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Sample preparation strategies for bioinorganic analysis by inductively coupled plasma mass spectrometry

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ABSTRACT

In this review the recent strategies of sample preparation are described and discussed for biomolecules separation/extraction for further metal(oid) or metal(oid)-containing compounds determination by inductively coupled plasma mass spectrometry (ICP-MS). Taking into account that ICP-MS can be considered a powerful analytical tool for the determination of metals and metaloids in animal and plant tissues, fluids and related samples, applications reported in the last fifteen years are described. This technique has been widely used in analytical procedures once it presents suitable sensitivity for trace analysis and could be coupled with several kinds of separation techniques such as liquid and gas chromatography, capillary and gel electrophoresis, among others. In this context, sample preparation is a crucial step in order to assure accurate results and non conventional sources of energy for sample preparation has been used, especially those related to ultrasound and microwaves. Thus, this review summarizes the sample preparation procedures regarding metal and metal-containing compound by ICP-MS, such as extraction and solubilization procedures, derivatization, pre-concentration, cleanup and also digestion for total metal analysis.

1. Introduction

Mass spectrometry (MS) can be considered one of the most important analytical techniques for analysis of element concentration down to the trace and ultra trace level, for isotope analysis, surface characterization and for structural analysis of bioinorganic compounds [1,2]. In order to understand toxicology and metabolic pathways of toxic and essential elements analytical strategies have been developed to obtain qualitative and quantitative information concerning the elements, element species, their interactions, transformations and functions in biological systems. In this sense, the analysis of metal(oid)s and their containing compounds in biological tissues is an important aspect to be considered in life science [3]. A great part of interest is attached to essential elements, which includes transition metals such as Cu, Fe and Zn and also toxic elements as Cd, Hg and Pb. Metals can be an integral part of proteins (metalloproteins) and enzymes (metalloenzymes), e.g. ferritin (Cu, Fe, Zn), β-amylase (Cu), alcohol dehydrogenase (Zn) and carbonic

Abbreviations: 2D-GE, two-dimensional gel electrophoresis; 2D-PAGE, two-dimensional polyacrilamide gel electrophoresis; AsB, arsenobetaine; AsC, arsenocholine; ASE, accelerated solvent extraction; CE, capillary electrophoresis; CE–ICP-MS, capillary electrophoresis inductively coupled plasma mass spectrometry; CHAPS, 3[3-cholamidopropyldimethylammonio]1-propanesulphonate; CRM, certified reference material; CVG, cold vapor generation; CZE, capillary zone electrophoresis; DMA, dimethylarsinic acid; DPAA, diphenylarsinic acid; DRC, dynamic reaction cell; DTE, dithioerythritol; DTT, dithiothreitol; DVB–CAR–PDMS, divinylbenzene–carboxen–polydimethylsiloxane; ESI, electrospray ionization; ETV–ICP-MS, electrothermal vaporization inductively coupled plasma mass spectrometry; F AAS, flame atomic absorption spectrometry; FAB, fast atom bombardment; GC, gas chromatography; GE, gel electrophoresis; GF AAS, graphite furnace atomic absorption spectrometry; GPC, gel permeation chromatography; HG, hydride generation; HPLC–ICP-MS, high performance liquid chromatograpy inductively coupled plasma mass spectrometry; ICP-MS, inductively coupled plasma mass spectrometry; IEF, isoelectric focusing; ITP, isotachophoresis; LA–ICP-MS, laser ablation inductively coupled plasma mass spectrometry; LC, liquid chromatography; LC–ICP-MS, liquid chromatograpy inductively coupled plasma mass spectrometry; MALDI, matrix assisted laser desorption ionization; MCGC, multicapillary gas chromatography; MECC, micellar electrokinetic chromatography; MMA, monomethylarsonic acid; MS, mass spectrometry; MT, metallotionein; PDMS, polydimethylsiloxane; PDMS–DVB, polydimethylsiloxane–divinylbenzene; PMSF, phenylmethylsulfonyl fluoride; PTFE, polytetrafluorethylene; PVDF, polyvinylidene fluoride; SDS, dodecyl sulphate; SEC, size exclusion chromatography; SEC–ICP-MS, size exclusion chromatography inductively coupled plasma mass spectrometry; SPE, solid-phase extraction; SPME, solid phase micro extraction; SRXRF, synchrotron radiation X-ray fluorescence; SSID, spike species-specific isotope dilution; TBP, tributylphosphine; TCEP, Tris–carboxyethylphosphine; TMAH, tetramethylammonium hydroxide; Tris, Tris(hydroxymethyl)aminomethane; UV, ultraviolet.

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anhydrase (Cu, Zn), or it can be less stronlgy bound to transport proteins (albumin, transferrin) [4]. In this sense, metal and metalloid species in a cell or tissue, their identity, quantity and localization are named as metallome. This issue is related to interactions and functional connections of metal ions and their species with genes, proteins, metabolites and other biomolecules within organisms and ecosystems [5].

In spite of the great importance, the information about the relation of proteins and metals has been disconnected and conclusions regarding to the importance of metals to proteins are sometimes fragmented. Comprehension of the roles involving metals and proteins will certainly depend on the quality of information obtained after metal-protein analysis [6]. The use of different technological processes and products in modern life results in an increasing number of elements that can be released into the environment entering in the food chain. On the other hand, biological effects related to this exposure often remain virtually unknown [7]. Then, the importance of metal species in biological materials has received special attention [6–13], since these species can be considered as signaling agents, can act as catalysts, or also modify gene expression, among other effects [6].

Interest in metal(oid) and its species in biological systems has led to more sophisticated methods for suitable investigation. These methods include usually one or more separation steps in order to isolate the biomolecules of interest or to eliminate disturbing matrices. In addition, methods are dependent on the availability of suitable detection systems [14,15]. In this sense, in order to investigate biomolecules and their interaction with elements, the integrity of metal complexes before and during identification/determination must be assured. Knowledge related to potential alterations of sample is essential to avoid misinterpretation of analytical results [14].

In the present review the strategies of sample preparation reported in the last fifteen years are discussed as analyte extraction and solubilization from biomolecules (the use of enzymes, buffers, accelerated solvent extraction, microwave and ultrasound-assisted extraction, among others), digestion for total metal(oid) analysis, pre-concentration and cleanup of the species and derivatization techniques (chemical vapor generation, ethylation and Grignard reaction). The main focus is on sample preparation methods for further metal(loid) and/or metal(oid)-containing compounds analysis in animal and plant tissues, fluids and related samples by ICP-MS or by ICP-MS coupled with separation techniques.

2. General remarks

2.1. Sampling and storage

Among the pre-treatment steps required to perform an investigation about specific metal-binding biomolecules or biomarkers, sampling and storage procedures could be considered as a key requirement in order to preserve the species information during the whole analytical process. Then, it could be possible to distinguish two main strategies in order to achieve this goal: firstly, species preservation may keep the chemical species of interest unchanged during all steps of analysis, and secondly, the species may be quantitatively transformed into suitable derivatives for further separation, accumulation and quantification [16]. In practice, there is usually a mixture of both strategies. In this sense, chemical stability and volatility of the analytes can be considered as critical as all aspects concerning sampling procedures [16]. Moreover, during storage step, some drawbacks can be pointed out such as degradation which will depend on the chemical nature of the species and may be influenced by biochemical processes as enzyme activity [17]. This is usually more critical for animal tissues in comparison with plant samples. An important parameter for chemical reaction rates is the temperature, which could determine the rate of species transformation [17]. Thus, it could be considered as decisive to reduce the temperature as much as possible in order to decrease species transformation.

Sample stabilization could be performed by drying, but there is no standardized method for this purpose. Freeze-drying (also known as lyophilization or vacuum drying) is one of the most common drying procedures used for trace element determination in biological materials. Lyophilization allows removing water or other solvents from sample through a process of sublimation. Care should be taken in order to avoid possible losses of volatile compounds or species [18]. Shock-freezing of the desired samples in the gas phase under liquid nitrogen seems to be the safest technique to prevent species changes and it can be performed immediately at the sampling site. It offers the additional advantage of an inert gas atmosphere for samples storage [17]. If this approach is not feasible within the specific project a short-term preservation of the biological material at −20 °C is recommended [16].

2.2. Separation methods and detection systems

Methods for separation and detection techniques have to be combined in bioinorganic analysis in order to achieve suitable and accurate and selective information. Therefore, element species must be separated before being analyzed. Methods used for separation step have been widely reviewed [19–26] and a variety of separation systems have been employed in metallomics analyses including gas chromatography (GC), liquid chromatography (LC), gel electrophoresis (GE) and capillary electrophoresis (CE) [24].

