Selectron filters, as a function of irradiation time. The chlorine concentration was $7.5 \mu\text{g}/\text{cm}^2$, the beam energy 3.05 MeV and the beam intensities 50 and 100 nA/cm². (After de Rooij et al., 1981).

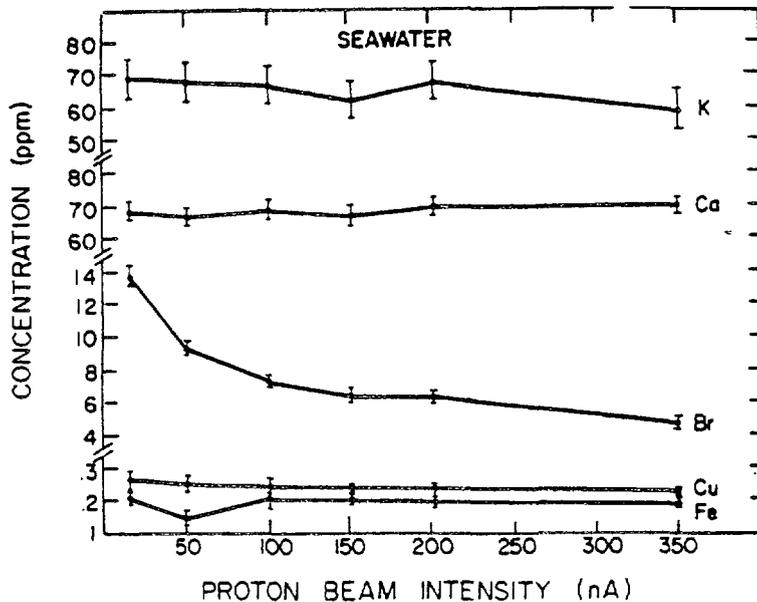


Fig. 3.9 Concentrations of different elements as determined from the bombardment of sea water targets by 3 MeV protons as a function of beam intensity.

Concentrations of different elements were determined from the bombardment of sea water targets by 3 MeV protons as a function of beam intensity. The results show the variations of K, Cu and Br concentrations with the beam intensity, with Br varying sharply from 5 to 15 ppm.

In order to reduce these losses De Rooij et al., (1981) have considered surface treatment of the target by addition of fixative agent or introduction of a shielding layer. In their studies dioxane was used as a fixative; this partly dissolves the selectron support, resulting in a collapse of the pore

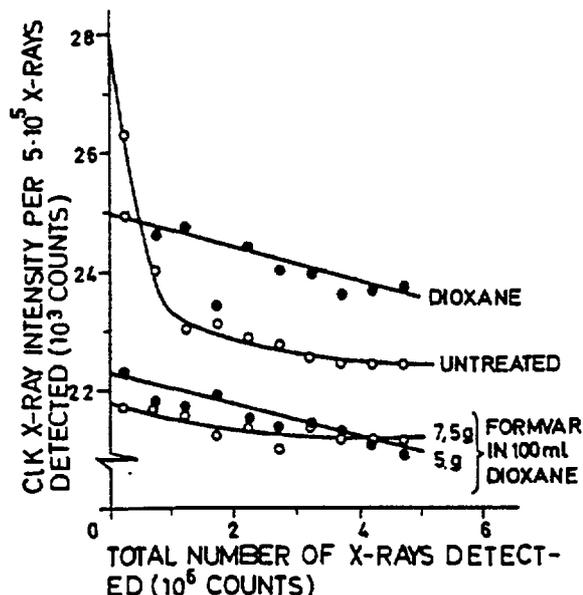


Fig. 3.10 The effect of different target treatments on the loss of chlorine for copper chloride on Selectron filters. The chlorine concentration was 7.5 $\mu\text{g}/\text{cm}^2$, the beam energy 3.05 MeV and the beam intensity 40 nA/cm². The Cl K X-ray intensity is shown per amount of 5×10^5 X-rays detected. (After de Rooij et al., 1981).

structure, with inherent inclusion of the elements. A shielding layer of Formvar may be introduced on both sides, by dispensing a solution of Formvar-dioxane onto a target. The Formvar layer was expected to prevent volatilisation of the elements due to its low permeability. The effect of different target treatments on the volatilisation of chlorine from a CuCl_2 target is shown in fig. 3.10, with a beam intensity of 40 nA/cm². Similar results were obtained for beam intensities of 25, 50 and 100 nA/cm². The beam intensity usually used by us is less than 100 nA/cm². As can be seen, the surface treatment is most effective for short analysis times (< 5 min). For a beam intensity of 40 nA/cm², the collection of 10^6 counts takes about 500 s.

Huda (1975) has investigated the effect of beam of charged particles on thick dried plasma pellets.

In a thick biomedical sample 2.5 MeV protons are completely stopped in a distance of about 10 mg/cm². The beam in these experiments covered an area of ~ 0.13 cm² and therefore a beam of 50 nA delivers about 125 mW to the sample mass of some 1.3 mg. Under vacuum conditions the temperature would begin to rise at over 10³ C/min and samples of low thermal conductivity become damaged by charring. Apart from loss of volatile elements, concentration of trace elements has been observed due to loss of the low Z organic matrix and there is also the possibility of contamination of the accelerator system.

The charge deposited onto a thick biomedical target, which is a good insulator, must leak away to the backing material which consisted of a pure aluminium foil. The

surface of the sample can thus get charged up to a high voltage during the proton bombardment, attracting free electrons in the sample chamber which in turn gives rise to electron bremsstrahlung x-rays.

Charge build up is also observed on the hair samples under proton bombardment. This appears to depend on the thickness of the hair, among other factors. It results in the presence of a high background in the spectrum, due to periodic discharging and should be avoided. For thick targets this can be accomplished by blending with graphite. For thin targets it may be necessary to coat with very thin layers of carbon or other suitable conducting material.

Other techniques of avoiding the problem are also recommended here. It is possible to place a heated tungsten filament near the sample. The emitted electrons prevent charge buildup. It is also possible to arrange the apparatus such that the sample is actually irradiated within the Faraday cage, and the beam enters via a small hole; the cage is differentially pumped and the pressure maintained at about 10^{-2} torr. The effect of this is to remove charge from the sample by carrying it on gas molecules.

3.5 Internal standards

Internal standards are materials added to sample under investigations in known chemical forms and predetermined concentrations. Very often is it useful to know the shape of the x-ray spectra for the targets of known composition. This is required when the composition of sample is being guessed without the aid of a computer. In order to help workers in the field of proton-induced x-ray emission spectroscopy the following three figures are shown:

Figure 3.11 is the x-ray spectrum obtained by proton irradiation of target with 949 ppm Hg (L-lines) and 65.5 ppm Y (K-lines); Cu and Pb are present as contaminants.

Figure 3.12 shows the iodine lines; ratio of I/Y concentration was 25.

Figure 3.13 shows the x-ray spectrum from Ni(50 ppm) to Pb (952 ppm) target. All three targets were prepared on Al-Formvar^R backing by drying a few drops of solution.

Determination of relative elemental sensitivities for any system based on the detection of characteristic x-rays requires the step of preparation of standards. Different authors use different approaches for this measurement. For example, in the work by Kubo¹²² the following chemicals were chosen for a standard solution: $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{K}_2\text{O}$, $\text{Sr}(\text{NO}_3)_2$, AgNO_3 and $\text{Pb}(\text{NO}_3)_2$. The Fe, Pb, Ca, Fe, Cu, Sr, and Ag compounds were dissolved in 200 cm³ doubly distilled water. A small amount of standard solution was pipetted onto the backing foil which was placed on the microbalance, and the target tare weight was then determined.

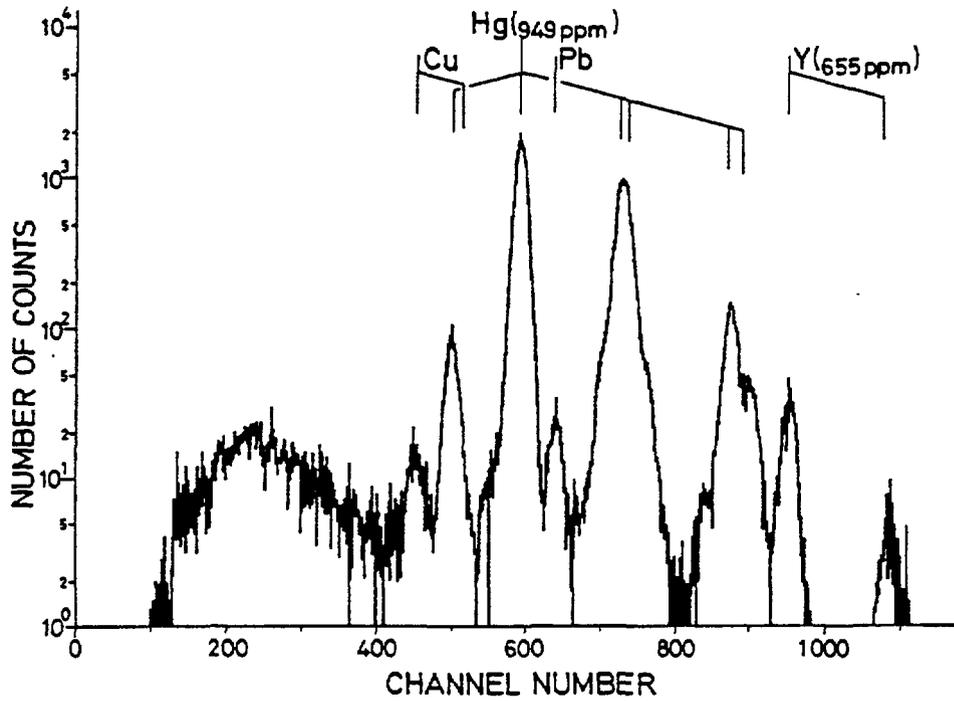


Fig. 3.11 Mercury spectrum (Yttrium is the internal standard).

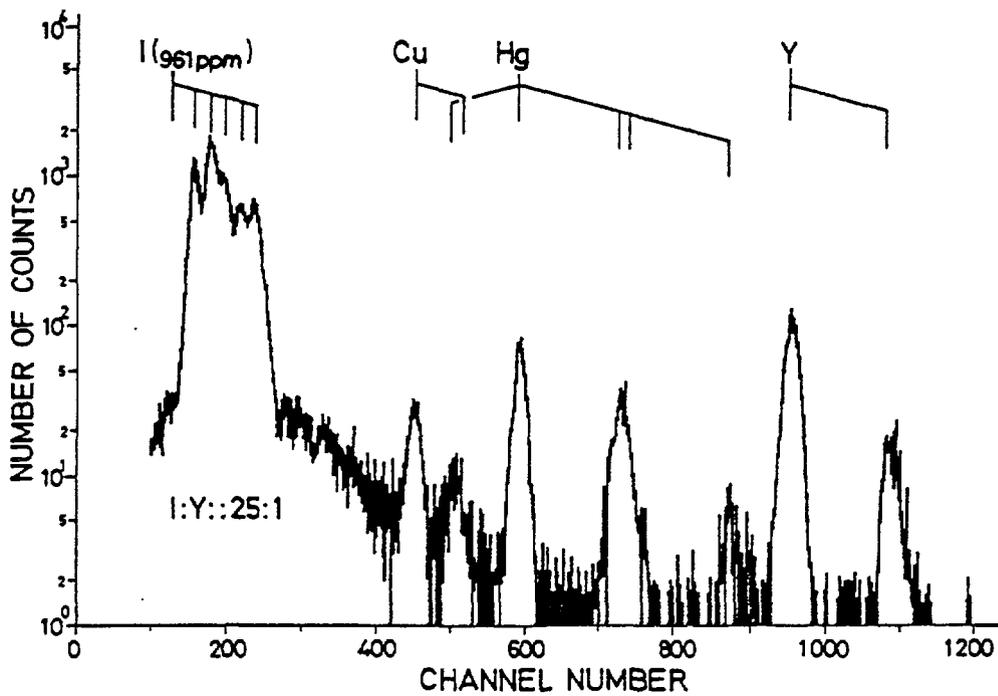


Fig. 3.12 Iodine spectrum.

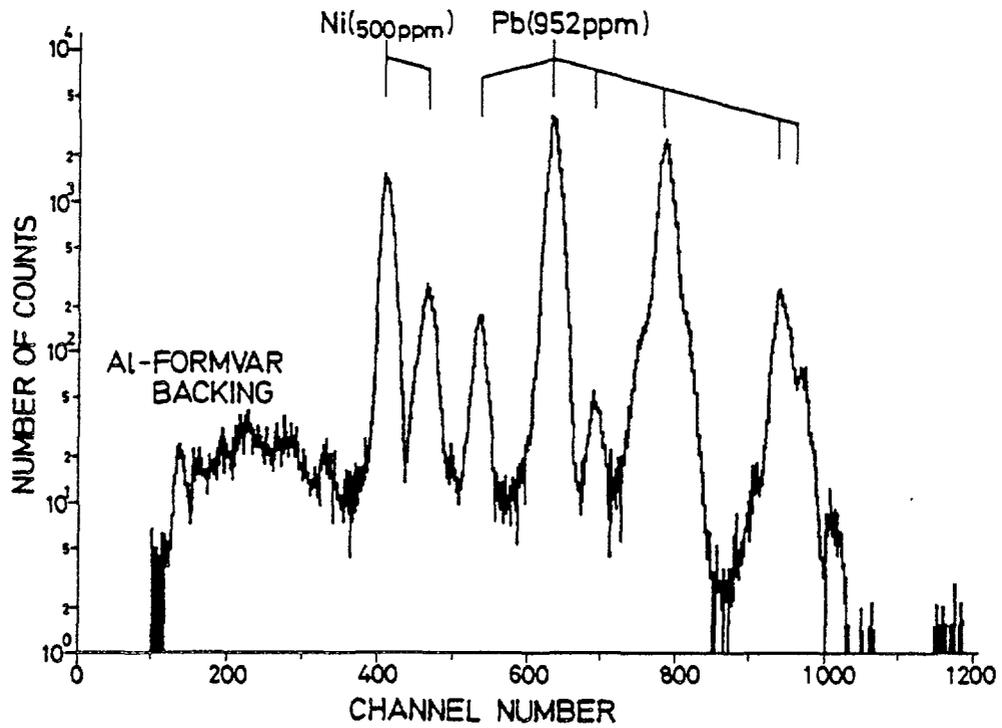


Fig. 3.13 Spectrum of lead
(nickel is added as dopant).

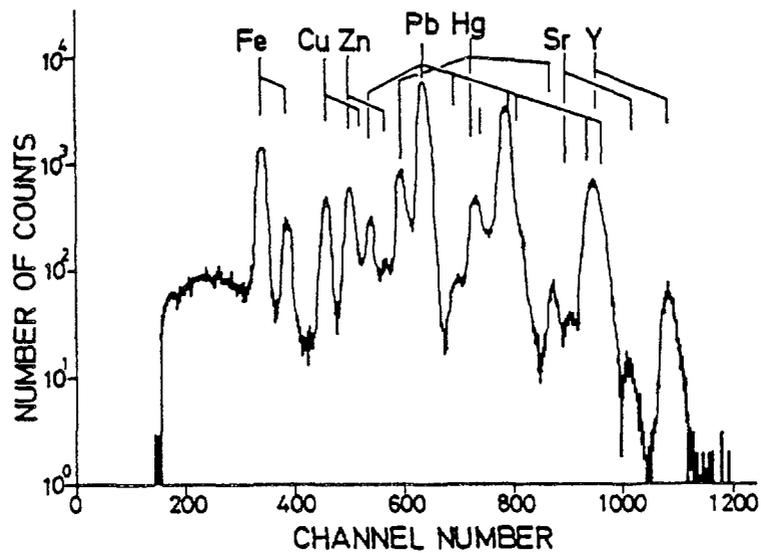


Fig. 3.14 X-ray spectrum of Fe-Cu-Zn-Pb-Hg-Sr-Y
solution (all elements 1000 ppm) as a
result of irradiation with 3 MeV
protons. All elements 1000 ppm, Al-
formvar backing.

These values were then used to obtain absolute concentrations of trace elements in biological samples.

Usually an element which does not appear in samples to be studied is chosen as an internal standard. This is often yttrium. Before measuring the samples, a set of standards which contain the known ratios of yttrium and other elements needs to be measured. For example, when the solution Fe-Cu-Zn-Pb-Hg-Sr-Y (all elements 1000 ppm) was used for the target preparation, and the target irradiated with 3 MeV protons, the spectrum shown in Figure 3.14 resulted. Intensities of peaks corresponding to different elements are not all the same because of the differences in:

- 1.. Detector efficiencies for different energies
2. Different cross sections for the production of x-rays.

The method of internal standards relies on the mass absorption coefficients cancelling out of the intensity ratio equations, so that

$$I_p/I_s = K Wt_p/Wt_s$$

where I_p , Wt_p = integrated intensity, weight of component p in the sample

I_s , Wt_s = integrated intensity, weight of internal standards

K = constant which depends on p and s, but is independent of the matrix

K can be found by calculating the slope of the graph of intensity ratios vs weight ratios. In our calculations a modified least squares linear fit is assumed, with the intercept forced to be the origin.

In the work by Roelandts et al., (1978) the internal standard technique was adopted in order to minimize target inhomogeneity effects and to correct for beam current fluctuations. Silver was selected for this purpose, as this element is very rare in usual samples. Its K_{α} lines ($K_{\alpha 1} = 22.1$ keV, $K_{\alpha 2} = 21.9$ keV) were used as internal monitors.

A 500 μ g Ag/ml stock standard solution was prepared by dissolving $AgNO_3$ in water. A few drops of dilute nitric acid were added for stabilization.

Individual stock standard solutions (1000 μ g/ml) of each element of interest (Y, La, Ce, Nd, Dy, Ho, Tm and Lu) were prepared by dissolving accurately weighed amounts of "Spec pure" oxides in nitric acid, evaporated nearly to dryness and taken up in very dilute nitric acid. By suitable dilutions of the stock solution, in 10 ml-graduate flasks, a range of required calibration standard solutions was made. 1 ml of Ag internal standard solution was added by pipette into each 10 ml-graduate flask before dilution to volume.

Aluminized mylar foils (0.5 mg/cm² mylar, 0.4 mg/cm² aluminum) inserted between two rings made of aluminum were adopted as support materials. Aluminum helps the prevention of charging of the samples.

Calibration targets were prepared by pipetting aliquots of 200 μl of the calibration standard solutions of Y(+Ag) and the individual REE(+Ag) onto these mylar disks and evaporating to dryness, exposed to the air. Afterwards they were placed in an oven at 40 C and finally stored in a desiccator. Thus five calibration targets were prepared for Y and individual REE: 5 μg , 10 μg , 20 μg , 50 μg and 100 μg , each of them containing 10 μg Ag as an internal standard element.

Buso et al., (1982) have used tellurium as an internal standard for determination of selenium in biological samples by PIXE.

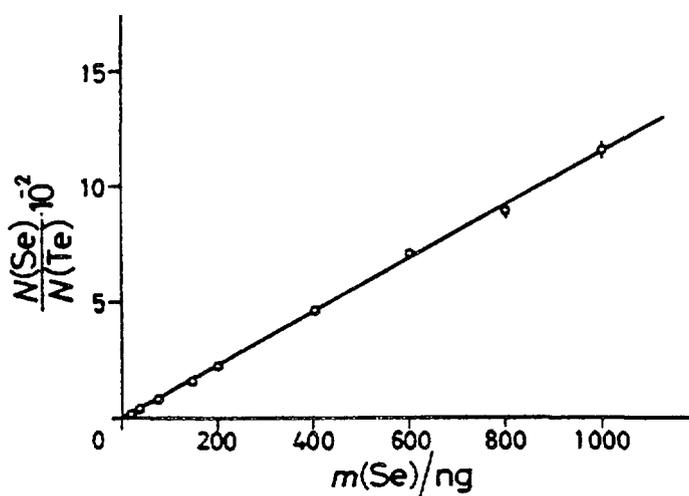


Fig. 3.15 Selenium determination at nanogram level without organic matrix (after Buso et al., 1982).

The first step of target preparation was the destruction of the organic matrix. The destruction was done in non-oxidative way, unlike usual methods, to allow the subsequent precipitation of selenium, which can take place only in reducing medium.

Tellurium (600 μg) was used both as internal standard and as coprecipitant. The suspension obtained is then filtered on 1 cm^2 millipore filter. Proton beams damage millipore filters even at very low current; therefore it was necessary to support these filters with a suitable backing during irradiation. Whatman filters were chosen for their good beam resistance and low background. First the authors have studied the efficiency of the precipitation at nanogram level without organic matrix.

Some results obtained by Buso et al., (1982) are shown in Figure 3.15. The reported errors are due to counting statistics. Similar correlations were obtained with different quantities of tellurium in the range 200-1000 μg ; 600 μg was chosen as a good compromise value, taking into account counting rate and target resistance.

The efficiency of the technique with organic matrix is shown in Figure 3.16: 1 cm^3 serum were doped with increasing

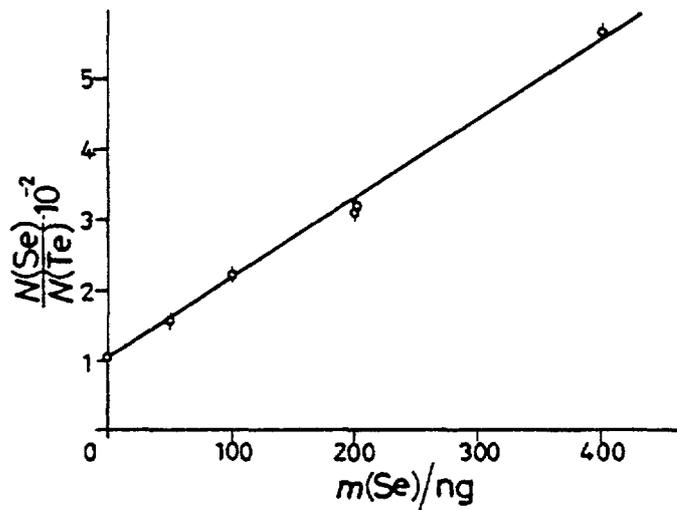


Fig. 3.16 Efficiency of selenium precipitation with organic matrix (1 cm³ serum). see text; after Buso et al.. 1982).

quantities of selenium (50-400 µg) and then treated as previously described. The efficiency measured with and without organic matrix is the same, it means that the recovery of selenium added is not affected by the organic matrix. One can therefore use Figure 3.15 as calibration curve for selenium determination with this preconcentration technique.

3.6 Examples of sample preparation for PIXE

3.6.1 General

Target preparation for irradiation by charged particles often requires that the sample be solubilized. For most biological substances, the two common procedures for organic matter destruction are wet digestion and dry ashing. The advantages and disadvantages of these two techniques have been researched by many. The best and most comprehensive work on this subject is by Gorsuch (1970). For routine plant analysis and most other biological materials, the dry ashing technique has been used successfully by many researchers. Losses of elements or contamination have been minimal. High wall ashing vessels and the placement of the vessel up off the floor of the muffle furnace are essential parameters necessary to minimize potential losses of the more volatile elements (Jones, 1976).

Thin specimens may be prepared directly from wet plant or animal tissue by various physical methods. Valković et al., (1974) crushed tissue in water and stored the resulting suspension until the cells were dissociated. However, the suspension was not particularly homogeneous and consistency for supposedly equivalent targets was not good. Jolly and White (1971) have described a nebulizer which directs a fine mist of droplets from sonicated material towards a backing, on which a thin film builds up.

Campbell et al., (1975) have prepared slices of 10 μm thickness by cutting with a stainless steel blade using a freezing microtome; freezing to enable slicing is preferable to embedding with a hardening agent (paraffin) from the contamination aspect. Slices of diameter 5 to 10 mm adhere satisfactorily to carbon foils.

Several workers have made thin targets by depositing a very small mass (≤ 1 mg) of lyophilized or ashed material on a backing foil, adhesion being effected by adding a drop of wetting agent or glue. This approach has also been used, by various authors, for specimens such as finely powdered rock, and seafloor sediments.

Such targets have thicknesses of the order of 1 mg/cm² and there are thus no x-ray self-absorption problems. Although one might expect the powder produced by lyophilization or ashing to be sufficiently homogeneous that 1 mg samples would be representative of the bulk, is not necessarily so. For example, the National Bureau of Standards warns users of its standard trace element reference materials SRM 1571 (orchard leaves) and 1577 (bovine liver) that 0.25 g is the minimum mass of powder that will afford a representative sampling of the bulk.

There are several methods of disintegrating cells, among which ultrasonic irradiation has been accepted as certainly one of the most effective. The disruption of living cells by ultrasound usually depends on the sound intensity in the medium being maintained above a critical value sufficiently high to induce the phenomenon known as "cavitation". Cavitation is a complex process which may be summarized as follows. When a liquid is subjected to sound intensity above the critical value the transport of the sound waves through the liquid causes very rapid alternations of pressure, resulting in the formation of large masses of minute gas-filled bubbles. These grow and pulsate through several sound cycles until a critical size is reached, the magnitude of which depends on the frequency of the sound. At this stage the bubbles disintegrate implosively, giving rise to intense local shock waves, high instantaneous temperatures and to microstreaming of the liquid around the points of collapse. The streaming around bubbles produces high shear gradients which are responsible for the degradative effects on cells.

