16th International Congress of Metrology, 10010 (2013)
DOI: 10.1051/metrology/201310010

Total Reflection X-Ray Fluorescence (TXRF) spectroscopy for environmental and biological analysis. - Réflexion spectroscopie des rayons X de fluorescence totale dans l'analyse biologique et de l'environnement.

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Abstract. This paper proposes the standardization of total reflection X-ray fluorescence (TXRF) analysis for environmental and biological samples. The importance of TXRF in environmental and biological analysis is now well established and it is continuously growing. However in these fields there are not standardized methods reported by the International Standard Organization (ISO). For this reason, the international collaboration for the development of new standard procedures and methods for TXRF analysis is required. Indeed, a project for "Interlaboratory comparison of TXRF spectroscopy for environmental analysis" was set up in the frame of the Versailles Project on Advanced Materials and Standards, with the aim of developing a new ISO standard for TXRF devoted to environmental and biological analysis.

1 Introduction

The importance of total reflection X-ray fluorescence (TXRF) analytical technique for chemical nanoscale metrology of environmental and biological samples is now well established and it is continuously growing [1].

The applicability of TXRF in most of the environmental fields is still under exploration. Studies about many different kinds of samples, such as natural waters [2], deionized water [3], heavily polluted waters and landfill leachates [4], waste waters [5], wastes and wastes leachates [6], sediments [7], soils [8,9], soils related materials such as fertilizers [10] and plants [11] have been reported. Atmospheric pollution studies were reported by means of TXRF analysis of different kinds of samples. Different bio-indicators, such as pollen [12], and honey [13], were considered; filter membranes for air particulate collection have been measured by conventional methods and new procedures for their analysis were also developed [14].

Recent trends in the use of TXRF for biological applications have been reviewed [15] and possibilities and limitations in the same field have been recently reported [16].

In those fields where standardized methods are required, almost everybody uses atomic spectroscopy methods for elemental analysis, i.e. atomic absorption spectroscopy (AAS), inductively coupled plasma optical emission spectroscopy (ICP-OES) or inductively coupled plasma mass spectroscopy (ICP-MS).

TXRF is competitive with those other techniques in many application fields [17]. For the detection of heavy metals, TXRF limits are comparable with those of AAS, while the former technique is more sensitive in terms of total sample amount [18]. Sample analysis without digestion is another advantage of TXRF that was also proposed for the direct analysis of industrial inlet and outlet water, for routine and screening analysis of waste water samples with respect to the analysis of digested sample with ICP-MS [5]. The sample amount required for TXRF analysis is considerably lower than that required for both AAS and ICPs techniques and another advantage of TXRF is the possibility to detect halogenides. Table 1 resumes the comparison among some of the characteristics of TXRF with respect to AAS and ICP.

The key advantages of TXRF are: simultaneous multi-element trace analysis including halogenides; analysis of very small sample amounts in nanograms or micrograms range; simple quantification using an internal standard; suitable for various sample types and applications; theoretically no matrix or memory effects; low operating costs; quick, a short time is required to perform sample preparation and TXRF measurement, high sensitivity and low detection limits in the order of ppb, depending on the sample (elements) and the matrix.

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Table 1. Comparison among TXRF, AAS and ICP characteristics

Property	TXRF	AAS	ICP
Technique used	Non-destructive technique	Destructive technique (digestion required)	Destructive technique (digestion required)
Calibration	Single standard calibration (internal)	Multi standard calibration (external)	Multi standard calibration (external)
Sample presentation	Solutions, suspensions, particles, thin films	Fully dissolved	Fully dissolved
Multi-element analysis	Yes	Sequential only	Yes
Measuring time	300 – 1000 s	< 10 s for each element	< 10 s for each element
Digestion procedure	Not necessary	Yes	Yes

However there are not standardized methods for TXRF analysis of environmental an biological samples reported by the International Standard Organization (ISO). For this reason a group of experts leaded by Italy started an international pre-normative research project on nanometrology, in the frame of the Versailles Project on Advanced Materials and Standards (VAMAS), whose aim is the development of new procedures to obtain valid measurements of environmental and biological samples. Then recommendations/guidance sheet will be written, leading to their international standardization.

A technical specification document, providing a framework on the uses of TXRF for environmental and biological elemental qualitative and quantitative analysis was accepted as a new project by ISO. This document is meant to help technicians, biologist, doctors, environmental scientists, and environmental engineers to understand the possible uses of TXRF for elemental analysis by providing the guidelines for the characterization of biological and environmental samples with TXRF.

2 Fields of application

Environmental and biological samples that can be analyzed by TXRF may have critical issues when the accurate and precise elemental content determination is required. Some examples are given in what follows.

2.1 Environmental samples

Environmental monitoring is one of the most important and most advanced analytical tasks. Monitoring of contaminated land, determination of hazardous elements in air and water, classification of waste materials, ashes, sludge and the specification of products for later recycling and disposal. Moreover new legislation forces the analysis of elements at lowest concentration levels in a huge variety of different material types.

