




Cite this: *Metallomics*, 2018, 10, 455

Metallomic study on the metabolism of RAPTA-C and cisplatin in cell culture medium and its impact on cell accumulation†

Hannah U. Holtkamp, Sanam Movassaghi, Stuart J. Morrow, Mario Kubanik and Christian G. Hartinger *

Metal-based anticancer agent development can be improved with advanced metallomics methods that allow for quick and efficient screening of metallodrugs for their metabolites in biological media. Cellular accumulation in *in vitro* settings is not always correlated with cytotoxicity; and protein binding, particularly with albumin and transferrin, can have an important influence on metallodrug transportation, selectivity, and efficacy. We contrast the time-dependent cellular accumulation of both cisplatin and the pre-clinically investigated RAPTA-C in terms of cell uptake and speciation in culture medium *via* CE-ICP-MS analysis. Despite RAPTA-C being administered at 40-fold higher dose than cisplatin, owing to its much higher IC₅₀ value, the accumulation over time was only 10-fold higher. An optimised CE-ICP-MS method, through the coating of the capillary to prevent protein–capillary surface interactions, resulted in superior resolution and metal–protein adduct identification. It was then used for extracellular speciation in conjunction with [tris(acetylacetonato)cobalt(III)] as an internal standard. RAPTA-C was found to be more inert to extracellular reactions than cisplatin which could be used to rationalise the observed cellular uptake patterns. While for cisplatin both transferrin and albumin were identified as the main binding partners, RAPTA-C was found to react nearly exclusively with albumin. Moreover, this behaviour was time-dependent and our results also demonstrate that cancer cells have an influence on metal species distribution in the cell culture medium over time.

Received 25th January 2018,
Accepted 19th February 2018

DOI: 10.1039/c8mt00024g

rsc.li/metallomics

Significance to metallomics

This study shows the impact of speciation of cisplatin and the organoruthenium developmental anticancer agent RAPTA-C on the accumulation in cancer cells. The metabolism of RAPTA-C in cell culture medium leaves it to a higher degree unreacted than cisplatin but cisplatin is taken up into cells more efficiently. Notably, the presence of cancer cells influenced the species detected for both cisplatin and RAPTA-C interaction with proteins.

1. Introduction

Metal-based drugs are essential pillars of cancer chemotherapy. A reason for the low development rate of novel anticancer metallodrugs is the limited knowledge about their modes of action beyond the DNA-targeting ability of cisplatin.¹ In particular newer generations of developmental metal-based anticancer agents are designed to target proteins which are overexpressed in tumour cells as compared to healthy cells.

Ruthenium complexes are some of the most widely studied non-Pt compounds and offer novel mechanisms of action, reduced toxicity, and a different spectrum of activity, which can provide alternative treatments for cisplatin-resistant cancers.^{2–4} Promising Ru compounds encompass the clinically-investigated Ru^{III} coordination compounds NAMI-A and KP1019/NKP-1339 as well as organometallic Ru^{II}(η⁶-arene) structures, such as RAPTA and RAED compounds. The organometallic Ru^{II}(η⁶-arene) scaffold provides a versatile platform for drug development because the choice of ligand allows modulation for particular biological targets and desired activity.⁴ RAPTA compounds of the general structure [Ru(η⁶-arene)(PTA)X₂] (PTA = 1,3,5-triaza-7-phosphaadamantane, X = halide; see Fig. 1 for RAPTA-C with arene = *p*-cymene) bind preferentially to proteins while the ethylene-1,2-diamine complexes RAED exhibit a preference for DNA.⁵

School of Chemical Sciences, University of Auckland, Private Bag 92019,

Auckland 1142, New Zealand. E-mail: c.hartinger@auckland.ac.nz;

Web: <http://hartinger.auckland.ac.nz>; Tel: +64 9 3737 599 ext. 83220

† Electronic supplementary information (ESI) available: ICP-MS operational values, calibration plots, components of the α-MEM cell medium and foetal calf serum and additional CE-ICP-MS data. See DOI: 10.1039/c8mt00024g



2.2.4. Separation conditions. Phosphate buffer (pH 7.4, 25 mM) was used as the BGE with PVP- and formic acid (50 mM) with polybrene-coated capillaries. The analysis procedure included pre-conditioning the prepared capillary by flushing with BGE (3 min) before each run and postconditioning with NaOH (1 M; 3 min) and H₂O (3 min) after each separation, in order to ensure repeatability and maintain a steady baseline. When using the polybrene coating, the NaOH rinsing step was omitted. Samples were injected hydrodynamically at 50 mbar for 5 s. The voltage applied for PVP-coated capillaries (70 cm, 75 μm ID) was 15 kV, producing a current of 30 μA; and for the PB-coated capillaries (70 cm, 75 μm ID) a voltage of 30 kV produced a current of 7 μA.

2.2.5. CE-ICP-MS. An Agilent 7700 ICP-MS was used as the detector hyphenated through a modified interface based on part number G1607B (Agilent, Waldbronn, Germany).⁵⁴ The CE-ICP-MS settings are provided in Table S1 in the ESI.† The sheath liquid was supplied using the ICP-MS inbuilt peristaltic pump [0.03 revolutions per second (rps)] *via* the internal standard line (blue-orange tubing, 0.25 mm ID) and in conjunction with an in-built alligator clip to close the electrical circuit. The ICP-MS was tuned with ⁴⁵Sc, ⁷²Ge and ¹⁸⁵Re in the sheath liquid until a stable signal (RSD <5%) was attained. Data analysis was carried out using Origin 2017 (OriginLab Corporation, Northampton, USA).

