SELECTED APPLICATIONS OF PROTON INDUCED X-RAY EMISSION TO TRACE ELEMENT ANALYSIS

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ABSTRACT

The capabilities of the equipment for proton induced X-ray emission analysis at the University of Surrey were examined with particular reference to the trace element analysis of biological samples. The study of samples arising from the Guildford Breast Screening Project was continued.

The requirements for trace element analysis of biological samples are outlined together with a review of the available physical analysis techniques. Detail is given of the factors to be considered in order to establish that the study of elemental concentrations by PIXE is feasible.

The apparatus used for PIXE analysis is described in detail together with recent improvements to the equipment. The development of a spectrum analysis code HISTO, which simulates the processes of 'manual' analysis, was undertaken. The performance of the code was compared favorably to that of the Ge(Li) spectrum analysis code SAMPO.

A preliminary study of 20 samples of human breast cyst fluid was undertaken. The minimum detection limits for those elements assayed by comparative analysis were calculated. The methodology of sample preparation was improved after the examination of target homogeneity and contamination. During a more detailed study of 30 samples the concentration of 9 elements relative to the concentration of potassium was calculated. Calculation of the minimum recommended sample size for elements with $Z \geq 26$ indicated that a much larger sample size would be required for reliable results.
The PIXE microprobe facility is described and the alignment and operation procedures are outlined. The microprobe was used to examine tree ring samples; examples of some 1D scans are presented.

Recommendation that the spectrum analysis code is developed to include a peak location capability is made. Progress with the breast cyst fluid analysis programme is reviewed. The requirement to improve the proton beam current measurement equipment and the data analysis facilities is noted.
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CHAPTER 1

INTRODUCTION

In this chapter the concepts of trace element analysis are described. The various analytical techniques available are outlined, with particular emphasis on the process of particle induced X-ray emission (PIXE).

1.1 Elemental Analysis

Determination of the elemental concentration of a sample may be achieved by many physical and chemical methods. It may be necessary to determine a wide range of concentrations of several different elements within one sample. The physical methods of analysis utilise the atomic and nuclear properties of the constituent elements to identify their species and concentrations. The range of samples which require or are available for analysis is comprehensive; such fields as, for example, archeology, metallurgy, forensic science, biology, environmental science, geology, solid state materials science and medicine all provide samples whose elemental composition is of interest.

The present work is restricted almost entirely to biomedical and certain environmental samples; such organic samples have a carbon based matrix. In total, 81 of the 92 elements in the classical periodical table have been detected in biological samples. With regard to their presence in animal organisms they are usually classified according either to their concentration or their essentiality. The divisions are therefore those of major elements (concentration >1% of body weight), minor elements (0.005-1%) and trace elements (<0.005%), or, separately, those of essential elements or non-essential elements. For the human, the 11 major and minor...
essential elements (H, O, C, N, P, S, Ca, Mg, Na, K and Cl) (63, 75) all have Z<21. The trace elements known to be essential have changed considerably over the past 30-40 years, but Vohora (75) takes these to be Co, Cr, Cu, F, Fe, I, Mn, Ni, Se, Si, Sn, V and Zn. This information is summarised in Figure 1.1 (after Labelle (41)). Potentially, therefore, widely ranging elemental concentrations may need to be measured. For example, ICRP Standard Reference Man (34) contains approximately $10^5$ ppm of body weight of carbon, 60ppm iron and <0.3ppm selenium. The samples for analysis are commonly available in small quantities and may be in a solid or liquid form. Some may be suitable for almost immediate analysis, whilst others must be appropriately prepared under carefully controlled conditions.

Elements in the body (81)

- Major and minor elements (11)
  - Essential
    - C, Ca, Cl, H, K, Mg, Na, O, P, S
  - Co, Cr, Cu, F, Fe, I, Mn, Ni, Se, Si, Sn, V, Zn
- Trace elements (70)
  - Essential (13)
  - Non Essential (54)
  - Toxic (3)
    - Ag, Al, As, Au, B, Ba, Be, Bi, Br, Ce, Cs, Dy, Er, Eu, Ga, Gd, Ge, Hf, Ho, In, Ir, La, Li, Lu, Mo, Nb, Nd, Os, Pd, Po, Pr, Pt, Ra, Rb, Re, Rh, Rn, Ru, Sb, Sc, Sm, Sr, Ta, Tb, Te, Th, Ti, Tl, Tm, U, W, Y, Yb, Z

Figure 1.1. Elements in the human body.
The technique used to analyse such samples should, therefore, have capabilities corresponding to these sample characteristics. These are, in summary;

- simultaneous determination of all or as many as possible of the elements of interest in the sample, (multielemental technique)
- requirement for small sample sizes
- accommodation of a variety of sample types with a minimum of sample preparation
- determination, where necessary, of ppm concentrations
- precise and reliable results

Of the physical analysis techniques available, proton induced X-ray emission (PIXE) was used in the present work. A general description of the technique is presented below, together with an outline of several complementary and competing techniques.

1.2 PIXE Technique

PIXE is now regarded as a proven technique for elemental analysis. It was first demonstrated by T. B. Johansson et al in 1970 (36) and has been the subject of several international conferences (60, 61, 62). There are several reviews of PIXE and its applications, for example Deconninck et al (1975), Johansson and Johansson (1976), Mitchell and Barfoot (1981) and Khan and Crumpton (1981), (21, 37, 69, 79).
1.2.1 Basic PIXE Technique

The emission of X-rays from a target or sample may be stimulated by bombardment with energetic charged particles. The cross section for X-ray production is relatively high and this, when coupled with the use of a high resolution Si(Li) detector, constitutes a powerful multielemental analysis method of high sensitivity.

The production of a vacancy in an inner electron shell by interaction with a charged particle such as a proton and the subsequent relaxation of the atomic shell by emission of characteristic X-rays is the physical basis of elemental analysis by PIXE, (Figure 1.2). The ionization of the inner shell is considered to arise by the electromagnetic interaction between the bound electron and the incident particle. The registration of the characteristic X-rays is the basis for the analysing process. The complete X-ray spectrum contains the lines of different electron transitions whose energies correspond to the atomic number of the target atoms. The intensity of each transition line is proportional to the concentration of that element in the target.

Typically, experimental samples of 1μm to 1mm thickness are irradiated under vacuum with a 1-3MeV proton beam of 1-500nA from a particle accelerator. The beam diameter at the target is typically 0.1-2.0 cm². A Si(Li) detector is used to examine X-rays in the range 1-25 keV.

The minimum detection limit is typically 0.001-10 ppm for elements with \( Z > 1 \frac{1}{2} \). The sensitivity is limited by the background continuum in the X-ray spectra; the specific sensitivity of PIXE for a given element is dependent on the ratio of the characteristic X-ray yield to the amount of background present in the region of the X-ray line of interest. The
Experimental factors affecting this ratio are the bombarding ion species and energy, the composition of the matrix, the thickness of the target and the atomic number and concentration of the element to be assayed. Enhanced sensitivity may therefore be achieved by suitably adjusting the experimental conditions so as to reduce the relevant background levels. Accuracy is approximately ±5-10%, and is generally assessed by comparison of results with those from other techniques or by examination of standard

Figure 1.2. Schematic diagram showing the production of characteristic K and L X-rays by MeV ion collision.
reference materials. Provided care is taken in the preparation of the samples, and that the various experimental parameters can be controlled and specified, the precision should be within ±3%.

A variety of samples may be accommodated with ease. If the examination is to be carried out in vacuum, wet samples must be dried. Inhomogeneous samples may be sliced or mounted directly onto a suitable target support. Homogeneous samples are usually pressed into a pellet before mounting. Thick samples must be electrically conducting; if the protons are stopped within the target a significant X-ray background contribution may be generated during electrical discharge. Non-conducting samples are coated with a thin layer of high purity carbon or aluminium. Elements with Z<13 are not detected by conventional PIXE; the thin beryllium entrance window of the Si(Li) detector stops the majority of the low energy X-rays from these elements. The trace elements most readily detected by PIXE are largely dependent on the composition of the bulk matrix, or more specifically, by the X-rays generated from elements within it that are detected. For the COHN based matrix of the majority of biomedical samples the best sensitivity is for the elements Ca, Al, Fe, K, Mn, P and Zn.

In summary, PIXE is a sensitive, fast, multielemental technique which requires small samples and relatively little sample preparation. It can be used to detect trace elements in the presence of other elements of high concentration. However, the apparatus is expensive both in terms of capital expenditure and of the necessary technical and scientific support. It is essentially a surface analysis technique as the proton penetration is small (≈ 20μm in a carbon based matrix for 2MeV protons) and peak overlap may hinder the detection of certain elements, particularly when the bulk matrix contains elements with Z>13.
1.2.2 Microbeam PIXE

In addition to the bulk analysis of a target sample with a charged particle beam of several millimetres diameter, it is possible to gain information about the spatial distribution of elements within the target by reducing the beam diameter to micrometre dimensions. The ion beam may then be positioned on the target as desired for single point information, or scanned across the surface of the target to provide line or area distribution information.

Such a system with the beam either focussed or collimated to the required diameter is referred to as a microbeam or micro-probe system. Microbeam PIXE provides, therefore, a multielemental surface analysis technique with high spatial resolution and high sensitivity. There are several other competing techniques, such as Auger spectroscopy and laser microscopy, but none can provide \( \mu m \) resolution with all of the other advantageous features of a PIXE microprobe. Detailed comparisons of such techniques are to be found elsewhere, (46, 76). Cookson (19) compares the capability of PIXE microbeam systems to other microanalysis techniques.

Discussion of microprobe facility development, operation and applications are to be found in reviews by Cookson (1979) and Cahill (1980), (17, 47).

1.3 Complementary Analysis Techniques

PIXE is only one of several analysis techniques resulting from the ion bombardment of a target. Several atomic and nuclear effects may take place, as illustrated in Figure 1.3 for an incident ion beam of MeV energy. Two other techniques, outlined below have been developed which can be used to obtain results complementary to those from PIXE, (22, 27).
Figure 1.3. Schematic diagram of possible reactions initiated by an ion beam of MeV energy.
1.3.1 Nuclear Reaction Analysis

Induction of nuclear reactions in the target nuclei by ion bombardment and the subsequent detection of emitted reaction products, which may be charged particles, neutrons or gamma rays, form the basis of elemental analysis by nuclear reaction analysis, (NRA) (22). Ions with sufficient energy to penetrate the nuclear coulomb barrier may induce nuclear capture or rearrangement reactions; for bombarding 2MeV protons these reactions are restricted to elements with Z<10. Alternatively, the ions may undergo inelastic scattering by the target nuclei. Many of the nuclear reactions exhibit resonances in the reaction cross sections resulting in particularly high reaction yields at specific ion energies. Usually a particular reaction, and therefore a particular element, is selected for analysis. Energy straggling of the bombarding ions in the target results in the variation of yield with depth and so depth profiling can be achieved by interpretation of these effects and by deconvolving the spectra.

NRA can therefore provide useful information which is complimentary to the results from PIXE, since it is primarily sensitive in the very low Z region where PIXE is not effective. Concentrations as low as 10^{-2}ppm can be determined. The technique can, in addition, be used to distinguish between the isotopes of an element. The cross sections for nuclear reactions are however, in general, lower than for PIXE and the technique is not generally considered as multielemental.

1.3.2 Elastic Scattering Spectrometry

The energy of an incident particle elastically scattered by the target nuclei is proportional to the mass of the scattering centre, according to the laws of conservation of momentum. In addition, the bombarding ions
will penetrate the target and suffer ion-electron straggling collisions. The energy spectrum of the scattered ions therefore provides information about the masses of the target nuclei, together with depth information as the ions first straggle through the sample and then undergo elastic scattering collisions. In many circumstances the reaction cross sections can be calculated from the Rutherford scattering formula and concentrations of $10^{-1}$ ppm can be detected. This technique, usually called Rutherford Backscattering Analysis (RBSA) (47) is insensitive to low Z elements in a heavier matrix, so the results are complementary to those from NRA. Crystalline samples may be examined by aligning the sample with the ion beam so that the ions travel along the planes of atoms. This technique (called channelling) results in the enhancement of the signal from atoms in interstitial sites, and it may be used to examine crystalline damage.

A technique related to RBSA but depending on scattering not described by the Rutherford formulae (non-Rutherford scattering) is High Energy Ion Spectrometry (HEIS). In this technique, resonances in the backscattering cross sections are exploited by selecting bombarding ions of an energy which will be preferentially scattered from an element of interest. This technique has recently been shown to be useful in the analysis of low Z elements in a heavier matrix, (33).

1.4 Competing Techniques

There are many analysis techniques available which can be used to determine the elemental composition of a variety of samples. These are all based on some physical property of matter and include neutron activation analysis, mass spectrometry and atomic emission and absorption spectrometry techniques. The techniques each have differing
characteristics and capabilities, as well as widely varying operational costs and availability. A brief summary of the principles of the most commonly used techniques is presented below.

1.4.1 Neutron Activation Analysis

The sample is exposed for a known period of time to a flux of thermal neutrons. A fraction of the constituent stable isotope nuclei will capture neutrons. The newly formed nuclei, which maybe radioactive, are in an excited energy state. Elemental analysis may be effected by detection of the gamma rays emitted as the isotopes decay to the ground state, (prompt analysis). More commonly, however, the induced activity of the radioisotopes is identified and measured by detection of the radioactive decay products. Expressions for calculating the absolute concentration of an element in an irradiated sample are available. The concentration is expressed in terms of the nuclear cross section, the magnitude of the neutron flux, the decay scheme of the radionuclide of interest, the isotopic abundance of the target nucleus as well as the experimental parameters describing the irradiation and counting procedures. Comparative, rather than absolute analysis may be more convenient when not all of the relevant parameters are known, and is the method more generally adopted.

Neutron activation analysis (NAA) (32), is essentially multi-elemental since the decay of all the activated nuclides may be determined simultaneously. It may, however, be necessary to carry out a number of irradiations and countings to determine all of the required elements. The technique may be optimised for the detection of a single element or of a group of elements under consideration. Post activation chemical separation of elements of interest from the sample is one method of
maximizing the sensitivity for those elements. The minimum detection limit under ideal conditions is about $10^{-4}$ ppm. The accuracy is reported to be ±1% provided reasonable care is taken with the experimental procedure, and precision lies between ±1-5%.

The samples may be either solid or liquid. Liquid samples requiring long irradiations may need to be dried to prevent the production of gaseous products during irradiation. The sample may be destroyed during analysis; this depends on the nature of the sample and the irradiation scheme used. Of the elements of interest in biological samples only Be, Tl and Pb cannot be determined. Sensitivity for the elements Na, Cl and Zn is highest; if radioanalytical separation is not performed, results from other elements may be obscured if the first of these elements in particular is present in medium to high concentrations.

NAA is a widely used technique of varied application. It is widely used due to its excellent analytical capabilities; however it is expensive, requiring the need of a research nuclear reactor and skilled operators. Alternatively, lower intensity radioisotopes can be used. Optimization of the experimental parameters and deconvolution of the analytical data require considerable familiarity with the technique.

1.4.2 Atomic Absorption Spectrometry

The sample is converted into an atomic vapour within which the majority of atoms are in their ground state. The atoms will then absorb radiation at wavelengths corresponding to the transitions from ground state to excited atomic states. Measurement of the amount of radiation of a particular wavelength that is absorbed by the sample is used to determine the concentration of corresponding element. The width of the resonance absorption lines is, however, very small ($\approx$10-3nm), and could not be
resolved if a continuous spectral source were used. Consequently, a source emitting the sharp atomic spectrum of the appropriate element is used. The technique is therefore not multielemental, since a separate analysis must be made for each element of interest.

Samples analysed by conventional AAS must be presented in solution. Solid samples are dissolved following wet or dry ashing. Various chemical treatments may have to be applied to the samples to suppress interferences and matrix effects. Obviously, the samples are destroyed during analysis.

AAS is a technique of high sensitivity, that is particularly good for determining traces of metals. The minimum detection limit is typically \( \approx 0.01 \text{ppm} \). The precision is dependent on the method of vaporisation used and lies between \( \pm 1-3\% \). The accuracy achievable is affected by sample contamination and elemental loss during the preparation procedures.

The elements Cl, F and I are not detected by AAS, and the determination of the remaining elements of interest is dependent on the sensitivity required. Adjustment of sample concentration must be made in order that the technique may be used for the analysis of both bulk and trace elements. AAS is however a relatively inexpensive analysis method, both in terms of capital and running costs. Practical provision must be made for the safe use of the cylinder gases required, and a clean laboratory environment is desirable to reduce contamination effects.

1.4.3 Inductively Coupled Plasma Atomic Emission Spectrometry

In this technique the sample is vaporised by introduction into a high temperature argon plasma. The spectra of the radiation emitted by the excited atoms as they return to their ground state is analysed for line
wavelengths and corresponding intensities, these being indicative of, respectively, the species and concentration of the elements in the sample. As with the technique of AAS the samples must be vaporised and so must be in a liquid form for analysis. The minimum detection limit is a function of the elemental species and of the experimental parameters and the technique maybe optimised for certain elements, concentrations and matrices. However, typical values of the minimum detection limit are 0.01-0.001ppm. The technique is multielemental and requires only a single analysis for all elements to be determined. ICPAES has similar precision and accuracy to NAA.

All of the 24 essential elements (Section 1.1) may be determined by this method; however the experimental parameters selected in order to effect multielemental analysis of a particular sample may preclude the detection of one or more of the constituent elements. The technique is particularly sensitive for the elements B, Be, Cd, Cr, Fe, P, Pb, S, Si, Tl and Zn.

Considerable improvement of the basic AES technique was seen on the introduction of the inductively coupled plasma. The technique can be used to complement results from NAA since many of the elements difficult to determine by ICPAES are more readily detected by NAA (e.g. As, Co, Ga, Ge, Hg, Mn, U, W and rare earths), and vice versa. The cost of ICPAES instrumentation is higher than that for AES, although the running costs are similar.

1.4.4 Mass Spectrometry

The mass spectrometer separates gaseous ions according to their mass to charge ratio and so provides output signals which correspond to the abundance of each ionic species. There are a number of different mass
spectrometric techniques, including source spark mass spectrometry (SSMS), isotope dilution mass spectrometry (IDMS), secondary ion mass spectrometry (SIMS) and inductively coupled plasma mass spectrometry (ICPMS).

IDMS and SIMS require expensive instrumentation and complex preparation of biological samples and are therefore rarely used for routine analysis. IDMS is a single element technique of high precision and accuracy (±0.1%) which is used for certification of standards. SIMS is a developing technique which can be used to study the distribution of elements within a sample, and is now routinely used in the analysis of semiconductor samples. The relatively new technique of ICPMS combines the advantages of a plasma source (originally developed for used with ICPAES) with those of mass spectrometry. It can be used to analyse 90% of all elements with detection limits in the range 0.01-1ppm (23). Currently, however, SSMS is the most commonly used technique. Here electrical sparking between the electrodes formed from the material to be analysed causes the vaporization and ionization of a small fraction of the sample. The positive ions are extracted and accelerated before passing through an energy selective electrostatic analyser and into the magnetic momentum analyser. The latter separates the ions according to their momentum to charge ratio. The analysed ions are recorded either photographically or electrostatically, the former being preferred for maximization of the information extracted. The technique is therefore multielemental.

