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An Efficient Analytical Technique for Research in Biology and Medicine

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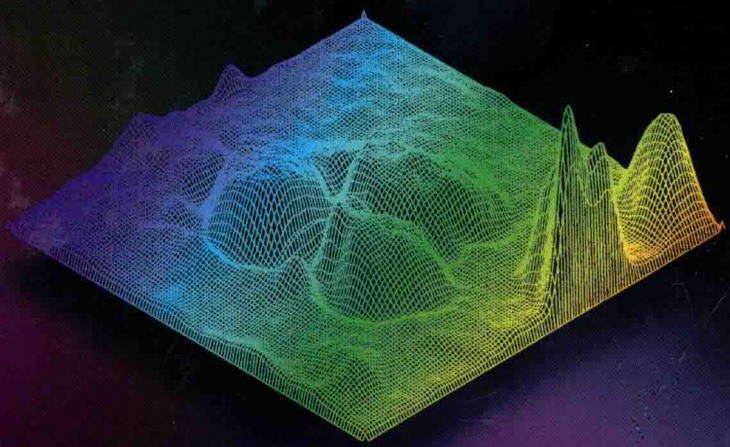

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APPLICATIONS OF

NUCLEAR MICROPROBES IN THE LIFE SCIENCES

An Efficient Analytical Technique for Research in Biology and Medicine

For
Monique
Gardrat
and
Florence
Moretto

PREFACE

Why should a nuclear microprobe be used in the field of life sciences? What are the advantages of using such a sophisticated technique? This book aims at answering these and other questions. In order to provide an adequate answer, we describe the instrument's capabilities and compare its performance with those of other methods. We also provide numerous examples of applications with a view to providing as complete a picture as possible.

The first nuclear microprobe with a focused ion beam was constructed at Harwell (England) twenty-five years ago and was the result of investigations undertaken by J.A. Cookson. The first facility, with a spatial resolution of a few microns, was already equipped for Rutherford Backscattering (RBS), Nuclear Reaction Analysis (NRA) and Particle Induced X-ray Emission (PIXE). It became rapidly evident that the analytical usefulness of such a tool was beyond that of other more familiar methods employed at that time by chemists. Since then, the number of nuclear microprobes established in the world has steadily increased. Today more than thirty microprobes are in operation not only in Europe, but also in North America, Africa, Australia and Asia. Most of these probes have been fitted in nuclear physics laboratories, close to Van de Graaff particle accelerators, which are widely used instruments operating in the 0.5–10 MeV energy range and were designed during the sixties for nuclear spectroscopy experiments.

Due to the success of nuclear physics during this period, most known nuclei were rapidly characterised and a wealth of information on nuclear and atomic interaction was accumulated. By then, nuclear physicists were studying higher energy instruments in order to investigate the nuclear structure more deeply. At the same time, the energy of particles available at current accelerators increased. It has now reached several hundred giga-electronvolts.

Low energy devices were thus progressively abandoned by fundamental physicists and became more accessible for other applications such as material analysis. However, nuclear data obtained in the domain of MeV energies during these years still remain a crucial interest, at least for elastic scattering or nuclear reaction analysis. The impressive development of ion beam microanalysis during the last two decades can be easily explained when both this large database and the increasing availability of low energy accelerators are being considered.

Given that nuclear microanalysis is still a relatively novel technique in the life sciences, we hope to show the uninitiated reader how nuclear microprobes may solve related problems in this field. First, we present a theoretical but simple approach to the numerous nuclear and atomic phenomena induced by MeV charged particles in matter. The information which can be deduced from such an interaction is also described bearing in mind the practical aspects. In Chapter 2 the reader is given a short description of the microprobe principle with special emphasis on the focusing system. Specific experimental details, including detectors, beam scanning and data acquisition, are also examined. From a microprobe user's point of view, the third chapter is presumed to be one of the most useful. It consists of a brief overview of alternative techniques of microanalysis usually employed in the biomedical field. For each method, a critical appraisal of the possibilities and limitations is given, as are references of the various applications. The microanalyst can expect to find here valuable help in the choice of a technique likely to solve his problem.

Chapters 4 and 5 are devoted to specific technical difficulties arising from a microanalysis of living tissues. The main sample preparation schemes are described with particular attention being paid to cryotechniques. Problems of irradiation damage, undoubtedly the main limitation of nuclear microanalysis, are then treated and advice is given on how to overcome the disastrous effects of this phenomenon. The last four chapters deal with examples of application in the respective fields of pharmacology, physiology, pathology and clinic, trace elements, and toxicology. Numerous references are included in these chapters. We believe that they will constitute a guide for new microprobe users. We hope that this book will not only furnish a good description of the usefulness of nuclear microprobes but will also indicate further applications in the biomedical field.

