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*LETTER*

# Mass Spectrometric Platforms to Study Cisplatin Resistance in Cell Models and Alternative Nano-Delivery Systems

**Maria Montes-Bayón[1,3]*, Lucía Gutiérrez Romero[1,3], Carlos López-Portugués[1,3], Paula Díez[2,3], Mario Corte-Rodriguez[1,3]**

[1]*Department of Physical and Analytical Chemistry, Faculty of Chemistry, University of Oviedo, Oviedo, Spain*
[2]*Department of Functional Biology, Immunology Area, Faculty of Medicine and Health Sciences, University of Oviedo, Oviedo, Spain*
[3]*Health Research Institute of the Principality of Asturias (ISPA), Oviedo, Spain*

The positive chemotherapeutic effect of cisplatin in most cancers (e.g. ovarian, prostate, etc.) is hampered by the inherent and acquired drug resistance, a multifactorial and still not well characterized process. Several mechanisms have been suggested to participate in conferring platinum-resistant properties to a tumor cell that need to be carefully studied in order to provide therapeutic alternatives. In this Letter, two different aspects are addressed: 1) the evaluation of molecular mechanisms involved in cisplatin resistance by the use of combined multi-strategy platforms based on mass spectrometry, and 2) the search for therapeutic alternatives to enhance efficacy and selectivity of cisplatin based on the use of nanotransporters. Both approaches are briefly discussed from the point of view of the analytical chemistry contribution.

Cisplatin, a platinum-based chemotherapeutic agent, exerts its cytotoxic effects primarily through the formation of irreversible nuclear DNA lesions that interfere with replication and transcription, ultimately yielding cell death. However, accumulating evidence indicates that cisplatin cytotoxicity is multifactorial, extending beyond direct DNA damage, and involving modulation of cytoplasmic and nuclear signaling pathways that influence cell survival, stress response, and metabolic adaptation.[1] Multiple mechanisms have been also implicated in conferring cisplatin resistance, including altered drug uptake and efflux, enhanced DNA repair capacity, dysregulated apoptosis, autophagy induction, and metabolic adaptation. These mechanisms can be classified as **pre-target**, mostly associated to a decrease on the drug cellular uptake or increase efflux, **on-target**, related to a lower binding to DNA or to an efficient repair of the formed adducts and **post-target**, due to deficiencies on the apoptotic route. In this work we have tried to compartmentalize the three types of resistance by combining strategies based on mass spectrometry and some, at the individual cell level.

### *Resistance pre-target: single cell ICP-MS*

For evaluation of the cellular incorporation of cisplatin in different cell models (sensitive and the resistant counterparts), an analytical strategy based on single cell ICP-MS was developed. Single cell analysis is crucial because it is now known that every cell can behave differently, also regarding cisplatin uptake, depending on many biological variables, such as cell type, cell cycle status, hypoxia, redox changes, etc. Therefore, cells were introduced individually into the ICP-MS using the instrumental approach shown in Figure 1, in which every cell containing Pt provided an independent cell event. Previous experiments revealed that

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most of the tested cells maintained an intact cellular morphology and stay in a monodisperse state during nebulization (only a low percentage of cells were disrupted during the transport and nebulization process), thus, each ICP-MS peak corresponds to the Pt in an individual cell.[2] Quantification of the cell-associated Pt content can be obtained by using inorganic Pt standards of increasing concentration to obtain a calibration curve. The mass of Pt per cell can be obtained using the equation plotted in Figure 1 where the transport efficiency of the inorganic standards into the plasma has to be calculated based on additional calculations. The obtained results in terms of Pt concentration revealed a heterogeneous behaviour among cell pairs (sensitive/resistant) revealing different molecular mechanisms governing resistance (see Figure 1).[3]