Sample preparation is time consuming and many staged. Usually biological samples cannot be analysed directly because of interferences arising from organic fragment ions. They must be ashed. The samples are first dried and homogenised before being ashed, preferably by a low temperature technique. Graphite, mixed with an internal standard, is then mixed with the sample before it is pressed into cylindrical electrodes. Precision and accuracy
are both approximately ±5-20%. Complete sample homogeneity is the most important consideration in reducing the precision to the lower limit. Absolute detection limits are, however, small (in the range 0.05-0.5ng), which represents better than 1ppm for most elements determined. The elements Ni, Se, and Hg are not detected and Cl, I, and F1 are lost during ashing. The major volatile elements (Na, K, Mg and Ca) are generally only determined with poor precision and a widely varying accuracy.

The SSMS technique is ideal for the simultaneous determination of a wide range of elements at very low concentrations with medium precision. It is versatile and provides information about both molecular structure and isotopes. Practically, sample preparation may be time consuming and several experimental re-runs may be required to verify the results produced. Equipment cost is comparable to that for the more expensive type of AAS and ICPAES apparatus and additional facilities for sample preparation are required.

1.4.5 X-ray Methods

X-ray emission from a sample may be stimulated by other than proton bombardment. Most commonly, energetic electrons or X-rays are used. Such analyses may be conducted in an air, helium or vacuum environment and can accommodate a range of sample types, sizes and shapes.

In XRF (X-ray fluorescence) analysis a cathode ray tube (CRT) or radioisotope provide the primary beam of photons. When used as a multielemental technique the detection limits are very poor (≈100ppm). In this instance a continuous energy X-ray source is used. Typically these are of low intensity and, combined with the relatively low reaction cross sections (compared to those for proton excitation) result in poor
The detection limits for selected single elements may be improved by matching the CRT target to the element of interest. The relatively high intensity beam of characteristic X-rays from such a target will be preferentially absorbed by this element and improved detection limits result. This technique is simple to use, the apparatus being readily available and inexpensive to run. The cost of equipment is similar to that for ICPAES but no facility exists for microanalysis.

The major advantage of electron excitation is the high spatial resolution (<1μm) that can be achieved by using a focussed beam. Electrons of energy 10-100kev are used which have a similar cross section for interaction as MeV protons. However, the Bremsstrahlung contribution to the X-ray spectrum background is very large, and results in a low sensitivity for most trace elements. The poor peak definition also adversely affects the precision and accuracy. Electron excitation is mainly used in conjunction with electron microscope facilities. These are widely available and, as with the XRF analysis equipment, they are compact and relatively inexpensive to operate.

1.5 Summary

PIXE can be seen to compare well with the other techniques commonly available for elemental analysis. Ideally more than one technique should be used to gain cross reference data and to expand the range of elements that may be assayed with maximum sensitivity. The present work was limited to the use of PIXE, the facilities for which were available at the University of Surrey. When appropriate, and experimentally practicable, analysis by NAA was undertaken by colleagues, for comparison with PIXE.
Several factors must be considered in order to establish that the study of sample elemental concentrations by PIXE is feasible. An examination of the sample characteristics must be made and compared with the potential capabilities of the technique. In addition, the type of study to be undertaken must be examined. Such considerations are basic to a successful analysis programme.

2.1 Biomedical Samples

2.1.1 Sample Characterization

Information about the elemental composition and distribution within biomedical samples is of use in the study of the processes of disease, growth and metabolism, together with the effect, quantity and distribution of such environmental factors as pollution. The samples examined during the present work are all of an organic type, obtained either from the environment of from the human body. As a percentage of weight, their bulk, or major, constituents are carbon, oxygen, nitrogen and hydrogen. Other elements which may be present include any of the minor and trace elements deemed essential for life (Ca, Cl, Co, Cr, Cu, F, Fe, I, K, Mg, Mn, Mo, Na, P, S, Se, Si, Sn, V and Zn), together with the inessential and toxic elements such as As, Br, Cd, Hg, Pb, Rb, Sr and Ti, (8). Of primary interest, in respect of these samples, is the simultaneous determination of all (or as many as possible) of the minor and trace elements which start in the atomic table with fluorine, Z=9. The concentrations of these elements can be expected to range from $10^4$ppm (e.g. Ca and K) to $10^{-3}$ppm.
It may be necessary to examine the samples for bulk elemental composition, i.e. concentrations within an homogeneous or homogenised sample, or to examine the distribution and concentration of elements on the surface of a structured sample. Ideally the analysis should be non-destructive to allow for the re-examination by PIXE or by an alternative technique.

2.1.2 Target Characterization

Targets prepared for examination by PIXE are defined according to their "areal density", as being either thick or thin. Thin targets are those for which both the energy loss of the incident particle and the self absorption of X-rays within the sample matrix is negligible. For biological samples this corresponds to an areal density of typically 1mg/cm², or approximately 10μm thickness. Targets with a higher areal density (thick targets) either attenuate or completely stop the incident particles or emitted X-rays within their depth. Thick targets, both homogenised and structured, were used throughout this work. Such targets have certain advantages over thin targets, but were mainly chosen because the design of the existing experimental apparatus severely limited the use of thin targets.

The quantitative analysis of elemental concentrations in thick targets is complicated by two factors. Firstly the need to take into account the variation of the X-ray production cross section (which is energy dependent) as the projectile straggles through the matrix. Secondly, compensation should be made for the variation in the X-ray self absorption since the X-rays are produced at different depths within the target. In addition, thick biomedical targets are invariably electrically non-conducting and so must be coated with a thin layer of carbon and then
earthed. The charge deposited on them by the ion beam may then be conducted away which prevents the local build up of charge and so avoids electrical discharge which can cause a significant addition to the X-ray spectra background. Earthing the target through a current integrator provides a means of measuring the total number of ions falling on the target during the analysis. Consideration must also be made of the possible loss of volatile elements from the target and of damage to the target caused by the heating effect of the deposited energetic ions (18). However, the advantages of using thick targets are considered by some workers to outweigh these disadvantages, (77). Most significantly, these targets require a minimum of preparation which considerably reduces both the risk of contamination and the overall analysis time. The choice of a suitable target backing material is considerably simplified as the ion beam does not interact with it.

Validation of analysis by PIXE requires careful consideration and assessment of the experimental factors that could lead to elemental contamination, loss or change. These will cause inaccuracies in the measured elemental concentrations. Contamination may occur at any stage of sample collection and storage, target preparation and mounting and even during the analysis itself. Attention should therefore be paid to the selection of containers and implements used to handle the samples, the laboratory environment and the cleanliness of the vacuum in the target chamber. Loss of elements may occur as a result of evaporation or leaching from the samples to the storage containers. Evaporation is a particular concern with respect to the volatile halogens, but significant losses of the elements Cr and Hg have also been reported. Time dependent change of elemental distribution may occur as a result of decomposition of incorrectly stored samples.
When examining homogenised samples, one must establish that the sample analysed is representative of the original sample source, as only then is it possible to assume that the measured elemental concentrations reflect those to be found originally. In the first instance, the sample collected may be only part of the total. This may then be divided for analysis, at which time the volume of interaction of the ion beams with the sample may represent as little as 0.05% of the total target volume. Attention must therefore be paid to the sampling technique, the homogeneity of the sample during target preparations and the homogeneity of the final target.

2.2 Specification of the PIXE Technique

There are four fundamental analytical performance parameters which may be considered in order to assess the capability of PIXE or any other analytical technique. These are sensitivity (detection limit), accuracy, precision and sampling factor.

2.2.1 Sensitivity

The sensitivity of a technique is most commonly defined as the minimum detectable concentration (MDC) of an element in a certain matrix. The criterion for reliably observing the characteristic X-ray peak from the ith element is taken to be

\[ s_i \geq f \sqrt{\frac{A}{B}} \]

Where \( s_i \) and \( A_i \) are respectively the number of counts in the peak arising from the ith element and from the background sources at the full width at tenth maximum (FWTM) of the peak. With \( f=3 \) this corresponds to a 99.9% probability of the element being present in the sample.
The sensitivity is therefore limited by the background signal from the sample matrix and consequently there is no general, simple expression which can be used to predict the sensitivity of PIXE in detecting a particular element. However, in the analysis of thick biomedical samples, or any thin samples mounted on a carbonaceous substrate, the matrix can be approximated by carbon, and the variation of sensitivity with various experimental parameters can be examined. Detailed discussion of the variability of sensitivity is made by Johansson and Johansson, (37), and Folkmann, (25). A summary of selected information is presented below.

Sensitivity is dependent in the first instance on the bombarding ion species and energy, but also on the atomic number of the element of interest. In addition, such parameters as detector resolution and solid angle, time of counting, target thickness and total incident charge are also significant. It is generally accepted that optimum sensitivity for the elements of interest in biomedical samples (i.e. 20<Z<40, 75<Z<92) is achieved by the use of 2MeV protons. For a given ion energy and species the sensitivity (or MDC) increases (worsens) in the low Z region (due to the decrease in fluorescence yield and increase in secondary electron Bremsstrahlung), then decreases (improves) through the region of medium Z and finally increases again at higher Z due to the decrease in the X-ray production cross section. However, it is an important feature of PIXE that the sensitivity across the whole range of target Z does not change by more than a factor of 2 to 3, and it is generally taken to be approximately 1x10^-8 or 1ppm for thin targets.
Further optimization of the sensitivity can be made by consideration of the following scaling factor for the minimum detection limit, (37)

\[ \Delta E^{1/2}(\Omega \phi l)^{-1/2} \]

where

- $\Delta E$: energy resolution of the detector
- $\phi$: total collected charge
- $l$: thickness of the target
- $\Omega$: detector solid angle

It should be noted, however, these factors are either effectively constant (i.e. the detector resolution is an intrinsic property) or they are limited by experimental or practical considerations. Nevertheless, Johansson and Johansson (37) report that with care the sensitivity maybe reduced to $10^{-7}$, again for thin targets. A more sophisticated approach to experimental optimization of PIXE is given by Campbell (9) who considers such factors as the X-ray background caused by scattering from the apparatus surfaces.

The sensitivity for thick targets is often not as good. Campbell and Cookson (10), report that a sensitivity of at least 100ppm is achievable for elements with $Z > 20$, but is greater than 100ppm for $Z < 20$. However, 1ppm may be possible for elements in the range $25 < Z < 40$ in a low Z matrix, (i.e. organic samples). Folkmann (25) discusses the effect on sensitivity of the variation in X-ray production cross section and electron and proton Bremsstrahlung with decreasing proton energy as the ions straggle through the matrix. He notes that self absorption of the low energy X-rays limits the sensitivity at low Z. Charging of non conducting thick targets will lead to a worsening of the sensitivity due to the increased electron Bremsstrahlung following localised potential discharge, as mentioned previously.
Sensitivity can also be expressed as the minimum detectable amount, (MDA). This can be calculated from the concentration of an element in the sample, assuming the sample density and the area of the incident ion beam is known. Provided the sample is representative and uniform, reduction in the beam spot diameter will significantly reduce (improve) the MDA for homogeneous samples. For example, assuming an MDC of 1ppm and that 2MeV protons penetrate 20μm into a pressed pellet of organic material with density 300mg/cm³. A uniform beam of area 1mm² corresponds to an MDA of 6x10⁻³mg whereas a beam area of 500μm² corresponds to 3x10⁻²mg.

2.2.2 Accuracy

Accuracy is a measurement of how closely the measured value of a parameter agrees with the true value, and is therefore a measure of systematic errors. Such errors arise, for example, from the errors in standard values and from instrumental errors. It is often very difficult to identify and eliminate such uncertainties. The analysis of well established standard reference materials such as those obtained from the National Bureau of Standards may be useful in estimating the accuracy of the technique. Alternatively, inter laboratory comparison of results obtained from identical samples may be made.

Typically, accuracy for PIXE is taken to be ±10%, when using standard reference materials to 'calibrate' the system. Mitchell and Barfoot (49) report that when calculating elemental concentrations by an absolute approach from X-ray production cross section data and other fundamental parameters, uncertainties of ±20-30% can be expected.
2.2.3 Precision

The precision of a measurement is an estimate of the uncertainty due to random errors and provides information on the reproducibility of the results. In most instances the precision may be improved by taking care in setting up the experiment, in preparing the target samples and by determining as accurately as possible the various experimental parameters that are used to calculate the results. In this context it is necessary to reduce possible sources of contamination of the samples and to ensure that the targets are homogeneous. The precision for PIXE analysis is taken to be between ±5-15%, and may be established experimentally by examining the variability of results from identical targets.

2.2.4 Sampling Factor

The sampling factor, or sampling constant, $F_i$ is an experimentally determined quantity which defines the mass of a sample required to reliably determine the $i$th element. It is defined by Heydorn (32) as

$$F_i = R_i^2 m$$

where $m$ is the analysed target mass. $R_i$ is the standard deviation of the signal arising from the $i$th element (in the target mass) expressed as a percentage. Spyrou and Al-Mugrabi (68) interpret $F_i$ as being the mass of the sample that must be analysed to reduce the error contributed by sample heterogeneity to one percent. The sampling factor for an element in a given target is specific to the analysis technique used. As discussed in Section 5.5.2, $F_i$ may prove to be a limiting factor in the quantitative determination of elemental concentrations.
2.3 Experimental Study

2.3.1 Qualitative Analysis

Identification of elements in the sample is the primary objective of qualitative analysis. Qualitative examination is often used as the first stage of a more detailed analysis or when undertaking 'finger printing' or comparison of samples. The sensitivity of the technique used is of obvious importance but, when undertaking analysis which has not been optimised for the detection of any one element, the detector system resolution will determine if different elements can be distinguished.

PIXE offers the advantages of a simultaneous multielemental technique with relatively high sensitivity. For the range of Z detected, overlapping spectral lines originating from K X-rays of adjacent Z elements or from the L and M X-rays of higher Z elements limits the capability of PIXE for a given type of sample or a given experiment. Nevertheless, PIXE provides a fast and reliable qualitative analysis technique, (33).

2.3.2. Quantitative Analysis

Analysis of samples to determine the concentration of elements present in them is referred to as quantitative. Optimisation of the experimental parameters may be required, depending on the requirements to examine, for example, a wide range of elements or just one or two of particular interest. Most frequently a compromise must be reached in terms of the trade off of one parameter against another and the time and cost of analysis. PIXE analysis can be used for the absolute determination of the concentration of an element since it is possible to relate the concentration to the number of counts in the corresponding X-ray peak as
discussed below. When one or more of the parameters in this relationship cannot be determined with sufficient accuracy, or when it is more convenient, comparative analysis may be undertaken. Here, the concentration of an element in the sample is related to the known concentration of the same element in a standard or reference material. The latter approach is most often adopted when using thick targets as will be seen below.

2.3.2.1 Absolute Analysis

The X-ray yield \( Y \) from the \( i \)th element of a target can be expressed by

\[
Y = \phi tN^B \int_0^R \sigma_{\alpha}^{(x)}(x) \, dx
\]

where

- \( \phi \) protons/unit time incident on the target
- \( t \) duration of the experiment
- \( N^i \) no of atoms of \( i \)th element/unit volume in target
- \( B \) proton beam area
- \( R \) range of protons in target
- \( \sigma_{\alpha}^{(x)}(x) \) \( K_{\alpha} \) X-ray production cross section for \( i \)th element
- \( dx \) fractional depth into target

Refer to Figure 2.1 for a schematic representation. The product \( \phi t \) is the total number of protons incident on the target and \( N^i B \) is the 'density' of the \( i \)th element per unit thickness of the target. It is assumed that the protons are uniformly distributed in the cross section as are the atoms of the \( i \)th element in the target. This equation can be rewritten as an
Figure 2.1  Schematic diagram of a thick sample showing the incident proton beam and emerging X-rays.

(a) Profile    (b) Cross section
integral over the fractional change in proton energy, \( \frac{dE}{dx} \),

using

\[
\frac{dY}{dE} = \frac{dY}{dx} \cdot \frac{dE}{dx}
\]

gives

\[
Y = \phi t N_{1} B \int_{0}^{E_{p}} \sigma_{p} (E) \left( \frac{-dE}{dx} \right)^{-1} dE
\]

Where \((-dE/dx)\) is the matrix stopping power and \(E_{p}\) is the initial proton energy. Then, the \( K_{\alpha} \) X-ray full energy photopeak area \( A_{K_{\alpha}} (E) \) is expressed by

\[
A_{K_{\alpha}} (E) = \phi t N_{1} B \epsilon_{K_{\alpha}} \frac{\Omega}{4\pi} \sum_{i} \mu_{ij} h_{j} \int_{0}^{E_{p}} \sigma_{\alpha} (E) \left( \frac{-dE}{dx} \right)^{-1} e^{-\mu_{j} x} dE
\]

where

- \( \epsilon_{K_{\alpha}} \): detector efficiency for \( K_{\alpha} \) X-ray from \( i \)th element
- \( \Omega \): solid angle subtended at detector
- \( \mu_{ij} \): attenuation coefficient of \( j \)th absorber for \( K_{\alpha} \) X-ray from the \( i \)th element
- \( h_{j} \): thickness of \( j \)th absorber
- \( \mu_{i} \): attenuation coefficient of matrix for \( K_{\alpha} \) X-ray from the \( i \)th element
- \( x \): path length of emerging X-ray

Note that \( \phi(x) \) is a function of \( x \) and therefore of \( E \). The equation can be evaluated numerically and illustrates the capability of PIXE as an absolute method of analysis. \( A_{K_{\alpha}} (E) \), \( \phi \), \( t \), \( B \), \( \Omega \), \( \epsilon_{K_{\alpha}} \), \( E_{p} \), \( \sum_{j} \mu_{ij} h_{j} \) and \( h_{j} \) can be measured at the time of experiment or with the experimental apparatus. The production cross section is related to the ionization
cross section $\sigma_{x_l}(E)$ by the following equation

$$\sigma_{pl}(E) = \sigma_{x_l}(E) w k_1$$

where

- $w$ fluorescence yield
- $k_1$ intensity ratio for ith element

Values of these parameters, $\mu_1$ and $-\text{dE/dx}$ are tabulated or can be calculated using empirical formulae. Khan and Crumpton (39) provide a useful list of references and discuss calculation of stopping power for a matrix containing more than one atomic component.

In practice, use of this formula is difficult and even if possible may result in a large uncertainty in the calculated value of $N_1$. Approximation to the formula (or acceptance of higher errors) must be made if the proton beam energy is not stable and single valued, if the beam is non-uniform in proton distribution or stable in position and area, and if the matrix composition is not a single element even if well defined. Difficulty may be encountered in measuring the parameters for and calculating the value of $\Omega$. The detector efficiency for low energy X-rays is difficult to measure and the source to detector attenuation factors have to be estimated or measured experimentally. In addition, the published values and formulae for $\sigma_{pl}$ all have attendant uncertainty, the magnitude of which is not always clear in the references.

