



## 1.41 Applications of NAA at Institute of High Energy Physics

Zhang Zhiyong Chai Zhifang

Laboratory of Nuclear Analytical Techniques, Institute of High Energy Physics,  
The Chinese Academy of Sciences, P. O. Box 918, Beijing 100039, China

### Abstracts

Recent achievements in application studies of neutron activation analysis (NAA) at Institute of High Energy Physics, The Chinese Academy of Sciences are briefly described. A small number of selected areas and problems, particularly in life sciences, are highlighted because they present challenges for NAA and its prospects in the future.

### Introduction

Neutron activation analysis is a well-established technique for the determination of elemental concentration at trace and ultratrace levels in a variety of matrices. It provides the sensitivity, selectivity and capability for multielemental determinations that are often required in studies involving the role of trace elements in biological, environmental and geological studies. Research using this technique has been carried out in Institute of High Energy Physics (IHEP), The Chinese Academy of Sciences (CAS) for over forty years and great achievements in developing NAA for a number of applicational purposes in research and industry have been made. Especially, in recent years, the methodology of molecular activation analysis (MAA) has been developed for studying the chemical species of essential and toxic elements in environmental and biological systems <sup>[1]</sup>. In this paper, the most recent progress made in our laboratory in several fields is briefly reported below.

### Applications of NAA

#### *Life sciences*

The study of trace elements in life sciences has been characterized by two stages: (1) determination of bulk composition of trace elements in samples of interest; and (2) correlation between total contents or concentrations of one element and another, and corresponding synergetic or antagonistic effects. During these two development stages, the neutron activation analysis has made a major contribution. However, with the development of trace element research, more and more emphasis is being oriented to individual chemical species rather than bulk analysis which is in many cases meaningless when assessing biological or environmental effects of elements.

In speciation analysis of trace elements, a number of nuclear and non-nuclear techniques were established among which the so-called molecular activation analysis (MAA) could play a unique role. The methodology of MAA, in fact, is a combination of conventional activation analysis with physical, chemical or biological separation procedure. For example, in order to study the chemical species of trace elements in biological samples, i.e., distribution patterns of trace elements in cells and subcellular fractions, and their combination with biological macromolecules (protein, enzyme or nuclear acid, etc.), the first step is to selectively separate various species fractions, followed by identification and determination. For this purpose, the physical or chemical characteristics of biological macromolecules, e. g. size, charge, solubility, mobility or specificity of biological functions are often utilized. Chemical stepwise

dissolution, phase separation, ion exchange chromatography, coprecipitation, ultracentrifugation, gel chromatography, PAGE gel electrophoresis, etc. are commonly employed methods that are combined with INAA. MAA inherits the merits of conventional NAA, e.g. high sensitivity, good accuracy and precision, sampling quantity and multi-elemental analysis ability, etc. and is able to provide information on the chemical species of elements in systems of interest.

*a. MAA for I in algae and human liver*

Up to now, the study on chemical species of iodine in marine algae is scarce and some available data are controversial. Taking into account of the fact that algae are becoming a well-received green food and a main source of dietary iodine for Chinese people, 40% of whom live in I-deficient areas, the chemical species of I in seven marine algae, *Codium fragile*, *Ulva pertusa*, *Monostroma nitidum*, *Gracilaria confervoides*, *Sargassum kjellmanianum*, *Dictyopteris divaricata* and *Laminaria japonica*, were studied by a newly established MAA procedure based on leaching, precipitation and NAA<sup>[2]</sup>. The experimental results indicated that the contents of total iodine and various species of iodine are different in different alga specimens. 99% of total iodine are soluble in *Laminaria japonica*, whereas in other algae, the soluble iodine contents range from 16 to 41%. In leachates of marine algae, 61 to 93% of soluble exist as I<sup>-</sup>, with less than 5% IO<sub>3</sub><sup>-</sup> and 5 to 37% organic iodine.

Besides thyroid, human liver is also an important target organ for iodine accumulation<sup>[3]</sup>. Some preliminary results from a study of the chemical species of iodine in human liver are given in Table 1.