Liquid chromatography is the most widely used mode of chromatography and it complains a liquid mobile phase to separate the components of a mixture [27–29]. The interactions between the analyte and mobile phase are based on dipole forces, electrostatic interactions and dispersive forces, which require a previous knowledge of the nature of chemical species to be separated [30]. On the other hand, GC could be applied for compounds separation according to their volatility and thermal stability. However, many compounds do not present those requirements for direct GC separation. In this way, chemical reactions can be used to transform non-volatile compounds (usually ionic) into volatile thermally stable compounds, which are known as derivatization reactions [31].

Capillary electrophoresis has been applied to protein and biomolecule analysis and it has become an important separation tool especially for life sciences [32]. It provides an efficient separation of species and even allows several different separation modes such as capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MECC), isoelectric focusing (IEF), isotachophoresis (ITP), among others. In addition, structural information about the interaction of metals and proteins, concerning the attached carbohydrate chains as well as degree of metal saturation can be obtained by both CZE and IEF methods [33–35]. In the same way, GE is an important technique used to separate biomolecules where molecules are forced across a span of gel motivated by an electrical current. The properties of molecules, such as size, electric charge, structure and others, can determine how rapidly an electrical field can move through the gel [27,28,34–36]. On the other hand, two-dimensional gel electrophoresis (2D-GE) has been widely used in proteomic studies due to its suitable separation power. Proteins are initially separated according to the respective isoelectric point in the first dimension, followed by separation in the second dimension related to the molecular weight [23,27].

Concerning the specific detection of a metal compound in a cell line, plant, animal or human tissue, it needs to be achieved before any subequent studies related to its identification and characterization. The actual problem can be simplified assuming that the analytical signal detected by an instrument is due to the presence of the metal compound, and not to that of a simple ion, or a ligand [7,9].

In order to perform a specific metal detection, traditional methods such as flame atomic absorption spectrometry (F AAS), graphite furnace atomic absorption spectrometry (GF AAS), or atomic emission spectrometry have been currently used [36]. Nevertheless, sensitivity is becoming a key issue when capillary separation systems are used or when the target sample is small (e.g., individual cells) [7]. In this sense, inductively coupled plasma mass spectrometry (ICP-MS) has been considered as a powerful technique for metal(oid) detection. It is based on the conversion of all the forms of an element present in a sample into the correspondent ions which are separated in a mass analyzer and counted by an ion detector [37]. This technique offers many advantages due to its isotopic specificity, high elemental selectivity regardless the metal coordination environment and sample matrix. In addition, the dynamic range is relatively large (at least six decades), and coupling with separation methods can be relatively easy [1,5,6,25,38,39].

Contrarily to the different mild ion sources – electrospray ionization (ESI), fast atom bombardment (FAB), matrix assisted laser desorption ionization (MALDI), etc. – [40,41], for commercial ICP-MS instruments there is no variation of the plasma ion source. However, in addition to the different types of nebulizers and desolvating systems, many sample introduction systems have been developed for ICP-MS, making this technique a versatile tool for many applications in bioinorganic analysis [39,42,43]. Systems based on ICP-MS can be used as versatile and sensitive detectors for compounds containing known or unidentified heteroatoms including metals and nonmetals. Therefore, this technique can be considered as a convenient elemental detector for high performance separations methods such as GC, LC and CE. Such hyphenated systems built for elemental speciation analysis are presenting an increased use for many applied research fields in bioinorganic analysis. Some particularities regarding these couplings, as e.g. the difficulty with the eluents from CE and LC systems as well as with the organic solvent from GC have been reported and special attention should be given to this point [44,45].

In order to make bio-molecules detectable by ICP-MS some alternatives have been used, as follows: the use of natural (hetero) element tags (covalently bound elements as S and P), the controlled labelling of bio-molecules with ICP-MS detectable elements using chelating agents and the chemical labelling of bio-molecules with nano-particles which contain elements detectable by ICP-MS. More information about these strategies is reported in a recent review [46]. Some alternatives that should be highlighted are the use of stable isotopes, as I [47], Eu, Tb and Ho [48] and the use of isotopic dilution analysis (IDA) [49] for quantitative protein determination combined to ICP-MS detection.

In general, the use of ICP-MS for molecule identification is possible when this system is on-line or off-line combined with a separation technique, once the ionization of the molecules does not retain any molecular information. In this point, the advantage of ICP-MS is that the molecular occurrence of the element of interest does not influence significantly the response for a given element. This technique shows limited matrix influence, resulting in a species-independent response and, as a consequence, stable elemental species can be used for quantification purposes for different species of the same element [50]. The total content of an element determined before separation allows the calculation of the portion missed by the separation technique used and the percentage of the individual peak recovered compared to the total amount [38].

In this sense, the use of microwave energy has been mainly used for total sample digestion for further analyte determination with applications in different matrices [51–53]. This procedure could be necessary for total metal or metalloid determination in different kinds of biomolecules, which could be normally required after separation step. When small sample amounts having low analyte concentrations are determined, some errors during total metal concentration can determine the success or failure of the analysis [54]. Such problems can be even more relevant when quantitative metalloprotein analysis is considered because all analytical information is taken from a sample localized, generally, in a two-dimension polyacrilamide gel electrophoresis (2D-PAGE) 1 spot (only a few milligrams) [6,55]. Although laser ablation (LA) may be used for metalloprotein analysis [56–60], this technique is still not easily available in most laboratories.

Wet digestion with oxidizing acids is the most common sample preparation procedure, which is performed in order to release the elements of interest from the sample matrix and transfer them to a liquid matrix for subsequent analysis [61,62]. Without an efficient digestion, modern analytical techniques, such as ICP-MS using conventional nebulization, would not give correct results in the determination of elements in complex samples, because the solubilization of analyte and removal of interfering molecules are essential for correct analyses [63]. In addition, microwave energy has been considered the most widespread source of energy for sample wet digestion, once it could improve both speed and efficiency of digestion for some types of sample considered difficult to bring into solution [61,64]. The advantages obtained using microwave-assisted wet digestion can be improved when the digestion procedure is performed in closed systems. The use of closed vessels can minimize the risk of contamination, digestion procedure can occur at relatively high temperature and, generally, it is much more efficient than conventional open wet digestion [61,64–66].

In order to evaluate the possibilities of metallomics to characterize metal-linking proteins in *Mus musculus*, which could be used in environmental assessment, a digestion procedure of different organs (liver, kidney, brain, heart, lung, spleen and muscle) was carried out in a microwave-assisted reaction system for further Cr, Cu, Mn, Ni, Pb and Zn determination. Digestion was performed in closed polytetrafluorethylene (PTFE) vessels with a mixture of concentrated HNO_3 and H_2O_2. Digests were analyzed by ICP-MS and a mass balance performed using Cu showed good recoveries in relation to a previously isolated brain fraction [67].

In the same way, total arsenic concentration was determined by ICP-MS after microwave digestion and results were compared with the values obtained after analysis by CE–ICP-MS in order to compare the total arsenic concentrations in fish samples. For this purpose, fish samples were digested with HNO_3 into a PTFE-coated vial for microwave heating. Results obtained for total As were in agreement with the sum of As species [68].

3. General aspects of sample preparation

A previous step for sample preparation could be considered as determinant in order to obtain accurate information. Considering that sample collection, handling and storage have been carefully performed, more manipulation could be required before determination and, as a consequence, the contamination risk becomes higher. Sample preparation step could be understood as any manipulation that modifies the sample matrix and one of the main objectives is to convert the sample to a more suitable condition to the analysis. It is commonly attained by reducing sample heterogeneity at a molecular level [32]. Conventional sample preparation methods involve several steps, being the isolation of the target analytes from a solid matrix one of the most critical. The main problems arise from the possibility of losses or contamination during sample preparation, the long time required for leaching step and large solvent consumption. Current tendencies are aimed to overcome these problems either by the development of new methods or by improving old solvent extraction methods [69].