For completeness, it should be noted that in the particular case of a target sufficiently thin not to attenuate the proton beam the expression relating photopeak area to volume density of target species is much simplified,
A_{\alpha_1}(E_p) = \phi t N_{1B} \varepsilon_{1\alpha} \frac{\Omega}{4\pi} T \sigma_{p1}(E_p) 1 \sum_{\mu_j}^j

where T combined X-ray transmission factor = \varepsilon

1 sample thickness

The product N_{1B} is the total number of target atoms 'seen' by the proton beam.

2.3.2.2 Comparative Analysis

Problems associated with the measurement and accuracy of some of the parameters in the formula for calculating absolute elemental concentrations mean that it is common practice to examine a standard reference material (R) at the same time as the unknown sample (S). The following formula expressing the volume density of the ith element may then be used,

\begin{align*}
A_{\alpha_1R}(E) &= \phi t N_{R1R} \int_0^{E_p} \sigma_{p1}(E) \left( \frac{-dE}{dx} \right)^{-1} \mu_{R}^{-1} \mu_{Y}^{-1} \frac{dE}{dx} \\
A_{\alpha_1S}(E) &= \phi t N_{S1S} \int_0^{E_p} \sigma_{p1}(E) \left( \frac{-dE}{dx} \right)^{-1} \mu_{Y}^{-1} \frac{dE}{dx}
\end{align*}

This assumes that the targets are examined sequentially under identical, or exactly scaling, experimental conditions when the quantities B, \varepsilon_{\alpha_1}, \Omega and the transmission factor cancel out. The targets must be homogeneous with a grain size small compared with the range of the protons in the matrix and the beam spot area. This still involves the numerical solution of the two integrals. If the reference and sample are well matched in the composition of their matrix and, less importantly, their trace element
composition, then approximations to the formula can be made

\[
\frac{A_{\alpha_{1R}}(E)}{A_{\alpha_{1S}}(E)} = \frac{\phi N_{1R}}{\phi S N_{1S}} \left(\frac{-dE/dx}{N}\right)_R
\]

where \(\phi\) is the total proton charge collected during the experiment. This formula is a useful approximation for examination of heavy elements in a light element matrix (when the attenuation of the X-rays will be negligible) provided the reference sample matrices are similar.

Systematic errors exist in this type of examination but it is an extremely simple approach, particularly if the absolute elemental concentrations are not of primary importance and relative concentrations can be used instead.

Another reference technique employed, although not during this work, is to introduce a known amount of a reference element to the sample during preparation. This is particularly useful when undertaking trace element analysis when the 'spiking' element can be carefully chosen so as not to produce interfering X-ray peaks and not to significantly change the matrix characteristics.

2.3.2.3 Reference Materials

A number of biological standard reference materials are available as reported by Kosta in an IAEA technical report (1980), (33). Such standards can be crudely classified as either biomedical or plant reference materials and may or may not have a certified elemental composition. The most commonly used certified standards are Bowen's Kale (5), NBS 1571 Orchard Leaves (51) and NBS 1577 Bovine Liver (52), the latter being the only fully certified biomedical standard available at the start of this work. The reference material selected should be chosen with
care and depends on the analysis technique used and the elements to be assayed. For quantitative comparative analysis a certified standard would be the most appropriate choice; the standard should have an elemental composition closely matching that of the samples for examination.

A comparison of the elemental composition of Bowen's Kale and NBS Bovine Liver with that of ICRP Standard Reference Man total body and blood plasma (34) is shown in table 2.1. The bulk (major) and minor elements are expressed as a percentage by weight, the trace elements in units of mg/Kg. Blood plasma is the body fluid most similar to the cyst fluid examined in this work. The histograms in Figures 2.2 and 2.3 illustrate respectively the relative elemental composition of the minor and trace elements that can be assayed by PIXE. Note the logarithmic scales which distort the relative concentrations of some elements in the different materials. Bowen's Kale was chosen as the reference material for the present work as it could be seen from this comparison that more of the minor and trace elements in Bowen's Kale had concentrations comparable with those in blood plasma than did Bovine Liver. Kosta notes an urgent requirement for a blood plasma standard.
<table>
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<th>ELEMENT TYPE</th>
<th>TYPE</th>
<th>BOVINE LIVER</th>
<th>BOWENS KALE</th>
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<th>BLOOD PLASMA</th>
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<td>ERROR</td>
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<td>ERROR</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>O (%) B</td>
<td></td>
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<tr>
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<tr>
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<tr>
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Table 2.1 The concentration of elements in bovine liver (BL), Bowen's Kale (BK) and ICRP Standard Reference Man total body (TB) and blood plasma (BP).
Figure 2.2 A comparison of the concentrations of bulk and minor elements in bovine liver (BL), Bowen's Kale (BK) and ICRP Std Reference Man total body (TB) and blood plasma (BP).
Figure 2.3 comparison of the concentrations of trace elements in bovine liver (BL), Bowen's Kale (BK) and ICRP Standard Reference Man total body (TB) and blood plasma (BP).
CHAPTER 3
EXPERIMENTAL ARRANGEMENT

This Chapter describes the experimental arrangement of the PIXE facility. The beam production and transport, target chamber, processing electronics and data storage are discussed. The microprobe forming elements used to focus the proton beam are described briefly but are discussed further in Chapter 6.

3.1 Apparatus Description

The beam, which may comprise H\(^+\), He\(^+\) or O\(^+\) ions was extracted from an RF source in the top terminal of a 2 MeV Van der Graaff accelerator. Accelerating voltages in the range 800 kV to 2 MV could be selected, and the beam was directed into the experimental beam line by a switching magnet. A vacuum of about 10\(^{-8}\) Torr was maintained throughout the system by the use of oil diffusion pumps, both water and liquid nitrogen cooled.

The principle components of the beam line are shown in Figure 3.1. Downstream from the switching magnet and the gate valve were the magnet exit slits. The ion voltage was stabilized in a feedback loop by the differential signal from the beam falling on the two control slits. The control slits were followed by a pair of electrostatic steering plates powered from two 2kV voltage supplies with variable voltage settings. These were used to achieve coarse system alignment by directing the beam onto the centre of the sample mounted in the target chamber. This feature was most important during the operation of the microprobe it was necessary to direct the beam along the axis of the quadrupole lens system.
Figure 3.1. Schematic diagram of the PIXE facilities at the University of Surrey.
Closely following the steering plates was the selectable aperture which forms the object in the ion optic system. The selected aperture could be moved into the beam path using micrometers with a positional accuracy of ±100μm in the X and Y directions. Niobium TEM apertures of 25μm, 50μm and 250μm diameter were available, plus a machined aperture of 1mm diameter, Figure 3.2. Next to the aperture was a viewing port, where the beam profile (beam spot) could be observed by the fluorescence it induced in a slip of glass or quartz. The glass slip was attached to a rod that could be pushed through a Wilson vacuum seal into the beam path. The divergence of the beam could be limited by a secondary aperture with machined holes of 1mm, 2mm or 5mm diameter which followed the object aperture plate in the beam line.

Downstream of the secondary aperture were the beam scanning plates. These comprised four pairs of plates which were arranged in two sets, to produce double deflection in both X and Y directions. The beam could be deflected by up to ±600μm at the target face. This configuration was of particular importance when scanning the microbeam and will be discussed further in Chapter 6.

Following the second set of deflection plates were the four magnetic lenses which were used to focus the beam at the target face as required. The magnetic lenses (quadrupoles) were mounted on a base which allowed positional adjustment of the lenses, (16). Movement in both the horizontal and vertical planes (by up to 50mm in each case), was effected by turning suitably placed screw threads at each corner of the rectangular base on which the quadrupoles were mounted; turning any one of these screws therefore affected the position of each of the four lenses. Each lens could be individually rotated on the base. The lens currents were supplied from four stabilised power supplies connected through a control
Figure 3.2. Schematic diagram of the ion beam object apertures.
unit and a switching unit. A remote control box was used to set the currents flowing through the electromagnets. The quadrupole coils were not actively cooled which limited the maximum current in each coil set to 0.6A. The switching unit allowed the lenses to be powered individually or with units 1 and 4 in series and 2 and 3 in series, thus operating them in the Russian Quadruplet configuration, (15).

The target chamber, described below followed the quadrupole lenses and could be isolated from the beam line by a manually operated gate valve.

3.2 Target Preparation

In the present work, the targets for analysis were mounted on to a plain, rectangular aluminium plate which fitted into the target chamber. Double sided sticky tape was selected to mount the thick targets on to the plate. The tape has considerable advantages; beside being easily available, cheap and quick to use, it was found to fluoresce when struck by the ion beam. This proved to be an extremely useful property when visually positioning the target under the beam spot. It would be inappropriate to use this tape to mount thinner samples which would be penetrated by the beam, as it contains a considerable number of the low Z elements (chlorine in particular) and would introduce contaminating signals to the X-ray spectra. The possibility of the elements in the tape leaching into the mounted samples was considered but this was not explicitly investigated and verified. Samples left in contact with the tape for longer than a few days (stored at 4°C) were discarded and not re-examined. Homogeneous samples were reduced to a powder and pressed into pellets a few milimetres thick which could then be stuck to the target plate. Structured samples were cut to a suitable size and dried if necessary before being mounted. The whole target plate was then coated with a thin layer of high purity...
carbon which was evaporated on to the surface under vacuum.

3.3 Apparatus Operation

Operation of the apparatus for PIXE analysis when the microbeam facility was not used was fairly straightforward. The experimenter had no direct control of the accelerator but selected the type of ion, the ion energy and the experimental beamline to be used by arrangement with the laboratory staff. The target chamber (isolated from the beam line) was opened and the Si(Li) detector was wound into the chamber on its screw threaded base. The detector filter (if used) was fitted.

The target plate for analysis was loaded into the target chamber which was then closed and evacuated. The target plate was positioned so that a glass slip attached to one end of the plate was approximately in-line with the expected beam path. The chamber isolation and gate valves were opened and the object aperture was wound out of the beam path. The beam position in the line was determined by observation of the fluorescence on the glass slips in the line and the target chamber. The voltages supplied to the first set of deflection plates were then adjusted to bring the beam to the required position in the target chamber. Care was taken to limit the beam current so that unduly high X-ray counts were not incident on the Si(Li) detector. The required aperture was then wound into the beam path and the steering voltages were again adjusted as necessary. Occasionally, the quadrupole lenses were used to define or change the shape of the beam spot at the target plate. These various adjustments may have required resetting several times a day to take into account both the short term drift in the accelerator performance and the fact the the accelerator would frequently go 'off line' following operation problems.
Once the beam position was correct, the beam current was set to the desired value by the accelerator operator and the target plate was re-positioned so that the sample analysis could commence.

3.4 Target Chamber

The rectangular target chamber was pumped by a 4 inch (102mm) oil diffusion pump. The chamber was machined from dural with internal dimensions of 0.33m x 0.24m x 0.17m. The front plate, also made of dural, had a conical section with six entrance ports arranged symmetrically round the front beam entrance. These ports accept the detectors and the front section of the optical microscope. The target chamber was illuminated by a light shining through a glass plate over one of the the lower ports. The backplate was completely removed to allow access to the internal features of the chamber.

An internal view of the target chamber is shown in Figure 3.3. The photograph was taken before the installation of the optical viewing system described below. The quadrupole lenses can be seen behind the target chamber. The facilities available in the target chamber are described below.

3.4.1 Target Manipulation

The target manipulator was attached by a hinge to a rectangular frame inside the target chamber. The frame was moved forwards to position the samples in the focal plane of the quadruplet and moved backward to allow the manipulator to be pivoted through 90° and the sample plates removed or loaded. The target manipulator had a linear drive and a two axis goniometer. These allowed the interchangeable sample plates to be
Figure 3.3 Target chamber internal view.
translated and rotated in both \( \theta \) and \( \phi \). Several samples could be mounted on each plate; the translational movement allowed each sample to be presented to the beam in turn. The \( \theta \) and \( \phi \) rotations were used primarily during channeling experiments with crystalline samples, often when using the microbeam. The driving stepper and dc motors were attached to the manipulator (and so were inside the vacuum); their controls were outside.

The total charge falling on the target was measured by earthing the targets through a current integrator. The carbon coated sample surface was electrically connected to the metallic target plate by silver paint and the target plate was partially retained in position by a copper clip which was in turn connected to a junction point in the chamber wall. Secondary electron emission from the target was suppressed by a negative voltage (\(-100\) V dc) applied to a loop of wire, positioned approximately 3mm above the target face, through the centre of which the ion beam passed.

3.4.2 Si(Li) Detector

The Si(Li) detector (PGT, model VZ-15) was mounted such that its axis was at approximately 35° to the beam axis. Externally, the liquid nitrogen dewar and the preamplifier were mounted on a screw thread which allowed the detector crystal to be positioned at a variable distance from the target face. The detector crystal had a 6mm diameter face, with a beryllium entrance window. The energy resolution was approximately 180eV at 59keV. The beryllium window was 0.7\( \mu \)m thick which allows 95% transmission of 7keV X-rays and 99% transmission of 15keV X-rays, (59). A collimator cap was designed to fit over the end of the detector snout. This had a window of the same diameter as the detector window, and X-ray filters of the desired composition and thickness could be mounted onto it.
3.4.3 Optical Viewing System

The viewing system was fitted in 1982 and was designed so that the front face of the target could be seen from outside while the target chamber was under vacuum. A wide range of magnifications were needed. For examining the target face directly and positioning an unfocussed beam, magnifications in the range X1 - X10 were required. Observation of the shape and size of the beam when focussed to micron diameters required magnifications of X100 and above. The conflict of these two primary requirements could only be resolved by the use of a high power objective lens mounted in the target chamber (for the high magnification use) which could be withdrawn from the viewing systems when lower magnification was required. The acquisition of a suitable objective lens was complicated by the need for a working distance of at least 10mm, which would allow it to be positioned without obscuring the ion beam from the target. The system installed is outlined below. A schematic diagram of the optical system showing the principle components is given in Figure 3.4.

The retractable objective lens (x25, 12.5mm working distance) was mounted inside the chamber on a pivot. When in place it coupled to the end of a light tight tube extending through one of the side ports in the target chamber face plate. Externally, (Figure 3.5), the light was deflected through 90° by a prism which was seated on an O-ring to provide the necessary vacuum seal. The light then passed along a channel into which lenses of various magnifications could be slotted, before passing into either the photocathode of an image intensifier and TV camera or being further deflected into an eyepiece lens where it could be viewed directly with the eye. Remote viewing of the camera image on a portable television would have made the operation of, particularly the microbeam, much easier. Practically, however, the image of the beam fluorescence on quartz or
Figure 3.4 Schematic diagram showing the principle components of the optical viewing system. (Not to scale).
glass (being one of a relatively dim spot in a dark field) did not have sufficient definition to be seen at a distance. In addition, meticulous care had to be taken not to damage the image intensifier by exposing it to ambient light conditions. These factors severely limited the use and application of the remote viewing facility. The available magnifications and fields of view are listed in Table 3.1.

3.4.4 Other Detectors

A connector inside the chamber allowed the mounting of a silicon surface barrier detector for Rutherford Backscattering experiments. In addition, a NaI(Tl) or Ge(Li) detector could be positioned outside the chamber, to the rear. Such gamma rays are the result of nuclear resonance reactions in the nuclei of the target atoms. These reactions were used during proton induced gamma ray experiments or during the energy calibration of the accelerator, (47).

<table>
<thead>
<tr>
<th>Lens Configuration</th>
<th>Secondary Lens</th>
<th>Magnification</th>
<th>Field of view</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objective Lens</td>
<td>Secondary Lens</td>
<td>Distance (mm)</td>
<td>Distance (mm)</td>
</tr>
<tr>
<td>out</td>
<td>in</td>
<td>from prism</td>
<td>from ph' cathode</td>
</tr>
<tr>
<td>out</td>
<td>125</td>
<td>87</td>
<td>280</td>
</tr>
<tr>
<td>in</td>
<td>60</td>
<td>190</td>
<td>97</td>
</tr>
<tr>
<td>in</td>
<td>60</td>
<td>130</td>
<td>157</td>
</tr>
<tr>
<td>in</td>
<td>32</td>
<td>70</td>
<td>217</td>
</tr>
</tbody>
</table>

Table 3.1 Magnification and field of view as a function of lens selection for the beam spot optical viewing system.
Figure 3.5 Beam spot optical viewing system.
A schematic representation of the data acquisition electronics is given in Figure 3.6. The detector output (suitably conditioned) and the current integrator output were input to the PDP-11 computer. The computer was used to control the microbeam scanning voltages.

The charge falling on to the target was earthed through the current integrator. The integrator converted the current signal to a frequency signal. On each of 5 range settings the generated frequency varied from 0Hz (0 A) to 110Hz (f.s.d) and was a linear function of the instantaneous current. The resulting pulse train from the integrator was input to the PDP-11 computer where the total number of pulses were counted and interpreted as a charge.

The signals from the Si(Li) detector were processed by a pre-amplifier and amplifier before being both displayed on an oscilloscope and fed into the ADC. An output from the ADC to a rate-meter provided a visual display of the counting rate. The digital signal passed through a handshake logic controller and into the PDP-11 computer.

The data collection programme on the PDP-11 ran with fully interactive command and simulated a hardware multichannel analyser. The detector output was 'sorted' into 512 channels and the spectrum was displayed on a VDU during the collection period at a terminal close to the apparatus. The programme was controlled by various user defined parameters, and in addition to displaying the X-ray spectrum it also calculated and displayed such information as the experimental run (live) time, total charge collected and count rate. Such information was logged with the spectrum data when it stored on to floppy disc. A second terminal was used for independent data processing and spectrum plotting.
Figure 3.6 Block diagram of processing electronics.
The data processing facilities available on the PDP-11 were designed for the analysis of RBS spectra. Analysis of the PIXE spectra was undertaken on the main frame computer by transferring the data from the mini-computer via a serial link. Once on the main frame, the data had to be reformatted for use with the two data analysis codes used to calculate the photopeak signal and background areas.
CHAPTER 4
SPECTRUM PRODUCTION AND ANALYSIS

The physical principles of proton/target interaction, X-ray production and X-ray detection are discussed below. Parameters to be considered when optimising the experimental conditions are outlined with particular attention to the sources of X-ray spectrum background. Examples of spectra are given and features are illustrated. The approach to spectrum analysis is outlined and the two computer analysis programmes used are discussed.

4.1 X-ray Production

In this context, the physical processes resulting in the collection of an X-ray spectrum are proton interaction and X-ray production in the target. An outline of the established theoretical description of these processes and their agreement with experimental results is presented here for completeness; more details are to be found in the references cited.

4.1.1 Proton Interaction

The mode of interaction of energetic protons with the target under discussion here is that which results in inner shell ionization. As described below, the filling of such vacancies gives rise to X-rays characteristic of the target atom species. Several theoretical models have been developed in an attempt to describe the available experimental data. These include the binary encounter approximation (26), the semi-classical approximation (29) and the plane wave Born approximation (45, 48). The latter approach has been found to be most versatile,
allowing the incorporation of corrections for relativistic electron motion, Coulomb deflection of the projectile and so forth. The most recent theory is followed by Brandt and Lapicki, (6). Paul (57) has compared experimentally determined values of \( \sigma_{\text{ion}} \) with the latter theory.