2.2.6. CE-ESI-MS. The capillary electrophoresis instrument was hyphenated to a Bruker microTOF-Q II ESI-mass spectrometer *via* a CE-ESI-MS interface (part number G1607B, Agilent, Waldbronn, Germany). The sheath liquid of isopropanol, methanol, water, formic acid and 3-nitrobenzyl alcohol (25/25/50/0.2/0.5, v/v/v/v%)⁵⁵ was delivered at 6 μL min⁻¹ with an isocratic pump (Agilent, Waldbronn, Germany). The MS was operated in positive mode with a scan range of *m/z* 150–2500. The dry temperature was 180 °C, the nebuliser pressure was set at 0.6 bar, and the dry gas flow was 4.0 L min⁻¹. Data was analysed with Compass DataAnalysis Version 4.0 (Bruker Daltonic, Bremen, Germany).

2.2.7. Sample preparation. Preliminary CE-UV studies were carried out to optimise the separation of the human serum proteins HSA and hTf (100 μM) and a mixture of HSA/hTf (1 : 1). The interaction of cisplatin and RAPTA-C with serum proteins was studied with incubation solutions consisting of 100 μM of either cisplatin or RAPTA-C, and 50 μM of either HSA, hTf or both HSA and hTf. The samples were prepared to contain a matrix of 100 mM NaCl and 10 mM phosphate buffer (pH 7.4) designed to simulate physiological conditions. The only exception was for the ESI-MS detector where Type 1 water was used. The samples were incubated on a Grant bio thermoshaker (37 °C, 260 rpm) and aliquots were collected after 0, 2, 4, 24, 48, and 96 h and were frozen until just prior to analysis.

Human serum samples were diluted 1 : 4 with the incubation solution before analysis. Cell medium samples collected from cellular accumulation experiment (compare 2.3) were analysed on CE-ICP-MS without additional workup, but for CE-ESI-MS samples needed to be filtered (Vivaspin[®] 500 Polyethersulfone, 10 kDa, Sartorius) and the low- and high-molecular weight (LMW and HMW) fractions were collected.

All samples contained [Co(acac)₃] (final concentration 10 μM) as an internal standard which was added just prior to analysis.

The calibration plots for Pt and Ru were prepared using cisplatin and RAPTA-C concentrations of 1.6, 3.1, 6.1, 12.5, 25, 50 and 100 μM with 10 μM of [Co(acac)₃] as the internal standard. The limits of detection (LOD) and quantification (LOQ) were calculated as described previously.³⁹

2.2.8. Data processing. The migration time corrected relative to the migration of the Co internal standards was termed relative migration time (*T_R*) and was calculated as $T_R = t_a/t_{Co}$ where *t_a* refers to the migration time of the analyte and *t_{Co}* refers to the migration time of the cobalt internal standard. Similarly, the ¹⁰¹Ru and ¹⁹⁵Pt traces recorded of the analysed samples were normalised with the ⁵⁹Co peak area recorded for [Co(acac)₃] before they were integrated.

2.3. Cell studies

HCT116 cells were supplied by ATCC, and were grown in αMEM (Life Technologies) supplemented with 5% fetal calf serum (Moregate Biotech) at 37 °C in a humidified incubator with 5% CO₂. The sulforhodamine B cytotoxicity assay to determine IC₅₀ values and the cellular uptake experiments were carried out as described previously.⁵⁶ To determine the intracellular metal concentrations, HCT116 cells (4 × 10⁵ per well) were seeded into 6-well plates and allowed to settle for 24 h time at 37 °C and 5% CO₂. The compounds RAPTA-C and cisplatin were dissolved in DMSO (10 000, and 250 μM, respectively) and diluted with medium to a concentration of 1% DMSO and the previously determined IC₅₀ values of the metal complexes. Cells were left to incubate at 4, 24, 48 and 72 h exposure times. The medium was collected and analysed by both direct infusion analysis ICP-MS to quantify the total metal content, and by PVP coated CE-ICP-MS for speciation analysis. The wells were washed twice with 1 mL of ice-cold PBS buffer. The cells were lysed with 2 mL of concentrated nitric acid (containing 0.1 μL of a 1000 ± 3 μg mL⁻¹ thulium standard as internal standard) and digested with an Ethos Up microwave digestion system (Milestone). Then the solutions were diluted with 10 mL of H₂O and the metal content was determined by ICP-MS. The ICP-MS (Agilent 7700) with an ASX-500 autosampler (CETAC Technologies) in a Serie SuSi laminar flow hood (SPECTEC) was equipped with a MicroMist nebuliser and a Scott double pass spray chamber. The carrier gas flow rate was 1 mL min⁻¹. The instrument was tuned for cerium, cobalt, lithium, magnesium, thallium, and yttrium. The calibration standards were matched to the samples with regard to HNO₃ concentration and the internal standard. The reported values are the mean of at least 3 independent uptake experiments conducted with blank wells for each substance to account for unspecific binding to the plastic of the well plates.

3. Results and discussion

3.1. Interaction of RAPTA-C with human serum proteins

CE-ICP-MS analysis was used to demonstrate that the Ru^{III} complexes KP1019 and NKP-1339/IT139 are predominantly bound to HSA in serum *in vivo*.^{45,47} A similar method was applied to compare these Ru(III) compounds and the organoruthenium(II)



complex RAPTA-C in terms of their ligand exchange kinetics with water and DNA models.⁴³ Such studies are of particular importance in the early stage of drug development,^{6,57} and CE-ICP-MS has significant potential in this research area. There have been a wide variety of reports elucidating the interactions between metal complexes and proteins and also on the impact of halido/aqua ligand exchange on biomolecule binding. The methods used are often based on liquid chromatography (LC) separation and several interesting results have been reported, contributing especially to the understanding of speciation and its impact on the biological activity of metal-based anticancer agents.^{14,26–28,58–61} LC provides low to high flow rates, which makes it suitable for hyphenation with molecular and element-specific detectors, and benefits from constant retention times for the analytes. However, separations based on solid phase interactions also suffer from the risk of shifts in the species present in the samples, especially for reactive metal complexes. Operating in purely aqueous conditions in CE reduces that risk and even allows the use of the capillary as a reactor to resemble the environment of specific biological tissue,⁶² while still using minute sample amounts and allowing for short analysis times.