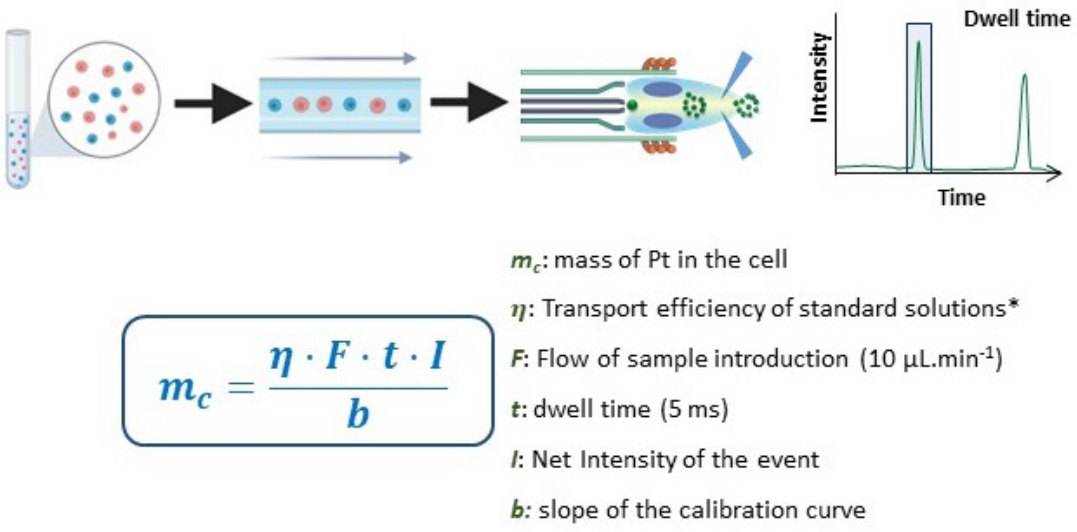


**Figure 1.** General scheme for single cell ICP-MS quantitative information.

### *Resistance on-target/post-target: ESI-MS for proteomics*

Since the resistance mechanisms can be associated to molecular pathways beyond drug incorporation, it is important to establish if these correspond to newly acquired molecular adaptations (developed upon exposure) or from pre-existing proteomic states that predispose cells to tolerate genotoxic stress. For this aim, the sensitive/resistant pairs were also tested to address proteomics variations using ESI-Q-ToF that could serve as fingerprints of molecular resistance. Our results demonstrate that resistance emerges from heterogeneous adaptive mechanisms governed by intrinsic proteomic states and stress-tolerance programs. The experiments serve to identify pharmacokinetic-, repair-, tolerance-and mixed-dominant resistance phenotypes highlighting the mechanistic diversity underlying cisplatin response.

### *Alternative drug-delivery systems: the use of nanocarriers*

The use of nanodelivery systems containing biocompatible components that are taken up by endocytosis instead of by specific cell transporters and preventing also the drugs from being recognized by efflux pumps is aimed. This would yield a higher intracellular cisplatin accumulation unaffected by the deregulation of specific membrane transporters. Cisplatin and also platinum(IV) prodrugs have been associated to nanoparticles as nanoplatforms for improving drug delivery to tumors and to promote preferential accumulation in cancer cells. Here, we explore the capabilities of the previously synthesized biocompatible ultrasmall iron oxide nanoparticles coated by tartaric and adipic acid, to be directly conjugated to the cisplatin(IV) prodrug cis-diamminetetrachloroplatinum(IV). The possibility of having a direct reaction between the two species dramatically simplifies the synthetic route. However, adequate analytical strategies that permit to quantitatively address the level of conjugation and release of the prodrug have to be developed to study the formation of these species. In this regard, the use of SDS-based reversed-phase chromatography

coupled to ICP-MS detection of Fe and Pt succeeded for this aim (see Figure 2 where Fe and Pt signals can be observed) confirming the elimination of the free drug and complemented with microscopy and light scattering experiments. Additionally, cellular uptake in ovarian cancer sensitive and resistant cell models was addressed at the individual cell level using single cell-ICP-MS strategies (see Figure 2) revealing the higher efficacy of the nanotransporter to increase the level of incorporation in both cell types.

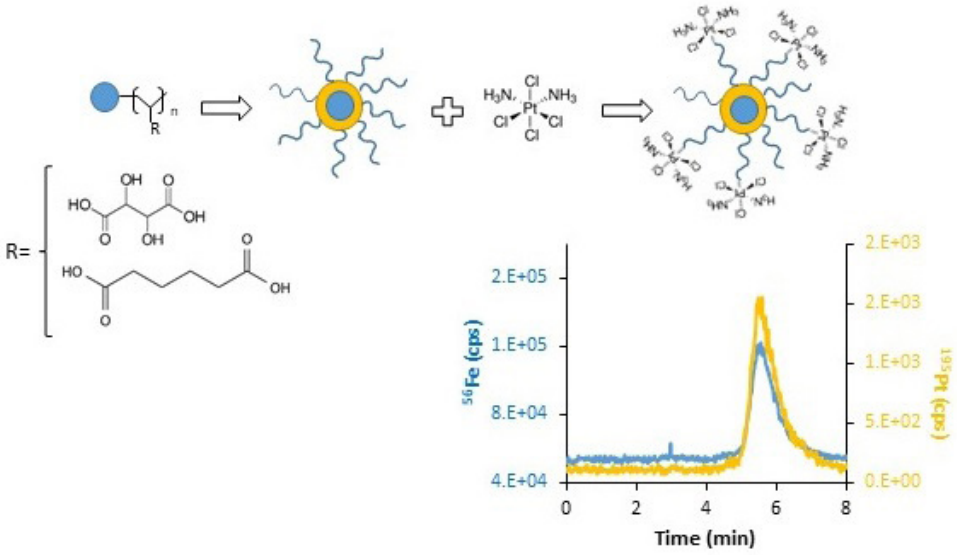


**Figure 2.** Drug delivery system for cisplatin(IV) transport into cells and assessment of the purity by HPLC-ICP-MS. (adapted from reference 3)