In accordance with first order perturbation theory, the ionization cross section \( \sigma_{\text{ion}} \) is expressed as the absolute value of the square of the matrix element of the Coulomb potential between the incident proton and the atomic electron, (the Hamiltonian function),

\[
\sigma_{\text{ion}} \propto |H_{sk}|^2
\]

\[
= \int \psi_f^* (R,r) \frac{Z_p e^2}{(R-R')} \psi_i (R,r) \, dr \, dR
\]

Where \( \psi_i \) and \( \psi_f \) are the initial and final wave functions, \( Z_p e \) is the projectile charge and \( R \) and \( r \) are illustrated in Figure 4.1.

---

**Figure 4.1** Schematic diagram of proton collision.
Note however, that the only collisions considered are those where the velocity of the proton is less than, or comparable with, that of the orbital electron. This is equivalent to saying that the binding energy of the electron, \( I \), is not much smaller than the maximum energy that can be transferred during the collision, i.e

\[
\frac{4mE_p}{M} \approx I
\]

Where \( M \) and \( m \) are the masses of the proton and electron respectively and \( E_p \) is the proton energy. The initial wave function may be written as

\[
\psi_i(R) = \psi_n(r) \cdot \phi(R)
\]

where, according to the first Born approximation, the unperturbed atomic wave function is assumed for the electronic wave function \( \psi_n(r) \). The condition for validity of the Born approximation is that

\[
\frac{Z_p e^2}{\hbar \nu_p} \ll 1
\]

where \( Z_p \) is the target atomic number and \( \nu_p \) is the proton velocity. This condition, when combined with the qualifying statement above, means that this treatment excludes both slow collisions and collisions with the lightest atoms. An expression for \( \sigma_{\text{ion}} \) follows.

\[
\sigma_{\text{ion}} \propto \frac{2^{20} \pi a_o^2}{45} \left( \frac{mE_p}{MZ_T^3 R_y} \right)^4
\]

where \( a_o \) Bohr radius
\( R_y \) Rydberg constant

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- 55 -
This shows the dependence of $\sigma_{\text{ion}}$ as $E_p^4$ and $Z_T^{-12}$. Such a model shows agreement to within 10% to 30% of the experimental data within the limits of $Z_T$ and $E_p$ as defined. Figure 4.2 illustrates the form of $\sigma_{\text{ion}}$, plotting $I^2\sigma_{\text{ion}}$ against $E_pM/\mu l$. The ionization cross section rises smoothly with $E_p$ until a maximum is reached near $E_p = Ml/m$. Beyond this $\sigma_{\text{ion}}$ falls off as $E_p^{-1}$.

Figure 4.2 K and L X-ray production cross sections as a function of target atomic number for 2MeV protons.
4.1.2 X-ray Production

The above section described the process of atomic ionization by charged particle excitation. The excited atoms are returned to the ground state by the transference of electrons from a higher shell; characteristic X-rays may be emitted from the atom as a result. The energy of the X-ray is a function of the particular transition and the atomic species of the excited atom. The competing process is radiationless transition (Auger effect). The probability that an excited atom will decay by K shell X-ray emission is expressed by the K shell fluorescence yield, $\omega_k$. This relates the X-ray production cross section $\sigma_p$ to $\sigma_{\text{ion}}$,

$$\sigma_p = \omega_k \sigma_{\text{ion}}$$

The theoretical models for $\sigma_{\text{ion}}$ produce insufficiently accurate data for many analytical purposes. An empirical approach to formulating $\sigma_p$ has therefore been adopted. A formula from Johansson and Johansson (37) has been widely used but is reported by Paul (58) as being insufficiently accurate for collisions with heavy target molecules. Paul's approach has been to normalise the available data for thin targets, average and reject data where indicated and then fit an analytical function. This fit is combined with an analytical function for the theory. The resulting analytical formula has an estimated minimum error of $< 5\%$ for $21 < Z_T < 30$ which rises to a maximum of $10\%$ for $31 < Z_T < 90$ and $21\%$ for $11 < Z_T < 20$.

4.1.3 Background Sources

The above sections describe the production in the target of X-rays whose energy is a function of the target atomic species. Detection of these events (as described in Section 4.2) results in the production of full
energy photopeaks which are characteristic of the atomic species. These peaks are superimposed on a continuous X-ray background. The processes giving rise to the background are described briefly below.

4.1.3.1 Bremsstrahlung

This is the major contribution at low and medium X-ray energies and has two sources. Proton Bremsstrahlung results from the acceleration of the incident protons in the field of the target nuclei. For the interaction of a single proton \( (E_p, Z_p, A_p) \) with a target nucleus \( (Z_T, A_T) \) leading to the emission of a single photon of energy \( E_x \), the cross section is given by

\[
\frac{d\sigma_B}{dE_x} = \frac{C Z_p^2 Z_T^2}{E_p E_x} \left( \frac{Z_p}{A_p} - \frac{Z_T}{A_T} \right)^2
\]

This shows the yield falling away as \( E_x^{-1} \). Proton Bremsstrahlung is the major high X-ray energy background source. The overall contribution decreases with increasing \( E_p \) and is the only background source to do so, contrary to the characteristic X-ray yield behaviour. The photon yield is near isotropic.

The acceleration of secondary electrons provides the second source of Bremsstrahlung radiation. This is a low energy contribution falling off rapidly above \( E_x = 4mE_p/M \) \((\approx 4.4 \text{ keV for } 2\text{MeV protons})\) which is the classical expression for the maximum energy transferred in a collision between incident proton and free electron. The cross section for electron Bremsstrahlung is found to decrease with \( E_p \) until the above limit is reached, when it falls off as \( \approx E_p^{-10} \). The requirement for minimising \( E_p \) to reduce electron Bremsstrahlung therefore clashes with the conditions for reducing proton Bremsstrahlung and maximising the characteristic X-ray
yield. In general, the proton energy will have to be optimised for
detection of a particular element or a compromise reached for
multielemental analysis, (9).

4.1.3.2 Compton Scattering

Protons of sufficiently high energy excite the emission of γ-rays from the
\( {\bar{K}} \)-rays of the target. These, if entering the \( {\bar{K}} \)-detector will deposit some of their energy
and cause a high energy tail to the spectrum. This effect is small
provided the proton energy is below the threshold for inelastic scattering
in the target matrix, (e.g. \( E_p = 4.43 \) MeV for a carbon matrix).

4.1.3.3 Target Charging

A poorly conducting target can develop large surface electrostatic charges
during bombardment. This can not only cause deflection of the proton beam
but also leads to discharges causing acceleration of electrons in the
target and so adding to the Bremsstrahlung background. Incomplete charge
\( e^- \) collection may make data normalization difficult. This problem can usually
be overcome by coating the samples with a thin layer of carbon or by
mixing a high purity electrical conductor with the target material.

4.1.3.4 Detector Background

Incomplete charge collection in the detector reduces the number of full
energy photopeak events counted. The resulting low energy tail to the
peaks becomes most significant when the peak of a low concentration
element lies in the tail of a higher \( Z_T \), higher concentration element.
4.1.3.5 Electronics

In order to achieve the best energy resolution the time constants of detector associated electronics are long, (10μs). This can lead to pulse pile up, particularly at high count rates, introducing high energy tail to the photopeak. This is reduced by using electronic pile up rejection. Note however that this affects the overall efficiency of the counting system and the efficiency becomes dependent on the count rate, (4).

4.2 Detector and Characteristics

The high resolution Si(Li) detector with high efficiency for detection of photons in the energy range 4-15keV is an essential feature of the PIXE apparatus. A summary of its structure and operation principle is given below followed by a discussion of experimentally measured characteristics.

4.2.1 Detector Structure and Operation Principle

A schematic cross-section of a Si(Li) detector is given in Figure 4.3. The detector consists of a deep layer of intrinsic material created by drifting lithium ions into high purity p-type silicon. Electrical contact to the surface layers allows the application of a reverse bias.

The X-rays are incident on the thin entrance window and pass into the semiconductor crystal. Here, interaction with valence band electrons in the intrinsic region excites them into the conduction band. The resulting electron-hole pairs (charge) are swept out by the DC voltage and pass into the pre-amplifier. An average of 3.8eV is required to produce one electron-hole pair at the temperature of liquid nitrogen (77K) which is
Figure 4.3 Schematic cross section of a Si(Li) detector. Not to scale.
The operational temperature of the detector. The number of electron-hole
pairs created is proportional to the X-ray energy \( E_x \) so the amplitude of
the charge pulse is also proportional to \( E_x \). An optical feedback FET
amplifier is positioned close to the crystal to reduce noise generation.
The use of pulsed optical feedback minimises the feedback resistance and
capacitance and so maintains the high count rate capability with a minimum
noise contribution. More information on the principle and operation of
such detectors can be found in references 40 and 78.

The manufacturer of the PGT Si(Li) detector used in these experiments
specified the detector window at 5mm diameter 0.007mm beryllium. The
crystal dimensions were given as 6mm diameter and 3mm depth. The depth of
the surface dead layer and window to crystal distance were not given.
Experimental measurement of the latter distance was undertaken by
application of the principle that the intensity of radiation reaching the
detector is inversely proportional to the source-detector distance. An
Fe-55 radioisotope source was mounted coaxially with the detector on a jig
that allowed it to be positioned at a distance ranging from 11 to 130mm
from the detector face. The number of detected photons \( P_d \) as a function
of source detector distance, \( d \), will obey the relationship

\[
P_d = \frac{A \exp(-\mu_a d)}{4\pi (d_0 + d)^2}
\]

where

- \( A \) proportionality constant
- \( \mu_a \) attenuation coefficient of air
- \( d_0 \) window-crystal distance

A plot of \( \sqrt{\exp(-\mu_a d)/4\pi P_d} \) vs \( d \) gave a straight line with intercept
equal to \( d_0 \). A least squares fit to the data gave \( d_0 = 0.71 \pm 0.15 \text{mm} \).
In addition to measuring the window-crystal distance, experiments were conducted to ascertain whether or not the detector crystal lay on the axis of the detector 'snout'. To this end the Fe-55 source was mounted approximately 40mm away from the detector face behind two brass collimators to produce a finely collimated beam of X-rays. The source was then traversed on a fine screw threaded stage (1 screw turn ≈ 0.42mm) across the face of the detector. The count rate as a function of distance from the axis of the detector snout is shown in Figure 4.4. The definition of the curve is affected by the low activity of the source which, after collimation resulted in a low count rate, (50 counts per second maximum). The indication is, however, that the crystal is located axially. Differentiation of this curve allowed the crystal diameter to be estimated as 5.6 ± 0.6mm. The manufacturer's value of 6mm diameter lies within this error band and was used in subsequent calculations.

The experimental performance of the Si(Li) detector and associated electronics is described by the energy calibration energy efficiency, solid angle and count rate performance.

4.2.2 Energy Calibration

Energy calibration relates the amplitude of the voltage pulses to the energy of the incident X-rays. Traditionally, Fe-55 and Co-57 radioisotopes are used to calibrate Si(Li) detectors; Am-241 was also used during this work. The amplifier was set to give approximately 26 eV per channel, i.e. about 13 keV across the available 512 channels. When calibrating using the radioisotope or Am-241 source (with various metal foils) the detector linearity was found to be excellent (±0.15%). Under those circumstances the peaks are well defined (with a minimum background)
Figure 4.4 Plot of count rate vs distance from detector axis.
and the count rate <1000 counts per second. Under typical experimental conditions with higher count rates and when using overlapping and statistically ill-defined peaks to calibrate the detector, linearity errors of up to 5% were seen. The calibration was found to drift slowly over periods of days or longer although no significant drift could be measured over an operation period of 12 hours. This drift meant that energy calibration of the detector on each day of operation was essential. The variation in the slope and intercept of the calibration curve was not found to follow any particular trend during this work; the drift is almost certainly due to environmental sensitivity and power supply instability. The instability may have been aggravated by the fact that the detector was powered only during the time it was in use for experimental work; the build up of condensation in the pre-amplifier caused the detector to become inoperative on several occasions.

4.2.3 Resolution

Energy resolution is a measure of the ability of the detector system to distinguish between two X-rays of similar energy. Resolution is energy dependent and is typically defined as the full width at half peak maximum (FWHM) at a given energy. As could be anticipated, the energy resolution of the detector system used in this work has degraded in time. Reported values are 154.4eV in 1979, 160 in 1983 and 182eV in 1984. By 1985 the resolution measured with the Fe55 source and the PIXE facility electronics was 215eV. Measurement of the resolution using a high quality MCA at the same time established the resolution as 183eV. This indicated that the system resolution was considerably degraded when using the beam line electronic equipment which may have been the result of poor performance of the ADC or of the computer. The energy dependence of the detector resolution was measured experimentally and the relationship subsequently
used in a spectrum analysis programme. The dependence of resolution,
R(E_x), on X-ray energy is known to be of the form (78),

\[ R(E_x) = 4.29 \left( F_f W E_x + (N_{\text{RMS}} W)^2 \right)^{1/2} \]

where \( 4.29 \) converts from \( 1\sigma \) rms deviation to FWTM

\( F_f \)  Fano factor

\( W \)  energy required per electron-hole pair

\( E_x \)  photon energy

\( N_{\text{RMS}} \)  rms electronic noise

Rearranging this equation gives,

\[ R(\text{Channels}) = k \left( N_{\text{RMS}} W \right)^{4.29} \left( 1 + F_f \frac{E_x}{N_{\text{RMS}}^2 W} \right) \]

where \( k \) is the spectrum channels per keV. This equation is of the form

\[ Y = A \left( 1 + BE \right)^{1/2} \]

which may be fitted to the experimental data. If \( W \) is taken to be \( 3.81 \times 10^{-3} \) eV/electron hole pair the value calculated for \( F_f \) is

\( 0.12 \pm 0.05 \) and for \( N_{\text{RMS}} \) is \( 24.4 \pm 0.5 \) both of which agree well with

commonly quoted values. The experimental data is shown in Figure 4.5
together with the fitted function of the form given above. Data is
presented for the detector with and without a 350 \( \mu \)m mylar filter in
place. The mylar filter was mounted on a collimating cap that could be
fitted over the end of the detector. The graph clearly shows the improved
resolution resulting from the collimating effect of the filter cap as
would be expected.
Figure 4.5 Resolution (FWTM) of Si(Li) detector as a function of photon energy.
4.2.4 Efficiency

Intrinsic efficiency is the ratio of the number of photons detected to the number of photons incident on the detector. Full energy photopeak intrinsic efficiency is energy dependent, limited at low X-ray energies by absorption in the detector window and at higher energies by the increasing transmission of photons through the detector.

Calculation of efficiency is effected by measurement of the number of detected photons when the detector is irradiated by a X-ray source of known activity. A well collimated source at known distance from the detector is used; corrections for attenuation and detector solid angle are applied. Detector efficiency degrades with age and with thermal cycling so regular experimental measurement is recommended. Use of both radioisotopes and fluorescent X-ray sources are reported, (39). The only suitable sources available during the present work were Am-241 (emitting photons of energy 11.9 (0.85%), 13.9 (13.3%), 17.8 (18.9%) and 20.8keV (4.9%) ) and Co-57 (6.4 (29.3%), 7.06 (29.3%) and 14.4keV (9.4%) photons).

The experimental results are shown in Figure 4.6; measurements above 20.8keV were not made as this is the approximate energy limit of the current work. As indicated by the error bars the uncertainties were large, mainly resulting from errors in calculating the detector area and the estimation of the radioisotope self-absorption. The use of fluorescent sources was considered and would have been particularly useful for X-ray energies below 6.4keV. However, the uncertainty in measuring the source-detector distance with the detector in-situ on the PIXE apparatus precluded such calculations. Repetition of these measurements is recommended; attention should be paid to calculating the detector efficiency both at lower and higher energies.
Figure 4.6 Intrinsic efficiency of Si(Li) detector as a function of photon energy.
4.3 Typical Spectra

The physical processes of spectrum production and the characteristics of the Si(Li) detector have been described above. A detailed description of the data collection electronics is given in Section 3. Some typical X-ray spectra collected during this work are shown in Figures 4.7 and 4.8.

The spectra are characterised by the many photopeaks superimposed on a continuously varying background. The photopeaks may overlap to form complex doublet and triplet shapes. The background typically rises to a maximum at approximately 2keV and falls off exponentially thereafter. The photopake shape is approximately Gaussian. Physical processes in the detector and the electronics result in a low energy tail to the peak shape. The width of the peaks increases slightly with increasing X-ray energy reflecting the decreasing resolution of the detector at higher energies.

The spectra illustrated were collected from a sample of human breast cyst fluid (BCF). Note the exponential scale on the y-axis. The sample preparation is described elsewhere, (Section 5.3). The thick targets were bombarded with 2MeV protons. The spectrum shown in Figure 4.7 was collected in approximately 200 seconds with a count rate of approximately 1500 counts per second. The beam current was 0.66 ± 0.04 nA; the total charge was 0.15µC. Characteristic peaks of elements with $Z_e$ up to 20 (calcium) are clearly significant. The high count rate arising from these lower atomic number elements limits the information available from the higher $Z_e$ elements. The use of a filter in front of the X-ray detector is a procedure commonly adopted to enhance the higher energy X-ray peaks. The filters used in this work were made from 350µm thick mylar. The
Figure 4.7 Example PIXE spectrum from a single position on a breast cyst fluid target bombarded by 2MeV protons.
Figure 4.8 Example PIXE spectrum from a single position on a breast cyst fluid target bombarded by 2MeV protons and using a 350μm mylar detector filter.
spectrum shown in Figure 4.8 was collected under the conditions described above with a mylar filter in place. The data was collected in approximately 350 seconds at a count rate of 630 counts per second. The beam current was $0.7 \pm 0.06\text{nA}$ with a total charge of $0.2\mu\text{C}$. The peaks arising from the elements Cu, Zn, Br and Rb are now apparent.

4.4 Spectrum Analysis

4.4.1 Introduction

Given a PIXE spectrum, the most basic requirement for analysis is to identify the elements giving rise to significant peaks. This corresponds to the requirements of qualitative analysis and requires reference to the detector energy calibration and to standard tables of X-ray energies. For quantitative analysis, when the concentration of the elements are to be determined, the area of the characteristic peaks must be calculated. Knowledge of the peak area is then combined with other system data to calculate elemental concentrations. Such analysis is ideally undertaken using computer codes, although most codes will only be useful in the first two stages (element identification and peak area calculation) due to the complex and sample specific problem of relating peak area to elemental concentration. A computer code, therefore, is unlikely to provide quantitative results directly.