The preference for the particular method of target preparation depends on many factors, most of them being subjective in nature. One should keep in mind that there is no universal prescription and that usually several methods will give equivalent results. This is indicated also in Figure 3.17 which shows the x-ray spectra from the targets of microorganisms, mold, which was harvested by the collection on filter paper. Spectrum A results from a direct exposition of such a filter paper with proton beam. Because of problems with insulating backings Scotch^R tape is used as a conductor (spectrum B). This has introduced bromine as a contaminant. Spectrum C was obtained by ashing filter paper with harvest and subsequent deposition of ash on Scotch^R tape. The last spectrum (D)

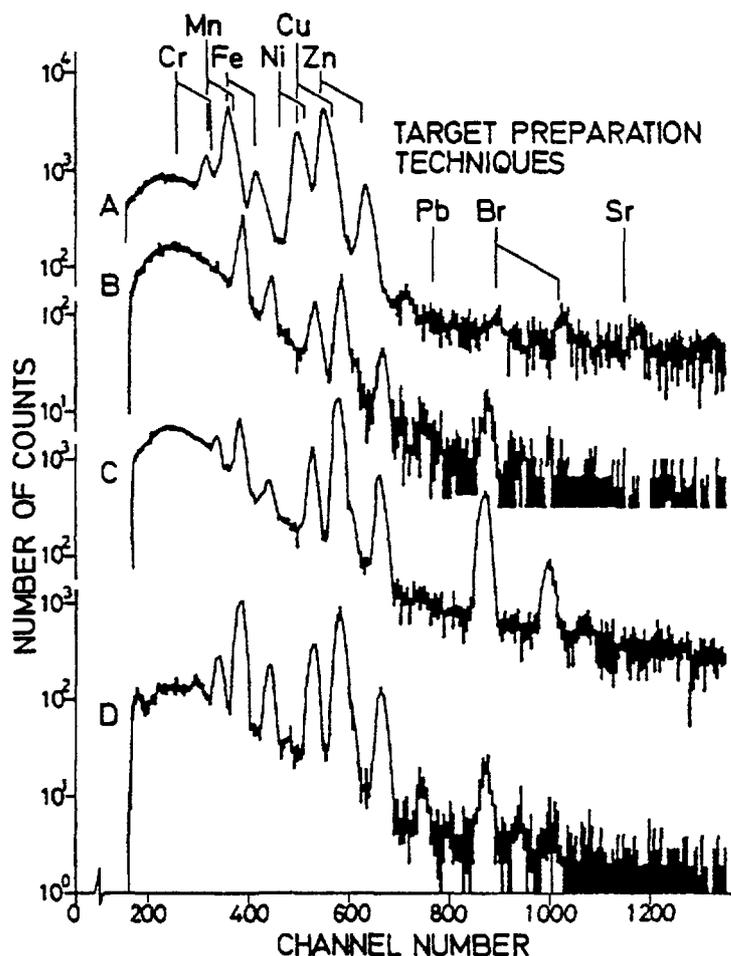


Fig. 3.17 X-ray spectra from the same biological target prepared on different backings (see text).

is obtained by irradiation of harvest which was transferred from filter paper onto Scotch^R tape. With careful analysis, and taking care of background radiation, information on the concentration of essential trace elements (Cr, Mn, Fe, Ni, Cu, and Zn) was obtained in all the cases.

3.6.2 Aqueous samples

Water and some other liquid samples can be prepared by drying a few drops on an appropriate backing when charged particles are used for excitation. This method is not good enough for the sample excitation with x-ray tube or radioactive sources, or when concentrations smaller than 0.1 ppm need to be measured. In cases like this preconcentration of elements to be analyzed should be determined. This is usually done

1. By evaporation
2. By complexing the metals with ammonium pyrrolidine dithiocarbamate and extraction with methyl isobutyl ketone (the concentrations can therefore be doped with an internal standard and evaporated on an appropriate foil)

3. By complexing some elements with oxine and absorbing the dissolved complexes on activated charcoal (Vanderborcht and Grieken, 1978).

There are some other procedures described in more detail in the chapter on trace elements in water.

Very often ammonium-1-pyrrolidine dithiocarbamate is used as chelating agent. It forms insoluble coordination complexes with more than 30 transition metals (for details, see Elder et al., 1975).

De Rooij et al., (1981) have described the method for various target preparation techniques for liquid samples.

This method can be shortly described as follows:

- a) Selectron cellulose-acetate foil (5 mg/cm²) is employed as a supporting material;
- b) The aqueous sample is diluted with ethanol 1:1 (v/v);
- c) Selectron filter is rotated at 18 000 rpm;
- d) 50 ul of the solution is dispensed as quickly as possible (< 0.2 s), yielding a wetted area of 570 mm²;
- d) The target is air dried at room temperature.

Sometimes, two-phase systems like emulsions and suspensions have to be analysed. Such systems may involve a more complex physical behaviour in target preparation. However, the physical behaviour of suspensions may be changed by chemical treatment into that of a homogeneous real solution. The advantage of thin targets is that the intensity of the proton beam, striking the target, can be measured.

Several authors use external beam for the analysis of liquid targets. For example in the work by Tsang-Lang Lin et al., (1979) Van de Graaff accelerator was used as a source of protons. The plastic target holder is mounted directly on the extension tube of the Al chamber. Dry nitrogen is continuously and slowly flushed in the volume between the exit window and the sample in order to suppress the x-rays iron argon in air. The argon K x-ray peaks, which were severe originally were suppressed effectively.

Liquids are contained in Chemplex XRF sample cups with a 6.35 μm thick mylar film as cover. Protons penetrate the mylar film and are then slowed down in the liquid. The x-rays were detected by a Si(Li) x-ray detector which was placed at a distance of 8 cm from the sample. Detector signals were amplified by an optical feedback preamplifier, a linear amplifier and then analysed by an MCA. A lead shield was used to prevent the x-rays produced at the proton exit window from reaching the detector. Proton beam currents can be measured beforehand by placing a metal target at the sample site to collect proton charges. Proton currents were extracted continuously from the isolated exit window which was served as beam monitor during the measurement.

Mylar films of 6.35 μm thickness were strong enough in tolerating proton bombardment up to total proton charges of 10 μC . However, it became fragile and deformed at the beam spot. A deformed surface will alter the absorption

factor of x-rays in the sample thus changes of the mylar film cover are necessary after it has been exposed to a cumulative proton beam of 10 μC charges.

In literature, several techniques for depositing a liquid onto a support are described. A multi-drop technique is described by Camp et al., (1974). A quantity of 220 μl is deposited in drops of 10-15 μl on a support, just enough to wet the entire surface. The reported standard deviation of the composition over the wetted area is 3% when measured with PIXE using a 5-mm diameter proton beam. In our experience it is difficult to obtain reproducible results with this preparation technique. This is probably due to fractional crystallisation or different migration speeds of the elements in the supporting material (of. paper chromatography).

The preparation method of dropping a solution on a Mylar foil of 10- μm thickness and drying in the open air at room temperature tends to give non-uniform targets, as was shown by Ishii et al., (1975).

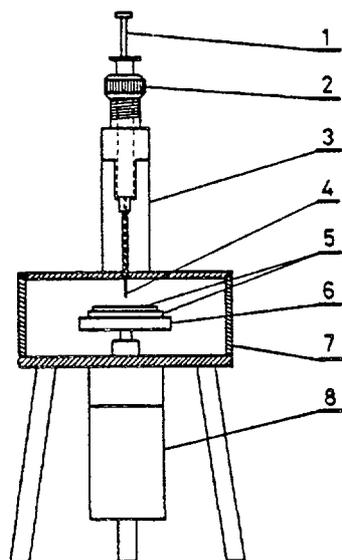
Baum et al., (1977) describe a method using a device consisting of 37 capillary tubes of equal and known volume. When dipped into a solution, the tubes fill by capillary action. The device is then placed on a filter, and the capillaries drain, simultaneously wetting the entire filter area. Freeze drying is necessary because otherwise the composition of different elements along a radius will not be constant due to different migration speeds, as is mentioned by the authors. Targets prepared according to this method are reported to be highly reproducible. However, besides the drawback of the need for freeze drying there is another disadvantage: the curling of the filter paper after the wetting-drying process. This implies a deterioration of the target geometry with inherent variable x-ray attenuation, especially for low-Z elements. Practical disadvantages are the fragility and the rather complex cleaning of the capillary device.

Let us describe in some detail, methods used by Kivits (1980) for target preparation for real solutions:

For target preparation of aqueous samples based on the use of a support Selectron OE67 foils (Schleicher and Schull, Dassel, F.R.G.) as supporting material were chosen. The area density of Selectron is about 5 mg/cm^2 , and elemental analysis gave an overall composition of $\text{C}_{32}\text{H}_{44}\text{O}_{23}$. By rapidly depositing the liquid on a rapidly rotating Selectron foil one can achieve uniform distribution of the solution over a constant area. It was found experimentally that the solution was uniformly spread over the entire wetted volume of the target. Besides the Selectron support, the author also investigated thinner supporting materials such as Nuclepore and Formvar. These supports proved to be inappropriate for the rotating-foil technique, because they are not easily wettable.

The apparatus developed for the preparation of the target is shown in 3.18. A microdispenser is centered above a rotating table on which a ring can be mounted. The outer part of the Selectron foil is clamped into the ring. The inner part of the foil which is also the area to be wetted, does not touch any part of the device. Air drying is applied

Fig. 3.18



Apparatus for target preparation from liquid samples as described by Kivits (1980):

- (1) Microdispenser,
- (2) height adjuster,
- (3) centering column,
- (4) tip of dispenser,
- (5) target (frame),
- (6) rotating table,
- (7) Perspex house,
- (8) motor.

before removal of the foil from the ring and is found to be very effective in preventing curling of the foil.

Parameters that can be adjusted in order to obtain maximum uniformity and homogeneity are the speed of rotation, the distance between the tip of the dispenser and the Selectron foil and the speed of depositing the liquid. Moreover, the parameters of the liquid (e.g. the viscosity and surface tensor) can be changed by the addition of certain chemicals.

The author has investigated aqueous samples of copper nitrate (1 gram copper per litre). Aliquots of 50 μ l were deposited on Selectron foils; in order to visualize the distribution of the liquid, methyl red was added. For targets prepared with a rotation speed of 15.000 rpm the distribution of the liquid was found neither uniform nor reproducible. Moreover, part of the sample was lost by sliding over the foil with a high speed and was found back on the edge of the apparatus. To investigate the influence of the surface tension, different kinds of synthetic detergents and aliphatic alcohol were added. The addition of ethanol proved to be most successful. Although addition of propanol decreases the surface tension more than ethanol, the author has opted for the latter, to avoid problems with precipitation of ionic constituents. Under the chosen working conditions a volume of ethanol equal to the volume of the aqueous solution gave, on visual examination, sufficiently reproducible uniform distribution with 18.000 rpm. Another parameter investigated is the rate of dispensation. The best results are obtained by rapid dispensing (< 0.2 s); slow dispensing results in ring-shaped distribution.

Some information about the transport mechanism of the solution on the Selectron foil was obtained from the following experiment: 25 μ l of red coloured solution was sucked into a 50 μ l pipet followed by 25 μ l of a yellow solution. The resulting 50- μ l volume was immediately dispensed on a foil in order to prevent the solution being mixed up. The resulting target showed the yellow colour in the inner-circle surrounded by a red ring, with

a sharp borderline between the two areas. Moreover, good rotational symmetry is clearly demonstrated. This phenomenon suggests a mechanism in which the first fraction of the dispensed solution is absorbed in the centre part of the foil, subsequent liquid fractions each time sliding over the previous fraction.

Measurements of the coloured area of 50- μ l targets (methyl red is used as dye) gave an average wetted area of 570 mm² with a standard deviation of 20 mm². Moreover, addition of a dye has another advantage: after irradiation, the proton-beam-exposed area can be determined accurately due to a change in colour. This may, for instance be helpful in outlining the proton beam.

Because of the constant amount of liquid deposited (50 μ l), the reproducible wetted area (570 mm²), the constant porosity of the Selectron foil and its low area density (5 mg/cm²), good analytical results are ensured.

Based on the results described above, Kivits (1980) adopted the following standard preparation procedure for real solutions:

- (a) Selectron is employed as a supporting material;
- (b) The aqueous sample is diluted with ethanol 1:1 (v/v) and coloured by methyl red;
- (c) Selectron filter is rotated at 18.000 rpm;
- (d) 50 μ l of the solution is dispensed as quickly as possible (< 0.2 s), yielding a wetted area of 570 mm²;
- (e) The target is air dried at room temperature.

The authors has also checked by PIXE analysis the retentivity of the target for volatile elements such as Cl and Br in different inorganic compounds (CuCl₂, CuBr₂, RbBr, RbCl). Storing the target at room temperature in the open air for one week did not influence the amount of analyte in the target; neither did vacuum pretreatment of 12 hours at 30°C.

The retentivity for CuBr₂ targets was also checked in a radioactive tracer experiment. CuBr₂ activated via neutron irradiation. The intensities of the ⁶⁴Cu and ⁸²Br γ -rays were measured in aliquots of 50 μ l CuBr₂ solution and in targets onto which 50 μ l was deposited. No significant differences were found. From this experiment the author has concluded that the whole aliquot is retained by the support, (?) either in the dispensing step or in the drying step.

The retentivity of the targets prepared with this radioactive CuBr₂ solution was also checked for a prolonged storage time. Targets were kept at 30°C in vacuum for 24 hours and some targets were kept at 57°C in the open air for the same period. Both treatments gave no significant losses.

3.6.3 Biological samples

Target preparations techniques for biological samples have been discussed in many papers. For example, Jundt et

al., (1972) have used Formvar^R backing and diffused proton beam. Techniques they have used in preparing biological tissue samples include:

1. Samples deep-frozen, then sliced with a microtome.
2. Washed, formalin fixed and paraffin embedded, then sliced with microtome, after removing paraffin with an appropriate solvent, deposited on target backing.
3. Homogeneous solution in distilled water deposited on backing and dried.

Renan and van Rensburg (1980) have described the procedures used for the preparation of sample from biological material (laboratory animals). Liver and oesophagus samples were obtained at autopsy; sections 1-2 mm in thickness were used for the trace element determinations. The tip of the middle lobe of the liver (after removal of the outer capsule), and the epithelium of the middle third of the oesophagus were analysed in each case. Care was taken to ensure that connective tissue and visible blood vessels were not included in the samples for analysis. All tissues analysed were infinitely thick in the sense that the projectile beam stopped completely in the material; the section thickness used was greater than the range of protons in an organic matrix at the energy used (approximately 15 mg cm^{-2} at 3 MeV). Although the sections were prepared with as uniform a thickness as possible, most of the x-ray yield originates from the outermost layers of the specimen (where the production cross-section is greatest and x-ray absorption least); thus small inhomogeneous patches will not greatly affect the accuracy of the analysis.

Numerous investigators have cautioned against contamination in the analytical laboratory; the well known work in this area is that of Sansoni and Iyengar (1978). The stringent protocol adopted should be based on the guidelines suggested by these authors.

In the work by Renan and Rensburg (1980) plastic working surfaces and gloves were used throughout, as were Perspex knives for the dissection. Unfortunately these knives were inadequate for the removal of the epithelial tissue from the oesophagus and stainless steel surgical instruments were necessary. The contamination so introduced is probably not significant but the results for Cr in this organ should not be regarded as reliable. Immediately after dissection, the samples were mounted on clean Perspex holders and freeze-dried at -50°C for 24 hours. The samples were then stored in sealed plastic containers in a vacuum desiccator prior to analysis. The tissue samples were analysed intact, i.e. no ashing, grinding or acid digestion was found to be necessary. As a consequence, differential losses of elements and inhomogeneities due to fragmentation were kept to a minimum.

Chen et al., (1977) have measured nickel concentrations in lung and kidney tissues. In their work samples (0.1 to 0.5 g) of tissue were weighed in sterile, capped plastic test tubes. Concentrated nitric acid (Baker "Ultrex") was added, and the samples were heated to promote digestion.

Portions (10 ul) of the solutions were evaporated to dryness and analyzed directly in a 2-MeV proton beam. Proton-induced characteristic x-rays, including those of nickel at 7.472 keV, were detected in a Si(Li) detector, and the nickel concentrations were determined. The quantitative calibrations were performed and cross-check measurements by analyzing samples to which known concentrations of nickel had been added.

Hair is one of the biological materials often analyzed by PIXE. Analysis by PIXE is easiest in two cases; when the sample is extremely thin, and when it is infinitely thick to the proton beam. Cases between these two extremes are analytically difficult, and it is recommended that sample preparation be used to convert samples to one of the two forms. This applies generally to biological samples, but specifically to hair, which is neither infinitely thin nor infinitely thick for protons at energy 2-3 MeV and in addition is heterogeneous. Special sample preparation is therefore essential for quantitative work, except where specific information is required about the distribution of elements within the hairs, or much less rigorous quantitative data is required.

Three possible methods of preparation can be recommended: (a) pulverisation and preparation of a thick target, (b) dissolution and evaporation on a thin backing, and (c) embedding and sectioning.

(a) Pulverisation is recommended to the level of approximately 50 μm . Various techniques are possible: (i) using liquid nitrogen and an ultrasonic vibrator, pulverisation in a teflon container with a teflon bail, or (ii) pulverising without freezing in an agate mortar. In each case about 1% of reactor-grade pure graphite may be added to avoid charging effects.

(b) The procedure of dissolving hair can be best done in hot concentrated nitric acid rather than concentrated alkali, but that the technique be checked for volatilization of mercury (and possibly other elements) before it be used on a large scale for determination of that element. The solution or a portion of it (with possible addition of an internal standard) should be dried on a thin film of some pure resistant material. Hostaphan and Kapton are already known to be satisfactory though the problem of flaking needs further investigation. Any other material should be checked carefully for purity before use on an extended scale.

3.6.4 Blood serum samples

Whole blood and serum samples are often investigated for trace elements using PIXE as the analytical techniques. We shall here describe some of the approaches taken in these studies.

It has been demonstrated by Valković (1973) that the blood serum target might be prepared on Al+ formvar backing with satisfactory reproducibility of results. In their studies the blood plasma was doped by 100 ppm solution of yttrium.

Berti et al., (1971) have determined selenium in the serum. Palladium was chosen as internal standard for the

determination of selenium. The targets were prepared in the following way: 1,2 cm³ of blood serum were doped with 100 ppm Pd as PdCl₂. Serum and internal standard were ultrasonically mixed, dry-ashed in borosilicate glass beakers for about 1 h at 60°C, ground into a fine powder using an agate mortar and then thoroughly mixed with 20% of nuclear graphite powder.

A self-supporting pellet was prepared from this mixture by pressing at 4×10^3 kg/cm² into a suitable die. The resulting pellets are 12 mm in diameter and have area density 50-60 mg/cm², which constitutes infinite thickness for protons in our energy range. By varying the graphite concentration in the mixture, it was found that about 20% allows the pellets to withstand the beam heating for the time necessary to reach the required sensitivity. (see also Calaritti et al., 1980; Perona et al., 1977).

One should take precautions to avoid contamination of serum. How to do that is well described by Versieck et al., (1978): Venous blood should be taken with a plastic cannula trocar (Intranule 110 16; Vygon) and collected in high-purity quartz tubes (Spectrosil; Thermal Quarz-Schmelze) previously cleaned with twice-distilled water, boiled in a mixture of equal volumes of nitric acid (min. 65%) and sulfuric acid (96%) (Suprapur^R; Merck), rinsed again and finally steam-cleaned with triple quartz-distilled water. Transport of the samples should be limited as much as possible and done in air-tight boxes. Spontaneous clotting can be allowed. Before the irradiation, further handling of the samples should be carried out in a dust-free room. After centrifugation the serum should be decanted into thoroughly cleaned polyethylene containers, frozen and lyophilized.

A detailed description of serum preparation for irradiation by protons can be found in the paper by Bearse et al., (1974): Capillary pipets, rinsed in heparin and air-dried, were used to draw 0.1-ml samples of whole blood from the sinus cavities of mice. Human blood samples were drawn into 5-ml syringes, potassium oxalate was added, and the samples were repipetted with an Oxford 0.1-ml autopipet. The samples were pipetted directly into 1-ml borosilicate glass beakers that had been previously weighed on a Torbal balance to an accuracy of 0.2 mg. About 30 samples were processed at one time. The filled beakers were arranged on a 7.6- by 15.2-cm borosilicate glass plate on the base of a bell-jar system, and all beakers were repeatedly filled with liquid nitrogen until the blood was thoroughly frozen. With the beakers still containing liquid nitrogen, the bell-jar was sealed and the system evacuated. The system was pumped for several hours, and an ultimate vacuum of about 0.06 Torr was achieved. The beakers were then removed from the bell-jar and reweighed to obtain the dry weight of the whole blood. The weight reduction from wet to dry blood was about a factor of 4.8. The samples were placed in Tracerlab LTA-302 asher on a 7.6- by 15.2-cm borosilicate glass plate which allowed the beakers to be on the diameter of the ashing chamber. The samples were ashed for 48-72 hours at a power of 100 watts, a pressure of 0.9 Torr, and an oxygen flow of 150-cm³/min.

The purpose of ashing is to increase the concentration of the elements with $Z \geq 26$ by removal of the elements H, C, N, and O which comprise the bulk of the blood mass. Targets with a higher concentration of trace elements can then be fabricated with a much smaller total mass. This decrease in mass reduces the amount of background due to secondary electron bremsstrahlung thus improving the detectability limit. It has been shown that plasma ashing is the method of choice to keep metal losses to a minimum.

After ashing, the samples were removed from the chamber, and 0.1 ml of a solution of 6 parts 1.8% HCl and 4 parts of 1000 ppm PdCl₂ (for normalization purposes) was added to each sample using the autopipet. A new disposable tip was used for each sample, and dissolution was effected by rapid inhalation and expiration of the solution, using the pipet. Occasionally, small back specks appeared in the solution and, so long as they were few and small, they did not seem to affect the accuracy of the analysis. A 0.02-ml autopipet was used to transfer that amount of the sample to the surface of a Formvar foil on which a still wet 0.02-ml drop of 3% NH₄OH solution had been placed. The NH₄OH neutralized the acidic blood solution which would otherwise have attacked the foil. The samples dried on the foils within a diameter of 5-6 mm. They were stored and transported in a desiccator and then transferred to the Los Alamos Scientific Laboratory trace-analysis chamber which had been modified to hold six 2.5- by 5-cm aluminum frames with 1.9-cm diameter holes. Larger frame holes were used so that the target and beam area could be increased to a reasonable and practical size (governed by the size to which the liquid drops dried) without stray beam striking the frame. Even a beam 10³ times smaller than the primary beam would cause an appreciable background spectrum upon hitting the frame because of the effectively infinite thickness of the frame.

Formvar foils were used in this work because they were relatively easy to prepare in quantity, withstood the required beam intensity, and were tough enough to endure the abuse of target preparation and subsequent handling. Another important reason for using Formvar was that it can be made very thin, thus contributing insignificantly to the secondary electron bremsstrahlung continuum. A Formvar solution was prepared which consisted of 16 grams of Formvar resin 15/95E (Monsanto Polymers and Petrochemicals Co.), 200 ml of methyl benzoate, 480 ml of toluene, and 320 ml of ethanol. The foils were made by placing a drop of this solution onto the surface of doubly distilled water. When the edges of the film began to wrinkle, it was picked up onto one of the aluminum frames, with care that double layers were not formed. These films were measured, by weighing and by the energy loss of alpha particles to be about 10 µg/cm². The foils were prepared ahead of time, allowed to dry, and stored in a desiccator containing CaSO₄ desiccant.

Blood consists of a liquid (serum) and cellular particles (e.g. erythrocytes and, leucocytes). In the target preparation scheme described by Kivits (1980) and De Rooij et al., (1981) cellular particles are destructed allowing blood to be treated as if it were a real solution. For this purpose, additon of

0.25 ml 1 M NaOH per ml blood gives a good solution, as could be microscopically observed. The method consists of following steps:

- a) To the blood sample, NaOH is added (1 ml blood: 0.25 ml 1M NaOH);
- b) propanol is added, such that the ratio of pre-treated sample to propanol is 5:1 (v/v);
- c) a Selectron filter is rotated at 18 000 rpm;
- d) 50 μ l of the solution is dispensed as quickly as possible, yielding a wetted area of about 10 cm²;
- e) the target is air dried at room temperature.

To test the blood samples for their reproducibility the amount of iron per unit area was measured by PIXE. For this purpose, ten targets were prepared from the blood of one person. The reproducibility of the targets amounted to about 1.6%.

Because of the low trace-element concentrations in blood, it could be favourable to use a thinner supporting material. The peak-to-background ratio in a PIXE spectrum will increase, for the same amount deposited per cm², resulting in a lower detection limit and more accurate peak-area determination. The quantity of blood on a Selectron foil of 5 mg/cm² is about 2 mg/cm². The support contributes to the Bremsstrahlungs continuum for about 70 per cent. If Formvar (0.2 mg/cm²) were used as support, this contribution would decrease to about 10%, provided the wetted area is equal to that for Selectron. Since attempts to prepare reproducible targets with blood solutions showed rather good prospects with Formvar supports, Kivits (1980) has developed a technique based on their use:

- a) to the blood sample, NaOH is added (1 ml blood: 0.25 ml 1M NaOH);
- b) propanol is added, such that the ratio of pretreated sample to propanol is 25:1 (v/v);
- c) a Formvar filter is rotated at 5,500 rpm;
- d) 50 μ l of the solution is dispensed as quickly as possible, yielding a wetted area of about 10 cm²;
- e) the target is air-dried at room temperature.

3.6.5 PIXE targets preparation for solid samples

De Rooij et al., (1981) have discussed targets preparation for solid samples. Solid samples may be analysed as such, or may be reduced to slices, powders or solutions. If the material can be analysed in the as-received state, the analyses are usually very rapid and convenient. However, since most solid samples are not homogeneous, problems may arise in the target preparation with respect to a representative bulk analysis. The representativity may be improved by powdering of the solid, in order to obtain a homogeneous sample and to reduce the particle size. Addition of an internal standard in the sample is then also feasible. A disadvantage of powdering a sample is that such pretreatment

is time-consuming and may be an extra source of contamination.

Powders may be spread into a thin layer, dry or as slurries. In the "dry technique" the powder can be dusted onto an adhesive surface, or can be spread out over a support adding a fixative later. In practise it seems to be difficult to obtain a uniform or thin layer of the powder by the "dry techniques" described. In the "slurry technique" commonly a small amount of powder and bonding material is slurried in an appropriate solvent. The slurry is spread over a clean microscope slide. The film is floated off on water, then scooped up and supported some other way. This technique however, involves many manipulations, which may introduce sources of analytical errors.

De Rooij et al., (1981) have developed a thin-target preparation technique via a "slurry procedure" requiring less manipulations and therefore less time. For powdering the solid, they use a "brittle fracture technique" similar to that of Iyengar. Thin targets ($\sim 1 \text{ mg/cm}^2$) have been chosen, since for such targets the requirements of uniformity and homogeneity are less stringent. On the other hand, for a good representativity of the bulk composition, preparation of several targets for irradiation from the same sample seems advisable. The steps involved are:

- a) A solution of 5 g Formvar in 100 ml dioxane is prepared;
- b) powdered solid sample is mixed with this solution (sample weight fraction about 10%) with the aid of a whirlimixer;
- c) a Selectron filter is rotated at 3,000 rpm and saturated with 0.5 ml water;
- d) 0.2 ml of the powder-Formvar-dioxane mixture is immediately dispensed on the wetted rotating filter;
- e) the resulting Formvar foil, in which the solid sample is embedded, is pulled from the Selectron filter.

The resulting foils have an area density of less than 1 mg/cm^2 , as determined with an α -particle set-up. The target preparation technique takes only a few minutes; the fraction of failures is less than 10%. The reproducibility in the amount of material irradiated and the reproducibility in the homogeneity of this material was tested using ten targets prepared from homogenised fish meal (elements considered: P, S, Cl, K, Ca and Fe). The reproducibility for the amount of material as well as the homogeneity was better than 5%.