Notably, a disadvantage of CE separation with bare fused-silica capillaries is that proteins such as HSA tend to adsorb on the capillary surface. Though experimental procedures analysing serum or HSA samples on bare fused-silica capillaries have been previously published,^{63–65} we have observed this phenomenon in previous studies looking at the kinetics and binding behaviour of HSA with metallodrugs. This strongly interferes with the analysis and may result in capillary blocking. Lucy *et al.* describe the fundamentals of this problem in detail in a recent review.⁶⁶

To overcome this issue, many procedures have been investigated that involve the coating of the capillary and high adsorption resistance was observed, while being even able to separate protein isoforms with high resolution.^{67–72} One approach of capillary coating is based on polybrene (PB) and polyethylene glycol,⁷³ which creates a cationic surface requiring the use of an acidic background electrolyte (BGE). This procedure was modified and then successfully applied to binding studies of Ru compounds with serum proteins.^{44,45} The practical limitation of this approach was that the polybrene coating is unstable and dynamic, which can lead to contamination of a hyphenated MS detector. While ICP-MS is robust, it can be problematic for ESI-MS.

Therefore, an alternative approach was investigated for the application of CE-MS in the elucidation of metallodrug protein interaction in serum and cell culture medium operating the CE in capillary zone electrophoresis mode (CZE). To avoid contamination with PB, poly(vinyl pyrrolidone) (PVP) was used to covalently coat the capillary. PVP was shown to be stable, insensitive to pH, and biocompatible.⁵³ To study the suitability of the coating, the separation performance of a PVP-coated capillary for the serum proteins HSA and hTf was evaluated and contrast with PB-modified capillaries. In PVP coated capillaries the BGE can be broadly varied for separation and it is possible to separate the principal serum proteins HSA and hTf using a physiologically compatible buffer (25 mM phosphate buffer, pH 7.4),

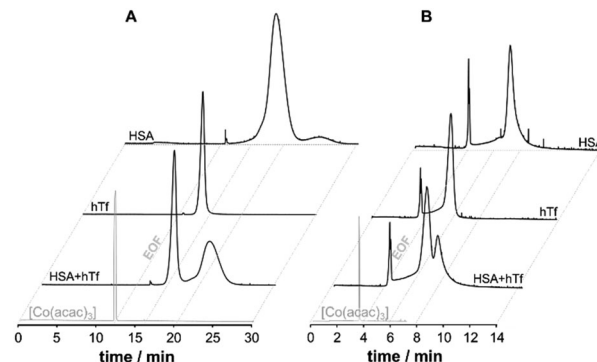


Fig. 2 The CE-UV (214 nm) electropherograms of $[\text{Co}(\text{acac})_3]$ (10 μM , internal standard, EOF marker) and the serum proteins (100 μM) HSA and hTf and a HSA/hTf mixture (1:1) using either (A) PVP- or (B) PB-coated capillaries.

while PB coatings require a low pH buffer (50 mM formic acid) for separation. A significantly higher signal-to-noise ratio was found with PVP, and a superior protein resolution was achieved for the HSA and hTf mixture (Fig. 2). $[\text{Co}(\text{acac})_3]$ was added to the samples as an internal standard to a final concentration of 10 μM prior to analysis to record the position of the EOF and to correct for run-by-run variation in migration time and peak area (Fig. 2).

The enhanced robustness to a broad pH range allowed the use of 1 M NaOH for rinsing to regenerate the EOF, while the coating remained stable for over two weeks. There was no indication of any loss of ion sensitivity in the ESI-MS confirming the PVP is fixed firmly to the exposed silanol groups.

Given these positive results, PVP-coated capillaries were employed in CZE^{PVP}-ICP-MS studies to evaluate the serum protein binding behaviour of the organometallic complex RAPTA-C, monitoring the Ru, Co (internal standard), Re (nebulisation stability), and P (capillary performance) traces. The total amount of measured Ru for the same sample was found to be higher with the PVP-coated capillary than when using PB (Table S2, ESI[†]). Although the peak area was initially more variable than with PB coating, correction with the internal standard $[\text{Co}(\text{acac})_3]$ decreased the variability to a level comparable with PB (Fig. S2 and Table S2, ESI[†]). Moreover, the peak areas for the Ru-serum protein adducts measured *via* PVP capillaries were comparatively larger. Therefore, this improved method made it possible to identify the principal binding partners of RAPTA-C in human serum (Fig. 3). Using pure samples of HSA and hTf incubated with RAPTA-C for 96 hours as standards helped to identify the migration behaviour of the respective Ru-serum protein adducts with the hTf adduct migrating quicker than the HSA conjugate. For a 1:1 HSA/hTf mixture, approximately a 2.5:1 ratio of adduct formation with the proteins was identified, with a preference for HSA. Studies in human serum, where HSA is present in about 10-times higher concentration support this outcome and the vast majority of Ru was found attached to HSA (Fig. 3), although the peaks were broadened in these studies. Importantly, only one cobalt peak was detected in these samples, which indicated that the $[\text{Co}(\text{acac})_3]$ not only remained intact but did not form adducts with any species present in the reaction mixture.



