MOLECULAR ACTIVATION ANALYSIS FOR CHEMICAL SPECIES STUDIES

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Abstract. The Molecular Activation Analysis (MAA) mainly refers to an activation analysis method that is able to provide information about the chemical species of elements in systems of interest, though its exact definition has remained to be assigned. Its development is strongly stimulated by the urgent need to know the chemical species of elements, because the bulk contents or concentrations are often insignificant for judging biological, environmental or geochemical effects of elements. In this paper, the features, methodology and limitation of MAA were outlined. Further, the up-to-date MAA progress made in our laboratory was introduced as well.

1. INTRODUCTION

The study of trace elements in biology, environment and geology has roughly experienced two stages: (1) on existence and bulk composition of trace elements in sample of interest; and (2) on correlation between total contents or concentrations of one element and another, and corresponding synergetic or antagonic effect. During these two development stages the Neutron Activation Analysis (NAA) and other nuclear analysis methods, e.g. PIXE, have made a major contribution. However, with development of trace element research, more and more emphasis is being oriented to their chemical species, rather than the bulk analysis, which is meaningless in many cases when assessing biological, environmental or geochemical effects of elements [1]. A literature survey clearly demonstrated an implicitly increasing tendency for speciation study of elements [2], from a few papers in this field in early seventies to hundreds nowadays annually. In literature one can quite often find two terms: species and speciation. Both are arbitrarily used without any difference. Strictly speaking, the "species" means a chemical form, state or valence of an element in a medium, e.g. Cr^{3+},CrO_4^{2-}, $Cr_2O_7^{2-}$ or low molecular weight chromium compound, etc. refer to the possible species of chromium element, whereas the "speciation" means an action resulting in transformation, alteration or variation from one species to another. Of course, more discussion on their definitions is desirable.

In order to meet this challenge in species analysis of trace elements, a number of nuclear and non-nuclear techniques were established, in which the so-called MAA can play a unique role in the species analysis (e.g. [3–10]). The term of MAA refers to an activation analysis method that is able to provide information about the chemical species of elements in system of interest, though its definition remains to be assigned. Since the MAA was first proposed in 1986, it has become one of the main techniques for species analysis. In this paper its features, methodology and limitation will be outlined. In the meantime, some practical MAA examples recently performed in our laboratory will be presented as well.

2. FEATURES OF MAA

In general, the MAA inherits the merits of conventional NAA, e.g. high sensitivity, good accuracy and precision, small sampling amount and multi-elemental analysis ability, etc. During analytical process, it is easier for MAA to keep the original chemical species of elements unchanged than for other non-nuclear methods. In some cases the MAA is even a sole choice to do speciation analysis.

However, the critical point of the MAA is that it is not permitted to alter the original chemical species of elements existing in system of interest, or the alteration must be under strict control and is able to be traced; in the meantime not allowed to form the "new artefact" originally not present in system.

3. METHODOLOGY OF MAA

The methodology of MAA, in fact, is a combination of conventional nuclear analysis methods with physical, chemical or biological separation procedures. For example, in order to study the chemical species of trace elements in biological samples, e.g. distribution patterns of trace elements in cell 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. The chemical stepwise dissolution, phase separation, ion exchange chromatography, coprecipitation, ultracentrifugation, gel chromatography, PAGE gel electrophoresis, etc. are of common practice combined with NAA. Besides, the PIXE is also a valuable nuclear analysis technique for chemical species research, which possesses the unique scanning characteristics providing a two-dimensional distribution information of element species [11, 12].

Another important factor which has to be taken account is the quality assurance for chemical species analysis. Unfortunately, only a few reference materials for species analysis are available [13, 14]. The preparation and certification of more species reference materials are desirable.

4. PRACTICAL APPLICATION OF MAA

Since 1986 the MAA has been widely used in environmental and biological fields to study the chemical species of trace elements in various samples, e.g. tissue, hair, urine, blood, sediment and water [2–10] and rarely in geology [15]. The recent progress made in our laboratory in this field is briefly introduced as follows:

4.1. MAA for iridium in Cretaceous and Tertiary boundary clay

Iridium, one of 6 platinum group elements, is often used as an extraterrestrial indicator in geochemistry and cosmochemistry [16]. Further, the chemical species of Ir is associated with its origin. According to the known chemical behaviors of Ir 5 possible iridium species in nature are available, i.e. (1) soluble complex ion; (2) sulfide; (3) metallic phase; (4) noble nugget; (5) organic complex. Thus, we attempted to reveal the origin of anomalous Ir at the Cretaceous and Tertiary (K-T) boundary samples via its chemical species analysis by a newly-developed MAA procedure based on a chemical stepwise dissolution and radiochemical and instrumental NAA [15]. Figure 1 shows the distribution patterns of Ir at 4 K-T boundary samples. It is clear from Fig. 1 that the residue phase is the main host phase of Ir for all K-T boundaries, no matter what they are marine or continent sediment. Our MAA results provide more evidence to favor the extraterrestrial origin of anomalous Ir at K-T, although the volcanic activity and geochemical processes also play more or less role in the Ir enrichment.

