Hyphenated Elemental Mass Spectrometry for the Biosciences

by Sarah Meyer

Thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy: Science

under the supervision of Dist. Prof. Philip Doble, Dr. David Bishop, Dr. David Clases, and Dr. Raquel Gonzalez de Vega

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July 2022

Certificate of Authorship

I, Sarah Meyer, declare that this thesis is submitted in fulfilment of the requirements for the award of Doctor of Philosophy: Science, in the Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

This research is supported by the Australian Government Research Training Program.

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Date: 19th of July 2022

Acknowledgements

I would like to thank all the people who supported, motivated, and inspired me along my path and who contributed to this work in multiple ways.

First, I would like to thank my supervisor Dist. Prof. Philip Doble for the opportunity to do my PhD in Australia in his group. Thank you for being a great mentor and supervisor, for your support, advice, and encouragements, for teaching me everything about CE, the challenging projects, and your funny and charismatic nature. I am very grateful for the last 3.5 years and have developed myself so much, both scientifically and personally.

Moreover, I would like to acknowledge my supervisors David Bishop, David Clases and Raquel Gonzalez de Vega. Dave – thank you for supporting me with all imaging and ICP-MS questions, and especially for being a great supervisor during my first visit that inspired me to come back to UTS for my PhD. David – for motivations and encouragements, for scientific and personal discussions, for teaching me everything you know, especially about single particle ICP-MS and ICP-MS operation modes, and last but not least for being a great friend. My experience at UTS would not have been the same without you. Raquel – for all the meetings, support, and advice, for always being available for me, for being a competent advisor for everything that has to do with immunohistochemistry, and for your friendship. I miss and wish you all the best for your future.

I would like to make a special mention to Ariane Roseblade, Matt Padula and technical staff members Anthea Harris, Dayanne Bordin and Tom Lockwood. Ariane – for being a great, fun, and smart person to be around, for bouncing ideas off each other, and for having a similar obsession with pretty Origin figures and

Excel spreadsheets. Matt – for your support in the protein project and for providing me with protein standards and surfactants. Anthea, Dayanne and Tom – for keeping the instruments running in the teaching and research labs, for somehow always finding spare parts or rare chemical equipment that is useful for my research, and for your willingness to help in many situations.

Furthermore, I gratefully acknowledge the philanthropic support from George Miklos and the Miklos Family.

Thank you to the members of the research group: David G., Dylan, Jacob, Jake, Karen, Monique, Prashina, Siobhan, and Tash for all the good times and making me feel welcome. I would like to mention Mika and Tom especially for the scientific discussions, regular check-ins, for your help, and all the restaurant and bar recommendations.

A special thank you to my fellow PhDs at UTS: Alisha, Amber, Ana, Blake, Brooke, Caleb, Camilla, Ciara, Dan, Eda, Edward, Freddy, Helen, Minh, Oksana, Sandi, Sharni, Simon, and Tom G. for the lunch breaks, birthday cakes, wine dates, picnics, good chats, weekend get-aways, being there in challenging times, and for making me feel so welcome even though I am such a busy bee running around.

I would further like to acknowledge Prof. Dr. Uwe Karst for inspiring me to get into analytical chemistry and for supporting me along my way, as well as my fellow students from Münster: Arne, Carina, Jordi, Patrick B., Patrick H., Niklas, Sabsi and Sommel for making the time in Münster so valuable and being the best friends I could wish for.

Thank you to all my friends outside from uni, specifically Aileen, Jessica M, Maike and Naomi - I know I can count on you and call at any time, in any situation.

A huge thank you goes to Diane and Van Wallace for your acceptance, interest, support, and being my Australian family. I don’t take this for granted and really appreciate you both.

Mein größter Dank gehört meiner Familie: meinem Bruder Nicolas, meinen Eltern Martina und Norbert Meyer sowie meine Großeltern Elfriede und Ewald Meyer, und Gerta und Josef Becker. Ich weiß, es nicht einfach für euch ist, dass ich nach Australien gegangen und daher ich bin euch dankbar, dass ihr mich meinen Weg gehen lasst. Ohne euch und eure Unterstützung wäre diese Arbeit nicht möglich gewesen. Danke für das Vertrauen, den bedingungslosen Rückhalt und eure Fürsorge. Euch ist diese Arbeit gewidmet.

Finally, I would like to thank my partner Cheyne Wallace, who has been an unwavering support since the day I have met you. You have such amazing listening and problem-solving skills, and often a great answer for all kinds of questions. Thanks for your encouragements, understanding, for showing me the fun side of life, and accepting me as the person I am. As they say in Bondi: “You are my rock, babe.”

List of Publications

Sarah Meyer, David Clases, and Philip A. Doble, *A Simple, Low-Cost and Robust CE-ICP-MS Interface for the Analysis of Gadolinium-based Contrast Agents in Biological Samples*, in preparation.

Sarah Meyer, David Clases, Raquel Gonzalez de Vega, Matthew P. Padula and Philip A. Doble, *Separation of Intact Proteins by Capillary Electrophoresis*, Analyst (**2022**), 147, 13, 2988-2996.

