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Toxic trace metal speciation: importance and tools for environmental and biological analysis

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1.- INTRODUCTION: CONCEPT AND IMPORTANCE OF TRACE ELEMENT SPECIATION

Total element determinations of toxic metals, both in environmental and in biological materials or in living organisms, are increasingly demanded by society. This determination of low levels of toxic metals, and also of essential and therapeutic trace elements, is normally accomplished by atomic spectrometry methods, very appropriate for this analytical task (e.g. concentrations below 10 $\mu g.g^{-1}$ in tissues, 10 $\mu g.l^{-1}$ in biological fluids or less than 1 $\mu g.l^{-1}$ in river waters, need today to be monitored). However, every chemist in his laboratory recognises that a trace element, and so a toxic metal, can exist in a given real sample, e.g. a sediment, urine, serum, etc, in a most varied number of physico-chemical forms (e.g. in the case of lead: Pb^{+2}, $Pb(OH)_2$, $Pb(OH)_4^-$, $(CH_3)_4Pb$, $(CH_3\text{-}CH_2)_4Pb$, etc). More recently, the scientific community has concluded that toxicity, bioavailability, bioactivity, transport in the organism, bio-geological distribution/transportation and, thus, the eventual impact of the toxic element in our body and environment, will be dictated by the particular species or form present in the sample. In this sense, total element determinations by atomic spectroscopy, as discussed before, of a toxic element as As, Hg or Sn are insufficient today, and sometimes misleading, to assess the toxicity of a food or sediment (e.g. arsenobetaine is not toxic, methylmercury is much more toxic than Hg^{+2} and tributyltin is a most potent biocide, while Sn(IV) is not). Therefore, additional "speciation" information to complement total toxic element determinations is being increasingly demanded, both in environmental and in clinical/biological issues (1,2).

In order to provide such type of information demanded in the most varied fields and for many chemical species (Figure 1) there is an enormous interest in the last five years or so to develop analytical strategies and methods able to tackle this modern challenge of "speciating" trace element concentrations in the different forms or species actually present in the samples under scrutiny.

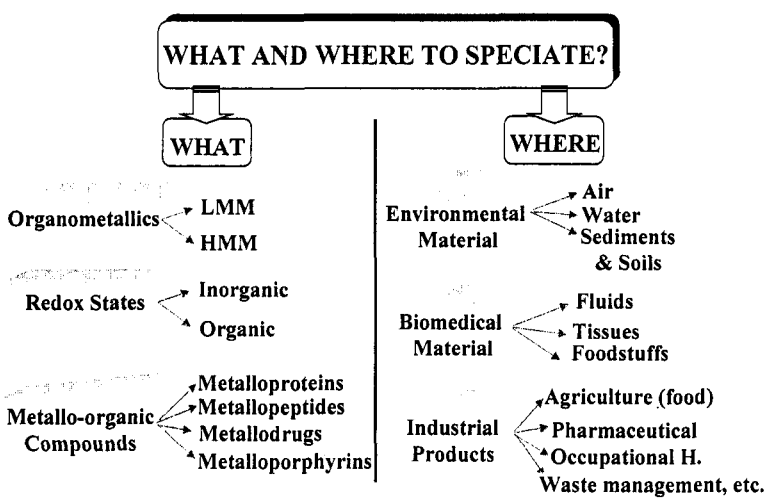

Fig. 1 What and where to speciate?

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Other presentations are published in this issue, pp. 2259–2336.

2.- THE ANALYTICAL CHALLENGE

Of course, atomic methods are by definition non-speciating methods as the atomiser destroys the molecules because measurement of atoms or ions is aimed at. Thus, for speciation analytical approaches are needed able to provide reliable molecular information of the sought particular species of the toxic metal studied. This is a real challenge to modern Analytical Chemistry, because:

a) It is very difficult to preserve the integrity of such species along the whole analytical process.

b) We have to deal with extremely low concentrations (down to $\mu g.l^{-1}$) and fractionate them for speciation.

c) Such minute concentrations are "buried" in a complex real matrix (e.g. of a sediment, serum, etc).

A few years ago, we proposed a useful classification (3) to order the species according to their lability. This could be useful to guide us on ensuring that the nature of the sought species is preserved all along the steps of the analysis (sampling, storage, pretreatment, separation and measurement).

Whereas reliable equipment for routine total trace element measurements is readily available on the market, there is hardly any commercial instrumentation to separate and measure species of trace elements in a reliable way. In fact, speciation of toxic metals is carried out at present almost exclusively in research laboratories.

3.- WHICH ANALYTICAL TOOLS CAN BE EMPLOYED TO FACE THIS MODERN CHALLENGE OF TRACE ELEMENT'S SPECIATION?