Many strategies have been adopted for sample preparation, such as solubilization, extraction, leaching, ashing, decomposition and

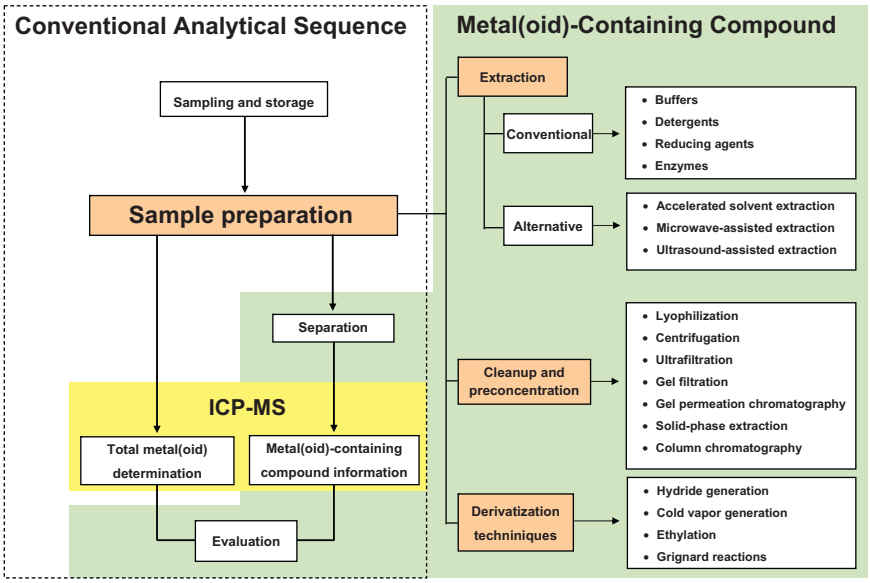

Fig. 1. Sample preparation strategies for bioinorganic analysis.

others, and each one could be more appropriated depending on the objective of the analysis. As an example, in terms of sample preparation for biomolecules, it is sometimes imperative the use of soft process for extraction in order to avoid analytes losses and/or interconversion during extraction [32]. It is important to mention that some techniques, as laser ablation inductively coupled plasma mass spectrometry (LA–ICP-MS) [2] and electrothermal vaporization inductively coupled plasma mass spectrometry (ETV–ICP-MS) could be performed avoiding some classical sample preparation steps [1]. Fig. 1 summarizes the strategies for sample preparation for bioinorganic analysis that will be discussed in the following sections. It is possible to observe that most of applications involve the use of extraction, derivatization and preconcentration or cleanup steps for further metal(loid) and/or metal-containing compounds analysis.

4. Cell disruption or solubilization methods for analyte extraction

The first step of a sample preparation procedure for metal(loid) analysis, considering the content of proteins, should involve the cell disruption (cell lysis) or a partial degradation of sample matrix in order to extract the analyte from the cell or tissue [32,69]. The most common procedures used to promote cell disruption are based on mechanical (different devices can be used to perform mechanical homogenization of tissues or cells that can be opened by hand grinding with a mortar and pestle with nitrogen or not), chemical (by addition of detergents, where cellular membranes are solubilized), lysing cells and releasing their components, enzymatic lysis (specific enzymes are used to remove cell walls or extract the analyte), osmosis, French press and freeze–thaw procedures, among others [15,32,70].

Sometimes the use of only nitrogen could be enough to promote the cell disruption for further analyte extraction. Plant tissues could be simply cut in pieces and extracted as fresh tissues with liquid nitrogen [71,72] and directly ground or freeze-dried and further ground prior to leaching with a cell lysis buffer [73]. On the other hand, the cell disruption method can be selected for mammalian tissues depending of the tissue type. For soft tissues (as liver) the most common method is liquid shear and a mechanical shear (using high velocity homogenizers) is the method of choice for relatively hard tissues (as muscle). Hard tissues may be rendered susceptible to liquid shear homogenization by treatment with hydrolytic enzymes. These methods do not require reagents addition, but fine cellular debris can be formed if mechanical lysis is too vigorous. Vascular tissues, as mouse liver, may require some form of perfusion to remove blood previous homogenization [73].

Mechanical methods could be performed using high velocity stirrers (e.g. turrax system) [74] or other types of stirring, as with the use of glass beads [75] and glass homogenizers [70] and also by ultrasonic extraction [70,76].

The extraction step could be considered more critical due to the high degree of proteins heterogeneity and the difficulty of break and releasing their structures. In addition, depending of the analytical techniques, different extractions procedures could be used [77]. In general, after tissue disruption, cells must be lysed and procedures depend on the presence (or not) of the cell wall. It can be more critical for plant cell lysis due to multiple layers of cellulose, making necessary to use more effective methods for disruption. In spite of most useful methods for cell lysis are based on physical lysis, in last years, many procedures using reagents, as e.g. detergents, have been used mainly due to its characteristics as availability, low cost, high efficiency and suitability for electrophoresis, chromatographic and MS techniques [15,77]. In addition, the use of alternative strategies, as ultrasound, could bring some beneffits and many works have reported better analyte extraction from proteins after a sonication step [70,76].

The extraction step is, in general, performed for a long time (several hours or overnight) at temperatures about 37 °C [15,78]. Many applications are related to As and Se speciation and the procedures used for sample preparation for these analytes have been widely discussed and reviewed in literature [73,78–81]. In the present section, extraction and solubilization procedures were divided according to the reagent used in order to achieve analyte extraction from biomolecules and its solubilization.

4.1. Buffers

Buffer salts are used to obtain the ion strength necessary for optimal protein solubilization and should be chosen according to the type of protein present in the sample [77]. The most commonly used buffer is Tris and it has been used for different elements, as Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Pb, Pt, Se and Zn [15,70–72,75,76,82]. In general, Tris buffer can be used in association with HCl solution

in order to liberate the free or weakly bounded inorganic analyte species and analyte-aminoacids species, as e.g, selenite and selenate and selenoaminoacids, respectively. It was observed that the use of only HCl or a solution composed by Tris–HCl as extractants only releases 50% of the total Se present in leaves and results can be slightly higher when only a solution of HCl is used instead of Tris–HCl. On the other hand, similar results have been obtained for Se extraction species using HCl or Tris–HCl where about 50% of the total Se content was extracted and no differences were observed between results obtained after the use of HCl or Tris–HCl [71].

4.2. Detergents

The use of SDS or 3[3-cholamidopropyldimethylammonio]1-propanesulphonate (CHAPS) is particularly recommended when element speciation is required, the element is incorporated or strongly complexed and the cell disruption is necessary [73]. In this case, detergents disrupt the cell membranes, breaking lipid–protein interaction and, consequently, solubilizing the metal-binding proteins and preventing hydrophobic interactions [83].

The selection of the more suitable detergent does not follow a general procedure. In most of cases, non-ionic and zwitterionic detergents are less denaturing than ionic detergents and they are used to solubilize proteins for functional studies. On the other hand, ionic detergents are strong solubilizing agents which produce protein denaturation [77].

The benefits of SDS complain the effective protein solubilization and it has been commonly used to recover selenoproteins from yeast and mammalian tissues, by increasing the yield of Se and releasing the aminoacids bound in selenoproteins [75,80,83] or to extract other metals from proteins, as Co, Cr, Cu, Fe, Mn and Pb [72].

In spite of some authors have reported that SDS could interfere with the first dimension separation and, if present it should be removed [77], other authors reported that if SDS is used in combination with an excess of nonionic or zwitterionic detergents (e.g. Triton X-100 and CHAPS), no interference was observed [23]. Concerning the MS analysis, many works have reported that SDS should be removed prior the analysis, since it could interfere in, e.g., MALDI, ESI, liquid chromatography inductively coupled plasma mass spectrometry (LC–ICP-MS) analysis, mainly due to signal suppression [19] and, then, it should be avoided. An additional peculiarity concerning the use of SDS as solubilization/denaturing agent is that it is not suitable for all types of proteins because strongly acidic proteins do not bind to SDS. In this case, the alternative is to use a cationic detergent that, on the other hand, is not commonly used for 2D-GE applications [23].

Nonionic detergents, as Triton X series and NP-40 are favored for non-denaturing protein in the first dimension 2D-GE. These detergents are used in concentrations ranging from 0.4% to 4%, although higher concentrations could be used [15,23]. The zwitterionic detergent CHAPS has been demonstrated to be more effective for solubilization and it belongs to the class of linear sulphobetaine surfactants [23] or shows better compatibility with the isoelectric focusing step of electrophoresis [76,83].

Nowadays, it is possible to find some commercial reagents ready to use, mainly composed by mixtures of detergents and protease inhibitor for cell lysis, as Poppers Cell Lysis™ Reagents (Pierce Biotechnology Inc. Rockford, IL), CyQUANT® cell lysis buffer (Invitrogen Co. Carlsbad, CA) and CelLytic™ (Sigma–Aldrich, St. Louis, MO). In addition, an extended description of the commercial available reagents and kits for protein extraction was previously reported [77].

4.3. Reducing agents

Some specific reagents could be added in order to reduce the disulfide bonds, helping in the protein denaturation process [23,77] and avoiding protein oxidation [84]. For this purpose, free thiol-containing reducing agents such as dithiothreitol (DTT), dithioerythritol (DTE) and β-mercaptoethanol are used. Other reagents, as phosphine compounds, as tributylphosphine (TBP) and Tris–carboxyethylphosphine (TCEP) have been used in order to improve the solubilization of proteins. In spite of the advantages of the use of these reagents, the ionization of the reductor, re-oxidation of S–S bonds, precipitation of some proteins are problems that could occur during the separation step [23,85].