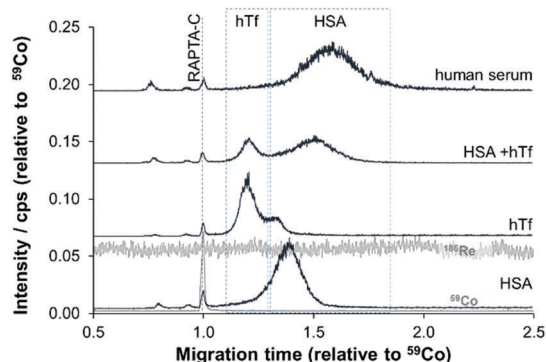


Fig. 3 The CZE-ICP-MS electropherograms for reaction mixtures of RAPTA-C with HSA, hTf and HSA/hTf (50 μM , 1:1) in saline solution and human serum after 96 h incubation, using PVP-coated capillaries. $[\text{Co}(\text{acac})_3]$ was added as an internal standard and used as the EOF marker.

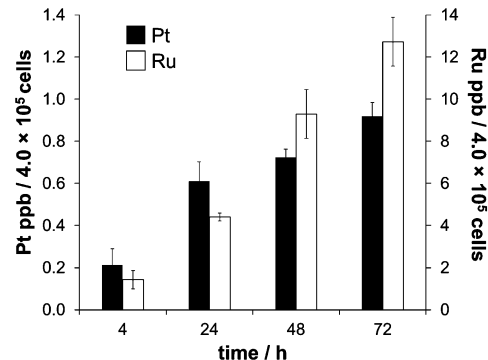


Fig. 4 The time-dependent uptake of cisplatin (2.5 μM) and RAPTA-C (100 μM) in HCT116 colon carcinoma cells, measured at time points of 4, 24, 48 and 72 h.

This is another example that confirms the broad applicability of $[\text{Co}(\text{acac})_3]$ as an internal standard in CE analyses.³⁹

3.2. Cell uptake and speciation of RAPTA-C and cisplatin in cancer cells

The biological activity of metal-based anticancer agents is often strongly dependent on the speciation and the accumulation in cancer cells.⁷⁴ Therefore, cellular uptake experiments for cisplatin and RAPTA-C were conducted in HCT116 colon cancer cells grown in αMEM supplemented with 5% fetal calf serum containing the bovine serum proteins bovine serum albumin (BSA) and transferrin (bTf). HSA has approximately the same molecular weight as BSA and they feature 76% sequence homology.⁷⁵ Transferrins in general have a significant degree of conservation in their amino acid sequence (>70%),⁷⁶ and hTf and bTf also have similar molecular weights. The concentrations of the complexes chosen were at or below the IC_{50} values for cisplatin (2.5 μM)⁵⁶ and RAPTA-C (100 μM) in HCT116 cells, respectively. The cell viability was confirmed under the light microscope before cells were collected for each time point of the cell uptake analysis. Analysis with direct infusion ICP-MS demonstrated that cisplatin was accumulated more efficiently inside the HCT116 cells, while the total amount of Ru was higher despite the lower antiproliferative activity of RAPTA-C (Fig. 4). While the uptake of Ru from RAPTA-C appears to increase steadily over the time course of 72 h, the concentration of Pt detected in the cells treated with cisplatin only slightly varies between 24 and 72 h. This may be related to the differing reactivity of the complexes with proteins or other medium components.

In order to investigate this differing behaviour further, we prepared samples of cell medium containing fetal calf serum spiked with both anticancer agents and analysed them with our newly optimised method based on PVP-coated capillaries with a particular emphasis on the influence of speciation on the uptake. Notably, analysis of the cell medium samples by CZE^{PVP}-ICP-MS was carried out without the need for prior work up or dilution, despite the significant matrix in such samples (Fig. 5, composition listed in Tables S3 and S4, ESI[†]).

For both cisplatin and RAPTA-C, the principal binding partner(s) in the cell culture medium were identified as the serum proteins. This confirms reports by Groessler *et al.* for cisplatin and an analogous RAPTA compound by size exclusion chromatography (SEC)-ICP-MS.^{3,28} It is also in line with a study by Gibson *et al.* who collected SEC fractions from cell extracts treated with cisplatin and determined two-thirds of the Pt content to be in molecular weight fractions of >3000 g mol^{-1} .⁷⁷ Heffeter and colleagues found large protein aggregates with high molecular weights for Ru^{III} anticancer agents in the cytosol, which was very different to the protein adducts cisplatin formed.²⁷

With the rising signals assigned to the BSA and bTf adducts for cisplatin and BSA for RAPTA-C, the peaks identified for the free metal complexes declined over the time of the incubation period (Fig. 5A and B). While for RAPTA-C a cationic metabolite, presumably $[\text{Ru}(\text{cym})(\text{PTA})\text{Cl}(\text{H}_2\text{O})]^+$ based on previous experiments,^{21,43,78,79} was identified, no cationic Pt-containing peaks were observed. For both compounds, several additional Ru- or Pt-containing anionic species were detected. Attempts of using ESI-MS in a hyphenated setup did not allow identification of any adducts as the salt content of the cell medium samples was too high. Therefore, the samples were further separated with a 10 kDa cut-off filter into low (LMW) and high molecular weight (HMW) fractions. CE^{PVP}-ESI-MS analysis of the HMW fraction successfully confirmed the identity of the principal serum protein binding partner as BSA for both cisplatin and RAPTA-C. Analysis of the LMW fraction by CZE^{PVP}-ICP-MS clearly shows that the unknown Ru species has a molecular weight below 10 kDa (ESI, [†] Fig. S4). This anionic species may be formed by a reaction with phosphate or sulfate ions, or the negatively-charged amino acids aspartic and glutamic acid, all present in the cell medium solution. Adducts with some of these were detected before for cisplatin, although in absence of proteins.³⁷