Table 1 Distribution of iodine in subcellular fraction of human liver

Subcellular Fraction	Iodine mg/g (d.w.)				A%	B%
	Pre-permeation		Post-permeation			
	Whole sample	Protein	Whole sample	Protein		
Liver	0.321	4.472	0.531	2.917	78.3	
Nuclei	0.932	6.413	1.194	5.346	12.4	48.0
Mitochondria	0.825	7.015	0.891	5.412	22.1	15.7
Lysosome	0.681	9.238	0.900	8.746	80.7	10.6
Microsome	0.171	1.471				1.0
Cytose	0.135	1.120				17.7

A Percent of I content of pre- over post-permeation;

B Percent of I content of subcellular fraction in whole liver

*b. MAA for Se in human liver*

Since Se is an essential element for humans, its biological effect and chemical species have been widely studied. However, data on its distribution and behavior in human liver are still scarce. For this reason, we recently used MAA to study the subcellular location of Se and cytosolic distribution of Se contained in human liver<sup>[4]</sup>. Our results indicated almost half of Se existed in the nuclear fraction, followed by cytosol and mitochondria. Very few percent of Se were present in lysosome and microsome. Further, in a Sephadex G-200 gel chromatographic experiment we found four Se-containing components with MW 335±20, 70±5, 45±1.5 and 14±3 kD in the soluble fractions of human liver (See Fig.1). The most abundant Se-containing component, peak II, accounted for 70% of total cytosolic Se. Peak II was subjected to further purification via DEAE Sepharose fast flow ion exchange chromatography with a linear gradient of 10 to 500 mmol/L (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub> buffers. It is clear that peak II consists of 4 Se-

containing proteins, whose identification is in process.

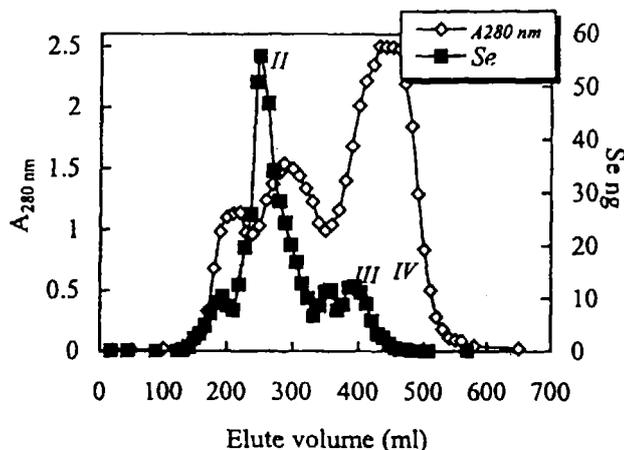


Fig. 1 Profiles of selenium content and protein of human liver cytosol in Sephadex G-200 gel chromatography

*c. MAA for Chromium (III) in rat organs*

Chromium (III) is an essential trace element in human glucose and lipid metabolism. Its deficiency can cause diabetic-like symptoms. In order to study the formation of Cr-related biological macromolecules in cells and its physiological and pathological roles, the enriched stable isotope  $^{50}\text{Cr}^{3+}$  tracer technique combined with NAA was used to study the subcellular distribution and chemical species in rats [5, 6].

The subcellular distribution of Cr in liver, pancreas, kidney and testes of normal rats was significantly different from that of diabetic ones. The nuclear fraction has the highest Cr concentration in the liver cell of both normal and diabetic rats. The diabetic rats retain more Cr in the mitochondrial and lysosomal fractions of liver homogenate.

The chemical species of Cr in liver cell cytosol, blood serum and urine of rats were studied by gel filtration chromatography and NAA. It was found that Cr (III) was mainly combined with a high-molecular-weight protein in liver cytosol. The Sephadex gel chromatography of liver cytosol, serum and urine of both normal and diabetic rats reveals a