Preparation of soil sample for analysis by PIXE is described by Navarrete et al., (1976). First, soil was dried and powdered to micro-millimeter fineness. The target used for 3.5 MeV proton was prepared by scattering a few milligrams of soil on $4 \mu\text{m}$ Mylar film and using a drop of 0.1 ml of polyvinyl acetate diluted with acetone as adhesive. It was ascertained that the film of soil was homogeneous and adequately thin. Energy loss of proton beam

in the sample was negligible as to cause error in determinations. Aluminium absorber of 50 μm thickness was used to decrease the low energy bremsstrahlung background.

Another target was prepared for bombardment using 200 keV proton. The soil sample was mixed with high purity graphite into 10:3 soil-graphite ratio and was compressed into a disk shape with 0.5 mm thickness and 10 mm diameter. A Mylar film of 20 μm thickness was placed in front of the Si(Li) detector as absorber to avoid the piling up effect of low energy x-rays due to the high concentration of Al and Si.

In the destructive method, 10 g soil sample was extracted with 50% HCl and 50% HNO₃ and diluted to 100 ml. An aliquot portion of 0.4 ml of the extract was dried on 4 μm Mylar foil.

Baker and Piper (1976) have described trace element analysis of marine suspensates. They have used a special holder with a Nucleopore^R filter attached to the tip of the syringe, and the necessary amount of water squeezed through the filter. Immediately after collection, the filters were rinsed free of sea salt by at least five flushes with filtered, distilled water and then stored in sealed plastic Petri dishes. In the laboratory, the filters were dried at room temperature and weighed to determine the total amount of suspensate collected. The filters were then mounted in the XRF sample chamber between two pieces of Mylar^R film, the top piece having a hole just smaller than the diameter of the sample filter so that the filter was held flat against the bottom piece without disturbing the collected particulate matter. This simplicity of preparation is particularly advantageous when dealing with very dilute suspensions, where the possibility of contamination is serious.

Separating the elements of interest from a large volume into the almost ideal matrix and volume of a resin-loaded paper disk has proven to be a versatile and effective approach to trace metals determination by x-ray spectrography (Law and Campbell, 1974). These papers are composed of approximately 50% cellulose and 50% powdered ion-exchange or chelating resin, providing a matrix of low-atomic-number elements that have little effect on the x-ray determination of most elements. Incorporation of the resin in a thin paper disk provides a convenient media for handling small quantities of resin and for supporting the resin in the x-ray spectrograph or energy dispersive system. Standards and unknowns can be prepared on similar resin-loaded papers, providing a match that is often impractical to achieve in direct x-ray analysis.

The general analytical procedure consists of the following steps (after Law and Campbell (1974)):

1. Dissolution of the sample, or selective dissolution of the element or elements of interest
2. Adjustment of pH, addition of complexing or masking agents, or other chemical treatment that may be necessary to achieve the selectivity desired in the ion exchange process

3. Collection of the desired elements on a resin-loaded paper disk by filtration or by suspension of the disk in the solution
4. X-ray determination of the elements on the dried resin-loaded disk, using disks containing known quantities of the elements as standards.

The combination of ion-exchange separation and collection greatly extends the range of fluorescent x-ray spectrometry. The chemical preconcentration reduces or eliminates problems of matrix correction, variations in physical properties of the sample, and increases sensitivity by several orders of magnitude. In addition, reliable standards are easily prepared.

4. SAMPLE PREPARATIONS FOR EXCITATION WITH RADIOACTIVE SOURCES AND TUBE EXCITATION

In this chapter we shall describe sample preparations for analysis with x-ray fluorescence (radioactive source and x-ray tube excitation). Only some characteristic procedures will be presented in the form as published by the authors.

4.1 Water

Monitoring elemental composition of water is of great importance to life. However this is a difficult problem because of concentration levels of about 10^{-3} ppm for most elements of interest. Many preconcentration methods have been described when preparing water samples for analysis by x-ray emission spectroscopy. Sample preparation for the analysis by tube system is often accomplished by precipitating the elements with the nonspecific chelating agent, ammonium-1-pyrrolidine dithiocarbamate (APDC), and filtering through membrane filter.

Simplicity of this method permits processing of large number of samples in relatively short time. The bidentate chelating agent forms insoluble coordination complexes with more than 30 transition metals: V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Nb, Mo, Ru, Rh, Pd, Ag, Cd, In, Sn, Sb, W, Te, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi and U. The alkali and alkaline metals are excluded. The high stabilities and low solubilities of the metal dithiocarbamate chelates are sufficient to permit quantitative recovery of many metals with little or no pretreatment of the natural water at a dithiocarbamate concentration of 10^{-1} M. The usefulness of APDC chelation is enhanced by its effectiveness over broad pH ranges.

Equipment for sample preparation consists of a Pyrex filter holder and includes a reservoir, a 17-mm circular fritted glass support and a metal clamp. The filter paper discs often used are white 25-mm Millipore paper discs of 0.45 μ m pore size. After insertion of the discs and installation of the reservoir the solution should be filtered under suction.

Let us describe measurements done by Marijanović et al. (1982). Initial pH of seawater was 7.96. Preparing a target of solutions with known concentrations of elements it was shown that the best preconcentration is obtained at pH 3. For particular elements complexation is possible over the wide pH range (2-14) but for simplicity and rapidity of process it is necessary to complexate all elements at one pH value. It is shown that results obtained are different and lower for other pH values.

Usually 200 ml of water sample is measured into suitable container. The pH is adjusted by adding of HCl or solution of NH₃ in water. Four ml of 1% - APDC solution is added. Solution is prepared fresh daily by dissolving ammonium-1-pyrrolidine dithiocarbamate in distilled water. APDC is added to sample while mixing with a magnetic stirrer for 20 minutes at room temperature to permit complexation. Suspension is filtered through a Millipore filter which is then allowed to dry on clean absorbent tissue for several hours. In that way the uniform thin samples suitable for x-ray analysis were prepared.

X-ray spectrum from seawater sample shown in Fig. 4.1 is obtained by using x-rays from Mo x-ray tube for excitation. Usual working conditions for Phillips x-ray apparatus Model PW 1010/30 were 26 kV, 12 mA with x-ray beam passing through Zr, Ti and Mo filters to reduce its intensity. Si(Li) x-ray detector used has an energy resolution of 180 eV, at $E_x = 6.4$ keV. Results are presented in Table 4.1.

Similar procedure is also described by Elder et al., (1975). A volume of water (usually 100 ml) free of particulate matter is measured into a suitable container. The pH can be adjusted, if judged necessary, by addition of a small amount of HCl or HNO₃. A 1.0% APDC solution is prepared fresh daily by dissolving ammonium-1-pyrrolidine dithiocarbamate in distilled water and filtering through a prewashed glass fiber filter (Whatman GF/C or equivalent). A minimum of 2 ml of this solution per 100 ml of water sample is introduced with thorough mixing, and the sample is allowed to stand at room temperature for 20 min to permit equilibration. The suspension is filtered through a prewashed membrane filter after which the filter is placed on a clean absorbent tissue and weighted around the edges with a plastic ring to prevent curling. The filter is allowed to dry for several hours then mounted on a target holder for x-ray analysis.

About 30 min are required to reduce a natural water specimen to a form suitable for analysis. A portion of this time is for the separation of the particulate matter from the aqueous phase. This may be accomplished by centrifugation or by filtration through a membrane filter. If the latter method is used, the particulate matter, if not too coarse, may also be analyzed by XRF (Elder et al., 1975).

Leyden et al., (1975) have described the use of ion exchange resin for sample preparation. In their recovery studies, the synthetic sea water doped with metal ions was used. The purpose of this study was to determine the recovery capability of the resin in saline solutions simulating sea water. The resin to be used was buffered to pH 6.0 by washing

INTENSITY

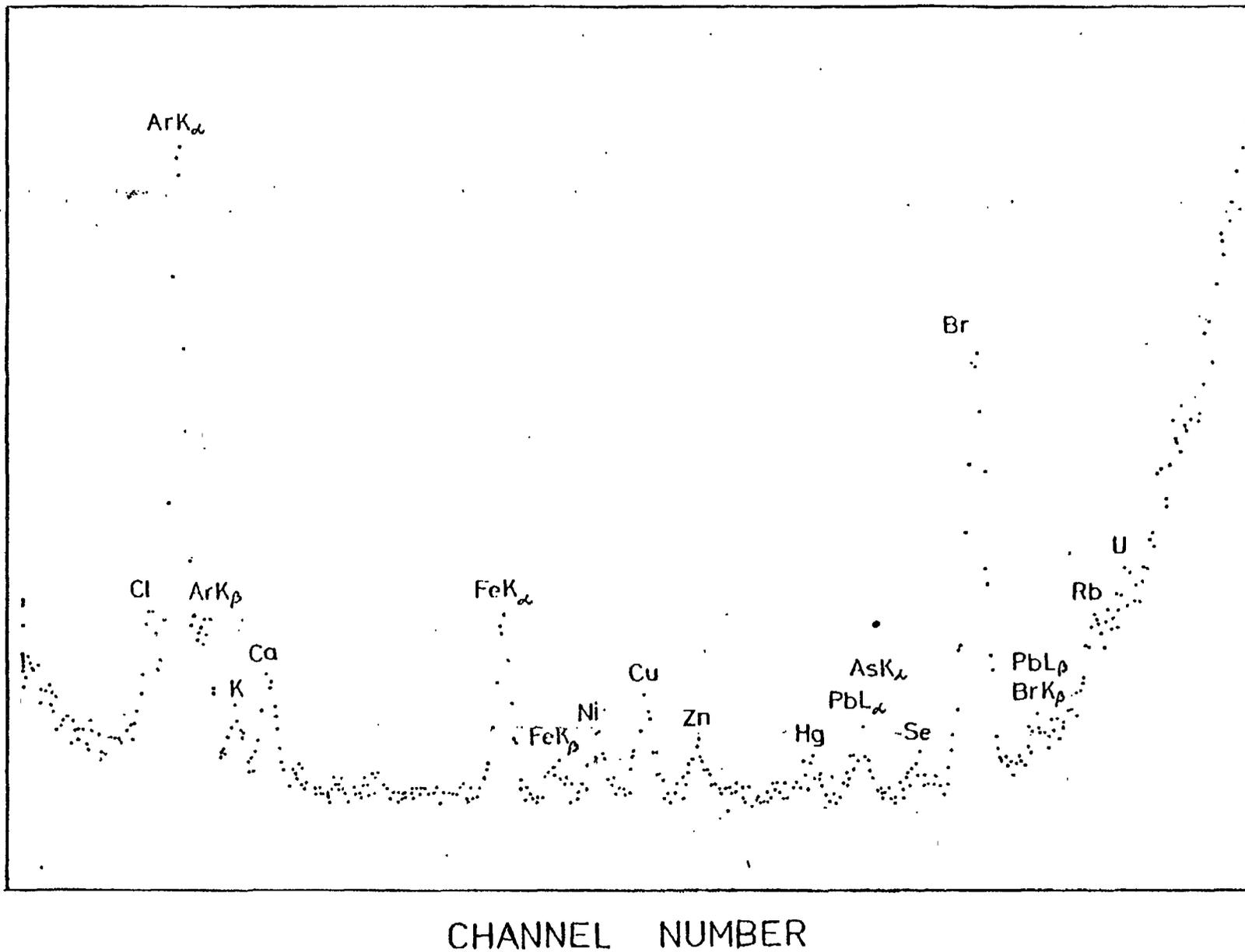


Fig. 4.1 X-ray spectrum from seawater sample.

Table 4.1
 Concentrations of the elements in seawater obtained by analysing of samples complexated
 at the different pH values

Element	pH 2	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9
V		2.34×10^{-3}						
Cr		0.68×10^{-3}						
Mn		0.39×10^{-3}						
Fe	4.51×10^{-3}	2.5×10^{-2}	1.86×10^{-3}	8.5×10^{-3}	31.0×10^{-3}	7.0×10^{-3}	2.6×10^{-3}	5.4×10^{-3}
Ni	0.29×10^{-3}	1.3×10^{-3}	0.1×10^{-3}	0.4×10^{-3}	0.5×10^{-3}	0.5×10^{-3}	0.4×10^{-3}	0.3×10^{-3}
Cu	0.69×10^{-3}	3.4×10^{-3}	1.8×10^{-3}	1.6×10^{-3}	2.2×10^{-3}	1.7×10^{-3}	0.8×10^{-3}	0.9×10^{-3}
Zn	0.29×10^{-3}	4.3×10^{-3}	1.6×10^{-3}	5.1×10^{-3}	2.8×10^{-3}	5.9×10^{-3}	0.34×10^{-3}	1.6×10^{-3}
Ge		0.68×10^{-3}	0.2×10^{-3}					
As		0.59×10^{-3}		0.2×10^{-3}	0.2×10^{-3}	0.1×10^{-3}		
Pb	0.69×10^{-3}	4.68×10^{-3}	1.4×10^{-3}	1.1×10^{-3}				
U		1.5×10^{-3}				0.3×10^{-3}		

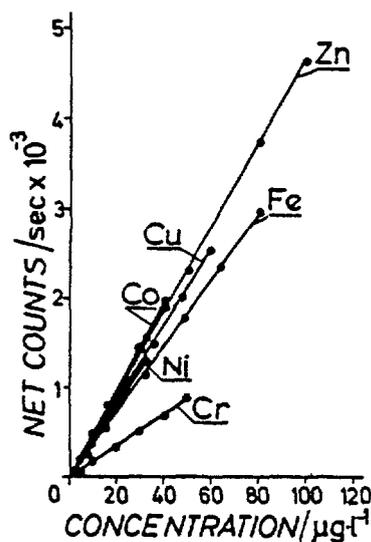


Fig. 4.2 X-ray counts versus concentration of some elements in synthetic (after Leyden et al.. 1975).

with a 0.05 M sodium acetate solution to which sufficient HNO_3 had been added to obtain pH 6.0 by pH meter reading. The studies were conducted by placing 150 ± 1 mg of the resin in 15-ml beakers and adding approximately 2 ml of 0.7 M NaCl. This slurry was allowed to sit for 24 hours to equilibrate with the salt solution. The resin was placed in a column (4x0.5 cm) which had been previously fitted with a glass wool plug. The resin in the column was washed with the pH 6 buffer until the emergent solution was pH 6. Each of several 500-ml plastic bottles were filled with the synthetic sea water, to these, various aliquots of the standard solutions were added. The synthetic sea water served as a blank. These solutions were then passed through the columns containing the resin at a flow rate of ~ 3 ml/min. After all of the solution had passed through the column, the bottle was rinsed with a 10-ml portion of the acetate buffer and this rinse was also allowed to pass through the resin bed. The resin was then transferred from the column into 30-ml sintered glass filtering crucibles of fine porosity and washed with three 5-ml portions of the acetate buffer using suction. The resin was pelletized in a 0.6-inch diameter hardened stainless steel die under a pressure of 15,000 pounds, then analyzed by x-ray fluorescence. The standard resin pellets were checked by grinding them and eluting the metals using HNO_3 . The metal ions in the eluant solution were determined by atomic absorption using a set of standards prepared independently of the synthetic sea water. In all cases, the concentration of the transition metals were within 8% of their calculated value. This test indicated that no significant error resulted from impurities in the reagents used to prepare the synthetic sea water solutions (Leyden et al., 1975). Fig. 4.2 shows x-ray counts versus concentration of some elements in synthetic seawater.

In the sampling of seawater Leyden et al., (1975) 30 liter Niskin bottles at depths above, in, and below the oxygen method. The water samples were drained into clean plastic containers and then 4-liter subsampels were transfer-

red into 1-gallon plastic jugs equipped with 4-cm columns of TEPA resin. The samples were allowed to pass through the resin columns by gravity feed until sufficient water had been samples (either the entire 4 liters or the amount that passed in 48 hours). The glass columns were sealed with para-film and rubber stoppers and transported to the laboratory in that manner.

Seils and Tisue (1979) have described procedures for preparation of samples from rain, snowmelt or lakewater. Here one is seldom limited by sample size. In other instances (sediment interstitial fluids) only small sample volumes are available, and some modifications of technique become necessary. These two circumstances are treated separately. It is demonstrable that the presence of cations other than H^+ or the alkali metals may interfere with the determination by acting to hold back sulfate in solution, or by coprecipitating with $BaSO_4$, thereby altering the stoichiometric relationship between Ba and S on which the indirect method depends.

For these situations, the authors have described a pretreatment of the sample with chelating resin. (The rain samples analyzed did not require this conditioning).

- (i) Procedure for samples and standards up to 0.1 l and 15 $\mu\text{mol } SO_4^{2-}$:

This procedure is applicable to sample volumes of 100 ml containing up to 15 $\mu\text{mol } SO_4^{2-}$. For more concentrated samples an aliquot portion containing 15 $\mu\text{mol } SO_4^{2-}$ should be diluted to 100 ml with 10 mM HCl. Reagents: 10 mM HCl; 0.5 M $BaCl_2$; 0.1% by weight "Tween-20" surfactant (Fisher Scientific Co.); 10 mM HCl/10% methanol by volume; and 6 M HCl. (All reagents are prepared with Barnstead "Nanopure" deionized water, or equivalent, passed through a 0.22 μm final filter. Likewise, all samples are filtered through a 0.4 μm membrane prior to further treatment).

A 100 ml aliquot portion of the filtered sample is pipetted into a 250 ml beaker. The pH is then adjusted ± 0 about 2 by the dropwise addition of 6 M HCl to eliminate CO_3^{2-} interference; pH paper is a suitable indicator. While stirring gently with a teflon-coated magnet, one then adds 5 ml of 0.1% Tween-20, and 1 ml of 0.5 M $BaCl_2$. The resulting mixture is stirred at 80 C for one hour, then cooled and allowed to stand overnight under a watch glass. It was found convenient to dislodge the precipitate from the beakers' walls by ultrasonication for 2-5 min, and then to suspend it by magnetic stirring. The precipitate is collected by passing the resulting slurry through a 25 mm diameter, 0.4 μm Nuclepore filter contained in a 5/8" ID glass Millipore vacuum filtration holder. To provide reliable sealing around the inner circumference of the funnel's base, one may place a 5 μm Millipore membrane behind the Nuclepore filter. The beaker and filtration funnel are washed with several small portions of 0.01 M HCl/10% methanol to complete the transfer and to remove excess Ba^{+2} . The collected precipitate is dried in clean air and mounted between 0.5 ml Mylar film in a cardboard slide holder.

If pretreatment to remove interfering cations is indicated by preliminary tests, the following technique is

applicable. Additional reagent: AG 50W-X8 cation exchange resin, 100-200 mesh, hydrogen form (BioRad Laboratories), or equivalent, washed first with 6 M HCl, then with 10 mM HCl. Sometimes the resin absorbs small amounts of SO_4^{2-} irreversibly. Samples containing 3 $\mu\text{mol SO}_4^{2-}$ gave low and erratic recoveries. The difficulty may be overcome in the main by pre-equilibrating the resin with a small amount of the sample being analyzed. However, in ultratrace analyses using the small spot geometry it is desirable to substitute Chelex-100 in the H^+ form, since that material does not appear to absorb SO_4^{2-} vide infra.

A 47 mm, 0.4 μm Nuclepore membrane is mounted under a vacuum filtration funnel of >100 ml capacity then covered for 5 min with ca. 1 g of prewashed cation exchange resin and 25 ml of the sample. Vacuum is applied and the resin drawn dry without washing. After changing the receiver, one adds the sample (about 100 ml) and allows 5-10 min for equilibration with the resin while stirring occasionally. The sample filtrate is then drawn off by vacuum without washing; the used resin is discarded. 100 ml of the filtrate is pipetted into a 250 ml beaker. The BaSO_4 precipitate procedure described earlier is followed in subsequent steps.

(ii) Procedure for samples up to 10 ml containing less than 1 $\mu\text{mol SO}_4^{2-}$:

This procedure is applicable to sample volumes up to ca. 10 ml containing 1 $\mu\text{mol SO}_4^{2-}$. The reagents are the same as were described for the large spot procedure.

A portion of the filtered sample is pipetted into a 50 ml centrifuge tube and adjusted to a volume of ca. 15 ml with 0.1 M HCl. To it is added ca. 0.1 g of Chelex-100 resin, 100-200 mesh, in the H^+ form. (This reagent may be omitted in the absence of interfering cations.) Resin and sample are allowed to react for 30 min with occasional stirring, then filtered through a 25 mm diameter, 0.4 μm Nuclepore membrane backed by a 25 mm diameter, 5 μm Millipore membrane. It is convenient to collect the filtrate in a 50 ml centrifuge tube. The collected resin should be washed with amounts of 0.1 M HCl sufficiently small that the combined volume of sample and wash liquid is 25 ml.

To the filtrate one then adds 1 ml of 0.1% of 0.5 M BaCl_2 . The resulting precipitate is digested for 1 hour at 80° in a water bath, covered, and allowed to stand overnight. Prior to filtration, it is desirable to dislodge the precipitate by ultrasonication for 2-5 min. It may then be collected on a 25 mm diameter, 0.4 μm Nuclepore membrane backed by a 5 μm Millipore membrane. To confine the precipitate to an area smaller than the x-ray beam in spectrometer, a 1/4" diameter filter holder and funnel were used. To obtain leak free operations, the upper and lower portions are clamped together tightly by means of a screw clamp; the usual spring-leaded clamps do not prevent loss of precipitate around the gasket seals.

Transfer and washing of the precipitate are completed by washing the centrifuge tube and filter with several small portions of 10^{-2} M HCl containing 10% methanol by volume. After drying in clean air, the filter containing the precipitate is mounted for x-ray fluorescence analysis.

Let us also describe procedures used by van Grieken et al., (1982) for trace element analysis of water. For the suspended matter, simple filtration through e.g. a 0.4 μm pore-size Nuclepore membrane was found to be a suitable collection step. For the dissolved material, the choice of the preconcentration method is not an obvious one and many alternative procedures have been proposed, most of which are included in Bächmann's survey (1981) on preconcentration methods.

Van Grieken et al., (1982) prefer following scheme:

- the use of cellulose filters with 2,2' -diamino-diethylamine (DEN) functional groups (Smits and van Grieken, 1981), which allows direct enrichment by a simple filtration step, leads to sub-ppb detection limits of most cations, almost independently of the major ion composition, but implies the synthesis of the DEN-filters;
- co-crystallisation on 1-(2-pyridylazo)-2-naphtol (PAN) (Vanderstappen and van Grieken, 1978) which is quite simple and leads to enrichment factors around 10^5 and detection limits near $0.1 \mu\text{g}/\text{l}$, but suffers somewhat from collection yield variations with speciation;
- co-precipitation on ferric hydroxide (Chakravorty and van Grieken, 1978) which allows a quantitative compromise between high enrichment factors and high collection yields;
- chelation by oxine and subsequent adsorption onto activated carbon (Vanderborcht and van Grieken, 1978) which collects also quantitatively the colloidal and organic forms of the trace metals, is dependent of the concentration and matrix composition and was found quite valuable in recent preconcentration intercomparisons (Leyden et al., 1979); Smits et al., 1979), in spite of its being a two step procedure.

Numerous other methods are, of course, available in the literature; each has specific advantages and drawbacks. Some authors prefer ion exchange material, in the column or batch mode, e.g. silylated glass beads (Leyden et al., 1976) and Hyphan (Burba and Liesen, 1977), or commercially available ion exchange filters (Campbell et al., 1966), or they use coprecipitation on sodium-diethyldithiocarbamate, ammonium-pyrrolidinedithiocarbamate or related compounds (Elder et al., 1975), or solvent extraction (Marcie 1976) or electrodeposition (Wundt et al., 1976) or other chelation and immobilisation procedures (Knapp et al., 1975).

Besides multi-element analysis procedures, various highly specific single element or elemental species preconcentration methods are available. Most of the chemical preconcentration procedures combine the following advantages: they lead to thin homogeneous targets that are ideally suitable for XRF and they allow very advantageous detection limits. On the other hand, the chemical speciation of the ions to be determined and the matrix composition can influence the collection yield, particularly in relatively concentrated samples like waste water.

Simple evaporation or freeze-drying of the water matrix obviously collects all non-volatile element, independent of the chemical and physical speciation, even in very complex samples like waste water and sewage sludge. For XRF analysis, the residues were hitherto either strongly diluted with a binder, to reduce matrix effects at the expense of the sensitivity, or the analysis was limited to one type of water sample only. Via the automated spectrum evaluation and matrix correction procedure highly variable evaporation residues of sewage sludge and waste water can be analysed in a very simple automatic and versatile way (Van Dyck; 1980, 1982).

The sample preparation thus includes freeze-drying of a 250 ml waste water volume, in the presence of 100 mg graphite if little dissolved or suspended matter is present, and with 100 ug Y as an internal standard. The residue is more or less quantitatively transferred to a mortar, homogenized, and pelletized on a thin Mylar carrier for XRF analysis.

4.2 Liquid Samples

Various target preparation techniques for liquid samples have been described in the literature. These techniques are based either on the use of liquid-specimen cells or on depositing the liquid on a supporting material and drying.

For liquid-specimen cells it is fairly easy to produce reproducible targets, but there are some practical problems (Kivits, 1980):

- the cell window must be uniform in thickness and impervious to the constituents of the solution;
- the cell window must be thin enough to transmit both the incident particles or photons and the induced x-rays without appreciable attenuation;
- some liquid cells are difficult to fill properly or are leaky and easily become messy;
- some solutions are volatile and must be contained in closed cells;
- liquid may be heated under irradiation causing expansion and in closed cells distension of the cell window;
- some specimens may undergo radiolysis with possible precipitation.

Liquid-specimen cells are easy to use and attractive for volatile analytes (e.g. Hg in water). However, the method is not useful for detecting low-Z elements, because of the absorption of x-rays by the cell window.

Another method is based on depositing the liquid on a thin support. This has the advantage of a better peak-to-background ratio and will be discussed below. However, it is more complex. Important criteria in the selection of a support are (Kivits, 1980):

- homogeneity;

- uniformity and low area density to minimise x-rays scatter and absorption;
- low concentration of elements with atomic number $Z \geq 11$;
- resistant to chemicals, mechanical stress and radiation damage;
- retention of the material to be analysed during the entire procedure of preparation and irradiation, to prevent losses;
- wettable by liquids.

The most commonly used support materials, in order of decreasing thickness (mg/cm^2) are: filter paper (~ 10); Millipore (5); Nuclepore (1); Mylar (0.5-1); collidion (0.02) and Formvar (0.02).

In addition to real solutions, one is often dealing with systems containing two phases, e.g. suspensions (solid-liquid) or emulsions (liquid-liquid). In general it is difficult to prepare homogeneous and uniform targets from these samples, due to their complex physical behaviour. In the case of suspensions, the volume fraction or weight fraction of the solid material and its particle size distribution may be critical parameters in target preparation.