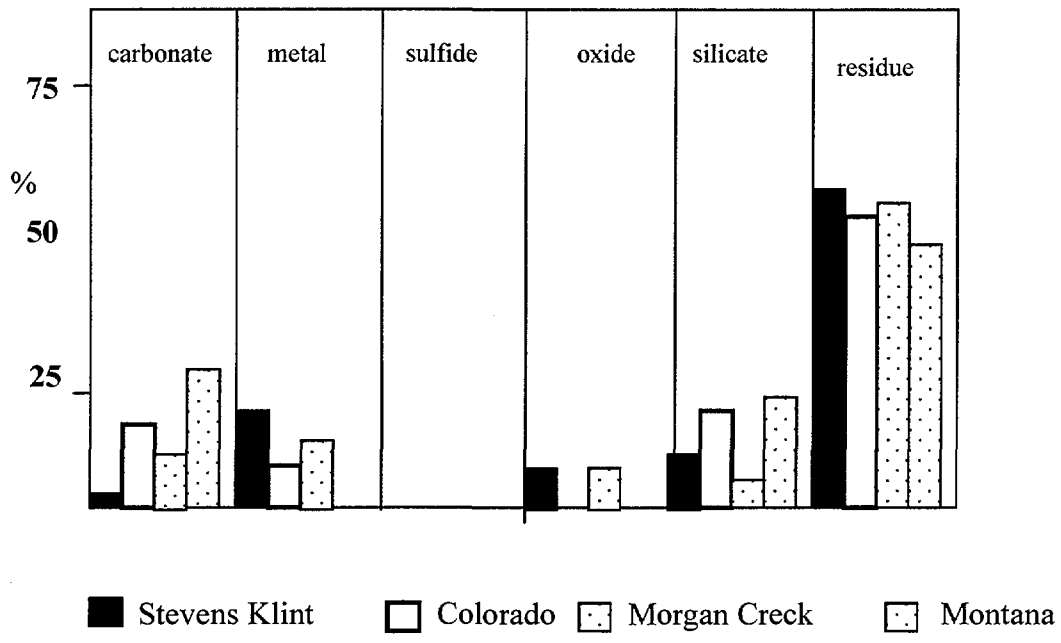

FIG.1. Distribution patterns of Ir in K-T boundary samples.

4.2. 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 [17, 18]. 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, of whom 40% live in the I-deficient area, we studied the chemical species of I in 7 marine algae *Codium fragile, Ulva pertuse, Monostroma nitidum, Gracilaria confervoides, Sargassum kjellmanianum, Dictyopteris divaricata and Laminaria japonica* by a newly-established MAA procedure based on leaching, precipitation and NAA. The experimental results for the I species in 7 algae indicate that the contents of total iodine and various species of iodine are different in different alga specimen. 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 iodine exist as I^- with less than 5% IO_3^- and 5 to 37% organic iodine [19, 20].

Besides the thyroid, the human liver is also an important target organ for iodine accumulation. At the moment we are studying the chemical species of iodine in human liver. The preliminary results are listed in Table 1.

4.3. MAA for Hg in human hair

It is known that the methylmercury (Me-Hg) has a strongly toxic effect on human embryo, which is evidently different both qualitatively and quantitatively from that on adult. This hazard can be seen during the prenatal and postnatal stages. In order to study the transfer mechanism of mercury, mainly Me-Hg, from pregnant women to their new-born infants, we developed two simple MAA procedures to determine the longitudinal variation of total, inorganic and organic mercury contents in their hair sample [21, 22]. One is based on the

Table 1. Distribution of iodine in the subcellular fraction of human liver (A = percentage of iodine content of pre- over post-permeation; B = percentage of iodine content of subcellular fraction in whole liver)

Subcellular fraction	iodine μg/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
cytrose	0.135	1.120				17.7

selective extraction of methylmercury from hair by hydrochloric acid (see Fig. 2) and other is to take advantage of the volatility of methylmercury cyanide. The hair sample is mixed with potassium hexacyanoferrate and sulfuric acid. Then the resulting methylmercury cyanide is absorbed by cysteine paper, which is irradiated in reactor and directly counted. The interlaboratory comparison demonstrates that their accuracy, precision and reproducibility are satisfactory.

