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Advances of CE-ICP-MS in speciation analysis related to metalloproteomics of anticancer drugs ☆

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ABSTRACT

The mode of action of metal-based anticancer drugs, including their accumulation in blood, transport, delivery to cancer cell, and cell processing (together with release of an active form and possibly targeting) is largely dependent on protein binding. Among analytical methods capable of providing a better understanding of metallodrug–protein interactions, capillary electrophoresis (CE) with inductively coupled plasma mass spectrometry (ICP-MS) detection is arguably a premier technique. Since its advent to the area of metallodrug proteomics in 2004 [1], the benefits of CE-ICP-MS became evident, stimulating further research and methodological developments. This hyphenated technique's merits comprise an ability to separate rapidly and efficiently the parent drug and protein-bound drug form(s), with no alteration of original speciation in the sample, to identify the metal-containing species due to specific ICP-MS response, to measure the binding parameters (e.g. rate and equilibrium constants), and finally to quantify the metal–protein adducts in real-world samples. This review is aimed on offering the reader a summary of applications of CE-ICP-MS to various metallodrug–protein systems, with a focus on experimental strategies in use for the assessment of binding reactivity and affinity, monitoring in vitro cellular transformations and serum binding profiles, and ex vivo metallodrug–proteomic analysis.

1. Introduction

In the recent years, capillary electrophoresis (CE) hyphenated with inductively coupled plasma mass spectrometry (ICP-MS) has become one of the preferred techniques for speciation analysis. This is the effect of a number of factors, including improvements in the methodology of interfacing CE and ICP-MS instruments, significant advances in the development of new approaches for the analysis of various element species in different types of samples, and the existence of a vast body of literature that supports understanding and implementation of this combined technique [2–5]. An area of great potential of CE-ICP-MS is identification and determination of different chemical species of an element in biological systems, where the method has attracted significant attention in the last decade [6–9]. A highly intensive, element-specific, and interference-free response provided by ICP-MS makes CE separations tailor-made to elucidating the essentiality, toxicity, and in general, biological functions of trace elements. Furthermore, when such an element is an active drug component, as, for instance, platinum in the case of platinum-based anticancer drugs, CE-ICP-MS appears to be one of few analytical tools that enable a better knowledge about the destiny of the drug in the body and its mechanism of action at the molecular level [10,11]. In this field of biospeciation analysis, CE offers an attractive alternative to HPLC-based speciation schemes. With the renowned capability of CE in the analysis of metal species [12,13], this platform does not only provide superior separation efficiency within shorter time, simplicity in separation hardware, and lower consumption of sample and reagents but more importantly, it minimizes an impact on the equilibrium between different metal species existing in a given model system or a real biological sample. An inferior sensitivity of CE compared to HPLC seems to be no great analytical challenge, as the administered levels of metallodrugs are fairly high to deal with in the direct analysis of clinical samples, even using quadrupole ICP-MS.

Abbreviations: BGE, Background electrolyte; CE, Capillary electrophoresis; EOF, Electro osmotic flow; ICP-MS, Inductively coupled plasma mass spectrometry

☆ Dedicated to Professor Yuri A. Zolotov on the occasion of his 80th birthday.

