10 Inductively Coupled Plasma Mass Spectrometry for Analysis of Metal-Containing Pharmaceuticals

BENTE GAMMELGAARD, STEFAN STÜRUP, and CHARLOTTE MØLLER

Department of Pharmaceutics and Analytical Chemistry, University of Copenhagen, Copenhagen, Denmark

10.1 INTRODUCTION

The inductively coupled plasma mass spectrometry (ICP-MS) technique was developed in the 1970s based on the need for a mass spectrometer capable of accepting solid mineral samples and providing multielement analyses at levels down to 10 ng/g in an analysis time of few minutes [1]. Thus, the technique was the successor of atomic absorption spectrometry and intensively used for analysis of geological samples.

In the last decades, the technique has evolved as hyphenation to different separation systems and has become the state of the art in the research field often referred to as *metallomics.* This research field has been described as "the comprehensive analysis of the entirety of metal and metalloid species within a cell or a tissue type" [2]. This field includes speciation analysis of essential and toxic elements as well as interaction of metal-based drugs (MBDs) with cell constituents and macromolecules.

The major advantages of the ICP-MS technique are the capability of multielement analysis, a very large sensitivity, and on-line sample introduction. The most

Encyclopedia of Drug Metabolism and Interactions, 6-Volume Set, First Edition.
Edited by Alexander V. Lyubimov.

important feature, however, is that the detector response of the ICP-MS, in contrary to UV- and ES-MS (electrospray-mass spectrometry) detection, is independent of the analyte structure. Thus, unknown metabolites can be detected and quantified based on the ICP-MS detectable element in the metabolites. This makes ICP-MS an obvious detector in analysis of MBDs.

10.2 ICP-MS

10.2.1 The Instrument

ICP-MS is a hard ionization mass spectrometric technique that produces positive ions of single atoms in a high temperature argon plasma. Most often, the sample is introduced in solution, although solid material can be introduced using laser ablation. For analysis of pharmaceuticals, however, liquid sample introduction is predominant. The sample is pumped into a nebulizer, where an aerosol is produced by a flow of argon and transferred to a spray chamber. In the spray chamber, large droplets are discarded allowing only a fine mist of liquid to enter the argon plasma where the aerosol is desolvated, molecules are vaporized and decomposed into atoms, and finally the atoms are excited and ionized.

The argon plasma is an electric conducting gaseous mixture of argon, argon ions, and electrons sustained by a fluctuating magnetic field produced by a radio frequency induction coil. The plasma temperature of 6–10,000 K gives rise to complete sample combustion and a very efficient ionization. Positively charged ions are extracted from atmospheric pressure into the vacuum of the mass spectrometer through a set of cones. In the mass spectrometer, the ions are separated based on their mass to charge ratio (m/z). In routine instruments, the mass spectrometer is most often quadrupole based, operating at mass ranges of 3–300 with unit resolution, although high resolution instruments with a resolution of up to 10,000 are also commercially available [3].

The ionization efficiency of the plasma is dependent on the first ionization energies of the measured elements (Table 10.1). Metals are, in general, efficiently ionized and ICP-MS is therefore intensively used in inorganic analysis as the detection limits for most elements are at the subparts per billion level.

10.2.2 Interferences

One major challenge in ICP-MS analysis is the presence or formation of interferences. These are often divided into spectroscopic and nonspectroscopic interferences. Spectroscopic interferences appear at the same m/z value as that of the analyte, for example, the overlap of ^{196}Hg on ^{196}Pt. Such an overlap can most often be overcome by the choice of another isotope of the analyte. Another group of spectroscopic interferences is the polyatomic ions derived from the plasma gas (e.g., $^{40}Ar_2{}^+$), atmospheric gasses, sample solvent, or sample matrix constituents (e.g., $^{12}C^{40}Ar^+$ on $^{52}Cr^+$). The number of possible interferences decreases with increasing atomic mass, hence few interferences compromise the measurement of metals such as platinum and gold compared to the lighter metals such as iron and chromium. A comprehensive list of possible interferences on most elements has been given by May and Wiedmeyer [4]. To avoid polyatomic interferences, instruments equipped with a collision or reaction cell can

TABLE 10.1 First Ionization Energy and Degree of Ionization for Selected Elements

Atomic Number	Element	Most Abundant Isotope	Percentage	First Ionization Energy (kJ mol)	Degree of Ionization (%)
12	Carbon	^{12}C	98.9	1086	5
15	Phosphorus	^{31}P	100	1012	33
16	Sulfur	^{32}S	95.0	1000	14
18	Argon	^{40}Ar	99.6	1520	0.04
22	Titanium	^{48}Ti	73.8	658	99
23	Vanadium	^{51}V	99.8	650	99
26	Iron	^{56}Fe	91.7	759	96
33	Arsenic	^{75}As	100	947	52
44	Ruthenium	^{102}Ru	31.6	711	96
51	Antimony	^{121}Sb	57.4	834	78
64	Gadolinium	^{158}Gd	24.9	593	93
74	Tungsten	^{184}W	30.7	770	94
78	Platinum	^{195}Pt	33.8	870	62
79	Gold	^{197}Au	100	890	51
83	Bismuth	^{209}Bi	100	703	92

be used. In a collision cell, the polyatomic ions are disintegrated by collision with an inert gas such as He, while in the reaction cell, the interference is removed from the m/z value of the analyte by reaction with a gas resulting in reaction products with m/z values different from the analyte [5]. These second-generation instruments are most commonly used today. Use of high mass resolution sector field instruments is an alternative, but a much more expensive solution to the interference problems. These instruments are capable of resolving minute differences in the masses of the analyte ion and the interfering ions [6].

The nonspectroscopic interferences are observed as changed sensitivity of the analyte signal induced by matrix constituents, for example, high salt concentrations. These interferences may be caused by changes in nebulization and ionization efficiency, in ion transport efficiency, in ion extraction, or combinations of these [7]. Elements with low mass or high ionization energies are most severely affected [8]. The nonspectroscopic interferences are often referred to as *matrix interferences*.

10.3 QUANTITATIVE DETERMINATIONS

The multielement capabilities of ICP-MS are routinely applied in the related area of determination of inorganic impurities in drugs and active pharmaceutical ingredients, an area not covered in this chapter, but that has been reviewed recently [9]. Drugs containing metals are often analysed by ICP-MS due to the low limits of detections, high sensitivity and few interferences for most drug related metal-ions (Pt, Ru and Au), few interferences are present. The multielemental capabilities of ICP-MS are rarely used, although an internal standard or a few other elements are sometimes measured along with the selected metal in order to correct for changes in instrument sensitivity over time or to monitor potential polyatomic interferences. The determination of total metal-containing drugs using ICP-MS is often applied in metabolism, distribution, and

excretion studies or in uptake experiments using cancer cell line models. This requires analysis of biological samples such as serum, urine, or cell samples. These are all difficult to analyze due to the small sample volume available or matrix effects from salts, proteins, and other biomolecules. Thus, sample preparation and quantification are critical in order to achieve accurate and precise results.

Two different approaches can be chosen for the sample preparation of biological samples. Most simple is the application of a dilution with an appropriate diluent [10–12], thereby keeping the drug in solution in its existing form. For dilutions, there is a risk of coprecipitation of the metal, as proteins and other such molecules might precipitate over time. Alternatively, acid digestion with concentrated acids (plus hydrogen peroxide) can be applied, reducing organic molecules to CO_2 and H_2O and bringing the metal ions in their ionic form [13–18]. In general, acid digestions should be preferred, resulting in more stable solutions of the metal ion of interest. Another advantage of acid digestions is that quantification using external calibration is possible, while the more laborious standard addition calibration or careful preparation of matrix-matched standard is needed, when samples are simply diluted before analysis. Acid digestions are, however, more time consuming and require more sample handling steps. Most accurate and precise quantification is achieved applying isotope dilution using an enriched isotope of the analyte as a standard (e.g., ^{194}Pt for the quantification of platinum). Isotope dilution quantification can be either unspecific applying a solution of the enriched isotope as standard or specific for which the enriched isotope is incorporated into a specific compound, for example, $[^{194}Pt(NH_3)_2Cl_2]$. These approaches were used by Sar *et al*. [19] for the accurate and precise determination of platinum in DNA samples.

Many of the drugs are lipophilic, and adsorption to the surfaces of sample preparation vials and sample wells can be a problem. This problem was addressed by Egger *et al*. [17], who developed a protocol for determination of the cellular uptake of metal compounds in adherent cancer cell lines, a protocol that takes into account adsorption and blank correction. Utilizing this protocol for determination of the cellular uptake of Pt and Ru drugs, they were able to measure the cellular uptake experiments with good precision and accuracy.

Certified reference materials (CRMs) are widely used for validation of ICP-MS methods for determination of total metal concentrations, but for Pt and Ru, only a few biological CRMs with certified concentrations exist, and there is a clear need for new CRMs in the metallo-drug area. Thus, accuracy and precision are most often evaluated using in-house quality control samples. Bettinelli [11] carefully evaluated the analytical uncertainty associated with the determination of total Pt in plasma, plasma ultrafiltrate, and urine using ICP-MS. The instrumental LOD was at submicrogram per liter level and the limits of quantification (LOQs) (10σ) were at low microgram per liter level for plasma, ultrafiltrate, and urine[11,12]. Similar detection limits led to an LOQ of 0.5 μg/kg in DNA samples [12]. Figures of merit in the same range have been found by others [13,15,16,18], while even better LODs can be achieved with high resolution ICP-MS instrumentation [14].