CONTENTS

CHAPTER 1

CONCEPTS AND PRINCIPLES

1.1 Introduction

Before we describe some of the biological applications of nuclear microprobes, it is necessary to present a summary of its principles and capabilities which allow the use of methods of nuclear analysis with a micron or sub-micron ion beam. We will therefore expose, in the first part, the different phenomena taking place during the interaction of such a charged particle beam with matter. For readers unfamiliar with ion beam techniques, we will also explain how it is possible to make use of these numerous processes for material analysis. The principles of the different methods based on X-ray fluorescence, elastic scattering and nuclear reactions, will then be presented followed by their advantages and limitations.

The reader will therefore be prepared to address, in the following chapter, a comparison between the nuclear microprobe with alternative microanalytical methods more commonly employed in the life sciences. Even though nuclear microanalysis is still regarded as a complex technique carried out only in laboratories able to afford such an expensive facility, it offers various unique capabilities and often remains the sole solution to some specific problems. It is, for instance, the sole method that allows, during the same run and on the same sample, the simultaneous use of different techniques of analysis. This point is particularly useful for the analysis of biological specimens. The measurement and the mapping of trace elements can be performed as well as the determination of the organic mass of the sample, thus giving the analyst the possibility to express quantitative results in terms of elemental concentration (in gram of element per gram of dry matter). No technical description is available here. It is dealt with in the next chapter which will be devoted entirely

to a purely technical description of the microprobe, including most key components for beam production and transport. The production of high energy ion beams by electrostatic accelerators and the arrangements generally employed for their focusing will be described in greater details.

Several publications have already dealt with this subject in a more or less exhaustive manner. The reader is referred to the excellent book edited by Watt and Grime (1987) entitled *High-Energy Ion Microbeams* or, for a more succinct approach, a review by Malmqvist (1991).

1.2 Interaction of charged particles with matter

Methods of nuclear analysis make use of the numerous occurring interactions when a particle beam impinges on a target. Atomic inelastic processes are concerned as well as nuclear elastic and inelastic collisions. Light nuclei with a mass in the range 1–4 (from protons up to alpha particles) are mostly employed, each of them being of particular interest for specific applications. One can, for instance, mention deuterons which are more particularly used, in the aim of inducing nuclear reactions. The energy of these particles generally ranges between 0.5 and 4 MeV (1 MeV=10^6 eV). Most interactions useful in analytical applications take place in this energy range. At a lower energy, the interaction probability becomes really too weak to produce a signal which can be really exploited and paradoxically the analysis is more destructive. Above 4 MeV, other nuclear phenomena may induce some extra background signal and may consequently give rise to eventual data misinterpretation.

Let us review the reasons why light particles are generally employed :

- These beams are easy to produce with high current intensity. Moreover, their transport and focusing do not necessitate very intense magnetic fields.
- The detection of these particles by means of classic silicon detectors does not present any technical problem since no pulse high defect is present.
- Their range in materials is longer and allows therefore an indepth analysis of the sample. In addition, the energy deposit is lower compared to that of heavier ions. Damage caused by the passage of the beam in the target is consequently limited.

- For the same energy, a heavier ion will have a lower particle velocity. Therefore it is interesting to use lighter particles because the emission yields of some interaction products (particles or photons) become greater when the velocity of the incident particle is raised. This is particularly true for X-ray fluorescence.

The quasi-totality of microprobes currently in operation in the world works with light ion beams. However, some very specific applications require heavier ions. This is, for example, the case of the following nuclear reaction:

$$^{15}\mathrm{N} + {}^{1}\mathrm{H} \longrightarrow {}^{12}\mathrm{C} + {}^{4}\mathrm{He} + \text{gamma-ray}$$

It is well-known for the analytical sensitivity it provides and is used for the detection of hydrogen in materials. This point will be developed later in the section on nuclear reactions. This is true also for techniques taking advantage, for light elements detection purpose, of the elastic recoil of target nuclei which may occur during scattering of projectiles. In that case, it is necessary to use a projectile heavier than the recoiling nucleus. Moreover, the incident energy must be high enough to ensure the ejection of the nucleus from the sample and consequently ensure its detection. This process is known as ERDA (elastic recoil detection analysis). In fact, this technique is hardly used with microbeams, at least in the area of life sciences.