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Reactions with various biological molecules in the human body indisputably play a key role in metabolism and therapeutic action of metal-based drugs. Many such interactions, particularly those with proteins as binding partners, have been evaluated in recent CE-ICP-MS research, as discussed below. Interest in these studies stems from the well-known fact that the rate and degree of the transformation upon binding toward serum proteins regulate the cellular uptake and accumulation of the drug in tumor tissue and determine differences in efficacy, activity and toxicity, as well as the overall distribution and the excretion of individual drugs [14,15]. There exist different ways how we can investigate this important component of preclinical development of anticancer metallodrugs by CE-ICP-MS. The binding behavior can be assessed (and compared) kinetically, in terms of rate constants. Next or in parallel, it is essential to analyze the binding equilibrium, with the aim of determining the association (binding) constant and/or the number of protein sites participating in drug binding, i.e. its stoichiometry. Such information would afford a more faithful interpretation of protein-mediated metabolism of anticancer agents after intravenous administration or after the uptake of an oral drug in the bloodstream. Stability of the protein–drug adducts in the presence of various bioligands and /or reductants at simulated extracellular or intracellular conditions or, oppositely, their inclination to respective speciation changes is another important line of CE-ICP-MS research. Using such in vitro screening, one may gain an insight into biotransformations accompanying the storage and delivery of metallodrugs, their intracellular activation, and interaction with drugable cell targets. Evolution of the protein–drug conjugates under real serum or plasma circumstances can be monitored directly, taking advantage of relative freedom of CE from proteinaceous sample matrices (compared to HPLC). There is no need to emphasize that experiments with blood constitutions give a more inclusive picture of the in vivo state. In situations when a specific protein-bound form dominates drug's speciation in blood (or its fractions), the drug of interest can be subject to quantification through measuring metal loading of the protein adduct, e.g. via drug/protein ratios. This actually moves CE-ICP-MS forward to the realm of quantitative biospeciation analysis.

In the following sections of the present review the aforementioned research domains will be critically scrutinized, after a brief consideration of advanced separation/detection methodology used in metallodrug–protein studies. Challenges that remain in applying CE-ICP-MS in investigations of protein-binding behavior of metallodrugs and future steps to be taken toward method's betterment for further narrowing the gaps in our understanding of the roles of proteins in the mechanism of action of metal-based anticancer drugs will also be brought into focus.

2. CE-ICP-MS methodology in metallodrug proteomics

2.1. Sample preparation

It makes no sense to accentuate that the interaction of metal-based drugs with serum transport proteins should be assessed under conditions as close as possible to extracellular physiological environment. Otherwise, all binding data acquired turn to be simply irrelevant. Therefore, the binding experiments are typically carried out in incubation solution comprising 10 mM phosphate buffer [1,16–18,21–24] or 4 mM disodium hydrogenphosphate–25 mM sodium bicarbonate buffer [19] at pH 7.4 and 100 mM NaCl (at 37 °C). Given the total salt concentration (about 0.154 M) and a pH of 7.43 in human plasma, these buffers appear to be a good choice to simulate a real-world situation. However, the carbonate-containing buffer was found to be unstable to perform prolonged binding [17]. It should also be mentioned that maintaining such ionic strength within the background electrolyte (BGE) presents a challenging problem when using ICP-MS detection (see below).

The initial concentration of proteins is commonly kept at 5×10^{-5} M, which approximates satisfactorily the physiological level for transferrin (3.5×10^{-5} M) but about ten times lower than the actual concentration of albumin (ca. 6×10^{-4} M). Notably, serum proteins other than albumin and apo-transferrin (hereafter referred to as transferrin) have not been examined (until a very recent attempt on holo-transferrin [20] and a single report exploring a partially Fe(III)-loaded transferrin [19]). A reasonable explanation is behind their relative abundance and pertinent transport functions. The drug concentration in the incubated mixture is varied from a 1:1 to 20:1 M ratio to the protein, being a reasonable estimate of the factual drug-to-protein proportion at the stage of intravenous administration (or entering the bloodstream). In most cases, this concentration range also guarantees the complete (equilibrium) binding conditions.

In case of real serum or plasma samples, their treatment should be performed in a gentle way so that the integrity of the protein–drug adducts is not deteriorated. This is accomplished by a moderate dilution (10-fold at maximum) with the physiological buffer [21,22] or water [23].