Briefly, the advantages of using a computer code are usually given as speed, accuracy and reliability. The disadvantages are the need for (an often large) computer, the analysis runtime and the need to learn how to use the code. It is clear, however, that these 'advantages' are not automatically obtained. Of the codes developed elsewhere and commonly used in spectrum analysis, many are extremely time consuming to use both
in terms of operator and computer time. Results are often assumed to be accurate but little work has been done to show this is true for many of the codes available. Specifically, use of codes designed for Ge(Li) spectra analysis for analysis of Si(Li) spectra must be questioned carefully. The analysis reliability is most often compromised by the need for considerable operator intervention when using some of the codes; the results for peak area in particular can be greatly affected by selection of fitting and background parameters. Different operators may introduce systematic errors due to the way in which they use the code.

An intercomparison of a number of computer codes for analysing Ge(Li) detector spectra was made by the IAEA in 1978, (56). The authors supplied some well defined (and mostly non-complex) spectra to various workers and compared the results returned. Interestingly, it was stated that, "Classification of (the analysis) methods into groups according to the principle underlying the method did not reveal any groups offering a significantly restricted range of performance, though some methods did appear, in the best hands, to be capable of producing better results than others". It was also noted that many methods gave no or poor estimates of errors. None of the programmes tested identified all of the significant spectral peaks in the sample spectra.

4.4.2 Data Analysis Techniques

In the late 1970's when work with Si(Li) detectors was started, analysis codes developed for Ge(Li) spectra were used for Si(Li) spectra. The recognition that this approach was inappropriate led several groups to develop codes designed for Si(Li) detector spectra. A review of such codes available to 1981 is given in 39. All of these codes, to a greater or lesser extent, take into account the basic physical processes of PIXE
and so aim to model the background in some way and to deconvolve the spectrum doublet and triplets. Such programmes are thus able to avoid the major problems of using codes developed for Ge(Li) spectra which are the inaccurate fitting of the background and poor resolution of multiplet peaks. The Si(Li) spectrum analysis programmes have been developed to run on both main frame and mini computers and involve several complex data reduction processes.

The steps involved are typically those of peak location and element identification, background fitting, multiplet identification and peak area calculation. These steps may be undertaken separately with data smoothing and background and/or peak stripping stages interposed. Alternatively, a complex function defined by the physical rules governing PIXE and the background processes may be fitted to the complete spectrum followed by peak identification and so forth. Such a function may have up to 20 independent variables.

No intercomparison of these codes is available to date. Little information of their absolute accuracy is available. Several codes are time consuming to run and require a considerable amount of operator intervention. Typically, such programmes cannot deal with a complete spectrum at one time; the spectrum is divided before analysis. The ideal of a fully automatic analysis programme of verified performance appears not to be available at the moment, although the programme PIXAN described by Clayton (14) and available from the Australian Atomic Energy Commission would appear to be worth investigating.

During the present work the Ge(Li) detector spectrum analysis programme SAMPO was the only code available. Experience with this code is described below. Concerns about the accuracy of the results led to 'manual'
examination of the spectra. This experience led to the development of a numerically simple programme HISTO that contained no fitting routines and relied solely on the detector energy calibration and operator input to identify peaks for analysis. Such a programme has serious limitations, but in the light of the comments made by the IAEA (56) about Ge(Li) spectrum analysis programmes and the lack of a widely accepted code for Si(Li) spectrum analysis this approach was felt to be justified.

4.4.3 SAMPO Analysis

SAMPO is a general purpose Fortran code written in 1969 by J.T. Routti, (65). At the University of Surrey a modified version of the code (12, 13) was available in compiled form only. This version of SAMPO was designed for a card input computer system wherein lay its major practical limitation. As described below, data was submitted to the programme together with 'control cards' which define the operations to be carried out on the data. The programme was non-interactive and generally required a minimum of 3 'runs' to reduce the data for PIXE spectra. In the IAEA intercomparison of computer codes mentioned above, SAMPO was said to produce the 'best' results, but the authors noted that the user played the major role in determining the quality of results. It was also noted that the error estimations calculated by SAMPO were generally too low. The error estimate on the peak position was low by up to a factor of 4 and for the peak area by 20%.

SAMPO is first used to calculate 'shaping parameters' for a given detector and set of experimental conditions. The operator defines suitable spectral peaks to which SAMPO then fits a Gaussian function with two exponential tails. The shaping parameters define the peak width and the points at which the exponential tailing functions are joined. These
parameters are used in the next programme run when peaks are identified by SAMPO and a fit to the data is made. Peak identification is by application of a complicated second difference routine. A significance level for peak identification may be set by the user. SAMPO will then make a fit to significant peaks. The programme output is in the form of a complex results file showing graphically and numerically the 'quality' of the fit. A further run is then usually required when various fitting intervals may be operator set to effect a 'better' fit or to fit peaks that were initially rejected by SAMPO for which results are required. The protocol used for this work is shown in Figure 4.9.

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Figure 4.9 SAMPO protocol.
SAMPO was found to be difficult to use; the time taken to analyse even a small amount of data was always counted in terms of days rather than minutes or hours. The inflexible data and command input format demanded attention to detail at all times; mistakes could lead to several days wasted time. The programme would take up to several tens of minutes to run due to the time share nature of the main frame computer. The results of each run would have to be examined closely and individually. Invariably, SAMPO was found to be unable to fit multiplets and produced inappropriate estimates of the spectrum background. An example of this is illustrated in Figure 4.10. Careful selection of fitting parameters rarely resulted in a satisfactory fit to such data. At all times the operator is aware of the very large number of parameters that can be user specified which influence the quality and accuracy of the fit to the data. Data analysis over a period of time inevitably leads to refinement and change in the operator’s approach to the use of the programme. Consequently, it can be anticipated that the results from SAMPO have significant systematic errors, the magnitude of which can be expected to change with time. It was found to be impractical to use the same fitting data for a number of spectra since differences between samples and changes in experimental conditions (including the detector energy calibration) meant that spectra from different target plates had to be examined individually.

4.4.4 HISTO Analysis

In order to check the results generated by SAMPO manual analysis of the data was undertaken. During manual analysis, peaks were visually identified from a plot of the spectrum. The signal area of each peak was then calculated by summing over the counts in the number of channels corresponding to the FWTM at the peak energy. The FWTM vs energy
Figure 4.10 Examples of SAMPO fit to X-ray spectrum triplet.
relationship had previously been determined, (see Section 4.2.3). The FWTM was taken to be the limit of the peak and the background is then a trapezium defined by these limits, (Figure 4.11). This is obviously a time consuming and tedious method of analysis but was found to yield reliable results. The computer code HISTO was developed to simulate the processes of manual analysis. The aim was to produce a simple analysis code that was fast, easy to use, requiring a minimum of operator intervention, providing output that was both useful and easy to interpret. Not all of these aims were perfectly achieved; as described below, development of the code resulted in a number of compromises being made.

The programming language selected was Pascal, (1). Pascal is a highly structured programming language first introduced in 1971. It does not have the universal acceptance as a scientific programming language as, for example, does Fortran, but offers the advantages of being more 'user friendly' and is fairly easy to learn. As BSI/ISO draft standard Pascal was used throughout, the programme was later transferred and run on a personal computer to great advantage and convenience.

In order to ensure that HISTO would be executed as fast as possible the programme was designed to be self contained in as much as no use was made of the comprehensive range of library routines available on the main frame computer. This meant that the time consuming stages of library loading and linking were not necessary each time HISTO was executed. The code assumes no shape for the peaks and contains no fitting or searching routines. A flow diagram showing an abbreviated outline of the main stages of HISTO is shown in Figure 4.12. The operator inputs the name of data file, results file, and the name of a file containing the slope and intercept of the detector energy calibration equation together with a list
Figure 4.11 Representation of HISTO analysis of a single spectral peak.

The peak is limited by the FWTM centred at the peak centroid.
Figure 4.12 Flow diagram of HISTO spectrum analysis code.
of doublet and triplet peaks occurring in the spectrum. The operator then selects from a list of elements those K X-ray peaks that are to be analysed. The peaks are analysed one by one. The X-ray energy is identified and the theoretical peak centroid is calculated from the energy calibration data. The FWTM at that energy is calculated from the experimentally determined FWTM vs energy relationship. The peak background level at the limits defined by the FWTM is then estimated and the total background signal is calculated, (see Figure 4.11). The background contribution is subtracted and the signal area is calculated. A simple test of left/right symmetry is undertaken by calculating and comparing the LHS and RHS signal areas. If the peak is not symmetrical then the smaller side is 'mirrored' onto the larger side and the calculations are repeated. Lack of symmetry is assumed to arise due to peak overlap. HISTO is not sensitive enough to detect a lack of symmetry due to low energy tailing of the essentially Gaussian peak shape. The calculated values for FWTM, total signal and background areas are written to the results file. If the peak for analysis is flagged as being one peak in a doublet or a triplet then the corresponding 2 or 3 peaks are analysed together. In the case of a triplet, the LHS peak is analysed first, followed by the RHS peak and then the central peak.

The complexity of this programme was not anticipated when programming started. The programme is some 800 lines long. Manual analysis requires very many decisions to be made by the operator that are largely unconscious. The operator is able to look at a spectrum and make judgments and estimations. Within the programme these complex operations must either be coded or suitable approximations found. It immediately found that it was not sufficient to describe the calculated central channel number and FWTM interval by integers as large errors in the peak area calculation were introduced by rounding (up to 80% for low amplitude
peaks). The small value for FWTM (typically 8-14 channels) means that significant errors in the background and signal estimation and the peak symmetry are made by ignoring the fractional parts of these variables.

The avoidance of the use of any fitting routines undoubtedly limits the performance of HISTO as small errors in the calculation of peak centroids (which may arise due to an inaccurately defined calibration equation or instability or non linearity in the detector energy calibration) were significant, as indicated above. Ideally, the calculated value for the peak centroid would act as a means of locating a peak and a local curve fitting operation would be used to more accurately determine the centroid. As a means of compensating for this problem, the option of operator input of peak centroids was added to the code. In this way, peak centroids generated by SAMPO could be input to HISTO as required.

The aims of this programme development were partially achieved. The programme is numerically simple although structurally complex and is available for modification to suit any application. The programme is easy to use and executes in a few seconds on the main frame computer. The amount of operator input is undesirably high but cannot be reduced without making the programme more 'intelligent', particularly by the introduction of peak fitting and automatic peak location facilities. The operator input does not, however, affect the values for peak area and so forth except when peak centroids are manually input. The performance of the program is, however, extremely sensitive to the accuracy of the energy calibration data.

Despite the limitations of HISTO, the results obtained were found to be reliable and the errors usually no worse than those given by SAMPO. Details of the intercomparison of the two codes is given below.
4.4.5 HISTO and SAMPO Intercomparison

The performance of HISTO was assessed by a comparison of the analysis results from several spectra produced by both HISTO and SAMPO. Four spectra were collected from each of four breast cyst fluid samples; the spectra used were similar to that plotted in Figure 4.8. The peak areas of all peaks present were calculated by both codes. The peak signal areas were then normalised to the area of the potassium \( K\alpha \) X-ray peak in the corresponding spectrum. After normalisation, the ratio of the normalised peak areas produced by SAMPO and HISTO was calculated for each peak in the 16 spectra. The values of the ratios as a function of element is shown in Figure 4.13.

It can be seen that agreement between both the individual values of the ratio and its average value varies from element to element, and is therefore a function of the individual peak characteristics. The best grouping between the values is for the higher intensity peaks (\( Ca_\alpha \) and \( Ca_\beta \)) indicating that the values returned by HISTO for the area of such peaks is reliably proportional to the area produced by SAMPO. Secondly, in cases where peaks of interest overlapped, as for example the \( K, Ca_\alpha \) and \( Ca_\beta \) triplet, the peak areas found by HISTO were always on average larger than from SAMPO. This reflects, as expected, the difference in the methods of estimation of the background by the two codes; HISTO is likely to produce lower values of the peak background as SAMPO has been found to yield underlying background shapes which are physically incomprehensible when resolving doublet and triplet areas. The correlation between the values of the ratio for the remaining, low intensity, peaks is not so marked. This may partly be accounted for by poor counting statistics associated with the X-ray peaks identifying the higher Z elements but, in addition, the possibility that either SAMPO or HISTO was producing values
Figure 4.13 The ratio of normalised peak area calculated by SAMPO to that calculated by HISTO plotted for the elements detected in four BCF samples.
for the peak areas largely uncorrelated to the "true" value was
investigated. Independently, the results from the two codes were used to
calculate the average and standard deviation of every peak area in the
sixteen spectra. Both sets of values yielded almost identical percentage
standard deviations, indicating the same spread in the values of the peak
areas from both codes. It can therefore be surmised that HISTO and SAMPO
produce results of the same reliability and that the spread in the value
of the area ratio is not due to an inadequacy in one or the other code.

HISTO, however, has the advantage that it can be used to calculate the
area of peaks that cannot be fitted using SAMPO. Such peaks usually
either have their centroids separated by only 3 to 4 channels from an
adjacent peak or are close to a much higher intensity peak which distorts
their shape. In either case SAMPO may be unable to fit these peaks
whereas HISTO can successfully separate them. HISTO is, in addition, more
simple and faster to use as indicated above. The performance of HISTO was
found to be better than anticipated. It would seem likely that systematic
errors in the calculated peak areas are introduced but, when the code is
used for analysis in a comparison study these errors are self cancelling.
PIXE was used to analyse the elemental composition of human breast cyst fluid samples which were available locally from a breast cancer screening programme. The methodology of sample preparation and examination by PIXE was established. The available standard reference materials were examined. A preliminary study of 20 samples was made and the results were used to establish sample homogeneity, contamination and sampling factors. A more detailed study of 30 samples was then undertaken.

5.1 Introduction

5.1.1 Trace Elements and Disease

That there is a relationship between the concentration of certain trace elements in biological systems with disease has long been established. Trace elements are utilised in many metabolic processes and may be present in abnormally elevated or deficient levels in specific tissues or organs as a result of disease. It is thought that the concentration of such elements may be used as an indication of the health of an organism.

Detection of abnormal levels of any element require that 'normal' levels be defined. This latter work has been undertaken by a number of workers and a compilation of results is reported by Iyengar (35) and a task group of the International Commission on Radiological Protection (34). There is, however, no single value for the 'normal' concentration of any element but an acceptable range of concentrations can be defined. At lower concentrations an element is said to be deficient in the biological system.
and at higher levels toxicity occurs. The range of elemental concentrations in any individual organism may change according to diet, environment, activity and state of health.

Several workers have paid particular attention to the role of trace elements in carcinogenesis. Schrauzer reports (66) that trace element deficiency may lead to a lowered resistance to carcinogenic stress. He also notes that some trace elements possess anti carcinogenic properties.

5.1.2 Breast Screening Project

The Breast Screening Project in the UK, supported by the DHSS and the MRC was run as a pilot scheme for a number of years both in Guildford and in Edinburgh. The project aim was to detect and treat malignant breast cancer as early as possible in women who were regularly examined at a specialist unit. During screening any cysts found in the patients’ breasts were aspirated and the fluid was routinely sent for histological analysis. A fraction of this fluid was available to the current project.

The onset and progress of breast cancer is marked by change in tissue elemental composition. It is not always clear whether such changes are causes or consequences of the disease, but the elemental examination of breast cyst fluid (BCF) may prove to be of help in the early detection and diagnosis of disease. A number of workers have reported studies on human mammary cancer; a lowered incidence of breast cancer in areas of high dietary selenium has been noted, as have elevated levels of copper in breast tissue of patients with breast cancer (20). In addition, it is widely thought that fibrocystic disease is associated with increased incidence of breast cancer. Davies et al (20) reported that breast cancer developed 1.73 times as often in women with fibrocystic disease as in the
The aim of the present work was to establish the suitability of using PIXE for the analysis of such samples. The sample preparation and analysis techniques were assessed and the contamination levels and sample homogeneity of the PIXE targets were studied. The range of elements found in BCF by PIXE and their corresponding detection limits were measured. Attempts to determine typical elemental concentrations by a comparator technique were made.

5.2 Previous Work

Liaison between the Physics Department and the Guildford Breast Screening Project has continued over a number of years and several research projects involving breast tissue and cyst fluid have been undertaken. Othman (54) reports the initial qualitative findings from the examination of breast tissue microcalcifications by both INAA and SEM techniques. This work was continued by Othman and Spyrou (55) when the quantitative analysis of surgically removed cancerous and normal tissue was undertaken by INAA. This latter study established a difference in the concentrations of both Rb and Zn between cancerous and normal tissue from the same patients to the 98% confidence level.

Cameron (11) undertook the first study of some 15 BCF samples by INAA and SEM techniques. This work was followed by a similar study of BCF from patients with more than one cyst using NAA, (53). Beside illustrating correlation between the concentrations of certain elements, both latter workers applied cluster analysis to their results and discovered evidence that the concentrations of some elements in the BCF samples fell into one of two groups.
5.3 Sample Preparation and Experimental Analysis

The cyst fluid was aspirated from the patients breast by medical staff at the Jarvis Clinic in Guildford. At the clinic, priority was given to the hystological examination of the cyst fluid so only when the aspirated volume exceeded approximately 3-4ml was the sample partitioned and a proportion made available for examination at the University. The samples were stored at 4°C until collection by the University researchers approximately once a week. Samples were at all times stored in re-sealable containers and handled by tools made from polyethylene that had been carefully washed and rinsed in deionised water before being air dried in a laminar flow cabinet.

The samples were weighed before being transferred to a centrifuge. Batches of samples were centrifuged at 3000 rpm for 45 minutes whereafter the supernatant was carefully removed by pipette. If the remaining solid debris was available in sufficient quantity it too was retained for analysis. The supernatant was then freeze dried at -15°C for approximately 24 hours and the resulting powder was thoroughly stirred before being homogenised by ultrasonic vibration for two hours. Careful note was made of the percentage weight loss (typically 92±2.7%) for future reference. The homogenised powder was then pressed into 7mm diameter pellets using a hydraulic press and a hard steel die. Typically, the whole of each sample was pressed into a single pellet resulting in pellets that were of the order of 1mm thick. The modal dry sample weight was approximately 0.1 to 0.2 grammes so that the target density was about 300mg/cm³. The pellets were then mounted on to an aluminium target plate prior to analysis. A target of the reference material (Bowen's Kale) was prepared and also mounted on the target plate. Between 4 and 6 BCF samples were mounted on each target plate. The target plate was then coated with a
layer of high purity carbon in a vacuum coating unit.

The target plate was introduced into the Van der Graaff beam line target chamber. The samples were analysed using an unfocussed beam of 2MeV protons. The beam diameter was about 1 to 2mm diameter at the target face. Variable beam currents up to a maximum of 5 to 6nA were used; above this current target damage and element loss may occur as a result of the heating effect of the incident protons.

5.4 Pilot Study

5.4.1 Introduction

A pilot study of some 20 BCF samples was undertaken to establish the experimental methodology. Behne notes (3) that the accuracy and precision of the trace element analysis of biological samples are predominately determined by sampling and sample preparation rather than the data analysis, so particular attention was paid to these aspects.

