Modern and Hyphenated Analytical Techniques for Metal Analysis of Environmental, Food, Pharmaceutical and Biological Matrices

GEORGE ZACHARIADIS
Department of Chemistry, Laboratory of Analytical Chemistry
Aristotle University of Thessaloniki
Panepistimioupoli, 54124 Thessaloniki
GREECE
zacharia@chem.auth.gr

Abstract: - Analytical chemistry offers today sophisticated techniques in order to estimate or accurately determine the levels of many hazards and, among them, of toxic heavy metals like lead, mercury, cadmium, tin, chromium etc. Modern hyphenated techniques usually combine the capabilities of a separation technique with the power of a sensitive and selective detector. The most sensitive hyphenated detectors are based on the mass spectrometric determination of chemical substances or the atomic spectrometric determination of elements through their spectral lines. Heavy metals are found in the environment or in food or even in biological tissues in various forms (i.e. species) and should be monitored in regular basis. Based on the long-term experience of our laboratory in relative applications, various examples showing the improved possibilities of the instrumentation of modern analytical techniques are presented and discussed in this paper, in relation to the complexity of the sample matrices.

Key-Words: - Analytical methods; hyphenated techniques; biological samples; pharmaceutical; environmental matrices; food samples; metals.

1 Introduction

Mercury (Hg) is highly toxic element and its toxicity is depending on its various chemical species. It tends to bioaccumulate in the living organisms and the food chain. In the past mercury pollution has been a major environmental issue. The methylmercury derivative of natural biotransformation processes is more toxic than its inorganic precursor, causing severe neurological problems and several lethal accidents were reported [1]. Both inorganic mercury and methylmercury species are extremely harmful and even low concentrations at the mg kg\(^{-1}\) level may lead to severe clinical symptoms and death. Direct contact with the skin is very dangerous and special care is required to avoid breathing methylmercury vapours.

Tin (Sn) is a very useful metal in industry, but its organometallic compounds are harmful for the organisms. Organotin compounds have been used as ship-color antifouling agents, as agricultural biocides, as stabilizers in polymers and to various other purposes. Short- to medium-term exposures have shown neurotoxicity, developmental toxicity, immunotoxicity, and endocrine disruption to be relevant end-points [2]. Since alkylation and dealkylation processes are likely to happen in the environment and in the living organisms, it is important to develop speciation methods to analyze several of the most toxic organotin species.

Lead (Pb) is a very toxic metal and many people were exposed to it for years, through the use of leaded fuels or paint formulations. Various biological effects including the central nervous system are currently considered as primary effects associated with increasing concentration and accumulation of lead in critical organs and tissues [3]. Lead can also inhibit some enzymatic activities of heme biosynthesis of human organism [4].

Cadmium (Cd) is another metal widely used in industry and many people are occupationally exposed to this metal. The main route of absorption is the respiratory tract and main target organs are kidneys and lungs. Cadmium concentration in kidneys is an estimator of medium- to long-term exposure while that in blood reflects mainly recent exposure.

Cobalt (Co) and trivalent Chromium (Cr\(^{III}\)) are known to be essential for humans in low-level concentration in metabolic procedures and may also affect the balance of pro-oxidants in blood. However, increased cobalt concentrations can be toxic to human and other organisms. The hexavalent chromium is highly toxic and its presence of Cr\(^{VI}\) in biological systems has been implicated in specific forms of cancer [5].
2 Applications and Discussion

2.1 Human blood samples analysis by Electrothermal Atomic absorption spectrometry (ETAAS).
Although there is a constant demand for accurate analytical methods for metal determination in whole blood, few detailed studies about the concentration in different blood fractions have been reported until now, which include Cu, Co, Cr, Fe, Mn, Ni, Zn [6].

The trace elements distribution in different human blood fractions can provide useful information with respect to various biochemical process and mechanisms. The use of blood fractions in medicine and blood transfusion makes the differential estimation of the heavy metal levels in separated fractions more interesting. Also, metal determination in blood fractions is of high interest in toxicology concerning acute and chronic exposure. Centrifugation as a separation technique is capable to enrich a specific fraction of a whole blood sample with high mass constituents and metal containing proteins [7].

Atomic spectrometric methods are frequently used for the estimation of the total content of blood samples in metals. Using for example electrothermal atomic absorption spectrometry (ETAAS) it is possible to determine trace elements in low levels requiring sample aliquots of only few µL. Several factors are known to affect trace elements determination by ET-AAS. Biological matrices like blood are known to cause interferences to the electrothermal atomization of trace elements, thus various matrix modifiers are used to improve the accuracy and selectivity of the analysis. Ashing and atomizing the sample in presence of a matrix modifier can improve the performance characteristics of the method.

The quantitative estimation of absorbance measurements, especially for biological samples, is important point of studies and it requires special attention to the calibration technique, which finally will be used. It is worth to mention that biological samples, which were acid digested, present substantially different behaviour from the acidified aqueous standards. To remedy this problem the standard addition technique is recommended among various calibration techniques. The slope of the reference curve (calibration or standard addition procedures) reflects the sensitivity of the determination.

The analysis of partitioned blood fractions is a more complicated process as compared to the analysis of whole blood matrix. The matrices of variable blood fractions affect the sensitivity of the determination. Of great importance is also the matrix modifier because the resulting digests from various blood fractions are not atomized in the same rate in the graphite tube conditions. With the proposed method it is possible to determine cadmium, chromium, cobalt and lead in blood fractions, which is useful for monitoring of blood levels in various toxicological analyses.