2.2. Separation

Ideally, the BGE composition is to be the same as incubation solution (or blood electrolyte composition). This is believed to preserve the protein-bound analytes of scrutiny against possible changes in speciation in a CE system and in addition, minimizes electrodispersion effects. However, high-salt electrolytes disturb the coupling to ICP-MS by producing unstable spraying conditions and frequently clogging the nebulizer by deposits. Next, the electric current may reach a value unacceptable to maintain stable performance of the interface. Moreover, use of simulated physiological buffer solution may lead to poor resolution, low intensity of adduct response, and lack of signal repeatability [16]. Therefore, neutral BGEs void of sodium chloride have been used in most of metallodrug–protein studies by CE-ICP-MS, with the preference given to phosphate buffer electrolytes (10–15 mM) [1,16–18], using bare fused-silica capillaries. These BGEs ensure good resolution of the target species (including adducts of different proteins) [16] and rather high electroosmotic flow (EOF) to transport them rapidly past the detector, as can be seen in Fig. 1. Another BGE based on a 10 mM ammonium carbonate solution showed less favorable separation ability [19]. Occasionally, a cationic surfactant is added to the BGE accompanied by polarity reversal of the power supply [16]. This favors the separation of different protein adducts (by virtue of ion-pairing interaction and possibly micellar partitioning effects) and improves the recovery of metal species from the capillary. For hydrophobic, oral metal-based drug, displaying high affinity to fused-silica capillary walls and hence poor recovery, a zwitterionic separation electrolyte of Good's buffer type was found more suitable than commonly used phosphate buffer system [23]. The rationale of employing such BGEs is that they can be applied at high concentration (without increasing the current) so that analyte adsorption is competitively suppressed.

When analyzing serum and plasma samples, protein adsorption onto the surface of uncoated capillary wall can also be overwhelming. The use of capillaries semi-permanently coated with a cationic polymer (hexadimethrine bromide) was proposed to eliminate this problem [21,22]. The coating led to a positively charged surface, a reversal of the EOF, and consequently, to a change of voltage polarity and requiring acidic BGE conditions.

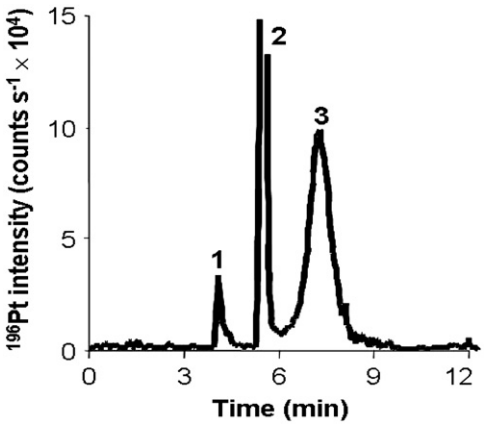

Fig. 1. Separation of platinum species induced by interaction of cisplatin with human serum albumin. BGE, 15 mM phosphate buffer, pH 7.4. Sample: cisplatin (1×10^{-3} M) and albumin (5×10^{-5} M) after 12 h of incubation. Peaks: (1) cis-diammineaquachloroplatinum(II); (2) cisplatin; (3) cisplatin–albumin adduct. Reprinted from [1], with permission.

While such separation system proved to be robust, non-physiological electrolyte pH (<3) may be detrimental for species quantification.

It is worthwhile of noting that despite the fact that a matrix-matching selection of BGE is not feasible in CE-ICP-MS, disequilibrium effects are most likely not the case. Even though electric field-induced dissociation of the protein–drug adducts might take place, the protein acting as a polydentate macroligand would not leave the sample zone throughout a CE run and hence keep up the complexation conditions. This consideration is due to very similar mobilities of free protein and its metal-bound form (i.e. the adduct) and in accord with a theoretical treatment on the role of disequilibrium effects in metal-speciation studies by CE-ICP-MS [25].

2.3. Detection

In fact, there are no marked differences in instrumental and operational settings compared with common speciation analysis by CE-ICP-MS. Since the metal concentrations in model or real samples are fairly high and the respective metals have usually low background signals, all reviewed work was done using a quadrupole-based ICP-MS system. The mass spectrometer was interfaced by means of a commercial microconcentric nebulizer (from CETAC Technologies, Omaha, NE, USA) that is employed in most of current CE-ICP-MS research. Superior suitability of the CETAC interface (over a Mira Mist configuration available from Burgener Research Inc., Mississauga, Ontario, Canada) was confirmed in a purposely designed study [26], centered on the quantification of carboplatin in plasma incubations.