Jolly and White (1971) describe a method for preparing uniform film deposits ($10-1000 \mu\text{g}/\text{cm}^2$) from solutions or colloidal suspensions of micron-size particles. The suspended or dissolved material is placed in a container of a nebuliser. A compressed gas forces the liquid through a small hole to form a spray which in turn impinges with high speed on an obstruction and thus breaks up into droplets of various sizes. The resulting mist is then allowed to deposit on a rotating substrate. The particle size of the suspended material in the original suspension and the microscopic size of the droplets is crucial to the success of this method in obtaining a thin uniform deposit. Valković (1975) reports that the reproducibility of these targets will be questionable because the finest droplets are swept along by the gas stream and do not impinge on the obstruction. From the results of Wilkniss and Bressan (1971) it may be concluded that the trace-element composition may not be constant for all droplet sizes.

In addition this method involves dividing the liquid into a super-fine mist and deposition onto the target substrate at a rate equal to the rate of evaporation. One can have difficulties with residue recovery due to "mist bounce-off". This can be corrected by the use of internal standardization however, the change of contamination is high and the rate of concentration is low with this method. Physical methods such as buretting onto Mylar and evaporation by heat lamp, produce surface gradients of elemental distribution since the solubility of the dissolved constituents varies greatly and the configuration of the water (droplet etc.) is constantly changing (i.e. the residue is a series of concentric closed contours). Brady and Cahill (1973) attempted to overcome this configuration change by thin film evaporation utilizing surfactants to reduce the surface tension of the water. The authors have tried Vatsol

OT and surfactant 77 but evaporation still proved to be variable since precise leveling of the substrate and a uniform drying environment are critical. They also attempted absorbing the liquids into Cellex and/or Somar spectroscopic grade powders followed by pelletization. This technique proved to be tedious and held merit only for the production of standards. In addition the sensitivity was greatly reduced due to the high matrix to trace metal ratio, which increases the background with which the x-ray signals must compete. Residue recovery can be also a problem for this method if the cellulose is over-saturated initially.

Physical techniques are essentially abandoned because of successful chemical means of liquid target preparation. Briefly this technique involves the formation of water-insoluble metallic complexes and filtration as in the case of suspended material. The flocculent distribution produced has proven to be very uniform and the collection efficiency has been shown to be essentially 100% for the transition series. This simple method is sensitive down to one ppb as can be seen in the spectrum of a natural water sample on the following page. The only critical phase of the preparation (besides using clean active reagents and clean glassware) is the complete separation of particulate and solute phases. The particulates can be analyzed separately (Brady and Cahill, 1973).

4.3 Solid Samples

Solid samples may be analysed as such, or may be reduced to slices, powders or solutions. If the material can be analysed in the as-received state, the analysis are usually very rapid and convenient.

Kivits (1980) has shown that thin targets ($< 1 \text{ mg/cm}^2$) are most favourable, and solid samples may therefore be divided into slices. This is for instance useful for analysis as a function of depth. One should take into account that surface treatment may result in unwanted contamination and/or in selective removal of certain constituents. Any surface treatment involving movement of a cutting tool across the surface may result in smearing of soft constituents. This causes a spectral enhancement for the smeared constituents and attenuation for the covered constituents. The latter effect is more severe the higher the absorption coefficient of the smeared material and the lower the x-ray energy of the covered material.

For an analysis of the bulk of a sample, the problems due to inhomogeneous solids must be solved. These may be overcome by powdering the solids, in order to obtain a homogeneous sample and to reduce the particle size. Targets prepared from homogeneous powders of small particle size are expected to be representative for the original sample. Addition of an internal standard in the sample is then also feasible. A disadvantage of powdering a sample is that such pretreatment is time-consuming and may be an extra source of contamination. Thin targets for powdered samples may be prepared by spreading into thin layers, dry or as slurries (Kivits, 1980).

In the "dry technique" the powder can be dusted onto an adhesive surface (e.g. Scotch tape), or can be spread out over a support, adding a fixative later. Rudolph et al. (1972) placed 0.3-0.5 mg of powder in a 4-mm diameter circle on a Formvar foil. Adherence is effected with polystyrene doped in toluene. Barnes et al., (1975) ashed whole blood and mounted it on Kapton backings with 5 μ l of albumin fixative.

Sometimes a measured amount of powder is placed on a Mylar film and distributed as uniformly as possible. A second Mylar film is then stretched over the powder and the first film, thus enclosing the powder layer (Rinsvelt, 1977).

In practice it seems to be very difficult to obtain a uniform or thin layer of the powder by the "Dry techniques" described.

In a commonly used slurry technique, a small amount of powder and bonding material is slurried in an appropriate solvent. The slurry is spread over a "scrupulously" clean microscope slide. The film is floated off on water, then scooped up and supported in some other way. Suggested solvents are: amyl acetate, chloroform, dioxane and dichloroethane. Bonding materials recommended are: nitro-cellulose, ethyl-cellulose and polystyrene. The techniques described above involve many manipulations, which may introduce sources of analytical errors, such as contamination and loss of elements to be analysed.

Kivits (1980) has described a procedure for the preparation of thin targets from the powdered materials. For the preparation of thin targets, the powdered samples should have particle sizes below about 10 μ m. For samples received as non-fine powders a pulverising technique is needed.

A "brittle fracture technique" (Iyengar and Kasperek, 1977) is often used to homogenise solid samples and to reduce their particle size. Particle size reduction is needed to obtain homogeneous and thin targets.

In the work by Kivits (1980), use is made of a ball in a vibrating Teflon vessel. Dry sample material is brought into this vessel. The vessel is cooled in liquid nitrogen for a few minutes and then vibrated for 1 minute at 3000 cycles per minute with a mechanical shaker, constructed in our workshop. If necessary, the cooling-shaking process may be repeated. Quantities of up to 50 grams can be pulverised and homogenised as one batch. Soft tissues (human tissue, hair) can be pulverised and homogenised using a Teflon ball or a high-purity quartz ball. Hard samples (teeth, bone, nails) are treated using a Teflon ball with a metal core or a tungsten carbide ball. Wet samples such as human tissue are better pulverised after previous lyophilisation.

The following steps are included in the sample preparation method used by Kivits (1980):

- a) a solution of 5 grams Formvar in 100 ml dioxane is prepared;
- b) powdered solid sample is mixed with this solution (sample weight fraction about 10%) with the aid of a whirlimixer;

- c) a Selectron filter is rotated at 3.000 rpm and saturated with 0.5 ml water;
- d) 0.2 ml of the powder-Formvar-dioxane mixture prepared is immediately dispensed on the wetted rotating filter;
- e) the resulting Formvar foil, in which the solid sample is embedded, is pulled from the Selectron filter.

The resulting foils have an area density of less than 1 mg/cm², as determined with an α -particle absorption set-up. The target-preparation technique takes only a few minutes; the fraction of failures is less than 10 per cent (Kivits, 1980).

Brady and Cahill (1973) used two techniques in their work. The first technique requires about 500 mg or more of homogeneous target material. Homogeneity is obtained via ashing, grinding, or lyophilization coupled with grinding for all biological type materials. This powder is then spread into a 1 1/4" die and pressed into a pellet with a 20-ton hydraulic press. A cellulose binder must be mixed into the target material prior to pressing for some materials. This technique is easy but self-absorption must be dealt with carefully for such thick targets, which are hardly optimum for the lightest elements.

The second technique requires only a few milligrams of material but only works well for dense insoluble powders (e.g. many geologic type samples). It involves placing the powdered sample temporarily into a turbulent suspension succeeded by rapid filtration onto a Millipore type filter. This method has resulted in quite good thin uniform targets. However the target must be coated with an acrylic spray after the mass of the deposition has been determined. This is to reduce flaking.

They have also tried to use static charge to hold thin layers of powder onto Mylar and also Mylar with an adhesive coating. These two methods have proven to be inconsistent as far as yielding reproducible depositions. There is a lower limit as to how thin one should make a target and that is on the order of the mean particle diameter.

In the analysis of material by x-ray fluorescence utilizing the powder method, particle size effects can be a cominant factor in an accurate and reproducible quantitative chemical analysis with variations in particle size often responsible for significant changes in the emitted intensity. In multiphase systems, as portland cement, the intensities from several elements may all increase, decrease, or one may increase while another decreases as a function of particle size. Here is the sample preparation method as recommended by ORTEC:

Prior to the analysis, a minimum grinding time was established by grinding one cement sample in a Spex Shatter Box (Tungsten carbide mill) for 1, 2, 3, 4, 5, and 6 minutes (5 g cement + 100 mg Ivory Snow). The resulting powders were pelletized at a pressure of 15 tons/sq in. and mounted

in the instrument and each analyzed for 200 seconds. A grinding time of five minutes was established since the intensity vs. particle size was stabilized and formed a plateau at approximately four minutes. As a result of the grinding curve, all samples for subsequent analysis were ground for the minimum grinding time plus one minute or for a total of six minutes.

4.4 Soil

Trace metal levels in soils are not only important from a geochemical or pedological point of view, but also with respect to environmental research, since atmospheric fallout, incidental contamination and deliberate waste dumping can be reflected.

In their work van Grieken et al., (1982) have prepared thin targets as follows: Aliquots of 1 g of soil or rock are mixed with 7 ml of twice-distilled water and pulverized for 1 min in a McCrone Micronizing mill, with corundum or agate grinding elements. Of the resulting suspension a 0.5 ml fraction is pipetted onto a Mylar carrier foil and dried at 80 C. Also thicker pellets can be compressed to obtain better sensitivities (for the high-Z-elements only), at the expense of more critical matrix effect corrections.

Here again the variations in particle size can cause serious errors in quantitative analysis. The pellets from soil samples can be prepared as follows:

Five grams of sample, plus 100 mg of calcium stearate as a grinding aid should be ground in a Spex Shatterbox for a period of five minutes. The resulting powder should be placed in a die with a boric acid backing added and pressed at 15 tons/sq. in.

4.5 Geological Samples

There is a large number of possibilities how to prepare geological samples for the analysis by XRF. Here we shall describe some.

Description of the technique used by Elsheimer and Fadbi (AXRA 17) contains the following instructions:

Mix samples by the hand-rolling technique just prior to analysis because high density sulfide and sulfate minerals may segregate during mechanical mixing, causing as much as a 15% error. For samples known to contain 10% or less of sulfur as sulfide or sulfate, take 100 mg for each analysis. Weigh 50-mg portions for samples having a sulfur content significantly greater than 10%, known to contain large amounts of ferrous iron, or thought to contain elemental sulfur. Mix each sample intimately on weighing paper with 200 mg of $\text{Ce}(\text{NH}_4)_2(\text{NO}_3)_6$ (99.97% pure, G.F. Smith Chemical Co.), prepared for use by grinding to 100 mesh and drying for 1 hr at $\leq 85^\circ\text{C}$. Lightly mix into this mixture 150 or 200 mg of pure quartz, depending on the sample size (100 or 50 mg). Transfer the resulting mixture to a 30-ml,

black-glazed porcelain crucible containing 1.7500 g of dried LiBO_2 .

Thoroughly mix the contents in the crucible and carefully transfer term to a 30-ml, preignited graphite crucible with a hemispherical bottom. Fuse the sample mixture for 15 min in a furnace at 900 C and cool the resulting bead in air.

Remove the bead from the crucible, weigh and place in a large steel ball mill (Spex Industries, Inc., Model No. 8001) containing two 1/2-inch-diameter steel balls. Grind for 4 min and transfer the resulting powder to a boron carbide mortar containing a weighed portion of chromatographic cellulose (Whatmin CF-I.I.) sufficient to bring the combined weight of bead and cellulose to 2.4000 g. After mixing to a uniform consistency, return the mixture to the ball mill and grind for an additional 5 min. To insure uniform particle size and homogeneity, transfer the ground mixture to the mortar again and hand grind for a few min. Split the finely ground mixture into approximately equal parts on weighing paper and prepare 2 pellets for XRF determination. Analyze each sample or standard in duplicate.

In the measurements described by King et al., (1976) a modified direct dilution method was used in which the sample-to-binder ratio was increased from 1:1 (Fabbi and Moore, 1970; Fabbi, 1970; Fabbi et al., 1975) to 85:15; the binder is a chromatographic cellulose. A mixture of 28% paraffin base powder and 72% methyl cellulose was used for the backing material. By increasing the amount of sample to be analyzed, it was possible to obtain sufficiently high x-ray intensities from the trace elements of interest to gain better precision and sensitivity.

Based on the results of the binder and backing experiment, Fabbi's direct dilution sample preparation method was modified by grinding 850 mg of sample instead of 500 mg in a vial for 10 min using a mixer mill. A binder consisting of 150 mg of 200 mesh chromatographic cellulose was mixed with the ground sample in a mortar and pestle. The mixture was transferred back into the vial, and ground for 5 min in the mixer mill. The finely ground powder was then pressed into a pellet at 30,000 psi, using a backing material which is a mixture of 28% paraffin base powder and 72% methyl cellulose (Mixture A).

The results of binder and backing investigations by King et al., (1976) are summarized in Table 4.2. When samples were not diluted or diluted with too small an amount of binder, the vials were hard to clean. The sample loss was great, and the ground rock powder either did not adhere or adhered poorly to the backing. With an increasing ratio of cellulose binder to the sample and by using methyl cellulose or mixture A, as a backing material, the vials can be easily cleaned, and the pellets obtained had homogeneous, mirror-like surface. With mixture A as a backing, the adherence was excellent, no cracking of surfaces or any other problems were encountered in the sample preparation. Using aspirin as a binder, the preparation was very cumbersome, and uneven shades of darkness appeared on the pellet surface. Therefore, it

Table 4.2
Results of binder and backing
experiments by King et al., (1976)

Sample-to-binder ratio	Binder	Backing	Results
100:0	-----	Methyl-cellulose	Vial hard to clean. Sample does not adhere to backing. Pellet surface cracks or pops up.
95:5	Chromatographic cellulose	Methyl-cellulose	Vial hard to clean. Sample does not adhere or adheres poorly to the backing.
85:15	Chromatographic cellulose	Methyl-cellulose	Vial easy to clean, homogeneous surface. Sample adheres to backing.
85:15	Chromatographic	Mixture A*	Vial easy to clean, homogeneous surface. Sample adheres rigidly to the backing.
85:15	Aspirin	Methyl-cellulose	Vial hard to clean. Heterogeneous surface.
85:15	Paraffin, spectroscopically pure	Methyl-cellulose	Vial easy to clean, heterogeneous surface. Sample deposits on the glass lens.
80:20	Chromatographic cellulose	Methyl-cellulose	Vial easy to clean, homogeneous surface. Sample adheres rigidly to the backing.
75:25	Chromatographic	Methyl-cellulose	Vial easy to clean, homogeneous surface. Sample adheres to the backing.

* Mixture A = 28% paraffin and 72% methyl cellulose.

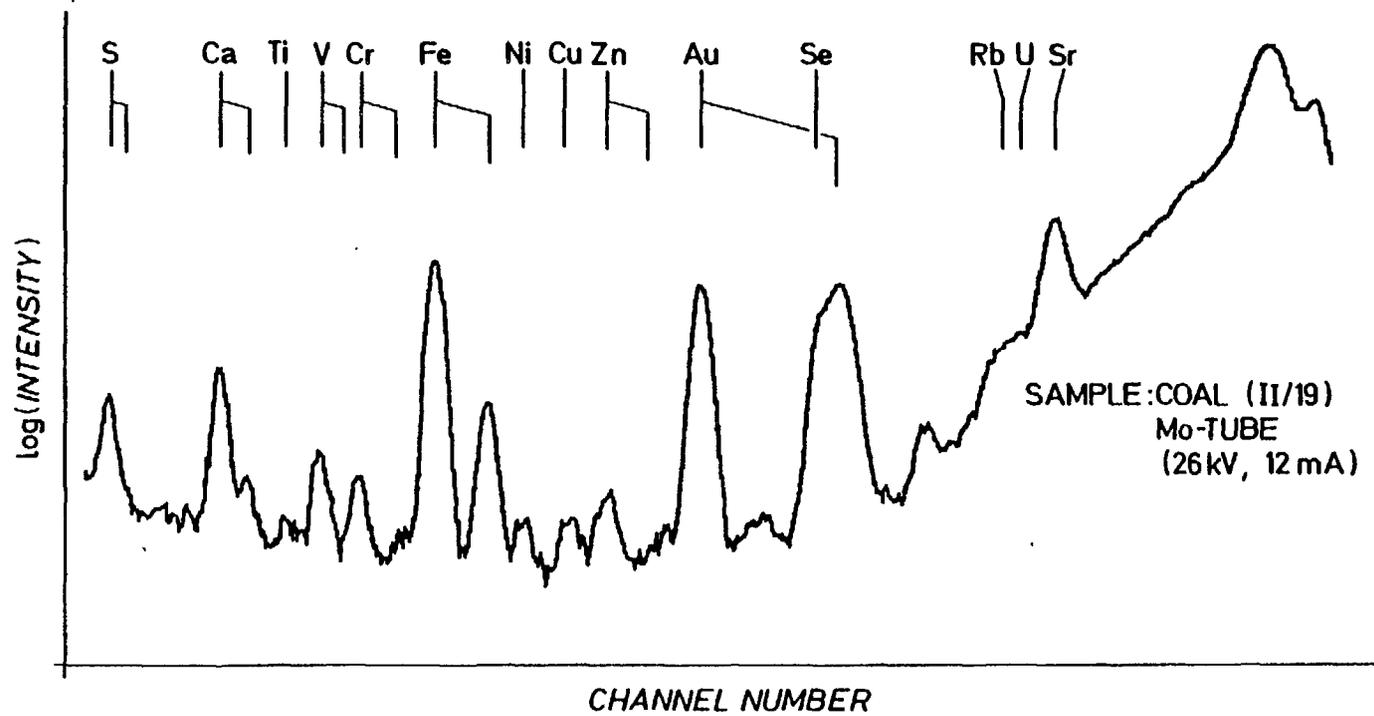


Fig. 4.3 X-ray spectrum from coal sample.

is not feasible to use aspirin as a binder, even though it is preweighed and can be directly crushed and mixed with the sample.

ORTEC procedure for the preparation of geological samples includes the grinding of samples (about 5 grams of material) in a tungsten carbide mill and pelletized under a pressure of 15 tons per square inch. (This grinding contaminated the samples with a couple hundred ppm of tungsten which complicated the spectrum in the region of Cu and Zn due to tungsten L-line interference). The standard rock samples (AGV-1, BCR-1, PCC-1, G2 and GSP-1) were pelletized without grinding. The pressed pellets were transferred directly to the TEFA analysis chamber which was evacuated prior to analysis.

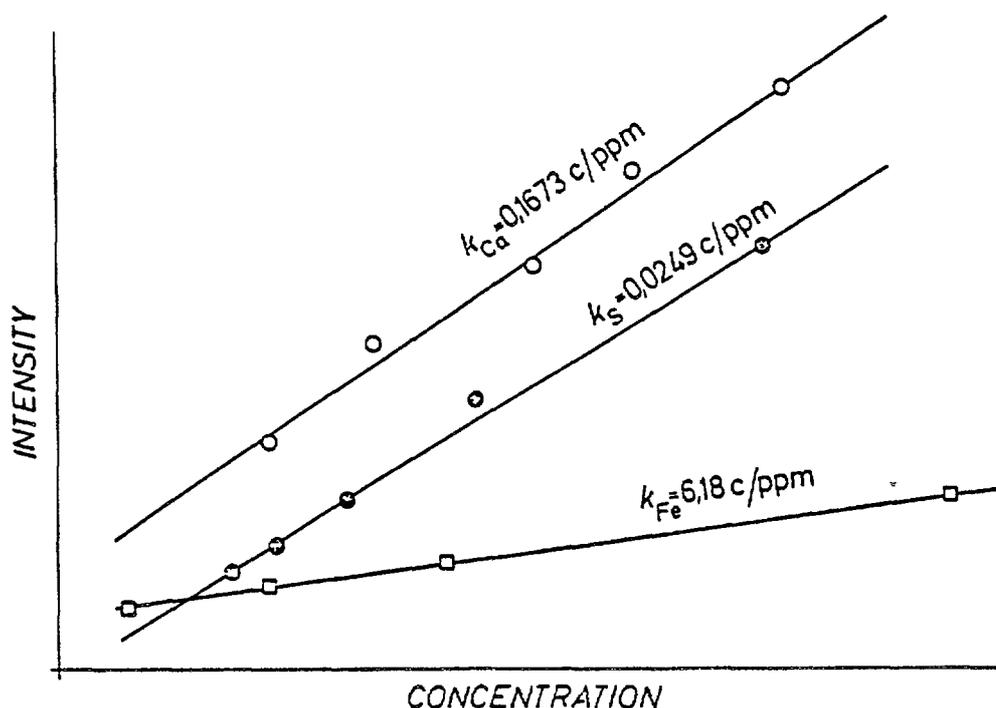


Fig. 4.4 Calibration curves for Ca, S and Fe in coal matrix.

Standard addition method is often applied to geological samples when a number of samples with identical (or similar) matrix has to be analyzed. As an example Fig. 4.3 shows elements which were measured in coal samples using Mo-tube for the excitation. In the experiment large number of coal samples from the same coal mine had to be analyzed. For each element of interest number of standards was prepared by adding to coal known concentrations of element. The analysis has performed after appropriate mixing and sample preparation and the x-ray intensities were measured as a function of concentration. This is shown in Fig. 4.4 for Ca, S and Fe. These calibration curves were than used in the concentration asignments for unknown coal samples from the same coal mine. There was no need to apply any matrix correction calculations afterwords.

4.6 Atmospheric particulates

X-ray emission spectroscopy is applied to elemental analysis of air particulates in all three forms of sample excitation: radioactive sources, x-ray tube, and charged particle beams. Here we shall first describe some systems using radioactive sources.

The analytical system used by Rhodes (1972) consists of the following main components: automatic sample and source changer with capacities of 30 samples and four sources, respectively with three annular radioisotope source assemblies: Fe-55, 120 mCi; Pu-238, 400 mCi; and Cd-109, 12 mCi.

Specimens in the form of discs of 47 mm diameter were cut out of the original filter papers and measured in batches of 27 unknowns and three standards. To obtain the best excitation efficiencies, the 17 elements to be determined were divided into three groups, each excited with a different source, namely: Ca, Ti, V with Fe-55; Cr, Mn, Fe, Co, Ni, Cu, Zn with Pu-238; and Hg, Pb, As, Br, Sr, Zr, Mo with Cd-109. Each batch of specimens was counted with each of the three sources. The counting period was that required to accumulate 200,000 counts in the reference peak, which amounted to about 10 min/specimen with Pu-238 and Cd-109, and 5 min with Fe-55.

The detection limits quoted in Table 4.5 are based on 10-min measurements of deposits on cellulose filters. Longer measurements improve detection limits while the presence of high blanks (e.g., Zn in glass fiber filters, Cl in PVC membrane filters) worsen them. Concentrations up to about 1 mg/cm² can be measured without interelement effects and nonlinearities. After special calibration even higher concentrations can be measured accurately.

More detailed literature is available on the topic of analysis aerosol loaded filters.

The major problems are in the correction for x-ray absorption by the aerosol matter and by the filter material, in which the particles always penetrate to a certain extent, especially when cellulose fiber filters are being used. The latter problems can often be reduced significantly by analysing the filter in a sandwich geometry, i.e. folded in two with the loaded side inwards (Van Grieken et al., 1982).

For quantitative XRF analysis, the aerosol particles need to be collected as a uniform deposit on a suitable filter medium. A filter medium of Teflon (Registered trademark of E.I. duPont de Nemours and Company, Inc., Wilmington, Delaware) is preferred by Dzubay and Rickel (1978) because of the high purity and minimal tendency of Teflon to react with gaseous pollutants. Polycarbonate is also fairly good as a filter medium for the same reasons. Esters of cellulose have a high purity but are only useful as filter media for applications where the tendency to absorb moisture and to react with certain gaseous pollutants is not of concern. Most types of filters that contain glass or quartz contain large amounts of impurities of many elements, seriously limiting their application to XRF analysis.

Teflon membrane filters are available in a variety of forms. A thin 1- μ m pore size Teflon membrane that is bonded

Table 4.5

DETECTION LIMIT FOR A SYSTEM USING
RADIOACTIVE SOURCES (AFTER RHODES, 1972)

Element	g/cm ²	g/M ³
Si	0.50	120
P	0.30	70
S	0.17	40
Cl	0.13	30
K	0.10	20
Ca	0.08	20
Ti	0.05	12
V	0.03	7
Cr	0.20	50
Mn	0.12	30
Fe	0.10	20
Co	0.08	20
Ni	0.06	14
Cu	0.04	9
Zn	0.03	7
As	0.10	20
Se	0.10	20
Br	0.10	20
Pb	0.16	40
Rb	0.06	14
Sr	0.05	12
Zr	0.04	9
Mo	0.03	7
Ag	0.20	50
Cd	0.15	35
In	0.20	50
Sn	0.15	35
Sb	0.15	35
I	0.15	35
Ba	0.20	50

CSI, Austin data. Criterion: 3 SD of background. Based on filter area of 420 cm² (8 in. x 10 in filter) and air volume of 1800 M³ (24 hr. HI-volume sample).

to a polyethylene support net is available from Millipore Corporation as FALP Fluoropore. It was reported that its collection efficiency exceeds 99.9% for all particles in the 0.03- μ m to 1- μ m range. A minor problem with this type of filter is the difficulty of making a good vacuum seal in some types of filter holders. A Teflon membrane without a support net is available from Ghia Corporation, Pleasanton, California. This membrane has an asymmetric pore size distribution with 1- μ m pores on one side and 10- μ m pores on the other. When particles are collected on the 10- μ m side, there is a tendency for submicron particles to penetrate into the filter. As a result, the filter medium attenuates the fluorescent x-rays from the particles, making it difficult to obtain quantitative results. Such attenuation is most pronounced for the lighter elements which emit soft x-rays. By collecting the particles in the 1- μ m side, the particles are collected on the surface, which eliminates the attenuation by the filter.

In a typical XRF spectrometer, the exciting x ray beam is nonuniform across the sample. Therefore, the aerosol

deposit must be uniform if an accurate analysis is to be obtained. An acceptable deposit can be achieved if a filter holder with a suitable aerodynamic design is used.

4.7 Plants

Preparation of plant material for the analysis by XRF requires a preconcentration step: ashing. This is early done by some of the methods described in chapter 2.

Here is procedure used by van Grieken et al., (1982) for the preparation of algae samples:

The algae samples were dried at 80°C for 24 h in plastic Petri dishes, then manually ground by means of an agate pestle and mortar, dried again for 24 h in an oven at 80°C and finally grounded for 1 min in a McCrone Micronizing Mill, which introduced minimal contamination. Microscope photographs showed that the powder is quite homogeneous with a grain size of only a few micrometers. About 15 mg of the powder was suspended in some bidistilled water. This slurry was pipetted onto a Mylar foil (< 4 µm thickness), which was glued to a Teflon ring, fitting in the sample changer of the XRF-unit. The target area within the Teflon ring was 9.6 cm². The slurries were dried carefully at 80°C, which resulted in targets of approximately 1.6 mg cm⁻² plant material, homogeneous to within a factor of two or better.

4.8 Tissues

Many authors have analyzed different tissues for trace elements using x-ray fluorescence as an analytical technique. Here we shall describe only some work, because there are no many variations in sample preparation techniques used.

Here we shall describe work by Forssen (1972) who did trace element analysis of different organs on autopsy samples. In this study the subjects were victims of accidental and other sudden deaths from external causes. The bodies were placed as soon as possible in a room at +4°C and stored there for 1-2 days before autopsy. From each body 43 different organ samples were dissected and placed in polyethylene containers. The samples were frozen immediately and kept at a temperature below -15°C until prepared for ashing. From the larger organs 20-70 g samples were taken. The smaller organs were used whole. Skin samples were taken from the middle of the surface of the abdomen and fat from below these samples. Polyethylene is a suitable container material for samples to be analyzed for trace elements, since the trace element concentrations in polyethylene are usually less than 1 mg/kg. Quartz crucibles were used for drying and ashing the organ samples. Contamination was minimized by using different sets of crucibles for each organ (brain, liver, etc.). The crucibles were washed with 32% HCl each time before use, and rinsed with a liberal amount of cold and warm water and finally with distilled water. They were then put in an oven for about 20 minutes at 100°C, cooled in a desiccator, and weighed. If cracks appeared in the glaze, the crucible was discarded. For dry weight measurements the tissues were

dried at 105°C in the same quartz crucibles in which they were to be ashed. Drying and ashing were performed in an electric quartz muffle furnace. The initial temperature of 200°C was maintained for some hours. It was then raised to 450°C for one night. After the crucibles had been cooled in a desiccator and weighed, the ash was stored in tightly stoppered plastic tubes.

The ash was ground in an agate mortar, which was cleaned between grindings with silicon dioxide. 30 mg of well-ground ash was spread evenly on round filter paper. The exact area supporting the ash was delimited in the middle of the filter paper by attaching another covering filter paper, with a central circular aperture 18 mm in diameter, to the supporting paper. The weighed ash was spread over the round area delimited by the covering filter paper. A thin condenser paper was placed on the ash, and the sample was pressed with a hydraulic press (10-15 tons), which attached the ash to the filter paper. 30 mg of ash was found to be a suitable amount for this method. The optimal diameter of the circle for spreading this amount of ash is 18 mm and this diameter was used in the present study.

Contact between the sample and metallic matter was carefully avoided. When a metal tool had to be used it was made of pure tantalum.

Another detailed description for tissue sample preparation is presented by Iyengar and Sansoni (1980).

Calcified tissues such as bone and tooth present formidable difficulties, especially when homogenization in the natural state (i.e. without ashing) is desired. This difficulty is exemplified by the absence of a certified reference material for bone in its natural form. Another difficulty is the variation in the mass of marrow in different types and parts of bone. According to Iyengar and Sansoni (1980) big samples of bone can be divided into small pieces by cooling in liquid nitrogen, wrapping in PVC sheets and then fracturing with the help of a nylon hammer. The conventional methods of powdering bone using agate, ceramic, tungsten carbide or steel mortars is unsuitable for trace element analysis. Both wet digestion and dry ashing at high temperature have only limited use for calcified tissues. Low-temperature dry ashing (100-150°C) is used by some investigators because of its simplicity and wide applicability for the non-volatile elements. Concerning the loss of volatile elements, the assessment is complicated by the unknown biochemical binding of trace elements in bone, which is a unique biological material.

In a recent attempt, the brittle fracture technique has been used to pulverize small pieces of bone at liquid nitrogen temperature, using a teflon-covered metal ball, teflon vessel and a microdismembrator. Because of technical difficulties, teflon-covered metal balls were not entirely successful, and had to be replaced by pure titanium balls. However, only 2-3 g of bone and single tooth samples can be homogenized by this method, which is therefore unsuitable for handling the much larger amounts needed for the preparation of analytical reference materials.

A primary difficulty with soft tissues results from the presence of unwanted components such as connective tissue, capsule, skin, visible fat, blood vessels, nerves, hair (in skin sampling), glandular parts, GI-tract contents such as food remains and faeces, residual blood, extracellular fluid, etc., which are intimately mixed with the sample material to be prepared for analysis and are difficult to remove completely.

Blood-rich organs, such as liver, heart, spleen, kidney and placenta, present more difficulties than other soft tissues. It is not possible to remove the residual blood completely without damaging in some way the originality of the parent tissue.

Sample preparation is much simpler, and more reliable, when sufficient material is available for multiple subsampling, which is normally the case for autopsy material but not for biopsy material. In the former case, after shaving off the outer layer with appropriate instruments, subsampling may be done either in the frozen state or after thawing. However, random subsampling in the frozen state does not permit the removal of interfering components, such as residual blood in blood-rich organs. Another problem is that, if frozen tissue is handled with a stainless steel knife, contamination from elements such as Cr, Co, Mn and Ni should be anticipated because of the greater friction in cutting the frozen solid.

Biopsy samples can be obtained from a number of organs and tissues such as liver, kidney, muscle, prostate, skin, tooth and bone. Only a few milligrams of the sample material can be obtained by needle biopsies. One problem with such small samples is the difficulty of removing fat, blood and connective tissue. For example, about 1% of blood can be found in muscle samples. Another difficulty is to determine the exact weight of the sample if information is needed on a fresh-weight basis. This is generally done by weighing the samples several times at short known intervals of time soon after removal and extrapolating to zero time.

Biological fluids are susceptible to bacterial growth in the unfrozen state and therefore sample preparation should preferably be finished within 24 h. Among the various biological fluids whole blood, serum, plasma, urine, milk and cerebrospinal fluid are easily accessible. Other less common specimens in this group include semen, tears, sweat, sputum and saliva. Samples from fluids such as synovial, amniotic, pancreatic, prostatic, bile and gastric juice require special procedures. Drying of biological fluids concentrates the residue by a large factor - about 5 for whole blood, 12 for serum and 30 for urine.

Let us describe procedure to be followed for some fluids:

Blood

Blood is conveniently obtained by venous puncture. Since various considerations limit the widespread use of non-contaminating needles made of alloys such as Pt-Rh for drawing blood, disposable needles and syringes are generally used. It is recommended to collect the blood in 10 or 20 ml fractions successively using the same needle, and to discard the first two or three fractions in order to limit contamination. However,

this method is not entirely satisfactory for elements such as Cr and Mn.

In addition to the difficulties listed above, the preparation of neonatal blood samples presents additional problems since only a small volume can be obtained because of certain paediatric considerations.

It is important to avoid haemolysis of blood samples, since elements such as K and Fe, which are present at a higher concentration in erythrocytes, may affect the serum value depending upon the degree of haemolysis. Visible haemolysis begins at about 50 mg of haemoglobin per 100 ml serum. Use of a dry syringe, slow transfer to a dry test tube, and sufficient time for clotting are all necessary. The use of fresh blood, blood from the newborn and blood which has not sufficiently clotted, increase the risk of haemolysis. Up to 0.3% haemolysis does not affect the Cu and Zn values in serum.

Simply drying a 200 ul serum aliquot on a thin carrier at ambient temperature can be chosen as a sample preparation step. This yields a preconcentration factor of about eleven and makes it possible to reach detection limits, for several elements, quite well below the normal concentration level in the serum. To correct for the distinct inhomogeneity of the serum deposits, scandium and yttrium spikes were added as early as possible in the sample preparation to serve as internal standards.

Serum

Serum should preferably be separated from clotted blood within one hour. The clotting of blood takes about 15 min at room temperature, but is delayed if siliconized glassware, teflon or PE-containers are used. Fractionation of blood by centrifugation is done at about 3000 rev/min for 5 to 10 min. The contents of the tubes should be kept closed to prevent contamination and loss by evaporation. Recentrifugation of the separated serum is sometimes necessary to spin down the residual erythrocytes. Disadvantages with serum are that haemolysis is greater than in plasma and clotting releases K from platelets. For this reason, normal K values are lower in plasma than in serum. Zn may also be affected in the same way.

In the work by Cesareo (1982) different kinds of serum samples have been compared: -0.5-1 ml of liquid serum samples, directly analyzed; -0-1-0.5 ml serum samples deposited on thin filters and dried; -0.5 ml of serum samples, ashed at about 500°C. The first preparation method, i.e. direct analysis of liquid samples, has been considered for completeness, since analysis of liquid samples gives rise to superior MDL.

In the second case, when the aqueous component of serum is evaporated, metals, proteins and other organic compounds remain in the filter, and fluorescent radiation is strongly attenuated. In the last case, proteins and other organic compounds split, and an ash residue of about 1-3 mg remains, which can be concentrated in a thin substrate and optimally analyzed.

For the analysis of iron in blood in toto, the concentration of which is about 500 ppm, high sensitivity is not required. In this case, XRF measurements require approximately 0.1 ml blood deposited on filter paper.

Plasma

The main difference between serum and plasma is the presence of fibrinogen in the latter. Plasma can be obtained by immediate centrifugation of heparinized blood, but the presence of the anticoagulant is undesirable for trace element analysis. However, it is possible to obtain plasma without an anticoagulant if fresh blood is immediately centrifuged. The conditions are the same as described under serum.

Erythrocytes

Careful removal of all the serum is necessary to separate the erythrocytes. However, a certain amount of trapped serum (usually 5-8%) is unavoidable and needs correction. A check on the haematocrit is recommended if erythrocyte values are used to compute whole blood values, or vice versa.

Urine

According to the procedure described by van Grieken et al., (1982) sample preparation procedure involves ashing 25 ml urine samples overnight in an automatic Tecator wet destruction unit. The program consists of heating at 130°C (for 2 h), 150°C (1 h), 250°C (1 h) 350°C (1 h), 400°C (1 h) and 460°C (3 h).

(The final ashing temperature was chosen after recording a thermogravimetric ashing curve for the urine residue. Since a significant weight loss was observed just below 460°C, resulting in more advantageous XRF detection limits, and since most authors do not report elemental losses below 500°C (except for, e.g., Hg, Se, As), the maximum ashing temperature was set at 460°C).

After cooling, the residue is transferred to an agate mortar for homogenizing with an agate pestle. Between two thin Mylar foils, the powder is then pressed into a pellet at 7000 kg cm⁻².

The pellet, of about 60 mg cm⁻² thickness, is fixed onto a Mylar foil in a teflon ring which fits into the XRF target holder.

Sweat

Sweat is usually collected either by the so-called arm bag method using a PE-bag around the arm or from the whole body collecting the free flowing drops from various points of the body. This is done in a specially created sweating environment after the subjects have showered. However, collection of a valid sweat sample is difficult because of numerous contamination hazards. In addition, unpredictable

dilution may occur since the profuseness of sweating varies greatly in different parts of the body. The sweat collected should be homogenized by vigorous shaking and prepared immediately for analysis. It is necessary to centrifuge it in order to obtain a cell-free sample.

5. STANDARDS

Whenever it is possible analytical methods should be validated through the use of certified standards. In their absence, independent analytical techniques should be used on a subset of samples in order to obtain a measurement of accuracy.

The number of institutions and agencies involved in the manufacturing, testing and distribution of standard reference materials (SRM) is increasing. Here are some addresses:

In the U.S.A. many standards are available from the U.S. National Bureau of Standards. In addition, the Federal Water Quality Administration, now a part of the Environmental Protection Agency, has made available a number of standard water samples for a few elements. Rock standards are available from the U.S. Geological Survey and include granites, basalts, and carbonates.

In Europe, a number of standard reference materials can be obtained from the Analytical Quality Control Services (AQCS) provided by International Atomic Energy Agency.

The purpose of AQCS is to enable laboratories engaged in the analysis of nuclear materials, radionuclides or trace elements for which nuclear methods may be used to their advantage, to check the quality of their work. Such a control is necessary since results of analytical activities may be the basis upon which economic, administrative, medical or legal decisions are taken; they must, therefore, be documented to be sufficiently reliable.

Reliability of results is a function of precision (reproducibility) and of accuracy. The precision of results can easily be determined by internal measures. The determination of accuracy, however, in most cases requires more detailed procedures such as (LAB/243 Circ., IAEA, 1982):

- Analysis to be carried out by as many different methods, analysts and instruments as possible. In cases where agreement is good, results are assumed to be accurate.
- Control analysis of so-called certified reference material, i.e. material of certified qualitative and quantitative composition which is as similar as possible to the materials to be analysed. Agreement between certified and observed values is then a direct measure of accuracy for the particular type of analysis.
- Participation in an interlaboratory comparison. Samples used in such an intercomparison should be,

as far as is possible, similar in composition and concentration to the samples to be analysed on a routine basis. The agreement of results from a particular laboratory with the most probable value obtained from a statistical evaluation of all results is a measure of the accuracy for the type of analysis under investigation.

For practical reasons, most analytical laboratories are not in a position to check accuracy internally, as

- frequently resources are available for only one method;
- certified reference material, particularly in the case of trace analysis, is not available and can be prepared by the institutes themselves only in exceptional cases;
- intercomparisons are organized rather seldom and many important types of analysis have not been covered so far.

To overcome these difficulties I.A.E.A. provides Analytical Quality Control Services (AQCS), which is involved in distributing certified reference materials (CRM), reference materials (RM), and samples for intercomparisons (I).

Reference materials and certified reference materials available in 1983 are listed in Table 5.1. Reference materials marked by "rm" are materials which have previously been distributed as intercomparison samples, and which can be stored for reasonable time without appreciable change. Although the concentrations of elements or radionuclides in those samples are in most cases reliably established by the results of the intercomparison, the materials are not issued as CRMs, either because they have not been analysed by a sufficiently large number of different analytical techniques, or because the individual intercomparison results are too divergent.

Certified reference materials marked "CRM" in the Table 5.1 are rigorously analysed or calibrated by well known laboratories, normally by many different methods. Some CRMs are certified on the basis of interlaboratory comparison runs provided that the number of participating laboratories and of technique used was sufficiently big and the results were sufficiently compatible. Each material is supplied on request with a certificate stating its composition, physical form, etc.

5.1 Standard Solutions

Standard solutions can be prepared in the laboratory and in most cases the needs of analyst using x-ray emission spectroscopy can be satisfied in such a way.

Sometimes standard solutions used for atomic absorption spectroscopy are available and this might be of great value. There are many manufactures from which such solutions are commercially available. As an example, we list on Table 5.2 compounds and matrices used in preparation of AA standard solutions by Hicol BV (P.O. Box 1151, 3000 BD Rotterdam, Holland).

Table 5.1

I.A.E.A. Reference Materials and Certified
Reference Materials

SAMPLE CODE	MATRIX	ELEMENTS OR NUCLIDES REFERENCED	CONCENTRATION OR ACTIVITY LEVEL	SAMPLE SIZE	CLASS OF SAMPLE
<u>Nuclear materials and stable isotope standards</u>					
S-7	Uranium ore: Pitchblende	U content	0.527% U_3O_8	100 g	CRM
S-8	Uranium ore: Pitchblende	U content	0.140% U_3O_8	100 g	CRM
S-12	Uranium ore: Pitchblende	U content	0.014% U_3O_8	100 g	CRM
S-13	Uranium ore: Pitchblende	U content	0.039% U_3O_8	100 g	CRM
S-14	Thorium ore	Th and U content	Th content below 0.1%	50 g	RM ^{2/}
S-15	Thorium ore	Th and U content	Th content below 0.5%	50 g	RM ^{2/}
S-16	Thorium ore	Th and U content	Th content above 1%	50 g	RM ^{2/}
V-SMOW	Water	Ratio: $^{18}O/^{16}O$; $^2H/^1H$		30 ml	RM
SLAP	Water	Ratio: $^{18}O/^{16}O$; $^2H/^1H$	$^{18}O = - 55.5\%^{1/}$ $^2H = - 428\%$	30 ml	RM

Table 5.1 (cont'd)

SAMPLE CODE	MATRIX	ELEMENTS OR NUCLIDES REFERENCED	CONCENTRATION OR ACTIVITY LEVEL	SAMPLE SIZE	CLASS OF SAMPLE
<u>Environmental materials</u>					
Air-3/1	Simulated deposition on air filter (containing also some major constituents of dust)	Trace elements: As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, U, V, Zn	Fe, Pb, Zn: 100-200 /ug others: 0.1-30 /ug per one filter	6 filters (+ 6 blanks)	CRM
Soil-5	Soil	Trace elements: Al, As, Be, Br, Ce, Co, Cr, Cs, Cu, Dy, Eu, Fe, Ga, Hf, Ho, K, La, Li, Lu, Mn, Na, Nd, Pb, Rb, Sb, Sc, Sm, Ta, Tb, Th, U, Yb, Zn	natural content	25 g	CRM
SL-1	Lake Sediment	Trace elements: As, Ba, Br, Cd, Ce, Co, Cr, Cs, Cu, Dy, Fe, Hf, La, Mn, Na, Nd, Ni, Pb, Rb, Sb, Sc, Sm, Th, U, Yb, V, Zn, Ti	natural content	25 g	CRM
F-1	Feldspar	U, K	natural content	25 g	RM

Table 5.1 (cont'd)

SAMPLE CODE	MATRIX	ELEMENTS OR NUCLIDES REFERENCED	CONCENTRATION OR ACTIVITY LEVEL	SAMPLE SIZE	CLASS OF SAMPLE
<u>Animal and plant materials</u>					
A-11	Milk powder	Trace elements: Ca, Cl, Cu, Fe, Hg, K, Mg, Mn, Na, P, Rb, Zn	natural content	25 g	RM
A-12	Animal bone	Sr-90, Ra-226	environmental levels	80 g	RM ^{2/}
A-13	Freeze dried animal blood	Trace elements ^{3/}	natural content	25 g	RM ^{2/}
V-8	Rye flour	Trace elements: Br, Ca, Cl, Cu, Fe, K, Mg, Mn, P, Rb, Zn	natural content	50 g	RM
V-9	Cotton cellulose	Trace elements ^{3/}	natural content	25 g	RM ^{2/}
<u>Materials for biomedical studies</u>					
H-4	Muscle	Elements: Br, Ca, Cl, Cs, Cu, Fe, Hg, K, Mg, Mn, Na, Rb, Se, Zn	natural content	20 g (2 vials)	CRM
H-5	Animal bone (including mineral and organic components)	Trace elements: Ba, Br, Ca, Cl, Fe, K, Mg, Na, P, Pb, Sr, Zn	natural content	30 g (2 vials)	RM
H-8	Horse kidney	Cd + other trace elements	natural content	30 g (2 vials)	RM

Table 5.1 (cont'd)

SAMPLE	MATRIX	ELEMENTS OR NUCLIDES REFERENCED	CONCENTRATION OR ACTIVITY LEVEL	SAMPLE SIZE	CLASS OF SAMPLE
<u>Materials of marine origin</u>					
MA-A-1	Dried copepoda	Trace elements: Cd, Cu, Fe, Hg, Pb, Zn, etc. and chlorinated hydrocarbons	natural content 100 $\mu\text{g/g}$	30 g	RM
MA-A-2	Homogenized fish flesh	Trace elements and chlorinated hydrocarbons	natural content level of $\mu\text{g/g}$	30 g	RM
MA-M-1	Oyster homogenate	Chlorinated hydrocarbons	100 $\mu\text{g/g}$	30 g	RM

1/
$$= \frac{R_{\text{sample}} - R_{V\text{-SMOW}}}{R_{V\text{-SMOW}}}$$
 (in parts per mil), where R denotes $^{18}\text{O}/^{16}\text{O}$ or $^2\text{H}/^1\text{H}$, respectively.

2/ According to the results of intercomparison the material will be classified as RM or CRM.

3/ Referenced elements will be listed according to the results of intercomparison.

Table 5.2

Compounds and Matrices for Standard Solutions

<u>Element</u>	<u>Compound</u>	<u>Matrix</u>
Aluminium	AlCl_3	HCl
Antimony	$\text{KSbO}_4 \cdot \text{C}_4\text{H}_4\text{O}_6$	H_2O
Arsenic	$\text{NaOH As}_2\text{O}_3$	HNO_3
Barium	BaCl_2	H_2O
Beryllium	BeSO_4	H_2SO_4
Bismuth	$\text{BiO}(\text{NO}_3)$	HNO_3
Boron	H_3BO_3	HCl
Cadmium	$\text{Cd}(\text{NO}_3)_2$	HNO_3
Calcium	CaCl_2	HCl
Cerium	$(\text{NH}_4)_3\text{Ce}(\text{NO}_3)_6$	H_2O
Cesium	CsCl	H_2O
Chromium	$\text{Cr}(\text{NO}_3)_3$	HNO_3
Chromium	$\text{K}_2\text{Cr}_2\text{O}_7$	H_2O
Cobalt	$\text{Co}(\text{NO}_3)_2$	HNO_3
Copper	$\text{Cu}(\text{NO}_3)_2$	HNO_3
Dysprosium	DyCl_3	HCl
Erbium	ErCl_3	HCl
Europium	EuCl_3	HCl
Gadolinium	GdCl_3	HCl
Gallium	$\text{Ga}(\text{NO}_3)_3$	HNO_3
Germanium	Ge	HCl
Gold	HAuCl_4	HCl
Hafnium	$\text{Hf}(\text{NO}_3)_4$	HNO_3
Holmium	HoCl_3	HCl
Indium	$\text{In}(\text{NO}_3)_3$	HNO_3
Iridium	H_2IrCl_6	HCl
Iron	$\text{Fe}(\text{NO}_3)_3$	HNO_3
Lanthanum	LaCl_3	HCl
Lead	$\text{Pb}(\text{NO}_3)_2$	HNO_3
Lithium	LiCl	H_2O
Lutetium	Lu_2O_3	HCl
Magnesium	MgCl_2	HCl
Manganese	$\text{Mn}(\text{NO}_3)_2$	HNO_3
Mercury	$\text{Hg}(\text{NO}_3)_2$	HNO_3
Molybdenum	Mo	HCl
Neodymium	NdCl_3	HCl
Nickel	$\text{Ni}(\text{NO}_3)_2$	HNO_3
Niobium	$\text{Nb}(\text{NO}_3)_5$	HNO_3

Table 5.2 (cont'd)

<u>Element</u>	<u>Compound</u>	<u>Matrix</u>
Osmium	OsO_4	H_2O
Palladium	PdCl_2	HCl
Phosphorus	$(\text{NH}_4)_2\text{HPO}_4$	H_2O
Platinum	H_2PtCl_6	HCl
Potassium	KCl	H_2O
Praseodymium	PrCl_3	HCl
Rhenium	KReO_4	H_2SO_4
Rhodium	RhCl_3	HCl
Rubidium	RbCl	H_2O
Ruthenium	RuCl_3	HCl
Samarium	SmCl_3	HCl
Scandium	ScCl_3	HCl
Selenium	Se	HNO_3
Silicon	Si	H_2O
Silver	AgNO_3	HNO_3
Sodium	NaCl	H_2O
Strontium	$\text{Sr}(\text{NO}_3)_2$	H_2O
Tantalum	$\text{Ta}(\text{NO}_3)_5$	HNO_3
Tellurium	H_6TeO_6	H_2O
Terbium	TbCl_3	HCl
Thallium	TlNO_3	HNO_3
Thorium	$\text{Th}(\text{NO}_3)_4$	H_2O
Thulium	ThCl_3	HCl
Tin	SnCl_2	HCl
Titanium	$\text{Ti}_2(\text{SO}_4)_3$	H_2SO_4
Tungsten	W	H_2O
Uranium	$\text{UO}_2(\text{NO}_3)_2$	H_2O
Vanadium	NH_3VO_3	HNO_3
Ytterbium	YbCl_3	HCl
Yttrium	YCl_3	HCl
Zinc	$\text{Zn}(\text{NO}_3)_2$	HNO_3
Zirconium	ZrOCl_2	HCl

Available for example from

Hicol BV, P.O. Box 1151, Rotterdam, The Netherlands

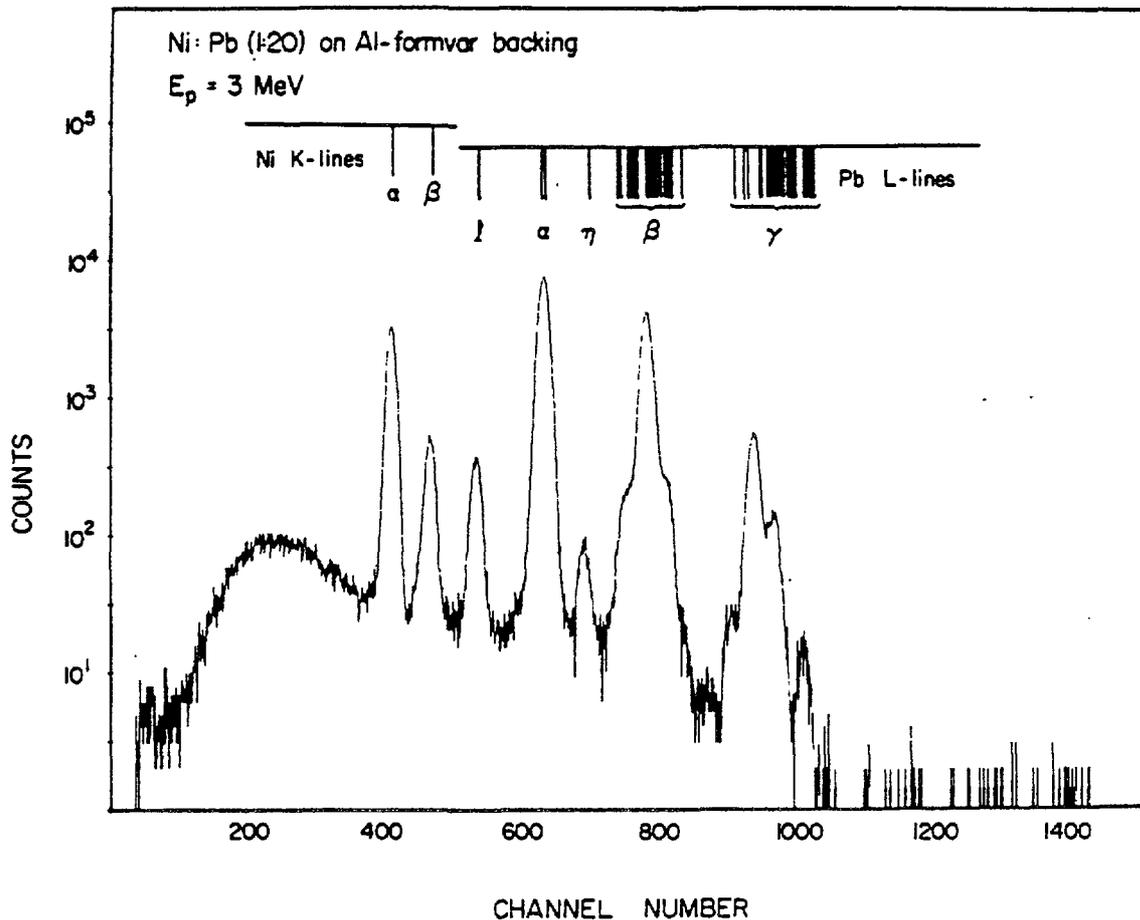


Fig. 5.1 X-ray spectrum from Ni:Pb (1:20) standard solution

Standard solutions should be used during the measurements of relative efficiency of system for different elements, as well when the internal standards case used. For example, Fig. 5.1 shows x-ray spectrum of Ni:Pb (1:20) standard solution. Other examples can be found in the literature.

