Single-step procedure for trace element determination in synovial fluid by dynamic reaction cell-inductively coupled plasma mass spectrometry

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ABSTRACT

A fast and single-step procedure for the dissolution of human synovial fluid in formic acid and further determination by dynamic reaction cell-inductively coupled plasma mass spectrometry (DRC-ICPMS) with a high-efficiency sample introduction system was developed. The samples were collected, treated and analyzed in the same screw-capped tubes. In order to overcome the effect of considerable carbon content, the sample introduction, nebulization and ICP operating conditions were carefully optimized. Furthermore, DRC technology with CH_4 as reaction gas was used for the elimination of spectral interferences due to polyatomic ions. The effect of the sample matrix was evaluated and mitigated through comparison of direct calibration against aqueous standards, direct calibration in formic acid media and analyte addition calibration. The recommended procedure involved low dilution and low detection limits (from 0.003 $\mu g\ L^{-1}$ for U to 13.3 $\mu g\ L^{-1}$ for Ti) with adequate precision (from 0.6% for Co to 6.6% for Ti). The proposed method was successfully applied to determine 16 trace elements in concentrations from 0.03 $\mu g\ L^{-1}$ (Cd) to 88.2 $\mu g\ L^{-1}$ (Cu) in human synovial fluid samples.

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1. Introduction

Trace element determination in body fluids is a routine protocol in clinical laboratories specialized in nutritional diagnosis and toxicology of chemical elements. Atomic absorption spectrometry (AAS) is still the dominant analytical technique used for trace element analysis [1,2]. Inductively coupled plasma optical emission spectrometry (ICP-OES) has also been employed to analyze biological and clinical samples [3–5]. However, more and more clinical laboratories are transitioning away from flame (FAAS), and Electrothermal AAS (ETAAS) methods toward those based on inductively coupled plasma mass spectrometry (ICPMS) [6]. Moreover, ICPMS has some distinct advantages, including sequential multielement measurement capability with very low detection limits; thus, this technique is suitable for biomonitoring studies [7,8].

Some trace elements are cofactors of enzymes in the organism and despite the fact that they are found at trace levels in the body, deficiencies of them could cause serious problems. Two general types of abnormality associated with trace elements are encountered: one is a result of a specific deficiency from dietary inadequacies and the second is abnormality related to other diseases [9,10]. Both kinds of abnormality can be diagnosed by analyses of trace elements in plasma or other tissues. For instance, secondary changes of trace elements that occur in inflammatory and non-inflammatory arthritis were studied since the 1970s, but the causes are not known [9]. In recent years, a great number of studies have investigated the possible role of trace elements in the etiology and pathogenesis of rheumatoid arthritis (RA) [11]. As seen in the literature, the alterations in trace element concentrations in the synovial fluid of patients with RA are inconsistent and, to our knowledge, there are no available reports of the profile element in these patients.

Sample preparation is a critical step of any analytical procedure, and despite all recent advances, it still requires further improvement to reach the same high standards of the instrumental techniques required for accurate determination [12]. Wet decomposition is among the most used methodologies for biological sample treatment. However, it is time consuming, can require high amounts of corrosive and toxic reagents that increase the cost of the analyses, and can cause sample contamination [12]. Besides, the high temperature frequently reached during the procedures can cause losses of the most volatile elements. More recently, microwave-assisted sample dissolution has been employed extensively for shortening the time required for sample dissolution, as well as to avoid analyte losses and contamination. Although microwave ovens of different designs are widely used in the analytical laboratories throughout the world, some problems which are not commonly described have arisen, e.g. high instrumentation costs, short lifetime of the digestion vessels operated at high pressures, long time required for cooling the digestion, sample throughput is not very high and the constant supervision required, among others [13].

As an alternative to the decomposition process, the solubilization with organic reagents, such as primary amines and tetramethylammonium

hydroxide (TMAH), has been proposed [14]. This treatment was applied with success to the determination of many elements in several samples by electrothermal atomic absorption spectrometry (ETAAS) [15–17], inductively coupled plasma optical emission spectrometry (ICPOES) [18], and inductively coupled plasma mass spectrometry (ICPMS) [12,19]. Recently, the use of formic acid (FA) as an alternative to TMAH has been suggested. However, it is not reliable introducing organic samples using the conventional cross-flow nebulizer and the double-pass Scott-type spray chamber, since the carbon loading may extinguish the plasma and/or may form carbon deposits on the cones and lenses [12]. Spectral interferences by carbon polyatomic ions are also a problem [20–22]. Some special sample introduction systems have been used to analyze organic samples, such as electrothermal vaporization (ETV), microconcentric nebulizers (MCN) [12,23–25], ultrasonic nebulization (USN) coupled to desolvation [26–28] and using oxygen as auxiliary gas [26,29]. New micromisting systems as used in this work are able to generate a stable fine mist spray allowing the formation of very low currents with a very good stability ICPMS.

In recent years, collision cells (CC) and dynamic reaction cells (DRC) have been increasingly used in ICPMS to reduce interferences in single-element, and multielemental analysis [30–32]. The DRC technology exploits ion–molecule reactions using a variety of reaction gasses. For instance, O_2 has been used to solve the interference of $^{40}Ar^{35}Cl^+$ over $^{75}As^+$ in high chlorine containing samples through the formation of $^{35}As^{16}O^+$ [33–35]. Other examples are the use of NH_3 for Fe determination in samples containing high Ca amounts [14,36,37], and the use of CH_4 to overcome the interference of the dimmer $^{40}Ar^{40}Ar^+$ over ^{80}Se [34,36,38].