4.4. MAA for rare earth elements (REEs) in fern

The fact that REEs are being widely used in China and other countries to modern industry and agriculture, is resulting in the higher level of REEs in environment. However, until now little work on their chemical species in natural plant has been reported. The information about whether there is REE-bound macromolecules in natural plant specimen is not available. Thus, we established a new MAA procedure based on pH variation , out salting, ultracentrifugation, gel filtration chromatography and electrophoresis, etc. and INAA, to study the REE-bound proteins in a natural plant fern, *Dicranopteris dichotoma*. Our results (Fig. 3) identified two new REE-bound proteins (RBP-1 and RBP-2) in this species of fern. The molecular weights (MW) of RBP-a and RBP-2 on Sephadex G-200 are about 8×10^5 and less than 1.24×10^4, respectively. Their SDS-PAGE graphs show that both contain two protein subunits with MW 14100 and 38700, that seem to be conjugated proteins, glycoproteins with different glyco-units [23, 24].

4.5. MAA for Se in human liver

Because of essentiality of selenium to human-being, its biological effect and chemical species have been substantially studied. However, the report on its distribution and behavior in human liver is 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. Our results indicated that almost half of Se existed in the nuclei fraction, followed by cytosol and mitochondria. A very few percentages of Se were present in lyzosome and microsome. Further Sephadex G200 gel chromatographic experiment found 4 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. 4). The most abundant Se-containing component, peak II, accounted for 70% of the total cytosolic Se. The peak II was subjected to be further purified via DEAE Sepharose fast flow ion exchange chromatography with a linear gradient of 10 to 500 mmol/L $(NH_4)_2CO_3$ buffer. It is clear that the peak II consists of 4 Se-containing proteins. The identification of these Se-containing proteins in human liver is in progress [25].

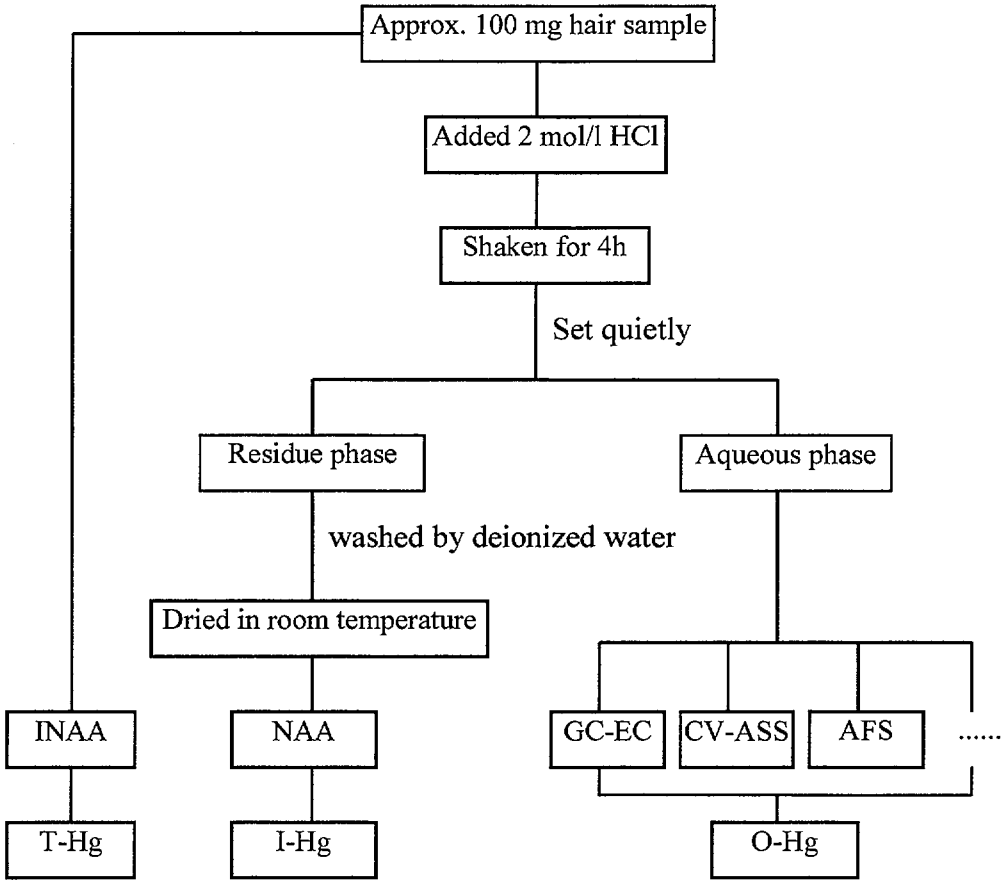

FIG.2. Flow chart of isolation of inorganic and organic Hg from the hair sample by HCl (T-Hg, I-Hg and O-Hg mean total, inorganic and organic mercury, respectively).