Diluted phosphate or bicarbonate buffer solutions are typically accommodated as a make-up liquid, with the pH matching the pH of both BGE and incubation solution (biofluid sample), i.e. pH 7.4. A diluted solution of NH_4NO_3 may be a good alternative to nonvolatile make-up electrolytes in view of possible problems of too much salt entering the ICP and nebulizer clogging (but with a certain risk of precipitation events) [16]. Signal quantifications are done in the peak-area mode by monitoring the total ion current of the major target metal isotopes. In some contributions [19,21–23], the traces of sulfur (^{34}S) or/and iron (^{57}Fe) isotopes (in the case of transferrin binding; see Fig. 5) are recorded simultaneously owing to the multi-elemental detection capability of ICP-MS. This allows for independent observation of the proteinaceous species (both unbound and bound), unambiguous identification of the latter ones, and quantification of the protein-bound drug fraction via the known sulfur content. However, because of strong isobaric interferences encountered when using low-resolution quadrupole ICP-MS, it is impossible to measure the most abundant sulfur (^{32}S) and iron (^{56}Fe) isotopes.

3. Kinetic characterization of protein binding

Species-friendly conditions of CE, particularly, no stationary phase involved in separation, and short analysis times are attributes that minimize alterations of the species under examination not associated with the binding process. This allows one to examine the kinetics of drug–protein interactions in a comparatively accurate way, also accounting for further development of the reaction during sample introduction and electrophoresis (until the drug and protein zones become spatially resolved). Otherwise, the reaction time can be taken as the period of time between the beginning of incubation and sample introduction (to exclude any effect of BGE on binding kinetics). The metal-specific nature of ICP-MS offers the possibility of distinguishing the signals of comigrating free and bound protein forms (in contrast to UV detection) and hence to monitor time-dependent concentration changes following the formation of the drug–protein adduct [1,16,18,21]. This is especially important in the case when the acquisition of kinetic information cannot be done as being based on the signal of unbound drug (because of its limited hydrolytic stability). To approach real-serum circumstances, the progress of drug interaction can be measured simultaneously for two major proteins, albumin and transferrin (at their physiological molar ratio [16,23] or concentrations [19]), until the entire drug became consumed and unevenly distributed between different protein fractions.

Apparent binding rate constant (k) is calculated from the adduct response–time plot, assuming the first-order character of the binding reaction:

$$\ln\frac{S_{drug}^{bound}}{C_{drug}^{0}} = -kt \quad (1)$$

where S_{drug}^{bound} is the adduct signal (or concentration) and C_{drug}^{0} is the initial concentration of the drug. To improve measurement precision and to ensure the comparability of results from different days, the peak area response of the monitored isotopes is normalized with respect of the abundance of one of metal isotopes and additionally with the total ion current of the internal standard (e.g., ^{72}Ge added to the make-up liquid). In view of the fact that the time required to complete a single run is no less than 15 min and cannot be reduced without impairing the quality of the separations, the points needed for establishing a representative kinetic profile can be obtained from several independent experiments.

Typical binding curves recorded at varied drug concentrations are shown in Fig. 2. Note that the authors built up these dependences by taking the number of metal-containing moieties attached to the protein, n, as a binding parameter. In this way, binding equilibrium can also be characterized at incubation time sufficient to reach the equilibrium state [1,16] (see the next section for more detail). Some kinetic trials have gone beyond assessment of kinetic binding constants (for the reason of extremely fast [19,23] or multiple [23] adduct formation) but revealed important aspects of comparative drug binding and selectivity toward a specific protein under physiological conditions.

Another important point to mention is that in all kinetic studies, the k values were acquired using the first-order rate expression, Eq. (1), for describing the kinetic changes of