In this work a fast and simple solubilization procedure with formic acid is proposed for the first time for the determination of 16 elements in synovial fluid samples in order to avoid issues related to sample preparation. The samples were treated with FA in the same tubes they were taken and analyzed by DRC-ICPMS with CH_4 as reaction gas. The goal of this method is offering a fast profiling tool to identify some marker trace elements associated with a specific physiological state by measuring Li, Mn, Co, Ni, Cu, Zn, Se, Sr, Cd, Ba, Tl, Pb, U, Ti, V and As and in human synovial fluid.

2. Material and methods

2.1. Instrumentation

An inductively coupled plasma mass spectrometer, PerkinElmer SCIEX, ELAN DRC-e (Thornhill, Canada) was used. The argon gas with minimum purity of 99.996% was supplied by Air Liquide (Córdoba, Argentina). An HF-resistant and high performance Teflon Nebulizer model PFA-ST, was coupled to a quartz cyclonic spray chamber with internal baffle and drain line cooled with the PC^3 system from ESI (Omaha, NE, USA) (Table 1).

Tygon black/black 0.76 mm i.d. and 40 cm length peristaltic pump tubing was used. The instrument conditions were: auto lens mode on, peak hopping measure mode, dwell time of 50 ms, 15 sweeps/reading, 1 reading/replicate, and 3 replicates. Nickel sampler and skimmer cones were used. Before changing to the microconcentric nebulizer, a performance check for sensitivity and oxide and doubly charged ion formation, using a conventional cross flow nebulizer and a Scott spray chamber was carried out (Table 1).

2.2. Reagents and samples

The used water was distilled and de-ionized, with a resistivity of 18.2 MΩ cm, produced by an Easy pure RF system from Barnstead (Dubuque, IA, USA). Concentrated nitric acid (65%v/v) from Sigma-Aldrich (Germany), tetramethylammonium hydroxide pentahydrate from Sigma-Aldrich (USA), dimethylformamide from Acros Organics (New Jersey, USA), and formic acid (98–100%v/v) from Fisher Scientific

Table 1
Instrument settings and data acquisition parameters for DRC-ICP-MS.

Instrument	ELAN DRC-e (PerkinElmer SCIEX, Thornhill, Canada)
Sample uptake rate (μL min^{-1})	200
Sample introduction	Nebulizer model PFA-ST, coupled to a quartz cyclonic spray chamber with internal baffle and drain line, cooled with the PC^3 system from ESI (Omaha, NE, USA)
RF power (W)	1200
Gas flow rates (mL min^{-1})	Nebulizer, 0.77
Interface	Ni cones (sampler and skimmer)
DRC gas	Reaction gas CH_4
Standard mode	^{7}Li, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{82}Se, ^{88}Sr, ^{111}Cd, ^{138}Ba, ^{205}Tl, ^{208}Pb, ^{238}U
DRC mode	^{47}Ti, ^{51}V, ^{75}As
Scanning mode	Peak hopping
Dwell time (ms)	50 in standard mode
Number of replicate	3

(Loughborough, Leicestershire, UK), were used. Multi-element calibration standard solution 1 containing 10 mg L^{-1} of Ag, Al, As, Ba, Be, Bi, Ca, Cd, Co, Cr, Cs, Cu, Fe, Ga, In, K, Li, Mg, Mn, Na, Ni, Pb, Rb, Se, Sr, Tl, U, V and Zn in 5% HNO$_3$, multi-element calibration standard solution 5 containing 10 mg L^{-1} of B, Ge, Mo, Nb, P, Re, S, Si, Ta, Ti, W and Zr in 5% HNO$_3$, Hg standard solution with 10 mg L^{-1} in 5% HNO$_3$, and setup/mascal solution from PerkinElmer Pure Plus, Atomic Spectroscopy Standard (Norwalk, USA), were used.

The analyzed sample was human synovial fluid taken previously from people with arthritis or evidence of disease by physician rheumatologists. The collected samples have been available after the relevant consents.

2.3. Analytical procedure

The samples (about 250 μL) collected in 15-mL polyethylene tubes from SARSTEDT AG & Co. (Nümbrecht, Germany) were added with 250 μL of the formic acid, and the mixture was hand shaken vigorously. In the sequence, the flask was kept at 90 °C in a water bath for 30 min. Then, the volume was completed to 2.5 mL with water and nitric acid to a final concentration of 1% v/v, obtaining transparent solutions. Spiked samples were also processed with this procedure to evaluate the recoveries. For the external calibration against aqueous standards, the standard solutions were prepared in 1%v/v nitric acid. For the calibration in the organic medium, the solutions were prepared in 10%v/v FA (discussed in Section 3.2) and 1%v/v nitric acid. The concentrations of the analytes were 5.0, 10, 20, 40, 80 and 100 μg L^{-1}. As internal standard, Rh was added to all solutions, including the samples, to a 20 μg L^{-1} final concentration.

3. Results and discussion

3.1. Effect of sample preparation and sample introduction conditions

Preliminary studies were performed to adequately dissolve HSF sample with low dilution factors and low sample handling in order to perform trace multielemental analysis. To these aims, different solubilization procedures were assayed involving formic acid, dimethylformamide and tetramethylammonium hydroxide, with and without heat.

The observation of the final solutions obtained enabled qualitative conclusions about the preferred reagent conditions. When the samples were added with TMAH (with and without heat) they remained as emulsions (turbid) and two phases appeared shortly. In the case of adding DMF (with heat), an homogeneous solution was observed, but it became turbid after several minutes. When using FA as dissolving agent, the HSF samples formed clear solutions, being clearer when heated