10.4 LC-ICP-MS

Coupling of LC (liquid chromatography) systems with flow rates of 0.1–1 mL/min to ICP-MS is quite simple, as these flow rates are readily compatible with the sample

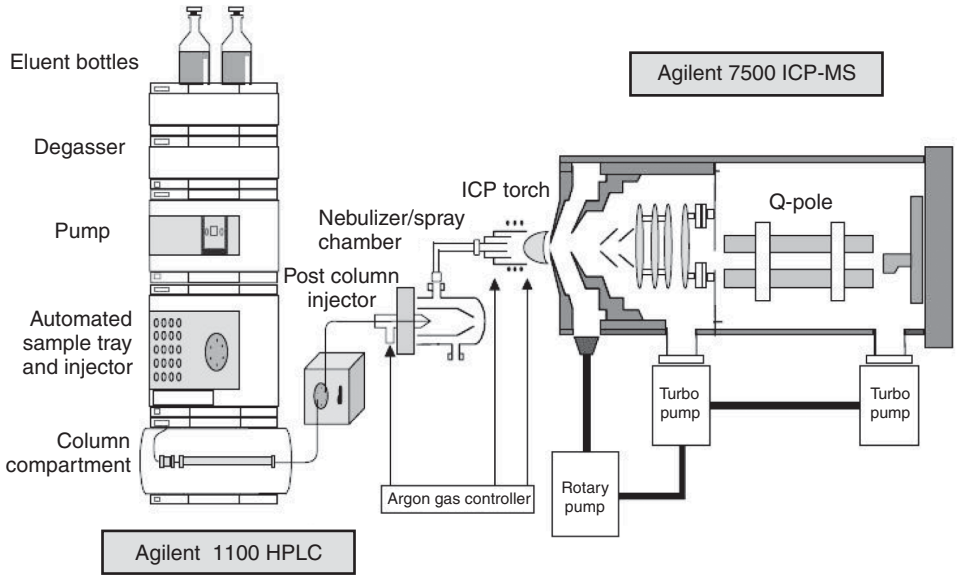

Figure 10.1 Schematic overview of LC-ICP-MS. *Source*: ©Agilent Technologies, Inc. 2010. Courtesy of Agilent Technologies, Inc., with permission.

uptake around 1 mL/min for the ICP-MS instrument. The coupling of an LC system to an ICP-MS instrument is illustrated in Fig. 10.1. Several reviews on this coupling have been published [20–22]. Most of the early work on LC-ICP-MS coupling was performed using ion-exchange chromatography, as the eluents in such systems are, most often, aqueous buffers and elution is regulated by the salt concentration. As the ICP-MS, in contrast to organic MS, is quite tolerant toward moderately high salt concentrations, ion-exchange columns were, for a long period, the most popular columns for the hyphenation.

10.4.1 Organic Solvents

When more lipophilic substances, such as pharmaceuticals and their metabolites, have to be separated, reversed phase (RP) chromatography involving eluents containing large amounts of organic solvents is the most common separation principle used. In most drug metabolism studies, linear gradient elution from 5% to 95% organic solvents is often applied as a standard procedure. This introduces several analytical challenges, as the plasma only tolerates small amounts of organic solvents. Severe suppression of the signal followed by plasma instability is observed by increasing the organic solvent load and eventually the plasma is extinguished. The influence of organic solvents on the plasma is complex and involves interaction between solvent and plasma as well as change of aerosol characteristics in the spray chamber [23,24]. Introduction of organic solvent cools the plasma and changes ionization characteristics, leading to decreased sensitivity. In contrast, the addition of organic solvent to an eluent decreases surface tension and improves the aerosol by changing the droplet distribution toward smaller droplets, which results in improved transport efficiency and increased sensitivity. However, the interaction between plasma and solvent most often dominates, and increasing the radio frequency (RF) power with increasing organic solvents is necessary.

As the sensitivity of the ICP-MS signal is highly dependent on the organic solvent content of the solution, and the sensitivity in general decreases when the amount of organic solvent is increased, gradient elution would compromise quantifications, as sensitivity would change with the elution gradient.

Several approaches for minimizing the influence of organic solvent on the sensitivity have been reported. Solvent combustion could be enhanced by adding oxygen to the nebulizer gas. A combination of 5% oxygen in the nebulizer gas and spray chamber cooling has been a common setup reported in many studies. The plasma load can be reduced by reducing the eluent flow rate or by flow splitting, cooling the spray chamber, or complete removal of the solvent before introducing to the ICP-MS. Complete removal of the solvent is used in membrane desolvation units, where the eluent is heated and removed. These systems are often used in combination with an ultrasonic nebulizer (Fig. 10.2). In principle, these systems should allow for using gradient elution. However, great care should be taken that the analyte itself does not escape with the solvent, and several studies have shown that different species show different sensitivities in such systems. A review of different approaches to circumvent the problems

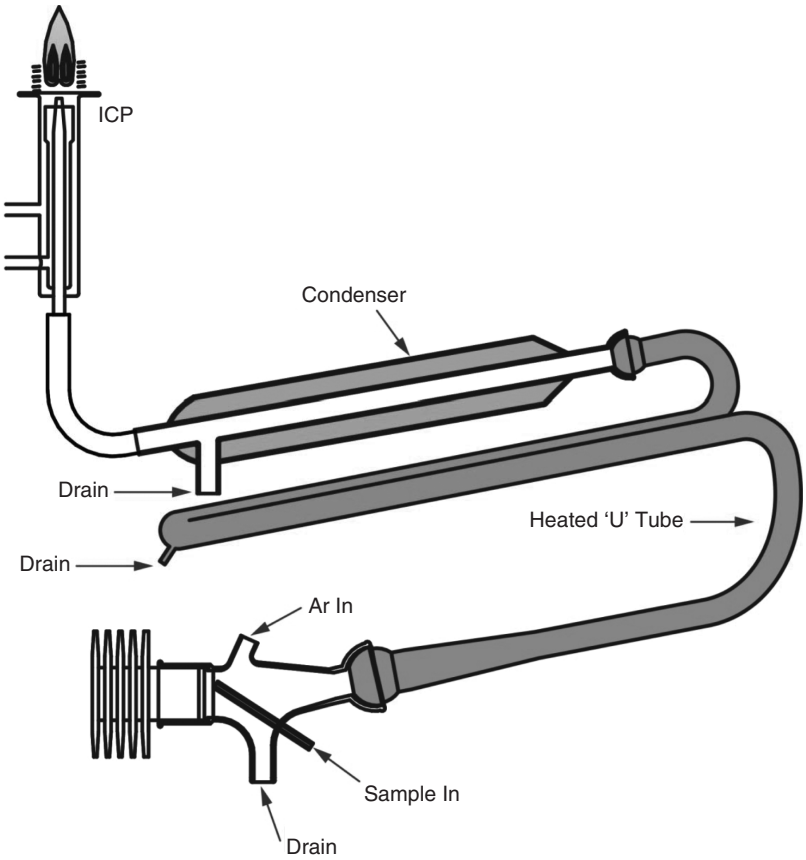

Figure 10.2 Schematic overview of membrane desolvation unit. *Source*: CETAC Technologies, with permission.

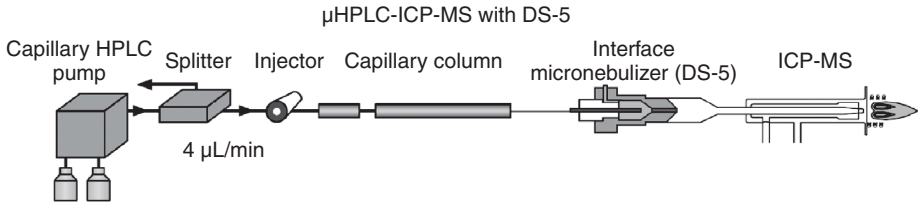

Figure 10.3 Schematic overview of capillary LC interfaced with ICP-MS via a micronebulizer system. *Source*: From CETAC, with permission.

introduced by organic solvents in drug metabolism studies of metal-containing and nonmetal drugs has recently been published [25].

10.4.2 Small Bore Columns and Low Flow Nebulizers

The common chromatographic column inner diameter was 4.6 mm just a decade ago, increasing interest in "green chemistry," and subsequent interest in diminishing the organic solvent consumption has led to decreasing column diameters to 2 mm, 1 mm, and even down to capillary sizes of <100 μm. The increasing interest in analyzing very small sample sizes has fortified this tendency. Thus, the use of microbore columns in combination with low flow nebulizers has become increasingly popular for hyphenation with ICP-MS.

The lower flow of microbore columns demands the use of low flow nebulizers capable of delivering stable nebulization of flow rates in the order of $1-100$ μL/min. These are known as the *direct injection high efficiency nebulizers* (DIHENs). Although the sample flow is lowered with these nebulizers, the sensitivity is still good, as the nebulizers introduce the whole sample flow into the plasma in contrast to the use of spray chambers where only a few percent of the sample reaches the plasma. The first of these nebulizers was very fragile and difficult to operate, while newer systems have become more robust. An example of a microflow sample introduction system is shown in Fig. 10.3. The handling of such systems, however, is still not a matter of routine [26].

Although the use of narrow-bore columns and low flow rates diminish the problem with plasma instability, stable signals are only obtained with isocratic elution schemes. A new approach to circumvent the influence of changing sensitivity is to oppose the gradient by introducing a counterflow, resulting in a constant load of organic solvent to the plasma[27].

Examples of LC-ICP-MS applications are listed under the individual elements in the following sections. The structures of the most commonly analyzed MBDs are given in Fig. 10.4.

10.5 CE-ICP-MS

Capillary electrophoresis (CE) is based on the migration of ions in an electric field. Thus, this separation principle is different from LC and could be a useful alternative technique. The main idea behind coupling of CE to ICP-MS is to exploit the sensitive and element-specific detection of ICP-MS with the high resolution of CE. Many MBDs have no or limited UV absorption limiting the use of CE-UV. Although hyphenation of CE and ICP-MS is not simple, it offers several advantages: a very low injection

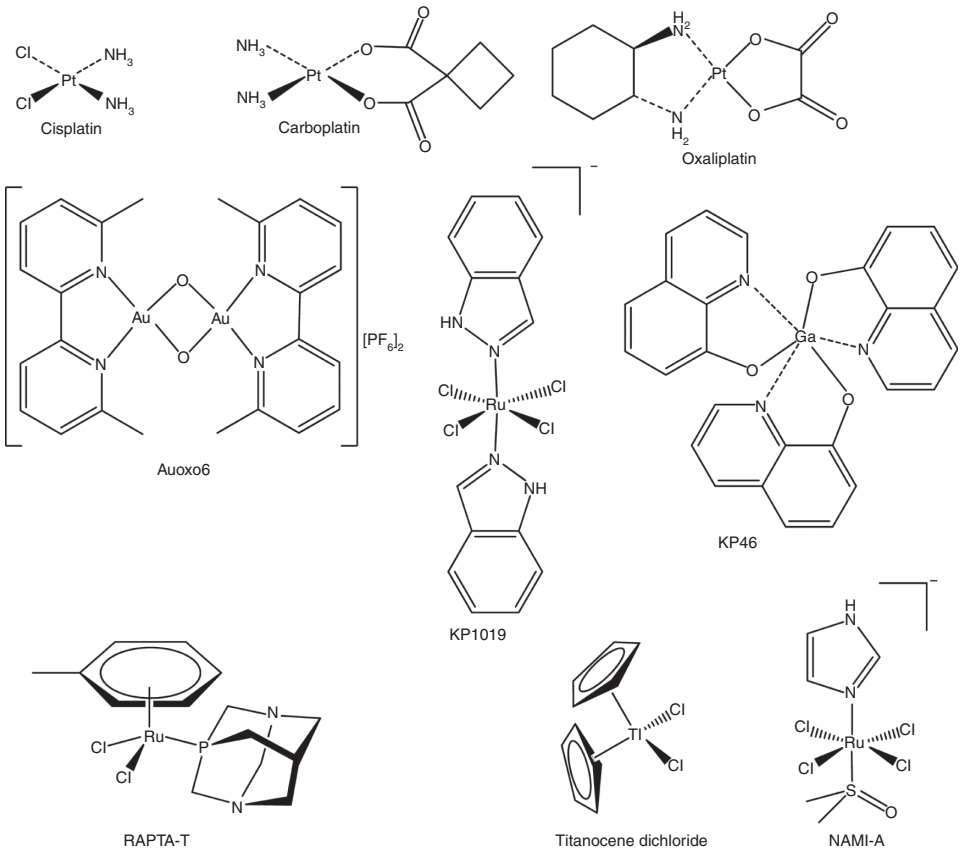

Figure 10.4 Structures of selected metal-based drugs.

volume in nanoliters, an alternative separation method to LC, low buffer consumption, and high number of theoretical plates.

10.5.1 Interfaces

The first paper on hyphenation of CE and ICP-MS was published by Olesik in 1995 [28], and since then, several papers have presented different ways of hyphenation. Today, two CE-ICP-MS interfaces are commercially available. In contrast to LC-ICP-MS, hyphenation is not straightforward as the low flow from the CE must be adapted to the flow acquired by the nebulizer, the electric connection must be maintained at the outlet of the CE capillary, and any laminar flow in the CE capillary must be avoided due to suction from the nebulizer. Figure 10.5 shows the hyphenation of CE and ICP-MS through a sheath flow interface. Here, a sheath flow is used to obtain a total flow of 5–10 μL/min, the electric connection is maintained through a grounded Pt electrode placed in a sheath liquid, a low consuming nebulizer is used, and liquid levels in the CE and sheath liquid are adjusted to avoid siphoning. A detailed description and technical aspects of hyphenation of CE and ICP-MS can be found in a recent review [29].

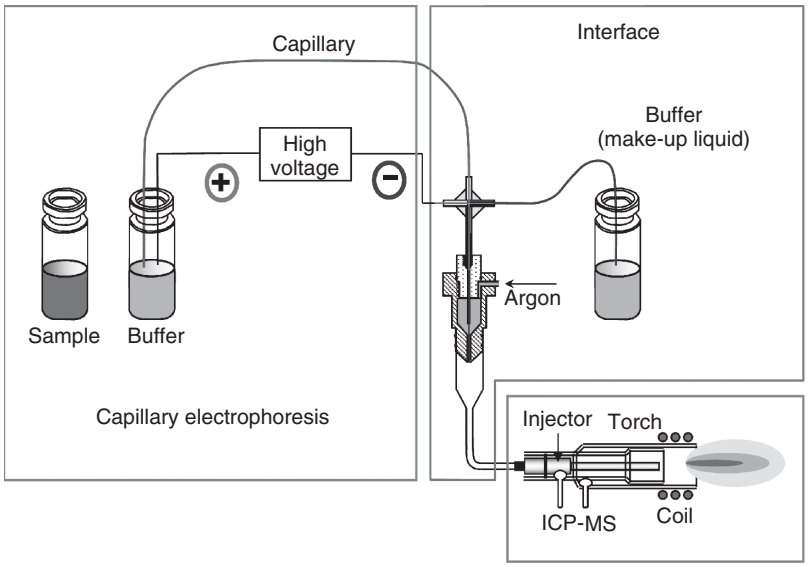

Figure 10.5 Schematic overview of CE-ICP-MS with the CEI-100 interface. *Source*: With permission from CETAC Technologies.

10.5.2 Quantitative Aspects

CE-ICP-MS has primarily been used for qualitative and semiquantitative analyses. A major drawback of the technique is changes in sensitivity during the day. Two ways of circumventing this problem has been proposed: addition of a trace element (external standard) to the sheath liquid [30] and the use of internal standard in the samples [31]. This leads to improved precision. Figure 10.6 shows an electropherogram of

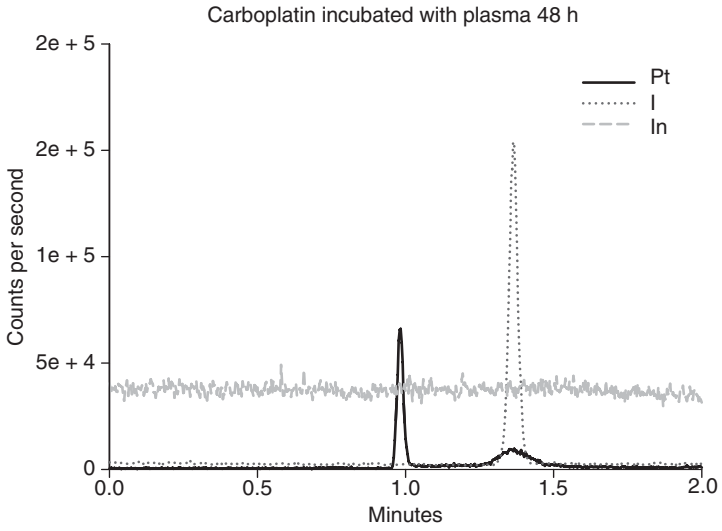

Figure 10.6 CE-ICP-MS analysis of incubation of plasma and carboplatin for 48 h at 37°C. Sodium diatrizoate is added as an internal standard before analysis, and In is used as an external standard. (See color insert.)

TABLE 10.2 CE-ICP-MS Application

Isotopes	Analyte	Subject	Interface	Buffer	Year	References
^{194}Pt ^{195}Pt ^{195}Pt ^{72}Ge	Cisplatin and cisplatin analogs	Interaction with HSA	CEI-100	15 mM phosphate pH 7.4	2004	[30]
^{34}S ^{56}Fe ^{69} Ga ^{71} Ga ^{72}Ge	KP46Ga(NO_3)	Interaction with HSA and Tf	CEI-100	Ammonium carbonate pH 7.4	2009	[34]
^{115}In ^{127}I ^{194}Pt ^{195}Pt ^{196}Pt	Carboplatin	Interaction with plasma	CEI-100	10 mM phosphate pH 7.4	2009	[31]
^{100}Ru ^{102}Ru ^{72}Ge	KP1019	Interaction with HSA and Tf and stability	CEI-100	10 mM phosphate buffer	2007	[35]
^{72}Ge ^{101}Ru ^{102}Ru ^{104} Ru	KP1019, RAPTA-C, KP418	Stability and interaction with 5′-dGMP	CEI-100	50 mM formic acid	2008	[36]
^{34}S ^{72}Ge ^{64}Zn ^{66}Zn	Zn(II) complexes	Interaction with HSA and apo-Tf and serum	CEI-100	20 mM Tris or 10 mM ammonium carbonate	2009	[37]
^{34}S ^{72}Ge ^{101}Ru ^{102}Ru ^{104}Ru	KP1019	Human plasma interaction with HSA and apo-Tf and serum/plasma	CEI-100	Polybrene-coated capillaries, 50 mM formic acid (pH 2.7).	2008	[33]
^{197}Au	$HAuCl_4$	Interaction with L-histidine	Laboratory made	50 mM Tris pH 7.5	2000	[38]
^{69} Ga ^{57}Fe ^{72}Ge	Ga drug	Stability and interaction with HSA, Tf, and serum	CEI-100	10 mM phosphate buffer, containing 10 mM NaCl	2009	[39]

carboplatin incubated in plasma. Indium was added to the sheath liquid, and sodium diatrizoate (iodine) was used as an internal standard to correct for changes in sensitivity.

ICP-MS is a mass sensitive detector, meaning the signal is dependent on the total amount of analyte in the plasma. This explains why the detection limit for CE-ICP-MS is higher than that for LC-ICP-MS, as the injection volume is in nanoliters and

microliters, respectively. Furthermore, the sample is diluted due to the use of a sheath flow [32].

CE-ICP-MS has been applied to the analysis of various MBDs, with primary focus on stability and interactions with biomolecules, particularly proteins. Most work is based on model systems using human serum albumin (HSA), transferrin (Tf), or plasma/serum. However, the method has been applied to the analysis of a plasma sample from a patient treated with the experiment Ru drug KP1019 [33]. Table 10.2 gives an overview of some of the recent research on MBDs using CE-ICP-MS.

The main challenge of analyzing proteins is to minimize adsorption due to hydrophobic or ionic interactions between the protein and the capillary surface, which leads to broad or tailing peaks. Using coated CE capillaries or adding a modifier to the buffer minimizes the problem. Furthermore, the background electrolyte should resemble the incubation buffer and physiological conditions should be used during sample preparation to avoid alternation of weak interactions during sample preparation and analysis [34]. A high background electrolyte concentration also increases the likelihood of clogging the nebulizer [32].

CE-ICP-MS analysis is still not a matter of routine. The technique has been compared to capillary LC-ICP-MS for analysis of metalloproteins. Using the same injection volume, similar performance of the two systems was observed. Capillary LC-ICP-MS was more robust and offered the opportunity to inject a larger sample volume and use on-line preconcentration of the sample before analysis. This is important when analyzing real samples with low concentration [40]. Thus, over time, CE-ICP-MS systems may be outperformed by capillary LC-ICP-MS in most applications.

10.6 LA-ICP-MS

In contrast to LC-ICP-MS, for which samples must be dissolved before analysis, laser ablation (LA)-ICP-MS offers the opportunity to measure solid samples by laser ablation of a small sample quantity, which is transferred to the ICP-MS. LA-ICP-MS has not gained great prevalence for the analysis of drugs. A general problem with LA-ICP-MS quantification is that it is difficult to obtain suited standards. The first application of LA-ICP-MS in the analysis of MBDs was to identify the platinum-containing proteins in *Escherichia coli* cells treated with cisplatin before separation with gel electrophoresis [41]. Examples of recent applications are quantification of Pt along a single hair strand from a patient treated with cisplatin [42] and scanning of kidney tissue from a mouse treated with cisplatin to create images of the distribution of ^{63}Cu, ^{64}Zn, and ^{196}Pt [43].

10.7 APPLICATIONS

The first application of LC-ICP-MS for MBD analysis is analysis of the gold-containing drug, auranofin [44]. The hydrolysis products of cisplatin and reaction products with methionine, cysteine, and glutathione were investigated using ion-pair LC-ICP-MS [45]. In addition to monitoring interactions with small biomolecules and metabolites, the interaction with proteins has been of general interest. In early investigations, the fraction of free and protein-bound metal was analyzed after ultrafiltration, after

which on-line-SEC-ICP-MS monitoring the interaction between proteins and Pt- or Ru-based drugs was introduced [46]. Following is an overview based on the individual elements.

10.7.1 Platinum

Since Rosenberg, in 1965 by accident, discovered cisplatin [47], various platinum compounds have been synthesized and tested for their cytotoxicity. Today, three Pt-based drugs are approved worldwide as anticancer drugs. The structures of cisplatin, carboplatin, and oxaliplatin are given in Fig. 10.4. Cisplatin is very efficient in the treatment of certain cancer types, particularly testicular cancer and some solid tumors. The limited application; severe side effects such as kidney damage, loss of hearing, damage to the nervous tissue; and intrinsic or acquired resistance to the treatment have led to the synthesis of many new Pt-based drug candidates. At first, most new drug candidates were cisplatin analogs, based on a square planar Pt(II) center with two monodentate or one bidentate leaving groups, but the oral Pt(IV) drug satraplatin (JM216) has also entered clinical trials [48]. In total, around 40 Pt-based drugs have been investigated in clinical trials and more than 1000 have been synthesized [49].

10.7.1.1 ICP-MS and Platinum. Platinum has six stable isotopes ^{190}Pt,^{192}Pt, ^{194}Pt, ^{195}Pt, ^{196}Pt, and ^{198}Pt. The three most abundant isotopes ^{194}Pt,^{195}Pt, and ^{196}Pt have a relative abundance of 32.9%, 33.8%, and 25.2%, respectively. They are all subject to isobaric interference of ^{178}HfO, ^{179}HfO, and ^{180}HfO/^{180}WO, respectively, but this is normally not a problem, as Hf and W are not present in biological samples and the formation of metal oxides can be controlled through plasma conditions. Furthermore, ^{196}Pt is subject to isobaric interference of ^{196}Hg. Instrumental detection limits for determination of total Pt in subnanogram per liter range have been reported for quadruple-based ICP-MS [18], while detection limits for LC-ICP-MS have been reported to be around or below 1 μg/L using a quadrupole and the detection limit improved by a factor of 10 using sector field ICP-MS [50,51]

10.7.1.2 DNA-Binding Mechanism. The general mechanism of Pt-based drugs involves binding to DNA in the cell nucleus. When cisplatin enters the cell, it undergoes hydrolysis because of low chloride concentration inside the cell, ~5 mM. The major binding site for cisplatin on DNA is on the guanine base, and it forms an intrastrand cross-link [52]. DNA is bent leading to inhibition of transcription and in the end to apoptosis or necrosis [53]. As formation of DNA adducts is believed to be crucial for cell death, studies of the nature of the binding have been conducted. Recently, an LC-ICP-MS method was developed to quantify cisplatin–DNA adducts formed after incubation of cisplatin cell carcinoma cultures. Three methods all applying isotope dilution analysis (IDA) and species-specific or unspecific spiking were used for quantification. A method for quantifying the amount of Pt bound to DNA, extracted from peripheral blood mononuclear cells and tumor tissue from patients treated with cisplatin, has been developed. The results were compared to the alternative ^{32}P-postlabelling method and found to be more precise, sensitive, and less laborious [54].

10.7.1.3 Interaction Studies. Pt(II) is a soft metal with a high affinity toward soft ligands such as sulfur. Therefore, interactions between biomolecules and Pt-based drugs are common. Table 10.3 gives an overview of some of the recent research obtained using LC-ICP-MS. Cisplatin, carboplatin, and oxaliplatin are all administered intravenously. Therefore, interactions with serum protein have gained particular interest.

One of the features of ICP-MS is multielement detection. This has been utilized in several papers on interaction with proteins and biomolecules. All serum proteins contain sulfur as a result of the presence of the sulfur-containing amino acids, methionine and cysteine. This gives the opportunity to simultaneously monitor Pt and S in interaction studies. In addition, Tf contains iron, and in serum, around 30% of the Tf is loaded with iron. This has been used to demonstrate that the incubation of transferrin with cisplatin did not displace iron from transferrin [19,65]. Recent studies, however, contradicted this observation [65]. Interaction between metallothionein (MT) and cisplatin has also been monitored with Zn, Cd, and Pt [56,66]. Sensitivity of S is much lower than that of Pt. This is due to the high ionization potential of S and the fact that the most abundant isotope ^{32}S (95%) is subject to interference from $^{16}O_2$. Instead, ^{34}S (4.2%) can be monitored or a dynamic reaction cell can be used to move $^{32}S^+$ to $^{32}S^{16}O^+$. Figure 10.7 shows the simultaneous monitoring of Pt, S, and Fe in iron-saturated apotransferrin incubated with cisplatin [19].

10.7.1.4 Stability of Pt-Based Drugs During Sample Preparation and Analysis. Whenever performing speciation analysis, the integrity of the sample is of primary concern. However, only few papers have dealt with this problem. Tran *et al*. [67] analyzed cell lysates from T289 malignant melanoma cells exposed to cisplatin using hydrophilic interaction chromatography (HILIC)-ICP-MS. Chemical lysis, osmosis, and freeze thaw were compared. Chromatograms of the lysates showed different results, stressing the need for critical evaluation of the lysis method. In addition, they also found a difference in the total amount of Pt extracted from the cells using the aforementioned lysis methods. Esteban-Fernandez *et al*. [60] evaluated the interaction strength between cisplatin biomolecules under denaturating conditions using LC-ICP-MS and found that the interaction was strong, as no changes were observed. As previously mentioned, conditions applied during separation may affect the binding of metals to proteins [34]. The choice of mobile phase may also lead to artifacts. Heudi *et al*. [68] found that phosphoric acid, in contrast to formic acid, interacted with monoaquacisplatin. The above-mentioned papers all demonstrated the need to carefully evaluate the sample preparation method and separation method in order to avoid altering the sample and creating artifacts.

10.7.1.5 Uptake in Cancer Cells—Total Determination of Pt in Various Fractions. In the search for new Pt drugs, there has been an interest in establishing a relationship between the cytotoxicity and the uptake of Pt in the cells and the amount of Pt bound to DNA. Also, the distribution of Pt between different cellular compartments has been investigated. After incubation of the drug, the cells were divided into nucleus, DNA, mitochondria, cytosol, and medium. The concentration was determined relative to the amount of protein, which was used as a measure of the amount of cells. In addition to the determination of distribution in the cellular compartments, ICP-MS has been used to determine the pharmacokinetic parameters. A recent review summarizes different investigations on the pharmacokinetic parameters for oxaliplatin [69]. An overview of selected papers on total determination of Pt can be found in Table 10.4.

TABLE 10.3 Application of LC-ICP-MS in the Analysis of Platinum-Based Drugs

Isotopes	Analyte	Subject	Separation	Year	References
^{195}Pt ^{34}S ^{54}Fe	Cisplatin	Interaction with HSA, Tf, IgG, and serum	AEC	2008	[19]
^{194}Pt ^{195}Pt ^{205}Tl	Cisplatin	Quantification of cisplatin and monoaquacisplatin in plasma UF and cell culture medium UF	RP	2006	[55]
^{66}Zn ^{111}Cd ^{194}Pt ^{195}Pt	Cisplatin	*In vitro* interaction with MT and GSH Analysis of rat kidney, liver, and inner ear cytosol	SEC	2007	[56]
^{194}Pt ^{195}Pt	Cisplatin	Quantification of isolated DNA adducts from cell carcinoma cultures and a larvae using different IDA strategies	RP narrow bore	2009	[19]
^{195}Pt ^{57}Fe	Cisplatin, carboplatin, and oxaliplatin	*In vitro* interaction with Hb and *in vitro* incubation with whole blood and analysis of red blood cells	SEC	2004	[57]
^{194}Pt ^{195}Pt ^{196}Pt	Cisplatin, carboplatin, and oxaliplatin	Incubation with whole blood Analysis of plasma fraction	HILIC	2009	[13]
^{195}Pt	Carboplatin	Incubation with HSA and IgG, analysis of human plasma, *in vitro* incubation with plasma	SEC	2007	[58]
^{194}Pt ^{195}Pt ^{196}Pt	Carboplatin	Human urine analyzed using isotope dilution	RP	2008	[59]
^{194}Pt ^{195}Pt ^{191}Ir ^{191}Ir	Cisplatin, (carboplatin, oxaliplatin)	Analysis of cytosol from rat ear and kidney after monodosage of cisplatin. Total determination in mitochondria, nucleus and ear, kidney, and liver cytosol	SEC	2008	[60]

TABLE 10.3 (*continued*)

Isotopes	Analyte	Subject	Separation	Year	References
^{195}Pt ^{56}Fe	Oxaliplatin	Quantification of profiling oxaliplatin–protein complexes in blood samples	SEC	2006	[61]
^{194}Pt ^{195}Pt ^{205}Tl	Satraplatin, metabolite of satraplatin, cisplatin	Incubation with human plasma, determination of reversible or irreversible binding and stability/degradation	RP	2008	[62]
^{194}Pt ^{195}Pt ^{205}Tl	Oxaliplatin	Human plasma, quantification of intact oxaliplatin	RP	2008	[63]
$^{32}S^{16}O$ ^{102}Ru ^{195}Pt	Cisplatin	Incubation with serum	SEC-AEX and CIM	2010	[64]

Abbreviations: CIM, convective interaction media; GSH, glutathione; AEC, anion-exchange chromatography.

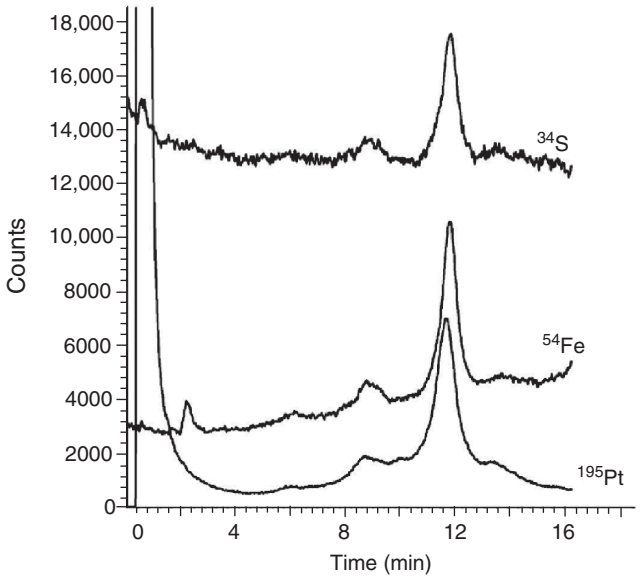

Figure 10.7 HPLC separation of iron-loaded transferrin incubated with cisplatin (1:10) with simultaneous detection of S, Fe, and Pt. *Source*: Reproduced from D. Esteban-Fernandez, M. Montes-Bayon, E. B. Gonzalez, M. M. G. Gomez, M. A. Palacios, A. Sanz-Medel. J Anal At Spectrom 2008;23:378–384. *Source*: By permission of the Royal Society of Chemistry. Ref. 19.

TABLE 10.4 Examples of Determination of Total Platinum

Isotopes	Analyte	Subject	Sample Preparation	Year	References
^{194}Pt ^{195}Pt	Cisplatin	Comparison of different cell lysis methods	Chemical lysis, osmosis and freeze thaw	2009	[67]
^{69} Ga ^{102}Ru ^{115}In ^{195}Pt	Cisplatin and KP1019	Development of protocol to determine uptake in adherent tumor cells	Acid digestion	2009	[17]
^{195}Pt ^{115}In	Cisplatin, carboplatin and oxaliplatin and experimental Pt drugs	Uptake/accumulation after prolonged incubation of cancer cells with various Pt compounds	Acid digestion	2003	[16]
^{195}Pt ^{193}Ir	Oxaliplatin	Validation of microwave-assisted mineralization method for Pt in plasma and PUF	Acid digestion	2008	[18]
^{195}Pt ^{194}Pt	Cisplatin	Determination of Pt DNA ratio	Buran lysis buffer	2007	[12]
^{194}Pt ^{195}Pt	Cisplatin, carboplatin, oxaliplatin	Determination of Pt DNA ratio at 37°C and 43°C	Acid digestion	2008	[70]
^{194}Pt ^{191}Ir	Cisplatin	Total Pt in DNA extracts from human blood mononuclear cells and tissue	Acid digestion	2008	[54]
^{195}Pt ^{193}Ir	Oxaliplatin	Pharmacokinetics, Pt in PUF, plasma, and whole blood	Acid digestion	2000	[71]
^{194}Pt ^{191}Ir	Cisplatin	The effects of sulfur-containing compounds and gemcitabine on the binding of cisplatin to plasma proteins and DNA	Acid digestion	2008	[72]

Abbreviations: PUF, plasma ultrafiltrate; UF, ultrafiltrate.

10.7.1.6 Drug-Delivery Systems. Owing to the side effects from treatment with Pt drugs, drug-delivery systems have gained attention. A targeted delivery of the drug to the tumor could enhance efficacy and minimize side effects. ICP-MS has been used to monitor the amount of encapsulated drug and the uptake of drug in cancer cells. Passive targeting takes advantage of the enhanced permeability and retention (EPR)

of macromolecules, nanoparticles, and liposomes. EPR arises because of both vascular permeability stimulated by extravasation and increased retention from the inefficient drainage of macromolecules by the lymphatic system [73]. Several ways have been explored: encapsulation of the drug in nanoparticles [74,75] or microspheres [76] and binding of the drug to macromolecules [77,78].

10.7.2 Gold

Gold-containing drugs were originally developed for the treatment of arthritis [79]. In recent years, gold complexes have also attracted attention as possible anticancer drugs. Numerous gold(I) complexes have been evaluated for cytotoxicity and antitumor activity [80]. Also, gold(III) complexes demonstrate antitumor properties, an example is Auoxo6, a binuclear gold(III) complex (Fig. 10.4) [80,81]. Gold(III) complexes typically display the same electronic configuration and similar structure and reactivity as platinum(II) complexes. However, apoptosis induced by gold(III) complexes seems to be triggered by mitochondrial damage and not by prevention of DNA replication, as in the case of cisplatin [80]. Despite the efforts in discovering and developing new gold complexes for cancer treatment, no drugs have yet been approved. Hence, gold-containing drugs are still limited to arthritis treatment. These comprise the polymers of sodium thiopropanolsulfonate-*S*-gold(I), sodium bis(thiosulfate), sodium aurothiomalate (myocrisin), and aurothioglucose (solganol) and the monomer triethylphosphine-goldtetraacetylthioglucose (auranofin and Ridaura). Recently, a new promising field applying gold nanoparticles as drug-delivery vehicles has emerged. Gold nanoparticles can be functionalized with a thiolated poly(ethyleneglycol) monolayer onto which a drug can be linked. Gold nanoparticles have been loaded with doxorubicin [82] and oxaliplatin [75]. In both cases, a high cellular uptake was observed along with improved or at least similar cytotoxicity to that of the free drug.

10.7.2.1 ICP-MS and Gold. Gold is monoisotopic (^{197}Au), and because of its high first ionization potential, the degree of ionization in the argon plasma is low (51%). But since polyatomic interferences of m/z 197 are virtually nonexistent when analyzing biological samples and the complete Au signal is seen on one isotope, instrument detection limits in the 1–3 ng/L range can still be achieved. However, one important prerequisite is to ensure that Au is in a soluble form during the sample preparation processes (e.g., as the $[AuCl_6]^{3-}$ ion) to prevent reduction and precipitation of solid Au particles. Furthermore, Au drugs are often lipophilic and are easily reduced to metallic Au, making sample preparation and ICP-MS analysis a challenge. As a consequence, very few studies on the application of ICP-MS for investigation of Au-containing drugs have been published. One exception is the study by Zhao *et al*. [83], who used LC-ICP-MS to determine Au-containing metabolites in urine sample from patients treated with myocrisin and auranofin, the main metabolite found was $[Au(CN)_2]^-$. Surprisingly, new studies on the application of ICP-MS technology in the investigation of Au-containing drugs have not been published in the last 10 years, but the new interest in the application of Au nanoparticles as drug-delivery vehicles may prompt a renewed interest in the use of ICP-MS for analysis of gold-containing drugs (Table 10.5).

TABLE 10.5 Application of LC-ICP-MS in the Analysis of Gold-Based Drugs

Isotopes	Analyte	Subject	Separation	Year	References
^{197}Au	Auranofin and myochrysine and their metabolites	Human urine	Ion-pair RP	1992	[83]
^{197}Au	Myochrysine	*In vitro* incubation with RBCs and analysis of human RBCs	SEC, FIA, ion-pair RP	1995	[84]
Cu, Cd, Zn, Au	Auranofin	Human blood	SEC, WAX	1989	[44]
^{197}Au	Auranofin, myochrysine, and solganol	Human urine and blood	Ion-pair RP	1993	[85]

Abbreviations: FIA, flow injection analysis; RBCs, red blood cells; WAX, weak anion exchange; SEC, size exclusion chromatography.

10.7.3 Ruthenium

In recent years, there has been a growing interest in ruthenium complexes as anticancer drugs. Two Ru(III) drugs with octahedral coordination chemistry, NAMI-A (imidazole Ru(III) dimethyl sulfoxide complex) and KP1019 (indazolium[transtetrachlorobis(1H-indazole)ruthenate(III)]), have successfully completed phase I clinical trials. NAMI-A showed efficacy as an antimetastatic agent, whereas KP1019 exhibited activity against colorectal carcinomas and primary human tumors. Other ruthenium complexes that exhibit favorable pharmacological profiles are under investigation, for example, RAPTA complexes that are ruthenium(II)-arene complexes bearing the 1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane ligand, the structures are shown in Fig. 10.4. Ruthenium binds strongly to DNA by mechanisms similar to the ones proposed for Pt(II) complexes. Protein binding is supposed to play a role in the mechanism of action, as Ru(III) uptake by cells is mediated by transferrin transport. This transport mechanism offers opportunities to deliver not only Fe but also Ru complexes inside the cancer cells improving tumor selectivity [86]. Ruthenium(II)–chloroquine complexes have recently been shown to be potential antimalaria drugs [87].

10.7.3.1 ICP-MS and Ruthenium. Ruthenium has seven stable isotopes ^{96}Ru, ^{98}Ru, ^{99}Ru, ^{100}Ru, ^{101}Ru, ^{102}Ru, and ^{104}Ru, the latter three being the most abundant with abundances of 17.1%, 31.6%, and 18.6%, respectively. Ruthenium is almost fully ionized (96%) in the argon plasma. Potential interferences are few, but since most originate from matrix elements (e.g., $^{40}Ar^{61}Ni^{+}$, $^{64}Ni^{37}Cl^{+}$, and $^{85}Rb^{16}O^{+}$ on $^{101}Ru^{+}$ and $^{62}Ni^{40}Ar^{+}$, $^{66}Zn^{36}Ar^{+}$, and $^{65}Cu^{37}Cl^{+}$ on $^{102}Ru^{+}$), interferences can be a problem when analyzing biological samples, as transition metals such as Ni, Cu, and Zn often occur in relatively high concentrations. Still, these interferences can often be avoided by the use of multiple Ru isotopes and careful monitoring of transition metal levels during analysis. Detection limits for total determination of Ru in biological samples below 0.1 μg/L can be obtained on a routine basis [10], while detection limits for LC-ICP-MS are in 2–10 μg/L range (Table 10.6) [88].

TABLE 10.6 Example of Application of LC-ICP-MS in the Analysis of Ruthenium

Isotopes	Analyte	Subject	Separation	Year	References
^{54}Fe ^{56}Fe ^{195}Pt ^{196}Pt ^{198}Pt ^{100}Ru ^{101}Ru ^{102}Ru	RAPTA and cisplatin	Interaction with HSA and transferrin (TF) at various ratio between drug and protein and different concentrations	SEC	2010	[65]
^{194}Pt ^{195} Pt ^{196}Pt ^{100}Ru ^{101} Ru ^{102}Ru ^{63}Cu ^{114}Cd ^{31}P	Three experimental Ru drugs and cisplatin	Interaction with serum proteins, release of protein-bound Ru after treatment with EDTA	SEC	1999	[46]
^{32}S^{16}O ^{56}Fe ^{102}Ru	KP1019	Human plasma and interaction with plasma *in vitro*	SEC + AEX	2005	[88]
^{32}S^{16}O ^{115}In ^{102}Ru	KP1019	Pioneer study of the use of immunoaffinity LC	Immunoaffinity SEC	2010	[89]

Abbreviations: AEX, anion exchange; SEC, size exclusion chromatography.

10.7.3.2 Interaction Studies. The first study on the interaction of a ruthenium-based drug with serum proteins by ICP-MS detection was performed by Szpunar *et al*. [46]. They observed that the ruthenium drug was bound to proteins with a molecular mass of 50–70 kDa. No free drug was observed, as neither the drug nor its hydrolyzed products could be eluted from the column. The same approach was taken by Sulyok *et al*. [88]. They analyzed the drug KP1019 by SEC-ICP-MS and also observed that the ruthenium signal appeared in the 50- to 70-kDa band. By LC-ES-MS, they identified two proteins in the band, HSA and Tf. The stoichiometry of the KP1019 protein binding was determined by the Ru/S ratio. Less than 2% of KP1019, in an incubated mixture of HSA and Tf, was found to bind to Tf. This example illustrates how the multielemental detection feature of ICP-MS provides qualitative as well as quantitative information on the drug–protein binding [88]. Recently, Groessl *et al*. [65] used SEC-ICP-MS to study the interaction of RAPTA complexes with transferrin and found that the affinity was higher for holotransferrin (iron saturated) than for apotransferrin (iron free), suggesting an iron-mediated Ru binding mechanism. Similar results were obtained by examination of the interaction of KP1019 with HSA and Tf using CE-ICP-MS. Hartinger *et al*. [90] developed a two-dimensional chromatographic scheme for studying interactions of KP1019 with serum proteins in plasma samples from a patient by monitoring Ru, $^{32}S^{16}O^{+}$, and $^{56}Fe^{+}$. This study highlights the strength of ICP-MSs multielemental capabilities.

10.7.3.3 Determination of Total Ru in Biological Samples. Brouwers *et al*. [10] successfully developed an ICP-MS method for the determination of Ru in human plasma filtrate, plasma, and urine samples from an NAMI-A phase I clinical trial. Sample preparation was a simple dilution with either 1% HNO_3 or 0.01% EDTA-triton before analysis. Potential polyatomic interferences were avoided by monitoring the ^{102}Ru isotope, which was the isotope least affected by interferences. Carefully correcting for the adsorption, an LOD of 0.44 fg Ru per cell was achieved along with good accuracy and precision on repeated cellular uptake experiments. These two studies clearly demonstrate that ICP-MS is a very powerful technique for the determination of Ru in biological samples and that controlled sample preparation is of utmost importance.

The availability of RAPTA complexes linked to recombinant HSA has been investigated with the purpose of exploiting the EPR effect [91].

10.7.4 Other Metal-Containing Drugs

Several other metal complexes have been investigated in search of new drugs. Some of these, such as Gd(III)-, Ga(III)-, and Ti(IV)-complexes, have entered clinical trials. Some of the most prominent examples of potential metal-containing drugs are outlined below and summarized in Table 10.7.

10.7.4.1 Gadolinium. Motexafine gadolinium is a member of the texaphyrins or "expanded porphyrins" class of macromolecules capable of coordinating large metal ions [96]. It has been subject to several clinical trials for its anticancer activity and is also under investigation as a site-directing molecule improving delivery of carboplatin [94,100]. Gadolinium complexes, for example, gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA) are widely used as magnetic resonance imaging (MRI) contrast agents due to gadolinium's ability to enhance the intracellular ^{1}H MRI signal. Contrast agents should be nontoxic and excreted rapidly, as they are often given in gram quantities. Gadolinium has seven isotopes, and ICP-MS sensitivity and accuracy are good, provided that potential interferences from various lanthanide oxides are resolved or minimized. ICP-MS has been used to study the pharmacokinetics and biodistribution of motexafin in plasma and tissue [94] and to follow the excretion and stability of Gd-DTPA in urine from a patient, using SEC-ICP-MS. No Gd-DTPA metabolites were found, and the complete dose (> 99%) was excreted within 24 h [95].

10.7.4.2 Gallium. A small group of Ga(III) complexes has entered clinical trials as oral anticancer drugs. One of these was designed to be similar to the iron(III)–maltol complex, which is known to provide iron in a bioavailable, readily absorbable form. These gallium complexes are interesting, as Ga^{3+} is more bioavailable than other metal ions (e.g., Ru^{3+}) and allows administration as an oral drug. In the cell, Ga(III) impairs DNA replication leading to apoptosis through the mitochondrial pathway [101].

Gallium has two isotopes, ^{69}Ga and ^{71}Ga. The ICP-MS analysis of gallium is hampered by many polyatomic interferences (various ClO_2^+ and SO_2^+ ions) plus interference from doubly-charged $^{138}Ba^{2+}$ ions, which makes accurate determination of total gallium at low levels in biological levels difficult. CE-ICP-MS has been used to investigate the binding of KP46 to plasma proteins. By simultaneous monitoring of $^{69}Ga^+$, $^{34}S^+$, and $^{56}Fe^+$, it was shown that KP46 preferably binds to transferrin [34].

TABLE 10.7 Examples of Application of LC-ICP-MS in the Analysis of Other Metals

Isotope	Analyte	Subject	Separation	Year	References
^{21}Sb ^{123}Sb ^{115}In	Glucantime (*N*-methyl meglumine antimoniate)	Human whole blood, plasma, urine, and hair	AEX	2002	[92,93]
^{160}Gd ^{159}Tb	Motexafin gadolinium	Stability of the drug in plasma	RP	2006	[94]
^{158}Gd	Gd-DTPA (DTPA: diethylenetriaminopentaacetic)	Human urine	SEC	2004	[95]
^{184} W ^{186} W ^{191}Ir	Sodium tungstate	Interaction with proteins, incubation with serum	SEC	2008	[96]
^{51}V	V(III) and V(V)	Interactions with human serum	AEX	2005	[97]
^{34}S ^{44}Ca ^{46}Ti ^{47}Ti ^{48}Ti ^{49}Ti	Ti citrate	Interaction with Tf and human serum, species-specific isotope dilution analysis	SEC and AEC	2008	[98]
^{207}Tl ^{209}Bi	Bismuth citrate (ranitidine)	Interaction with transferrin and albumin, competition	Off-line LC	2003	[99]

Abbreviations: AEX, anion exchange; SEC, size exclusion chromatography.

10.7.4.3 Titanium. The organometallic agent titanocene dichloride [Cp2TiCl2] (Fig. 10.4) has entered clinical trials as an anticancer drug [102–104]. To our knowledge, no ICP-MS analysis has been carried out on titanocenes. Titanium analysis using ICP-MS in biological samples is, in general, difficult due to heavy interference from polyatomic ions and isobaric overlap in the mass range of Ti isotopes (^{46}Ti, ^{47}Ti, ^{48}Ti, ^{49}Ti, and ^{50}Ti); furthermore, careful sample preparation is critical as titanium can be difficult to maintain in solution. As a consequence, there are few papers describing the ICP-MS analysis of Ti in biological samples, one exception is the paper by Sarmiento-Gonzalez *et al*. [98], who studied the binding of Ti(IV) to transferrin in blood serum using LC-ICP-MS.

10.7.4.4 Vanadium, Zinc, Rhodium, and Osmium. Vanadium and zinc complexes have been suggested to be involved in the regulation of glucose level in the body and thereby mimic the biological effects of insulin. Studies on binding of vanadium and zinc to transferrin and other human serum proteins utilizing various hyphenated ICP-MS techniques have been reported [37,97,105]. Like for Ti and Ga, ICP-MS analysis of V and Zn is hampered by a large number of polyatomic interferences and can

be difficult. By applying hyphenated techniques and by careful monitoring of blank levels/contamination, ICP-MS analysis of low levels of V and Zn in biological samples is possible.

Recently, rhodium and osmium analogs to the ruthenium RAPTA drugs have been synthesized, these complexes show similar cytotoxicity in cancer cell lines as that of the Ru RAPTA complexes [106]. However, no ICP-MS studies on these compounds have been reported.

10.8 SUMMARY

ICP-MS is a hard ionization MS technique characterized by a high and structure-independent sensitivity as all MBDs and their metabolites are converted into the M^+ ions in the plasma. When interferences have been overcome by use of collision or reaction cell instruments, instrumental detection limits at the nanogram per liter level are obtained for most metals.

A distinctive feature of MBDs is their interactions with proteins and other biomacromolecules. LC-ICP-MS is the method of choice for analysis of these interactions. The system tolerates well high salt concentrations used in ion-exchange chromatography, while the high organic solvent loads used in RP chromatography is not readily compatible with plasma. This problem is most easily solved by diminishing column dimensions, resulting in lower flow rates of the organic solvent introduced. Microbore and capillary column separations will probably become the method of choice before long.

CE coupling to ICP-MS is not as straightforward as the LC coupling and has not gained a broad use presumably because of the difficulties of hyphenating and the higher LODs than those of LC-ICP-MS. However, as CE offers a complementary separation technique to LC, it still finds applications in certain research areas.

Hyphenated ICP-MS systems have been applied to numerous experiments on elucidation of metal–drug reactions with plasma proteins and DNA, which is a target for several of these drugs. These comprise primarily platinum-containing drug compounds, as cisplatin has been used for decades, and ruthenium and gold are gaining increasing attention as alternative metals in new anticancer agents. The multielement capability of the technique is exploited by simultaneous monitoring of the metal and sulfur to examine protein binding, in general, and simultaneous monitoring of iron or phosphorus to examine binding to the iron-containing transferrin and DNA, respectively. As the interest in MBDs involving a variety of possible metals for different purposes grows rapidly, the use of ICP-MS will increase correspondingly.

ABBREVIATIONS

AEX	Anion Exchange
CE	Capillary Electrophoresis
CIM	Convective Interaction Media
DNA	Deoxyribonucleic Acid
GSH	Glutathione
FIA	Flow Injection Analysis
Hb	Hemoglobin

HILIC	Hydrophilic Interaction Chromatography
HSA	Human Serum Albumin
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IDA	Isotope Dilution Analysis
IgG	Immunogobulin G
IP	Ion Pair
LA	Laser Ablation
LC	Liquid Chromatography
MRI	Magnetic Resonance Imaging
MT	Metallothionein
PUF	Plasma Ultrafiltrate
RP	Reversed Phase
RBCs	Red Blood Cells
SEC	Size Exclusion Chromatography
UF	Ultrafiltrate
WAX	Weak Anion Exchange

REFERENCES

1. Gray AL. J Anal At Spectrom 1986;1:403–405.
2. Szpunar J. Analyst 2005;130:442–465.
3. Jarvis KE, Gray AL, Houk RS. Handbook of inductively coupled plasma mass spectrometry. London: Blackie Academic & Professional; 1992.
4. May TW, Wiedmeyer RH. At Spectrosc 1998;19:150–155.
5. Koppenaal DW, Eiden GC. J Anal At Spectrom 2004;19:15 N.
6. Jakubowski N, Moens L, Vanhaecke F. Spectrochim Acta, Part B-At Spectrosc 1998;53:1739–1763.
7. Evans EH, Giglio JJ, Castillano TM, *et al*. Inductively coupled and microwave induced plasma sources for mass spectrometry. Cambridge: The Royal Society of Chemistry; 1995.
8. Tan SH, Horlick G. J Anal At Spectrom 1987;2:745–763.
9. Rao RN, Talluri MVNK. J Pharm Biomed Anal 2007;43:1–13.
10. Brouwers EEM, Tibben MM, Rosing H, *et al*. Rapid Commun Mass Spectrom 2007;21:1521–1530.
11. Bettinelli M. Microchem J 2005;79:357–365.
12. Bjorn E, Nygren Y, Nguyen TTTN, *et al*. Anal Biochem 2007;363:135–142.
13. Falta T, Koellensperger G, Standler A, *et al*. J Anal At Spectrom 2009;24:1336–1342.
14. Hann S, Koellensperger G, Kanitsar K, *et al*. Anal Bioanal Chem 2003;376:198–204.
15. Yamada K, Kato N, Takagi A, *et al*. Anal Bioanal Chem 2005;382:1702–1707.
16. Ghezzi A, Aceto M, Cassino C, *et al*. J Inorg Biochem 2004;98:73–78.
17. Egger AE, Rappel C, Jakupec MA, *et al*. J Anal At Spectrom 2009;24:51–61.
18. Breda M, Maffini M, Mangia A, *et al*. J Pharm Biomed Anal 2008;48:435–439.
19. Sar DG, Montes-Bayon M, Gonzalez EB, *et al*. Anal Chem 2009;81:9553–9560.
20. Sutton KL, Caruso JA. J Chromatogr A 1999;856:243–258.
21. Szpunar J, Lobinski R, Prange A. Appl Spectrosc 2003;57:102A–112A.
22. Montes-Bayon M, DeNicola K, Caruso JA. J Chromatogr A 2003;1000:457–476.
23. Pan CK, Zhu GX, Browner RF. J Anal At Spectrom 1990;5:537–542.
24. Hu ZC, Hu SH, Gao S, *et al*. Spectrochim Acta, Part B-At Spectrosc 2004;59:1463–1470.
25. Gammelgaard B, Packert Jensen B. J Anal At Spectrom 2007;22:235–249.
26. Giusti P, Lobinski R, Szpunar J, *et al*. Anal Chem 2006;78:965–971.

27. Profrock D, Prange A. J Chromatogr A 2009;1216:6706–6715.
28. Olesik JW, Kinzer JA, Olesik SV. Anal Chem 1995;67:1–12.
29. Michalke B. Electrophoresis 2005;26:1584–1597.
30. Timerbaev AR, Aleksenko SS, Polec-Pawlak K, *et al*. Electrophoresis 2004;25: 1988–1995.
31. Moller C, Sturup S, Hansen HR, *et al*. J Anal At Spectrom 2009;24:1208–1212.
32. Timerbaev AR. Trac-Trends Analyt Chem 2009;28:416–425.
33. Groessl M, Hartinger CG, Polec-Pawlak K, *et al*. Electrophoresis 2008;29:2224–2232.
34. Groessl M, Bytzek A, Hartinger CG. Electrophoresis 2009;30:2720–2727.
35. Timerbaev AR, Foteeva LS, Rudnev AV, *et al*. Electrophoresis 2007;28:2235–2240.
36. Groessl M, Hartinger CG, Dyson PJ, *et al*. J Inorg Biochem 2008;102:1060–1065.
37. Bytzek AK, Enyedy EA, Kiss T, *et al*. Electrophoresis 2009;30:4075–4082.
38. Cuadrado JA, Zhang WX, Hang W, *et al*. J Environ Monit 2000;2:355–359.
39. Abramski JK, Foteeva LS, Pawlak K, *et al*. Analyst 2009;134:1999–2002.
40. Montes-Bayon M, frock D, Sanz-Medel A, *et al*. J Chromatogr A 2006;1114:138–144.
41. Allardyce CS, Dyson PJ, Abou-Shakra FR, *et al*. Chem Commun 2001;24:2708–2709.
42. Pozebon D, Dressler VL, Matusch A, *et al*. Int J Mass spectrom 2008;272:57–62.
43. Zoriy M, Matusch A, Spruss T, *et al*. Int J Mass spectrom 2007;260:102–106.
44. Matz SG, Elder RC, Tepperman K. J Anal At Spectrom 1989;4:767–771.
45. Zhao Z, Tepperman K, Dorsey JG, *et al*. J Chromatogr Biomed Appl 1993;615:83–89.
46. Szpunar J, Makarov A, Pieper T, *et al*. Anal Chim Acta 1999;387:135–144.
47. Rosenberg B, Van Camp L, Krigas T. Nature 1965;205:698–699.
48. Bhargava A, Vaishampayan UN. Expert Opin Investig Drugs 2009;18:1787–1797.
49. Esteban-Fernandez D, Moreno-Gordaliza E, Canas B, *et al*. Metallomics 2010;2:19–38.
50. Vacchina V, Torti L, Allievi C, *et al*. J Anal At Spectrom 2003;18:884–890.
51. Hann S, Koellensperger G, Stefánka Z, *et al*. J Anal At Spectrom 2003;18:1391–1395.
52. Egger AE, Hartinger CG, Hamidane HB, *et al*. Inorg Chem 2008;47:10626–10633.
53. Cepeda V, Fuertes MA, Castilla J, *et al*. Anti Canc Agents Med Chem 2007;7:3–18.
54. Brouwers EEM, Tibben MM, Pluim D, *et al*. Anal Bioanal Chem 2008;391:577–585.
55. Bell DN, Liu JJ, Tingle MD, *et al*. J Chromatogr B 2006;837:29–34.
56. Esteban F, as B, Pizarro I, *et al*. J Anal At Spectrom 2007;22:1113–1121.
57. Mandal R, Kalke R, Li XF. Chem Res Toxicol 2004;17:1391–1397.
58. Xie R, Johnson W, Rodriguez L, *et al*. Anal Bioanal Chem 2007;387:2815–2822.
59. Koellensperger G, Stefanka Z, Meelich K, *et al*. J Anal At Spectrom 2008;23:29–36.
60. Esteban-Fernandez D, Verdaguer JM, Ramirez-Camacho R, *et al*. J Anal Toxicol 2008;32:140–146.
61. Mandal R, Sawyer MB, Li XF. Rapid Commun Mass Spectrom 2006;20:2533–2538.
62. Bell DN, Liu JJ, Tingle MD, *et al*. Clin Exp Pharmacol Physiol 2008;35:1440–1446.
63. Ip V, McKeage MJ, Thompson P, *et al*. J Anal At Spectrom 2008;23:881–884.
64. Hann S, Falta T, Boeck K, *et al*. J Anal At Spectrom 2010;25:861–866.
65. Groessl M, Terenghi M, Casini A, *et al*. J Anal At Spectrom 2010;25:305–313.
66. Mandal R, Jiang G, Li XF. Appl Organomet Chem 2003;17:675–681.
67. Tran MQT, Nygren Y, Lundin C, *et al*. Anal Biochem 2010;396:76–82.
68. Heudi O, Cailleux A, Allain P. Chromatographia 1997;44:19–24.
69. Levi F, Metzger G, Massari C, *et al*. Clin Pharmacokinet 2000;38:1–21.
70. Gabano E, Colangelo D, Ghezzi AR, *et al*. J Inorg Biochem 2008;102:629–635.
71. Morrison JG, White P, McDougall S, *et al*. J Pharm Biomed Anal 2000;24:1–10.
72. Brouwers EEM, Huitema ADR, Schellens JHM, *et al*. Anticancer Drugs 2008;19: 621–630.
73. Gabano E, Ravera M, Osella D. Curr Med Chem 2009;16:4544–4580.
74. Patra CR, Bhattacharya R, Mukherjee P. J Mater Chem 2010;20:547–554.
75. Brown SD, Nativo P, Smith JA, *et al*. J Am Chem Soc 2010;132:4678–4684.

76. Poggi G, Quaretti P, Minoia C, *et al*. Anticancer Res 2008;28:3835–3842.
77. Garmann D, Warnecke A, Kalayda GV, *et al*. J Controlled Release 2008;131:100–106.
78. Aronov O, Horowitz AT, Gabizon A, *et al*. Bioconjugate Chem 2003;14:563–574.
79. Fricker SP. Dalton Trans 2007;43:4903–4917.
80. Magherini F, Modesti A, Bini L, *et al*. J Biol Inorg Chem 2010;15:573–582.
81. Ronconi L, Aldinucci D, Dou QP, *et al*. Anti Canc Agents Med Chem 2010;10:283–292.
82. Asadishad B, Vossoughi M, Alamzadeh I. Biotechnol Lett 2010;32:649–654.
83. Zhao Z, Jones WB, Tepperman K, *et al*. J Pharm Biomed Anal 1992;10:279–287.
84. Zhang YF, Hess EV, Pryhuber KG, *et al*. Inorg Chim Acta 1995;229:271–280.
85. Elder RC, Zhao Z, Zhang YF, *et al*. J Rheumatol 1993;20:268–272.
86. Hartinger CG, Jakupec MA, Zorbas-Seifried S, *et al*. Chem Biodivers 2008;5:2140–2155.
87. Martinez A, Rajapakse CSK, Naoulou B, *et al*. J Biol Inorg Chem 2008;13:703–712.
88. Sulyok M, Hann S, Hartinger CG, *et al*. J Anal At Spectrom 2005;20:856–863.
89. Hann S, Boeck K, Koellensperger G. J Anal At Spectrom 2010;25:18–20.
90. Harrington CF, Hann S, Koellensperger G, *et al*. Int J Clin Pharmacol Ther 2005;43: 583–585.
91. Ang WH, Daldini E, Juillerat-Jeanneret L, *et al*. Inorg Chem 2007;46:9048–9050.
92. Miekeley N, Mortari SR, Schubach AO. Anal Bioanal Chem 2002;372:495–502.
93. Hansen HR, Hansen C, Jensen KP, *et al*. Anal Chem 2008;80:5993–6000.
94. Miles DR, Mesfin M, Mody TD, *et al*. Anal Bioanal Chem 2006;385:345–356.
95. Loreti V, Bettmer J. Anal Bioanal Chem 2004;379:1050–1054.
96. Rodriguez-Farinas N, Gomez-Gomez MM, Camara-Rica C. Anal Bioanal Chem 2008; 390:29–35.
97. Fernandes KG, Montes-Bayon M, Gonzalez EB, *et al*. J Anal At Spectrom 2005;20: 210–215.
98. Sarmiento-Gonzalez A, Encinar JR, Cantarero-Roldan AM, *et al*. Anal Chem 2008;80: 8702–8711.
99. Sun HZ, Szeto KY. J Inorg Biochem 2003;94:114–120.
100. Arambula JF, Sessler JL, Fountain ME, *et al*. Dalton Trans 2009;48:10834–10840.
101. Timerbaev AR. Metallomics 2009;1:193–198.
102. Hannon MJ. Pure Appl Chem 2007;79:2243–2261.
103. Kaluderovic GN, Perez-Quintanilla D, Sierra I, *et al*. J Mater Chem 2010;20:806–814.
104. Kaluderovic GN, Perez-Quintanilla D, Zizak Z, *et al*. Dalton Trans 2010;39:2597–2608.
105. Nagaoka MH, Akiyama H, Maitani T. Analyst 2004;129:51–54.
106. Dorcier A, Ang WH, Bolano S, *et al*. Organometallics 2006;25:4090–4096.