Spectrochimica Acta Part B 130 (2017) 53–59

Contents lists available at ScienceDirect

Spectrochimica Acta Part B

journal homepage: www.elsevier.com/locate/sab

Total reflection X-ray fluorescence as a fast multielemental technique for human placenta sample analysis

E. Marguí [a,*], P. Ricketts [b], H. Fletcher [c], A.G. Karydas [d,e], A. Migliori [d], J.J. Leani [d,f], M. Hidalgo [a], I. Queralt [g], M. Voutchkov [b]

[a] Department of Chemistry, University of Girona, C/M.Aurèlia Campmany 69, 17003 Girona, Spain
[b] Department of Physics, The University of the West Indies, Mona, Kingston 7, Jamaica
[c] Department of Obstetrics and Gynecology, The University of the West Indies, Mona, Kingston 7, Jamaica
[d] Nuclear Science and Instrumentation Laboratory, IAEA Laboratories, A-2444 Seibersdorf, Austria
[e] Institute of Nuclear and Particle Physics, National Center for Scientific Research "Demokritos", 153 10 Aghia Paraskevi, Athens, Greece
[f] IFEG, National Scientific and Technical Research Council (CONICET), X5000HUA, Córdoba, Argentina
[g] Institute of Environmental Assessment and Water Research, IDAEA-CSIC. Jordi Girona 18-26, 08034 Barcelona, Spain

ARTICLE INFO

Article history:
Received 24 November 2016
9 February 2017
Accepted 13 February 2017
Available online 15 February 2017

Keywords:
Placenta
TXRF
Multielemental analysis
Slurry
Suspension
Synchrotron

ABSTRACT

In the present contribution, benchtop total reflection X-ray fluorescence spectrometry (TXRF) has been evaluated as a cost-effective multielemental analytical technique for human placenta analysis.
An easy and rapid sample preparation consisting of suspending 50 mg of sample in 1 mL of a Triton 1% solution in deionized water showed to be the most suitable for this kind of samples. However, for comparison purposes, an acidic microwave acidic digestion procedure was also applied. For both sample treatment methodologies, limits of detection for most elements were in the low mg/kg level.
Accurate and precise results were obtained using internal standardization as quantification approach and applying a correction factor to compensate for absorption effects. The correction factor was based on the proportional ratio between the slurry preparation results and those obtained for the analysis of a set of human placenta samples analysed by microwave acidic digestion and ICP-AES analysis. As a study case, the developed TXRF methodology was applied for multielemental analysis (K, Ca, Fe, Cu, Zn, As, Se, Br, Rb and Sr) of several healthy women's placenta samples from two regions in Jamaica.

1. Introduction

The human placenta is a temporary tissue that is formed at the onset of conception. The purpose of placenta is to facilitate the exchange of elements and other substances between the mother and fetus. Therefore, inadequate or excess intake of certain nutrients and toxic elements during pregnancy can affect fetal development [1]. For this reason it is of significance the development of analytical methodologies for multielemental analysis in human placenta samples. High K concentrations in pregnancy (maternal hypotension) have been associated with a low birthweight [2]. Other essential element such as Ca, Br, Fe and Zn are also relevant in human placenta samples. For instance, Zn is considered as one of the key elements in new-born health [3] and Br has been associated with a small but statistically significant increase in risk of birth defects [4]. In addition, the monitoring of toxic elements such as Pb, Cd and Hg has been also performed in this type of biological samples [1].

Commonly used techniques for element determination in placenta samples include atomic absorption spectrometry (with both flame and graphite furnace atomization) [5–6], inductively coupled plasma atomic emission spectrometry (ICP-AES) [7] and inductively coupled plasma mass spectrometry (ICP-MS) [8]. This kind of instruments are basically designed for the analysis of liquid samples and thus, biological samples such as placenta have to be brought into solution by means of a wet digestion procedure before the spectroscopic analysis. Although less used, solid state techniques such as neutron activation analysis (NAA) [9,10] and energy dispersive X-ray fluorescence spectrometry (EDXRF) [3,11] have been also employed for multielement analysis of placenta samples.