"Homogeneous" standard samples can be prepared in this way:

A measured volume of a gravimetrically-prepared solution standard is micropipetted by the multidrop technique onto a cellulose fiber or cellulose membrane filter so as to homogeneously impregnate the filter. This is rapidly dried yielding a deposit having a submicron particle size, a homogeneity of 5% or better on a scale of 5 mm, and an over-all concentration known to better than 2%.

Thin layered sample can be prepared using Snap-on ring sample cap. First, one has to affix thin-film sample support; then a slight concavity in the thin-film with a round-ended glass rod. Gentle heating will restore thin-film to original tautness. Insert solution droplet, evaporate if desired and overlay with another piece of thin-film of suitable gauge with Snap-on ring.

5.2 Reference Materials

There is a number of reference material available to the analyst. They can be classified into groups, i.e. biological, geological, etc. reference materials. For example, biological reference materials for trace element studies are currently being prepared and issued by a number of different international and national laboratories. Very commonly, when such materials are submitted as unknown samples to a diverse group of analytical laboratories, the results reported cover a wide range - in some cases so wide that no consensus values can be derived. In part this may be due to the inexperience of some of the laboratories concerned. However, for some elements and some matrices, even well-experienced laboratories may have great difficulties in producing mutually consistent results. Such problems, as evidenced by results for reference materials, are probably also very typical of what may be encountered in analysing any biological material. Thus they provide a very useful demonstration of the practical problems that analysts working in this field must be prepared to face (Kosta, 1980).

Biological CRMs for trace element studies are presently available from two main sources, the US National Bureau of Standards (Office of Standard Reference Materials, National Bureau of Standards, US Department of Commerce, Washington, D.C. 21234, USA) and the IAEA (Analytical Quality Control Services, Laboratory Seibersdorf, International Atomic Energy Agency, POB 100, A-1400 Vienna, Austria). In addition, one of the earliest and most important of all biological CRMs, Bowen's kale, is still available from its originator (Prof. H.J.M. Bowen, Department of Chemistry, Reading University, Whiteknights Park, Reading, Berks RG6 2AD, United Kingdom). Table 5.3 gives details of all presently available CRMs and the elements for which they are certified; the concentrations of these elements (except nitrogen) and other details taken from the relevant certificates of analysis or information sheets are given in the Table 5.4 (Parr, 1980).

The data in Table 5.4 are listed in alphabetic order of the chemical symbol of the element, and within each element, in order of decreasing value of the concentration. The number of available CRMs for each element can thus be seen at a glance as well as the range of concentration of the element.

Here we shall present spectra from some reference materials obtained by sample irradiation with x-ray tube (Mo-anode).

Fig. 5.2 shows the x-ray spectrum obtained from the irradiation of animal muscle, IAEA H-4 standard reference material. The spectrum showed was obtained in few minutes measurement with a sample prepared in the form of formvar-tissue-formvar sandwich. Two measurements were performed; thin and thick target measurement. Concentration ratios were determined from thick target measurement while the absolute Zn concentration was determined in a thin target measurement.

Table 5.3

List of presently available CRMs. See text
for addresses of suppliers (after Parr 1980)

Supplier	Material	Elements certified
NBS	Oyster	Ag, As, Ca, Cd, Cr, Cu, Fe, Hg, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, U, Zn
	Wheat flour	Ca, Cd, Cu, Fe, Hg, K, Mn, Na, Se, Zn
	Rice flour	As, Ca, Cd, Co, Cu, Fe, Hg, K, Mn, Na, Se, Zn
	Brewers yeast	Cr
	Spinach	Al, As, Ca, Cr, Cu, Fe, Hg, K, Mn, P, Pb, Rb, Sr, Th, U, Zn
	Orchard leaves	As, B, Be, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, N, Na, Ni, P, Pb, Rb, Sb, Se, Sr, Th, U, Zn
	Tomato leaves	As, Ca, Cr, Cu, Fe, K, Mn, P, Pb, Rb, Sr, Th, U, Zn
	Pine needles	Al, As, Ca, Cr, Cu, Fe, Hg, K, Mn, P, Pb, Rb, Sr, Th, U
	Bovine liver	As, Ca, Cd, Cr, Cu, Fe, Hg, K, Mn, Mg, N, Na, Pb, Rb, Se, Zn
	IAEA	Animal blood
Fish Solubles		As, Co, Cr, Cu, Fe, Hg, Mn, Se, Zn
Milk powder		Ca, Cl, Co, Cu, Fe, Hg, K, Mn, Mg, Na, P, Rb, Se, Zn
Animal muscle		Br, Ca, Cl, Cs, Cu, Fe, K, Mg, Mn, Na, Rb, Se, Zn
Human serum		I (total and protein bound)
Copepod		Cd, Cu, Fe, Hg, Zn
Fish flesh		As, Cu, Fe, Hg, Mn, Zn
Wheat flour		I
Bowen	Kale	As, Au, B, Br, Ca, Cd, Cl, Co, Cs, Cu, Fe, Hg, K, La, Mn, Mg, Mo, N, Na, P, Rb, Sb, Sc, Se, Sn, V, W, Zn

As another example Fig. 5.3 shows x-ray spectrum obtained by the irradiation of fish sample, IAEA A6 standard reference material. It should be mentioned 0.5 mg of material was used in sample preparation, therefore there is a question of how representative that is.

Figures 5.4 and 5.5 show the measured x-ray spectra from the irradiation of IAEA hair, HH1 standard with Mo-tube (26 kV, 12 mA) using 3 filters. Targets were prepared on formvar backing and in the form of formvar-hair (or hair ash) - formvar sandwich. Thick targets (20 mg/cm²) were used for the determination of element concentration ratios, while thin targets (0.2 mg/cm²) were used for determination of absolute concentrations of Zn

Table 5.4

Elemental composition of some CRMs in
order of the chemical symbol of the element
(after Parr, 1980)

Element	Material	Code	C*	Conc. (ppm)	Error (%)
Ag	Oyster tissue	NBS-SRM-1566	C	0.89	10
Al	Spinach	NBS-SRM-1570	C	870	5.7
Al	Pine needles	NBS-SRM-1575	C	545	5.5
As	Fish solubles	IAEA-A-6	R	14.5	15
As	Oyster tissue	NBS-SRM-1566	C	13.4	14
As	Orchard leaves	NBS-SRM-1571	C	10	20
As	Fish flesh	IAEA-MA-A-2	Q	2.5	18
As	Rice flour	NBS-SRM-1568	C	0.41	12
As	Tomato leaves	NBS-SRM-1573	C	0.27	19
As	Pine needles	NBS-SRM-1575	C	0.21	19
As	Spinach	NBS-SRM-1570	C	0.15	33
As	Bowen's kale		I	0.14	14
As	Bovine liver	NBS-SRM-1577	C	0.055	9.1
Au	Bowen's kale		I	0.0023	17
B	Bowen's kale		I	46	8.7
B	Orchard leaves	NBS-SRM-1571	C	33	9.1
Be	Orchard leaves	NBS-SRM-1571	C	0.027	37
Br	Bowen's kale		R	24	5.8
Br	Animal muscle	IAEA-H-4	R	4.07	15
Ca	Bowen's kale		R	41400	4.3
Ca	Tomato leaves	NBS-SRM-1573	C	30000	1.0
Ca	Orchard leaves	NBS-SRM-1571	C	20900	1.4
Ca	Spinach	NBS-SRM-1570	C	13500	2.2
Ca	Milk powder	IAEA-A-11	R	12900	6.2
Ca	Pine needles	NBS-SRM-1575	C	4100	4.9
Ca	Pine needles	NBS-SRM-1575	C	4100	4.9
Ca	Oyster tissue	NBS-SRM-1566	C	1500	13
Ca	Wheat flour	NBS-SRM-1567	C	190	5.3
Ca	Animal muscle	IAEA-H-4	R	188	13
Ca	Rice flour	NBS-SRM-1568	C	140	1.4
Ca	Bovine liver	NBS-SRM-1577	C	124	4.8
Cd	Oyster tissue	NBS-SRM-1566	C	3.5	11
Cd	Bowen's kale		I	0.89	10
Cd	Coperod	IAEA-MA-A-1	P	0.75	8.1
Cd	Bovine liver	NBS-SRM-1577	C	0.27	15

Table 5.4 (ctn'd)

Element	Material	Code	C*	Conc. (ppm)	Error (%)
Cd	Orchard leaves	NBS-SRM-1571	C	0.11	9.1
Cd	Wheat flour	NBS-SRM-1567	C	0.032	22
Cd	Rice flour	NBS-SRM-1568	C	0.029	14
Cl	Milk powder	IAEA-A-11	R	9080	19
Cl	Bowen's kale		I	3500	8.6
Cl	Animal muscle	IAEA-H-4	R	1890	4.4
Co	Animal whole Blood	IAEA-A-2	R	0.42	24
Co	Fish solubles	IAEA-A-6	R	0.22	23
Co	Bowen's kale		R	0.06	17
Co	Rice flour	NBS-SRM-1568	C	0.02	50
Co	Milk powder	IAEA-A-11	R	0.005	20
Cr	Spinach	NBS-SRM-1570	C	4.6	6.5
Cr	Tomato leaves	NBS-SRM-1573	C	4.5	11
Cr	Pine needles	NBS-SRM-1575	C	2.6	7.7
Cr	Orchard leaves	NBS-SRM-1571	C	2.6	12
Cr	Brewers yeast	NBS-SRM-1569	C	2.12	2.4
Cr	Fish solubles	IAEA-A-6	R	0.71	24
Cr	Oyster tissue	NBS-SRM-1566	C	0.69	39
Cr	Bovine liver	NBS-SRM-1577	C	0.088	14
Cs	Animal muscle	IAEA-H-4	R	0.12	12
Cs	Bowen's kale		I	0.075	6.7
Cu	Bovine liver	NBS-SRM-1577	C	193	5.2
Cu	Oyster tissue	NBS-SRM-1566	C	63.0	5.6
Cu	Animal whole Blood	IAEA-A-2	R	45	8.8
Cu	Spinach	NBS-SRM-1570	C	12.0	17
Cu	Orchard leaves	NBS-SRM-1571	C	12	8.3
Cu	Tomato leaves	NBS-SRM-1573	C	11.0	9.1
Cu	Copepod	IAEA-MA-A-1	P	7.6	5.2
Cu	Fish solubles	IAEA-A-6	R	5.25	12
Cu	Bowen's kale		R	4.9	8.6
Cu	Fish flesh	IAEA-MA-A-2	Q	4.6	18
Cu	Animal muscle	IAEA-H-4	R	3.96	8.3
Cu	Pine needles	NBS-SRM-1575	C	3.0	10
Cu	Rice flour	NBS-SRM-1568	C	2.2	14
Cu	Wheat flour	NBS-SRM-1567	C	2.0	15
Cu	Milk powder	IAEA-A-11	R	0.84	20
Fe	Tomato leaves	NBS-SRM-1573	C	690	3.6

Table 5.4 (ctn'd)

Element	Material	Code	C*	Conc. (ppm)	Error (%)
Fe	Fish solubles	IAEA-A-6	R	565	7.7
Fe	Spinach	NBS-SRM-1570	C	550	3.6
Fe	Orchard leaves	NBS-SRM-1571	C	300	6.7
Fe	Bovine liver	NBS-SRM-1577	C	268	3.0
Fe	Pine needles	NBS-SRM-1575	C	200	5.0
Fe	Oyster tissue	NBS-SRM-1566	C	195	17
Fe	Bowen's kale		R	115	5.2
Fe	Fish flesh	IAEA-MA-A-2	Q	61	13
Fe	Copepod	IAEA-MA-A-1	P	60	6.6
Fe	Animal muscle	IAEA-H-4	R	49.1	4.2
Fe	Wheat flour	NBS-SRM-1567	C	18.3	5.5
Fe	Rice flour	NBS-SRM-1568	C	8.7	6.9
Fe	Milk powder	IAEA-A-11	R	3.7	21
Fe	Animal whole Blood	IAEA-A-2	R	3.41	6.4
Hg	Fish solubles	IAEA-A-6	R	73.9	18
Hg	Fish flesh	IAEA-MA-A-2	Q	0.48	8.5
Hg	Copepod	IAEA-MA-A-1	P	0.28	7.3
Hg	Bowen's kale		R	0.18	17
Hg	Orchard leaves	NBS-SRM-1571	C	0.155	9.7
Hg	Pine needles	NBS-SRM-1575	C	0.15	33
Hg	Oyster tissue	NBS-SRM-1566	C	0.057	26
Hg	Spinach	NBS-SRM-1570	C	0.030	17
Hg	Bovine liver	NBS-SRM-1577	C	0.016	13
Hg	Animal muscle	IAEA-H-4	R	0.014	27
Hg	Rice flour	NBS-SRM-1568	C	0.0060	12
Hg	Milk powder	IAEA-A-11	R	0.0025	29
Hg	Wheat flour	NBS-SRM-1567	C	0.000	80
I	Human blood serum	IAEA-H-6	R	0.068	16
I	Wheat flour	IAEA-V-5	R	0.0029	43
K	Tomato leaves	NBS-SRM-1573	C	44600	0.7
K	Spinach	NBS-SRM-1570	C	35600	0.8
K	Bowen's kale		R	24300	5.3
K	Milk powder	IAEA-A-11	R	17200	5.8
K	Animal muscle	IAEA-H-4	R	15840	3.7
K	Orchard leaves	NBS-SRM-1571	C	14700	2.0
K	Bovine liver	NBS-SRM-1577	C	9700	6.2
K	Oyster tissue	NBS-SRM-1566	C	9690	0.5
K	Pine needles	NBS-SRM-1575	C	3700	5.4

Table 5.4 (ctn'd)

Element	Material	Code	C*	Conc. (ppm)	Error (%)
K	Wheat flour	NBS-SRM-1567	C	1360	2.9
K	Rice flour	NBS-SRM-1568	C	1120	0.2
La	Bowen's kale		I	0.08	13
Mg	Orchard Leaves	NBS-SRM-1571	C	6200	3.2
Mg	Bowen's kale		R	1560	5.1
Mg	Oyster tissue	NBS-SRM-1566	C	1280	7.0
Mg	Milk powder	IAEA-A-11	R	1100	7.3
Mg	Animal muscle	IAEA-H-4	R	1050	5.6
Mg	Bovine liver	NBS-SRM-1577	C	604	1.5
Mn	Pine needles	NBS-SRM-1575	C	675	2.2
Mn	Tomato leaves	NBS-SRM-1573	C	238	2.9
Mn	Spinach	NBS-SRM-1570	C	165	3.6
Mn	Animal whole Blood	IAEA-A-2	R	123	17
Mn	Orchard leaves	NBS-SRM-1571	C	91	4.4
Mn	Rice flour	NBS-SRM-1568	C	20.1	2.0
Mn	Oyster tissue	NBS-SRM-1566	C	17.5	6.9
Mn	Bowen's kale		R	15	8.0
Mn	Bovine liver	NBS-SRM-1577	C	10.3	9.7
Mn	Wheat flour	NBS-SRM-1567	C	8.5	5.9
Mn	Fish solubles	IAEA-A-6	R	4.73	12
Mn	Fish flesh	IAEA-MA-A-2	Q	1.0	21
Mn	Animal muscle	IAEA-H-4	R	0.52	7.1
Mn	Milk powder	IAEA-A-11	R	0.38	21
Mo	Bowen's kale		R	2.3	9.1
Mo	Orchard leaves	NBS-SRM-1571	C	0.3	33
Na	Oyster tissue	NBS-SRM-1566	C	5100	5.9
Na	Milk powder	IAEA-A-11	R	4420	7.5
Na	Bovine liver	NBS-SRM-1577	C	2430	5.3
Na	Bowen's kale		I	2300	10
Na	Animal muscle	IAEA-H-4	R	2060	6.1
Na	Orchard leaves	NBS-SRM-1571	C	82	7.3
Na	Wheat flour	NBS-SRM-1567	C	8.0	19
Na	Rice flour	NBS-SRM-1568	C	6.0	25
Ni	Orchard leaves	NBS-SRM-1571	C	1.3	15
Ni	Oyster tissue	NBS-SRM-1566	C	1.03	18
P	Milk powder	IAEA-A-11	R	9100	11
P	Spinach	NBS-SRM-1570	C	5500	3.6

Table 5.4 (ctn'd)

Element	Material	Code	C*	Conc. (ppm)	Error (%)
P	Bowen's kale		I	4450	5.8
P	Tomato leaves	NBS-SRM-1573	C	3400	5.9
P	Orchard leaves	NBS-SRM-1571	C	2100	4.8
P	Pine needles	NBS-SRM-1575	C	1200	17
Pb	Orchard leaves	NBS-SRM-1571	C	45	6.7
Pb	Pine needles	NBS-SRM-1575	C	10.8	4.6
Pb	Tomato leaves	NBS-SRM-1573	C	6.3	4.8
Pb	Spinach	NBS-SRM-1570	C	1.2	17
Pb	Animal whole Blood	IAEA-A-2	R	0.97	23
Pb	Oyster tissue	NBS-SRM-1566	C	0.48	8.3
Pb	Bovine liver	NBS-SRM-1577	C	0.34	24
Rb	Bowen's kale		R	52	10
Rb	Milk powder	IAEA-A-11	R	31	20
Rb	Animal muscle	IAEA-H-4	R	18.7	7.8
Rb	Bovine liver	NBS-SRM-1577	C	18.3	5.5
Rb	Tomato leaves	NBS-SRM-1573	C	16.5	0.6
Rb	Spinach	NBS-SRM-1570	C	12.1	1.7
Rb	Orchard leaves	NBS-SRM-1571	C	12	8.3
Rb	Pine needles	NBS-SRM-1575	C	11.7	0.9
Rb	Oyster tissue	NBS-SRM-1566	C	4.45	2.0
Sb	Orchard leaves	NBS-SRM-1571	C	2.9	10
Sb	Bowen's kale		I	0.07	14
Sc	Bowen's kale		I	0.008	11
Se	Fish solubles	IAEA-A-6	R	3.07	18
Se	Oyster tissue	NBS-SRM-1566	C	2.1	24
Se	Wheat flour	NBS-SRM-1567	C	1.1	18
Se	Bovine liver	NBS-SRM-1577	C	1.1	9.1
Se	Animal whole Blood	IAEA-A-2	R	0.59	24
Se	Animal muscle	IAEA-H-4	R	0.28	12
Se	Bowen's kale		I	0.14	7.1
Se	Orchard leaves	NBS-SRM-1571	C	0.080	13
Se	Rice flour	NBS-SRM-1568	C	0.040	25
Se	Milk powder	IAEA-A-11	R	0.034	21
Sn	Bowen's kale		I	0.21	14
Sr	Spinach	NBS-SRM-1570	C	87	2.3
Sr	Tomato leaves	NBS-SRM-1573	C	44.9	0.7

Table 5.4 (ctn'd)

Element	Material	Code	C*	Conc. (ppm)	Error (%)
Sr	Orchard leaves	NBS-SRM-1517	C	37	2.7
Sr	Oyster tissue	NBS-SRM-1566	C	10.36	5.4
Sr	Pine needles	NBS-SRM-1575	C	4.8	4.2
Th	Tomato leaves	NBS-SRM-1573	C	0.17	18
Th	Spinach	NBS-SRM-1570	C	0.12	25
Th	Orchard leaves	NBS-SRM-1571	C	0.064	9.4
Th	Pine needles	NBS-SRM-1575	C	0.037	8.1
U	Oyster tissue	NBS-SRM-1566	C	0.116	5.2
U	Tomato leaves	NBS-SRM-1573	C	0.061	4.9
U	Spinach	NBS-SRM-1570	C	0.046	20
U	Orchard leaves	NBS-SRM-1571	C	0.029	17
U	Pine needles	NBS-SRM-1575	C	0.020	20
V	Bowen's kale		I	0.36	11
W	Bowen's kale		I	0.06	12
Zn	Oyster tissue	NBS-SRM-1566	C	852	1.6
Zn	Copepod	IAEA-MA-A-1	P	158	2.6
Zn	Bovine liver	NBS-SRM-1577	C	130	10
Zn	Animal whole Blood	IAEA-A-2	R	89	10
Zn	Animal muscle	IAEA-H-4	R	86.3	3.9
Zn	Tomato leaves	NBS-SRM-1573	C	62	9.7
Zn	Spinach	NBS-SRM-1570	C	50	4.0
Zn	Milk powder	IAEA-A-11	R	38.9	5.9
Zn	Fish flesh	IAEA-MA-A-2	Q	36	17
Zn	Bowen's kale		R	31	7.1
Zn	Orchard leaves	NBS-SRM-1571	C	25	12
Zn	Rice flour	NBS-SRM-1568	C	19.4	5.2
Zn	Fish solubles	IAEA-A-6	R	18.9	7.0
Zn	Wheat flour	NBS-SRM-1567	C	10.6	9.4

* Type of Certified value specified by issuing organization

C = certified concentration

I = indicated concentration

P = probable concentration derived by application of Chauvenet's outlier test

Q = probable concentration (preliminary value; no outlier test applied)

R = recommended concentration

ANIMAL MUSCLE, H-4

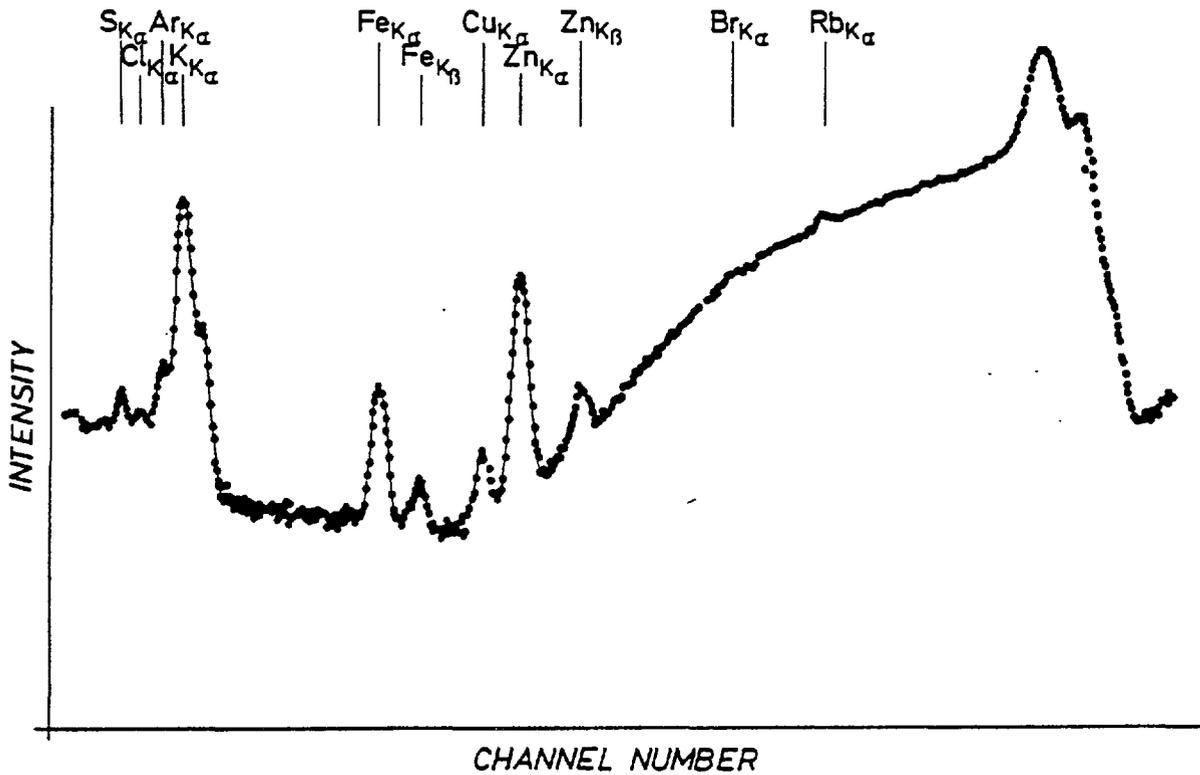


Fig. 5.2 X-ray spectrum from animal muscle, IAEA H-4 SRM.

FISH, A 6

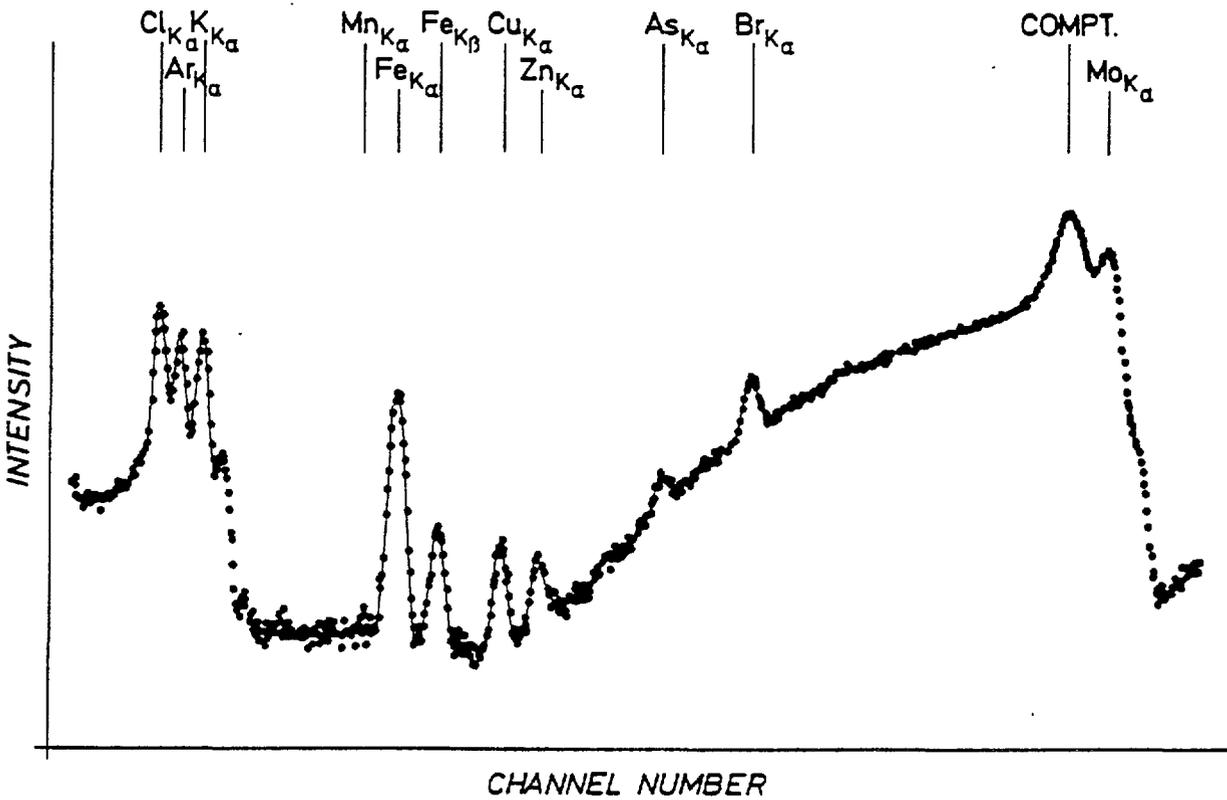


Fig. 5.3 X-ray spectrum from fish sample, IAEA A6 SRM.