The use of an extraction procedure for isolation of water-soluble Se containing proteins based on DTT and phenylmethylsulfonyl fluoride (PMSF) was studied [86]. In this study, samples were extracted with aqueous solution containing DTT and PMSF under sonication. This procedure minimized the risk of protein oxidation and sample loss but the Se recovery accounted was about 14% of the total Se in the yeast. Using a further precipitation step, the recovery was about 80% of the Se present in the aqueous extract. Other authors also reported the use of DDT and PMSF as antioxidant and protease inhibitor for Se speciation analysis [87,88]. On the other hand, some authors reported that no difference was found between the results obtained using only SDS in comparison with SDS and PMSF for Se species extraction [80].

4.4. Enzymes

In a general way, enzymes could be used to promote matrix degradation or to release selectively the analyte present in the sample, as e.g. selenoaminoacids [73,80]. In last case, enzymes have been used for Se-species extraction and it could be an attractive method of choice since organic molecules are sensitive to extreme conditions of pH and temperature and the use of enzymes excludes these circumstances which might cause changes (e.g. oxidation, decomposition) in Se-species. As enzymes can catalyse specific and well-known processes, no additional or undesired effects are expected [89]. In this sense, a commercial enzyme reagent is available (Driselase) containing laminarinase, xylanase and celullase to release selenium compounds trapped in the cell walls [80]. Some authors reported that using non-proteolytic enzymes (Driselase), the amount of Se released was more than twice in comparison with the procedure without enzyme. In addition, extraction procedures were performed with and without the use of SDS and it was observed that results were not changed probably because (i) the enzymes in the dry sample analyzed had already lost their activity or (ii) the Se compound recovered was not a protein and it was resistant to the degradation by proteolytic enzymes. On the other hand, using proteolytic enzymes almost 90% of Se present in the yeast sample was solubilized and this type of proteins has been widely used in the extraction step [87,90,91].

A study of different enzymes, as protease XIV and proteinase K used to extract Se species from plant tissues was performed [71]. It was observed by analysis using high performance liquid chromatography inductively coupled plasma mass spectrometry (HPLC–ICP-MS), that inorganic forms of Se or Se-cystine and some species of selenoaminoacids were extracted. Se-methionine was cleaved from the protein structure and a high signal was observed in the chromatogram. When different enzyme extraction procedures (protease XIV and proteinase K) were compared, proteinase K provided a higher signal for Se-methionine and also for the unknown peaks. The use of protease XIV and proteinase K was also evaluated [84] for release Se-methionine from proteins. Samples were mixed with Tris–HCl buffer and proteinase K and protease XIV. This procedure was compared with the acid hydrolysis using methanesulfonic acid and β-mercaptoethanol. Results indicated the formation of different methionine-containing peptides, but not the release of free amino acids. Furthermore, it was observed that proteinase K cleaved the peptide bonds containing amine groups, while the

bonds containing the carboxyl groups remained intact. The authors also reported that better results were achieved using acid hydrolysis [84].

In another study it was described the use of proteolytic (pepsin, trypsin and pronase) and cell wall digestion enzymes (Lysing enzymes and Driselase) to improve Se extraction from mushrooms, with and without inhibiting proteolysis during cell digestion. The highest Se extraction (89%) was achieved using Lysing enzyme and pronase [92]. Other authors also observed that proteolytic enzymes, as pepsin and tripsin were suitable for Se extraction, with recoveries about 75%. On the other hand, with the use of pronase recoveries over 69% were achieved. Concerning the use of pronase, some authors have reported that the cause of recoveries lower than 100% of Se species could be due to the presence of some protein structure (resulting from the application of some procedures for pretreatment) that can not be degraded by proteolytic enzymes [89].

The use of enzymes for analyte-protein extraction has been described in literature [71,89]. Recently, enzymatic extraction has been performed under focused microwave radiation in order to improve analyte-protein extraction and recoveries better than 99% were reported with a substantially reduced time for the procedure [93].

The use of α-amylase enzyme to partial degradation of the sample matrix was studied in order to extract As species from apple samples [78]. In addition, only a solvent extraction procedure was also used for result comparison. It was observed a significantly improvement in the extraction efficiency with the use of α-amylase due to break of the starch structures of the sample matrix.

5. Alternative methods for analyte extraction

5.1. Accelerated solvent extraction

Accelerated solvent extraction (ASE) was proposed for sample preparation [94] using high temperatures and pressures and liquid solvents. In this system, a solid or semi-solid sample is enclosed in a sample cartridge that is filled with an extraction fluid for further extraction at temperatures in the range of 50–200 °C and pressures about 500–3000 psi for short periods of time (5–10 min). Compressed gas is used to purge the sample extract from the cell into a collection vessel and it is performed using solvents commonly used for Soxhlet or ultrasound-assisted extraction.

The use of ASE could be also performed for analyte recoveries from the sample matrix. This procedure is based on static extractions steps at elevated temperatures (above the boiling point) and pressures. The pressure could be programmed independent of the temperature that should be suitable in order to avoid decomposition of thermally unstable compounds [73]. Some advantages related to this technique were firstly reported by Goenaga-Infante et al. [87] where high sample throughput and automation, in comparison with the standard ultrasound-assisted extraction, were obtained. This system has been applied for As or Se speciation in different types of matrices as fish tissues [95], yeast-based selenium supplements [87,88], watercress [90] and seaweed [96]. Methanol and water are commonly used and extraction is currently performed at ambient temperature in order to prevent degradation of As species [96].

Extraction procedures based on ASE and sonication for arsenic species from fish tissues were evaluated [95] and it was reported that results obtained for As species using ultrasound were lower in comparison with those obtained by ASE. In more recent works, the use of ASE has been proposed for extraction of Se compounds from yeast-based selenium supplements and watercress [87,88,90]. In these procedures, samples were weighed into stainless-steel extraction cells previously loaded with an inert diatomaceous material as a dispersing agent. The cell content was extracted with aqueous solution containing PMSF and DTT using temperatures about 100 °C and pressure of 1500 psi. After extraction, cells were purged with nitrogen to aid the complete recovery of the extraction solution [87,88,90]. For Se species extraction using ASE procedure the results were similar to those obtained using the standard method using ultrasound. In addition, it was observed that the increase of the temperature did not affect the Se pattern observed for the low molecular weight compounds and also increases the efficiency of the high molecular weight fractions. However, the use of temperatures higher than 115 °C caused a degradation of the major low molecular weight Se compounds [87].

It is important to point out that other types of extraction procedures based on solvents, as supercritical fluid and cloud point have not been used for metallomics field, but for proteomics (determination and purification) [97,98] and in this sense, these procedures will not be detailed in this review.

5.2. Microwave-assisted extraction

Recently, the use of microwave radiation was described for different applications such as drying, leaching, clean-up, as well as adsorption/desorption, extraction and digestion [32,51,99–102,62]. One of the main reasons of the efficiency of microwave heating could be attributed to molecular motion by migration of ionic species and/or rotation of dipolar species under action of microwave radiation field [64]. Thus, samples or solvents containing dielectric material (substances that presenting induced or permanent dipolar moment) could absorb microwave radiation and, as a consequence, it can result in a fast and uniform heating [103,104].

Microwave extractions have been recognized as an efficient and suitable way for extraction of labile components from various complex matrices. Particles are heated uniformly by microwaves and also, the undesirable effects of higher temperature can be avoided by adjusting power levels, exposure times and number of repeats of irradiation. It has been demonstrated that high temperatures which can be generated using microwave energy are not required in order to facilitate rapid desorption from matrices [51]. As a result of this fast and controlled heating, microwave radiation as an auxiliary energy can greatly speed up the extraction in relation to the procedures employing conventional heating [8]. However, preliminary tests must be performed before extraction in order to avoid undesirable effects that could result in sample degradation and loss of analyte identity as well as species losses due to excessive microwave heating [8,51,99].

Microwave energy was used for arsenic species extraction from fish and oyster tissue for further separation and determination using capillary electrophoresis inductively coupled plasma mass spectrometry (CE–ICP-MS). Arsenic species in biological tissues were extracted using 80% (v/v) methanol–water mixture, put in a closed centrifuge tube and kept in a water bath, using microwave heating. The extraction efficiency of individual arsenic species added to the sample at 0.5 mg As/g level was between 96% and 107%, except for As(III), where efficiencies of 89% and 77% were achieved for oyster and fish samples, respectively [105].

Samples containing iodine (I^- and IO_3^-) and bromine (Br^- and BrO_3^-) species were subjected to electrophoretic separation before determination by ICP-MS. In this point, current sample preparation methods using wet digestion and concentrated acids are not widely applicable due to iodine losses by volatilization, as HI or I_2, which can result in nonquantitative recoveries [61,100,106–108]. In addition, memory effects in the ICP-MS introduction system have been reported and sample preparation approaches using sample dilution or dispersion in alkaline solution as ammonia, sodium hydrox-

ide, tetramethylammonium hydroxide (TMAH) and water-soluble tertiary amines solution (CFAC) are recommended [108,109]. In this sense, microwave-assisted extraction was used for extraction of iodine and bromine species from tomato leaves and seaweed [107]. Samples were treated with TMAH solution under heating by microwave radiation at 90 °C. The authors observed that the extraction efficiency of iodine and bromine species in tomato leaves and seaweed were better than 87% and 83%, respectively. It was considered a fast and easy extraction method allowing a rapid and sensitive procedure for iodine and bromine speciation analysis [107].