In the present contribution, for the first time, total reflection X-ray fluorescence spectrometry (TXRF) is proposed for the direct analysis of human placenta samples. TXRF has several advantages over other multielemental spectrometric techniques such as low amount of sample to perform the analysis (μL, μg) and easier quantification by internal standardization (external calibration is not needed). Besides, new low power benchtop TXRF systems are really cost-effective since they do not require gas or cooling media [12].

Most of the papers published so far for direct multielemental analysis of biological samples by TXRF are dealing with the analysis of liquid

* Corresponding author at: Department of Chemistry, University of Girona, C/M.Aurèlia Campmany 69, 17003 Girona, Spain.
E-mail address: eva.margui@udg.edu (E. Marguí).

http://dx.doi.org/10.1016/j.sab.2017.02.008
0584-8547/

fluids (i.e., amniotic fluid [11], human serum [13] and saliva [14]) but for the analysis of biological solid samples a chemical decomposition is carried out prior to TXRF analysis. In fact, a recent review about sample pretreatment strategies for TXRF analysis highlights that slurry preparation of solid samples (without digestion) accounts only for the 15% of the sample treatment procedures used [15–17]. In the field of human placenta analysis by TXRF, only few scientific contributions have been published and in all of them a previous digestion procedure using nitric acid was performed before TXRF analysis. Moreover, in these studies, large-scaled TXRF systems with high-power X-ray tubes (3 kW) were used [18–19].

The aim of this research was to develop a cost effective and fast method for multielemental analysis of human placenta samples by benchtop TXRF instrumentation. For that, preparation of the sample as slurry was tested as preparation strategy for TXRF analysis. Evaluation of method trueness was performed by comparing the obtained results with those obtained after microwave acidic digestion and further TXRF and ICP-AES analysis. The developed methodology was applied for multielemental analysis of several healthy women's placenta samples from two regions in Jamaica.

2. Experimental section

2.1. Sample collection

A total of twenty placental samples were collected from healthy women in the age group 18 to 42 years in two different parishes in Jamaica, Manchester and St. Andrew. The parish of Manchester is characterized with terra rossa (red limestone) soils, while St. Andrew has mainly rendzinas (black limestone) soils [20]. For this study, ethics approval was granted from the Faculty of Medical Sciences ethics committee at the University of the West Indies. Placental samples were collected from participants of the University of the West Indies (UHWI) in St. Andrew and the Mandeville Regional Hospital in Manchester.

2.2. Sample treatment

The whole placenta was thoroughly examined for any abnormalities. It was washed to remove maternal blood. The weight of the placenta was recorded and approximately, a quarter of the flat part of the placenta from the region of the umbilical cord was severed with a surgical blade. It was then placed in a sealed plastic bag and stored in a freezer at −20 °C. The samples were placed in a drying oven (Memmert, Schwabach, Germany) at 60 °C. The oven was set to maintain a constant temperature for approximately 96 h until a constant dry weight of each placenta was obtained. These dried samples were ground to fine powder for later analysis. Two different sample treatment procedures (digestion and suspension) were tested in order to analyse human placenta samples by TXRF.

2.2.1. Sample digestion

A microwave acid digestion, based on the EPA method 3052, was employed for the preparation of human placenta samples. About of 500 mg of sample was added in PTFE vessel with 8 mL of nitric acid (69%, HIPERPUR, Panreac) and 2 mL of hydrogen peroxide (≥30%, TraceSELECT®, Sigma-Aldrich). The vessels were closed and heated following a two-stage microwave digestion program consisting of a first step of 5 min to reach 180 °C and a second step of 10 min at 180 °C (Ethos Plus Milestone microwave with HPR-1000/10S high pressure rotor (Sorisole, Bergamo, Italy)). After cooling, digested sample solutions were transferred to a 30 mL flask and brought to volume with ultrapure de-ionized water. From each sample digest, an aliquot of 1 mL was fortified with a suitable volume of a Y solution (internal standard) to have a final concentration of 8 mg/L. After that, 5 μL of the internal standardized sample was deposited on a quartz glass reflector and dried using an infrared lamp for the later TXRF analysis.