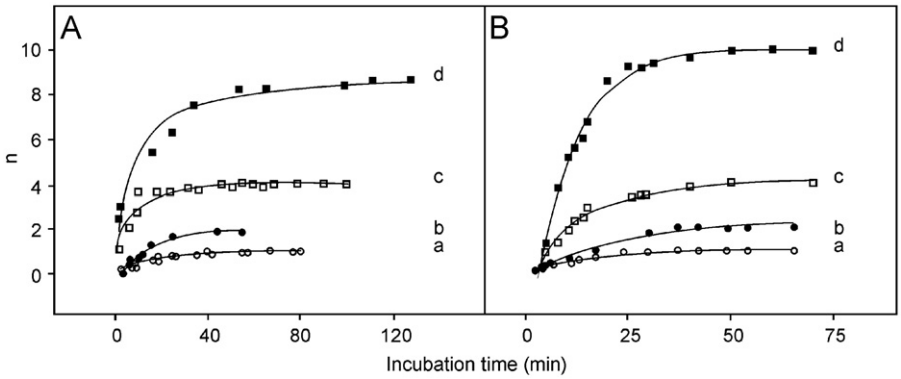

Fig. 2. Binding of indazolium trans-[tetrachloridobis(1*H*-indazole)ruthenate(III)] to (A) albumin and (B) transferrin at variable reaction time and drug/protein ratio: (a) 1:1; (b) 2:1; (c) 4:1 and (d) 10:1. Reprinted from [16], with permission.

Table 1
Summary of protein-binding information obtained by CE-ICP-MS for different metal-based drugs.

Drug	Protein	Binding data			Ref.
		k ($\times 10^5$, s^{-1})	K ($\times 10^3$, M^{-1})	n	
Cisplatin	Albumin	5.4	–	5[a]	[1]
(*SP*-4-2)-bis[(*R*)-(−)-2-aminobutanol)-dichloridoplatinum(II)	Albumin	2.2	–	–	[1]
(*SP*-4-2)-bis(4-aminobutanol)-dichloridoplatinum(II)	Albumin	3.9	–	–	[1]
Indazolium trans-[tetrachloridobis(1*H*-indazole)ruthenate(III)]	Albumin	110	10.6	8[a]	[16]
		53	–	–	[17]
		68	–		[21]
	Transferrin	290	5.6	10[a]	[16]
		155	–	–	[18]
	Serum	67	–	0.7	[21]
	Plasma	147	–	1.3	[21]
Sodium trans-[tetrachloridobis(1*H*-indazole)ruthenate(III)]	Albumin	53	–	–	[18]
	Transferrin	156	–	–	[18]

[a] At 10-fold drug excess.

protein-binding reaction. Strictly speaking, such treatment is only valid, when actual metal-to-protein ratios attain a 10-fold (or higher) excess which is not always the case (although minor deviations in k measured at different drug molar surpluses suggest the contrary [16]). Therefore, the kinetic data listed in Table 1 should be considered only for comparative estimation of the reactivity toward proteins, the efficiency of metallodrug transport or the speed of the deactivation of a drug by interactions with proteins. This option was intentionally demonstrated for two ruthenium-based drugs tested in the same kinetic study [18] that revealed similar binding kinetic behavior.

4. Analysis of binding equilibrium

As the subsequent step of a lead-drug selection program, the equilibrium binding information is to be acquired. Depending on the rate of adduct formation, protein–drug interaction can be analyzed using different CE approaches [11]. However, since anticancer metal-based drugs interacting with proteins are classified as binding inert (based on time scales of complete adduct formation relative to the time scale of a typical CE separation), the binding parameters can be measured only after the reaction attains the equilibrium. This prerequisite makes mandatory incubation of reaction mixture prior to analysis for a period of time determined from kinetic trials.

There are basically three types of equilibrium parameters that can be measured by CE-ICP-MS [1,16]. The number of active metal functionalities bound per protein molecule (n) is calculated from the following equation:

$$n = \frac{C^0_{drug}}{C^0_{protein}} \times \frac{S^{bound}_{drug}}{S^{free}_{drug} + S^{bound}_{drug}} \quad (2)$$

where $C^0_{protein}$ is the initial concentration of the protein while S^{free}_{drug} and S^{bound}_{drug} denote the signal responses of free and the protein-bound drug forms, respectively. It is important to mention that from the CE-ICP-MS experiments on evaluating n, one can judge the possibility of multiple drug binding and even manifesting a two-step binding process [1]. The conditional binding constant, K, is commonly determined utilizing the relationship between the protein-bound drug fraction (r) and the concentration of free drug, C^{free}_{drug}:

$$r = \frac{C^{bound}_{drug}}{C^0_{protein}} = \frac{nKC^{free}_{drug}}{1 + KC^{free}_{drug}} \quad (3)$$

at constant total concentration of the protein and varying C^0_{drug}. The drug bound to the protein, expressed as a percentage, is also an essential characteristic [1], as it illustrates the amount of the drug that may occur free in blood a prolonged time after administration.