Before we detail the different interactions, the definition of some terms widely used in nuclear physics are provided hereafter.

1.2.1 Cross-section

In the field of nuclear physics, the probability for a nuclear reaction or another kind of interaction is generally expressed in terms of cross-section. This cross-section represents the area of a hypothetical disc associated with each target nucleus. According to a classic image, this area is calculated in such a way that the reaction does take place with a 100% probability if the incident particle crosses this surface. For example, the geometric cross-section that a nucleus presents to an incident particle is πr^2 (r is the radius of the nucleus). If the particle penetrates into this disk it will result in a direct collision with the

nucleus. However, for most interactions the effective cross-sections are higher than the geometric cross-sections. The effective cross-section is always expressed in terms of square centimetres. If we assume 6×10^{-13} cm as an average value for the nuclear radius, an order of magnitude may be given for the geometric cross-section: $3.14(6\times10^{-13})^2 \approx 10^{-24}$ cm^2. This value is consequently reflected in the dedicated unit which is the *barn*, where 1 $b = 10^{-24}$ cm^2.

On the other hand, the cross-section is numerically equal to the reaction rate (number of events per second) calculated for one nucleus placed in a flux of one incident particle.cm^{-2}.s^{-1}. A realistic order of magnitude can be estimated for the yield of events susceptible to be achieved with the techniques which will be further described. For example, a mono-elemental atomic layer for carbon represents a number N of about 10^{15} atoms per square centimetre. If we assume an incident flux of 10^{10} particle.cm^{-2}. s^{-1} (1.6 nano-ampere for mono-charged ions) and a cross-section of σ =1 barn, the number N_{ev} of events per second is given by the following relation :

$$N_{ev} = N \sigma \Phi$$

The result of a calculation carried out with the previous values gives a number of 10 events per second.

Sometimes, the interaction does not lead to an isotropic emission of radiation. The probability of emission varies with the angle of emission and this angular distribution must be taken into account for the selection of the optimum geometry of detection. It is then useful to define a so-called differential cross-section which is representative of the probability of emission in a given direction. Usually, the differential cross-section is related to a solid angle portion $d\Omega$ centered on a given direction θ with respect to the incident beam. The number dN_{ev} of emission products issued from the reaction and emitted in the direction θ, within the solid angle $d\Omega$, is given by the formula :

$$dN_{ev} = N\Phi\left[\frac{d\sigma}{d\Omega}\right]d\Omega$$

where $\dfrac{d\sigma}{d\Omega}$ represents the differential cross-section (in barn/steradian unit).

Integrating over the whole solid angle, we have obviously:

$$\sigma = \int \left[\frac{d\sigma}{d\Omega} \right] d\Omega$$

1.2.2 Energy loss and ranges

Although not relativistic, the velocity of particles generally employed for ion beam analysis remains high. For example, the velocity of an alpha particle accelerated to an energy of 4 MeV is about 1/30 the velocity of light, namely 10^9 cm/s. When these particles impinge on the target, they usually undergo numerous interactions with target electrons that slow them down and finally stop them.

The range of these particles in the target is a function of their initial energy. Numerous theoretical studies of the energy loss of charged particles in matter have been carried out in the past. In 1930 Hans Bethe found a quantum relation valid for most cases in the MeV energy range. We give here this formula with relativistic corrections added some years later by Bloch (1933):

$$\left(-\frac{dE}{dX} \right) = \left(\frac{4\pi e^4 z^2 NZ}{mv^2} \right) \left[Log\left(\frac{2mv^2}{I} \right) - Log\left(1 - \frac{v^2}{c} \right) - \frac{v^2}{c^2} \right]$$

where dE/dX is the stopping power of the material with an effective nuclear charge Z, an ionisation potential I and an atomic density N. The two letters z and v represent the charge and the velocity of incident particles respectively while m is the electron mass. This formula is at the basis of numerous other works on this subject. From this formula, it must be pointed out that the stopping power is not related to the mass of the incident particle. Only its initial velocity is taken into account. This means that two particles of different masses but with the same initial velocity will have approximately the same range. For example, a 1 MeV proton will have the same range as that of a 4 MeV α particle, namely 15 μm in silicon.

This concept of stopping power and range is widely used for ion beam analysis, more so when Rutherford scattering or resonant nuclear reactions are