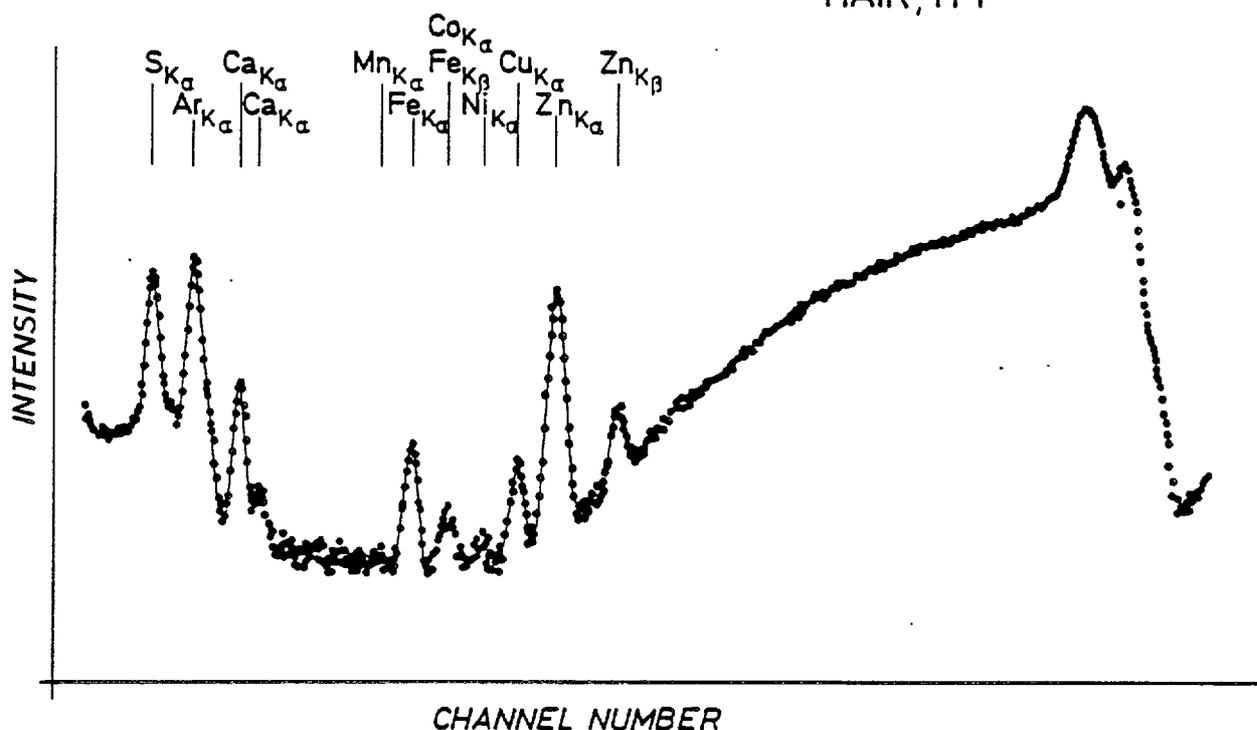


Fig. 5.4 X-ray spectrum from hair sample.
IAEA HH1 standard.

and Cu. The results obtained are shown in Table 5.5. Errors shown are statistical ones.

Fig. 5.6 shows x-ray spectrum obtained with the same system from SOIL-5 SRM.

A number of other standard reference materials has been prepared by different agencies. Very active in this field is office of Standard Reference Materials in National Bureau of Standards (Washington, D.C. USA). As an illustration we enclose the description of NBS SRM No 1635 (trace elements in subbituminous coal) in Table 5.6.

This Standard Reference Material is intended for use in the calibration of apparatus and the evaluation of techniques employed in the trace element analysis of coal and similar materials. The material should be dried without heat to constant weight before use.

The recommended procedures for drying are either vacuum drying at ambient temperature for 24 hours, or freeze drying in which the drying chamber is kept at room temperature. The moisture content of this material is approximately 20%. Because of this moisture level, it is recommended that small individual samples be dried immediately before use. Drying of large samples may result in a violent discharge of water vapor and resultant loss of sample. When not in use, the material should be kept in a tightly sealed bottle and stored in a cool, dark place. Long-term (>1 year) stability of this SRM has not been rigorously established.

The certified values given in Table 5.6 are based on at least a 250-mg sample of the dried material, the minimum amount that should be used for analysis. During the preparation

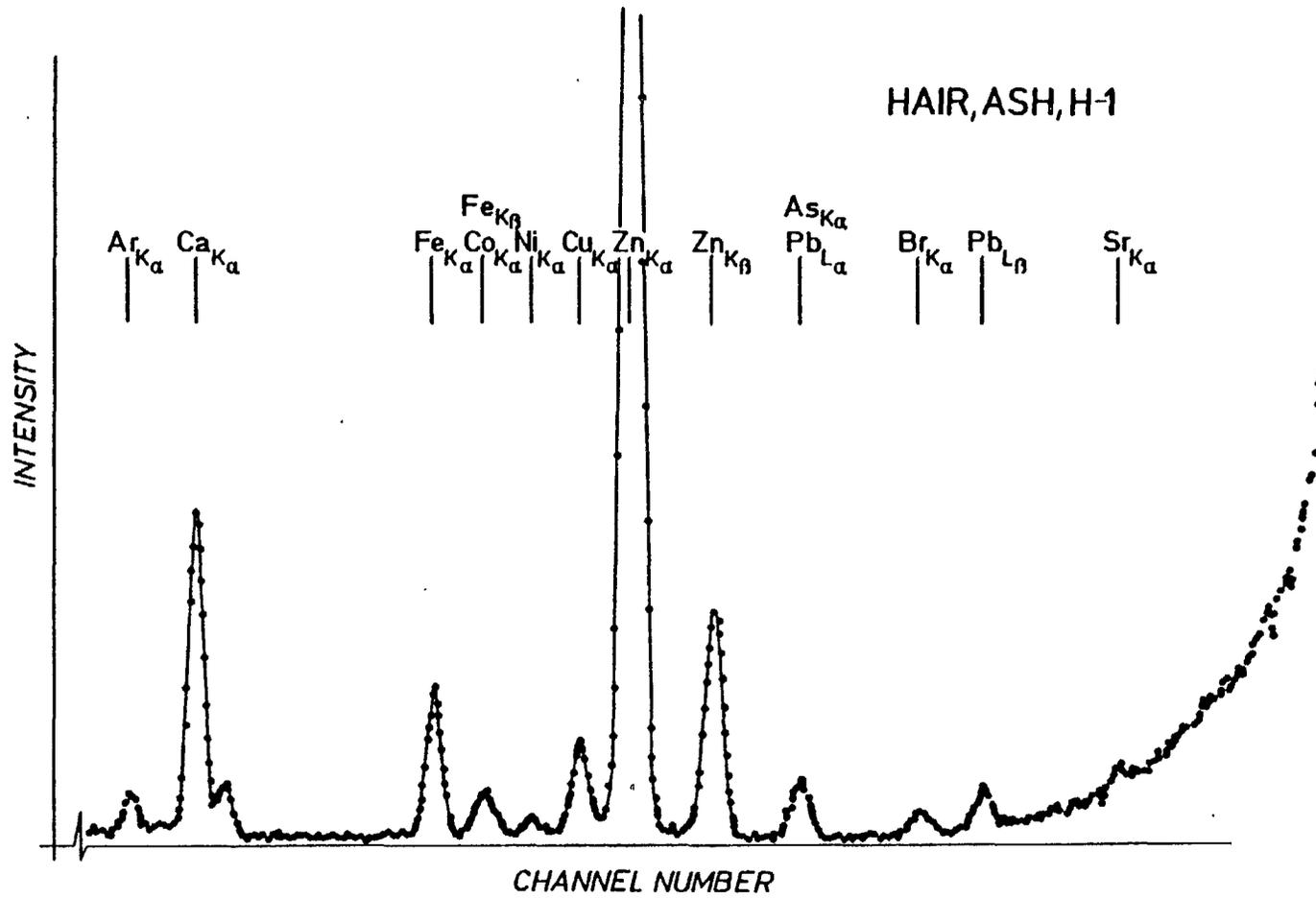


Fig. 5.5 X-ray spectrum from hair ash sample.

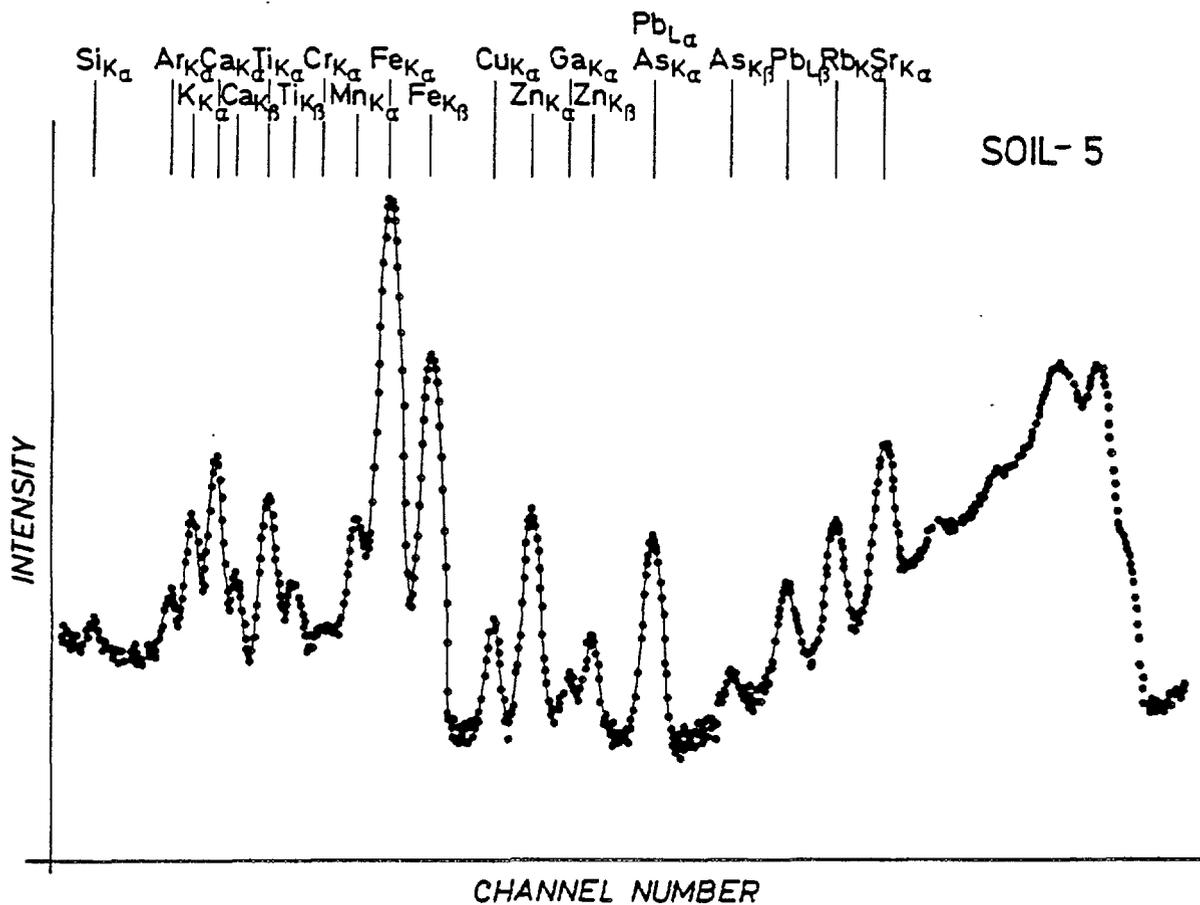


Fig. 5.6 X-ray spectrum from soil sample, IAEA SOIL-5 SRM.

Table 5.5

Trace element concentrations in IAEA hair HH-1 standard

Element	Concentration ppm wet weight
Zn	= 180 ± 1%
Cu	= 12.3 ± 4.8%
Ni	= 2.68 ± 16.7%
Co	= 4.02 ± 16%
Fe	= 19.6 ± 15.2%
As	= 1.34 ± 19.5%
Br	= 5.14 ± 6.9%
Ca	= 198 ± 3.8%
Cl	= 1643 ± 14%
S	= 35823 ± 2%
Pb	= only in ash possible contamination

Table 5.6

National Bureau of Standards (Washington D.C. USA)
 Standard Reference Material 1635
 Trace Elements in Subbituminous Coal

Element ¹	Content, ug/g ²	Element ¹	Content, ug/g ²
Arsenic ^{a,b}	0.42 ± 0.15	Thorium ^{c,e}	0.62 ± 0.04
Cadmium ^{c,d,e}	0.03 ± 0.01	Uranium ^c	0.24 ± 0.02
Chromium ^{c,e}	2.5 ± 0.3	Vanadium ^{e,g}	5.2 ± 0.5
Copper ^{a,c,e}	3.6 ± 0.3	Zinc ^{c,d}	4.7 ± 0.5
Lead ^{c,d}	1.9 ± 0.2		
Manganese ^{a,e}	21.4 ± 1.5	<u>Element¹</u>	<u>Wt %²</u>
Nickel ^{c,d}	1.74 ± 0.10	Iron ^{c,d,e,f}	0.239 ± 0.005
Selenium ^{a,e}	0.9 ± 0.3	Sulfur ^{f,h}	0.33 ± 0.03

1. Methods of Analysis:

- a. Atomic Absorption Spectrometry
- b. Photon Activation
- c. Isotope Dilution Mass Spectrometry
- d. Polarography
- e. Neutron Activation
- f. Spectrophotometry
- g. Flame Emission Spectrometry
- h. Gravimetry

2. The estimated uncertainty is based on judgment and represents an evaluation of the combined effects of method imprecision, possible systematic errors among methods, and material variability for samples of 250-mg or more. (No attempt was made to derive exact statistical measures of imprecision because several methods were involved in the determination of most constituents.)

of this material crushed and ground coal was sieved through a 250 um (No. 60) sieve and thoroughly blended in a V-type blender. Samples for homogeneity testing were taken from the top, middle, and bottom of three bulk containers of coal and analyzed by neutron activation analysis for scandium, chromium, iron, cobalt, cerium, and thorium. Replicate analyses of 250-mg samples indicated the material variability for these elements to be within ± 2% (relative). The homogeneity measurements were also performed.

The concentration values for some elements were not certified because they were based on a non-reference method, or were not determined by two or more independent methods.

5.3 Other Standards

A large number of standard materials have been developed by different agencies. For example, Columbia Scientific Industries (11950 Jollyville Road, P.O. Box 9908, Austin, Texas 78766) is marketing thin specimen x-ray spectrometry calibration standards. Single element or multielement dried solution deposits on filters; also deposits of sized pure minerals or sized quartz spiked with trace elements.

In addition this company's marketing standard reference strips for air particulate analysis: 8 x 3/4 in. glass fiber or other filter strips impregnated with dried solution and/or particulate deposits. Sulfate, nitrate, chloride, lead and arsenic available as dried solution deposits; also nine trace metals available spiked on sized quartz particulates. Ammonium ion is available on cellulose filters only.

Standard samples are prepared gravimetrically from pure materials under carefully controlled conditions. All procedures are rigorously monitored to insure a high standard of purity, accuracy and homogeneity of the reference samples. Special deposition techniques and non-interfering additives have been developed to insure homogeneity of the x-ray calibration standards. Most of the calibration samples offered have been independently tested in interlaboratory comparisons.

A number of geological material standards are available. For example, standards used in the work by Beitz et al., (1970) are listed in Table 5.7. The first six standards of this table are official standards of the U.S. Geological Survey Washington, D.C. They are supplied in the form of powders. The standards T1 to T3 were supplied by a university together with analysis data; the standards T12, T13, and T23 were obtained by mixing the first three standards.

The melts were obtained by mixing 0.7 g of analysis material, 2.975 g of lithium tetraborate, and 0.525 g of lanthanum oxide, yielding melts at the ratio of 1:5.

The lanthanum oxide is used to reduce the interelement effects on the measurement result, because the element La has a high absorption capacity for the radiations of the elements. Its use was due to a request made by the university which commissioned the investigations. Actually, the lanthanum oxide could be dispensed with, because modern computer programs are capable, even without such a damping agent, to carry out matrix corrections with a high degree of accuracy. Moreover, the element La reduces the intensities of the characteristic x-ray radiation to be measured.

Let us discuss the difficult problem of trace element analysis of oils. The analysis usually involves the addition of an accurately known amount of an oil-soluble compound of the element in question to an oil which contains one of this element. The resulting standard solution is then carried through all the steps of the method and the amount of the element added is determined. The same procedure is applied to the "unknown" oil, and its content of the element is determined by comparison.

The problem of finding suitable metallo-organic compounds to be used as standards has increased as more additives have

Table 5.7

Geological Standards

Designation of standard	Compound	% MgO	% CaO	% K ₂ O	% Na ₂ O	% MnO	% TiO ₂	% P ₂ O ₅	% SiO ₂	% Al ₂ O ₃	% Fe ₂ O ₃
PCC-1		43.562	0.534	0.019	0.053	0.122	0.023	0.014	41.870	0.8563	8.537
DTS-1		49.805	0.158	0.023	0.046	0.126	0.023	0.013	40.455	0.552	8.850
BOR-1		3.281	6.952	1.684	3.313	0.176	2.234	0.363	54.485	13.657	13.508
G-2		0.782	1.988	4.510	4.156	0.037	0.531	0.141	69.192	15.345	2.768
GSP-1		0.957	2.034	5.488	2.880	0.044	0.699	0.285	67.278	15.115	4.334
AGV-1		1.492	4.982	2.898	4.334	0.098	1.082	0.487	58.997	17.014	6.804
T-1		9.410	15.880	2.650	3.65	0.240	2.970	0.980	39.910	11.660	12.630
T-2		0.930	3.240	5.340	4.720	0.170	1.190	0.420	57.860	16.180	9.950
T-3		2.160	2.190	4.810	3.670	0.060	0.580	0.290	67.620	14.960	3.640
T 12		5.170	9.560	3.995	4.185	0.205	2.080	0.700	48.885	13.920	11.290
T 13		5.785	9.035	3.730	3.660	0.150	1.775	0.635	53.765	13.310	8.135
T 23		1.545	2.715	5.075	4.195	0.115	0.885	0.355	62.740	15.570	6.795

been introduced and as a more exact knowledge of the trace constituents of petroleum and its products has been needed. Commercial metallo-organic compounds available were found not to be satisfactory as standards because (Isbell et al., 1962):

1. Metallic and other contaminants are often present in these compounds in the amount greater than that of the trace element to be determined in the petroleum product;
2. Some of the compounds are not sufficiently soluble in petroleum products;
3. Some of the compounds are sufficiently volatile in that considerable loss occurs during (or even before) the analysis; and
4. When combinations of the elements (as their respective compounds) were dissolved in a single oil, the compounds often gave incompatible mixtures.

Isbell et al., (1962) have selected from 150 prospective compounds described in their monograph the ones having suitable physical properties as standards in the determination of the following 24 elements: Al, Ba, B, Cd, Ca, Cr, Co, Cu, Fe, Pb, Li, Mg, Mn, Hg, Ni, P, K, Si, Ag, Sr, Sn, V, and Zn. In their paper Isbell et al., (1962) give methods for preparation of the compounds and for spectrographic and chemical analysis of the chosen standards.

Procedures are described for the preparation of stable solutions thereof in petroleum oils. Xylene, together with 2-ethylhexanoic acid, 6-methyl-2, 4-heptanedione, and 2-ethylhexylamine are used as additives to render the various samples soluble. The resulting solutions are all compatible with each other, and as a result blends containing a known amount of several elements can be prepared.

The U.S. National Bureau of Standards (NBS) has undertaken the development of Standard Reference Materials (SRM) for trace metals in gasoline and fuel oil (Von Lehnden et al., 1974); certified values have been established for Fe, Ni, Pb, V, and Zn in the fuel oil (SRM-1634). In addition, information values (non-certified) are given for As, Cr, Hg, and Mn.

Metallo-organic standards, specially prepared organic sulfonates in al oil base, are available commercially (Conostan standards, produced by Continental Oil Company, P.O. Box 1267, Ponca City, Oklahoma 74601). Individual standards at 5.000 ppm metal in hydrocarbon oil solution are available for each of the following elements: Ag, Al, B, Ba, Be, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, La, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Sb, Si, Sn, Ti, V, Y and Z.

The matrix oil for these standards is a paraffinic hydrocarbon oil with an 80 SSU viscosity at 100°F (37.8°C) and a flash point of 340°F (171.1°C). These standards are readily soluble in ketones as well as aromatic and paraffinic hydrocarbons without the aid of solubilizers.

In addition, blended standards are also available. Blends of equal amounts of 12 elements: Ag, Al, Cr, Cu, Fe, Mg, Na, Ni, Pb, Si, Sn, and Ti are available in concentrations from

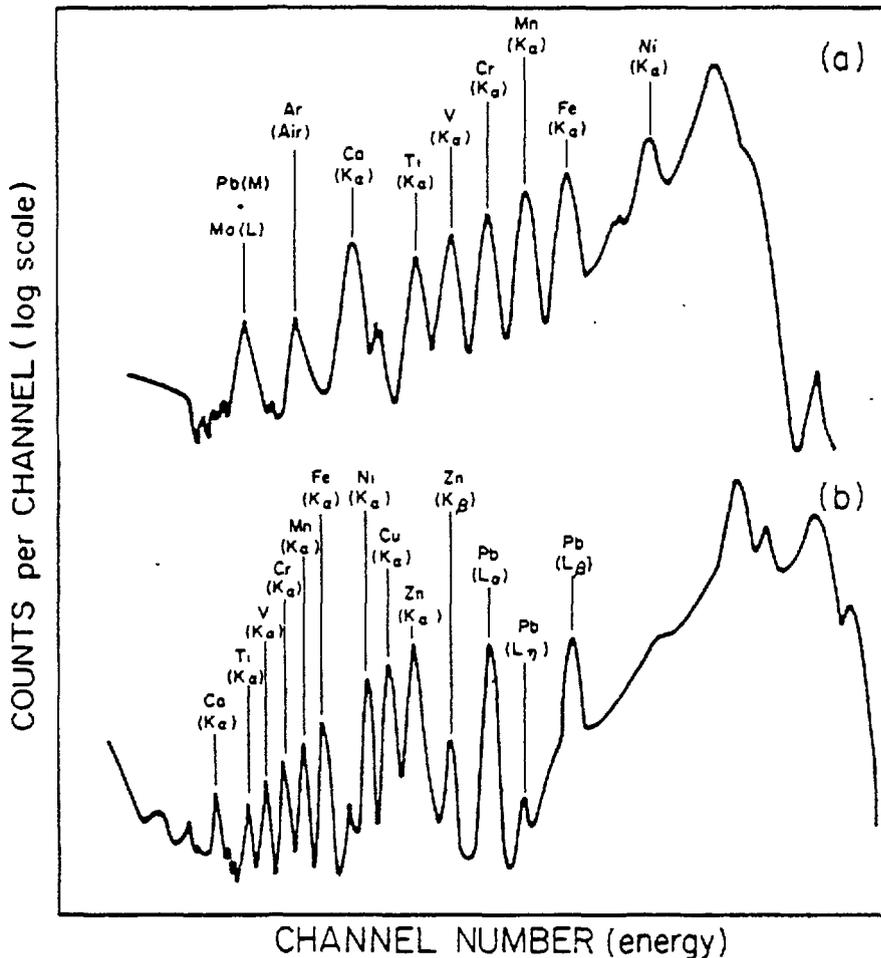


Fig. 5.7 X-ray fluorescence spectra from the C-20 Conostan standard: (a) excited by Cu filtered bremsstrahlung radiation from W anode; (b) excited by Mo-filtered radiation.

1C ppm to 900 ppm. Standards which are blends of equal amounts of 20 elements are also prepared. C-20 are 20-element standards with calcium.

Fig. 5.7 shows the characteristic x-ray spectrum from the 3 MeV proton bombardment of Conostan D-12 target. The target was prepared by simple deposit of a few drops on filter paper.

Fig. 5.8 shows examples of the x-ray spectra of the 200 ppm C-20 multielement Conostan standard obtained by the x-ray fluorescence (Ortec, 1975). The 0 to 10 keV of the spectrum excited with Cu filtered bremsstrahlung radiation from a W anode is shown in Fig. 5.7(a). This spectrum was accumulated in only 200 seconds with anode voltage of 20 KV and anode current of 20 uA. The 0 to 20 keV portion (Fig. 5.7(b)) of the x-ray spectrum excited with Mo-filtered radiation shows clearly defined peaks for Ca, Ti, V, Cr; Mn, Fe, Ni, Cu, Zn, and Pb in a counting interval of only 400 seconds. This logarithmic plot shows a uniformly increasing peak intensity for all of the elements except for Ca which is five

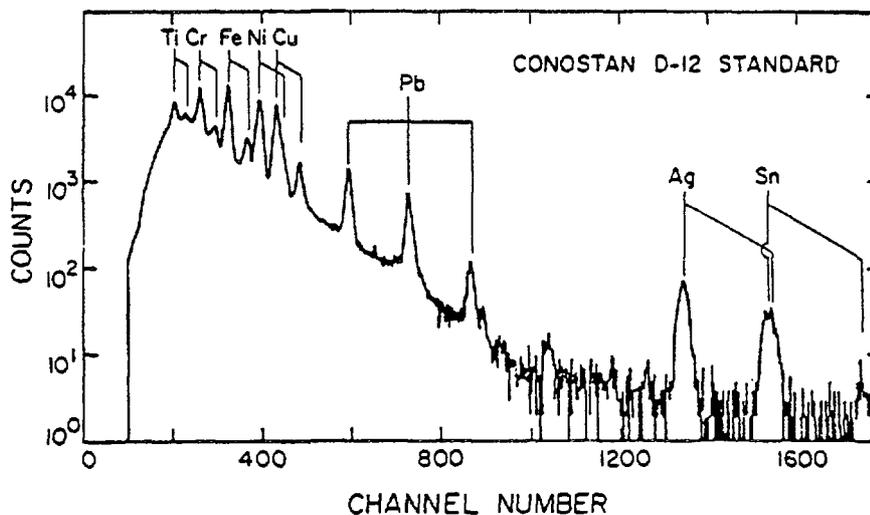


Fig. 5.8 Characteristic X-ray spectrum obtained by the bombardment of Conostan D-12 target by 3 MeV protons. The target was prepared by depositing a few drops on filter paper.

times more concentrated, and Pb which is represented by its L lines. The anode voltage in this case was 35 kV, while the anode current was 50 μ A.