The speciation data collected by CZE^{PVP}-ICP-MS showed a peak variability of ca. 7 and 5% for Pt and Ru after correction using $[\text{Co}(\text{acac})_3]$ as the internal standard. This was especially beneficial for samples containing cisplatin which behaved more irregularly, probably influenced by the low concentrations of the analytes. The high reproducibility of measurements involving RAPTA-C gave LOD and LOQ values of 1.3 and



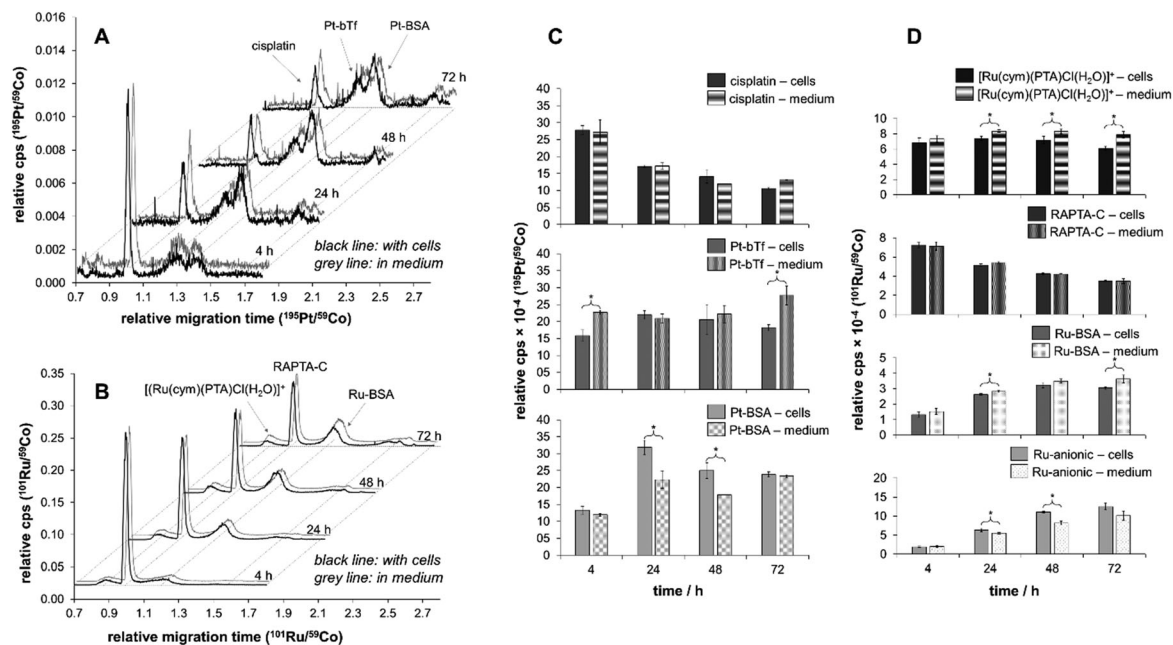


Fig. 5 The speciation studies carried out by CZE^{PVP}-ICP-MS. (A) ¹⁹⁵Pt and (B) ¹⁰¹Ru electropherograms of cisplatin and RAPTA-C speciation over time in cell medium both in the presence of HCT116 cells (black line) and absence (grey line). The principal species of interest are identified by arrows and comparisons of cisplatin and RAPTA-C species in the presence and absence of HCT116 are made in C and D, respectively. *t*-Tests were carried out on the species amount in the presence of cells and in medium. Statistically significant differences at the 95% confidence level are indicated with an asterisk.

3.9 μM (ESI,† Fig. S2), while for cisplatin with this method 2.6 and 8.0 μM were obtained. Similar observations for cisplatin have been reported previously,³⁹ and in a laser ablation (LA)-ICP-MS study contrasting the spatial distribution of cisplatin and KP1339 in animal tissue.⁸⁰

Importantly, the CZE^{PVP} method used allowed superior separation of albumin and transferrin in combination with both UV and ICP-MS detectors. This is a significant advantage as compared to PB-coated capillaries. Therefore, it was possible to use CZE^{PVP}-ICP-MS and show significant differences in the peak areas over time in the cell medium samples that could be assigned to the presence of HCT116 cells. Overall, the presence or absence of cells did not impact the decrease of the peaks assigned to cisplatin and RAPTA-C (Fig. 5C and D) but it had a significant influence on the amount of individual species detected, probably because of different interactions between the metal complexes and the proteins present in the samples. For example, for cisplatin the amount of bTf adduct formed after 4 h was significantly higher in the sample without cells than in presence of cells (Fig. 5C). While it remains fairly constant in the presence of cells over the whole period of the experiment, in case of the cell medium samples it significantly increased after 72 h. In contrast, the amount of Pt attached to BSA was higher in the samples incubated with cells for 24 and 48 h while it was constant for the cell medium samples. Overall, the relative abundance of the BSA adduct decreased from 24 to 72 h in presence of cell. The relative distribution between original complex and protein adducts showed that RAPTA-C is present to a higher degree as unreacted complex while much of the Pt is bound to proteins and only a minor amount of

unreacted cisplatin was detectable. We suggest that this is the reason for RAPTA-C to accumulate consistently over time, while the increase of Pt concentration in the cells beyond 24 h was limited.