PIXE analysis is also, however, fundamentally affected by the performance of the Van der Graaff accelerator. Measurement of the proton energy, the energy stability and so forth must be made if absolute analysis is to be undertaken. The stability and spread of the proton beam energy were estimated during NRA calibration experiments undertaken in 1983. During these tests the $^{18}$F (p,αγ) $^{16}$O resonance reactions were utilised in an attempt to calibrate the Van der Graaff energy settings. The combined energy resolution and stability in the region 0.8 to 1.3 MeV was estimated as being no greater than 6x10^{-3} keV/keV which was significantly worse than the reported values for the Harwell and Oxford systems, 1x10^{-3} and 5x10^{-4} respectively. The results obtained were disappointing and were limited by
gross instability of the accelerator when operated at the various different proton energies.

The spatial distribution of protons in the ion beam was examined by scanning the beam in two dimensions across a target edge. Provided the distance between sequential beam spot positions is smaller than or comparable with the dimensions of any non-uniformities in the beam profile it should be possible to 'map' the proton density in the beam. Once again the results were disappointing. The generally poor stability of the accelerator energy control loop resulted in a lack of positional stability of the beam spot during the experiments. It was also observed that the accelerator stability varied from day to day and was dependent on such factors as proximity to a servicing period, the accelerator operator, laboratory humidity and so forth. The usefulness of continuing the attempts to quantify these various experimental parameters was questioned in the light of the very limited experimental time available, (typically one day per month). Such experiments were very time consuming and would have had to be repeated several times in order to gain meaningful information about the accelerator stability. This information would have been fundamental to the absolute analysis of samples and the problems outlined above were some of the major reasons why a comparative analysis study was subsequently undertaken.

The sample preparation technique developed by previous workers was adopted but modified (to the protocol described in Section 5.3) after due consideration of sources of contamination, target homogeneity and sample fractionation. The process of centrifuging the samples was added after it was noted that the amount of solid debris in the samples varied considerably. The solid matter comprises mostly squamous cells shed from the cyst wall into the cyst cavity. If these cells were not removed, the
elemental composition of the cyst fluid would be changed during preparation by the addition of the intercellular fluid. The length of time that the samples were homogenised by ultrasonic vibration was increased following tests of target homogeneity, (see Section 5.4.2). The target preparation technique was improved by the introduction of the hard steel die with optically prepared surfaces, (to reduce target surface non-uniformities) and the use of a hydraulic press, (to provide a reproducible force with which to press the target pellets).

The type of experimental study to be undertaken was considered. The ability to generate qualitative results was immediately apparent and depended only on the ability to calibrate the Si(Li) detector and to identify photopeaks in the X-ray spectra. Quantitative determination of the elemental concentrations by absolute analysis would have been difficult to undertake because of the problems in experimentally measuring both the accelerator parameters and the detector solid angle. Consequently, the various reference materials available were examined to assess their suitability for a comparative study. The equations given in Section 2.3.2.2 would be used to calculate the elemental concentrations.

At the beginning of this work Bowen's Kale, although a plant rather than biomedical reference, was chosen as the comparison standard. It was noted that more of the trace elements in Bowen's Kale had concentrations matched with plasma than did Bovine Liver. The composition of the bulk CHO matrix of Bowen's Kale was unknown but was assumed to be close enough to that of cyst fluid to be useful as a reference material. In addition, Bowen's Kale was freely available to the laboratory and commonly used during NAA by my colleagues. As the emphasis of this work moved away from its original aims of absolute analysis and toward aspects of validation of the
technique and apparatus and a more comparative description of the cyst fluid, the use of Bowen's Kale was not reassessed. Retrospectively, examination in greater depth of the available standards should have been undertaken, as well as consideration of the use of a non-certified reference material. Similarly, the use of internal standards was not investigated and would bear examination, particularly in the event of a requirement for the absolute analysis of just one or two selected elements.

5.4.2. Qualitative Results

Of the 30 minor and trace elements known to be present in Bowen's Kale, 12 were detected by the PIXE arrangement used (Figures 5.1 and 5.2). These are the elements Mg (6), Al (10), P (16), S (1), Cl (3), K (2), Ca (5), Fe (7), Zn (9), Br (14), Rb (11) and Sr (23). The ranking in brackets refers to the 'position' of the element in a list of increasing concentration. The elements Zn, Br, Rb and Sr can only practically be detected when using a mylar detector filter to reduce the count rate from the lower Z elements. The element sodium (ranked 4 in increasing concentration) was detected but because the detector intrinsic efficiency for sodium K$_\alpha$ X-rays is less than 50% it is difficult to distinguish from the low energy background. The reason for the failure to detect Cu (8) was not clear; the concentration of copper in Bowen's Kale and the energy of its K$_\alpha$ X-ray lies between that of Fe and Zn.

The elements detected in all the BCF samples were Na, P, S, Cl, K and Ca. The trace elements occasionally detected were Ni, Fe, Cu, Zn, Br, Rb and Sr, (Figures 4.7 and 4.8). Considerable variation in the peak areas arising from the latter elements was seen. Ni in particular was rarely detected whereas Zn and Cu were almost always seen. Of the elements
Figure 5.1 Example PIXE spectrum from a single position on a Bowen's Kale target bombarded with 2MeV protons.
Figure 5.2 Example PIXE spectrum from a single position on a Bowen's Kale target bombarded with 2MeV protons and using a 350µm detector filter.
detected in BCF, Na, Cu and Ni were not detected in Bowen's Kale. The concentrations of these three elements could therefore not be determined by a comparator method as no reference signal was available.

Solid residue of sufficient quantity for analysis was obtained from only 3 out of the 20 samples in the pilot study. One of these samples was notably coloured red and undoubtedly contained blood. Subsequent PIXE analysis showed elevated levels of Fe, Cl, and Rb as expected. Analysis of the other two samples showed the presence of the same range of elements as in the fluid samples but that they were present in significantly different proportions. The concentrations of Al, P, S and Cl were found to be elevated with respect to the concentrations of K and Ca.

5.4.3 Detection Limits

The definition of reliable peak detection (to 99.9% certainty) given in Section 2.2.1 is

$$A_1 > 3 \sqrt{B_1}$$

where $A_1$ and $B_1$ are respectively the photopeak signal and background areas of the ith element. Using this definition, the typical minimum detectable concentration (MDC) for the elements detected in BCF (and also found in Bowen's Kale) was calculated. The simplifying assumption that the stopping power of Bowen's Kale and BCF matrices are the same was made.
For the ith element, the MDC in BCF is given as

\[
MDC_i = 3 \frac{\Phi_R N_{RI}}{\Phi_S A_{RI}} \sqrt{\frac{A_{SI}}{B_{SI}}}
\]

where \( \Phi_R \) and \( \Phi_S \) are the total incident charge on the reference and sample (BCF) targets respectively, \( N_{RI} \) is the concentration of the ith element in Bowen's Kale giving rise to \( A_{RI} \) signal counts. \( A_{SI} \) is the number of background counts in the BCF spectrum. The summary of results is given in Table 5.1. The errors shown include those arising from the quoted values of elemental concentrations in Bowen's Kale, the estimated error in charge collection and peak area calculations. The relatively poor MDC for low Z elements arises from the reduced counting efficiency of the Si(Li) detector at low X-ray energies.

<table>
<thead>
<tr>
<th>Element</th>
<th>Detector Filter</th>
<th>MDC mg/Kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>N</td>
<td>220 ± 20</td>
</tr>
<tr>
<td>S</td>
<td>N</td>
<td>53 ± 13</td>
</tr>
<tr>
<td>Cl</td>
<td>N</td>
<td>78 ± 21</td>
</tr>
<tr>
<td>K</td>
<td>N</td>
<td>49 ± 7</td>
</tr>
<tr>
<td>Ca</td>
<td>N</td>
<td>0.11 ± 0.01</td>
</tr>
<tr>
<td>Fe</td>
<td>Y</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>Zn</td>
<td>Y</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>Br</td>
<td>Y</td>
<td>2.5 ± 0.6</td>
</tr>
<tr>
<td>Rb</td>
<td>Y</td>
<td>4.1 ± 0.9</td>
</tr>
<tr>
<td>Sr</td>
<td>Y</td>
<td>1.2 ± 0.8</td>
</tr>
</tbody>
</table>

Table 5.1 Minimum detectable concentration of elements in BCF.
5.4.4 Sample Homogeneity

Homogeneity of the samples was tested by collecting X-ray spectra from typically 6-8 positions from the surface of several targets. The areas of the various peaks were determined using HISTO and the statistical variation in the peak areas from position to position was examined. If the target was homogeneous then the standard deviation in the area of any given peak for any one target should be less than or equal to the statistical uncertainty of the peak area, (i.e. $\sqrt{\frac{A_1 + 2A_2}{s_1}}$ where $A_1$ is the signal area and $A_2$ is the background area).

The first targets tested for homogeneity did show evidence of non-uniformity. Examination of the samples using the microbeam suggested that there were small areas of non-uniformity in the target thought to be caused by 'clumping' during the freeze drying process. The homogeneity of the targets was improved by increasing the amount of stirring of each sample after freeze drying and by introducing the use of the ultrasonic bath. In addition, the beam area at the target was increased from approximately 0.2mm$^2$ (as used initially) to about 3mm$^2$. During subsequent analysis typically 2-4 spectra were collected from each target in order to check target uniformity. Similar tests on the reference materials indicated that the powder was homogeneous.

5.4.5 Sample Contamination

The possible contamination of samples during the stages of homogenisation, target and target plate preparation (including target handling and storage) could be examined by an intercomparison of results from the analysis of reference materials. Simultaneous examination of Bowen's Kale, NBS Bovine Liver and NBS Orchard Leaves was undertaken and once again a
The number of spectra from each target were taken. Cross referencing the results between the standards yielded errors in the peak areas of the order of the expected statistical uncertainty. No evidence of contamination by elements not expected in the samples was found. The indication was that care in these latter stages of target preparation prevented sample contamination.

Assessment of contamination of the BCF samples in the 'wet' stages was more difficult. Contamination during the aspiration process was a possibility but was outside the control of this analysis programme. Contamination in the laboratory could arise from the storage and handling containers and tools and would probably be caused by the cleaning agent, Decon 90. A sample of Decon 90 was freeze dried and analysed by PIXE. The elements Na, Cl, K and Ca were found to be the major components with no significant spectrum peaks arising from any element with Z greater than that of Fe. Two large samples of BCF were partitioned into 4 sections and each section was prepared using tools and containers that had been washed using the recommended concentration cleaning liquid and were subsequently rinsed from 1 to 4 times in deionised water. Analysis of the peak areas in spectra from the sample tarets indicated slightly elevated concentrations of Na, Cl and K in the sample sections prepared using tools that had been rinsed only once after washing. Subsequently tools and containers were rinsed a minimum of three times prior to use for sample preparation.

5.5 Main Study

The pilot study of BCF samples provided information about the sample homogeneity and contamination levels as well as establishing the detection limits of PIXE with thick targets. The examination of the BCF samples was
continued by detailed analysis of some 30 fluid samples and 4 samples of solid residue. The aim of this study was to establish with reference to elemental concentrations in Bowen's Kale the concentration of elements detected in BCF. As discussed below the aim was only partially achieved.

5.5.1 Quantitative Analysis.

Using the equation (Section 2.3.2.2)

\[
\frac{A_{xR1}(E)}{A_{xS1}(E)} = \frac{\phi_{R} N_{R 1} B_{R} (-dE/dx)_{R}}{\phi_{S} N_{S 1} B_{S} (-dE/dx)_{S}}
\]

the number of atoms of the ith element per unit volume in the sample target \( N_{SI} \) can be determined. Note that, in particular, the assumption that the beam areas at the target (\( B_{R} \) and \( B_{S} \)) are equal can be assumed only for data collected during one experimental run. The usefulness of this equation was tested by again making the assumption that \((-dE/dx)_{R}\) and \((-dE/dx)_{S}\) were equal. This assumption will introduce a systematic error into the calculation of \( N_{SI} \). Concentration of the ith element in units of \( \text{g/g} \) (or similar), \( C_{i} \), is related to the number of atoms per unit volume by the equation

\[
N_{i} = \frac{C_{i} N_{A}}{W_{i} \rho}
\]

where \( W_{i} \) is the atomic weight of the ith element, \( N_{A} \) is Avagadros number and \( \rho \) is the target density. Measurement of the density of the reference material and sample targets were made as the elemental concentrations in Bowen's Kale are quoted in ppm or in percent of dry weight.

Table 5.2 lists the range of elemental concentrations of 14 elements in 5 BCF samples examined during one analysis period. These and similar
results showed greater than expected variability and agreement with the previous workers results was poor (11, 53). Furthermore, examination of results from a few samples which were large enough to be formed into more than one target pellet and which were then mounted onto different target plates showed that errors were introduced by the 'normalisation' of data by the total collected charge.

In order to find the source of variability in the measurement of the beam current, the current meter and its interface with the computer were tested and found to be in working order. The variability was found to be due to three factors which all caused a change in the electrical resistance to ground from the surface of the targets and so affected the current reading. Firstly, the thickness of the conducting coat of carbon laid down in the coating unit was variable and uncontrolled. Secondly, the carbon film thickness was not uniform across the whole target plate which was a consequence of the relative size of the carbon 'source' and the

<table>
<thead>
<tr>
<th>Element</th>
<th>Range of Concentrations ( \mu g/g ) (wet)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximum</td>
</tr>
<tr>
<td>P</td>
<td>84 ± 30</td>
</tr>
<tr>
<td>S</td>
<td>106 ± 28</td>
</tr>
<tr>
<td>Cl</td>
<td>880 ± 237</td>
</tr>
<tr>
<td>K</td>
<td>3362 ± 604</td>
</tr>
<tr>
<td>Ca</td>
<td>37 ± 10</td>
</tr>
<tr>
<td>Fe</td>
<td>182 ± 53</td>
</tr>
<tr>
<td>Zn</td>
<td>47 ± 11</td>
</tr>
<tr>
<td>Br</td>
<td>5.7 ± 1.9</td>
</tr>
<tr>
<td>Rb</td>
<td>4.7 ± 2.1</td>
</tr>
<tr>
<td>Sr</td>
<td>137 ± 119</td>
</tr>
</tbody>
</table>

Table 5.2 Range of concentration (\( \mu g/g \), wet) of 10 elements in BCF calculated by comparative analysis.
target plate area. Thirdly, and probably most importantly, the 'dryness' of the various BCF samples was variable and changed with time. It had been observed that some BCF samples were not completely dry even after repeatedly being freeze-dried and such samples were not analysed. Variability in dryness of those targets tested was therefore to be expected. Certain samples were also observed to be hygroscopic and could absorb enough water in the period of 2-3 days between target preparation and examination that the base pressure of the target chamber would be limited. Practical limitations on the use of the freeze drier, carbon coater and the clean room facility, combined with the need to respond quickly to the availability of the Van der Graaff accelerator meant that it was impractical to attempt to prepare the target plates immediately before analysis. Data normalisation by total incident charge was therefore unreliable and inaccurate. As described below, an alternative means of normalisation was therefore developed for the considerable amount of data that had been collected.

The peak areas in each spectrum were normalised to the area of the potassium Kα X-ray peak in the spectrum. This line was chosen since it was a peak that was present in all spectra, could be fitted by both SAMPO and HISTO and showed the greatest correlation between the results from SAMPO and from HISTO. The elemental concentrations would therefore be expressed in terms of concentration of the ith element per unit concentration of potassium. For the ith element the above equation may be written

\[
\frac{A_{\alpha R}}{A_{\alpha S}} = \frac{F_{1R} N_{1R} B_R}{F_{1S} N_{1S} B_S}
\]

where F is a constant of proportionately. Repeating this expression for potassium, dividing the two equations and rearranging gives
\[ \frac{N_{1S}}{N_{KS}} = \frac{P_1}{A_{1S}} \frac{A_{1S}}{A_{KS}} \]

where the constant \( P_1 \) is defined as

\[ P_1 = \frac{A_{KR} N_{1R}}{A_{1R} N_{KR}} \]

\( P_1 \) was evaluated for each of the elements under consideration in Bowen's Kale. The concentration (in atoms/unit volume) of the \( i \)th element in BCF is expressed as a function of the concentration of potassium in that sample. Alternatively, the relative concentration in units of g/g (or similar), \( C_{\text{wr}} \), can be calculated from

\[ \frac{C_{1S}}{C_{KS}} = \frac{P_1}{A_{1S} W_1} \frac{A_{1S} W_1}{A_{KS} W_k} \]

where \( W_1 \) is the atomic weight of the \( i \)th element.

The calculated values for \( P_1 \) are shown in Table 5.3. These values were generated by first summing the peak areas from spectra taken at 2-3 points on a single Bowen's Kale target before calculating the values of \( P_1 \) for that target. This process was repeated for a number of targets and the values of \( P_1 \) were averaged. The quoted uncertainties arise from the spread of the calculated values of \( P_1 \), rather than from the error which could be estimated from the peak signal and background areas and the uncertainty in the value of \( C_1 \) in Bowen's Kale. The relatively small spread in \( P_1 \) for the elements P, S and Cl reflect the correspondingly good counting statistics for those peaks. The small value of \( P_1 \) for calcium was to be expected as the detection limit for the element was the lowest of the higher Z elements. The uncertainties in \( P_1 \) for the elements above Z=26 (iron) reflect the worsening counting statistics encountered.
Table 5.3 Proportionality constant $P_1$ calculated for elements detected in Bowen Kale.

The relative concentrations of these elements in the 30 BCF samples were then calculated. Table 5.4 shows the results from 10 of these samples for reference. The mean, maximum and minimum values of the relative concentrations are given in Table 5.5. Note that the values quoted are the ratio of the concentrations in units of mass/mass in the dried BCF samples. The spread in most values, though large, was smaller than the spread in the previously calculated concentrations and shown in Table 5.2. Further discussion of these results is made in Section 5.6.

5.5.2 Sampling Factor.

Late in the current study, attention was drawn to the applicability of the experimentally determined sampling factor, $F_i$, which is described in Section 2.2.4. The definition of $F_i$ for the $i$th element is

$$
\frac{1}{R_i^2} = \frac{1}{F_i} \text{ m}
$$
Table 5.4 Concentration of 9 elements detected in 10 BCF samples relative to the concentration of potassium in the samples.