However, two-dimensional cell models do not seem to be the final stage to characterize the efficacy of newly designed drug delivery systems and the use of tri-dimensional cell models seem to be also required. Multicellular spheroids containing different number of cells (1000, 2000 and 4000 cells) were obtained using the “hanging-drop” procedure previously described. All of them were exposed to the Pt-loaded NPs and to the free cisplatin at the same concentration (20 μM Pt). To address the internal distribution of the different elements, the spheroids were sliced in thin sections (10 μm thickness). The elemental distribution maps of multiple elements obtained from a representative thin section of the spheroids after incubation with the different Pt compounds was obtained by laser ablation with ICP-MS detection. To obtain quantitative information of Pt profiles in the spheroids’ sections, gelatin embedded calibration standards of Pt were used according to previous publications. This work was conducted in collaboration with the laboratory of Prof. U. Karst (University of Münster, Germany). LA-ICP-MS analysis permitted to establish the penetration capabilities of the Pt-loaded nanoparticles in comparison to this of cisplatin, reflecting that the nanoparticulated form presented specific hot spots within 100-125 μm from the surface of the spheroid (outer shell and most proliferative) where highest Pt/Fe signals could be detected. This is also in agreement with previous publications revealing the size-dependent penetration capabilities of Au nanoparticles through spheroids.[4]

## SUMMARY

Out of the presented results, the most important conclusion is that the complexity of cisplatin resistance requires the development of adequate analytical strategies that permit to tackle the problem from different viewpoints. The characterization of the response from the patient at the individual cell level as well as the proteome of the cells before initiating the therapy could help to predict the response and the outcome in patients. In addition, the use of Pt(IV)-loaded ultrasmall Fe oxide NPs allow efficient drug penetration into cells in both, two-dimensional and three-dimensional cell cultures as established using SC-ICP-MS and LA-ICP-MS.

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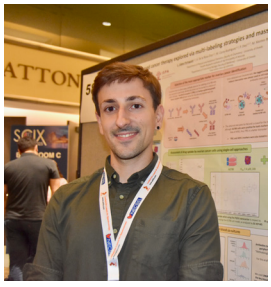
**María Montes-Bayón** is Full Professor of Analytical Chemistry at the Department of Physical and Analytical Chemistry, University of Oviedo, Spain. Her research focuses on ICP-MS-based analytical methodologies for single-cell analysis, metallodrug evaluation in chemotherapy, clinical biomarker determination, and biomedical applications of nanoparticles. She has authored numerous high-impact publications in analytical and bioanalytical chemistry and currently serves as President of the Spanish Society for Analytical Chemistry.

**Lucía Gutiérrez Romero** is a researcher at the Department of Physical and Analytical Chemistry, Faculty of Chemistry, University of Oviedo, Spain. Her research interests include bioanalytical chemistry, single-cell ICP-MS, nanoparticle-based drug delivery systems, and platinum-based anticancer therapies, with emphasis on analytical strategies for evaluating nanodelivery systems at the cellular level.



**Carlos López-Portugués** is a researcher affiliated with the Department of Physical and Analytical Chemistry at University of Oviedo, Spain. His research activities are focused on analytical and bioanalytical chemistry, particularly ICP-MS-based methodologies, nanoreporters, and biomarker quantification in clinical samples.

**Paula Díez** is a researcher at the Department of Functional Biology, Immunology Area, Faculty of Medicine and Health Sciences, University of Oviedo, Spain, and associated with the Health Research Institute of the Principality of Asturias (ISPA). Her research focuses on cancer biology, platinum resistance mechanisms, ovarian cancer biomarkers, and translational biomedical applications involving metallodrugs and nanomedicine.

**Mario Corte-Rodríguez** is Assistant Professor in Analytical Chemistry at the Department of Physical and Analytical Chemistry, University of Oviedo, Spain. His research is centered on mass spectrometry and ICP-MS applications in biomedical analysis, including metallodrugs, nanoparticle characterization, single-particle and single-cell analysis, and analytical approaches for studying cellular incorporation and reactivity of metal-based anticancer agents.