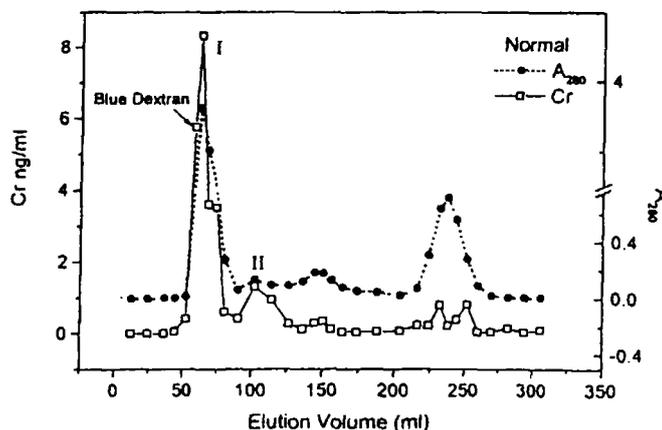


Fig. 2 Sephadex G-25 chromatography of the liver cytosol of the normal rat

similar low-molecular weight and Cr-containing compound in the same fraction of elutes. The excretion rates of this compound of diabetic rats are much higher than the normal group. This might be a main reason to explain why the diabetic rats retain low Cr in their bodies than the normal group.

*d. MAA for rare earth elements in plant and animal cells*

The fact that rare earth elements (REEs) are being widely used in China and other countries in modern industry and agriculture is resulting in higher levels of REEs in the environment. However, little is known about the physiological function of these elements, and there is no information available on their distribution in cellular or subcellular level.

A preliminary study on the distribution of REE in plant cells was carried out by neutron activation analysis and biochemical separation techniques. Young leaves of *Brassia napus* were treated with cellulase and mesophyll protoplasts were obtained. Isolated protoplasts were washed with osmotic solution containing EDTA for several times and purified by density gradient centrifugation. Then, the contents of REEs in the protoplasts were determined by INAA. The results show that REEs can enter the cells of the plant. A further study shows that the REEs within plants cells mostly localize in chloroplasts, which account for 30% of REEs in leaves and LREEs were enriched relative to the heavier ones [7, 8].

The distribution of Sm and Yb in subcellular fractions of rat livers and the metabolism of Sm and Yb in rats in  $\mu\text{g}$  level were studied by enriched stable isotope tracer  $^{152}\text{Sm}$  and  $^{168}\text{Yb}$  combined with INAA. The results showed that livers and bones were two main organs in which Sm and Yb were accumulated, the contents of Sm and Yb in kidney, spleen, lung, heart, testicle were very low. In livers, about 30% of Sm and Yb localized in mitochondrion, but only <10% for nucleus [9].

*Geo/Cosmo-sciences*

*a. Ir in the K-T boundary clays*

Iridium, one of six platinum group elements, is often used as an extraterrestrial indicator in geochemistry and cosmochemistry. Further, the chemical species of Ir is associated with its origin. According to the known chemical behaviors of Ir five possible iridium species are available in nature, i. e., (1) soluble complex ion; (2) sulfide; (3) metallic phase; (4) noble

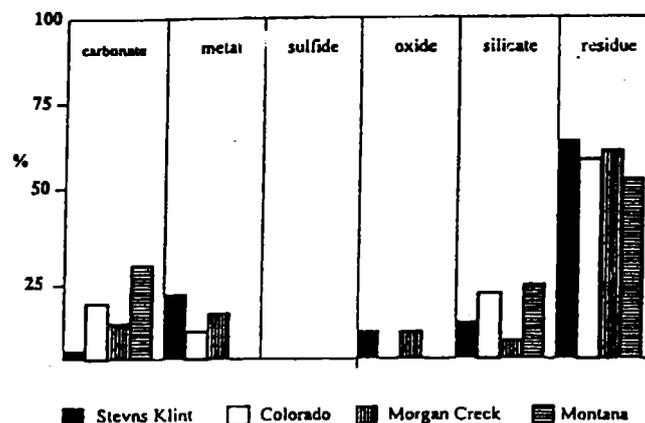


Fig. 3 Distribution patterns of Ir in K-T boundary samples

nugget; (5) organic complex. Thus, we attempted to reveal the origin of anomalous Ir in the Cretaceous and tertiary (K-T) boundary samples via chemical species analysis by a newly-developed selective chemical dissolution procedure combined with INAA and RNAA<sup>[10]</sup>.