<table>
<thead>
<tr>
<th>Sample No</th>
<th>58</th>
<th>62</th>
<th>63</th>
<th>71</th>
<th>73</th>
<th>74</th>
<th>78</th>
<th>80</th>
<th>88</th>
<th>89</th>
</tr>
</thead>
<tbody>
<tr>
<td>Element</td>
<td>P</td>
<td>S</td>
<td>Cl</td>
<td>Ca</td>
<td>Fe</td>
<td>Zn</td>
<td>Br</td>
<td>Rb</td>
<td>Sr</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.2E-01</td>
<td>6.1E-01</td>
<td>7.4E-01</td>
<td>7.2E-02</td>
<td>1.6E-03</td>
<td>2.8E-03</td>
<td>4.9E-04</td>
<td>3.2E-03</td>
<td>7.0E-05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.0E-02</td>
<td>5.7E-01</td>
<td>9.2E-01</td>
<td>9.8E-02</td>
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<td>4.5E-03</td>
<td>3.9E-03</td>
<td>2.6E-03</td>
<td>8.1E-04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.4E-01</td>
<td>2.4E-01</td>
<td>8.4E-01</td>
<td>9.8E-02</td>
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<td>4.5E-04</td>
<td>5.9E-05</td>
<td>2.6E-03</td>
<td>8.1E-04</td>
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<tr>
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<td>3.8E-01</td>
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<td>2.8E-03</td>
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<td>8.9E-05</td>
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<td>2.6E-06</td>
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<td>1.4E-02</td>
<td>5.7E-04</td>
<td>7.2E-02</td>
<td>1.4E-03</td>
<td>1.4E-03</td>
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</tr>
<tr>
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<td>1.2E-01</td>
<td>3.2E-01</td>
<td>8.1E-01</td>
<td>2.7E-03</td>
<td>5.7E-03</td>
<td>5.7E-04</td>
<td>6.4E-04</td>
<td>2.7E-03</td>
<td>1.6E-03</td>
<td></td>
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<tr>
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<td>2.7E-01</td>
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<td>4.1E-03</td>
<td></td>
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<tr>
<td></td>
<td>9.0E-02</td>
<td>4.3E-01</td>
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<td>8.2E-02</td>
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<td>8.2E-02</td>
<td>0.0E+00</td>
<td>0.0E+00</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.5 Mean, maximum and minimum concentration of 9 elements relative to the concentration of K in 30 samples of BCF.

<table>
<thead>
<tr>
<th>Element</th>
<th>Mean</th>
<th>Maximum</th>
<th>Minimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>0.14 ± 0.09</td>
<td>0.67</td>
<td>0.08</td>
</tr>
<tr>
<td>S</td>
<td>0.39 ± 0.19</td>
<td>0.65</td>
<td>0.04</td>
</tr>
<tr>
<td>Cl</td>
<td>0.65 ± 0.45</td>
<td>1.12</td>
<td>0.16</td>
</tr>
<tr>
<td>Ca</td>
<td>0.084 ± 0.018</td>
<td>0.19</td>
<td>0.0032</td>
</tr>
<tr>
<td>Fe</td>
<td>0.0011 ± 0.00073</td>
<td>0.0068</td>
<td>0.00021</td>
</tr>
<tr>
<td>Zn</td>
<td>0.01 ± 0.023</td>
<td>0.072</td>
<td>0.00032</td>
</tr>
<tr>
<td>Br</td>
<td>0.00039 ± 0.0002</td>
<td>0.00049</td>
<td>9.2.10^{-6}</td>
</tr>
<tr>
<td>Rb</td>
<td>0.0011 ± 0.0013</td>
<td>0.0033</td>
<td>8.9.10^{-5}</td>
</tr>
<tr>
<td>Sr</td>
<td>0.00026 ± 0.00031</td>
<td>0.00081</td>
<td>7.2.10^{-5}</td>
</tr>
</tbody>
</table>
The sample mass \( m \) in PIXE is defined by the volume of interaction at the target face so (for a given proton beam area) \( m \) can be defined as equal to one unit. For a unit incident charge let the corresponding X-ray photopeak signal area from the \( i \)th element be \( s_i^H \) and the background area be \( b_i^H \). \( R_i \) can then be expressed by

\[
R_i = \sqrt{\frac{s_i^H + 2b_i^H}{s_i^H}} \times 100\%
\]

When data is collected from \( j \) points on the target surface the expression for \( F \) becomes

\[
F = \frac{1 \times 10^4 \sum_{i=1}^{j} \frac{s_i^H + 2b_i^H}{s_i^H}}{\left( \sum_{i=1}^{j} s_i^H \right)^2}
\]

and \( F \) can be seen to be fundamentally limited by the counting statistics. An estimate for \( F \) can be obtained by setting \( j=1 \) and obtaining typical values for \( s_1^H \) and \( b_1^H \). This formula can be tested by obtaining spectra from a number of target positions, calculating \( s_i^H \) and \( b_i^H \) for each spectrum and plotting the corresponding values of \( 1/R^2 \) against \( m \). A straight line with slope \( 1/F \) should be obtained. Increasing heterogeneity in the samples will cause increasing error in the slope of the plotted line; Spyrou (68) warns against analysis protocols that give rise to a slope error < 20% or an intercept of greater than 0.2 on the y-axis.

Table 5.6 shows the numerical analysis for the Cl and the Rb X-ray lines in a sample of BCF. Figures 5.3 and 5.4 are the corresponding plots of \( 1/R^2 \) against \( m \), with \( m \) shown in units of one target sample. The signal and background areas were normalised by the total collected charge, a
procedure acceptable for intra-target measurements. The assumption that the beam spot area was the same for all samples was made. For calcium a fit to the data gave $F_{Ca} = 0.65 \pm 1\%$ with an intercept of $-0.14$.

Estimating $F_{Cl}$ for $j=1$ (i.e., just the first data point) gives

$$F_{Cl} = \frac{10^4 \times 36096 + (2 \times 25924)}{36096^2} = 0.67$$

or for $j=8$, (i.e, all 8 data points), gives

$$F_{Cl} = \frac{10^4 \times 700239 \times 8}{(292817)^2} = 0.65$$

This indicates that, for the definition of $F_1$ given above, the signal and background area from one sample may be used to estimate the value of $F_1$ with some confidence.

The average value of $F_1$ over some 10 samples was calculated for elements in the BCF and these are shown in Table 5.7. The indication is that, for the elements P, S, Cl, K and Ca (when no Si(Li) detector filter is used) the sampling protocol previously adopted of collecting data from 3 to 4 places on the target with a beam spot diameter of $2-3\,\text{mm}^2$ and a typical count rate of 1000-2000 cps was adequate to collect sufficient data to satisfy the requirements of the definition of $F_1$. For the higher Z elements, however, where spectra were collected with a similar beam spot area and with a count rate typically <1000 cps and a mylar filter in front of the detector, that the required sample size was approximately 10-100 times greater.
Table 5.6 Calculation of the sampling factor for chlorine and rubidium in BCF target.

<table>
<thead>
<tr>
<th>Element</th>
<th>( F_1 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>2.7 ± 1.4</td>
</tr>
<tr>
<td>S</td>
<td>0.38 ± 0.1</td>
</tr>
<tr>
<td>Cl</td>
<td>1.0 ± 0.6</td>
</tr>
<tr>
<td>K</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>Ca</td>
<td>1.8 ± 0.5</td>
</tr>
<tr>
<td>Fe</td>
<td>32 ± 15</td>
</tr>
<tr>
<td>Zn</td>
<td>46 ± 38</td>
</tr>
<tr>
<td>Br</td>
<td>207 ± 152</td>
</tr>
<tr>
<td>Rb</td>
<td>56 ± 32</td>
</tr>
<tr>
<td>Sr</td>
<td>104 ± 82</td>
</tr>
</tbody>
</table>

Table 5.7 Sampling factor, \( F_1 \) for 10 elements in BCF.
Figure 5.3 Sampling factor for chlorine. Plot of reciprocal of variance against number of sampling units.

BCF SAMPLING FACTOR, CHLORINE

RECIPIROCAL OF VARIANCE

NUMBER OF SAMPLES

0 1 2 3 4 5 6 7 8 9
Figure 5.4 Sampling factor for Rubidium. Plot of reciprocal of variance against number of sampling units.
5.6 Discussion

The target preparation technique adopted was found to be broadly adequate for the preparation of thick targets for PIXE analysis. The minimum handling requirements are ideal when attempting to minimise contamination. Potential elemental losses during the freeze drying process were not examined. The hygroscopic nature of the samples caused some problems both in terms of charge collection and the limit placed on the interval between sample preparation and analysis. It was thought to be inadvisable to attempt the re-analysis of the BCF PIXE targets by any other analysis technique as contamination of the lower face of the target pellets was thought to be likely. Preparation of thin targets was attempted by allowing the evaporation of about 1ml of BCF after placement on a small area of mylar film. The facility for the analysis of such targets was severely limited, however, by the design of the target chamber; only one sample could be analysed on each target plate. Re-hydrating Bowen's Kale for similar treatment as a reference was found to be impracticable. The use of thin targets was not investigated further.

Target homogeneity was improved by the methods detailed above but it should be noted that the relevant tests were applied to the relatively high concentration elements (K, Cl and Ca) since the areas of those peaks were most reliably determined by the spectrum analysis codes. Applying the same tests to the lower concentration elements might retrospectively be considered a better test of target homogeneity. The tests for target contamination were not exhaustive and relied mainly on intercomparison of results from the reference materials available. Contamination of the samples prior to collection at the screening clinic was a possibility that could not be investigated.
Of the 13 elements detected in the BCF samples by PIXE, all but P and Ni are detected by short or long lived isotope NAA protocols and Ni was only detected in 4 out of 50 BCF samples examined by PIXE. Detection of Ni, Fe, Cu, Zn, Br, Rb and Sr by PIXE can only be effected by use of a detector filter; the target chamber must be opened to reposition the filter. The 'finger printing' of samples may not therefore be as fast as might be anticipated as a minimum of 2 spectra from any one target must be obtained with the target chamber being opened to atmospheric pressure in between. Peak, and therefore element, identification must be done 'by hand' or by using SAMPO. The latter approach may take up to 3 hours per spectrum if the stages of data transfer and reformatting are taken into account.

The use of Bowen's Kale as the standard reference material allowed examination of 10 of the detected elements in BCF. As a plant rather than a biological reference material, however, the assumptions made during some calculations about the stopping power of the reference and sample targets must be expected to introduce a systematic error. The viability of using an internal reference (by 'spiking' the sample with a solution of known elemental content) was not experimentally tested as this approach would be most useful only when undertaking an absolute analysis. Such a technique might be useful if the determination of just one or two elements were required.

The minimum detectable concentration calculated for the 10 elements in BCF and in Bowen's Kale give an indication of the sensitivity of PIXE for these elements in a thick target. It is to be expected that the detection limits would be improved if thin targets were used. The detection limits for BCF targets analysed by NAA is not quoted so it is not clear that PIXE offers an advantage in this respect.
A method of calculating the relative elemental concentration of a limited number of elements detected in BCF by PIXE was established. The concentration was expressed as a function of the potassium concentration in the sample. It may be possible to determine the concentration of potassium by another technique (eg. NAA) and so calculate the absolute concentration of the elements in BCF. Calculating the relative concentration avoided problems with data normalisation by the usual method of total collected proton charge but, as the concentration of potassium in BCF is a variable itself, an analysis programme based on the examination of relative elemental concentrations is of questionable relevance. Indeed, previous workers have suggested that BCF samples may fall into one of two groups (one group of samples resembling intracellular fluid and the other resembling extracellular fluid) so valuable information could be lost by normalising to the potassium concentration. This situation would be best addressed by reliable beam current measurement. The use of a beam chopper or similar device might be investigated. Some other means of data normalisation may be found. The possibility of using the RBS signal from the BCF targets was briefly investigated toward the end of the current work. Richter (64) reports the use of the RBS signal from a thin metal foil inserted into the beam path a few millimetres before the target.

Calculation of the sampling factors for the various BCF elements produced some of the most interesting results and, if undertaken earlier in the study, would have significantly affected the experimental approach. Indeed, these calculations cast into doubt the viability of using PIXE for determination of trace elements in BCF. If one accepts the definition of the sampling factor (which is arbitrary) then it can be seen that the required sample size (ie number of spectra collected) for elements with Z greater than or equal to that of iron is approximately 30-200 times greater than the typical sample size used in the current work. The
practice of collecting an X-ray spectrum from 2-4 locations on a target pellet was clearly inadequate. The typical sample size, when a detector filter is used, is limited only by the maximum proton beam current that can be used to avoid damaging the targets. It is possible to defocus the proton beam at the target face which would allow slightly higher beam currents to be used but under those conditions the beam location and its boundary became more difficult to see and so the chance of the beam being incident 'off-target' was increased. Conversely, calculation of the sampling factor indicated that the protocol adopted was sufficient for the reliable assay of the elements P, S, Cl, K and Ca. The error in the slope of the graph of $1/R^2$ against $m$ was found to be a parameter that could be used to clearly express the sample homogeneity.

In summary, the sampling factor for elements with $Z \geq 26$ would appear to be the most fundamental limit to the applicability of PIXE for reliable quantitative elemental determination. For elements with $Z \leq 20$ the protocol described in earlier sections would appear to be acceptable. The MDC for those elements has been established and the range of concentrations relative to that of potassium in 30 BCF samples has been measured.
CHAPTER 6
MICROPROBE OPERATION AND USE

The PIXE microprobe facility is described and its alignment and operation are outlined. The microprobe was used to examine tree ring samples; examples of some 1D scan are given.

6.1 Introduction

A microprobe system is an arrangement for producing a beam spot with a diameter of a few micrometres at the target surface; such a facility can be used to assess the concentration of elements within a sample that have spatial distributions on a micrometer scale. A small diameter beam may be produced either by simple collimation (2, 67) or by using a lens system which demagnifies a beam passing through an object aperture; the latter method was employed here.

A focussing system is generally based on a series of magnetic quadrupole lenses. The magnetic field generated by such lenses is of sufficient strength to deflect the beam of high rigidity (ie relatively high mass and high energy) ions. Typically, from two to four quadrupoles are arranged to form a thick lens system, which is used to form a demagnified image of an object aperture located several metres upstream of the lenses. The number of lenses, their size, position and excitations are all variables that can be adjusted to produce a lens system with the desired characteristics and capabilities. A detailed examination of lens design and function and an assessment of various experimental configurations can be found in Blower (4) and Grime and Watt (27). A complete description of
the principles and properties of the design and operation of a microprobe facility are to be found elsewhere, (15, 17, 18, 42).

Degradation in the image spot size, shape and intensity must be minimised by careful consideration of lens aberrations and of general system design. Aberrations are described as either intrinsic or parasitic. Intrinsic aberrations are those which relate to a perfect quadrupole system and are only dependent on the position, angular divergence and fractional energy shift of the particles in the beam at the object aperture. Parasitic aberrations occur as the result of imperfect lens construction and misalignment or of variations in lens excitation. Other important factors include the presence of ferromagnetic materials close to the beam line, line vibrations and the vacuum maintained in the line. Variations in beam energy, energy spread and angular divergence of the charged particles caused by Van der Graaff instability or fluctuations in the power supply to the switching magnet will contribute to the intrinsic (chromatic) aberrations.

6.2 Microbeam Description and Operation

The microprobe facility was added to the apparatus in 1978, but it was not until 1985 after further development work that the full potential of the system was realised with the routine achievement of beam spot diameters of 10μm, (72). The design of the microprobe system (31, 50) is closely based on the Harwell system (15); the later additions were the pre-lens deflection system and the novel optical viewing device attached to the target chamber. The practical layout of the apparatus was described in Chapter 3, here an outline of the ion optics involved in the production of the microprobe are presented together with a description of the microprobe operation.
6.2.1 Ion Optics

A schematic diagram of the basic ion optical system is given in Figure 6.1. The proton beam was electrostatically deflected on to the beam line axis by the beam steering plates downstream of the gate valve. The beam then passed through the object aperture. It travelled down the beam line, diverging, until passing into the thick lens system where it was focussed on to the image plane in the target chamber. The beam trajectories in the two principle planes are also shown in Figure 6.1.

The magnetic lenses were excited in the Russian Quadruplet mode (17, 24). In this configuration the magnets were placed closely adjacent to each other and were excited so as to be alternatively diverging and converging in the XZ plane. The first and fourth lenses were excited equally, as were the second and third lenses. The resulting thick lens has several characteristics of note:

(i) The XZ and YZ principle planes are coincident

(ii) The demagnification is relatively low, X5.6, and is equal in both the XZ and YZ planes.

(iii) The acceptance angle is high, since the dependence of the aberration coefficients on the beam divergence is not too great.

(iv) The image is formed 210mm from the exit of the fourth lens.

These interrelated features meant that:

(i) Beam scanning on the target was achieved by using a pre-lens deflection system, as suggested by Heck (30). Scanning in
Figure 6.1: Schematic diagram of the ion beam optics.

- Diverging lens
- Converging lens
- Quadrupole

(Y scanning plates only)

Object aperture
Antiscatter aperture

N.T.S.

Angular divergence exaggerated

Image plane

X±Y principle planes

X plane trajectory

Y plane trajectory

21 cm

4.5 m
both X and Y directions could be effected with low aberrations. The alternative of positioning the deflection system after the quadruplet results in overcrowding of the limited space between it and the target chamber. In addition, this latter configuration has a considerably shorter lever arm and so correspondingly higher electric fields are required to produce similar beam spot deflections in the target chamber.

(ii) The microprobe was suitable for work involving beam channeling experiments. The low demagnification produced a beam with a correspondingly low convergence at the target face and, additionally, the beam could be scanned over a large sample area while deflecting it through a relatively small angle.

(iii) Although the small object aperture (which was required to produce a micrometer size image with low demagnification) limited the beam intensity, an acceptable beam throughput was achieved due to the large acceptance angle of the quadruplet.

(iv) There was adequate room after the quadruplet for a gate valve (to isolate the target chamber) and for forward facing detectors.

The scanning system (Figure 6.2) consisted of four pairs of scan plates which were arranged in two sets, to provide double deflections in both X and Y directions. The voltage applied to each set was derived from the same supply, but was scaled by the use of a voltage divider. The pair of plates nearest to the object aperture were twice the distance from the entrance principle plane of the thick lens system and carried half the voltage of the second pair of plates in the set. The deflected beam therefore crossed the axis of the lens at the entrance principle plane. By this geometry there are no additional contributions to the deterioration
Figure 6.2 Schematic diagram of the ion beam scanning facilities.
in beam spot size from spherical (intrinsic) aberrations, but the virtual
displacement of the object aperture introduces additional distortion and
astigmatic (parasitic) aberrations. Heck (30) suggests that this geometry
would cause a contribution of less than 0.1μm to a 3μm spot size for
deflections up to ±500μm. Note, however, that limiting the maximum angle
between the beam and the major axis of the quadruplet to ±0.6 mrad (a
recommended operating parameter for the Harwell system to achieve a 4μm
beam spot diameter) allows a movement of the beam spot in the image plane
of ±380μm. A scan area of 2mm X 2mm was, however, possible with this
deflection system.

6.2.2 Microprobe Alignment and Operation

The system regularly required alignment and adjustment for operation with
minimum spot sizes. Alignment reduces degradation of the image by the
elimination and reduction of the intrinsic and parasitic aberrations. The
principle aim of alignment is to arrange for the major axes of both the
beam line and its facilities to be concurrent and for the beam emerging
from the switching magnet to travel along this axis. Alignment was
achieved by allowing the beam into the line and observing the position,
shape and size of the beam spot fluorescence viewed in the target chamber
while adjusting relevant experimental parameters.