The four different possible approaches to trace metal speciation are given below (2):

a) Computational approach (very limited)

b) Direct species-specific techniques (sensors and biosensors)

c) Hybrid techniques

d) Spectral characterization techniques (bioinorganic scientists)

However, it is undebatable today that the so-called "hybrid" techniques (that is, the coupling of a powerful separation with an element-specific atomic detector) are preferred to solve real -life speciation problems. Of course, the separation can be "off-line" (non-chromatographic) and there are important examples of the use of such methodology in the field of biological materials (e.g.: mini-column separation of the sought species and final "off-line" analysis, after its release from the column, by electrothermal atomic absorption spectrometry, ETAAS (4)). However, it appears much more convenient to couple on-line a powerful separation unit (Gas Chromatography, GC, High Performance Liquid Chromatography, HPLC, Capillary Electrophoresis, CE) with an adequate atomic detector operating in a continuous manner to provide on-line, real-time, sensitive element-specific detection (e.g. analytical plasmas).

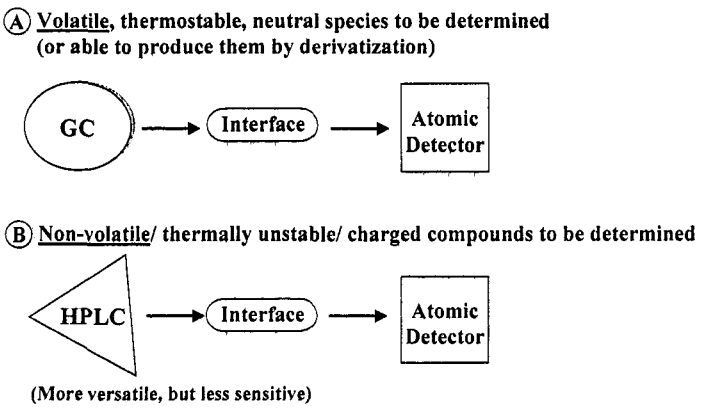

Fig. 2 Separation techniques.

The selection of the separation technique depends on the nature and physical properties of the species of the toxic element to be determined. Fig.2 shows schematically the more used "hybrid" approaches, with the main criteria used to select the more adequate strategy for speciation of a given form of the trace element under study.

4.- CHEMICAL SPECIATION TO ASSESS ENVIRONMENTAL POLLUTION

As a rule, the low levels of toxic metals (Hg, Cd, Pb, Tl, Sn, etc.) in the environment do demand a preconcentration step of the sought species. To do so, such species have to be usually derivatized to a volatile form (e.g. with $NaBH_4$, $NaBEt_4$, etc) which is adequately trapped (e.g. by a cryogenic trap, as described in the numerous environmental papers by the group of Olivier Donard (5)). Then, the derivatized species are desorbed/released by a proper heating of the trap and are injected in a Gas Chromatograph to be detected by Microwave Induced Plasma (MIP)-Atomic Emission Spectrometry (AES) with multielement capability (1)) or a gas chromatographic column coupled to an Atomic Absorption Spectrometer, AAS, with a hydrogen-enhanced atomiser flame (5). Water, atmospheric particulate and sediments can be analysed for toxic species in this way.

As an illustration, Fig. 3b shows the type of results obtained trying to determine different organotin species of natural sediments in our laboratory. Derivatization with sodium tetraethylborate of an acetic acid extract and liquid-liquid extraction of the ethylderivatives into hexane was selected for the tin compounds mild extraction from the sediment. This organic extract (200 µl) was then analysed by Capillary-GC-ICP-MS (where the interface has been built and designed in our laboratory). Comparison of Figs 3a (same organotins speciation in a sediment but using cryogenic trapping in a packed column with heating separation (5) and ICP-MS detection) and 3b clearly indicates the importance of a good chromatography to obtain a satisfactory resolution for the different organotins (MBT: monobutyltin; DBT: dibutyltin; TBT: tributyltin).

The present trend is to reduce analysis time by integrating the steps of digestion/derivatization/extraction from the solid (the more time-consuming steps) in a single operation carried out in a focalised microwave oven (6). Important developments expected in sample preparation procedures, enhanced by microwave heating, could push present research in speciation of environmental samples into future simple, fast and convenient determinations of toxic species in sediments, soils, atmospheric particulate, biota which could be implemented in routine laboratories to solve environmental problems created by toxic metals.

5.- CHEMICAL SPECIATION IN MEDICINE AND BIOLOGY

The problem of speciation of toxic/essential trace elements in biological material is more complex than that described above to speciate "metal contaminants" (usually well known organometallics of Hg, Pb, Sn, etc. having an initial antropogenic origin well established by now). Such contaminants are not "integrated" in soils and sediments, while in living organisms they are metabolised and transferred into metal-biomolecules of most varied nature and chemical complexity. The extreme possible complexity of biological systems, and so of the metabolised biocompounds, renders very often extremely difficult a correct interpretation of results obtained by usual speciation analytical techniques. There are dozens of

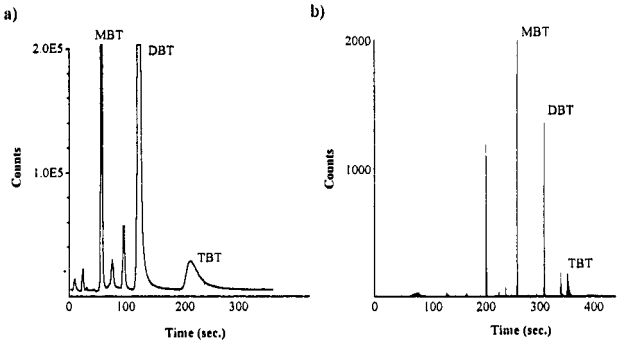

Fig. 3 Results obtained trying to determine different organotin species of natural sediments.