Fig.3 shows the distribution patterns of Ir at 4 K-T boundary samples. It is clear from Fig. 3 that the residue phase is the main host phase of Ir for all K-T boundaries, no matter whether in marine or continent sediment. Our results provide more evidence to favor the extraterrestrial origin of anomalous Ir at K-T, although volcanic activity and geochemical processes also play a role in Ir enrichment.

*b. Relationship between Ir concentration in hen eggs and the Ir-enriched feed*

At the beginning of 1960s, the upper Cretaceous dinosaur bone and egg fossils were discovered in large quantity in the Nanxiong basin, Guangdong Province of China. Inspired by this discovery, further investigations were carried out including the analysis of trace element content of the dinosaur eggshells. The results show that the concentration of some trace elements, such as Co, Cr, Cu, Mn, Ni, Pb, Sr, V and Zn, is very high in dinosaur eggshell. Moreover, Ir enrichment was found in the fossil samples of dinosaur eggshells at the K-T boundary. A hypothesis was proposed that trace element enrichment might play a very important role in the event of dinosaur extinction and Ir content in dinosaur eggshell could stem, directly or indirectly, from dinosaur food or intake. In order to verify this hypothesis, a model experiment was carried out in a group of fowls for studying the origin of the Ir enrichments in eggshell fossils of dinosauria<sup>[11]</sup>.

Four hens (A1-A4) were fed by adding ammonium hexachloroiridate into their feeds for 14 days. The Ir concentration in three fractions (eggshell, albumen, egg yolk) of their eggs was determined by INAA. The results indicated that Ir was present in all the three parts of the eggs and the Ir concentration was about  $2\sim7\times 10^{-10}$  g/g in the eggshell fraction (Fig. 4) compared to  $5.6\times 10^{-7}$  g/g in feed. Therefore, we can estimate the ratio from the feed over the eggshell via gastrointestinal pathway to be about 0.08%. The new result is useful to evaluate the Ir-enriched eggshell fossils of dinosaur and to interpret the origin of the mass extinction of dinosaur at the end of Cretaceous.

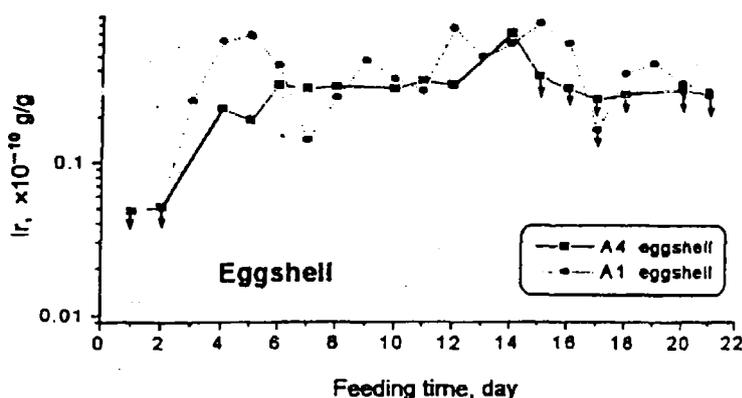


Fig. 4 Variation of Ir concentrations with the feeding time in the eggshells  
(↓ Below the detection limit)

*Environmental sciences**a. Biomonitoring trace element atmospheric deposition using lichens in China*

Lichen is one of the biomonitors with good accumulation property for determining the heavy metal deposition in terrestrial ecosystems. This well-established technique has been widely used in European countries for evaluating atmospheric deposition status since late 1960s. This work is the first attempt in China to apply this technique to retrospective study of the atmospheric heavy metal deposition trend for the period of nearly thirty years (1960s-1990s) in a remote Southwestern China area by using the early preserved lichen samples. The purpose of this study is two-folded: (1) Assessment of atmospheric background deposition; (2) Evaluation of the long-term anthropogenic emission impact on this area.

Concentrations of 32 elements in lichen samples were determined by INAA. The concentrations of Ag, As, Ce, Cr, Cs, Eu, Fe, Hf, La, Nd, Ni, Sc, Se, Sm, Tb, U, Yb and Zn were dropped down by the temporal prolongation. The results indicate that the extent of heavy metal atmospheric deposition among the sampling sites was declining during the past decades [12].