Smith et al., (1975) have analyzed diluted Conostan D-20 standard by x-ray fluorescence and atomic absorption method for Fe, Ni, Cu, and V at the sub-ppm level. Their results indicate that precise analysis at low levels is still not always possible. The results they obtained are shown in Table 5.8.

Several manufactures are producing powdered standards formulated from selective high-purity inorganic compounds. For example, CHEMPLEX industries (140 Marbledale Road, Eastchester, N.Y. 10707, U.S.A.) are marketing 53 different analyses in different matrices (graphite 74 μ m/-200 mesh, zinc oxide, lithium carbonate) with concentrations 0.1, 0.05, 0.01, 0.005 and 0.001 per cent.

Their product called 1-2-3 Powdered SpectroStandard is formulated to provide 1.23 wt.% of each of the 53 listed analytes; its matrix may be considered as the total combination of the oxides of the constituent elements. Powdered SpectroStandard Sets are comprised of a series of standards and a blank of a specific matrix as defined as the diluent. Individual standards of a set contain the same indicated 53 elements diluted to furnish the same concentration value for each analyte. The standards in a set differ from each other by incremental concentration values of the analytes. Every set provides calibration function for the 53 different analytes extending from 0.1 to 0.001 wt.%. Powdered SpectroStandards are furnished in 2 gram quantities contained in glass vials with plastic-lined screw caps to ensure airtight protection against the environment.

Table 5.8
 Analysis of Conostan D-20 standard
 (after Smith et al., 1975.)

Element	X-ray fluorescence		Atomic absorption	
	20 ppm expected	0.5 ppm expected	1.0 ppm expected	0.5 ppm expected
Ni	0.77 ± 0.24	0.45 ± 0.12	0.88	0.33
Cu	1.22 ± 0.45	0.66 ± 0.26	0.94	0.45
Fe	0.61 ± 0.21	0.34 ± 0.10	0.95	0.39
V	0.36 ± 0.14	0.26 ± 0.16	1.00	0.35

The concentration of the analyte of interest in a weighted powdered sample is increased by a known amount by the addition of a weighted quantity of the powdered standard. The resulting mixture consists of the initial unknown concentration of the sample analyte plus the known analyte in the SpectroStandard. Analyte-line intensities are measured from both the original sample and standard mixture and the concentration of the analyte of interest in the original sample is calculated from the following relationship:

$$C_X = \frac{(I_X/I_M) C_{SS}}{1 + (W_X/W_{SS}) (1 - I_X/I_M)}$$

- where, W_X = sample weight
 W_{SS} = standard weight
 W_M = standard mixture weight
 I_X = intensity of sample
 I_M = intensity of standard mixture
 C_{SS} = weight fraction of analyte added
 C_X = weight fraction of analyte in sample

It is presumed that a linear relationship exists between analyte-line intensity and analyte concentration with the calibration curve intercepting the origin. In low concentration ranges (0-5%) the calibration function is usually linear and the application of a correction factor is rarely necessary. For higher concentration levels, linearity may be limited to a narrow range and the amount of analyte added becomes critical. Experimental errors may become too large if an insufficient quantity of analyte is added; with excessive amounts, the approximation of linear correlation may be poor. Depending on required analytical accuracy, successive additions of analyte to the sample or dilutions of the standard mixture with the

sample represent approaches to cover the anticipated concentration range of analytical interest concurrent with considering nonlinearity and the slope of the calibration curve. In this regard, a series of standard mixtures containing known increases in analyte concentration are developed according to the pre-described procedure in preparation of establishing a calibration curve. The curve is extrapolated to zero analyte addition to determine the analyte concentration in the original sample. Alternatively, successive dilutions of the initially prepared standard mixture with the original sample is also used to generate a series of standard mixtures of known analyte concentrations. The concentration of the analyte of interest in the original sample is determined by extrapolation of the calibration curve to zero dilution. A variation in treating the intensity measurement data involves the assignment of relative intensity strength values using an arbitrary scale to the analyte of interest for each prepared standard mixture. The concentration of the analyte in the original sample is represented at the point where no further change in intensity is indicated.

The admixture of the Powdered SpectroStandard to the powdered sample is critical for exacting results inaccuracies associated with the standard mixture preparation procedure are reflected and significantly magnified in applying dilution correction factors. Dilutions greater than a factor of 10 frequently tend to produce inhomogeneous standard mixtures. The magnitude of error becomes obvious when considering that the dilution factor (DF) is a function of the weights of the standard mixture and the original sample:

$$DF = \frac{W_X + W_{SS}}{W_X} = \frac{W_M}{W_X} \quad \text{eq(5.1)}$$

where, W_X = sample weight
 W_{SS} = SpectroStandard weight
 W_M = standard mixture weight

CHEMPLEX industries are also manufacturing briquetted single-element and multi-element powdered standards.

Chemplex SpectroSet-up Standards are single analyte-enriched powdered briquettes used to establish optimum instrumentation conditions, periodically verify equipment performance, and to serve as semi-quantitative reference materials.

Each SpectroSet-Up Standard is formulated to contain 1 wt.% of an analyte by homogeneous admixing of a chemically stable inorganic compound with Chemplex X-Ray Mix Powder. The characteristic energy generated at the 1 wt.% concentration level is ample to produce a distinct spectral line distribution and analyteline intensities for integrations to intensity comparisons to "unknowns".

SpectroSet-Up Standards are formed into briquettes encased in pellet cups for ease of handling and protection. Individual SpectroSet-Up Standards of complete sets consisting

of 20 standards contained in a Chemplex XRS sample storage box are available. The sets are custom assembled to contain only the elements of interest requested by the analyst.

Chemplex Multi-Element SpectroPellet Standards provide the analyst with a readily available, convenient and uncomplicated analyte-line intensity comparison method for assigning reasonable concentration values to as many as 53 different elements in an "unknown" powdered specimen. For qualitative analysis, "unknown" sample spectral distributions may be compared to those generated by the Multi-Element SpectroPellet Standard to confirm the presence or absence of a multitude of elements.

Each Multi-Element SpectroPellet Standard is formulated with chemically stable inorganic compounds and diluted with 74 micron Powdered Graphite to a weight percent concentration of 1 for each of the 53 different contained analytes. Under briquetting pressure, firm durable pellets are formed encased in Pellet Cups for ease of handling and protection.

Chemplex Organo-Elemental SpectroStandards are synthesized from spectrographically pure starting reagents and diluted by weight to concentration with an exceptionally pure water-white base oil. They are readily miscible with each other for preparing special formulations, all petroleum products and a host of non-aqueous matrices for compositional compatibility to unknowns for spectrochemical applications in varied fields of interest.

Chemplex SpectroStandards are manufactured without solubilizers, in small batch quantities for extended shelf life, and packaged in 2 fl. oz. (60 ml) amber glass bottles to minimize potential photochemical reactions. Single-element SpectroStandards are formulated by weight to 5000 ppm. For fuel and motor oil additive studies, a multi-element SpectroStandard containing proportional concentrations of different elements is furnished. SpectroStandard Base Oil is also available for in-lab dilutions to suit specific concentration levels or formulations.

The use of Organo-Metallic Salts in powdered form permits the analyst to select the appropriate organic solvent or diluent oil for preparing standard reference solutions matrix-matched to the unknown. Certification of assayed metal content of each SpectroStandard allows for the accurate calculation and formulation of standard reference solutions in the anticipated analyte concentration range of investigation.

Chemplex Organo-Metallic SpectroStandard Salts are specifically suitable for all spectrochemical analytical applications requiring metal standards soluble in non-aqueous media. They are traceable to NBS values and are particularly selective to the analysis of petroleum products for trace wear-metals, crude oils, synthetic oils, fuel and motor oil additives, lubricating oils, food fats and oils, and greases with applications extending into other non-aqueous spectrochemical areas.

The list of Chemplex Organo-Metallic SpectroStandard Salts is shown in Table 5.9.

Table 5.9

Organo-Metallic Salts used as standards

Cat. No.	Metal	Organo-Metallic Salt	Formula	% Metal*
7047	Ag	Silver Cyclohexanebutyrate	$C_6H_{11}(CH_2)_3COOAg$	38.93
7013	Al	Aluminum Cyclohexanebutyrate	$C_6H_{11}(CH_2)_3COO_2AlOH$	7.05
7005	B	DL-Menthyl Borate	$(CH_3)_2CHCH(CH_2)_2CH(CH_3)CH_2CHO_3B$	2.27
7056	Ba	Barium Cyclohexanebutyrate	$C_6H_{11}(CH_2)_3COO_2Ba$	28.86
7020	Ca	Calcium 2-Ethylhexanoate	$CH_3(CH_2)_3CH(C_2H_5)COO_2Ca$	12.28
7048	Cd	Cadmium Cyclohexanebutyrate	$C_6H_{11}(CH_2)_3COO_2Cd$	24.93
7027	Co	Cobalt Cyclohexanebutyrate	$C_6H_{11}(CH_2)_3COO_2Co$	14.83
7024	Cr	Chromium (III) Benzoylacetone	$C_6H_5COCH:C(CH_3)O_3Cr$	9.71
7029	Cu	Copper Cyclohexanebutyrate	$C_6H_{11}(CH_2)_3COO_2Cu$	15.80
7026	Fe	Ferric Benzoylacetone	$C_6H_5COCH:C(CH_3)O_3Fe$	10.35
7080	Hg	Mercury Cyclohexanebutyrate	$C_6H_{11}(CH_2)_3COO_2Hg$	37.21
7019	K	Potassium Cyclohexanebutyrate	$C_6H_{11}(CH_2)_3COOK$	18.77
7003	Li	Lithium Cyclohexanebutyrate	$C_6H_{11}(CH_2)_3COOLi$	3.94

Cat. No.	Metal	Organo-Metallic Salt	Formula	% Metal [*]
7012	Mg	Magnesium Cyclohexanebutyrate	$C_6H_{11}(CH_2)_3COO_2Mg$	6.70
7025	Mn	Manganous Cyclohexanebutyrate	$C_6H_{11}(CH_2)_3COO_2Mn$	13.96
7011	Na	Sodium Cyclohexanebutyrate	$C_6H_{11}(CH_2)_3COONa$	11.96
7028	Ni	Nickel Cyclohexanebutyrate	$C_6H_{11}(CH_2)_3COO_2Ni$	14.78
7015	P	Triphenyl Phosphate	$(C_6H_5O)_3PO$	9.49
7082	Pb	Lead Cyclohexanebutyrate	$C_6H_{11}(CH_2)_3COO_2Pb$	37.97
7014	Si	Octaphenylcyclotetrasiloxane	$C_{48}H_{40}O_4Si_4$	14.15
7050	Sn	Dibutyltin Bis(2-Ethylhexanoate)	$CH_3(CH_2)_3CH(C_2H_5)COO_2Sn(CH_2)_3CH_3_2$	22.85
7038	Sr	Strontium Cyclohexanebutyrate	$C_6H_{11}(CH_2)_3COO_2Sr$	20.56
7023	V	Vanadium Oxobis- (1-Phenyl-1, 3-Butanedionate)	$C_6H_5COCH:C(CH_3)O_2VO$	13.09
7030	Zn	Zinc Cyclohexanebutyrate	$C_6H_{11}(CH_2)_3COO_2Zn$	16.19

* Theoretical Value based on the anhydrous salt.

5.4 Intercomparisons

In recent years many different intercomparison exercises were organized; sometimes including only few laboratories and one method, sometimes including large number of laboratories covering a spectrum of methods. In this latter group studies organized by IAEA should be mentioned, especially different biological reference materials for trace element studies. Results for these materials indicate that only a rather small group of the essential trace elements (Cu, Fe, Mn, Se and Zn) can be determined satisfactorily by most of the analytical laboratories that reported results. For other important essential trace elements, however, such as Co, Cr, I, Mo and Ni (and also for important toxic elements such as Cd and Pb) the results reported generally cover such a wide range that it is impossible to deduce a meaningful "recommended value" for the element concerned. For other essential trace elements such as F, Si, Sn and V, there are too few data available by which to judge the reliability of presently available analytical techniques. These problems do not usually appear to depend on the analytical technique employed. On the basis of these findings it is difficult to avoid the conclusion that many of the data reported in the literature for trace elements in biological materials are of extremely doubtful validity.

Parr (1980) has recently summarized situations with respect to biological material. This is illustrated in Fig. 5.9 and Fig. 5.10 representing the distribution of values obtained for cobalt in IAEA animal muscle and chromium in the same material. Different symbols refer to different analytical techniques used.

Results of different intercomparison exercises are published in the scientific literature. We shall here mention only some to illustrate the methodology used.

For example, Fukai et al., (1978) have reported results of intercalibration exercise on oyster homogenate. Approximately 2 t, gross weight, of oysters were collected, the soft part (~140 kg) were dissected, freeze-dried, powdered and sieved. The sieved fraction, between 63 and 500 μm , was retained and then mechanically homogenized. Precautions were exercised to minimize contamination of the sample during these operations, although some degree of contamination was inevitable in handling the large quantities of the material. Measurements of several trace elements on random fractions of the sample showed that homogeneity varied according to the elements measured, but the standard deviations of the measurements were normally less than $\pm 10\%$ at sample sizes around 100 mg; for many elements, standard deviations of less than $\pm 5\%$ were not uncommon. Changes of the trace element content during storage were tested for more than 2 years by repeated measurements and found negligible.

Samples were sent to 127 laboratories at world-wide locations. These laboratories are oriented to various aspects of environmental studies, including oceanographic and fisheries institutions, universities, analytical centers, nuclear study centers, etc. The laboratories were requested to carry out determinations for trace elements of their preference by using the normal procedures employed in their work. Altogether, the results of 85 laboratories from 25 countries and of 2 IAEA laboratories were made available by the end of 1977.

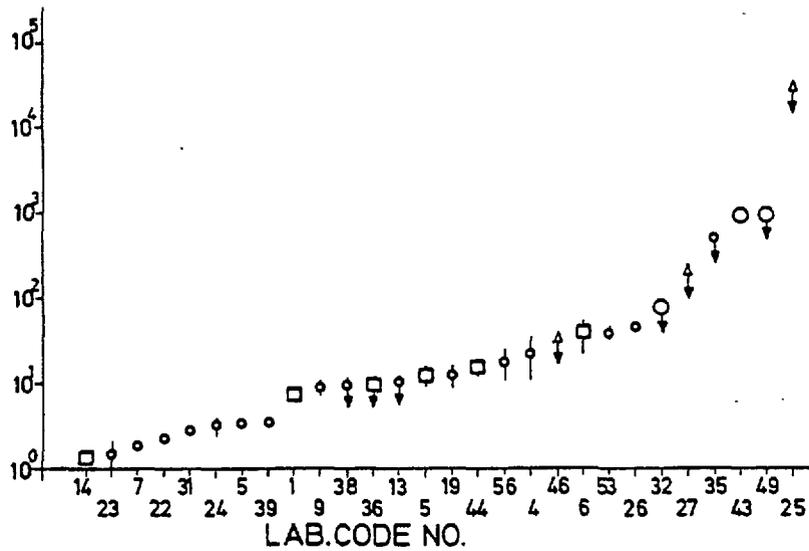


Fig. 5.9 Cobalt in IAEA animal muscle, ug/kg dry weight. Each point represents the mean value reported by one laboratory (points with downward facing arrows represent limits of detection). These results have been arranged from left to right of the figure in order of increasing value. The different symbols represent different analytical techniques (after Parr, 1980).

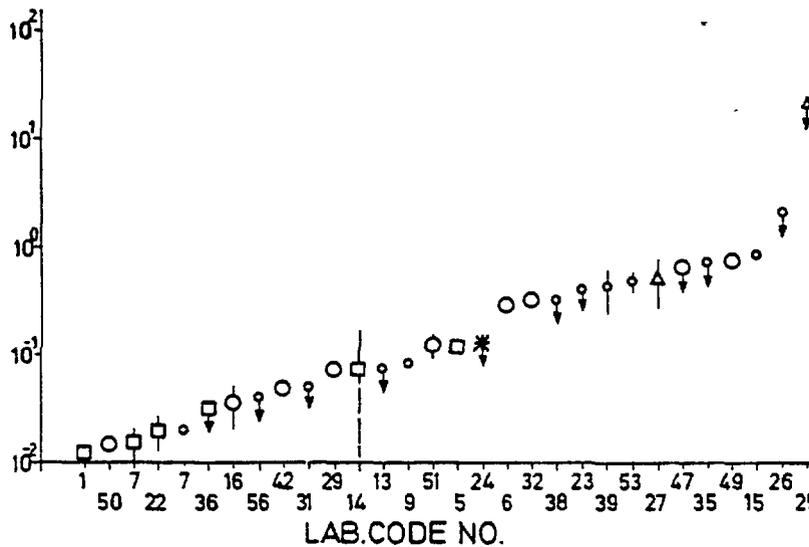


Fig. 5.10 Chromium in IAEA animal muscle, mg/kg dry weight. Each point represents the mean value reported by one laboratory (points with downward facing arrows represent limits of detection). These results have been arranged from left to right of the figure in order of increasing value. The different symbols represent different analytical techniques (after Parr, 1980).

Table 5.10

Ranges, overall averages and averages after applying Chauvenet's or Dixon's test for intercalibration exercise on oyster homogenate (after Fukai et al., 1978)

Elements	Cr	Mn	Fe	Co	Cu	Zn
No. of reported results(*)	30	49	54	26	66	77
Range reported ($\mu\text{g/g-dry}$)	0.304-75	0.094-110	0.21-2 800	0.29-5.7	55.5-480	2.8-5 400
Overall average (**) ($\mu\text{g/g-dry}$)	4 ± 2 (50%)	68 ± 2 (2.9%)	360 ± 50 (14%)	1.0 ± 0.3 (30%)	311 ± 9 (2.9%)	$2\ 700 \pm 100$ (3.7%)
Chauvenet's test	25	42	42	22	60	64
No. of accepted results	1.2 ± 0.1	72 ± 1	302 ± 6	0.46 ± 0.03	322 ± 6	$2\ 830 \pm 30$
Average (**) in acceptable range ($\mu\text{g/g-dry}$)	(8.3%)	(1.4%)	(2.0%)	(6.5%)	(1.9%)	(1.1%)
Dixon's test	28	47	52	26	66	77
No. of accepted results	1.5 ± 0.2	69 ± 2	300 ± 10	1.0 ± 0.3	311 ± 9	$2\ 700 \pm 100$
Average (**) in acceptable range ($\mu\text{g/g-dry}$)	(13%)	(2.9%)	(3.3%)	(30%)	(2.9%)	(3.7%)

Element	As	Se	Ag	Cd	Hg	Pb
No. of reported results (*)	23	21	28	50	44	34
Range reported (µg/g-dry)	0.016-163	0.05-3.9	0.0058-17.66	0.4-21	0.059-1.6	0.2-12.73
Overall average (**) (µg/g-dry)	17 ± 7 (41%)	2.0 ± 0.2 (10%)	6.1 ± 0.6 (9.8%)	2.7 ± 0.4 (15%)	0.27 ± 0.04 (15%)	3.0 ± 0.5 (17%)
Chauvenet's test	19	21	26	48	39	26
No. of accepted results	10.7 ± 0.6	2.0 ± 0.2	5.8 ± 0.4	2.2 ± 0.1	0.19 ± 0.01	1.5 ± 0.2
Average (**) in acceptable range (µg/g-dry)	(5.6%)	(10%)	(6.9%)	(4.5%)	(5.3%)	(13%)
Dixon's test	21	21	27	49	39	32
No. of accepted results	10 ± 1	2.0 ± 0.2	5.6 ± 0.4	2.3 ± 0.1	0.19 ± 0.01	2.3 ± 0.3
Average (**) in acceptable range (µg/g-dry)	(10%)	(10%)	(7.1%)	(4.3%)	(5.3%)	(13%)

(*) Results showing only detection limits of methods used are not included.

(**) Associated uncertainties represent standard errors.

Table 5.11

IAEA 1983 - 1984 PROGRAMME FOR INTERCOMPARISON RUNS

SAMPLE CODE	MATRIX	ELEMENTS OF NUCLIDES TO BE DETERMINED	CONCENTRATION OR ACTIVITY LEVEL	SAMPLE SIZE	SCHEDULED YEAR OF DISTRIBUTION	
					1983	1984 (tentat.)
<u>Nuclear materials and stable isotope standards</u>						
S-17	Uranium ore (phosphate matrix)	U	Low U content	25 g	I(2)	RM
S-18	Uranium ore (phosphate matrix)	U	Medium U con- tent	25 g	I(2)	RM
S-19	Uranium ore (phosphate matrix)	U	High U content	25 g	I(2)	RM
S-20	Uranium ore (phosphate matrix)	Ra/U ratio and U-235 content	Low U natural content	10 g	-	I
SR-60	Uranium dioxide	U content and iso- topic composition	Depl. UO ₂	10 g	I(3)	-
SR-70	Uranium dioxide	U content and iso- topic composition	Depl. UO ₂	10 g	-	I
SR-61	Uranium trioxide	U content and iso- topic composition	Depl. UO ₃	20 g	I(3)	-

SAMPLE CODE	MATRIX	ELEMENTS OR NUCLIDES TO BE DETERMINED	CONCENTRATION OR ACTIVITY LEVEL	SAMPLE SIZE	SCHEDULED YEAR OF DISTRIBUTION	
					1983	1984 (tentat.)
SR-71	Uranium trioxide	U content and isotopic composition	Depl. UO_3	20 g	-	I
SR-52	Uranovanadate	U content	70-80%	50 g	-	I
SR-54	U_3O_8	Impurities	total 2000 ppm	10 g	running I	RM
SR-64	U_3O_8	Impurities	total 2000 ppm	10 g	-	I
GISP	Water	$^{18}O/^{16}O$; $^2H/^1H$	under calibration	30 ml	running I	running I
<u>Environmental materials</u>						
W-3/2	Fresh water (containing also principal constituents of fresh water)	Trace multielement analysis: Pb, Hg, Mn, Cr, As, Cd, U, V, Fe, Ni, Zn, Co, Cu, Mo, Se, Ba, Au	as for natural fresh water	concentr. solution to be diluted to 2 litres with bi-distilled water	-	I(2)
SOIL-6	Soil	Sr-90, Cs-137, Mn-54, Pu-239	natural level	250 g	I(2)	RM
SOIL-7	Soil	Trace multielement analysis	natural content	25 g	I(2)	RM

Table 5.11 (cont.)

SAMPLE CODE	MATRIX	ELEMENTS OR NUCLIDES TO BE DETERMINED	CONCENTRATION OR ACTIVITY LEVEL	SAMPLE SIZE	SCHEDULED YEAR OF DISTRIBUTION	
					1983	1984 (tentat.)
<u>Animal and plant materials</u>						
V-10	Hay (powder)	Trace multielement analysis	natural content	25 g	I(4)	RM
A-14	Milk powder	Sr-90, Cs-137, Na, K, Ca, Sr	environm. level natural content	250 g	running I	RM
<u>Materials for biomedical studies</u>						
H-9	Mixed human diet	Trace multielement analysis	natural content	20 g	I(3)	RM
<u>Materials of marine origin</u>						
SD-N-1/1	Marine sediment	Natural sample for low- level transuranic analysis	Fallout level	100 g	running I	RM
SD-N-1/2	Marine sediment	Trace multielement analysis, U, Th and their decay products	Natural content	25 g	I	RM

SAMPLE CODE	MATRIX	ELEMENTS OR NUCLIDES TO BE DETERMINED	CONCENTRATION OR ACTIVITY LEVEL	SAMPLE SIZE	SCHEDULED YEAR OF DISTRIBUTION	
					1983	1984 (tentat.)
SD-N-2	Marine sediment	Natural sample for low-level trans- uranic analysis	Fallout level (lower activity than SD-N-1)	100 g	I	RM
SW-N-2	Natural sea water	Sr-90, Cs-137, trans- uranics	Elevated fallout level	50 g	Running I	RM
AG-B-1	Seaweed	Neutron-induced radionuclides and transuranic elements	$10^{-1} - 10^2 \text{ Bq.Kg}^{-1}$	50 g	I	RM
MA-M-2	Mussel tissue homogenized	Trace multielement analysis and chlori- nated hydrocarbons	natural content 100 μg	30 g	I	RM

The results of the analyses, reported on 12 selected trace elements, Cr, Mn, Fe, Co, Cu, Zn, As, Se, Ag, Cd, Hg and Pb, were treated statistically to deduce the "consensus values" of these elements in the sample. The results of selected laboratories were treated similarly to estimate "probable concentrations". These "probable concentrations" agree with the "consensus values" for most of the elements. On the basis of the "probable concentrations", ranges for acceptable values were estimated for each element. More than 80% of the results reported were acceptable for Mn and Cu by applying these ranges, more than 70% for Cr, Zn, Se, Cd and Hg, more than 60% for Fe, Co and Ag, while 59% were acceptable for Pb and only 43% for As.

The results are presented also in Table 5.10. Running and future intercomparison runs organized by IAEA are listed in Table 5.11 and marked "I". The number in brackets after this symbol indicates the quarter of the year in which the sample will presumably be distributed. Participation is free of charge, but all participants will be requested to report their analytical results to the IAEA before the announced deadline, usually 3 to 6 months from receipt of the intercomparison sample. About 6 months after the deadline, a report containing the statistical evaluation of the intercomparison data will be issued and sent to the participants.

Participating laboratories will be coded by number in these reports and each participant will be informed only of his own code number. The remainder of the sample still in the possession of each laboratory after the intercomparison has been completed, can be considered as reference material, provided it can be stored for a considerable period of time without appreciable change.

6. LITERATURE ON SAMPLE PREPARATION TECHNIQUES

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