Table 1 gives the selection of equilibrium binding parameters extracted from the experimental data obtained by CE-ICP-MS. While still limited in numbers, these data underscore the differences in binding affinity between various metallodrugs or investigational compounds as well as for different proteins. Note that in the case of simultaneously monitoring the sulfur isotope

(see Section 2.3), quantification of the metal loading, i.e. *n*, can also be done for real serum and plasma [21].

5. Probing the stability of protein–drug adducts

The fact that the protein-bound form is the major appearance of anticancer metallodrugs in the bloodstream does not necessarily mean that no transformation (i.e. by a redox route) or impair of the protein–drug adducts takes place until they are delivered to cancer cell. The mode in which the release and activation of a drug bound to protein(s) occurs in tumor tissue is another crucial research issue. This was the objective to establish the CE-ICP-MS methodology for in vitro screening the possible bioconversion accompanying drug delivery and cell processing.

Basically, there are two setups for monitoring the electrophoretic profiles of protein adducts in the presence of pertinent biological ligands and/or reductants at simulated extra- or intracellular conditions. One is based on the offline measurement of reaction kinetics when it proceeds outside a CE system. This implies that reaction components are present in the properly incubated sample and its aliquots are continuously taken for analysis to record the ICP-MS signal of the protein-bound metal species. Using this approach, it was found out that a promising anticancer drug, indazolium trans-[tetrachloridobis(1*H*-indazole) ruthenate(III)], bound to albumin or transferrin experienced no metal-speciation alterations under the action of ascorbic acid at its physiological concentration in blood [17]. Similarly, the ruthenium ion (within the appropriately complexed scaffold) did remain securely sequestered in the adduct, being unaffected by glutathione, ascorbic acid or citric acid, each tested at the cancer cytosol level [20]. However, one has to be aware of that ICP-MS detection technique is tentative of providing explicit information on the metal oxidation state (and consequently its possible change) in the protein adducts. On the other hand, the method affords a favorable means of recognition of alterations in their integrity by a mechanism of ligand exchange [25].

Another, in situ approach assumes that the development (if any) of the respective reaction can be followed when the equilibrium mixture, containing the adduct, is introduced into the capillary filled with the BGE which contains a relevant bioadditive. In this setup, the capillary is used not only as a separation chamber but also as microreactor, with benefits in that the reaction completely occurs within the capillary (without a contact with the atmosphere), there is no time delay from the time when reaction starts to the time of analysis of the reaction mixture, and the sample dilution is minimized. However, the reaction under scrutiny should be fast enough to notably proceed within the time scale of a typical CE run. This held true in the case when the same drug adduct with transferrin was in-line monitored using a BGE mimicking intracellular fluid of tumor cells with respect to the pH and sodium chloride concentration [24]. Fig. 3 depicts the results of this proof-of-principle study (cf. the behavior of transferrin and albumin adducts). In our opinion, the concept of a CE reactor permits a new frontier in the analysis of metallodrug–protein systems to be crossed, and it will be refined in our ongoing research to discover the route of a drug inside the cell and chemistry by which it targeted on cell structures.

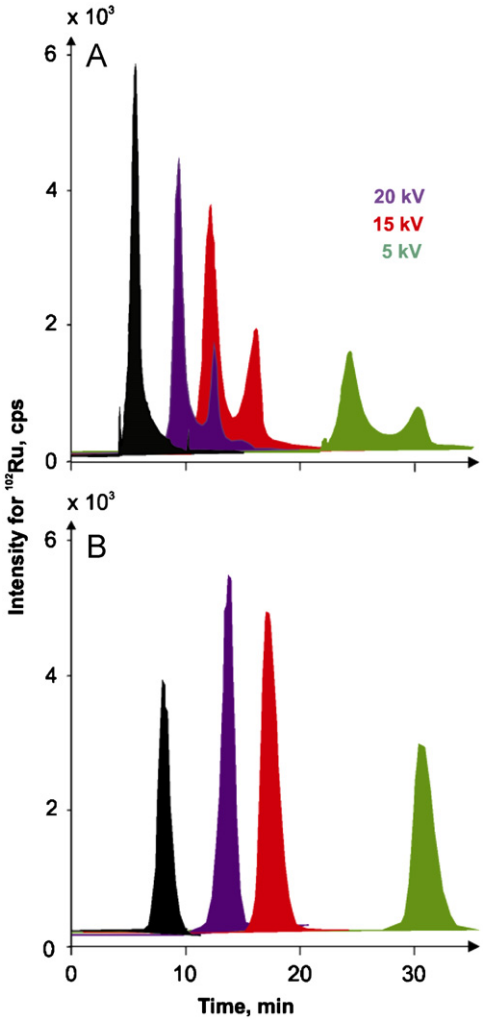

Fig. 3. CE-ICP-MS electropherograms revealing different stability of ruthenium-based drug adducts with (A) transferrin and (B) albumin in simulated cancer cytosol. BGE: 10 mM phosphate buffer, 4 mM NaCl, pH 6.0 (grew traces). Black traces show the same species recorded in 10 mM phosphate buffer with pH 7.4. Reprinted from [24], with permission.

6. Monitoring of drug transformations in serum and plasma

CE coupled to ICP-MS has a considerable potential of producing information on complex metal-speciation patterns resulting from biological samples [9]. Until recently, however, the method was limited to probing the protein-mediated speciation of metallodrugs using model compounds and simulated physiological conditions. In a pioneering study under real-biofluid environment [21], CE-ICP-MS has been applied to follow the formation of a Ru(III) drug–albumin adduct in human serum and plasma samples. From comparison of equilibrium binding profiles for whole serum and a serum from which the high-abundant proteins, albumin and immunoglobulin G, were depleted prior to incubation, it was found that only minor fractions of ruthenium are attached to other proteins than albumin. The same adduct dominates binding in plasma but surprisingly, the drug interacts about two times faster compared to serum or individual protein (see Table 1; data from [21]). However, it is a controversial matter whether such kinetic comparison can be made on the basis of *k* values. In the form in which it enters Eq. (1), the rate constant does take into account the occurrence of parallel reactions (with different blood proteins).

The time course of protein binding in human serum was also recorded for a gallium(III) drug [23]. As can be seen in Fig. 4, the peaks of two adducts are observed at concentrations above the limit of detection of the CE-ICP-MS technique (about 2×10^{-7} M). However, further characterization is required to delineate their identity.

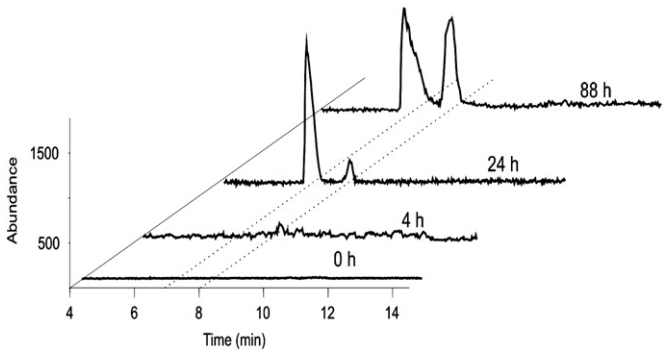

Fig. 4. Evolution of gallium–protein adducts during the incubation of tris(8-quinolinolato)gallium(III) in human serum. Serum was diluted 10 times when mixed with the drug. Reprinted from [23], with permission.