4. Conclusions

We have introduced a new CE-based method for the analysis of metal-based anticancer agents and we have applied it in conjunction with ICP-MS detection to study the impact of speciation in cell culture medium on the cellular accumulation of cisplatin and the developmental anticancer agent RAPTA-C. The PVP coating used in these studies has been demonstrated to show considerable robustness to high salt content samples, as often found in biological environment, with no loss of peak resolution. Given these characteristics, this method has high potential for application in speciation analysis of anticancer metal complexes. Being able to generate informative results from the analysis of complex serum sample with no sample work up is an important feature of the new method as it limits the potential of alteration in speciation. With this 1D CZE method, it was possible to separate proteins such as albumin and transferrin, despite their similar molecular weights, and we obtained LOD and LOQ values that allow application of the method in biological samples. Therefore, it enabled us to quickly and confidently assess the principal protein binding partners of cisplatin and RAPTA-C with enhanced resolution. Despite using low concentrations in the cell culture experiments, especially for cisplatin, it was possible to show that the presence



of cancer cells had an impact on the detected species distribution of anticancer metal complexes in terms of the amounts of protein adducts present in cell culture medium. While cisplatin was found to react with both albumin and transferrin, for RAPTA-C only an albumin adduct was detected, accompanied by a minor amount of hydrolysis product. Notably, the amount of free RAPTA-C was significantly higher than found for cisplatin, which may explain the different cellular accumulation behaviour of the two compounds.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

We thank the University of Auckland (University of Auckland Doctoral Scholarship to H. H. and M. K.) for the financial support. We would also like to thank Tony Chen for useful discussions and technical expertise.

References

- 1 A. R. Timerbaev, C. G. Hartinger, S. S. Aleksenko and B. K. Keppler, *Chem. Rev.*, 2006, **106**, 2224–2248.
- 2 R. E. Aird, J. Cummings, A. A. Ritchie, M. Muir, R. E. Morris, H. Chen, P. J. Sadler and D. I. Jodrell, *Br. J. Cancer*, 2002, **86**, 1652–1657.
- 3 M. Groessl, O. Zava and P. J. Dyson, *Metallomics*, 2011, **3**, 591–599.
- 4 B. S. Murray, M. V. Babak, C. G. Hartinger and P. J. Dyson, *Coord. Chem. Rev.*, 2016, **306**, 86–114.
- 5 Z. Adhireksan, G. E. Davey, P. Campomanes, M. Groessl, C. M. Clavel, H. Yu, A. A. Nazarov, C. H. Yeo, W. H. Ang, P. Droge, U. Rothlisberger, P. J. Dyson and C. A. Davey, *Nat. Commun.*, 2014, **5**, 3462.
- 6 A. Jouyban and E. Kennedler, *Electrophoresis*, 2008, **29**, 3531–3551.
- 7 H. Li and Z. M. Qian, *Med. Res. Rev.*, 2002, **22**, 225–250.
- 8 F. Kratz, A. Warnecke, K. Scheuermann, C. Stockmar, J. Schwab, P. Lazar, P. Drückes, N. Esser, J. Drevs, D. Rognan, C. Bissantz, C. Hinderling, G. Folkers, I. Fichtner and C. Unger, *J. Med. Chem.*, 2002, **45**, 5523–5533.
- 9 J. Ghuman, P. A. Zunszain, I. Petitpas, A. A. Bhattacharya, M. Otagiri and S. Curry, *J. Mol. Biol.*, 2005, **353**, 38–52.
- 10 F. Kratz, *J. Controlled Release*, 2008, **132**, 171–183.
- 11 T. R. Daniels, T. Delgado, G. Helguera and M. L. Penichet, *Clin. Immunol.*, 2006, **121**, 159–176.
- 12 W. H. Ang, E. Daldini, L. Juillerat-Jeanneret and P. J. Dyson, *Inorg. Chem.*, 2007, **46**, 9048–9050.
- 13 V. Pichler, J. Mayr, P. Heffeter, O. Domotor, E. A. Enyedy, G. Hermann, D. Groza, G. Kollensperger, M. Galanski, W. Berger, B. K. Keppler and C. R. Kowol, *Chem. Commun.*, 2013, **49**, 2249–2251.
- 14 M. Hanif, A. A. Nazarov, A. Legin, M. Groessl, V. B. Arion, M. A. Jakupec, Y. O. Tsybin, P. J. Dyson, B. K. Keppler and C. G. Hartinger, *Chem. Commun.*, 2012, **48**, 1475–1477.
- 15 F. Kratz and B. Elsadek, *J. Controlled Release*, 2012, **161**, 429–445.
- 16 S. Moon, M. Hanif, M. Kubanik, H. Holtkamp, T. Söhnel, S. M. F. Jamieson and C. G. Hartinger, *ChemPlusChem*, 2015, **80**, 231–236.
- 17 M. Hanif, S. Moon, M. P. Sullivan, S. Movassaghi, M. Kubanik, D. C. Goldstone, T. Söhnel, S. M. F. Jamieson and C. G. Hartinger, *J. Inorg. Biochem.*, 2016, **165**, 100–107.
- 18 N. S. Sommerfeld, D. Strohhofer, K. Cseh, S. Theiner, M. A. Jakupec, G. Koellensperger, M. Galanski and B. K. Keppler, *Eur. J. Inorg. Chem.*, 2017, 4049–4054.
- 19 J. D. Holding, W. E. Lindup, C. van Laer, G. C. Vreeburg, V. Schilling, J. A. Wilson and P. M. Stell, *Br. J. Clin. Pharmacol.*, 1992, **33**, 75–81.
- 20 F. Kratz, *Expert Opin. Ther. Pat.*, 2002, **12**, 433–439.
- 21 C. Sclaro, A. Bergamo, L. Brescacin, R. Delfino, M. Cocchietto, G. Laurenczy, T. J. Geldbach, G. Sava and P. J. Dyson, *J. Med. Chem.*, 2005, **48**, 4161–4171.
- 22 P. Nowak-Sliwinska, J. R. van Beijnum, A. Casini, A. A. Nazarov, G. Wagnières, H. van den Bergh, P. J. Dyson and A. W. Griffioen, *J. Med. Chem.*, 2011, **54**, 3895–3902.
- 23 A. Weiss, R. H. Berndsen, M. Dubois, C. Muller, R. Schibli, A. W. Griffioen, P. J. Dyson and P. Nowak-Sliwinska, *Chem. Sci.*, 2014, **5**, 4742–4748.
- 24 M. V. Babak, S. M. Meier, K. V. M. Huber, J. Reynisson, A. A. Legin, M. A. Jakupec, A. Roller, A. Stukalov, M. Gridling, K. L. Bennett, J. Colinge, W. Berger, P. J. Dyson, G. Supertifurga, B. K. Keppler and C. G. Hartinger, *Chem. Sci.*, 2015, **6**, 2449–2456.
- 25 M. Groessl and C. G. Hartinger, *Anal. Bioanal. Chem.*, 2013, **405**, 1791–1808.
- 26 M. Sulyok, S. Hann, C. G. Hartinger, B. K. Keppler, G. Stingeder and G. Koellensperger, *J. Anal. At. Spectrom.*, 2005, **20**, 856–863.
- 27 P. Heffeter, K. Böck, B. Atil, M. A. Reza 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.
- 28 M. Groessl, M. Terenghi, A. Casini, L. Elviri, R. Lobinski and P. J. Dyson, *J. Anal. At. Spectrom.*, 2010, **25**, 305–313.
- 29 I. Khalaila, A. Bergamo, F. Bussy, G. Sava and P. J. Dyson, *Int. J. Oncol.*, 2006, **29**, 261–268.
- 30 L. Hu, T. Cheng, B. He, L. Li, Y. Wang, Y.-T. Lai, G. Jiang and H. Sun, *Angew. Chem., Int. Ed.*, 2013, **52**, 4916–4920.
- 31 Y. Wang, L. Hu, X. Yang, Y.-Y. Chang, X. Hu, H. Li and H. Sun, *Metallomics*, 2015, **7**, 1399–1406.
- 32 C. G. Hartinger, M. Groessl, S. M. Meier, A. Casini and P. J. Dyson, *Chem. Soc. Rev.*, 2013, **42**, 6186–6199.
- 33 H. U. Holtkamp and C. G. Hartinger, *Drug Discovery Today: Technol.*, 2015, **16**, 16–22.
- 34 A. Prange and D. Pröfrock, *Anal. Bioanal. Chem.*, 2005, **383**, 372–389.
- 35 H. Cheng, P. Li, J. Liu and M. Ye, *J. Anal. At. Spectrom.*, 2016, **31**, 1780–1810.