The following environmental samples can be considered for interlaboratory tests:

- water
- soils
- environmental bioindicators, i.e. plants, mussels
- air particulate matter filters

There are different critical aspects that may be addressed for each one of these samples. Water is considered as the most simple environmental sample and it will be the subject of the first interlaboratory test. About soils the concern is due to their heavy matrix content, mainly Fe, which can lower the accuracy of quantitative TXRF analysis[19]. This problem less affects environmental bioindicators [20], for which absorption effects are negligible from Ca. Direct analysis as well as that of digested samples may be considered [1]. Suitable protocols will be proposed for the analysis of soils and environmental bioindicators on the basis of the most successful reported studies.

Particular interest is addressed to air particulate matter filters, since a new reliable procedure for their quantitative elemental by TXRF was developed in recent years [14,21]. This lead up to a patent [22] and the development of a dedicated instrumentation for sample preparation.

2.2 Food samples

Food analysis is becoming one of the most important analytical tasks since there is an increasing demand for safety and quality control in food industry. Indeed reliable and reproducible techniques and protocols are needed to satisfy consumers requests and to fulfill the target level for certification and valorization of products, as well as to drive food processes and production innovation.

The following food samples can be considered for interlaboratory tests:

- honey
- wine
- milk
- salads
- cereals

The considered samples may be separated in two different groups. The first three may be directly analyzed by TXRF, since they are liquid and/or can be diluted in water. The last two must be digested to be analyzed. Direct TXRF was already successfully applied for fingerprint analysis and geographical/biological origin determination of honey [12, 13, 23, 24]. Other studies were reported about elemental determination of wine [25, 26,27], milk [28], salads and cereals [29]. These studies may be useful sources for the set up of protocols for future round robin tests.

2.3 Biological samples

Metallomics refers to "the study of all possible species of a metal or metalloid in a specific cell or a tissue by means of their qualitative and quantitative analysis". It is becoming a new frontier in the investigation of trace elements in biology and is expected to develop as an interdisciplinary science. Indeed, trace elements are increasingly used as dietary supplements in the prevention of disease and as clinically effective therapeutics. Considering the most common types of samples that has to be measured in medical and scientific

The following biological samples can be considered for interlaboratory tests:

- tissue homogenate and powdered tissue, i.e. liver
- serum or plasma

Four different methods of direct analysis of biological samples are mainly used: 1. Direct analysis with internal standardization; 2. The method of Compton peak standardization; 3. In situ microwave digestion; 4. In situ chemical modification. The first method will be considered for the set up of protocols for future interlaboratory tests.

3 The VAMAS project

VAMAS is an international organization supporting trade in products using advanced materials through pre-normative research and providing structure to stimulate international collaboration and consensus.

VAMAS Project [30] A10(C), named "Interlaboratory comparison of TXRF spectroscopy for environmental and biological analysis", belongs to the technical working area of surface chemical analysis (TWA 2: Surface Chemical Analysis).

The project objectives are:

- the evaluation of results variability of environmental samples analysis performed by different TXRF instrumentations;
- the evaluation of the importance of the preparation method of the laboratory having the same instrumental configuration;
- the comparison of TXRF results with those obtained by standardized method for elemental chemical analysis of environmental samples;
- the preparation of a document to submit to ISO to pose the basis for the further standardization of procedures for TXRF analysis of environmental and biological samples.

3.1 The first TXRF round robin test: water samples

In this frame the "1st TXRF round robin test: water samples" was proposed. Water was considered as the most simple and significant environmental sample to test. Different kinds of water samples were considered: drinking water, waste water, leaching water and purified water.

Nineteen research laboratories were involved, from ten participant countries.

Table 2. Countries participating to the 1st TXRF round robin test: water samples

Country	Number of Laboratories
Italy	2
Spain	2
Germany	6
Belgium	1
Chile	1
Argentina	1
Japan	2
Kenya	2
Austria	1
Greece	1

Two certified laboratories were involved to perform inductively coupled mass spectroscopy (ICP-MS) measurements according to a standardized method.

The aims of the 1st round robin test on water samples were:

- to assess the reproducibility of TXRF measurements for different kinds of water samples across different laboratories;
- to highlight the influence of sample preparation procedure and/or instrumental contribution on data dispersion
- to identify other possible sources of eventual data dispersion
- to compare the results with those obtained from ICP-MS measurements.

Data have been collected and they are still under evaluation.

3 Conclusions and perspectives

The interpretation of the results obtained from the first round robin test of water samples will help to understand the critical aspects of TXRF measurements and analysis performed by different instrumental set up. On this basis new protocols for other environmental and/or biological samples will be set up for further interlaboratory test on the most interesting identified samples.

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