possible bioelements to follow and we have also hundreds of low molecular, medium size, high molecular, etc, metal-biocompounds which could, in principle, contain the metal. In these instances, speciation of a trace element in living organisms calls for a knowledge and judicious choice of different-principle based separations assisted by different -complementing-each-other specific detectors (7). The use of complementary analytical appproaches to get speciation information of toxic metals in biomedical materials and biochemical problems (an information validated by a good matching of speciation results obtained by the different techniques/ methods employed in the examination of the same sample) will be illustrated by resorting to the progress made in my research group during the last ten years or so studying Al and Si speciation in human serum (8).

Experiments carried out with non-chromatographic separations (ultramicrofiltration) and "off-line" ETAAS detection clearly showed that only 11 ± 2 % of serum Al is ultrafiltrable, while for silicon this figure amounted to 45 ± 5 % for fresh human serum samples. Addition of desferrioxiamine (DFO), a low molecular mass chelating drug used for Fe(III) and Al(III) detoxication, rendered 75 ± 2 % of total serum aluminium into an Al-Low Molecular Mass compound (which can now be ultrafiltered). Carrying out complementary speciation experiments using Chromatographic Separation - ETAAS detection we observed that trasferrin seemed to be the only serum protein binding quite strongly Al(III). Silicon, however, seemed to form weak bonds with all the main serum proteins (8). Anionic exchange HPLC and Fast Protein Liquid Chromatography with ETAAS and ICP-MS detection have been used in our laboratory (Fig.4) providing additional confirmation that transferrin is the single Al(III) transporting protein in human serum (9) and two different Al-Transferrin complexes have been recently identified using ICP-Sector Field MS detection (10).

The mechanism of Al detoxication in renal failure patients during hemodyalisis sessions can be understood in the light of all these matching speciation results and the readers interested in more details of this speciation research (and of its biochemical/clinical implications regarding Al toxicity to renal failure patients) are referred to my recent review in Analusis (8).

CONCLUSIONS

It is clear that there is an urgent need of chemical speciation to solve environmental and ecotoxicological modern problems posed by toxic metals in environment. Their toxicity (e.g. organometals) depends on speciation and therefore a reliable assessment of real environmental risk posed by toxic metals demands metal speciation information. Similarly, bio-geochemical transport mechanisms of metals are determined by the real species present (e.g. biomethylation/ volatilisation of Hg^{+2} by algae). The more toxic species can be accumulated in living organisms (e.g. fish accumulates methylmercury) which could be then used as human food (posing a much greater toxicological threat).

Similarly, there is an urgent need of chemical speciation information of toxic metals and semimetals in Biology and Medicine because bioavailability, metabolism and excretion, toxicity, biological activity and detoxifying processes will depend on the particular species considered; of course, transport of the metal in the body and its final deposition/storing in target organ (e.g. brain and bone for Al(III)) will also be conditioned by the speciation of the element.

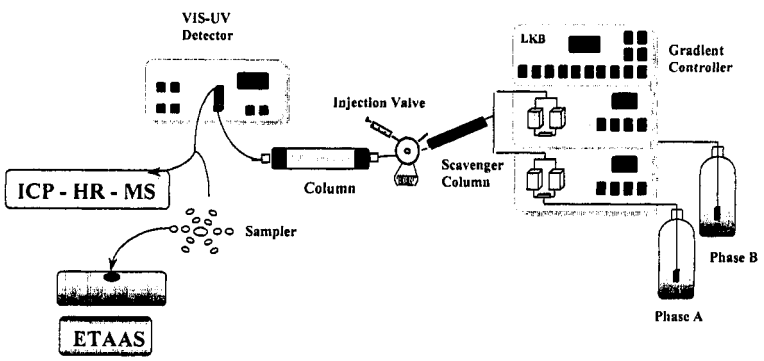

Fig. 4 Fast protein liquid chromatography.

At present it seems that the so-called "hybrid" techniques, having a powerful separation unit coupled with an atomic detector, offer the more powerful analytical approach to provide such required speciation information. In any case, orthogonal or complementary techniques providing matching results are invaluable to face speciation problems were the identity of the species is unknown, as demonstrated for aluminium and silicon speciation in human serum (8).

Finally, the complex, multidisciplinary character of speciation of trace elements becomes obvious, both in environmental and in life sciences, calling for a real cross-fertilization among different scientists (including metallo-organic chemists, biochemists, pathologists, analytical chemists,etc). In order to allow a more effective and faster development of basic elemental speciation knowledge, and its application in Environmental Sciences and Bio-Medicine, there is no doubt that practical actions should be undertaken inmediately to overcome the present lack of interactions among the different professionals involved, affected or needing trace element speciation information.

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