In order to characterize metal-linking proteins in laboratory mice (*Mus musculus*), a metallomic approach using size exclusion chromatography inductively coupled plasma mass spectrometry (SEC-ICP-MS) was applied to cytosolic extracts from different *Mus musculus* organs such as lung, liver, spleen, kidney, brain, testicle, hearth and muscle. In this way, extracts obtained from separations techniques were digested in a similar way but using a mixture of concentrated nitric acid and hydrogen peroxide in closed vessels assisted by microwave radiation. Arsenic, Cu, Fe, Mn, Ni and Pb were analyzed by ICP-MS [67].

5.3. Ultrasound-assisted extraction

Extraction can be assisted by auxiliary energies in order to improve its efficiency and to reduce treatment times. Thus, selective solubilization of analytes can be accomplished with the assistance of ultrasound, which could be dictated by both solubility mechanisms and transport phenomena. The use of ultrasound as an auxiliary energy is one way of increasing the extraction efficiency, mainly by boosting the transport phenomena [110]. The source of high-energy vibrations in ultrasonic equipments is a transducer designed to convert either mechanical or electric energy into ultrasound. The widespread ultrasound transducer (electromechanical) in analytical devices is most commonly used to power bath- and probe-type sonicators [110].

The effects of ultrasound are primarily related to the cavitation phenomenon, which involves the generation, growth and violent collapse of vapor bubbles during ultrasound application [110–112]. The implosion generates rapid adiabatic compression of gases and vapors within the bubbles or cavities and, as a consequence, high effective temperature and pressure are generated with heating and cooling rates in order to 10^{12} K s^{-1} [112,113]. The high temperature results in an increased solubility of the analytes in the extractant medium and in diffusivity of the analytes from the sample matrix to the outer region. The increased pressure favors the penetration of the extractant into the sample and transport between the solid matrix and liquid phase at the interface. Besides, the oxidative energy of radicals created by sonolysis of the solvent during cavitation could increase the efficiency of ultrasound-assisted extraction [110,112–114]. In addition, some effects could be involved during extraction procedure assisted by ultrasound such as: (i) collapse of bubbles generated in the proximity of the solid surface produces high-speed microjets which can raise transport rates and increase the surface area through surface pitting; (ii) particle fragmentation through collision also increases the surface area and, in addition, it facilitates the extractant penetration into the matrix; (iii) acoustic streaming disrupts the diffusion layer on the surface; and, (iv) ultrasonic energy facilitates diffusion of the analytes to the outer zone [110,115]. Nevertheless, extraction procedures assisted by ultrasound energy could result in some undesirable effects promoted by different phenomena that can occur in extracting medium as already discussed. In this sense, optimization must be performed in order to avoid analyte losses and sample degradation [110,116].

Ultrasound probe leaching and separation by 2D-GE was used for the characterization of Se-containing proteins in selenium-rich yeast. After centrifugation, the total Se content in the supernatant was measured by ICP-MS [76]. Ultrasound extraction was used for speciation of nickel in a plant tissue by LC with parallel ICP-MS and electrospray MS–MS detection. The authors reported that quantification of species showed that in the water extract, after ultrasound-assisted procedure of latex, more than 99% of the nickel were complexed by citrate and 0.3% were complexed by nicotianamine [117]. In another work, arsenobetaine, arsenite, arsenate and dimethylarsinic acid were identified and determined by CE–ICP-MS and capillary electrophoresis electrospray mass spectrometry (CE–ESI-MS) with a previous step of ultrasound-assisted extraction [68]. Using an ultrasound bath, Hg species were extracted from hair [118] and seafood [119] samples with mercaptoethanol, L-cysteine and HCl for further analysis by LC–ICP-MS.

A water-soluble selenium-containing protein in selenium-rich yeast was ultrasonically extracted for subsequently separation using 2D-GE and detection by an approach based on the consecutive use of nano-HPLC–ICP-MS and nano-high performance liquid chromatography electrospray ionization mass spectrometry (HPLC–ESI-MS). A sample of selenium-rich yeast was extracted with an ultrasonic probe in water containing DTT (anti-oxidant) and PMSF (inhibitor of protease activity) in ice bath. In order to measure the total selenium extraction recovery, the supernatant was analyzed by ICP-MS. The efficiency of different extraction steps was evaluated by total selenium analysis and SEC-ICP-MS. The authors observed that it was possible to preserve the protein integrity after each extraction and purification step for further separation by 2D-GE, mapping by nano-HPLC–ICP-MS and identification of selenium-containing peptides by nano-HPLC–ESI-MS [86].

6. Cleanup and preconcentration

After or during separation procedures, interfering compounds have to be inactivated or removed. Interfering compounds are substances present in the sample that interact with metal and metalloid species present in a cell or tissue type or can cause problems in the separation step [32]. Especially for analytical separations, as GC, LC and electrophoresis, sample cleanup is particularly important. Many solid matrixes and biological samples can contain a large amount of compounds of different nature, as hydrocarbons, polysaccharides, lipids, amino acids and glycerides, resulting in complex chromatograms [120,121]. It makes difficult the identification of analytes when they are present at a much lower concentration than the interfering species and a cleanup step prior to the analytical measurement must be performed to separate the species of interest from matrix components [73,121]. As an example, very rapid lipid decomposition is observed in biological samples and then they are commonly completely extracted from the sample. In addition, as a result of a nonspecific extraction method, some extracts can present high amount of non analytes compounds, making necessary to apply cleanup methods [120]. Lyophilization, centrifugation, ultrafiltration, gel filtration, gel permeation chromatography (GPC), solid-phase extraction (SPE) and column chromatography are some of techniques used for cleanup and purification of extracts [121]. A selective precipitation of some components of solution can be sometimes performed. Based on the precipitation phenomena, coprecipitation can be used to concentrate an analyte by coprecipitating it with species which are more abundant into the medium [122].

The simplification of sample matrix often allows the preconcentration of the analyte in the isolated fraction by means of off-line procedures. In this way, lyophilization could be required when complexity of biological matrices often makes it necessary to isolate the molecular mass fraction containing the species of interest in order to separate it from matrix components such as high-molecular weight polysaccharides or inorganic salts that would

otherwise foul the analytical column used in the subsequent analysis [73]. Other process used to interferences removal from matrix involves the use of the centrifugal force (by centrifugation) for the separation of mixtures and it is widespread used in analytical laboratories. It was used off-line for blood plasma separation prior to Se determination in order to evaluate the *in vivo* bioavailability of this element when mouses were fed with Se enriched mushrooms [123]. In addition, a subcellular fractionation was performed by centrifugation of cell homogenates suspended in Tris–HCl buffer in order to enable a metallomics approach for Cr, Cu, Fe, Mn, Ni and Pb determination in mushrooms (*Ustigo maydis*) by GF AAS, and the molecular weight distribution of compounds containing target elements was achieved as an additional aspect of the metallomics approach by SEC with UV and ICP-MS detection [72]. Centrifugation was also used for separation of plasma from whole blood for analysis of monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) by CE–ICP-MS after the intake of disodium monomethylarsonate in horses [124].

Ultrafiltration is an off-line membrane separation technique used to separate substances according to the respective molecular weight and size. It is ideally suitable to separate salts and other low-molecular weight solutes from high-molecular weight species. Ultrafiltration is based on a pressure differential across the semipermeable membrane to drive permeable materials through the membrane. The centrifugal force is used to create a pressure differential on the sample to facilitate the separation process. Membranes used in molecular filtration have pore diameters ranging from 1 to 1000 Å and usually separate particles up to 10^6 Da [73]. Particles with molecular weight or size less than the membrane molecular weight cut-off pass through the membrane and emerge as permeate. Solutes with greater molecular weight or size are retained by the membrane and are concentrated during the molecular filtration process. Ultrafiltration offers a fast way to promote the separation with minimal denaturation of molecules compared to precipitation methods [73]. Using ultrafiltration process as a cleanup method prior to chromatographic analysis for arsenic species in alga samples, it was possible to determine the extracted arsenic fraction with a molecular weight lower than 10 kDa, which accounts for about 100% for all analyzed samples [125]. Another application of ultrafiltration was the fractionation of different blood compartment (whole blood, plasma, residue, plasma-ultrafiltrate and protein-residue fraction) for further evaluation of the cisplatin, carboplatin and oxaliplatin distribution by ICP-MS measurement of total platinum after microwave digestion [126]. In addition, arsenic species (As(III), As(V), MMA, DMA, and arsenobetaine (AsB)) were determined in diluted whole blood using LC–ICP-MS with previous step of larger molecules removal (larger than 3000 Da) by ultrafiltration [127]. Ultrafiltration was also used as an alternative technique for paired human cerebrospinal fluid and serum samples for subsequent total metal concentrations (Ca, Cu, Fe, Mg, Mn and Zn) by SEC–ICP-MS [128].