- 36 A. Týčová, V. Ledvina and K. Klepárník, *Electrophoresis*, 2017, **38**, 115–134.
- 37 G. Grabmann, B. Keppler and C. Hartinger, *Anal. Bioanal. Chem.*, 2013, **405**, 6417–6424.
- 38 T. T. T. N. Nguyen, J. Østergaard, S. Stürup and B. Gammelgaard, *Anal. Bioanal. Chem.*, 2013, **405**, 1845–1854.
- 39 H. U. Holtkamp, S. J. Morrow, M. Kubanik and C. G. Hartinger, *J. Biol. Inorg. Chem.*, 2017, 1–10.
- 40 T. T. T. N. Nguyen, J. Østergaard, S. Stürup and B. Gammelgaard, *Anal. Bioanal. Chem.*, 2012, **402**, 2131–2139.
- 41 G. Grabmann, S. M. Meier, Y. Y. Scaffidi-Domianello, M. Galanski, B. K. Keppler and C. G. Hartinger, *J. Chromatogr. A*, 2012, **1267**, 156–161.
- 42 T. T. T. N. Nguyen, J. Østergaard, S. Stürup and B. Gammelgaard, *Int. J. Pharm.*, 2013, **449**, 95–102.
- 43 M. Groessl, C. G. Hartinger, P. J. Dyson and B. K. Keppler, *J. Inorg. Biochem.*, 2008, **102**, 1060–1065.
- 44 M. Groessl, C. G. Hartinger, K. Polec-Pawlak, M. Jarosz and B. K. Keppler, *Electrophoresis*, 2008, **29**, 2224–2232.
- 45 A. K. Bytzek, K. Boeck, G. Hermann, S. Hann, B. K. Keppler, C. G. Hartinger and G. Koellensperger, *Metallomics*, 2011, **3**, 1049–1055.
- 46 O. Dömötör, C. G. Hartinger, A. K. Bytzek, T. Kiss, B. K. Keppler and E. A. Enyedy, *J. Biol. Inorg. Chem.*, 2013, **18**, 9–17.
- 47 A. K. Bytzek, G. Koellensperger, B. K. Keppler and C. G. Hartinger, *J. Inorg. Biochem.*, 2016, **160**, 250–255.
- 48 A. K. Bytzek, É. A. Enyedy, T. Kiss, B. K. Keppler and C. G. Hartinger, *Electrophoresis*, 2009, **30**, 4075–4082.
- 49 M. Groessl, A. Bytzek and C. G. Hartinger, *Electrophoresis*, 2009, **30**, 2720–2727.
- 50 É. A. Enyedy, O. Dömötör, K. Bali, A. Hetényi, T. Tuccinardi and B. K. Keppler, *J. Biol. Inorg. Chem.*, 2015, **20**, 77–88.
- 51 T. T. T. N. Nguyen, J. Østergaard and B. Gammelgaard, *Anal. Bioanal. Chem.*, 2015, **407**, 8497–8503.
- 52 C. S. Allardyce, P. J. Dyson, D. J. Ellis and S. L. Heath, *Chem. Commun.*, 2001, 1396–1397.
- 53 L. Tan, X. Zheng, L. Chen and Y. Wang, *J. Sep. Sci.*, 2014, **37**, 2974–2982.
- 54 H. U. Holtkamp, S. J. Morrow, M. Kubanik and C. G. Hartinger, unpublished data.
- 55 C. Artner, H. U. Holtkamp, W. Kandioller, C. G. Hartinger, S. M. Meier-Menches and B. K. Keppler, *Chem. Commun.*, 2017, **53**, 8002–8005.
- 56 M. Kubanik, H. Holtkamp, T. Söhnel, S. M. F. Jamieson and C. G. Hartinger, *Organometallics*, 2015, **34**, 5658–5668.
- 57 D. C. Crans, K. A. Woll, K. Prusinskas, M. D. Johnson and E. Norkus, *Inorg. Chem.*, 2013, **52**, 12262–12275.
- 58 S. Hann, A. Zenker, M. Galanski, T. L. Bereuter, G. Stinger and B. K. Keppler, *Fresenius' J. Anal. Chem.*, 2001, **370**, 581–586.
- 59 T. Falta, G. Koellensperger, A. Standler, W. Buchberger, R. M. Mader and S. Hann, *J. Anal. At. Spectrom.*, 2009, **24**, 1336–1342.
- 60 T. T. Morris, Y. Ruan, V. A. Lewis, A. Narendran and J. Gailer, *Metallomics*, 2014, **6**, 2034–2041.
- 61 M. Sooriyaarachchi, G. N. George, I. J. Pickering, A. Narendran and J. Gailer, *Metallomics*, 2016, **8**, 1170–1176.
- 62 A. R. Timerbaev, L. S. Foteeva, K. Pawlak and M. Jarosz, *Metallomics*, 2011, **3**, 761–764.
- 63 A. R. Timerbaev, S. S. Aleksenko, K. Polec-Pawlak, R. Ruzik, O. Semenova, C. G. Hartinger, S. Oszwaldowski, M. Galanski, M. Jarosz and B. K. Keppler, *Electrophoresis*, 2004, **25**, 1988–1995.
- 64 K. Polec-Pawlak, J. K. Abramski, O. Semenova, C. G. Hartinger, A. R. Timerbaev, B. K. Keppler and M. Jarosz, *Electrophoresis*, 2006, **27**, 1128–1135.
- 65 M. Matczuk, K. Anecka, F. Scaletti, L. Messori, B. K. Keppler, A. R. Timerbaev and M. Jarosz, *Metallomics*, 2015, **7**, 1364–1370.
- 66 C. A. Lucy, A. M. MacDonald and M. D. Gulcev, *J. Chromatogr. A*, 2008, **1184**, 81–105.
- 67 N. Iki and E. S. Yeung, *J. Chromatogr. A*, 1996, **731**, 273–282.
- 68 X. Huang, L. J. Doneski and M. J. Wirth, *Anal. Chem.*, 1998, **70**, 4023–4029.
- 69 H. Stutz, *Electrophoresis*, 2009, **30**, 2032–2061.
- 70 Y. Alahmad, N. T. Tran, I. Le Potier, E. Forest, S. Jorieux and M. Taverna, *Electrophoresis*, 2011, **32**, 292–299.
- 71 R. Huang, A. R. Ferhan, L. Guo, B. Qiu, Z. Lin, D.-H. Kim and G. Chen, *RSC Adv.*, 2014, **4**, 4883–4888.
- 72 N. N. Poulsen, J. Østergaard, N. J. Petersen, K. Daasbjerg, J. Iruthayaraj, A. Dedinaite, R. Makuska and H. Jensen, *J. Sep. Sci.*, 2017, **40**, 779–788.
- 73 J. F. Kelly, S. J. Locke, L. Ramaley and P. Thibault, *J. Chromatogr. A*, 1996, **720**, 409–427.
- 74 A. Levina, D. C. Crans and P. A. Lay, *Coord. Chem. Rev.*, 2017, **352**, 473–498.
- 75 B. X. Huang, H.-Y. Kim and C. Dass, *J. Am. Soc. Mass Spectrom.*, 2004, **15**, 1237–1247.
- 76 M. D. Retzer, A. Kabani, L. L. Button, R.-H. Yu and A. B. Schryvers, *J. Biol. Chem.*, 1996, **271**, 1166–1173.
- 77 Y. Kasherman, S. Sturup and D. Gibson, *J. Med. Chem.*, 2009, **52**, 4319–4328.
- 78 C. Gossens, A. Dorcier, P. J. Dyson and U. Rothlisberger, *Organometallics*, 2007, **26**, 3969–3975.
- 79 C. Scolaro, C. G. Hartinger, C. S. Allardyce, B. K. Keppler and P. J. Dyson, *J. Inorg. Biochem.*, 2008, **102**, 1743–1748.
- 80 A. E. Egger, S. Theiner, C. Kornauth, P. Heffeter, W. Berger, B. K. Keppler and C. G. Hartinger, *Metallomics*, 2014, **6**, 1616–1625.