First the ion beam parameters were selected, i.e. the ion type, energy and
approximate current as well as the switching magnet Gauss (current). The
beam was focussed on the object aperture by the Van der Graaff operator.
This creates maximum current density at the object and therefore maximises
the throughput. The position of the feedback slits was adjusted to
optimise the proton energy control. After selecting the largest available
aperture (1mm diameter), the beam spot was observed in the target chamber,
and the voltages on the steering plates adjusted to deflect the spot into the centre of the field of view of the optical viewing system set to x25 magnification. The field of view of the objective lens had been designed to be at the intersection of the focal plane of the quadruplet and the major beam line axis. A smaller object aperture was now selected and the beam spot brought back into the field of view by adjusting the aperture X and Y micrometers only. A change in any of the Van der Graaff parameters may necessitate the apparatus being re-aligned from this stage.

The quadrupoles were aligned according to the scheme suggested by Cookson, (16). This utilises the fact that charged particles entering an excited quadrupole off-axis will suffer a net translation force, i.e. the beam will be steered either horizontally or vertically depending on the excitation mode of the lens. The positions of the quadrupoles with respect to the beam line were adjusted until increasing the current in any one of them did not move the beam spot. Rotational alignment of the quadrupoles was necessary if the image formed by certain parts of excited quadrupoles was a skew ellipse when viewed in the target chamber. The lenses 2, 3 and 4 were rotated until the quadrupole doublets 1 and 2, 2 and 3 and finally 1 and 4 all produced a satisfactorily shaped image spot (70). The quadrupoles were then switched from being powered individually to being powered by two supplies, with lenses 1 and 4 and lenses 2 and 3 in series in accordance with the criteria for the Russian Quadruplet system.

The quadrupole lens currents were then adjusted to bring the beam to a focus. The relationship between the current in the coils and the ion type
and energy is

\[ I = K \sqrt{\frac{E M}{q}} \]

where

- \( I \) coil current (A)
- \( E \) particle energy (MeV)
- \( M \) particle mass (amu)
- \( q \) particle charge (in units of the electron charge)

The approximate value for \( K \) for lenses 1 and 4 is 0.063, and for lenses 2 and 3 is 0.176, \(^{16}\). The values for the coil currents predicted by this equation agreed well with experimental values. The small variation (\( \approx 5\% \)) is most likely due to the slight differences in geometry which would affect the values of \( K \).

The coil currents were then optimised to produce the smallest spot when seen on the glass/quartz target viewed through the target chamber microscope. To measure the spot size the target plate was translated until a copper grid (commonly used with electron microscopes) was in the beam path. The beam was scanned over the grid so that it described either a horizontal or vertical line and it passed over both the spaces and bars of the grid. The copper X-ray signals from the Si(Li) detector were stored by the on line PDP-11 as a function of the scan voltage, (i.e. distance). Typical results of such scans are shown in Figures 6.3 and Figure 6.4. The known distance between the copper bars was used to calibrate the scan by equating this to separation of the scan peaks. The peaks in the scan are a convolution of the beam spot profile and the grid bar cross section. Accurate deconvolution of the data would require a precise description of the bar cross section which is known to be
Figure 6.3 Number of copper K X-ray counts vs channel number (distance) from a vertical scan of a copper grid.
Figure 6.4 Number of copper K X-ray counts vs channel number (distance) from a horizontal scan of a copper grid.
non-simple. A worst case estimate of the beam profile can, however, be made by evaluation of the full width at half maximum of a Gaussian curve fitted to the first difference of the data. The horizontal and vertical dimensions of the beam spot may both be evaluated in this way.

6.2.3 Data Collection

When using the microbeam facility the data collection programme was a modified version of the one described in Chapter 3. The programme 'MICRO' had the same facilities which allowed the collection of a single spectrum from a fixed point, but in addition it was used to control the beam scanning voltages and to store the resulting line scan data. The beam could be scanned in either the X or Y directions, the relevant scan voltage supply being controlled by the PDP-11. The beam was deflected to the start of the scan ('scan offset') and would stay there until an operator defined charge had been collected, at which time it would be deflected on to the next pixel and so on. The scan voltage (length), scan offset and charge per pixel were user defined. The width of each pixel was proportional to the scan length divided by 256, (i.e. the number of channels in a single scan). The line scan produced was one of total X-ray count against distance (uncalibrated). The count was the number of X-rays detected integrated across the total spectrum or across a user defined energy window. Lack of computer memory prevented the storage of the complete energy spectrum from each pixel, which would have been the ideal case as this would have effectively allowed the simultaneous scan of all elements whose X-rays were detected.

It is anticipated that future developments to the data collection facility will include a 2D scan capability which was prevented both by the limited computer memory and an additional computer control port.
6.3 Microbeam Development

Development work on the beam scanning facility and optical viewing system was undertaken during the current project with the aim of improving the performance and usefulness of the microbeam facility. These features were described in earlier sections. The microbeam facility was therefore not available for routine operation until 1985 and late in this project. The opportunity for using the facility was therefore limited and there was insufficient time to carry out an extended study of element distribution in samples of interest. The microbeam was, however, used to examine the distribution of selected elements in some geological samples and was shown to be of use in RBS channeling experiments, (71). To complement the BCF analysis project, and as no suitable biomedical samples were available, some tree ring samples were analysed.

6.4 Tree Ring Analysis

Tree ring analysis is commonly used in the study of pollution. PIXE offers the advantages detailed previously of requiring minimum sample preparation and small sample size. Tree ring analysis may be achieved by sectioning the sample into annual growth samples and analysing the bulk elemental content of each years sample or by mounting a complete section and scanning the proton beam across the surface. Workers at the University of Surrey had previously studied wood samples in pollution studies. NAA was used to examine homogenous samples and SEM to line scan structured samples, (73, 74). Expansion of the project to examine the penetration of preservatives into the wood when applied at the surface was anticipated. It was thought appropriate to test the suitability of using the microbeam to scan similar samples and to determine the spacial distribution of elements within them.
6.4.1 Sample Preparation and Analysis

The samples used were from a branch of an oak tree growing in the grounds of the University about 10m from a major road. The branch had a diameter of 34mm at the sample point. A section approximately 5mm thick was sawn from the branch. The section was cut into longitudinal 3mm wide strips and then further reduced in thickness to about 2mm. The various cuts were all made using cleaned stainless steel tools; the possibility of contamination was acknowledged but not investigated. The sample strips were then freeze dried before being mounted on to the target plate with the rings running approximately vertical. Prior to freeze drying the mean width of the growth rings was measured and noted. There were found to be 9 growth rings of thickness from 0.84 to 2.96mm (±0.05mm). The target plate was coated with high purity carbon as previously described.

The target plate was mounted into the beam line chamber and the 350μm mylar filter was fitted to the detector. The bulk elemental composition of the targets was examined by focussing the proton beam to a diameter of 2mm at the target face and collecting spectra from the central region of the target. The beam was then focussed to a diameter of approximately 500μm and X-ray spectra were collected from the bark. Figures 6.5 and 6.6 show typical examples. The spectra were collected with a beam current of approximately 4nA and a count rate of between 1000 and 1300 cps. Spectra were also collected from the Bowen's Kale reference target.

The beam of 2MeV protons was then focussed to micrometre dimensions at the target face and scanned both horizontally and vertically across a copper grid mounted on the target plate. The resulting scans were later deconvolved to determine the beam spot dimensions and to calibrate the scanning voltages. The beam spot was found to be an ellipse with 'width'
Figure 6.5  Example spectrum from a single position from the middle of a tree ring target bombarded with 2MeV protons and using a 350μm mylar filter.
Figure 6.6 Example spectrum from the bark of a tree ring target bombarded with 2MeV protons and using a 350\(\mu\)m mylar filter.
14±1µm and 'height' 31±8µm. The beam was then scanned horizontally across
the tree rings. The scan length was approximately 2mm; each channel
(pixel) was 0.0078 ± 0.0008mm wide. The energy window for the scan data
collection was set to correspond to some of the elements detected in the
bulk sample. The resulting scans for the elements calcium, iron and
Manganese are shown in Figure 6.7. Each scan was collected with 0.005µC
charge per pixel in about 45 minutes.

6.4.2 Results and Discussion

The elements detected in the bulk analysis of the wood sample were K, Ca,
Cr, Mn, Fe, Cu and Zn. In addition to these elements, As, Br, Rb and Sr
were detected in the bark. It is possible that some or all of these elements
were present due to local pollution or other near surface factors. The presence of bromine in particular is related to pollution form vehicle exhaust. PIXE is clearly a useful tool for sampling data
from different parts of a structured sample; the beam spot diameter can
be adjusted to suit the size of the feature to be examined. This is
comparable to the process of extracting and analysisng homogenised cores
of tree ring samples as mentioned above. Using PIXE in this way however,
requires a minimum of target preparation and is a flexible way of
analysing different sections of the sample.

The scans of the tree ring structure cross approximately 2 years growth.
It was not possible to define exactly where on the growth cycle the scans
started and finished. However, a periodicity of the signal from both
calcium and iron can be clearly seen. The manganese signal shows little
correlation with the change in the calcium signal. The number of X-ray
counts is proportional to the number of atoms of the element of interest
in the volume of interaction. The signal periodicity may therefore be due
Figure 6.7  PIXE microprobe scans of a tree ring sample for the elements calcium, iron and manganese. Plot of counts per pixel vs pixel number (distance). One pixel = 0.0078mm.
to a change in concentration of the element during the annual growth cycle, a change in target density or a combination of these two factors. A change in the concentration of minor and trace elements in the wood during the annual growth cycle might be expected but similarly the density of the wood varies with growth activity.

The opportunity for determining which was the important factor here was not available. This could have been achieved by setting the scan window to collect data from the complete energy spectrum. The major contribution to the integrated count would have been from the low Z minor elements; the integrated count would then have been used to normalise the scan data.

Despite the inconclusive results from these scans the indications were that the microprobe could be used to examine the distribution of elements in wood samples and would certainly be useful in the qualitative examination of the penetration of chemicals provided an element suitable for detection by PIXE is present in the preservative. Calculation of the sampling factor would show the potential for reliable quantitative analysis by PIXE. The PIXE scan width was limited to 2mm X 2mm and so the technique would be ideal for examining variations on this scale. It may be possible to use the results from a series of overlapping scans to examine features larger than this.
CHAPTER 7

DISCUSSION AND RECOMMENDATIONS FOR FURTHER WORK

7.1 Summary

As described in Chapter 1, PIXE is one of a number of elemental analysis techniques available for the examination of biological samples which offers the major advantage of being a high sensitivity multielemental analysis tool with a microanalysis capability. Discussion of the important parameters to be considered when comparing sample characteristics with the capabilities of the analysis technique (which is fundamental to any analysis programme) was made in Chapter 2, with reference to biomedical samples and to PIXE. This approach has not been found elsewhere and is a useful starting point for any analysis programme. Later practical experience was to suggest that the performance of the apparatus is frequently limited by parameters beyond the control of the experimenter and that this factor is itself important when considering using PIXE for an analysis programme.

The layout of the PIXE apparatus used was given in Chapter 3 and developments made to the optical viewing system were summarised. As described in Chapter 4, time was spent in measuring the performance of the Si(Li) detector; the relationship of the detector resolution to the X-ray energy was subsequently used in the development of the spectrum analysis programme HISTO. The methodology of examining breast cyst fluid samples by PIXE was established and reported in Chapter 5. The microbeam facility was developed and its usefulness for elemental micro analysis was established. The subsequent analysis of tree ring samples was detailed in Chapter 6.
7.2 Spectrum Analysis

The importance of a reliable and fast X-ray spectrum analysis programme to the success of a PIXE analysis programme cannot be over emphasised and should be seen as a priority for future work. Up to 40 spectra can be collected in one experimental day when undertaking PIXE analysis. If the planned improvements to the microprobe and data collection facilities are realised, the number of spectra collected in a similar period may increase by a factor two or three. Ideally, it should take no more than a few days to reduce this data to the required format so that time can be spent on results analysis rather than on the data processing stages. SAMPO manifestly failed to satisfy these requirements and in addition was both difficult to use and required considerable operator input. The development of HISTO went some way toward satisfying the spectrum analysis requirements and, being simple in principle, could be adapted for the analysis of other types of spectra. HISTO was tailored for analysis of spectra from the Si(Li) detector used in this project and, to some extent, for the particular samples analysed.

The limitation of HISTO lay in the assumption that the location of a peak could be reliably defined by the detector energy calibration. Short term instability of the Si(Li) detector gain and peak distortion caused by pulse pile up could result in considerable errors in peak area calculation as the peak would be wrongly identified as being non symmetrical. This problem could be resolved in the future by including in the code a method of peak location. As described in Section 4.4.2, a number of such methods have been used by other workers but most of them involve complex numerical calculation using library routines which were avoided in the development of HISTO. A method that could be considered here is a simple $\chi^2$ mimimisation fit of a Gaussian function to a small number of channels
close to the anticipated peak location. Such a peak location procedure may be successful for defining the centroids of statistically significant and well defined peaks arising from the lower Z elements but may prove inadequate in 'locating' the higher energy peaks. A further advantage of peak location would be the facility for internal energy calibration which would reduce the amount of manual intervention required in the data analysis procedures.

7.3 BCF Analysis Project

PIXE analysis has been established as a suitable technique for the qualitative analysis of BCF samples. The sample and target preparation protocol adopted was simple and data collection for 'finger printing' of one sample would take of the order of 20 minutes. Data analysis (to identify the peaks) may have taken up to two to three hours with the computers and codes available during this project. At first glance it would appear that PIXE offers little advantage over NAA in the number of elements detected, phosphorous being the only additional element regularly found by PIXE. However, NAA data collection for the long lived isotopes of, for example, zinc and iron may take several days, so PIXE is clearly a faster technique. In addition, the analysis of sulphur by NAA is complex and is sensitive to the concentration of other elements in the samples. PIXE may therefore be the preferred method for qualitative analysis.

The situation for quantitative analysis is less clear. Although the detection limits calculated were attractive, the indication was that the sample size required for accurate determination of the concentration of the higher Z trace elements was much larger than initially anticipated. The sample size is limited by the maximum beam current that can be used without causing damage to the target (when using a detector filter) and by
the practical limit to the detector countrate of 2000cps when no filter was used. In the latter case the dead time with the long electronic shaping time increases rapidly to the point where pulse pile up occurs and the system live time is dramatically reduced. The counting statistics for the lower Z elements could be improved by collecting data for longer periods; the maximum analysis time would be limited by the stability and availability of the accelerator and, of course, the number of samples that could be examined in the available time would be reduced. Absolute determination of elemental concentrations was found to be impractical because of the inability to measure the required experimental parameters to a tolerance that would have made subsequent calculations meaningful. Bowen's Kale was selected as the most suitable reference material available for a comparative study, and its use allowed the determination of 10 elements in BCF.

Problems with data normalisation further limited the capability of quantitative analysis although a partial solution was found by calculating the relative elemental concentrations. The relative concentrations calculated for the four elements P, S, Cl and Ca agreed well with the results obtained by previous workers. The values for the relative concentrations of Fe, Zn, Br, Rb and Sr should be treated cautiously following the calculation of sampling factor for these elements which indicated that the sample size for reliable results should be considerably larger.

PIXE may, therefore, have some role to play in any future analysis programme for BCF or similar samples, though note should be taken of the discussion of the PIXE facility below. It must be acknowledged, however, that the overall approach to the underlying aim of the BCF analysis work (i.e. to test the hypothesis that elemental levels in the fluid may act as
an indication of the disease state of the patient's breasts) must be completely reviewed if significant progress is to be made. Testing such an hypothesis would require both the reliable examination of many more samples than were analysed during this study and a much greater liaison with the medical staff interested in the tests. Use of an analysis technique that is practically as well as theoretically capable of analysing a large number of samples with the fastest possible turnround and the best degree of automation of spectrum analysis would be ideal. A greater degree of co-operation with the medical staff would facilitate the study of contamination introduced during sampling and would also provide invaluable help in relating the results of the physical analysis to the medical condition and history of the patients.

Future work on the project, particularly if PIXE is to be used, should involve further investigation of the reference materials available. A blood plasma reference material would be ideal for this work where the matrix composition of the sample and reference should be as closely matched as possible. If no suitable material is available it may be possible to manufacture a suitable reference that can be used for this project. Alternatively, the method of 'spiking' the samples with known concentrations of one or more elements could be investigated. Such techniques may facilitate the analysis of those elements not assayed during the current study.

7.4 PIXE and Microbeam Analysis

The use of the accelerator and beam line facilities at the University of Surrey have been assessed for use in the trace element analysis of biological/organic samples by PIXE. The equipment is easily used for the qualitative analysis of thick targets. Equipment restrictions limit the
practicality of analysing thin targets. Absolute quantitative analysis was found to be impractical during the current project and comparative analysis was found to be restricted by the reference materials available, by the problems with data normalisation and by the required sample size.

PIXE has been available as an analysis technique since the late 1970's and some facilities are reported to have reached a high degree of sophistication. The theoretical and practical capabilities and applications of the technique are well documented. As a result the expectations for PIXE analysis are high. The author's experience has suggested, however, that the overall support for the PIXE facility had the greatest impact on the quality of work that could be achieved in practice. Specifically, it was possible to engineer solutions to problems with the beam line apparatus but it was not possible to improve the poor performance of the Van der Graaff accelerator or to compensate for the lack of time available for PIXE analysis. Far from being a dedicated PIXE analysis facility or even a facility freely available for development, the accelerator was effectively dedicated to RBS analysis and was released for about 1 to 1 1/2 days per month for PIXE development and analysis purposes. It must be accepted that much of the work that can be recommended as requiring attention in the future to improve the capabilities of the system for both quantitative analysis and microprobe scanning is unlikely to be practical unless considerable more time and resource can be made available to an experimenter. If this is not so the viability of using the PIXE facility must be questioned.

The most important parameter requiring attention in the future is the procedure for data normalisation. Use of the total collected charge was shown to be inadequate. Alternative normalisation methods mentioned in an earlier Chapter include the use of the RBS signal from a thin metal foil in
the beam path before the target or the RBS signal from the target itself. The latter method would only be appropriate during the analysis of homogeneous targets. The present equipment however, does not allow the simultaneous input of signals from two detectors so the RBS signal cannot be sampled automatically and must be collected by manually switching the electronics used for counting. A more satisfactory solution, particularly if absolute analysis is to be considered, would be beam current measurement by some other means such as a rotating metal vane which intercepts the beam.

As mentioned above, the data processing and transfer facilities were limited and the spectrum analysis software available was inadequate. HISTO provides only a partial solution for this work and the data analysis facilities should be seen as an integral part of the PIXE apparatus. The installation of a semi-automatic analysis programme that could be used to analyse the spectra at the point of collection would be ideal, particularly when qualitative analysis is undertaken. Under those circumstances the results obtained from such a programme may be sufficient and further analysis may not be necessary. Improvement to the target chamber facilities to allow the examination of a number of thin targets at one time would be useful and would increase the potential application areas of PIXE.

Improvement to the data collection software for the microbeam operation was underway at the end of this work. Such improvement was necessary to allow greater flexibility in the use of the microbeam. The addition of 2-dimensional scanning facilities together with appropriate data display software would significantly improve the quality of information available from the PIXE equipment.
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