The use of size-exclusion gel filtration (low pressure) process performed on-line can result in a finer resolution for subsequent separation step. It could act as a cleanup step and allows the elimination of high molecular mass biopolymers (polysaccharides, proteins) and other compounds that might be absorbed on the chromatographic stationary phase or co-eluted with metal compounds during speciation analysis. In this sense, it could be possible to avoid the degradation of chromatographic resolution, reduction of the column lifetime and interference with MS detection [73].

Iron speciation in solutions containing physiological concentrations of citrate and human serum albumin at physiological values of pH and ionic strength was performed by gel filtration linked to ICP-MS [129]. A 2D micro-HPLC–ICP-MS system consisting of a gel filtration column and an anion-exchange column was constructed to separate two Cd-enriched metallothionein isoforms [130]. Besides, a multi-mode capillary gel filtration prior to HPLC columns of capillary size coupled with ICP-MS were evaluated for the separation of naturally occurring selenocompounds in selenized garlic and mouse urine samples. A distinct peak could be observed during subsequent selenocompounds separation and Se determination by HPLC–ICP-MS [131].

Gel permeation chromatography is a size-exclusion method performed on-line that uses organic solvents (or buffers) and porous gels for macro-molecules separation. The choice of packing gel is determined by its exclusion range and pore size, which must be larger than the analytes. The use of GPC is recommended for both polar and nonpolar analytes, for elimination of cellular components, lipids, proteins, polymers, natural resins and other dispersed high-molecular-weight compounds from the sample. Usually, GPC is more efficient for removal of high-boiling point materials that condense in the injection port of a GC or in the front of GC column [121]. A method for determining diphenylarsinic acid (DPAA) in human hair and nail samples samples was developed based on a combination of hydrophilic polymer-based gel permeation HPLC and ICP-MS [132].

Solvent extracts can be also cleaned up by traditional column chromatography or by SPE cartridges, common cleanup methods used for biological and clinical sample preparation. Solid-phase extraction is based on the nonequilibrium, exhaustive removal of chemical constituents via retention on a contained solid sorbent from a flowing liquid sample and a further recovery of selected constituents is performed by elution from the sorbent. This method provides efficient cleanup of steroids, esters, ketones, glycerides, alkaloids and carbohydrates as well as cations, anions, metals and inorganic compounds [8,121]. As example, high saline or ionic compounds can be retained by passing the extract through an alumina column [133]. Solid phase micro extraction (SPME) has been also used and some advantages have been recently highlighted: SPME can be considered as a non-exhaustive extraction method, it requires low cost and it is a solventless sample preparation strategy, it results on the reduction of the matrix effects and also allows the fully automation of the whole process [134].

Removal of organic matrix using C_{18} cartridges has been performed in human urine samples for quantification of selenium species by HPLC–ICP-MS [135]. Using anion exchange cartridges, SPE was used for elimination of picric acid, following crown-ether extraction, for the determination of selenium species in urine by LC–ICP-MS [136].

Headspace SPME coupled to LC–ICP-MS determination was used for speciation of methylated and ethylated mercury in urine. The mobile phase, a mixture of L-cysteine, 2-mercaptoethanol and methanol was used for desorbing organomercury species from the polydimethylsiloxane–divinylbenzene (PDMS–DVB) fiber [137]. For the speciation of organotin species by GC–ICP-MS, three different SPME coatings were evaluated and better results were obtained with using divinylbenzene–carboxen–polydimethylsiloxane (DVB–CAR–PDMS) fiber [138]. A method combining SPME and multicapillary gas chromatography (MCGC) hyphenated to ICP-MS was developed for simultaneous speciation analysis of Hg, Pb and Sn organometallic compounds in biological certified reference material (CRM). A comparison of seven SPME fibers was carried out in terms of extraction efficiency after *in situ* derivatization with $NaBEt_4$. Fiber with mixed coatings (PDMS–DVB and CAR–PDMS) enabled the highest overall extraction efficiency for all investigated species [139].

Some solvent extraction methods have a common disadvantage of yielding large volume of extract (usually about 1–5 mL). As a result of the extractions procedures, analytes can be often diluted in large volumes of extraction solvents and an additional preconcentration step is required for extracts containing the analytes prior to analysis [4]. This aspect is particularly important, especially when

the analysis is being performed at the trace level and an additional step is necessary to increase analyte concentration in extract. If the analyte is nonvolatile and the amount of solvent to be removed is not so large one option is the solvent vaporization using nitrogen or helium stream flowing across the surface or through the solution. In this point care should be taken that the solvent is lost only by evaporation and not as aerosol to avoid analyte losses along such fine droplets [121]. During preconcentration of derivatized species losses for more volatile alkyl derivatives may occur and better recoveries are obtained when preconcentration is performed prior to derivatization step [4]. When large volume reduction is required this method is not efficient and a rotatory evaporator is used. In this procedure, sample is placed in a round-bottomed flask which is heated in a water bath, a water-cooled condenser is attached at the top and the flask is continuously rotated to expose maximum liquid surface to evaporation. With a small pump or a water aspirator, the pressure inside the flask is reduced, and combined with a mild heating, the solvent is slowly removed by distillation and collected in a separate flask [121].

7. Derivatization techniques

Most metallic compounds present low volatility and hence are not suitable for GC analysis. In addition, physical-chemical properties of many metallic species make direct detection impossible. As example, some organoselenium and organomercurials are thermally sensitive and some metallic species were observed to be UV transparent making difficult their analysis without a previous conversion step [140]. Another problem is that the sensitivity achieved by direct detection does not meet the requirements for real sample analysis [140,141]. Therefore, chemical modification of the target analytes converting the existing states of the analyte to another states can be applied to facilitate its analysis [140]. This change, commonly called derivatization, needs to retain the structure of the element-carbon bonds to ensure that the identity of the original moiety remains conserved [4].

The most common use of derivatization has been mainly focused on the enhancement of detection sensitivity and the treatment of polar compounds to convert them into more easily extractable, thermally stable, more volatile analytes or with better chromatographic behavior. New developments are mainly focused on novel configurations and less consumption of samples and reagents [8,36,120,142]. On column derivatization is an absorbing mode in the analysis of samples with limited volume as CE and capillary LC. Derivatization can be also carried out in a sample matrix before or simultaneously with the extraction step. It can be considered a simple approach but it is prone to side reactions and interferences and can be dependent of the matrix and also of the derivatization efficiency. In addition, previous steps must be carried out in some cases to convert not reactive species by a derivatization reaction into a reactive one [143]. Chemical modification allows speciation analysis using CE, LC or GC. By converting the analytes from their existing forms, a suitable separation and detection can be achieved. Moreover, most of derivatization methods can be used combined with other sample preparation strategies as extraction and enrichment procedures [140].

The most common methods used for derivatization are hydride generation (HG), cold vapor generation (CVG), ethylation and Grignard reactions. These methods can be considered as versatile regarding the organometallic species to be derivatized. Selection of HG, CVG or Grignard reactions is dependent on the concentration of interest and of the matrix and the sample throughput required for analysis. Commonly, the derivatives are concentrated by cryotrapping or extraction into an organic solvent [120,144].

Derivatization strategy is also commonly used during sample preparation for Se-containing proteins. Iodoacetamide or iodoacetic acid are able for alkylation of thiol groups, preventing their oxidation. The reaction for derivatization introduces a mass increase of 58.0055 (C_3H_5NOSe for aminoacid residue composition and C_5H_7NOSe for residue composition after derivatization) [83]. The formation of volatile hydrides is applicable to compounds of several elements such as As, Bi, Cd, Hg, Ge, Pb, Sb, Se, Sn and Te [44,140,145,146]. The reaction of inorganic forms of these elements with sodium tetrahydroborate ($NaBH_4$) results in the formation of simple hydrides. However, not all species react equally fast under same reaction conditions and this behavior can be suitable in some cases for separation of different species. In addition, under similar conditions, some alkyl derivatives can also generate volatile hydrides. Then, its suitability is restricted by thermodynamic inability of some species and or considerable kinetic limitations for some species [120,140]. One advantage of HG is the separation of the analyte from matrix that is obtained as a consequence of the analyte release from solution. Hydride generation can be connected with a preconcentration step in a trap of liquid nitrogen, allowing the release of analytes by slowly raising the temperature [120,147,148]. However, the disadvantage that is related to HG includes the interference in the liquid phase as the inhibition of the hydride formation or its transformation due to interaction with other species in the solution. On the other hand, CVG is similar to HG and the volatile product is the volatile metal. It has been used mainly for Hg species determination in biological samples due to the ability of these species to react with reductant (borohydride) [149,150].

Derivatization with alkyl(aril) borates is another alternative, using mainly sodium tetraethylborate ($NaBEt_4$), as for the determination of Sn, Se, Hg, and Pb species. Ethylation with $NaBEt_4$, which is water soluble and stable in water, can be performed in aqueous medium and then derivatization and extraction can be carried out in the same step [120,140]. Derivatization with $NaBEt_4$ has been used for methylmercury species in biological tissues for analysis by GC–ICP-MS. Previously, methylmercury was recovered from sample by leaching with HCl [151] or by solubilization with TMAH [152]. After derivatization, purge and trap [152] or solvent extraction into toluene or nonane [139] were performed. Organotin speciation analysis in biological tissues has also been performed using $NaBEt_4$ as derivatization reagent [153–155]. Following derivatization reaction, a classical extraction approach can be used, mainly with isooctane [153] and also novel techniques such as stir bar solid phase extraction [154] or headspace SPME [155] were reported. Speciation analysis of Hg, Pb and Sn organometallic compounds in biological CRMs was carried out in terms of extraction efficiency for different SPME fibers after *in situ* derivatization with $NaBEt_4$. Derivatization with $NaBPr_4$ was also studied but for some fibers ethylation provided higher efficiency [139].

Speciation of tin compounds was performed using spike species-specific isotope dilution (SSID) coupled with GC–ICP-MS using $NaBEt_4$ as derivatization agent [156]. Different extraction procedures for speciation of phenyltin compounds were investigated in order to observe possible interconversion reaction of Sn species. It was observed that degradation of phenyltin compounds was affected by the acidity of the extractant, the presence of complexing reagents and the use of ultrasound and microwave. On the other hand, no formation of phenyltins through phenylation and degradation of monophenyltin to inorganic Sn were observed [156].

Reactions with Grignard reagents were applied as derivatization step for Sn, Hg and Pb speciation [120]. Some drawbacks that are related to the use of derivatization with Grignard reagents are their atmospheric instability and their ability to be hydrolyzed in water producing $Mg(OH)_2$. Reagents must be stabilized in ether and stored under inert atmosphere and water needs to be removed with complexing agents. As a consequence of this instability, sample preparation can be time consuming [120,157].

Table 1
Applications of ICP-MS for total metal(oid) and metal(oid)-containing compounds analysis.

Element/species	Sample	Sample preparation	Separation/detection technique	Ref.
As	Freeze-dried apple	For total As determination: Samples were mixed with concentrated HNO_3 and digested under microwave radiation. For As species determination, different procedures were evaluated using: (i) ultrasound and different solvents as extracting media. After sonication, the tubes were centrifuged and (ii) sample treatment with α-amylase solution, mechanical stirring and ultrasound extraction	HPLC–ICP-MS and ICP-MS	[78]
As	Seaweed	Seaweed was dried by lyophilisation and homogenized. For total As determination sample was digested with HNO_3 and H_2O_2 using a hot plate. Accelerated solvent extraction was used for As species extraction	ICP-MS	[96]
As species	Whole blood and urine	As(III), As(V), MMA, DMA, and AsB were determined after dilution with $HgCl_2$ and ultrafiltration for removing species which are likely to degrade LC column. Total As content was determined after microwave-assisted wet digestion with HNO_3	LC–ICP-MS	[127]
As species	Whole blood	After the intake of disodium monomethylarsonate in horses, plasma was separated from whole blood by centrifugation for analysis of MMA and DMA	CE–ICP-MS	[124]
As species	Fish	Accelerated solvent extraction and sonication methods were investigated for extraction of As species (AsB-AsC, As(III), As(V), MMA and DMA) from fish. Total digestion with HNO_3 and H_2O_2 was performed to evaluate the recoveries obtained by both extraction procedures	LC–ICP-MS	[95]
As species	Fish and oyster tissues	Samples were lyophilized, homogenized and ground. Arsenic species extraction was performed by microwave radiation with methanol and water. After microwave heating, samples were allowed to cool and directly centrifuged. The supernatants were diluted and filtered through a PVDF filter	CE–ICP-MS	[105]
Br and I species	Foodstuff	A procedure based on microwave-assisted extraction using TMAH as extractant medium was used. After microwave heating, samples were centrifuged and the supernatants were diluted and then filtered through a PVDF filter	CE–ICP-MS	[107]
Cd, Cr, Cu, Fe, Ni, Pb and Zn	Rat tissues (liver and kidney)	Samples were lyophilized and homogenized to isolate the water-soluble proteins. The proteins were extracted in water by using an ultrasonic probe and further separated by ultracentrifugation. Before metals determination by LA–ICP-MS, gel electrophoresis analysis was performed	MALDI-TOF–MS and LA–ICP-MS	[56]
Co, Cr, Cu, Fe, Mn, Ni, Pb and Se	*Ustilago maydis*	Samples were homogenized in liquid nitrogen and suspended in Tris–HCl, containing protease inhibitors and the extract was submitted to centrifugation steps. For total elements determination, samples were digested using heating block and concentrated nitric acid was added. For metal-binding to cells, a portion of sample was mixed with SDS, Tris–HCl containing PMSF and further centrifuged	SEC-UV–ICP-MS and AAS	[72]
Cu and Zn-superoxide dismutase	Human serum samples (red blood cells)	Samples were initially centrifuged and the cells were hemolyzed by addition of ice-cold water and mechanical shaking. After, a solution of ethanol/chloroform was added and further shaking and centrifuged	HPLC–ICP-MS and MALDI-TOF–MS	[74]
Cu, Ca, Fe, Mg, Mn and Zn	Paired human serum and cerebrospinal fluid	Ultrafiltration prior to ICP-MS analysis is investigated as an alternative fractionation technique. Ultrafiltration devices with 10 kDa molecular weight cut-off were used and total metal contents were determined in the original samples, in the permeate samples and in the retentate samples to establish a mass balance for quality control	ICP-MS and SEC–ICP-MS	129
Diphenylarsinic acid	Human (hair, nail and urine)	Solid samples were digested with alkali (NaOH), and diphenylarsinic acid (derivative) was extracted with diethyl ether, centrifuged, redissolved in water and analyzed. Liquid samples were analyzed directly after filtration	HPLC–ICP-MS	[132]
Fe species	Human serum albumin	Gel filtration associated with ICP-MS was used to separate the albumin-bound iron from the small molecular weight iron-citrate complexes and quantitatively determine the concentration of both iron pools	Gel filtration and ICP-MS	[129]
Hg compounds	Rice and horehound	Samples were ground in a mortar under liquid N_2 and extraction was performed at low temperature using formic acid	HPLC with simultaneous detection by ICP-MS and ES-MS	[12]
Hg species	Urine	Different SPME fibers were evaluated for extraction of Hg species (MeHg and EtHg): polydimethylsiloxane, polydimethylsiloxane/divinylbenzene and polyacrylate. After sample homogeneization, extraction was started by exposing the fiber to the headspace of the. After sampling, the fiber was withdrawn and inserted into the desorption chamber filled with mobile phase for analyte desorption	SPME coupled with LC–ICP-MS	[137]
Hg species	Biological tissues (dogfish liver)	Three derivatization approaches were optimized and compared for Hg derivatization for analysis by GC–ICP-MS (i) anhydrous butylation using a Grignard reagent; (ii) aqueous ethylation using $NaBEt_4$ and (iii) aqueous propylation with $NaBPr_4$. Ethylmercury was used as internal standard	GC–ICP-MS	[151]
Hg species	Biological materials (lobster hepatopancreas, dogfish muscle and fish tissue)	Organic and inorganic Hg species were extracted with TMAH and derivatized with $NaBEt_4$. After cryotraping, species were separated by multicapillary GC prior to analysis by ICP-MS	GC–ICP-MS	[152]
Hg species	Hair	A procedure based on ultrasound extraction with mercaptoethanol, L-cysteine and HCl was developed for the determination of inorganic Hg, MeHg and EtHg	LC–ICP-MS	[118]
Hg species	Seafood	Ultrasound was applied for inorganic Hg and MeHg extraction prior to analysis. Different extraction times were evaluated	LC–ICP-MS	[119]

Table 1 (*Continued*)

Element/species	Sample	Sample preparation	Separation/detection technique	Ref.
Hg species	Whole blood	Alkaline extraction with TMAH was used for total Hg and inorganic Hg using a flow injection system.	CVG–ICP-MS	[150]
Ni species	Latex form *Sebertia acuminate* tree	Size-exclusion and CZE were investigated for the separation of Ni-complexes. Samples of the dried latex were frozen, ground with pestle and mortar, extracted with water in an ultrasonic bath and centrifuged. Six stable Ni species were identified by ES–MS after preparative scale SEC	LC–ICP-MS and ES–MS	[117]
Organometallic compounds (Hg, Pb and Sn)	Biological tissues and road dust	Different SPME fibers were evaluated for extraction/preconcentration of 10 organometallic compounds of Hg, Pb and Sn. After derivatization ($NaBEt_4$ and $NaBPr_4$ were evaluated) samples were separated by a MCGC column and analyzed by ICP-MS	Multi capillary GC–ICP-MS	[139]
Organotin compounds	Potatoes and mussels	Potatoes and mussels were immersed in liquid nitrogen and crushed. Extraction was performed by SPME after digestion using TMAH	Capillary gas chromatography and ICP-MS	[155]
Organotin compounds	Biological tissues (muscle and fih)	Different extraction procedures for the speciation analysis of phenyltin compounds were investigated. Derivatization ws performed with $NaBEt_4$. Multi-isotope spike SSID calibration strategy was used	GC–ICP-MS	[156]
Organotin compounds	Mussels	Organotin compounds (tributyltin and triphenyltin) were extracted from their aqueous matrix using a stir bar coated with PDMS. After extraction, the stir bar was desorbed in a thermal desorption unit and then the compounds were cold-trapped on a precolumn. After flash heating the compounds were rapidly transferred to the GC where they were separated on a capillary column. Derivatization was carried out with $NaBEt_4$	GC–ICP-MS	[154]
Organotin compounds, Hg and Pb	Seafood tissues	Extraction was performed with TMAH solution under microwave radiation	MCGC and ICP-MS	[153]
Pb and Zn	Metalloproteases of bacterial origin	Bacterial cells were solubilized with Tris–HCl, NaCl and they were disintegrated with glass beads and further submitted to centrifugation, ultracentrifugation	HPLC–ICP-MS	[75]
Pt	Human malignant cells	Chemical lysis using Tris–HCl, EDTA, DTT, among others and sample was stand on ice. For sample preparation, cells were submitted to freeze–thaw cycles and thawing or to osmosis and mechanical shaking	ICP-MS	[15]
Pt species	Whole blood and plasma	The distribution of cisplatin, carboplatin and oxaliplatin in human plasma samples was investigated. Ultrafiltration and precipitation of proteins with acetonitrile followed by membrane filtration were studied. Samples were digested by microwave-assisted wet digestion with HNO_3 and H_2O_2 for the determination of total Pt content	Hydrophilic interaction liquid chromatography (HILIC) with ICP-MS	[126]
Se	Selenium-rich yeast	Samples were extracted with aqueous solution containing DTT and PMSF and submitted to ultrasound extraction using a probe and the supernatant was analyzed by ICP-MS. Further, supernatant was precipitated with acetone, centrifuged and dissolved in water for total Se determination	2D-GE, nanoHPLC–ICP-MS, nanoHPLC–ESI MS/MS	[86]
Se	Selenium-rich yeast	For total Se: samples were mixed with concentrated HNO_3 and digested under microwave radiation. For preparation of protein extracts samples were extracted using an ultrasound probe in water, Tris–HCl. After, a centrifugation step was applied and a further centrifugation with ultrasound using CHAPS, DTT was used	ICP-MS	[76]
Se	Selenium-rich yeast	Eight sample preparation procedures were evaluated based on the solubilization in different solvents, use of mechanical stirrer and further centrifugation	HPLC–ICP-MS	[80]
Se	*Brassica juncea*	Several procedures were evaluated for Se extraction based on digestion/extraction procedures, using Tris–HCl, HCl and enzymes	LC–ICP-MS and ES–MS	[71]
Se	African catfish	For total Se determination, ultrasound extraction was performed using protease. Different extraction procedures were applied: (i) ultrasound extraction and (ii) glass tissue homogenizer.	ICP-MS and LA–ICP-MS after SDS–PAGE	[70]
Se	Selenium-enriched plants	Different sample preparation strategies were evaluated for Se determination and speciation in plants. The procedures evaluated were based on: (i) acid extraction (using HCl), (ii) buffer extraction with ammonium acetate buffer and (iii) enzymatic hydrolysis. In addition, ultrasound extraction was investigated	HPLC–ICP-MS and ICP-MS	[81]
Se	Selenium-rich yeast	Total Se determination was performed in samples after acid digestion with nitric acid. Extraction was performed in two steps: with aqueous buffer using Tris–HCl and using a SDS-containing buffer (Tris–HCl). Ultrasound extraction and centrifugation were also used during the sample preparation.	LA–ICP-MS, 2D-GE and ESI–MS	[83]
Se	Selenium-enriched supplements	Total Se determination was performed after sample digestion in a microwave oven, using nitric acid and hydrogen peroxide. For extraction of Se compounds, ASE procedure was applied using water containing PMSF and DTT. After extraction, cell were purged with nitrogen and extracted again by ASE. An enzymatic hydrolysis was also investigated using protease, lipase, Tris–HCl and the mixture was submitted to extraction by mechanical homogenization and ASE. A further centrifugation step was performed	Reversed phase ion pair-HPLC–ICP-MS and ESI–MS/MS, ICP–MS	[87]
Se	Watercress extracts	Total Se determination was performed after sample digestion in a microwave oven, using nitric acid and hydrogen peroxide. Extraction of Se compounds was performed by ASE and by enzymatic hydrolysis with protease, lipase and Tris–HCl	HPLC–ICP-MS	[90]
Se	Human serum selenoalbumin	All serum samples were filtered before the analysis and an enzymatic hydrolysis using lipase-protease was evaluated with and without sample stirring	HPLC–ICP-MS	[91]

Table 1 (*Continued*)

Element/species	Sample	Sample preparation	Separation/detection technique	Ref.
Se	Selenized yeast	Se compounds were extracted from proteins using a two-step enzymatic hydrolysis with protease, lipase and driselase in a focused microwave reactor. In addition, conventional enzymatic hydrolysis was also performed for comparison of results. Samples were ground and mixed with a mortar with pestle and a Tris–HCl buffer was added containing protease and lipase and further stirring. The mixture was submitted to microwave radiation and further centrifuged. The supernatant was filtered and the residue was subjected to microwave-assisted proteolytic digestion with buffered enzymatic solution containing driselase. Finally, the two supernatants were pooled, filtered and diluted prior analysis	HPLC–ICP-MS using IDA	[93]
Se (water-soluble gamma-glutamyl-Semethylselenocysteine)	Yeast-based selenium supplements	For extraction of Se compounds, ASE procedure was applied using water containing PMSF and DTT. After extraction, cell were purged with nitrogen and extracted again by ASE. An enzymatic hydrolysis was also investigated using protease, lipase, Tris–HCl and the mixture was submitted to extraction by ASE and further centrifuged	RP HPLC–ICP-MS and RP HPLC–ESI-MS/MS	[88]
Se compounds	Selenized garlic and urine from seleno-toxicosis rat	Multi-mode gel filtration HPLC columns of capillary size coupled with ICP-MS were evaluated for the separation of naturally occurring selenocompounds. Samples were homogenized with water using a mechanical homogenizer under an atmosphere of nitrogen with ice-water cooling. The homogenate was centrifuged and the supernatant was filtered through a PVDF membrane filter	HPLC–ICP-MS and ESI–MS/MS	[131]
Se species	Urine	Crown ether extraction was performed in human urine samples, mixture was centrifuged and submitted to anion-exchange solid phase extraction for further analysis	ICP-MS	[136]
Se species	Urine	Human urine samples were processed in a vacuum manifold system by passage through C18 cartridges previously conditioned with methanol and water. The cartridge was washed with phosphate buffer prior to analysis	HPLC–ICP-MS	[135]
Seleno-methionine	Yeast and nuts	Extraction step was performed using methanesulfonic acid and β-mercaptoethanol and the mixture was submitted to heating at reflux. For protein hydrolysis, samples (defatted or ground) were mixed with Tris–HCl, proteinase and further incubation and centrifugation	HPLC–ICP-MS	[84]

Table 1 summarizes the main applications of ICP-MS for further total metal(oid) or metal(oid)-containing compounds analysis with remarks on sample preparation strategies according to the discussion presented in this review.

8. Conclusion

In this review several approaches that have been used as sample preparation for further detection and speciation of a wide range of analytes were discussed in order to understand the interaction of trace elements in biomolecules. A summary of developments and applications in the last fifteen years was reported. Sample preparation methods for bioinorganic analysis could be still considered as a challenge step in order to preserve the analyte (or its species) information during the analytical process. On this aspect, recent applications which have been published concerning metal(oid) and metal(oid)-containing compounds analysis in biological systems were reviewed. Alternative sample preparation strategies regarding the use of enzymes, accelerated solvent extraction and the use of ultrasound and microwave radiation have been currently reported. Finally, the continued advancement of sample preparation strategies has been considered as extremely important to the metallomics field.

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