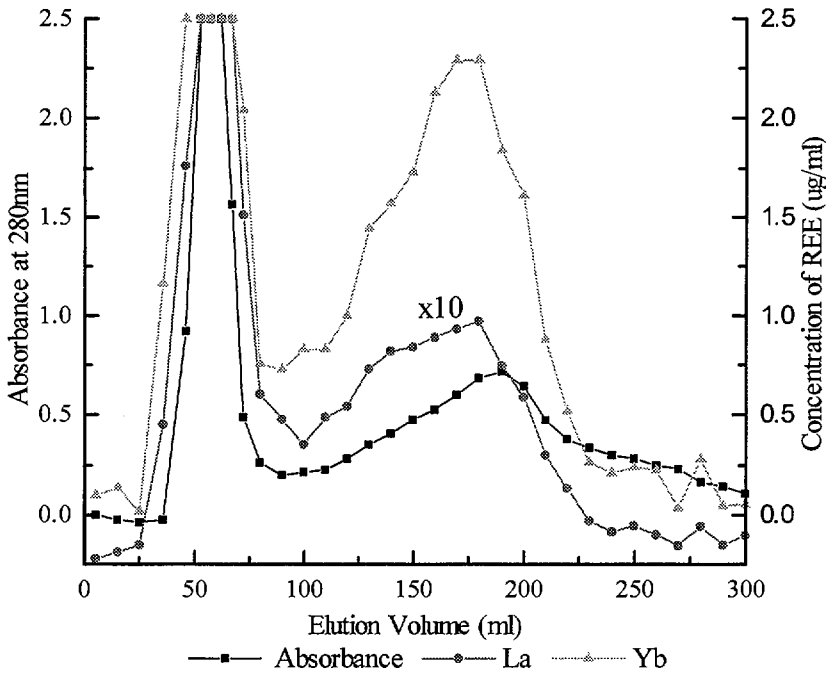

FIG. 3. Two new REE-bound proteins found in a species of fern by a combination of NAA and UV absorption spectrometry.

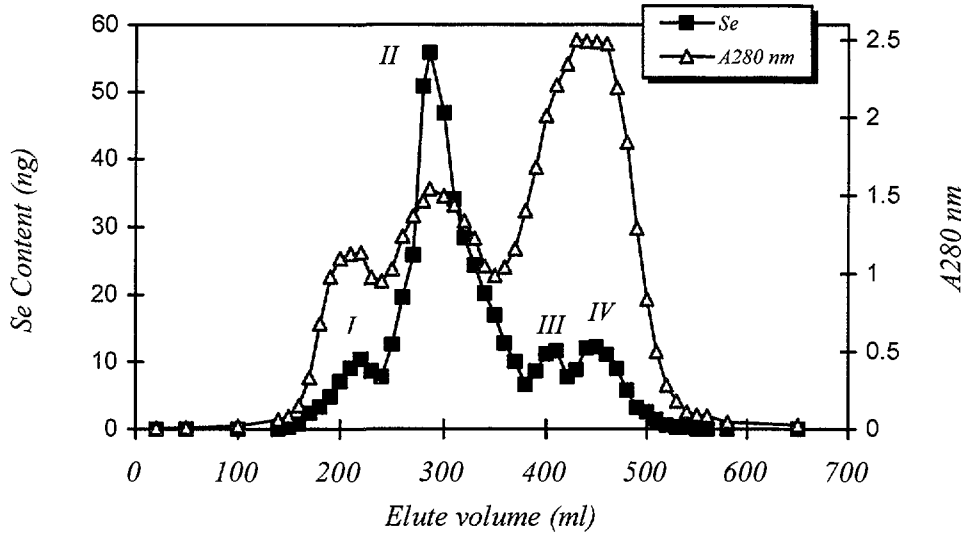

FIG. 4 Profiles of selenium content and protein of human liver cytosol on Sephadex G-200 gel chromatography

The above-mentioned examples have clearly demonstrated that the important biological effects of chemical species of essential and toxic elements in environmental and biological systems and geochemical explanation of indicative elements have given a strong impetus to develop the MAA and will further stimulate its necessity, and for the foreseeable future it is hard to imagine how such studies can be pursued without a heave reliance on the molecular activation analysis. The new application topics will need to improve the available methodology of MAA and more new MAA procedures will come out under this impact.

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