*b. Extractable organic halogen in milk*

Man-made toxic organic halogen compounds viz., PCBs, DDTs, HCHs, HCB and chlordanes (CHLs) are ubiquitous in meat, egg and milk due to their lipophilic characteristics. In spite of the capabilities of modern separation and mass spectrometry methods, the known compounds account for only 2-15% of the total amount of extractable organic halogen (EOX). The only practical method for the measurement of total Cl, Br and I in EOX is NAA.

In this study, EOX in milk samples which were collected from different areas of China was separated and therein concentrations of Cl, Br and I were simultaneously determined by INAA.

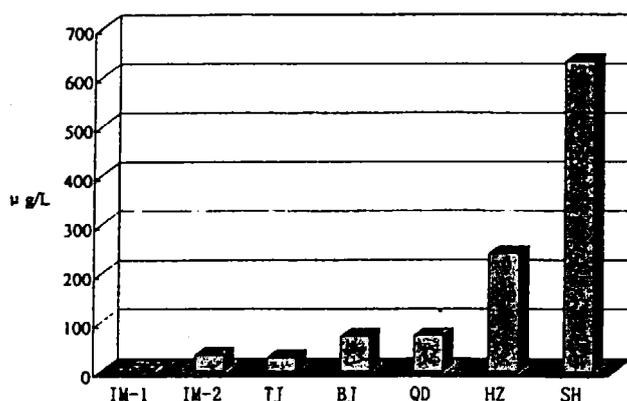


Fig. 5 Concentrations of EOX in milk (IM, Inner Mongolia; TJ, Tianjin; BJ, Beijing; QD, Qingdao; HZ, Hangzhou; SH, Shanghai)

Results indicated that organic halogen concentrations in milk decrease in the order of Cl >> Br > I. The EOX contents in samples collected from south China especially from coastal area were much higher than those collected from north and inland areas (Fig 5). This phenomenon can be explained by the dosage difference of organic chlorine used in different areas.

*c. Study on the elemental species of atmosphere aerosol*

Knowledge of the overall elemental composition and chemical species of aerosol is essential for understanding of their source and influences to the environment. For this purpose, a five-phase sequential extraction procedure has been applied for elemental composition and species in aerosol samples. Analyses were carried out by INAA <sup>[14]</sup>.

Experiment data show that elements have various distribution complexions in different phases. Among the lithospheric elements, Sc, Al, Fe and Ti are mainly enriched in the residual phase; REEs are mainly present in the oxide and residual phase. The residual phase does not readily react after their deposit, and has little effect to the environment, while oxide phase can react with other compounds. Distribution proportions of elements from anthropogenic sources in five phases have also some difference due to various sources. These elements have large proportion of environment mobile phases; that is, when deposit on the earth's surface by dry and wet removal processes, exchangeable phase can directly enter water circulation. In proper condition, carbonate phase, oxide phase and organic phase can react with other compound in environment to enter ecosystem as water dissoluble compound, thus have strong influences to environment.

*Archaeological sciences*

Ancient ceramics is an important part of Chinese traditional culture. However, it is difficult to identify the provenance and age of these cultural relics. Fortunately, trace element spectrum of ancient ceramics shows not only some information of producing area, but also the time-dependent characteristics.

Nuclear analytical methods, such as INAA, PIXE and XRF, are particularly suited to the analysis of elemental composition in ceramics because the matrix of silica, aluminum, and other light elements mostly do not interfere with the determination. At present the major problem which block the application of nuclear analytical methods in ancient ceramics study is the relation and consistence of experimental data from various methods <sup>[15]</sup>.

We have collected ancient potsherd from typical regions of Zhejiang, Anhui, Jiangxi, Henan, Hebei, Shanxi, Shaanxi, Hunan and Fujian province. At first, the ancient porcelain specimens with accurate layer and time will be analyzed by non-destructive method such as XRF, SRXRF and external beam PIXE. Afterwards, they will be analyzed by INAA. After having studied the experimental data gained from various nuclear analytical methods, we will try to find the relation between undestructive and destructive analysis data to offer scientific basis and method for appraise of ancient ceramics.

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