2.2.2. Sample suspension

A preliminary study was performed to select the most suitable amount of sample and disperser agent to suspend biological samples. According to the obtained results (see Section 3.1 for details), finally, sample suspensions were prepared by weighing 50 mg of sample and adding 1 mL of Triton X-100 1% (v/v) containing 10 μg of Y as internal standard. Duplicates were prepared for each sample. The sample deposition volume and drying mode to perform TXRF analysis were the same as for the digested samples.

2.3. Instrumentation

TXRF analysis of all samples was performed using a benchtop TXRF system (S2 PICOFOX, Bruker AXS Microanalysis GmbH, Berlin, Germany) equipped with a 50 W X-ray tube with a tungsten (W) anode. The characteristic radiation emitted by the elements present in the sample is detected by a silicon drift detector with an active area of 10 mm^2 and a resolution of 147 eV (Mn Kα). The measurements were performed working at 50 kV and 1000 μA and in air environment. Energy values and analytical lines used for TXRF measurements were: K (K_α: 3.314 keV), Ca (K_α: 3.692 keV), Cr (K_α: 5.415 keV), Mn (K_α: 5.900 keV), Fe (K_α: 6.405 keV), Co (K_α: 6.931 keV), Ni (K_α: 7.480 keV), Cu (K_α: 8.046 keV), Zn (K_α: 8.637 keV), As (K_α: 10.543 keV), Se (K_α: 11.224 keV), Br (K_α: 11.924 keV), Rb (K_α: 13.396 keV), Sr (K_α: 14.165 keV), Cd (K_α: 23.173) and Pb (L_α: 10.551 keV).

A stereoscopic optical microscope from (NIKON SMZ-1000) was used for morphological study of sample suspensions deposited on quartz reflectors.

In order to study if elements at ultra-trace concentrations (<1 mg/kg) were present in the target human placenta samples, one of the samples was analysed by synchrotron based TXRF (SR-TXRF) at the IAEA end-station of the XRF beamline at Elettra Sincrotone Trieste [21]. The end-station consists of an ultra-high vacuum chamber that includes as main instrument a seven-axis motorized manipulator for sample and detectors positioning, different kinds of X-ray detectors and optical cameras. The beamline end-station allows performing measurements in different X-ray spectrometry techniques including TXRF measurements [22]. This beamline offers, at its present configuration, tunable SR excitation in the energy range from 3.6 to 14.5 keV by means of a double crystal Si(111) monochromator with a resolving power of 1.4×10^{-4}. The beam size at the sample position is equal to around 260 μm (H) × 130 μm (V) and XRF spectra are acquired by a SDD (Bruker nano GmbH, X-Flash 5030) with a nominal area of 30 mm^2, 450 μm crystal thickness and an energy resolution of 131 eV (FWHM) at the Mn Kα (5.9 keV) line.

For comparison purposes, digested samples were also analysed by means of an Agilent ICP-OES 5100 Synchronous Vertical Dual View (SVDV) spectrometer. Element wavelengths (nm) used were: K (766.491), Ca (422.673), Fe (238.204), Cu (324.754), Zn (213.857), Rb (780.026) and Sr (407.771). The plasma was operated with 12 L/min plasma gas and the plasma configuration elements were: radial (K, Ca) and axial (other elements). The type of detector was silicon based multichannel array CCD (charge coupled device). Other parameters were: 1200 W RF power, concentric nebulizer type and polychromatic wavelength selector.

3. Results and discussion

3.1. TXRF method development

As stated in the Introduction, one of the aims of this contribution was the development of a simple, fast and cost-effective TXRF method for multielemental analysis of human placenta samples. For that,