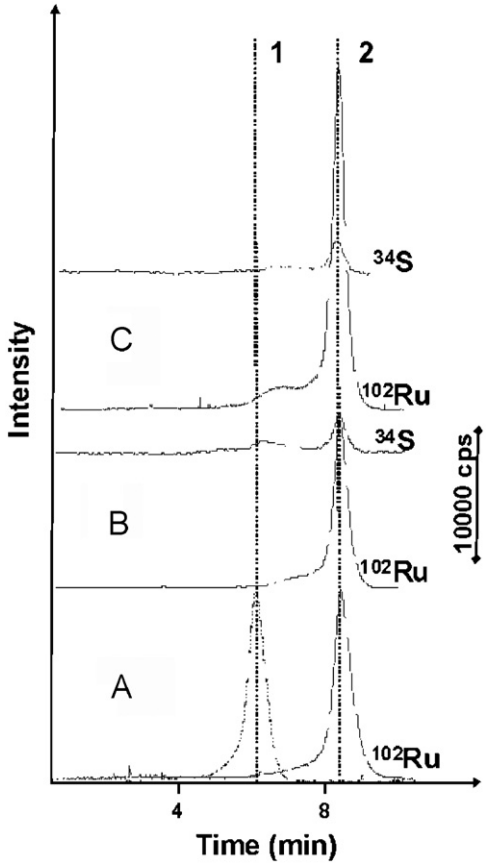

Fig. 5. CE-ICP-MS analysis of plasma samples taken on days (B) 2 and (C) 18 from the beginning of repeated intravenous drug administration. Trace A shows in vitro drug binding to transferrin and albumin (peaks 1 and 2, respectively). Reprinted from [21], with permission.

7. Quantification of protein–drug adducts in real samples

To further advance CE-ICP-MS methodology, the above-mentioned separation/detection conditions were implemented to analyze clinical samples from a cancer patient undergoing treatment with indazolium trans-[tetrachloridobis(1*H*-indazole)ruthenate(III)] during a clinical trial [21]. Fig. 5 demonstrates this first practical metallodrug–proteomic analysis. Monitoring drug/albumin ratios in samples taken at different time after subsequent drug infusions revealed pharmacokinetically significant changes in the amount of the albumin-bound Ru. A following contribution was aimed on characterization of the in vivo protein binding of a structurally analogous drug, sodium trans-[tetrachloridobis(1*H*-indazole)ruthenate(III)], in mouse plasma [22]. The results showed a similar drug speciation, with the majority of Ru being attached to albumin (as was quantified in terms of the molar Ru/S ratio) but other bound forms falling below method's limit of quantification.

It is important to point out that another common shortcoming of CE-ICP-MS, when intraday changes in sensitivity impel precision to go beyond acceptable level, could be more challenging in real biofluid analyses. In the above studies, an external standard was added to make-up solution to circumvent this problem. Alternative approach leading to improved reproducibility implies the addition of an internal standard directly to plasma samples [26].

8. Conclusions

CE with ICP-MS used as an on-line detector has emerged as a recognized analytical tool to map metallodrug–protein interactions. This review has highlighted how CE-ICP-MS, continually being developed in past few years to streamline the existing procedures, contributes in the development of metal-based anticancer drugs via shedding light on the fate of the drug upon intravenous administration or entering the bloodstream. Even at the present stage of its development, the method can be widely adopted in research and possibly clinical laboratories for the high-throughput screening and selection of drug candidates in the early phase of development, given that binding to transport proteins is often considered as a prerequisite for tumor-inhibiting activity.

However, being applied alone, CE-ICP-MS cannot tackle all the challenges of identification, localization, and quantification of drugs bound to proteins, especially at the level of natural abundance in biofluid samples. The future, therefore, points toward more widespread applications of multidimensional hyphenated methodology, in which CE-ICP-MS is synergistically supported from both detection and separation parts by CE-ESI-MS and HPLC coupled with elemental and molecular MS, respectively. Insofar, this approach has not become a common place [19,22]. We also anticipate much progress from a before-long implementation of advanced ICP-MS technology, using collision/reaction cells, isotope dilution techniques, and high-resolution mass spectrometers, that has already found a growing-in-importance niche in biospeciation analysis [9]. This would require a good deal of effort, taking into consideration a limited number of research groups working in the area under consideration. Still, there are undoubtedly grounds for optimism with respect to the practicability and the robustness of CE-ICP-MS assays.

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