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REVIEW

A tutorial and mini-review of the ICP-MS technique for determinations of transition metal ion and main group element concentration in the neurodegenerative and brain sciences

Yonghwang Ha · Olga G. Tsay · David G. Churchill

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Abstract New techniques in the biosciences are always welcomed and important. Reviews help in updating and disseminating information in scientific fields, especially when there are many advances such as in the vital area in neuroscience. Herein is a recent review of inductively coupled plasma mass spectrometry (ICP-MS) in the context of trace elements, relating to neurodegenerative diseases. The accurate determination of such elemental distributions in Alzheimer's disease, Parkinson's disease, etc. allows for a better understanding of such diseases and relates to the sensitivity and scope of the ICP-MS technique. The elements detected are often "trace" and can be analyzed for both body tissues and fluids. We discuss the practical use of ICP-MS. This includes the explanations of the instrumental setup, elements and their detection limits, a brief comparison of ICP-MS with other inorganic analysis instruments, sample preparation, and the analysis method. Next, we discuss neurodegenerative disease and metal ion analysis with ICP-MS. This includes introductions to neurodegenerative diseases, tissue analysis, fluid analysis, and bioimaging of metals in brain tissue samples, and protein analysis application with metals and ICP-MS, broken down into the subtopics of (1) isotope dilution analysis, (2) related immunoassay techniques, and (3) hyphenated instrumental applications. This article is meant to be a primer for a synthetic chemist interested in utilizing this technique and is current through the middle of 2010.

Keywords Alzheimer's disease · Parkinson's disease · Iron · Copper · Zinc

Y. Ha · O. G. Tsay · D. G. Churchill (✉)
Molecular Logic Gate Laboratory, Department of Chemistry, Korea Advanced Institute of Science and Technology (KAIST), 373-1 Guseong-dong, Yuseong-gu, Daejeon 305-701, Republic of Korea
e-mail: dchurchill@kaist.ac.kr

Y. Ha
Hydro-Technology Research Laboratory, Convergence Science and Technology Research Center (CSTRC), Jungwon University, Goesan-gun, Chungbuk 367-805, Republic of Korea

Abbreviations

APP	Amyloid precursor protein
Aβ	Beta amyloid
AES	Atomic emission spectroscopy
ALS	Amyotrophic lateral sclerosis
AS	α-Synuclein
BBB	Blood–brain barrier
BSA	Bovine serum albumin
cps	Counts per second
CRC	Collision reaction cell
CSF	Cerebrospinal fluid
cys	Cysteine
CZE	Capillary zone electrophoresis
DRC	Dynamic reaction cell
DOPAC	3,4-Dihydroxyphenylacetic acid
EDS	Energy dispersive spectroscopy
FI	Flow injection
HPLC	High-performance liquid chromatography
Htt	The huntingtin protein (gene)
ICP	Inductively coupled plasma
IDA	Isotope dilution analysis
LA	Laser ablation
LA-ICP-MS	Laser ablation inductively coupled plasma mass spectrometry
MCI	Mild cognitive impairment
MS	Mass spectrometry
NAA	Neutron activation analysis

NMR	Nuclear magnetic resonance
6-OHDA	6-Hydroxydopamine
OES	Optical emission spectroscopy
PE	Polyethylene
PP	Polypropylene
ppb	Parts per billion
ppm	Parts per million
ppq	Parts per quadrillion
ppt	Parts per trillion
PTFE	Polytetrafluoroethylene
SF-ICP-MS	Sector field inductively coupled plasma mass spectrometry
SN	Substantia nigra
SOD	Super oxide dismutase
ZnT	Zn transporter

Introduction

There are many lines of research relating to the neurodegenerative disorders that include Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), and Creutzfeldt–Jakob disease. Such disorders are commonly related to human protein misfolding and aggregation; this accumulation commonly occurs in, or near, neurons or neurologically related cells. AD is commonly related to amyloid-β and tau proteins. PD and Lewy body dementia are considered as synucleinopathy due to their abnormal accumulation of α-synuclein in the brain. HD is related to the Huntingtin protein; Creutzfeldt–Jakob disease is related to the prion protein. Depending on the point in time in a biological system and conditions present in the organism, unwanted protein dimers, trimers, oligomers, plaques, fibrils, and other morphologies of networks may be formed. Many researchers think certain such phenomena somehow are the cause of the disease, but there are still many open questions relating to this enormously complex issue. Many researchers are trying to unearth the exact mechanism of disease and pinpoint disease-causing chemical species. Other chemical aspects suspected of being involved in such disease etiology include oxidative stress, certain toxic chemicals, enzyme malfunctioning, and the simple act of aging. Also, particular metals can bind into the proteins folded or misfolded quite preferentially (e.g., Cu^{2+} into histidine-rich fragments). Some researchers seek to link a disease to metal ions in some way. Certain transition metals such as Cu, Zn, and Fe have important roles in catalytic activities or transport in the active site of enzymes. When these metals are found abundantly in their free form (uncomplexed, labile), toxic effects can be imparted to the cell, e.g., induction of covalent linkages between monomer proteins. And some researchers support that the abundance/activity/function of Fe, Cu, and Zn (and previously Al) are importantly related to AD and PD.

For the study of metal ions in such diseases, there is a possibility to prepare model systems. There are of course both advantages and technical limitations in studying real living systems. Some instruments can directly analyze tissue (brain tissue, neuron samples, cerebrospinal fluid), and various instruments (HPLC–MS, IC, ICP-MS, MALDI-MS, ICR-MS, etc.) have been developed. In particular, ICP-MS is broadly used. ICP-MS has many advantages: small sample volumes are allowable; short analysis times are possible ($\sim\mu$s for one metal in one sample); good hyphenated utility exists with other instruments (HPLC, capillary electrophoresis, SDS-PAGE, laser ablation (LA) systems). ICP-MS is increasingly becoming an essential instrumental technique for researchers needing knowledge of metal concentration in biological samples of brain, neurons, blood, and CSF. Thus, a review about this technique in the context of neurodegenerative diseases is presented here.

ICP-MS analysis and its practical use

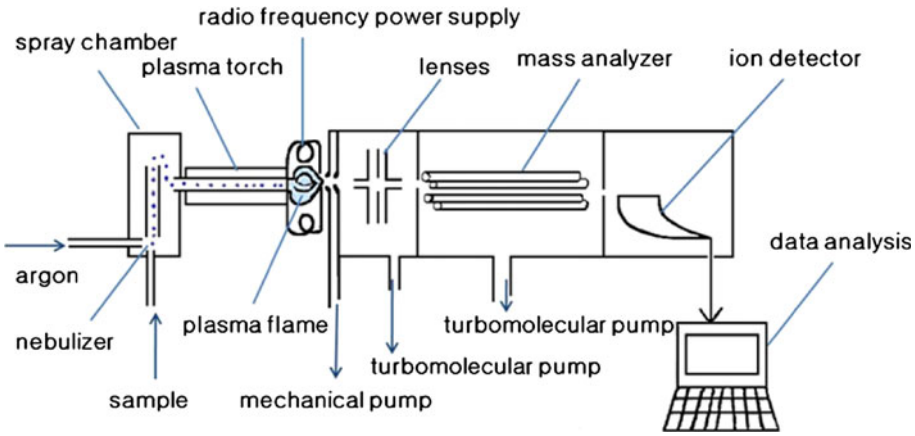
Instrumental setup

Interestingly, ICP-MS is not a very new technique as it was first used commercially in 1983. At the time of this review, a crudely estimated 8,000 or more systems are used around the world [1].

There are five major parts relating to the ICP-MS technique: the sample injection portion, ICP portion, interfacing, mass separation, and detection (Scheme 1). The sample injection parts are usually composed of a nebulizer and sample chamber. This portion can be connected to many kinds of instruments, including LA systems, liquid chromatography, and capillary electrophoresis. The injector system is designed to analyze liquid samples. When solid samples need to be analyzed, the solid sample must first be dissolved in or otherwise prepared by using extra pure grade acid. The LA system can analyze solid surfaces, clinical biological tissues, and 1- or 2-D gel electrophoresis.

ICP generates plasma from Ar gas and radio frequency power. Its temperature reaches 6,000–10,000 K. In this part, the sample converts into the 1+ positive ion streams. The mechanism is like that found in Scheme 2. The positive ion stream is directed to the interface which connects the ICP part (1,000 mbar) and the mass separation part (10^{-6} mbar). Usually there are several regulating devices of the positive ion stream: the sampler cone, the skimmer cone, and the ion optics portion. The vacuum is maintained

Scheme 1



Scheme 2

at ~1–2 mbar. It is cooled because it should be protected from the ICP. The ion stream passes through the sampler cone, skimmer cone, and ion optics. The ion optics functions to keep those things which are non-ionic, such as photons and neutral species, from reaching the detector and causing artifacts [1].

Positive ions streams also contain many kinds of interference molecules produced by Ar^+ or O. So, the dynamic reaction cells (DRC) or collision cells have been developed to separate the analysis elements from the interfering molecules [2]. Compared with the ICP-OES, removing interference molecules is one of the most important aspects in ICP-MS analysis [3]. There are many kinds of hyphenated instruments for mass separation, e.g., time of flight (TOF), double focusing sector field (SF), and quadrupole. At the detector, quantifying and amplifying the signal are needed from very low (~ppq) to relatively high concentration levels (~ppm). Commercially used apparatuses include the channel electron multiplier, Faraday cup, and a discrete dynode electron multiplier [1].

Elements according to their limits of detection and normative value

ICP-MS is engineered to accommodate the analysis of more than 70 elements to the best of our knowledge; almost all are commonly positively charged elements, whereas some are commonly negatively charged elements (e.g., halogens, sulfur) [1]. Experimentally, different elements can be detected with different detection limits. In Table 1, these elements are grouped according to the common range of detection limits [1]. These are general guidelines; particular ones may exist for each instrument or new instruments. As seen from Table 1, most elements (65) can be analyzed at a concentration that is below 1 ppt. In particular for Be, B, and Hg, this detection limit is less, at 1–100 ppt. Finally, Si, P, and S have detection limits of 0.1–1 ppb with Br and I having limits of 1–50 ppb.

Molecules commonly considered as important for understanding molecular neurodegeneration such as Cu, Zn, and Fe have been analyzed by ICP-MS. Importantly, various ions are usefully found in the body above these levels of detection. A range of normative values of metal ions are provided below in Table 2. For instance, levels of these elements in cerebrospinal fluid for healthy human subjects are usually in the range of 20–30 μg dm^{-3} [4]. This range is suitable for ICP-MS analysis.

At this point, a chemist might ask whether there is a difference between detecting an ion or a neutral atom. The ions are separated by their mass-to-charge ratio. Various mass separators can be used including the time-of-flight, sector field double focusing, and quadrupole. In the neutral atom case, detection is also possible by spectroscopic methods, e.g., optical emission spectroscopy (OES), energy dispersive spectroscopy (EDS), and nuclear magnetic resonance spectroscopy. But the precision of neutral atom detection is a little lower than for ion detection [1].

Comparison with other forms of inorganic analysis

There are several atomic spectroscopic techniques, such as flame atomic absorption spectroscopy (AAS) and atomic emission spectroscopy (AES) (Fig. 1). The flame atomic absorption is a single-element technique. The flame is generated from a graphite furnace, not ICP, at a temperature in the range of 2,500–4,000 K and generates the ground state analyte atom. When the sample enters the flame via the instrumental nebulizer, the ground state atom absorbs at an element-specific wavelength (λ_{abs}). The amount of absorption is measured and detected by using a

Table 1 Groups of elements according to their limits of detection with the ICP-MS technique

Detection limit	Elements	No. of elements
Below 1 ppt	Li, Na, Mg, Al, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Ge, As, Se, Rb, Sr, Y, Zr, Nb, Mo, Tc, Ru, Rh, Pd, Ag, Cd, In, Sn, Sb, Te, Cs, Ba, La, Hf, Ta, W, Re, Os, Ir, Pt, Au, Tl, Pb, Bi, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Th, U	65
1–100 ppt	Be, B, Hg	3
0.1–1 ppb	Si, P, S	3
1–50 ppb	Br, I	2

Adapted from Ref. [1]

Table 2 Trace element concentrations of Cu, Fe, and Zn found for normal human subjects

Element (μg dm^{-3})	Plasma		Whole blood		CSF	
	Mean	Error	Mean	Error	Mean	Error
Cu	478	37	859	30	21.7	2
Fe	458	36	454,000	15,100	30.5	9.7
Zn	342	15	5,642	249	24.6	2.5

Adapted from Ref. [4]; Error, standard error of the mean (SEM)

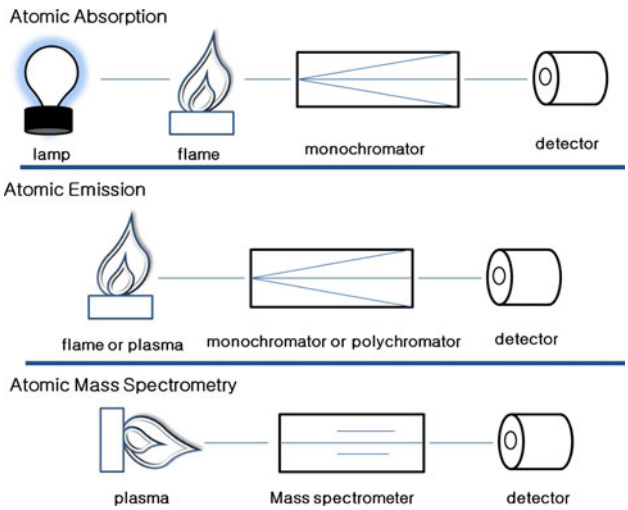


Fig. 1 Schematic illustration of the instrumental setup for atomic absorption and emission, and atomic mass spectrometry. Adapted from Ref. [1]

monochromator, photomultiplier, and a solid-state detector in order to calculate the concentration of the element. This technique is relatively inexpensive, and easy to handle without excessive technical training; also, it is a fast detection method. Flame AAS can be hyphenated with HPLC directly. However, the sensitivity is somewhat low. Detection limits at the ppm level are common, meaning that ICP is superior in sensitivity [1, 5–7].

Atomic emission spectroscopy or OES usually involves the use of an inductively coupled plasma source, 6,000–7,000 K. ICP-OES is multi-elemental. In plasma, the atoms of the sample are excited to a higher energy level. The amount of light (wavelength signal λ_{em}) emitted when the excited electron falls back down to the ground state is then detected. By measuring the intensity and integration of the spectral signal, practitioners can obtain the concentration of any of the aforementioned elements. There are two kinds of ICP-OES: radial and axial. Usually, in the axial type, the plasma is horizontally positioned, so more photons can be procured than for the radial type; in fact, the detection limit is 2–10 times better [1]. Because a cone is not used, the tolerance to matrixes is good, and it can more easily be "hyphenated" with other instrumental techniques, e.g., with HPLC. Furthermore, it is possible to detect sulfur or phosphorus (see Table 1), because there is no need to remove the interfering molecules, when compared with ICP-MS [6].

Sample preparation and analysis method

We addressed the general method for ICP-MS analysis for three types of samples (fluid, tissue, and tissue for bioimaging). The key point when undertaking sample preparation is to protect the sample from metal or element contamination. So, exquisite cleaning of the instrumental tubing is very important. For this procedure, high- or extra-pure grade acids are used; most commonly nitric acid is selected. All processes are performed in a clean room [8]. For sampling, transferring, and storing, plasticware is often used [8]. Generally, glass is not used because of the possibility for metal dissolution to occur out of the glass matrix and into the sample [9]. Usually apparatuses made of quartz, Teflon, polyethylene (PE), polypropylene (PP), polysulfide [10], or polytetrafluoroethylene (PTFE) are used [1].

Fluid analysis

Stainless steel needles are used to sample biological fluids which can be transferred into precleaned polymer tubing (vide supra). Samples are stored at −20 °C [4]. In some cases, digestion by microwave irradiation is needed. Samples in Pyrex tubes are placed into steel bombs with inner tubes of Pyrex and sealed with a Teflon lid [11]. In

this way, certain proteins in the buffer system can be directly analyzed [12].

Tissue analysis

All sample containers should first be cleaned with aqueous HNO_3 for several hours prior to use. Tissues are cut in thin slices by an acid-washed quartz knife on an acid-washed PE surface. Samples can then be homogenized by motor-driven Teflon pestles or dried in a vacuum drier or freeze-drier. In some cases, the samples may be digested in acidic solution in a sealed Teflon "bomb" in a microwave oven and diluted with ultra pure water [13, 14].

Bioimaging analysis

Usually, biological sectioning is performed with a ceramic or stainless steel knife. For micrometer-thick samples, certain kinds of instrumental dissectors, microtomes, or cryo-cutting tools are used. Sectioned tissues are mounted on sodium-free glass slides and dried [15–17].

Neurodegenerative diseases and metal ion analysis with ICP-MS

Neurodegenerative diseases

Alzheimer's disease

This disease is progressive and irreversible and involves eventually grave injury to the brain characterized by extensive neuronal death. Symptoms include memory loss and personality changes. A precursor disorder termed mild cognitive impairment (MCI) is thought to be the disease at its earliest detectable stage. Major physiological/pathological characteristics include extracellular senile plaques and intra-neuronal neurofibrillary tangles in the brain. Senile plaques arise from beta amyloid (Aβ) deposition from excessive scission of the amyloid precursor protein (APP), and neurofibrillary tangles from the hyperphosphorylated tau protein. Metal-catalyzed oxidation relating to transition metal ions of Cu, Fe, and Zn may lead to mitochondrial dysfunction and molecular processes of aging. Copper can enter the cell via the copper transporter 1 (Ctr1) specific for Cu(I) in the intestinal absorptive cell. Inside the cell, copper ions are transferred to certain cellular organelles or molecules including the Golgi complex, superoxide dismutase (SOD), and the mitochondria. These processes must be mediated by metallochaperones. These are specialized proteins that can protect metals from scavengers: the trans-Golgi network (Atx1), copper chaperones for SOD (CCS), and cytochrome c oxidase (Cox17) in the mitochondria [18]. Genetically, MURR1 mouse (U2af1-rs1), COMMD1 (copper metabolism gene MURR1) have been studied and discovered to be important for regulation of copper concentration. COMMD1 can bind copper ions to induce conformational changes; this relates to the interaction with other regulatory proteins. Metallothioneins (MTs) also participate in copper regulation. Four distinct MTs are known. MT1–MT3 are found primarily in the brain. But, MT4 exists outside of the brain. These molecules are cysteine-rich and can bind a range of ions (e.g., Cu, Zn). If copper regulation is not efficient, these ions may impart a cytotoxic effect. Copper incorporation into the cell sometimes can fail. In Menkes disease (copper deficiency) this is true. In the case of the failure of copper to become excreted from the cell, Wilson's disease (copper accumulation) may be present. When the Cu(II) level is not controlled in the cell, Cu(II) can bind to the Aβ protein which can turn on the production of hydrogen peroxide. Cu(II) reduction into Cu(I) is made possible through the use of biological reducing agents such as cholesterol, catecholamines, NADH, NADPH, and ascorbate. Aβ–Cu(I) complex reacts with H_2O_2 to make hydroxyl radical and reactive oxygen species (ROS) [18]. These reactions are known as the Haber–Weiss or Fenton reactions [18–20]. Hydrogen peroxide can produce various reactive oxygen species [21] that can induce covalent links between proteins (protein–protein cross links) and oxidations of protein backbone (Fig. 2) [20].

Zn^{2+} homeostasis is understood to be highly regulated, but many aspects of this regulation are still in the process of being discovered; resulting misregulation may be related to AD. Various specialized biomolecules such as at least 10 members of the Zn transporter (ZnT), 15 members of ZIP (Zn(II)-regulated metal transporter, iron-regulated metal transporter-like proteins), and 3 isoforms of metallothionein [18]. Usually Zn^{2+} efflux is related to the action of the zinc transporter, Na^+/Zn^{2+} exchanger, and ZIP [22]. Zn^{2+} buffering in the cell is regulated by metallothioneins. MTs are composed of 61–68 amino acids, which have a highly conserved sequence of 20 cysteine residues (vide infra). Such Zn^{2+} sequestration from the cytosol is controlled by mitochondria [23]. Free Zn^{2+} ion may induce toxic effects. When Zn^{2+} ion enters the postsynaptic neuronal region through Ca^{2+}-A/K channels, it is observed to generate oxygen species rapidly [24]. These ROS can induce mitochondrial dysfunction and oxidative stress to increase the activity of neuronal nitric oxide synthase and NADPH oxidase which in turn generates peroxynitrite ($O{=}NOO^-$). This allows the mitochondria to decrease the Zn^{2+} sequestering ability in the cytosol. This "positive feedback" is thought to induce cellular apoptosis or necrosis; eventually neuronal cell death ensues [18]. A diseased state such as that of AD involves neuronal cell death.

The physiological role of iron in the brain is often considered with such topics as (1) embryonic neuronal development, (2) myelin formation, (3) production of neurotransmitters, (4) DNA synthesis, (5) oxidative

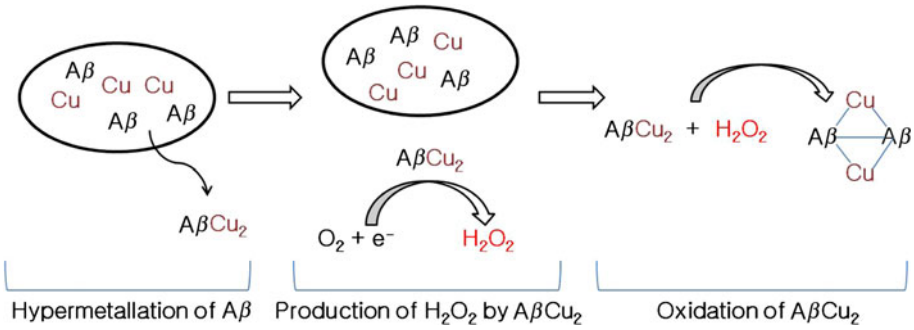


Fig. 2 Formation of cross-linked Aβ using copper. Adapted from Ref. [20]

phosphorylation and ATP synthesis [18, 25, 26]. Iron homeostasis is regulated via transferrin/transferrin receptor, divalent metal transporters, the ferritin protein, ferroportins, mitochondria, endosomes, etc. Iron trafficking is mediated by transferrin/transferrin receptors, cubilin, divalent metal transporter, and hemoglobin scavenger receptor (CD163). Inside the cell, iron levels are regulated by endosomes and mitochondria. Iron is usually stored in ferritin which can store up to 4,500 Fe ions. This protein is also a highly symmetrical entity, a container composed of 24 light and heavy subunits. Iron release is mediated by ferroportin [27]. Iron toxicity largely occurs by oxidative stress based on the Fenton reaction. ROS react with membranes, proteins, and DNA strands alike; the expression of p53 and caspase 3 are stimulated. Cytochrome c is released from mitochondria; eventually, cell apoptosis or necrosis ensues [28].

Parkinson's disease

PD is the most common neurodegenerative disorder that impairs motor skills, cognitive processes, and other functions. A sufferer may experience symptoms that include muscle rigidity, bradykinesia (slow movement), resting tremor, as well as cognitive impairment. These stem from the progressive dopaminergic neuronal cell death in the substantia nigra (SN) region in the brain [29]. The change of Fe content in the SN is of special interest. Other characteristics are Lewy bodies and Lewy neuritis which are eosinophilic and round cytoplasmic inclusion bodies in which the main component protein is α-synuclein filaments. α-Synuclein (AS) is strongly, but not exclusively, linked to PD. It is commonly and simplistically considered that AS is to PD as Aβ is to AD (but AS is also found in AD). Oligomer forms of α-synuclein are known to be more toxic to the neuronal cell than fibrillar forms are [30]. α-Synuclein is one of the most abundant proteins at the presynaptic terminal. AS is composed of 140 amino acids and usually found in its unfolded form (in the cytosol) [31]. There are many areas of research regarding aggregation of α-synuclein, e.g., metal-catalyzed oxidation [32]; certain toxic aldehydes have also been considered as being linked to PD (4-hydroxynonenal [33], oxidized cholesterol [34], 3,4-dihydroxyphenylacetaldehyde (DOPAL) [35], etc). Long-term exposure to certain transition metals, stress, or aging can also influence the general homeostasis of metals. Uncontrolled iron and copper contamination can generate reactive oxygen species that can allow α-synuclein to cross-link and produce aggregates that include oligomers and fibrils. Dysregulated free metal ions in neuronal cells can influence mitochondrial dysfunction, effect changes in antioxidant systems of monoamine oxidase type-B (MAO-B), SOD activity, and allow for abnormal interaction of neuromelanin with iron. The main reactions are related to mitochondrial respiration, based on the Fenton reaction, and metabolism of the catecholamine dopamine (Scheme 3) [36].

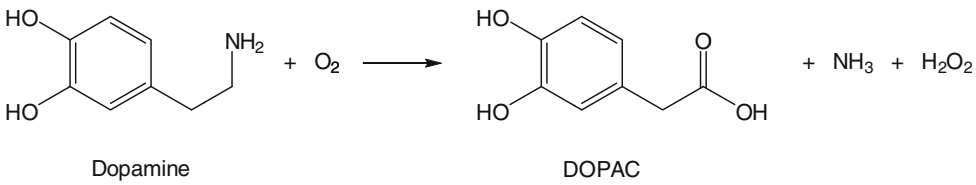
Menkes and Wilson's diseases

Menkes and Wilson's diseases are genetic diseases. They stand as examples of well-recognized disorders in which atypical copper transport and homeostasis exists. They exhibit marked neurological manifestations. Mutations in the P-type copper transporting ATPases, ATP7a (MNKP, Menkes protein) in the case of Menkes disease and ATP7b (WNDP, Wilson protein) are found in the case of Wilson's disease. The disruption of cellular copper trafficking results, and is given as the main reason for the disorders [19, 37]. In Menkes disease, copper transport across the blood–brain barrier (BBB) is blocked; thus, there is copper deficiency in the body. Wilson's disease is caused by copper overload; as a result, patients suffer from the somewhat opposite effect: excessive copper accumulation (intestinal). Thus, the brain and liver are copper-deficient. Furthermore, there is holoceruloplasmin biosynthesis and a marked impairment in biliary copper excretion [18, 26].

Amyotrophic lateral sclerosis

ALS (sometimes referred to as Lou Gherig's disease) is a progressive and fatal neurodegenerative disease caused by the degeneration of motor neurons. About 10% of cases are caused by genetic factors; these are termed as cases of familial ALS (fALS). The others are termed sporadic (sALS) [19]. Approximately 20% of fALS cases are caused

Scheme 3



by inherited mutations in the gene encoding superoxide dismutase 1 (SOD1). SOD1 normally functions as an antioxidant by detoxifying the potentially damaging superoxide radical anion. The enzyme is a homodimeric protein consisting of 153 amino acid residues per monomer. Each subunit is composed of an eight-stranded "Greek key" β-barrel fold; each one binds one zinc and one copper ion; there is one intrasubunit disulfide bond as well. The copper ion in the enzyme catalyzes the disproportionation of the superoxide anion ($O_2^{\cdot -}$); here, alternating cycles of reduction and oxidation exist. Copper and zinc in the enzyme play not only a catalytic role (Cu is the active site), but are also involved in preserving correct protein folding and in retaining "negative design". As a consequence, the loss of these ions may induce partial or global unfolding transitions leading to misfolding and aggregation of protein [19, 38–40].

Prion diseases

Prion diseases, also known as transmissible spongiform encephalopathies, are degenerative neurological diseases that relate to a "contagious" protein and its misfolding. These disorders include the classic Creutzfeldt–Jakob disease, Gerstmann–Sträussler–Scheinker syndrome, fatal familial insomnia as well as Kuru in humans. Prion diseases are associated with the accumulation of misfolded forms of cellular prion protein (PrP^C). Prion protein is considered a copper-binding protein and much research is related to the interaction of it with copper ion. Two main biological functions of the prion protein appear to be copper transport and antioxidative activity. Besides copper, an imbalance of other metal ions such as iron, manganese, and zinc is also associated with prion diseases [19]. Many papers have investigated the pathogenic role of manganese including conformational changes of protein and non-homeostatic redox activity [41, 42].

Huntington's disease

HD is a neurodegenerative genetic disorder caused by the mutation of the gene coding for a natural protein named Huntingtin (Htt) [43]. Although the exact function of Htt is unclear, the protein is thought to impact myelin integrity and plays a role, albeit indirectly, in the striatal iron accumulation observed in HD. The possible mechanisms of involving iron homeostasis in HD are reviewed in the book *Neurodegenerative diseases and metal ions* edited by Sigel et al. and in a mini-review by Rivera-Mancia et al. [44]. Copper imbalance is also observed in HD patients. A contribution of copper–protein interaction to HD pathogenesis was also studied [45].

Tissue analysis

There are carefully classified regions of the human brain below the broad regions of the cortex and medulla (Fig. 3). The organ subdivisions in the brain include the hindbrain (myelencephalon, metencephalon), midbrain, and forebrain (diencephalon, epithalamus, third ventricle, thalamus, hypothalamus, subthalamus, pituitary gland, telencephalon, white matter, rhinencephalon, lateral ventricles, and cerebral cortex). There is much research and analysis about the concentration of metals in many of these tissues—the metal concentration can be telling about pathology.

ICP-MS can be used in the assessment of trace element brain tissue. These multi-element analyses involve the identification and quantification of metal ions that relate to neurodegenerative disorders. Ejima et al. [46] measured concentrations of 16 elements (Mg, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Hg, Pb) in three regions of the human brain (frontal cortex, frontal white matter, and cerebellum) by using ICP-MS. These analyses from samples from control subjects, who had died and did not possess incidence of neurological disease, were compared with those of the tissues of a patient with fALS. Data obtained were in good agreement with those from other techniques such as neutron activation analysis (NAA) and ICP-AES. Also, concentrations of Mn were higher in the samples of the ALS patient than those in the controls.

In AD studies, specifically, the ICP-MS technique was also widely used in the determination of the concentration of certain metals. For example, concentrations of Zn and Cd were estimated through analysis of human brain tissues samples (the superior frontal gyrus, superior parietal gyrus, and medial temporal gyrus, the hippocampus and thalamus of both hemispheres) from AD subjects and compared with "normal" subjects [47]. The ranges and the arithmetic means with standard deviations were given for all regions of the brain, including both hemispheres. Concentrations of Cd and Zn in "normal" samples were in the 5–145 ng g^{-1} and 6.4–29.3 mg g^{-1} ranges with means of 26.7 ± 22.2 ng g^{-1} and 13.8 ± 4.0 mg g^{-1}, respectively. The corresponding values for AD samples were in the 4–104 ng g^{-1} and 7.1–19.4 mg g^{-1} ranges with means of

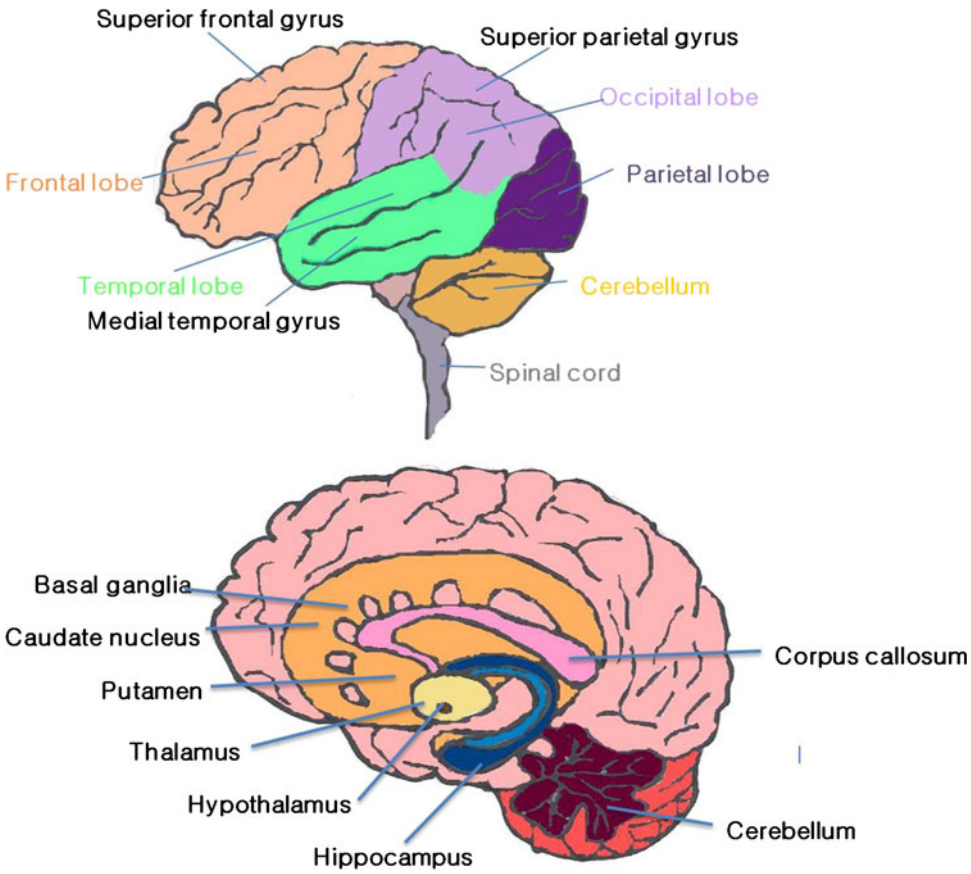


Fig. 3 Some regions of the human brain

32.1 ± 19.0 ng g^{-1} and 13.8 ± 2.7 mg g^{-1}, respectively. Importantly, variations in element concentration are justified when considering differences between (1) regions of the brain, (2) effect of disease duration, (3) age, and (4) gender.

Corrigan et al. [48, 49] analyzed 38 different elements by NAA and ICP-MS methods. Experiments were conducted with brain tissue acquired post-mortem from 11 cases of persons previously diagnosed with Alzheimer's disease, and from six controls. Four brain regions (temporal lobe, frontal cortex lobe, caudate nucleus, and putamen) were analyzed. An increase in tissue concentration for aluminum, silicon, and calcium for the cortical areas and for the basal ganglia was observed. On the other hand, zinc, selenium, cesium, and cerium showed lower concentration values. Alzheimer's plaque cores, specifically, can be successfully analyzed by ICP-MS as well. The ICP-MS technique was combined with flow injection (FI) and a selective extraction procedure. Concentrations of several metals in the plaque sample were measured, including Cr, Mn, Ni, Cu, and Pb which were in the 0.2–0.8 mg dm^{-3} range and Al, Fe, and Zn which were in the 2–20 mg dm^{-3} range [14].

Furthermore, the metal ions of molybdenum and especially manganese have attracted much recent attention in the AD [50, 51], PD [43, 52, 53], ALS [13], and prion disease research field [41, 42]. In one study by Csaszma et al. [50] a total of five brain regions were studied, and the mean concentration of manganese was found to be in the range of 1.1–1.3 ppm, while that for molybdenum was between 90 and 150 ppb for dry weight tissue (both control and AD subjects). The highest concentrations of Mo and Mn (330 ± 42 ppb and 2.90 ± 0.07 ppm through dry weight measurements, respectively) were found for AD patients in the putamen brain region [50]. Higher concentrations of manganese, as well as Si, Sn, and Al, in the parietal cortex of the AD brain compared with control values were measured by the research group of Srivastava [51]. Other ions of metals such as Na, Te, Cr, Fe, and the non-metal B showed moderate increases in concentration in the same brain region. Changes in Mn, Fe, Cu, and Zn concentration in the 6-hydroxydopamine (6-OHDA)-induced parkinsonian rat brain were observed in all brain regions that lay along the dopaminergic pathway [43]. Measurements were performed by ICP-MS at 1, 3, 7, 10, 14, and 21 days after treatment with 6-OHDA. Stable ^{55}Mn, ^{56}Fe, ^{63}Cu, and ^{64}Zn isotopes were measured simultaneously with the procedural detection limits of 8, 70, 27, and 30 pg g^{-1}, respectively. In studies relating to the effects of manganese exposure on neurotoxicity, and

the subsequent exacerbation of parkinsonism, concentrations of manganese in the whole brain were measured with an average accuracy of 94%; here the analytical detection limit was 0.16 ppb [53]. Concentration profiles of Fe and Cu upon manganese exposure were obtained in various brain tissues for juvenile and adult mice [52]. Influence of manganese uptake on pathogenesis of prion disease was also investigated [42]. Data obtained by ICP-MS indicate the increase in intracellular Mn levels for the mouse brain slice cultures upon chronic manganese exposure; the levels of the divalent cations of copper, iron, and zinc were not significantly altered by the aforesaid manganese treatment. Through the use of ICP-MS, decreases of Mn levels by up to 50% in treated mice compared with untreated controls in manganese chelation therapy were observed [41]. Copper, iron, zinc, and cobalt levels remain unchanged.

Concentrations of several metals (Rb, V, Mn, Fe, Co, Cu, Zn, and Cd) were estimated in formalin-fixed brain tissue from eight Guamanian (i.e., inhabitants of Guam) patients who suffered from ALS; four subjects were confirmed to have Parkinsonism–dementia complex (PDC), and five were used as control subjects [13]. Two metals (Zn and Cd) were reported at different values; for the other metals, no significant differences were observed. An increase in the concentration of Zn in both gray and white matter for both patient groups was observed. Cadmium gives higher values in ALS, but not in PDC patients. Alkali metals (Li, Na, K, Rb, and Cs) also can be detected in several AD brain parts [54]. Magnesium content in AD brain was determined by Andrasi et al. [55]. A decrease in Mn concentration in the basal ganglia was detected by several instrumental methods including ICP-MS, ICP-AES, and NAA.

Environmental pollutants including heavy metals can enter the food chain and eventually contaminate the central nervous system (CNS) [56, 57]. An impact of nickel and vanadium exposure on nasal and brain areas in dogs was also studied and reported [57]. ICP-MS data showed higher vanadium concentration in exposed animals over those in control animals (0.36 ± 0.3 μg g^{-1} vs. 0.19 ± 0.17 μg g^{-1}, respectively).

Fluid analysis

There are certain important fluids that relate to CNS diseases: brain cerebrospinal fluid (CSF), blood, etc. Fluid analysis is very straightforward in ICP-MS. Usually it is possible to analyze the liquid sample directly, e.g., in its neat liquor form. In several studies, the involvement of metal ions in pathogenesis of neurodegenerative diseases was supported by analysis of body fluids. ICP-MS is widely used for quantification of several metal ions in CSF, urine, serum, and whole blood. In most cases, sector field inductively coupled plasma mass spectrometry (SF-ICP-MS) was used. This was undertaken to avoid several disadvantages that exist with the conventional quadrupole mass filter (Q-ICP-MS). Such disadvantages include signal interferences during the experiment originating from the plasma, water, reagents, and matrix of the biological samples used [58]. Samples of cerebrospinal fluid from individuals of a large group of PD patients ($n = 42$) were analyzed for metal content by the research group of Alimonti in 2007 [8]. A total of 20 metallic elements (Li, Be, Sr, Ba, Zr, V, Cr, Mn, Co, Ni, Mo, W, Cd, Hg, Al, Sn, Sb, Bi, Pb, and Tl) were detected by SF-ICP-MS. Additionally, six elements (Si, Mg, Ca, Fe, Cu, and Zn) were quantified by ICP-AES. The researchers presented statistical values for the elements found in PD patients and controls and a plot of the elements allowing discrimination between controls and patients. Data indicated abnormally low levels of Si, Cr, Fe, Co, Sn, and Pb in the CSF of PD patients compared with values from control samples. Earlier, the same research group detected significantly increased concentrations of Pb and V in blood and urine ($p \leq 0.03$, for both metals) and reduced levels for Al, Cd, Hg, and Pb in serum and Cr, Co, Cd, Hg, and Pb in CSF ($p \leq 0.003$) [59]. The limits of detection for most of the elements ranging from 0.001 ng cm^{-3} for Cd to 0.05 ng cm^{-3} for Al were satisfactory and suitable for the intended purposes. Here, the applied ICP-MS method provided good accuracy; for example, in urine these values ranged from 92.4% for Ni to 105% for Hg; in the case of serum from 97.8% for Mn to 104% for Al; and in blood it varied between 97.0% for Cr and 103% for Pb.

Forte et al. [60] determined concentrations of aluminum and manganese in urine, blood, and CSF for 26 PD human cases. Both metals gave no statistical difference in concentration levels for patients and controls in any of the analyzed fluids. One exception was the aluminum level in blood serum; this was lower in PD patients and a slight decrease, albeit not statistically relevant, in manganese concentration for whole blood and CSF was also found for PD patients. A similar trend for aluminum was reported by Bocca et al. [61]. Here, concentration values of 3.0 ± 1.8 in PD patients versus 5.3 ± 3.4 ng cm^{-3} for the control group in serum and 11.7 ± 7.05 versus 20.7 ± 12.6 ng cm^{-3} in urine, respectively, were detected. In a separate study, the analysis of 31 elements in samples of serum for 45 PD patients and 42 controls showed that aluminum, copper, iron, manganese, and zinc concentration are the main distinguishing element concentrations which can be used to separate PD cases from control cases [62]. Concentrations of 31 elements in the cerebrospinal fluid, blood plasma, and whole blood were determined in living human subjects suffering from multiple sclerosis (MS) [63], so-called Skogholt's disease (a demyelinating disorder of both the central and the

peripheral nervous system involving adult onset) [64] and those from a control group [4]. SF-ICP-MS was used to detect a three-fold increase of Cu and Fe, and a two-fold increase in Zn concentration in the cerebrospinal fluid of Skogholt's disease patients compared with those of controls. Other elements such as Rb, Co, P, S, Th, and Se are also found at higher concentration levels in the Skogholt's disease subjects. Interestingly, a decrease of Mo, Cd, Hg, and Tl levels was observed in CSF of subjects with Skogholt's disease and MS compared with the control group.

Bioimaging of metal ions in brain samples and the advent of LA-ICP-MS

In recent years, bioimaging or metal distribution of the brain has been widely researched by way of LA-ICP-MS. LA-ICP-MS is one of the most widely used analytical spectrometric imaging techniques for trace metal ion concentration/distribution determinations in brain tissues via imaging. Several examples of practical application of LA-ICP-MS in neurodegenerative disease studies are presented in several papers, including reviews [65, 66]. In comparison to the tissue or fluid cases, it is possible to determine the microstructure of the sample, as well as the metal distribution. Zoriy et al. analyzed human brain tissue for $^{13}C^{+}$, Cu, and Zn distributions in five adjacent sections. The reproducibility was 5.4–6.5, 5.8–8.2, and 5.1–6.7%, respectively, for these elements [15]. Becker et al. investigated the insular region and brain tumor tissue to detect the distribution of Cu, Zn, and certain other elements. The detection limits were 340 ng g^{-1} and 140 ng g^{-1}, respectively [67].

In a Parkinson's disease study, the distribution of several metal ions (iron, copper, and zinc) in a (Parkinson's) mouse brain model was compared with control values [65]. The images obtained by LA-ICP-MS clearly show significantly higher iron content in the SN region in the Parkinson's brain than for in the control. In similar research conducted by Matusch et al. [16] quantitative images for Mn, Fe, Cu, and Zn in the Parkinson's brain in a "time-course" series were obtained. The images involved tissue exposure with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). The most pronounced changes were observed for Cu and Fe (Fig. 4). There was an increase of Cu (38% vs. control) in the periventricular zone at 28 days, and 38 and 39% increase of Fe in the interpeduncular nucleus at a time of 7 and 28 days, respectively.

A report by Hare et al. [68] discussed an observed increase in the concentration of ^{56}Fe in a 6-hydroxydopamine (6-OHDA) lesion mouse brain model within the SN compared with control animal subjects. Because of the notions that (1) neurodegenerative diseases are age-related and that (2) copper may play an important role in the brain

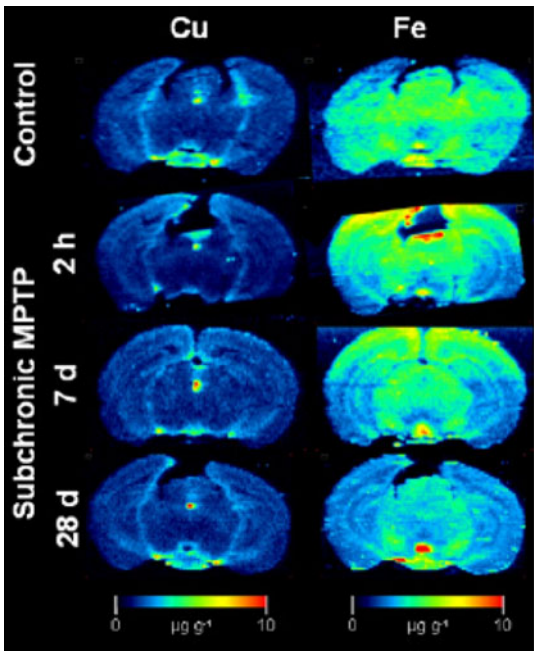


Fig. 4 Images of brain cross-sections and analysis of Cu and Fe content for the control (*top row*), 2 h (*second row*), 7 days (*third row*), and 28 days (*bottom row*) after the last of five daily MPTP injections. Reprinted with permission from Ref. [16] (License from Rightslink/Copyright Clearance Center. Licensee: David G. Churchill, License Date: Nov 18, 2010, License Number: 2551800410899, Type Of Use: reuse in a journal/magazine.)

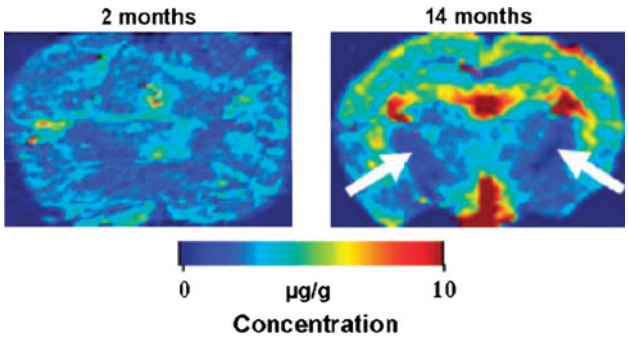


Fig. 5 Images demonstrating distributions of Cu in cross sections of brains of 2- and 14-month-old mice. Reprinted with permission from the publisher of Ref. [69]

in aging, the quantitative Cu distribution was analyzed for 2- and 14-month-old mice [69]. While total Cu content in brain after intravenous (i.v.) administration of ^{67}Cu is increased in both cases, in the striatum and ventral cortex, Cu showed an approximately 50% reduction in 14-month-old mice compared with those who were 2 months old (Fig. 5).

Iron and zinc distributions also were investigated [70, 71]. Zinc does not give any changes, whereas iron gives increased values in the SN, the thalamus, and the CA1 region of the hippocampus. In an AD study, LA-ICP-MS

was also used for metal distribution and also in imaging the localization of Aβ peptide in the brain tissue. For the imaging of amyloid plaques, ^{60}Ni and ^{153}Eu radioisotopes were used [17]. Concentrations of metal ions were different in plaque and non-plaque tissue samples; important plaque to non-plaque ratios were calculated, and some are provided as follows: 3.5:1 for Mg, ~3:1 for Ca, 2:1 for Fe, 3.5:1 for Cu, and 2:1 for Zn.

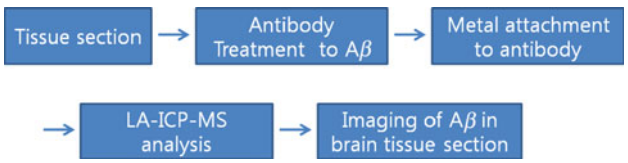
Protein analysis applications with metals and ICP-MS

Isotope dilution analysis

Isotope dilution analysis (IDA) is an important scientific technique that allows one to obtain the exact concentrations of samples by adding (spiking) an isotopic sample involving an exact isotope ratio or concentration to an unknown sample. The experimental isotope ratio of the mixture is then obtained. Through this method, there are many advantages because the results are not influenced by (1) the mode of sample preparation, (2) various instrumental instabilities, (3) potential signal drift, (4) possible matrix effects, and (5) potential sample loss after complete equilibrium mixing between the sample and the isotope "spike" [72]. There are two modes for this method: "species specific" and "species unspecific". In the species specific case, the sample species is the same as the isotope chemical compound. It is necessary to know the exact composition and the structure of the sample. The isotope sample can be added at the beginning point of the analytical procedure. In the species unspecific case, sample species can be different from the isotope spike chemicals. This method can be used when the structure and composition of the sample species are not accurately known. Often it is expensive to purchase certain commercially available reagents or even synthesize them. In this case, the isotope spike could be added to the sample after the sample preparation and a treatment are completed, e.g., performing chromatography immediately before the ionization process [72]. It has been reported that the IDA method has some challenges related to (1) instruments, (2) spectral interference, (3) the dead time of the detector, (4) mass discrimination, etc. Spectral interference occurs from molecules of H, O, Ar, and so on with the same mass. To overcome this problem, a double-focusing spectrometer at high resolution can be used. In the quadrupole case, there is a removable apparatus: a DRC or collision reaction cell (CRC). A DRC uses certain kinds of reactive gases, e.g., O_2, CO, NH_3, CH_4, among others; CRC uses colliding gases, usually H_2 and He [72]. Usually, an electron multiplier type detector has "dead time" during which the signal cannot be processed completely at high counting rates such as those of 10^6 cps or more. In practice, a high abundance state of isotope can be influenced by dead time. Thus, this is a limitation with some correction being necessary [72]. Heavier elements can be detected better than lighter elements because of the space-charge effect. So, heavier isotopes show more high intensity than light isotopes. This can be corrected through known methodology with certified isotopic composition materials [72]. Jakubowski et al. tried to quantify three different kinds of proteins, bovine serum albumin (BSA), chicken egg white lysozyme, and porcine gastric mucosa protein, all via iodine labeling. This method involves covalently attaching an iodine atom to a histidine or tyrosine residue of the protein under analysis. The researchers used the stable isotope ^{127}I and detection by ICP-MS. The investigation range was from 0.015 pmol (BSA) to 105 pmol (lysozyme) [73]. So the ICP-MS is essential for IDA. This method can decrease the discrepancy of the matrix effect or sample preparation [72, 74]. Becker et al. [75] attempted to perform isotope dilution of $^{63}Cu^+$ and $^{65}Cu^+$ to analyze human brain fluids, and ^{65}Cu, ^{67}Zn, and ^{54}Fe for tissue of AD brain [76]. Heumann et al. discussed the possibility to use the isotope dilution method with ICP-MS as a routine analysis for trace elements. Hyphenated techniques, e.g., liquid chromatography, and coupling of gases can help in the difficulty stemming from the lack of commercially available isotope-labeled spikes. If the isotope can be generated in a closed system, there is no problem of losing or evaporating the spiked sample. Isotope dilution techniques are useful for their accuracy in detecting elements of $MeHg^+$ and Cr^{6+} [77].

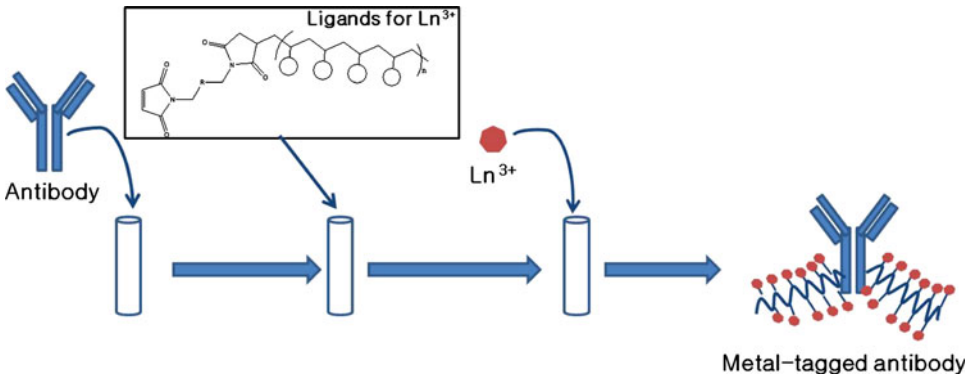
Immunoassay techniques

Through the use of ICP-MS, there are various technical advantages, including increased signal sensitivity, wide analysis dynamic range, minimized interference from complex matrices, and the ability to perform high-throughput analysis. Therefore, protein quantification can be more thoroughly exploited. There are techniques for protein quantification by analyzing metal ions tagged with antibodies (Scheme 4). For this method it is very important for the metal to be attached to an antibody. Lou et al. reported the use of certain kinds of polymers that bear metal-chelating functional groups. The researchers first tried to reduce the S–S cross-linked bond in the protein which allowed for the reaction of polymers. Upon addition of metals, the purified metal ion attached antibodies were analyzed [78] (Scheme 5).



Scheme 4

Scheme 5



In 2008, Razumienko et al. compared the ICP-MS-based immuno-protein quantification with conventional immunoassays (colorimetric ELISA and indirect ELISA). The samples included protein (hPDGF-AA), p53 in cell line (A431, 293T, KG1a, and K562). Whether Tb- or Tm-attached antibodies were used, results of analyses were similar to the conventional colorimetric ELISA or indirect ELISA method [79]. There is another case in which specific element-tagged antibody methods have been reported. Baranov et al. in 2002 connected the methods of centrifugal filtration, protein A affinity, size-exclusion gel filtration, and ELISA with ICP-MS through the use of specific element-tagged antibodies. The investigators reported that target protein levels of 0.1–0.5 ng cm^{-3} can be measured, involving a linear response that can be reported to over three orders of magnitude. The ICP-MS hyphenated method revealed more precise results and were obtained more conveniently [80].

Hyphenated instrumental applications

There are many kinds of hyphenated instruments whose designs are often considered in the realm of engineering but can also be briefly mentioned here. Usually the injection part and mass separation parts are connected. The injection part uses capillary zone electrophoresis (CZE), LA, HPLC, and gel electrophoresis. The mass separation portion uses a quadrupole, DRC, CRC located between plasma and quadrupole. Another mass separator, the sector-field analyzer can be used and proven to higher accuracy. But in this case, a prospective high instrument cost and significant maintenance cost are expected. When using hyphenated instruments, it is required to optimize the replicate time, "dwell time", number of sweeps per readings, and other parameters between instruments and the ICP-MS technique [6]. Helfrich et al. succeeded in coupling the gel electrophoresis technique with that of the SF-ICP-MS. They attempted to detect the $^{32}S^{+}$ and $^{31}P^{+}$ of α- and β-caseins so as to better understand the biological phosphorylation levels. They obtained results which matched well with anticipated values [81].

Conclusion

In this review, we endeavored to summarize the utility of the ICP-MS techniques and related techniques with respect to the determination of trace element concentrations of tissues in neurodegenerative studies. The practical use of ICP-MS was presented in a tutorial style. As discussed, the accurate determination of elemental distributions (metals and non-metals) in cases where AD or PD were diagnosed allows for a better understanding of such diseases and relates to the sensitivity and scope of the ICP-MS technique. Furthermore, element analytes detected involved tissues and fluids. This discussion includes the instrumental setup, elements and their detection limits, brief comparison of ICP-MS with other inorganic analysis instruments, and sample preparation and analysis methods. Also, material about neurodegenerative diseases and metal ions analysis were presented in the context of the ICP-MS technique. Subtopics in this section included neurodegenerative disease, tissue analysis, fluid analysis, and bioimaging of metals in brain tissue samples, and protein analysis application with metals and ICP-MS. This last subtopic was further divided into isotope dilution analysis, related immunoassay techniques, and hyphenated instrumental applications. Thus, from the topical list above, the reader should find that this review has touched on a lot of important biophysical, analytical, and clinical studies that underscore how ICP is used and how it has been developed practically. This review, while current through the summer of 2010, was not meant to be exhaustive as much as it was meant to be user-friendly. Along with the inclusion of various studies is also the mention of related techniques and improvements and limitations of current techniques.

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