2.2 Honey samples analysis by Inductively Coupled Plasma Atomic Emission Spectrometry
Metal determination in sugar-rich foodstuffs has been a challenging analytical task due to the interference arising from the matrix. Sample pretreatment is usually required to destroy the organic matrix and to extract the metal ions bound in organic complexes. When matrix mineralization is applied [8] for the determination of heavy metals in honey and sugars, there is a considerable risk of contamination or analyte loss during sample pretreatment. The simultaneous metal determination using ICP-AES without any dry or wet sample dissolution was applied in honey samples and it was previously reported [9]. The sensitivity of the method with respect to each metal was evaluated and matrix effects on the atomization process were examined using aqueous matrix, mixture of glucose and fructose, commercial sugar, and finally honey. The diluted solution was directly nebulized and the maximum concentration was estimated. Higher sample concentrations caused plasma instability. The accuracy and the precision of the developed method were calculated and also the obtained recoveries were very good and the detection limits of several metals like Cd, Ni, Mn, Co, and Cu were allowed the routine analysis of commercial samples of honey, sugar, glucose and fructose.

2.3 Pharmaceutical samples analysis by Inductively Coupled Plasma Atomic Emission Spectrometry
Various pharmaceutical products like antitussive syrups, antibiotic powders, antibacterial formulations, drugs etc. are extensively used to treatment of various diseases and infections. The extensive application of drugs and consumption leads to the establishment of fast analytical methods for determination of heavy metals in routine analysis. Considering the maximum allowable exposure, national and international pharmacopoeias
have established limits and recommended tests of the concentration of various metals [10, 11].

The most common approach to analyze pharmaceutical samples by atomization techniques is the application of a preliminary wet or dry sample digestion step and aspiration of the resulting solution into the nebulizer. Direct introduction of solid sample in the form of slurry is an alternative technique, which offers significant advantages such as eliminating time-consuming digestion steps and avoiding possible losses or contamination.

In this context we have developed a method of aspiration of slurries into the nebulization system of inductively coupled plasma atomic emission spectrometer, in order to achieve quantitative multielement analysis of powdered anti-inflammatory drugs and of antibiotic formulations as well as multivitamin supplements [12, 13, 14]. The slurries were prepared by mixing the ground pharmaceutical tablets with dilute nitric acid and a surfactant solution. Two different nebulization systems were tested, one with a cyclonic spray chamber and another with a scott-type double-pass spray chamber. The recovery obtained and other analytical figures of merit were evaluated and compared to those obtained employing the wet-acid digestion technique. The robustness and applicability of the method can be approximately demonstrated by the sensitivity variation of the method when applied to various drug matrices, as it is illustrated in the example of Figure 6.

### 2.4 Biological samples analysis by Gas Chromatography Microwave Induced Plasma Emission Spectrometry (GC-MIPAED)

An example of a speciation study concerning the presence of organotin compounds in human urine is selected to highlight the efficiency of the hyphenation of gas chromatography combined to a microwave induced plasma atomic emission detector, GC-MIPAED [15, 16]. The method was based on liquid-liquid extraction (LLE) in hexane and subsequent gas chromatographic separation of seven organotin species, namely monobutyltin, dibutyltin, tributyltin, tetrabutyltin, monophenyltin, diphenyltin and triphenyltin. After in-situ ethylation to form ethylated less polar derivatives they could be determined directly in the urine matrix. Alkylation of the ionic species by sodium tetraethylborate reagent is required in order to render the ionic and less volatile species to fully alkylated and more volatile ones. After comparative investigation, hexane extraction was proved as a better alternative to the headspace SPME. The critical parameters which have a significant effect on the yield of the successive liquid-liquid extraction procedure were examined, and the method was optimized for use in direct analysis of undiluted human urine samples. The total analysis time for the seven species was less than 10 min. GC-MIPAED was proved efficient to achieve detection limits in the sub-µg L\(^{-1}\) concentration level, the overall method precision was less than 12 %, and the detection limits at the sub-µg L\(^{-1}\) (as Sn) scale. This makes the method suitable for rapid sensitive screening analysis of human urine samples without dilution of the sample.

### 2.5 Environmental and biological samples analysis by Gas Chromatography Mass Spectrometry (GC-MS)

Mercury determination in the environment is always a very interesting task. Analysis of environmental and biological liquid samples by GC-MS, needs to isolate the mercury species from the rest of the matrix constituents, at least the low volatile ones. Derivatization reagents like sodium tetraalkylborate (NaBR\(_4\)) can be employed for aqueous phase and derivatization while Grignard reagents can be used for organic phase derivatization. After the alkylation step there are two alternative procedures to extract the fully alkylated mercury compounds: liquid-liquid extraction or solid phase microextraction. In the latter case one can apply either the direct immersion of the
solid phase into the liquid phase or the so called headspace microextraction. With the developed method [17] methylmercury could be determined in presence of inorganic mercury in liquid biological samples as well in several surface and wastewaters. Among the biological liquids analysed, urine was proved the less difficult matrix and the sensitivity obtained in this case is similar to that obtained for aqueous solutions. For serum and saliva the sensitivities are lower, due to the protein present in the matrix, which affect the SPME procedure.

4 Conclusion
There is strong and urgent demand for accurate and sensitive analytical techniques, capable to measure traces of toxic substances in environmental, food and biological matrices. As a result to this global trend, the state, research or academic laboratories are shift to be self-consistent, equipped and experienced in application of various methodologies concerning the analysis of human environment, foods and biological matrices and the determination of essential and trace metals as well as heavy metals which are highly toxic for the living organisms. The trend is always towards lowering the detection limits of the applied methods, increase the quality of the obtained results in terms of accuracy and precision and improve the capability to detect new substances in complicated matrices with minimum cost and time consume.

References: