JAAS

Accepted Manuscript

This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/jaas

Recent progress of ICP-MS in the development of metal-based drugs and diagnostic agents

Andrei R. Timerbaev

Drug discovery and development is a long, expensive, and multiplex process, most of the steps (if not all of them) being unfeasible without use of different analytical techniques. In the case of metal-based drugs, their preclinical development and clinical testing are increasingly relied on ICP-MS, having no-match analytical features in this seemingly 'killer' application. Applied with the standalone or combined (hyphenated) setup, the method allows robust, sensitive, and precise determinations of drug-comprising metals as well as specific and often multielemental detection of the biomolecular metabolic forms. This analytical information is invaluable for the assessment of drug-like properties, metabolite fingerprinting and profiling, monitoring the drug–biomolecule interactions, cellular uptake and pharmacokinetic studies, *etc*. but above all, for a better understanding of drug's mechanisms of delivery and action. This review is mainly focused on the emerging role and current challenges of ICP-MS-based methodology in the field. Consistently with the title matter, special emphasis is placed on investigational metal-containing compounds that not only exhibit certain pharmacological or diagnostic properties but also hold promise of being advanced to (or already entered) clinical studies. It also provides a brief outlook of how the potential of ICP-MS is to be exploited in the future so as to accelerate the metallodrug development and reduce enormous accompanying costs.

Vernadsky Institute of Geochemistry and Analytical Chemistry,
Russian Academy of Sciences, Kosygin Str. 19, Moscow, Russia.
E-mail: andrei.timerbaev@univie.ac.at; Fax: +7 495-9382054;
Tel: +7 495-9397035

Introduction

Although pharmacology is dominated by organic compounds, many inorganic compounds, particularly those containing metal atoms, are highly promising as therapeutic drugs.[1,2] Inspired to a great extent by the success of cisplatin and its few analogs, considerable effort is being

stepped up to developing novel anticancer drugs, with greater efficacy and reduced toxic side effects. These chemotherapeutics may include not only platinum but other metals, mostly ruthenium, gallium, gold, and tin.[3–7] Contemporary medicine also reserves promising future for metal compounds that can find application as diagnostic agents, *e.g.*, for magnetic resonance (gadolinium) or radioisotope (cobalt, technetium) imaging, drugs with anti-diabetic (zinc, vanadium), anti-flammatory (copper), anti-rheumatic and arthritis (gold) functions (to mention a few), as well as markers of certain diseases (such as aluminum for neurodegenerative disorders). However, it should be emphasized that the process of creating new metal-based medicines is far from efficient and effective. For instance, in the field of cancer chemotherapy, at average only one new metallodrug per decade has been launched for clinical use and a rough cost estimate hits as much as one billion US dollars. This implies that there is serious lack of productivity in drug discovery and development, as the main reason of such enormous time-lags and costs is behind high failure rate.

In the last years, this demanding challenge has received much attention of drug developers who critically reconsidered the arsenal and design of analytical techniques and methodologies in use. These activities paved the way of ICP-MS to become the method of preferential choice for determining the intact drug and its bioconversion products in relevant model and real-world samples. Importantly, modern ICP-MS instruments enable virtually interference-free response when dealing with such samples and quantification of not only the target metal but also metalloids and nonmetals originated from drug interactions with biomolecules. Furthermore, being by its nature a non-specific (single element-species) method, in combinations with a suitable separation technique, such as HPLC or capillary electrophoresis (CE), ICP-MS gains potential of a powerful speciation tool. This is a particularly valuable asset as the samples of interest can comprise a variety of metal forms resulting from various metabolic transformations.

A great deal of research directed toward adopting the ICP-MS methodology in metallodrug research and development has been the subject of copious review work. The issues most pertinent to the present review are those by Brouwers *et al.*[8] and Gammelgaard *et al.*[9] which provide a wide-ranging coverage of contributions published before 2008. Several more recent overviews are available in which highlighting of ICP-MS takes a prominent place among other standalone and combined techniques. In particular, Timerbaev and coworkers[10] critically assessed analytical methodology used in anticancer metallodrug proteomics, Ge *et al.*[11] apprised the applicability of hybrid techniques to identification of metallodrug metabolites, while Timerbaev and Stürup[12] evaluated ICP-MS-based technology for assaying metallodrugs in biological samples. However, despite the large body of review literature, none of the reviews

focuses on ICP-MS to aid the discovery and development of metal-based drugs, from the initial estimation of pharmacological properties for drug candidates to systematic cellular uptake and pharmacokinetic investigations as well as optimization of dosage schedules for drug-lead compounds in pragmatic studies.

This review article is written to fill this gap and to offer critical analysis of method's current capabilities and shortcomings as well as some developmental trends. In order to avoid duplication and reexamination of material and also to provide an update of benchmark reviews from 2008,[8,9] only publications concerning investigational compounds and promising prototype medicines, coming out as from that year, are considered here. To this end, no discussion is given to clinically approved metallodrugs (with few exceptions when they were utilized as test compounds to advance the ICP-MS methodology) but those who are interested are referred to recent reviews,[13–15] accounting for ICP-MS applications to metal-containing pharmaceuticals (see also the Supporting information; Table S-1). For benefit of the ICP-MS users who figure attractive their research diversion, we highlight robust workflows required for accurate measurements of target metal analytes, with the focus on species quantification from biomatrices (but with no effort to rival the dedicated overviews on sample preparation strategies to avoid matrix effects in ICP-MS[16,17]). On the other hand, with regard to expertise of the readership of this journal, basics and instrumental aspects of ICP-MS and its hyphenations with separation techniques are excluded from consideration.

Analytical measurements to the effect of drug development

There are several application domains of ICP-MS in the area of drug discovery and development, presented in Table 1. Yet before a novel pharmacologically challenging entity enters extensive preclinical testing, its drug-like properties have to be thoroughly evaluated. It should be mentioned that medicinal chemists are often missing that point and keep their efforts up as being inspired only by a certain biological activity of a given compound, *e.g.* cytotoxicity (especially when it is favorably compared with that of approved metallodrugs). Therefore, the first objective within a successful lead-drug candidate selection program is initial characterization with respect of desirable drug parameters such as solubility (not always all sufficient for organic ligand complexes!), stability (many metal complexes are prone to hydrolytic degradation!), lipophilicity (as a prerequisite of the efficient penetration through cell membrane!), *etc*. Most of these characteristics can be assessed by direct ICP-MS measurement of intact drug in fairly easy matrices.

Another motive stems from the need of elucidating biospeciation profiles that for intravenously administrated drugs are dominated by protein-bound forms. Reactivity and affinity toward plasma proteins are strongly related to drug delivery mechanism, being also a mandatory component of evaluating the adsorption, distribution, and metabolism triad. Here monitored are different metal species originating from simulated or real biosamples. This makes necessary incorporation of a separation procedure prior to ICP-MS analysis, to differentiate free and bound drug fractions, or its combination with a more powerful separation technique, to distinguish various protein-containing species. In the latter case, care is to be taken to avoid troubles coming from the proteinaceous analytes and high-salt matrix.

Systematic pharmacokinetic studies, including the excretion constituent, take place *in vivo*, after an early hit compound identification stage leads to selection of a drug nominee. Drug pharmacokinetic characteristics, such as the maximum concentration or free plasma concentration, are derived from the ICP-MS data obtained by analyzing, respectively, plasma and its ultrafiltrate samples. These are taken from experimental animals, then from the patients who received different dose levels. Similarly, drug clearance, which is in the most cases identical with renal clearance, is determined by monitoring drug's urinal levels.

In their turn, analyses of tissues and organs samples are answerable for the issues of drug distribution, accumulation, and long-term retention, while knowledge of metal speciation in explanted cell compartments shed light on the drug uptake and mechanism of action at the molecular level. It is obvious that dealing with such biosamples may pose difficulties to the ICP-MS analysis and hence requires pretreatment to alleviate the matrix interferences or to isolate the target analytes from interfering matrix components. This important analytical matter will be given consideration when discussing specific applications.

There is another reason why changes in concentrations of investigational metal-based drugs after administration require accurate measurements. Toxic side effects limit clinical treatment with a good deal of chemotherapeutics, especially those based of extraneous metals. Optimal use of such drugs in clinical settings implies developing advanced administration protocols to avoid overdosing and inhibit the dose-limiting toxicity. The corrected dosages would also open the floodgates to personalized treatment (likewise, for already approved drugs).

As follows from the scrutinizing of available literature, the ICP-MS technique, at the present stage of its adaptation, experiences a dissimilar application rate in different phases of drug development program. To a considerable extent, this reflects the thinking style of drug developers to whom the method is still seen as a sophisticated analytical tool in spite of its evident points of excellence. Nonetheless, for the sake of consistency, the following sections are

seriated in the succession of the course of drug development process (see Table 1), not in order of publication abundance ratio.

Characterization of drug properties

The core properties required to estimate drug's transport in the body, uptake and distribution are solubility, stability, lipophilicity, as well as interaction with transport plasma proteins (the latter subject will be considered in the following section). Evaluation of these properties is of crucial importance in metallodrug research as it helps to select a lead candidate for further preclinical development, guarding against failures, and to provide guidelines for designing more efficient compounds.

Solubility testing

Regardless of the administration route, solubility is to be high enough to render a drug candidate sufficient bioavailability in order to express the *in-vivo* activity. This is of special concern for pharmacologically active compounds intended to use as oral drugs. As a matter of fact, ICP-MS can be straightforwardly applied for such measurements, with due account for dealing with saturated drug solutions. To prevent a bias, these should be diluted at once, *e.g.* by 1% HNO_3. Usually such sample pretreatment also reduces polyatomic interferences when solvents are other than water. However, the opposite can be true in the case of fairly light metals, such as gallium, being a principal component of an oral investigational anticancer drug, *tris*(8-quinolinolato)gallium(III) (GaQ_3). In testing the solubility of GaQ_3 in simulated intestine juice, the blank signal at the mass of gallium-71 isotope was observed using high-resolution ICP-MS and attributed to the formation of $^{36}Ar^{35}Cl^+$ and $^{40}Ar^{31}P^+$ ions.[18]

Drug stability

As soon as a metal complex comes into solution, it would be the subject of hydrolysis. Hydrolytic decomposition can take place not only at body-fluid circumstances but already in pharmaceutical formulation. Furthermore, complexes of metals in higher(st) oxidation states, *e.g.*, ruthenium(III) or platinum(IV), may undergo bioreduction. Both hydrolytic and redox transformations are believed to bring about more active metallodrug species. However, they are generally considered as unwanted processes (at least at the stage of administration), posing

clinical limitations. Therefore, assessing survival rates is a basic requirement in systematic metallodrug discovery. ICP-MS coupled to CE offers a specific, sensitive, and reliable screening tool for such measurements. The intact drug and its degradation products can be well resolved, importantly in a fast manner and without notable conversion in a CE system. The time-dependent stability behavior may be assessed quite easily by means of relative peak-area measurements. These options were demonstrated by profiling the hydrolysis patterns and monitoring the hydrolysis kinetics for several ruthenium(III) drug candidates[19] and examination of the stability of GaQ_3 in simulated intestine juice[20] and liposomal metallodrug formulations under the action of an enzyme[21,22] or in human plasma.[23,24] In these trials, the integrity of the liposomes comprising phospholipids and the release of a platinum drug were recorded simultaneously by concurrent monitoring of the phosphorus and platinum isotopes.

The same detection strategy was employed for the purpose of investigation of liposome stability and metallodrug liberation from liposomes using HPLC–ICP-MS as an alternative stability-indicating assay.[25] In similar HPLC–ICP-MS studies related to drug formulation stability, the release of a Pt(II) species from biodegradable polymers chosen as a drug carrier was recorded.[26,27] In order to isolate the target analytes from micelles, into which the anticancer drug-containing polymer self-assembles, these were subjected to dialysis against different aqueous solutions. By applying the same combined technique, satraplatin, an investigational Pt(IV) antitumor drug, and its active Pt(II) metabolite were found unstable in human plasma.[28] It should be noted, however, that the number of platinum-containing degradation products formed *in vitro* (six and three, respectively) seems to be superfluous. This makes fairly suspicious chromatographic conditions chosen for this assessment, in view of the fact that metal complexes may lack the ability to withstand separation using the reversed-phase columns. Similar complications were faced when the stability of novel nanoemulsion-based formulations of gadolinium contrast agents for magnetic resonance imaging (MRI) was tested by HPLC–ICP-MS with on-line isotope dilution (using a ^{157}Gd-enriched spike solution).[29] One of such agents, the Gd complex of a lipophilic polyaminocarboxylic acid, appeared to be irreversibly retained (and partially degrade) on the reversed-phase column. Mass balance was also not achieved on the size-exclusion column because of a significant on-column loss of gadolinium and inter-species conversions; use of species-specific isotope dilution, implying the addition of laboratory-prepared enriched gadolinium–ligand standards, only partly rectified this drawback.

Direct ICP-MS analysis may also be useful to monitor the release of a Pt drug from cross-linked polymeric micelles into which it is encapsulated (in order to be protected against deactivation by proteins)[30] or a partial transformation of platinum nanoparticles, separated from

human colon carcinoma cells by ultracentrifugation, into soluble Pt species that could be responsible for moderate cytotoxic effect.[31]

Lipophilicity measurements

Lipophilicity is an essential factor in metallodrug development equation as it effectively determines transport through membranes (until the drug binds to the target and induces the desired response). It is important to emphasize that the way how the drug developers are evaluating the cell uptake has not always to do with the ability of a potential drug to penetrate membranes. This is because the compound tested for bioavailability is quite vigorously introduced into the cell culture, typically in its native form and occasionally in an aqueous–organic medium (as the case for sparingly soluble compounds).

Most often lipophilicity is measured using octanol–water partition, with the partition coefficient in the logarithmic form, log *P*, serving as a widely used lipophilicity parameter in medicinal chemistry. When assisted by ICP-MS, a typical procedure comprises equilibrium partition of a given compound between water and *n*-octanol (so called shake-flask method), and metal concentrations in the aqueous phase (before and after partitioning) or in the aqueous and organic phase are quantified to calculate log *P*. Appropriate dilution necessary in both situations may be a matter of special concern in the analysis of the organic phase. One needs to realize that the same very measurements can be carried out by means of a less expensive atomic spectroscopic technique, *e.g*. ICP-AES or even AAS. Actually, the advantage of ICP-MS becomes evident only for the assessment of a compound with extreme log *P* values, when its concentration in one of the phases tends to be exceptionally low.

The suitability of ICP-MS has been recently proven for antiproliferative thiosemicarbazone complexes of gallium(III) and iron(III)[32] and *cis*- and *trans*-configured Pt(II) complexes with cytotoxic properties,[33] whose partition coefficients varied four orders of magnitude. The precision of experimental results was verified by consistent correlations with the data of independent methods.

Protein-mediated transformations

Conceivably, plasma proteins performing transport functions are the top priority binding partners for metallodrugs in the bloodstream. Interaction with proteins would particularly affect the bioavailability and the metabolite profiles of therapeutics administered intravenously, though

oral drugs eventually also find their way in blood circulation system. In author's opinion, characterization of metallodrug interaction with plasma proteins belongs to drug-like assets and is to be examined yet before a drug candidate enters the *in-vivo* testing (which is not all the time true). The speed at which the drug substance converts into the protein-bound fraction and the stability of drug–protein adducts, especially in comparison with the existing drugs, would allow easy sorting-out of the most promising candidates, without performing expensive test animal or human experimentation. Note that binding to proteins (even in real-serum environment) is not traditionally referred to drug metabolism that will be particularized below. As will be detailed hereafter, ICP-MS found a rich niche in proteomics of therapeutic and diagnostic agents containing metals. This is due to method's 'killing' features in species identification, quantification and measuring the binding parameters after separation of free parent drug and its protein-bound forms using the principles ultrafiltration, electrophoresis, or chromatography.

ICP-MS following ultrafiltration

Perhaps the easiest way to determine the protein-mediated speciation of a drug in blood is to subject the plasma fraction to ultrafiltration (Fig. 1). Similarly, distribution between free and bound fractions can be characterized by ICP-MS after ultrafiltrating the incubated mixture of drug and protein. Ultrafiltrates are most often acquired using a 30 kDa cut-off filter, and probably the only complication from this straightforward sample handling may present the nonspecific adsorption onto the filter membrane or/and a plastic device. Dealing with metal complexes may give rise to artifacts, and mass balance studies should therefore be implemented to prove that the nonspecific binding does not affect a measurable amount of the tested compounds.

From the measurement part of the ultrafiltration–ICP-MS scheme, plasma-matrix components can interfere with different isotopes related to a drug (but unlikely platinum whose major isotopes, ^{194}Pt and ^{195}Pt, are virtually free from isobaric overlap). For instance, molecular ions formed by isotopes of argon or matrix chloride ion with metallic blood constituents can be disturbing, particularly in the case of using low-resolution quadrupole-based instruments. Spectral interferences have to be carefully addressed when validating an analytical procedure. In turn, nonspectral signal disturbances caused by the presence of organic components and salts are to be corrected using an internal standard. Otherwise, their impact on nebulization efficiency and energy stability of the plasma source can be reduced in a more rigorous way, by microwave-assisted digestion (MAD). On the other hand, sensitivity is no issue in the ultrafiltration-based assays as the sample is not diluted (as is the case of separation by HPLC and especially CE), but

oppositely, metal analytes are rather concentrated. However, in binding studies undertaken with real plasma samples (taken *e.g.* from dogs[34]), the concentration of free metal can fall below the LOQ after a prolonged time after administration.

Table 2 summarizes the diversity of metallodrug–protein systems studied using ICP-MS in combination with ultrafiltration, as well as other separation principles as expanded in the following subsections.

CE–ICP-MS

CE interfaced on-line with ICP-MS is deemed to be one of the most useful tools for the characterization of metallodrug–protein interactions.[48] Here polyatomic interference from the matrix components is an event of rare occurrence, as these are separated from the analytes in a CE system and additionally, greatly diluted by make-up liquid in the interface. Unless the detection power is hence compromised, changes in the metal speciation following the formation of protein adducts can be trustfully monitored. This is in a great part due to a range of attributes due to which CE is arguably regarded as superior to HPLC.[10] Most cited advantages of CE include no stationary phase involved in separation, use of electrolyte compositions compatible with real or simulated physiological conditions, and often shorter analysis times. Together these merits help to preserve the species under investigation against alterations that are not associated with the binding process. Also importantly, distinguishing different protein–metal adducts is feasible, in contrast to ultrafiltration enabling only total serum-protein fraction to assay.

However, when analyzing the protein-mediated speciation of metallodrugs in real serum or plasma samples, protein adsorption onto the surface of capillary walls can be a challenge. To circumvent this problem, a moderate sample dilution with water[20] or the physiological buffer[40,41] and the use of capillaries coated with a cationic polymer[40,41] was proposed. In addition, the excessive proteins, such as albumin and immunoglobulin G, can be depleted from serum to monitor metal loading of less abundant proteins.[40] Another common shortcoming of CE–ICP-MS, intraday changes in sensitivity impelling the signal precision, can be largely overcome by adding an external standard (*e.g.* ^{72}Ge) to make-up solution.

In most of the contributions under the current examination, the sulfur (^{34}S) or/and iron (^{57}Fe) isotopes were recorded simultaneously with the objective of unambiguous identification and quantification of the protein-bound species of ruthenium or gallium (*via* the known sulfur content of the protein). It should be noted that these are not the most abundant isotopes. Because of strong isobaric interferences (mainly from ^{16}O^{16}O and ^{40}Ar^{16}O, respectively), it was impossible to measure ^{32}S and ^{56}Fe that certainly reduced the sensitivity.

HPLC–ICP-MS

This hybrid technique is much more robust to put into practice and in most cases it can provide adequate sensitivity for characterization of interactions of metallodrugs with proteins (and other biomolecules) in real-world samples. As such, HPLC–ICP-MS has enjoyed a decent application record in the area, particularly in situations where the efforts made to attain complete recovery of the analytes of interest from the column and prevent metal contamination (*e.g.*, by using the metal-free chromatographic system) turned out to be successful. However, as compared with the previously reviewed period,[10] there has been less progress in methodological developments.

Size-exclusion chromatography (SEC) coupled to ICP-MS remains a trademark method for indicating proteins or high-molecular fractions of plasma (or serum) containing the bound metal. Its recent advancement comprised use of a two-dimensional chromatographic scheme in which SEC serves as the first separation dimension.[49] Each fraction isolated by SEC was sequentially loaded on one of two small monolithic anion-exchange columns connected on-line with an SEC column through a two-position switching valve. Notwithstanding that the two-dimensional approach offers more information concerning identification of the protein–drug adducts (Fig. 2), incomplete drug recovery can pose obstacles.[49] Mentioned in this regard should be an efficient procedure proposed to check whether the adduct is stable in an SEC system.[50] Anion-exchange HPLC used alone also works well for mapping the protein affinity of drug candidates bearing fairly hydrophilic character.[46,47]

Cellular uptake and distribution

The easiness with which ICP-MS can be utilized to quantify total intracellular metal levels facilitated the method's footing in drug uptake studies. Direct measurements of the metal content in drug-exposed cells (after their lysis and dilution of lysates) indicate the uptake rate and the intracellular fate that are essential indicators when evaluating the therapeutic potential of a drug. Among the plethora of investigational metal-based medicines that have been thus tested within the reviewed period, are tumor-inhibiting picoplatin[45] and bis-indazole tetrachloridoruthenate(III) compounds,[52,53] cytotoxic *cis*- and *trans*-configured acetone oxime Pt(II) complexes,[54] an adamantane–platinum(II) conjugate encapsulated in ß-cyclodextrin,[55] a Pt(IV) complex and its polymer conjugate,[28] the polymer–Pt(II) micelles,[29] organometallic complexes of Ru(II), bearing π-bonded arene ligands,[56–58] photocytotoxic nitrosyl

phthalocyanine ruthenium(III) complex (hosted in liposome as a drug delivery system),[59] luminescent (or phosphorescence) thiolato gold(I)–phosphane[60] and cyclometalated Ir(III) polypyridine[61–63] or polyamine[64] complexes, a carbohydrate drug containing an indium–DTPA–hexa-lactoside complex,[65] anti-diabetic bis(maltolato)oxovanadium,[66] gold nanoparticles of different surface charge and size,[67] nanoparticles of gadolinium oxide embedded in a polysiloxane shell[68] or gold, functionalized with different DNA oligomers,[69,70] copper powder and different surfaces evaluated for the antibacterial activity,[71] and arsenic trioxide encapsulated in nano-sized liposomes.[72] However, experimental protocols reported in support of assessing the cellular accumulation of metal compounds should be regarded with caution. First of all, to the best of author's knowledge, in none of these (or early published) contributions drug internalization has been tested using its metabolic or active (but parent) form (see also the Lipophilicity measurements section). This circumstance makes the ability to penetrate the cell inferred in such a way quite provisional. Second, there are certain indications that some drugs may exhibit unspecific adsorption onto cell culture dishes (typically made of plastic),[52] which is often overlooked by researchers. Therefore, in the case of lysis performed directly in the culture dishes, it is indispensible to correct the results for metal levels of a blank well containing no cells (as a negative control)[52–54,58] to avoid the risk of generating artifacts.

Important information on cellular localization of a drug presents the metal distribution between different cell fractions. Some of these, *e.g.*, cytoplasm and nucleic fractions, can be separated prior to the ICP-MS analysis merely by ultracentrifugation,[53] while more differential fractionation, into the cytosol, membrane/particulate, cytoskeletal, and nuclear fractions, required using a cell fractionation kit.[56,57] Apparently, such measurements are less interference-proof as the quadrupole-based system used was equipped with a dynamic reaction cell[53] or alternatively, the subcellular fractions were at first digested[56,57] (more potently, in a closed pressurized MAD unit[57]). A further insight into the metal speciation in the cytosolic fraction of Ru drug-treated cancer cells may be gained by adapting SEC. For this purpose, an SEC × SEC system was designed, in which two columns with different exclusion limits were assembled on-line and connected to an ICP mass spectrometer in order to increase the range of analyzed molecular masses.[53] Large protein complexes and/or membrane protein aggregates (above 700 kDa) were identified as initial major binding partners (Fig. 3A), followed by the Ru redistribution to the soluble protein fraction (below 40 kDa; see the LMW signal in Fig. 3B). This study is one of a very few examples where ICP-MS-based methodology is applied to probe the metallodrug affinity toward protein targets other than plasma proteins. However, incomplete column recovery (70%) implies uncertainties in authors' characterization of the intracellular speciation of Ru, by a rule of thumb, of 30%. No such complications were though perceived in

another SEC–ICP-MS study focused on the profiling of ruthenium drug distribution in the subcellular fractions.[56]

Cell processing

Most of metallodrugs exert their therapeutic effects at the cellular level, with the plausible scenario that they are activated inside the cells and thence commence targeting.

Activation

Once it is inside the cell, the drug is supposed to get activated. Activation of the drug might involve the release of an active metal functionality, *e.g.*, from the protein-bound form, accompanied by reduction (often coined as 'activation by reduction' and is believed to occur for Pt(IV) and Ru(III) prodrugs) or structural transformations (in particular, due to pH differences between the blood and tumor tissue). Provided that one knows the mechanism of activation, strategies for increasing the efficacy of a drug could be suggested.

There is a recent trend of preclinical studies toward recognition of cell activation chemistry by means of metallomic techniques associated with ICP-MS.[42,73,74] An integrated ICP-MS approach, comprising ultrafiltration and/or CE separation, has been applied to addressing kinetics of alterations in the metal speciation for one of two bis-indozole tetrachloridoruthenate(III) compounds that are progressing in clinical trials. These trials were basically addressing conditions that mimic intracellular fluid of tumor cells with respect to the pH and chloride concentration[73] and cytosol components that display reductive and complex-formation functions (such as glutathione and ascorbic acid).[42,74] As can be seen in Fig. 4, the formation of novel ruthenium species was indeed revealed, being presumably released from the drug–transferrin adduct. However, the fact that no cytosol derived from cancer cell lines has been yet tried by the authors makes it uncertain whether these very species would be involved in cell processing of the drug.

Targeting

As a matter of fact, synthetic molecules containing metals are designed so that they are capable of selectively affecting (typically perturbing) the function of individual biomolecules. Evaluation of the drug binding properties to such molecules, in the first instance, nucleic acids but also

cellular proteins, is a crucial step toward identifying the ultimate targets of the compound and consequently toward understanding its mode of action.

DNA is shared by all types of cells, and quite reasonably that ICP-MS found most of claim in assessing the extent of drug binding to DNA. The method can be straightforwardly applied to quantify the DNA-associated metals after the isolation of genomic DNA from cells exposed to (or incubated with) a drug and checking the purity of the isolated DNA (*e.g.* against proteins). This is commonly accomplished using purpose-made commercial kits and occasionally followed by acid digestion.[30,56,75] Alternatively (but perhaps less reliably), the amount of metal (platinum) incorporated to DNA can be determined after solvent extraction (*e.g.* from liver tissues) and acidic digestion of extracts[76] or by relating the platinum and phosphorus concentrations measured by ICP-MS.[54] The latter approach also implicated a rather tedious sample preparation procedure, including an excessive dilution with HCl to minimize the impact of $^{15}N^{16}O$ interference with ^{31}P. The level of DNA metallation has been specifically measured (and often compared to that of the approved metallodrug, cisplatin) for RAPTA-T, an organometallic Ru(II)–arene complex,[56] miriplatin or *cis*-(((1*R*,2*R*)-cyclohexanediamine-*N*,*N*')bis(myristato))platinum(II),[76] developed as a chemotherapeutic agent for hepatocellular carcinoma, cytotoxic tri-functional mononuclear,[77] binuclear,[78] and *cis*- and *trans*-configured[54] complexes of Pt(II), lipoplatin (a liposomal formulation of cisplatin),[79] as well as platinum nanoparticles[30] and a nanoparticular Pt(II) compound in which the platinum moiety is complexed with a polymer.[75]

Recognition of the DNA-binding profiles requires a more sophisticated methodology to use. For instance, this can be achieved by combining a highly specific enzyme-based procedure (to extract the adducts from drug-exposed tumor cells) with HPLC coupled to the collision cell ICP-MS instrument.[80] When applied to patient samples, this protocol made it possible to detect a Pt–DNA adduct (Fig. 5) but its quantification was feasible only after quite a prolonged drug treatment. In another study aimed at quantitative profiling of *in-vivo* generated Pt–DNA adducts,[81] two different isotope dilution strategies were attempted: species-unspecific (with the post-column addition of a ^{194}Pt-enriched solution) and species-specific (using an isotopically enriched ^{194}cisplatin adduct with a custom oligonucleotide, spiked before enzymatic digestion). Species-specific method was shown to be more accurate and precise to differentiate between *Drosophila* larvae and carcinoma cell culture samples treated with cisplatin; however, it implied the synthesis and characterization of an isotopically labeled spike. It seems that it is for this reason that specific-unspecific isotopic dilution was given preference in the following related research by the same group.[82,83]

As another potential nucleic target, RNA was investigated with regard of binding to an antimetastatic Ru-based prodrug NAMI-A.[84] A RNA purification kit was used to isolate the metallated nucleic acid from cells, followed by its desalting and acid digestion prior to the ICP-MS analysis (*cf*. the DNA isolation as above).

The trafficking of metal species after they are taken up by cells may also include exocytosis, a process by which a cell directs its content back into the extracellular space. A recent case study, demonstrating the feasibility of ICP-MS to measure the metal content expelled by cells, has concerned gold nanorods.[85]

Pharmacokinetic studies

The next requested step in drug development is acquiring information on how a specific drug candidate is affected by the whole body through the mechanisms of absorption, distribution, and metabolism and then is eliminated from it, which is the subject of pharmacokinetics. Detalization of various models used to simplify understanding of the many processes that are involved in the interaction between an organism and a pharmacological substance is beyond the scope of this work. Here, we only emphasize that pharmacokinetic examinations are based on the determination of the concentration of a drug in samples taken mainly from laboratory animals after administration of different dose levels. Most often, these are plasma and plasma ultrafiltrate, the ICP-MS analysis of which – following certain pretreatment (usually acid digestion) – enables the calculation of drug pharmacokinetic metrics, as exemplified in Table 3. In should be underlined that no attempt on comprehensiveness was made when collecting these data, since only developmental metal-containing compounds were the focus of consideration.

In order to assess the tissue distribution of a drug by common ICP-MS technique, the accumulated metal is to be brought into solution. This can be achieved by using MAD.[47,76,79,87,89,93] While this is a standard means to treat solid biological samples, such tissue handling increases the complexity of the analytical procedure and, as any sample preparation step, might be a source of uncertainty. A more effective approach pursued by an increasing number of researchers is due to combination of ICP-MS with laser ablation (LA). LA–ICP-MS requires no extensive sample preparation, as the tissues of interest are ablated by irradiating with a laser beam and the metal under scrutiny is transported (in the form of aerosol) to the ICP torch. Using LA–ICP-MS, it is possible to visualize the *in-vivo* distribution of metals originating from metallodrugs or metal-based nanoparticles of diagnostic or drug delivery relevance by measuring the target metal not only in various organs taken as whole (*e.g*. kidney, liver, lungs, brain, *etc*.[94])

but also its layered or 2D metal distribution.[95–102] This allows one to evaluate drug distribution properties and Figure 6 shows a representative example with high sensitivity (50 pg) and high spatial resolution (down to 8 μm). Although the potential of LA–ICP-MS as an elemental bioimaging method does not admit of doubt, reliable quantification strategies are still feeble. This challenge leads to poor precision and recoveries, especially for low analyte and semi-solid samples such as drug-affected tissues (that are prone to fast compositional changes). Internal standardization seems to be not always the clue whereas use of matrix-matched calibration standards presents apparently the most workable approach for quantification in LA–ICP-MS. In response to another bottleneck, stemming from non-specificity of LA–ICP-MS (as actually every ICP-MS-based technique), a common LA system has been hyphenated in parallel with an ICP and a molecular mass spectrometer (*via* a flow splitted interface, see Fig. 7 for a detailed setup) in order to accomplish simultaneous elemental and molecular spatially resolved analysis.[101]

The matter of the chemical changes of the drug substance in the body, or metabolism, is also coming to the front of ICP-MS assaying. Still, only a few contributions as commented below are devoted to metabolite profiling at real-world circumstances, *i.e.*, by analyzing clinical samples. An HPLC–ICP-MS method has been developed for quantification of a putative active biotransformation product of oxaliplatin, dichlorido(*R*,*R*-diaminocyclohexane)platinum(II), in blood plasma.[103] However, despite a fairly low LOD attained (1.9 μg L^{-1}) the method was not able to detect this metabolite in samples of patients treated with the drug. Trace levels of the volatile $(CH_3)_3Bi$ were detected by low temperature-gas chromatography–ICP-MS in blood and exhaled air samples of healthy volunteers who received colloidal bismuth subcitrate (as tablets).[104] This technique was given preferential choice over isotope dilution or standard addition quantification procedures because Bi is a monoisotopic element while standards tended to be unstable. SEC with combined elemental and molecular MS detection was applied to metabolite characterization of a vanadium anti-diabetic agent.[66] The challenge of the nearly monoisotopic character of vanadium was partly overcome by using a ^{50}V-enriched drug compound. However, this study has been limited to a liver cell model.

Surprisingly, urine and faces samples are only occasionally analyzed by ICP-MS with the objective to determine drug clearance.[87] It should be noted that the drug levels in urine are deemed to be more representative, as renal excretion is the major pathway by which metal therapeutic agents are excreted.

It is important to conclude this section with that the fate of a drug from the moment that it is administered involves yet one component, liberation. However, to attempts to discover the process of drug release from the pharmaceutical formulation under *in-vivo* conditions using ICP-

MS could be traced in recent literature. Therefore, we cross-index the reader to several examples, confined to the simulated settings, which were mentioned above (see Drug stability section).

Miscellaneous

There are a number of reports in which biofluid analyses have been carried out without immediate purpose to assess drug pharmacokinetic parameters, rather to confirm the applicability and to validate the ICP-MS method. For instance, the LOD of gallium (deriving origin from GaQ_3) was found to be as low as 20 ng L^{-1} in human serum[30] and 60 ng L^{-1} in urine.[16,105] Serum and plasma samples taken from a cancer patient, undergoing treatment during clinical trials of a Ru investigational drug, were analyzed by CE–ICP-MS.[40] However, the results showed a systematic (slightly positive) discrepancy when verified with regard to an independent HPLC–ICP-MS approach. Advantageously, in mouse plasma analyses of a similar ruthenium(III) drug candidate, good agreement between the same hyphenated techniques was later achieved by the same group.[41]

The enumeration of absolute cell numbers in clinical samples is important for diagnostic purposes and as cell enumeration methodology, ICP-MS opens the possibilities unattainable by other bioanalytical techniques. In combination with metallointercalators, such as Ru(III) or Ir(III) complexes that irreversibly bind DNA, ICP-MS proved to be an extremely sensitive means for determining cell numbers as well as for cellular DNA detection.[106] LA–ICP-MS was also shown capable of targeting human cells, labeled with commercial Gd-based MRI contrast agents, importantly at a single cell level, and hence enumerating labeled cells.[107] Measured in a similar fashion was the distribution in rat brain sections of labeled receptor-targeted nanocomplexes devised for the delivery of therapeutic DNA to the brain.[108] Another area where LA–ICP-MS could find arguably more widespread use is high-sensitivity detection of proteins, to which metallodrugs bind, separated by gel electrophoresis.[109,110] However special care should be taken to preserve the metal–protein bonding while unfolding the rest of the protein in order to maintain the separation efficiency. With the aim of monitoring a similar type of biointeractions, continuous elution gel electrophoresis has been coupled to sector-field ICP mass spectrometer.[111]

Other ICP-MS applications to be listed out only briefly encompass quality control of a Sb(V)-based drug for a trace Sb(III) impurity using HPLC–ICP-MS,[112] determination of chemical composition of anti-inflammatory Co(II)–oxicam complexes[113] and multifunctional Gd-based nanoparticles intended for theranostic use,[114] the dose-dependent ability of silver

nanoparticles to cross the blood-brain barrier (using an *in-vitro* model),[115] evaluation of gold nanoparticle binding with a prodrug Pt(IV) complex in terms of the amount of platinum bound and the equilibrium binding constant,[116] and a systematic multielemental serum analysis undertaken to help in diagnosis of Parkinson's disease.[117]

Conclusions

It is not an overstatement to say that ICP-MS is by far a mature analytical technique and even with its hyphenated, metallomics-directed configurations, the method's progress largely depends on how important is its application base. In author's opinion, metallodrug development presents one of the 'killer' applications to capture the attention of the ICP-MS practitioners – and this is the main message of this review. From the examination of recent literature, as demonstrated above, the reader will gain an appreciation that due to the considerable effort devoted to adding to the analytical capabilities of ICP-MS in the field, the method has greatly supplanted other atomic spectrometry and traditional bioanalytical techniques. This welcome situation is owing to successful interdisciplinary collaboration, with close interactions between analytical and medicinal chemists. It should be repeated, however, that drug development is a multiplex process and even bioinorganic chemists, discovering the new metal-based compounds (using ICP-MS as well), may not take into due account assessment of some important components of drug development program. To help avoid any screening pitfall and also to attract consideration of those who are just switching their research interests to the thrilling world of developing new metallotherapeutic drugs and metal-based diagnostic agents, or acquiring the necessary working experience with ICP-MS methodology, there was an obvious need to organize, codify, and critically assess the continuing advances of ICP-MS. The author believes that this task has been at least in part accomplished.

It will be exciting to watch the advancement of ICP-MS, also as a detector in the hyphenated systems, as the field continues to evolve. Apparently, most of forthcoming research endeavors are to be given to streamline the drug-development output of ICP-MS measurements, *e.g.*, by a wider acceptance of high-resolution and triple quadrupole mass spectrometers, isotope-dilution methods, and multidimensional separation technology. Many expectations are also from further progress of LA–ICP-MS for imaging/detection of metal species, including their protein-bound forms. However, instrumentation should be reduced in price and made simpler to use by non-spectroscopists. In this context, the biomedical community is requested to be willing to

implement properly the results acquired by ICP-MS, in the light of the significant resources being put into generating such data.

There is one issue which was not overlooked but purposely given no consideration in this work. Somebody reasonably called ICP-MS 'a mother that tells you the truth but not the all.' In other words, an inherent lack of ICP-MS in providing structural information means that for in-depth characterization of pertinent metal biospecies, the method should be complemented by a harmonized use of molecular-specific MS (also in combinations with separation techniques). Molecular MS techniques did find acceptance in a good proportion of metallodrug-related studies. However, it is a different story.

References

1 C. X. Zhang and S. J. Lippard, *Curr. Opin. Chem. Biol*., 2003, **7**, 481–489.

2 M. Gielen and E. R. T. Tiekink (Editors), *Metallotherapeutic Drugs and Metal-Based Diagnostic Agents*, Wiley, New York, USA, 2005, p. 598.

3 D. Esteban-Fernández, E. Moreno-Gordaliza, B. Cañas, M. A. Palacios and M. M. Gómez-Gómez, *Metallomics*, 2010, **2**, 19–38.

4 A. Levina, A. Mitra and P. A. Lay, *Metallomics*, 2009, **1**, 458–470.

5 L. Messori and C. Gabbiani, in: J. M. Perez, M. A. Fuertes and C. Alonso (Eds.), *Metal Compounds in Cancer Chemotherapy*, Research Signpost, Kerala, India, 2005, p. 355.

6 A. R. Timerbaev, *Metallomics*, 2009, **1**, 193–198.

7 A. Alama, B. Tasso, F. Novelli, F. Sparatore, *Drug Discov. Today*, 2009, **14**, 500–508.

8 E. E. M. Brouwers, M. Tibben, H. Rosing, J. H. M. Schnellens and J. H. Beijnen, *Mass Spectrom. Rev*., 2008, **27**, 67–100.

9 B. Gammelgaard, H. R. Hansen, S. Stürup and C. Møller, *Expert Opin. Drug Metab. Toxicol*., 2008, **4**, 1187–1207.

10 A. R. Timerbaev, K. Pawlak, C. Gabbiani and L. Messori, *Trends Anal. Chem*., 2011, **30**, 1120–1138.

11 R. Ge, Z. Sin and Q.-Y. He, *Curr. Drug Metabol*., 2011, **12**, 287–299.

12 A. R. Timerbaev and S. Stürup, *Curr. Drug Metabol*., 2012, **13**, 272–283.

13 B. Gammelgaard, S. Stürup and C. Møller, in *Encyclopedia of Drug Metabolism and Interactions,* A. V. Lyubimov, A. D. Rodrigues and M. S. Sinz (Eds.), Wiley, New York, USA, 2012, part V, 287–311.

14 F. van Heuveln, H. Meijering and J. Wieling, *Bioanalysis*, 2012, **4**, 1933–1965.

15 B. Meermann and M. Sperling, *Anal. Bioanal. Chem*., 2012, **403**, 1501–1522.

16 M. F. Mesko, C. A. Hartwig, C. A. Bizzi, J. S. F. Pereira, P. A. Mello and E. M. M. Flores, *Int. J. Mass Spectrom*., 2011, **307**, 123–136.

17 C. Agatemor and D. Beauchemin, *Anal. Chim. Acta*, 2011, **706**, 66–83.

18 D. G. Filatova, I. F. Seregina, L. S. Foteeva, V. V. Pukhov, A. R. Timerbaev and M. A. Bolshov, *Anal. Bioanal. Chem*., 2011, **400**, 709–714.

19 M. Groessl, C. G. Hartinger, P. J. Dyson and B. K. Keppler, *J. Inorg. Biochem*., 2008, **102**, 1060–1065.

20 J. K. Abramski, L. S. Foteeva, K. Pawlak, A. R. Timerbaev and M. Jarosz, *Analyst*, 2009, **134**, 1999–2002.

21 U. Franzen, T. T. T. N. Nguyen, C. Vermehren, B. Gammelgaard and J. Østergaard, *J. Pharm. Biomed. Anal*., 2011, **55**, 16–22.

22 T. T. T. N. Nguyen, J. Østergaard, S. Stürup and B. Gammelgaard, *Anal. Bioanal. Chem*., 2012, **402**, 2131–2139.

23 T. T. T. N. Nguen, J. Østergaard, S. Stürup and B. Gammelgaard, *Int. J. Pharmaceutics*, 2013, **449**, 95–102.

24 T. T. T. N. Nguen, J. Østergaard, S. Stürup and B. Gammelgaard, *Anal. Bioanal. Chem*., 2013, **405**, 1845–1854.

25 T. T. T. N. Nguen, S. Stürup, J. Østergaard, U. Franzen and B. Gammelgaard, *J. Anal. At. Spectrom*., 2011, **26**, 1466–1473.

26 H. Xiao, R. Qi, S. Liu, X. Hu, T. Dian, Y. Zheng, Y. Huang and X. Jing, *Biomaterials*, 2011, **32**, 7732–7739.

27 H. Xiao, D. Zhou, S. Liu, Y. Zheng, Y. Huang and X. Jing, *Acta Biomaterialia*, 2012, **8**, 1859–1868.

28 D. N. Bell, J. J. Liu, M. D. Tingle, B. Rattel, T. U. Meyer and M. J. McKeage, *Clin. Exp. Pharmacol. Physiol*., 2008, **35**, 1440–1446.

29 D. Cleveland, S. E. Long, L. C. Sander, W. C. Davis, K. E. Murphy, R. J. Case, C. A. Rimmer, L. Francini and A. K. Patri, *Anal. Bioanal. Chem*., 2010, **398**, 2987–2995.

30 H. T. T. Duong, V. T. Huynh, P. de Souza and M. H. Stenzel, *Biomolecules*, 2010, **11**, 2290–2299.

31 H. Gehrke, J. Pelka, C. G. Hartinger, H. Blank, F. Bleimund, R. Schneider, D. Gerthsen, S. Bräse, M. Crone, M. Türk and D. Marko, *Archiv. Toxicol*., 2011, **85**, 799–812.

32 L. S. Foteeva, D. A. Trofimov, O. V. Kuznetsova, C. R. Kowol, V. B. Arion, B. K. Keppler and A. R. Timerbaev, *J. Pharm. Biomed. Anal*., 2011, **55**, 409–413.

33 K. Ossipov, Y. Y. Scaffidi-Domianello, I. F. Seregina, M. Galanski, B. K. Keppler, A. R. Timerbaev and M. A. Bolshov, *J. Inorg. Biochem*., in the press.

34 D. Zhao, Y. Zhang, C. Xu, C. Dong, H. Lin, L. Zhang, C. Li, S. Ren, X. Wang, S. Yang, D. Han and X. Chen, *Biol. Trace Elem. Res*., 2012, **148**, 203–208.

35 Y. Wang, X. Wang, J. Wang, Y. Zhao, W. He and Z. Guo, *Inorg. Chem*., 2011, **50**, 12661–12668.

36 K. Ossipov, L. S. Foteeva, F. Seregina, S. A. Perevalov, A. R. Timerbaev and M. A. Bolshov, *Anal. Chim. Acta*, 2013, **785,** 22–26.

37 F. Biba, M. Groessl, A. Egger, A. Roller, C. G. Hartinger and B. K. Keppler, *Eur. J. Inorg. Chem*., 2009, 4282–4287.

38 F. Biba, M. Groessl, A. Egger, M. A. Jakupec and B. K. Keppler, *Chem. Biodiversity*, 2009, **6**, 2153–2165.

39 K. Połeć-Pawlak, J. K. Abramski, J. Ferenc, L. S. Foteeva, A. R. Timerbaev, B. K. Keppler and M. Jarosz, *J. Chromatogr. A*, 2008, **1192**, 323–326.

40 M. Groessl, C. G. Hartinger, K. Polec-Pawlak, M. Jarosz and B. K. Keppler, *Electrophoresis*, 2008, **29**, 2224–2232.

41 A. Bytzek, K. Boeck, G. Hermann, S. Hann, B. K. Keppler, C. G. Hartinger and G. Koellensperger, *Metallomics*, 2011, **3**, 1049–1055.

42 S. S. Aleksenko, M. Matczuk, X. Lu, L. S. Foteeva, K. Pawlak, A. R. Timerbaev and M. Jarosz, *Metallomics,* 2013, **5**, 955–963.

43 M. Groessl, A. Bytzek and C. G. Hartinger, *Electrophoresis*, 2009, **30**, 2720–2727.

44 A. Bytzek, E. A. Enyedy, T. Kiss, B. K. Keppler and C. G. Hartinger, *Electrophoresis*, 2009, **30**, 4075–4082.

45 M. Groessl, M. Terenghi, A. Casini, L. Elviri, R. Lobinski and P. J. Dyson*, J. Anal. At. Spectrom*., 2010, **25**, 305–313.

46 T. Jakusch, D. Hollender, E. A. Enyedy, C. Sánchez González, M. Montes-Bayón, A. Sanz-Medel, J. Costa Pessoa, I. Tomaz and T. Kiss, *Dalton Trans.*, 2009, 2428–2437.

47 T. Iglesias-González, C. Sánchez-González, M. Montes-Bayón, J. Llopis-González and A. Sanz-Medel, *Anal. Bioanal. Chem*., 2012, **402**, 277–285.

48 A. R. Timerbaev, K. Pawlak, S. S. Aleksenko, L. S. Foteeva, M. Matczuk and M. Jarosz, *Talanta*, 2012, **102**, 164–170.

49 S. Hann, T. Falta, K. Boeck, M. Sulyok and G. Koellensperger, *J. Anal. At. Spectrom*., 2010, **25**, 861–866.

50 C. Møller, H. S. Tastesen, B. Gammelgaard, I. H. Lambert and S. Stürup, *Metallomics*, 2010, **2**, 811–818.

51 C.-H. Tang, C. Parham, E. Shocron, G. McMahon and N. Patel, *Cancer Chemother. Pharmacol*., 2011, **67**, 1389–1400.

52 A. E. Egger, C. Rappel, M. A. Jakupec, C. G. Hartinger, P. Heffeter and B. K. Keppler, *J. Anal. At. Spectrom*., 2009, **24**, 51–61.

53 P. Heffeter, K. Böck, B. Atil, M. A. R. Hoda, W. Körner, C. Bartel, U. Jungwirth, B. K. Keppler, M. Micksche, W. Berger and G. Koellensperger, *J. Biol. Inorg. Chem*., 2010, **15**, 737–748.

54 C. Bartel, A. K. Bytzek, Y. Y. Scaffidi-Domianello, G. Grabmann, M. A. Jakupec, C. G. Hartinger, M. Galanski and B. K. Keppler, *J. Biol. Inorg. Chem*., 2012, **17**, 465–474.

55 D. Prashar, Y. Shi, D. Bandyopadhyay, J. C. Dabrowiak and Y.-Y. Luk, *Bioorg. Med. Chem. Lett*., 2011, **21**, 7421–7425.

56 D. A. Wolters, M. Stefanopoulou, P. J. Dyson and M. Groessl, *Metallomics*, 2012, **4**, 1185–1196.

57 L. Côrte-Real, A. P. Matos, I. Alho, T. S. Morais, A. I. Tomaz, M. H. Garcia, I. Santos, M. P. Bicho, and F. Marques. *Microsc. Microanal*., 2013, **19**, 1122–1130.

58 A. Grau-Campistany, A. Massaguer, D. Carrion-Salip, F. Barragán, G. Artigas, P. López-Senín, V. Moreno and V. Marchán, *Mol. Pharmaceutics*, 2013, **10**, 1964–1976.

59 Z. A. Carneiro, J. C. B. de Moraes, F. P. Rodrigues, R. G. de Lima, C. Curti, Z. N. da Rocha, M. Paulo, L. M. Bendhack, A. C. Tedesco, A. L. B. Formiga and R. S. da Silva, *J. Inorg. Biochem*., 2011, **105**, 1035–1043.

60 E. Vergara, E. Cerrada, C. Clavel, A. Casini and M. Laguna, *Dalton Trans*. 2011, **40**, 10927–10935.

61 K. Y. Zhang, H.-W. Liu, T. T.-H. Tong, X.-G. Chen and K. K.-W. Lo, *Inorg. Chem*., 2010, **49**, 5432–5443.

62 S. P.-Y. Li, H.-W. Liu, K. Y. Zhang and K. K.-W. Lo, *Chem. Eur. J*., 2010, **16**, 8329–8339.

63 P.-K. Lee, W. H.-T. Law, H.-W. Liu and K. K.-W. Lo, *Inorg. Chem*., 2011, **50**, 8570–8579.

64 S. P.-Y. Li, T. S.-M. Tang, K. S.-M. Yiu and K. K.-W. Lo, *Chem. Eur. J*., 2012, **18**, 13342–13354.

65 C.-C. Chang, C.-N. Chen, W.-L. Liao, T.-Y. Lo, S.-L. Lei and F.-D. Mai, *Surf. Interface Anal*., 2013, **45**, 251–254.

66 V. Nischwitz, J. T. Davies, D. Marshall, M. González, J. L. Gómez Ariza and H. Goenaga-Infante, *Metallomics,* 2013, **5**, 1685–1697.

67 X. Liu, N. Huang, H. Li, Q. Jin and J. Ji, *Langmuir*, 2013, **29**, 9138−9148.

68 J.-L. Bridot, D. Dayde, C. Rivière, C. Mandon, C. Billotey, S. Lerondel, R. Sabattier, G. Cartron, A. Le Pape, G. Blondiaux, M. Janier, P. Perriat, S. Roux and O. Tillement, *J. Mater. Chem*., 2009, **19**, 2328–2335.

69 Y. Ikeda, D. Kubota and Y. Nagasaki, *Colloid Polym. Sci*., 2013, **291**, 2959–2964.

70 S. Hong, S. Park, J. Park and J. Yi, *Colloids Surfaces B*, 2013, **112**, 415–420.

71 M. Ibrahim, F. Wang, M. Lou, G. Xie, B. Li, Z. Bo, G. Zhang, H. Liu and A. Wareth, *J. Biosci. Bioeng*., 2011, **112**, 570–576.

72 H. Chen, R. Ahn, J. Van den Bossche, D. H. Thompson and T. V. O'Halloran, *Mol. Cancer Ther*. 2009, **8**, 1955–1963.

73 A. R. Timerbaev, L. S. Foteeva, K. Pawlak and M. Jarosz, *Metallomics*, 2011, **3**, 761–764.

74 M. Matczuk, M. Prządka, S. S. Aleksenko, Z. Czarnocki, K. Pawlak, A. R. Timerbaev and M. Jarosz, *Metallomics*, 2014, **6**, 147–153.

75 C. L. King, S. Ramachandran, S. G. Chaney, L. Collins, J. A. Swenberg, K. E. de Krafft, W. Lin, L. Cicurel and M. Barbier, *Cancer Chemother. Pharmacol*., 2012, **69**, 665–677.

76 K. Yasui, H. Takashima, M. Miyagawa, K. Miyazawa, T. Ochiai, K. Mukaisho, H. Amaike, K. Ueda, J. Morikawa, E. Otsuji and T. Yoshikawa, *Hepatology Res*., 2013, **43**, 1093–1099.

77 J. Zhang, Y. Gong, X. Zheng, M. Yang and J. Cui, *Eur. J. Med. Chem*., 2008, **43**, 441–447.

78 J. Zhang, Y. Li, J. Sun, W. Li, Y. Gong, X. Zheng, J. Cui, R. Wang and J. Wu, *Eur. J. Med. Chem*., 2009, **44**, 4772–4777.

79 T. Tippayamontri, R. Kotb, B. Paquette and L. Sanche, *Anticancer Res*., 2013, **33**, 3005–3014.

80 C. F. Harrington, R. C. Le Pla, G. D. D. Jones, A. L. Thomas and P. B. Farmer, *Chem. Res. Toxicol.*, 2010, **23**, 1313–1321.

81 D. García Sar, M. Montes-Bayón, E. Blanco González, L. M. Sierra, L. Aguado, M. A. Comendador, G. Koellensperger, S. Hann and A. Sanz-Medel, *Anal. Chem.*, 2009, **81**, 9553–9560.

82 D. García Sara, M. Montes-Bayón, E. Blanco González, L. M. Sierra Zapico and A. Sanz-Medel, *Chem. Res. Toxicol*., 2011, **24**, 896–904.

83 D. García Sara, L. Aguado, M. Montes Bayón, M. A. Comendador, E. Blanco González, A. Sanz-Medel and L. M. Sierra, *Mutation Res*., 2012, **741**, 81–88.

84 A. A. Hostetter, M. L. Miranda, V. J. DeRose and K. L. McFarlane Holman, *J. Biol. Inorg. Chem*., 2011, **16**, 1177–1185.

85 W. Zhang, Y. Ji, X. Wu and H. Xu, *ACS Appl. Mater. Interfaces*, 2013, **5**, 9856−9865.

86 Q. Su, Q. Liu, G. Luo, W. Liu, Y. Yao, Y. Wang and K. Bi, *Drug Develop. Ind. Pharmacol*., 2008, **34**, 472–477.

87 Q. Liu, X. Li, Q. Su, G. Luo, Y. Wang and W. Liu, *Arch. Pharm. Res*., 2009, **32**, 1621–1628.

88 K. A. Selting, X. Wang, D. L. Gustafson, C. J. Henry, J. A. Villamil, D. L. McCaw, D. Tate, M. Beittenmiller, C. Garnett and J. D. Robertson, *J. Vet. Intern. Med*., 2011, **25**, 909–915.

89 M. Pernot, T. Bastogne, N. P. E. Barry, B. Therrien, G. Koellensperger, S. Hann, V. Reshetov and M. Barberi-Heyob, *J. Photochem. Photobiol. B*, 2012, **117**, 80–89.

90 M. K. Chaffin, V. Fajt, R. J. Martens, C. E. Arnold, N. D. Cohen, M. O'Conor, R. J. Taylor and L. R. Bernstein, *J. Vet. Pharmacol. Therap*., 2010, **33**, 376–382.

91 C. Arnold, M. K. Chaffin, N. Cohen, V. R. Fajt, R. J. Taylor and L. R. Bernstein, *Am. J. Vet. Res.*, 2010, **71**, 1371–1376.

92 J. J. Hostynek, F. Dreher and H. I. Maibach, *Inflamm. Res*., 2010, **59**, 983–988.

93 N. Dewi, H. Yanagie, H. Zhu, K. Demachi, A. Shinohara, K. Yokoyama, M. Sekino, Y. Sakurai, Y. Morishita, N. Iyomoto, T. Nagasaki, Y. Horiguchi, Y. Nagasaki, J. Nakajima, M. Ono, K. Kakimi and H. Takahashi, *Biomed. Pharmacother*., 2013, **67**, 451–457.

94 J. M. Madeira, C. J. Renschler, B. Mueller, S. Hashioka, D. L. Gibson and A. Klegeris, *Life Sciences*, 2013, **92**, 1072–1080.

95 D. Pozebon, V. L. Dressler, A. Matusch and J. S. Becker, *Int. J. Mass Spectrom*., 2008, **272**, 57–62.

96 E. Moreno-Gordaliza, C. Giesen, A. Lázaro, D. Esteban-Fernández, B. Humanes, B. Cañas, U. Panne, A. Tejedor, N. Jakubowski and M. Milagros Gómez-Gómez, *Anal. Chem*., 2011, **83**, 7933–7940.

97 E. White, A. Bienemann, J. Pugh, E. Castrique, M. Wyatt, H. Taylor, A. Cox, C. McLeod and S. Gill, *J. Neurooncol*., 2012, **108**, 77–88.

98 D. Gholap, J. Verhulst, W. Ceelen and F. Vanhaecke, *Anal. Bioanal. Chem*., 2012, **402**, 2121–2129.

99 O. Reifschneider, C. A. Wehe, I. Raj, J. Ehmcke, G. Ciarimboli, M. Sperling and U. Karst, *Metallomics,* 2013, **5**, 1440–1447.

100 O. Reifschneider, C. A. Wehe, K. Diebold, C. Becker, M. Sperling and U. Karst, *J. Anal. At. Spectrom.*, 2013, **28**, 989–993.

101 C. Herdering, C. A. Wehe, O. Reifschneider, I. Raj, G. Ciarimboli, K. Diebold, C. Becker, M. Sperling and Uwe Karst, *Rapid Commun. Mass Spectrom*., 2013, **27**, 2588–2594.

102 Y.-K. Hsieh, H.-A. Hsieh, H.-F. Hsieh, T.-H. Wang, C.-C. Ho, P.-P. Lin and C.-F. Wang, *J. Anal. At. Spectrom*., 2013, **28**, 1396–1401.

103 V. Ip, M. J. McKeage, P. Thompson, D. Damianovich, M. Findlay and J. J. Liu, *J. Anal. At. Spectrom*., 2008, **23**, 881–884.

104 J. Boertz, L. M. Hartmann, M. Sulkowski, J. Hippler, F. Mosel, R. A. Diaz-Bone, K. Michalke, A. W. Rettenmeier and A. V. Hirner, *Drug Metab. Dipos*., 2009, **37**, 352–358.

105 D. G. Filatova, I. F. Seregina, K. B. Ossipov, L. S. Foteeva, V. V. Pukhov, A. R. Timerbaev and M. A. Bol'shov, *J. Anal. Chem*., 2013, **68**, 106–111.

106 O. I. Ornatsky, X. Lou, M. Nitz, S. Schäfer, W. S. Sheldrick, V. I. Baranov, D. R. Bandura and S. D. Tanner, *Anal. Chem*., 2008, **80**, 2539–2547.

107 A. J. Managh, S. L. Edwards, A. Bushell, K. J. Wood, E. K. Geissler, J. A. Hutchinson, R. W. Hutchinson, H. J. Reid and B. L. Sharp, *Anal. Chem*., 2013, **85**, 10627–10634.

108 G. D. Kenny, A. S. Bienemann, A. D. Tagalakis, J. A. Pugh, K. Welser, F. Campbell, A. B. Tabor, H. C. Hailes, S. S. Gill, M. F. Lythgoe, C. W. McLeod, E. A. White and S. L. Hart, *Biomaterials*, 2013, **34**, 9190–9200.

109 C.-N. Tsang, J. Bianga, H. Sun, J. Szpunar and R. Lobinski, *Metallomics,* 2012, **4**, 277–283.

110 E. Moreno-Gordaliza, D. Esteban-Fernández, C. Giesen, K. Lehmann, A. Lázaro, A. Tejedor, C. Scheler, B. Cañas, N. Jakubowski, M. W. Linscheid and M. M. Gómez-Gómez, *J. Anal. At. Spectrom.*, 2012, **27**, 1474–1483.

111 W. Brüchert, R. Krüger, A. Tholey, M. Montes-Bayón and J. Bettmer, *Electrophoresis*, 2008, **29***,* 1451–1459.

112 F. Séby, C. Gleyzes, O. Grosso, B. Plau and O. F. X. Donard, *Anal. Bioanal. Chem*., 2012, **404**, 2939–2948.

113 S. Nita, A. A. Andries, L. Patron, R. Albulescu, F. Radulescu, C. Tanase and M. Vintila, *Rev. Chim*., 2011, **62**, 549–553.

114 A. Mignot, C. Truillet, F. Lux, L. Sancey, C. Louis, F. Denat, F. Boschetti, L. Bocher, A. Gloter, O. Stéphan, R. Antoine, P. Dugourd, D. Luneau, G. Novitchi, L. Carlos Figueiredo, P. Cesar de Morais, L. Bonneviot, B. Albela, F. Ribot, L. Van Lokeren, I. Déchamps-Olivier, F. Chuburu, G. Lemercier, C. Villiers, P. N. Marche, G. Le Duc, S. Roux, O. Tillement and P. Perriat, *Chem. Eur. J*., 2013, **19**, 6122–6136.

115 J. Tang, S. Wang, L. Liu, C. Wang and T. Xi, *Wuji Huaxue Xuebao*, 2012, **29**, 1025−1030.

116 Y. Shi, J. Goodisman and J. C. Dabrowiak, *Inorg. Chem*., 2013, **52**, 9418−9426.

117 S. S. S. J. Ahmed and W. Santosh, *PLoS One*, 2010, **5**, e11252.

Figure captions

Fig. 1 General scheme of sample preparation for measuring the distribution of a metallodrug in different blood fractions by ICP-MS. Centrifugation of the blood (with the outcome of erythrocytes in a residue) results in the plasma as a supernatant, which is then ultrafiltrated to obtain protein-bound drug fraction (IIa) and free drug in ultrafiltrate (IIb). The stream width (corresponding to Sankey diagram) is proportional to a typical amount of metal in each fraction. Adapted with permission from ref. 10.

Fig. 2 2-D ICP-MS chromatograms of fetal calf serum ex vivo incubated with cisplatin. Anion-exchange chromatograms shown in the upper insets were obtained on-line for the corresponding SEC fraction. Peaks were identified as cisplatin adducts with PB1 – albumin dimer; PB2 – transferrin, and PB3 – albumin. PB4 and PB5 are positively charged or neutral low-molecular Pt species. Adapted with permission from ref. 49.

Fig. 3 Comparative performance of (A) two- and (B) one-dimensional SEC–ICP-MS in elucidating ruthenium–protein binding patterns in cytosolic fractions of ruthenium drug-treated cancer cells. Note that the latter system could not differentiate the high molecular weight (HMW) fraction. Reproduced with permission from ref. 53.

Fig. 4 Electropherograms illustrating the formation of low-molecular-mass species of Ru (peaks 1–3) upon incubation of a Ru drug–transferrin adduct with (A) ascorbic acid and (B) glutathione at varying times. Reproduced with permission from ref. 42.

Fig. 5 HPLC–ICP-MS chromatogram of the Pt–DNA intrastrand adduct from leukocytes of patients received cisplatin. The response is for ^{195}Pt. Reproduced with permission from ref. 80.

Fig. 6 High resolution LA–ICP-MS image for ^{195}Pt monitoring on kidney sections of a rat treated with cisplatin. Reproduced with permission from ref. 96.

Fig. 7 Schematic of the LA-ICP-MS/atmosphere pressure chemical ionization-MS setup. Reproduced with permission from ref. 101.

Table 1 Application of ICP-MS techniques for preclinical metallodrug development

Step	Technique(s)	Information on drug candidate obtained
Evaluation of drug-like properties	ICP-MS CE–ICP-MS HPLC–ICP-MS	Solubility, lipophilicity, *etc*. Stability
Metabolite profiling	HPLC–ICP-MS CE–ICP-MS	Metal speciation in blood plasma; determination of major metabolites
Reactivity and affinity toward plasma proteins	CE–ICP-MS HPLC–ICP-MS ICP-MS	Data on binding kinetics and equilibrium, as well as composition (stoichiometry) of protein–drug adducts
Cell uptake and distribution assessment	ICP-MS	Cellular accumulation and uptake rate; distribution between different cell fractions
Cell processing investigations	ICP-MS CE–ICP-MS	Identification of possible active metallic forms and target molecular entities; quantification of the degree of target metallation
Pharmacokinetic studies	ICP-MS	Pharmacokinetic characteristics
Tissue distribution measurements	ICP-MS LA–ICP-MS	Metal content in different tissues and organs; 2-D distribution mapping; drug accumulation and long-term retention
Quality control of pharmaceutical forms	HPLC–ICP-MS	Active drug and impurity(ies) content

Table 2 Outline of investigational metallodrugs characterized by ICP-MS with regard to interaction with blood plasma or plasma proteins

Drug[a]	Plasma (protein)	Binding information	Ref.
Ultrafiltration			
(*SP*-4-2)- and (*SP*-4-1)-dihalidobis(2-propanone) oxime-κ*N*)platinum(II)	Human plasma, albumin	Albumin binding kinetics and constants, degree of binding to albumin and total serum proteins	33
cis-Diammine(1,1-cyclobutane dicarboxylate)platinum(II)	Dog plasma	Unbound drug fraction as a function of time after administration	34
N,*N*,*N*′,*N*′,*N*″,*N*″-hexakis(2-pyridylmethyl)-1,3,5-tris(aminomethyl)benzene-trichlotidotriplatinum(II) perchlorate	Albumin	Binding kinetics	35
Tris(8-quinolinolato)gallium(III)	Human plasma, albumin, transferrin	Protein binding kinetics and constants, degree of binding to individual and total serum proteins	36
Tris(1,10-phenanthroline)tris(thiocyanato-κ*N*)lanthanum(III)	Human plasma	Degree of binding	37
Aquatrichloridobis(1,10-phenanthroline)cerium(III)	Human plasma	Degree of binding	38
CE			
Sodium and indazolium *trans*-[tetrachloridobis(1*H*-indazole)ruthenate(III)]	Albumin, apo-transferrin	Binding kinetics	39
Indazolium *trans*-[tetrachloridobis(1*H*-indazole)ruthenate(III)]	Human serum and plasma, albumin, transferrin	Binding kinetics, degree and stoichiometry (for albumin)	40

Sodium *trans*-[tetrachloridobis(1*H*-indazole)ruthenate(III)]	Mouse plasma	Binding stoichiometry (to albumin)	41
Indazolium *trans*-[tetrachloridobis(1*H*-indazole)ruthenate(III)]	Holo-transferrin	Binding kinetics	42
Tris(8-quinolinolato)gallium(III), gallium(III) nitrate	Albumin, transferrin	Degree of binding	43
Tris(8-quinolinolato)gallium(III)	Human plasma, albumin, apo-transferrin, transferrin	Binding kinetics	20
Bis(maltolato)-, bis(2-picolinato)- and bis(2,6-dipicolinato)zinc(II)	Human plasma, albumin, apo-transferrin	Degree of binding	44
HPLC			
(*OC*-6-43)-bis(acetato)amminedichlorido-(cyclohexylamine)platinum(IV), (*SP*-4-2)-amminedichlorido-(cyclohexylamine)platinum(II)	Human plasma	Binding kinetics for irreversibly bound drugs and degree of binding for reversible binding	28
Dichlorido(η^6-toluene)(1,3,5-triaza-7-phosphaadamantane)ruthenium(II)	Albumin, apo-transferrin, holo-transferrin,	Degree of binding as a function of drug-to-protein ratio	45
Bis(maltolato)-, bis(2-picolinato)-, bis(1,2-dimethyl-3-hydroxy-4(1*H*)-pyridinone)oxovanadium(IV), vanadyl sulfate	Human serum, apo-transferrin	Preferential binding to transferrin	46
Bis(maltolato)oxovanadium(IV)	Rat serum	Exclusive binding to transferrin	47

[a] Names as stated in original papers.

Table 3 Selection of investigational metal-based drugs and diagnostic agents and their pharmacokinetic parameters determined by ICP-MS

Drug[a]	Expected clinical use	Sample	LOD / LOQ	Main pharmacokinetic characteristics	Ref.
cis-3,5-Diisopropylsalylic cyclohexanodiaminoplatinum(II) (saliplatin)	Antitumor chemotherapy	Rabbit plasma	0.4 μg L^{-1} / 1.0 μg L^{-1}	Initial concentration; area under concentration–time curve; elimination half-life; clearance; mean retention time; volume of distribution	86
cis-3,5-Diisopropylsalylic cyclohexanodiaminoplatinum(II) (saliplatin)	Antitumor chemotherapy	Rat plasma	0.01 μg L^{-1} / 0.03 μg L^{-1}	Area under concentration–time curve; elimination half-life; clearance; volume of distribution	87
cis-Diammine(1,1-cyclobutane dicarboxylate)platinum(II) (dicycloplatin)	Antitumor chemotherapy	Rat plasma, dog plasma and plasma ultrafiltrate	- / 1.0 μg L^{-1}	Time to reach the peak plasma concentration; peak plasma concentration; area under concentration–time curve; clearance; mean retention time	34
(*OC*-6-43)-bis(acetato)amminedichlorido-(cyclohexylamine)platinum(IV) (satraplatin)	Antitumor chemotherapy	Dog plasma and plasma ultrafiltrate	0.003 μg L^{-1} / 0.01 μg L^{-1}	Time to reach the peak plasma concentration; peak plasma concentration; area under concentration–time curve; clearance	88
Dichlorido(η^6-*p*-cymene)(5-(3-pyridyl)-10,15,20-triphenylporphyrin)ruthenium(II),	Antitumor photodynamic therapy	Mice plasma	0.3 μg kg^{-1} / -	Elimination half-life and constant; mean retention time	89

octachloridotetra(η^6-*p*-cymene)(5,10,15,20-tetra(3-pyridyl)porphyrin)tetraruthenium(II)					
Gallium(III) maltolate	Anti-pneumonic chemotherapy	Horse serum	0.5 μg L^{-1} / 1.5 μg L^{-1}	Time to reach the peak serum concentration; peak serum concentration; area under concentration–time curve; elimination half-life; volume of distribution	90, 91
Diacetate(glycyl-L-histidyl-L-lysine) copper(II)	Anti-inflammatory chemotherapy	Receptor liquid after diffusion through skin tissue	- / 1.0 μg L^{-1}	Skin permeability coefficient, flux at steady state	92

[a] Given in parentheses is the trivial name.

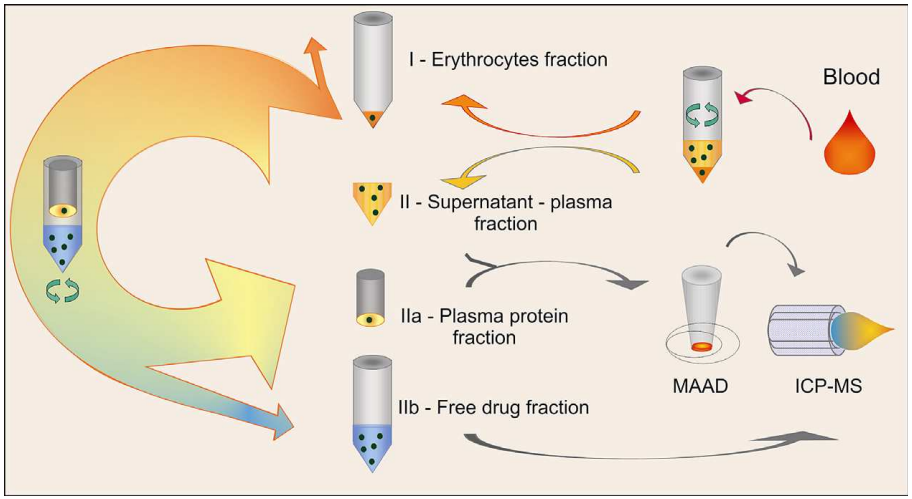


Fig. 1

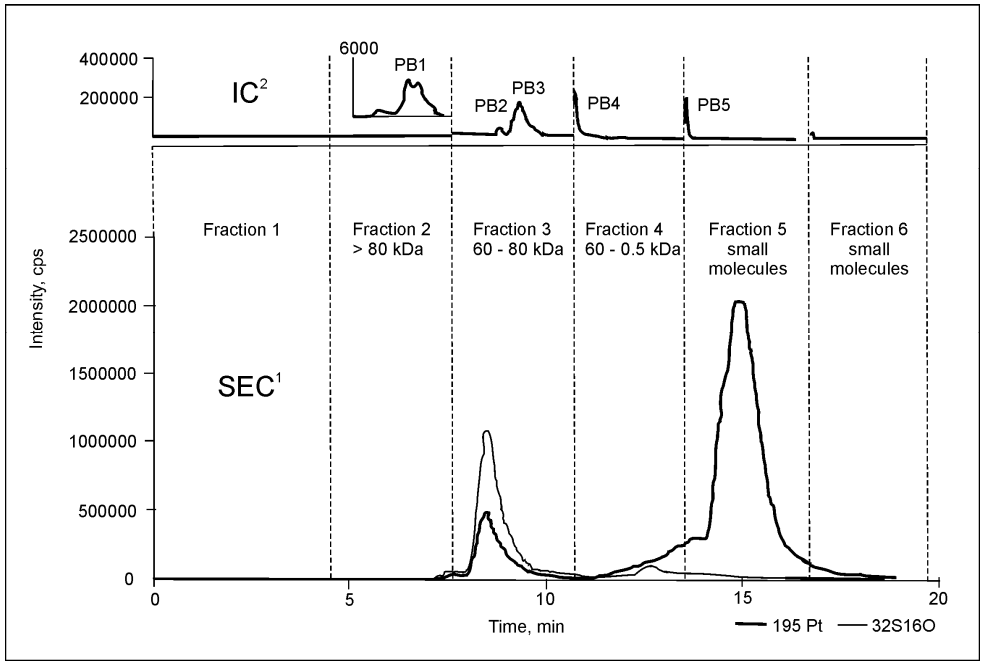


Fig. 2

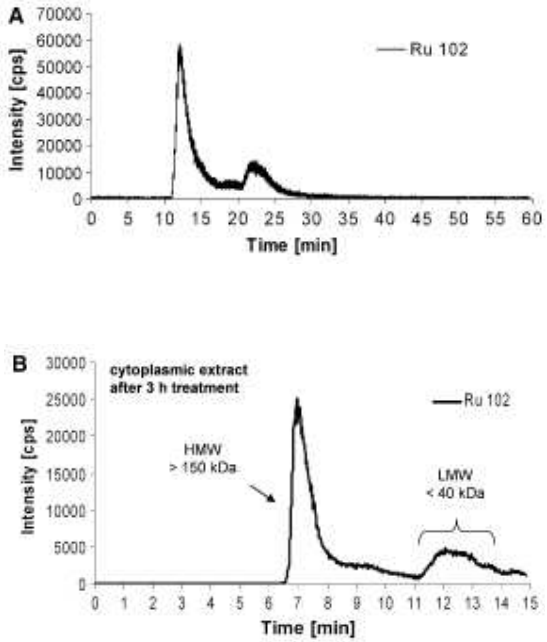


Fig. 3

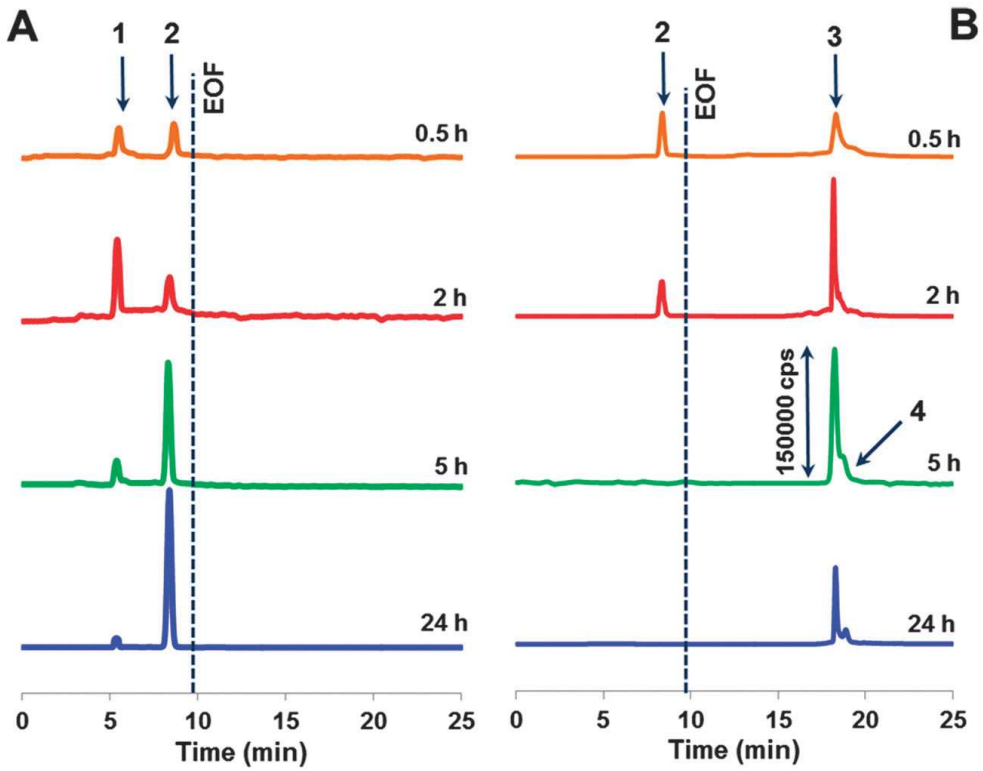


Fig. 4

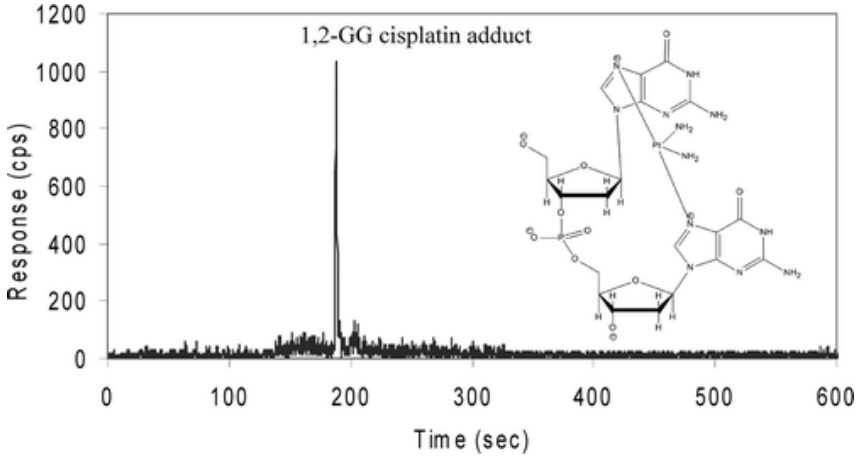


Fig. 5

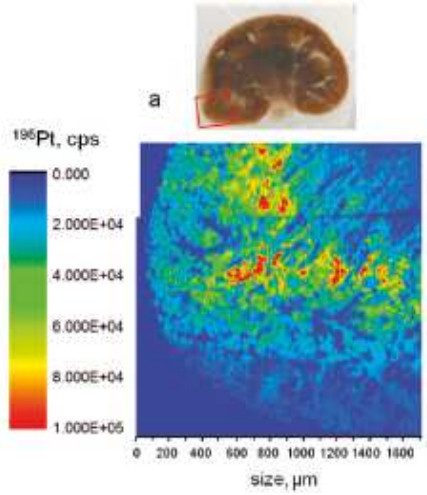


Fig. 6

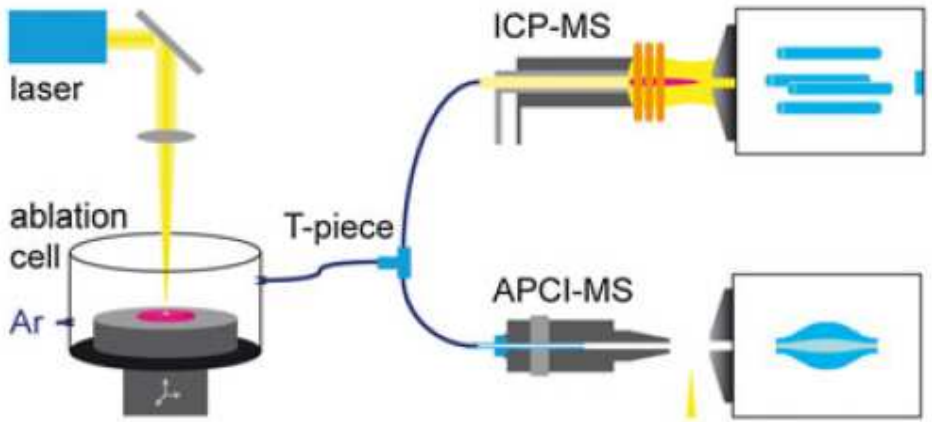
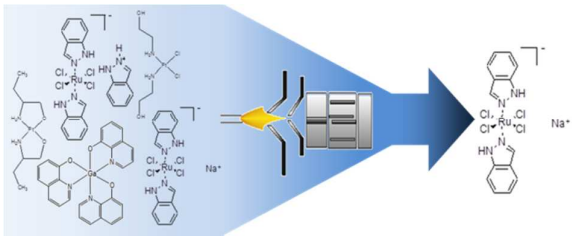


Fig. 7



Critical analysis of current capabilities, limitations, and developmental trend of ICP-MS applied to the development of metal-based medicines is conducted.