Sarah Meyer, Raquel Gonzalez de Vega, Xiaoxue Xu, Ziqing Du, Philip A. Doble, and David Clases, *Characterisation of Upconversion Nanoparticles by Single-Particle ICP-MS Employing a Quadrupole Mass Filter with Increased Bandpass*, Anal. Chem. (**2020**), 92, 22, 15007–15016.

Sarah Meyer, Callum Clarke, Robson Oliveira dos Santos, David Bishop, Marco A. Krieger and Lucas Blanes, *Developing self-generated calibration curves using a capillary-driven wax-polyester lab on a chip device and thermal gates*, Microchemical Journal 146 (**2019**) 709-712.

“Studies derived from manganese-based atomic research have resulted in a profound new understanding of cancer radiotherapy. The data obtained are totally unexpected. They are of fundamental importance and will certainly have deep implications for patient treatment.”

Edmond H. Fisher

Nobel prize in Physiology and Medicine, 1992

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Abbreviations

A1 β-CN	A1 β-casein
A2 β-CN	A2 β-casein
α-CN	α-casein
α-Lac A	α-lactalbumin a
BFS	bare fused silica capillaries
BGE	background electrolyte
BPM	bandpass mode
BSA	bovine serum albumin
β-Lg	β-lactoglobulin
β-Lg A	β-lactoglobulin a
β-Lg B	β-lactoglobulin b
CA	citric acid
CE	capillary electrophoresis
CEI	capillary electrophoresis interface
CIC	compound-independent calibration

CK	creatine kinase
CMC	critical micellar concentration
CTAB	cetyltrimethylammonium bromide
DC	direct current
DIHEN	direct injection high efficiency nebulizer
DMT1	divalent metal transporter 1
DNA	deoxyribonucleic acid
DOTA	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid
DTPA	diethylenetriaminepentaacetic acid
DTTA	diethylenetriaminetetra-acetic acid
EBI	elemental bioimaging
EOF	electroosmotic flow
Fab	fragment antigen binding
Fc	fragments crystallizable
FFT	fast fourier transform
Fpn	ferroportin
FWHM	Full width at half maximum
GBCA	gadolinium-based contrast agent
Gd-BT-DO3A	gadobutrol (Gadovist®)
Gd-DOTA	gadoteric acid (Dotarem®)

Gd-DTPA	gadopentetic acid (Magnevist®)
Gd-DTPA-BMA	gadodiamide (Omniscan®)
Gd-EOB-DTPA	gadoxetic acid (Primovist®)
GC	gas chromatography
His	histidine
HPLC	high performance liquid chromatography
HPMC	hydroxypropylmethylcellulose
HSA	human serum albumin
ICP-MS	inductively coupled plasma-mass spectrometry
IgG	sheep igg antibody
IHC	immunohistochemistry
iMSI	immuno-mass spectrometry imaging
IR	ionising radiation
LA	laser ablation
LOD	limit of detection
LOQ	limit of quantification
m/z	mass-to-charge ratio
Mb	myoglobin
MeCAT	metal coded affinity tags
MRI	magnetic resonance imaging

MW	molecular weight
NCX	sodium-calcium exchanger
NP	nanoparticle
NRAMP	natural resistance-associated macrophage protein
NSF	nephrogenic systemic fibrosis
OIO	optimised ion optics
PBS	phosphate buffered saline
PDMS	dimethyl polysiloxane
pI	isoelectric point
PM	plasma membrane
RF	radio frequency
RNase	ribonuclease a
ROI	regions of interest
ROS	reactive oxygen species
rpm	revolutions per minute
RSD	relative standard deviation
sDL	size detection limit
SDS	sodium dodecyl sulfate
SEC	size exclusion chromatography
SLG	scan line gain

SLS	scan line slope
SM	standard mode
SMIL	successive multiple ionic polymer layers
SP	single-particle
SQ or Q	single quadrupole
TEM	transmission electron microscopy
Tf	transferrin
TfR	transferrin receptor
TQ or QQQ	triple quadrupole
TRIS	tris(hydroxymethyl)aminomethan
UCNPs	upconversion nanoparticles
UV	ultraviolet
ZIP	zrt- and irt-like proteins
ZnT10	zinc transporter 10

Abstract

The underlying biological mechanisms of widespread radioresistance of many human tumours remain elusive despite decades of investigations. Research efforts have largely focussed on the genomics/proteomics-based enzymology of DNA repair and free radical scavenging enzymes such as the superoxide dismutases. A recent novel hypothesis is that radiation resistance is predominantly underpinned by non-enzymatic complexes of manganese and small molecular metabolites. These complexes are thought to act as free radical scavengers which provide metabolic radioprotection that render cells variably resistant to the products of ionising radiation.

Multiple influx and efflux metal transporters are involved in manganese homeostasis and are potentially differentially expressed on the surface of cancer cells, leading to variable concentrations of manganese within tumours. Uncovering the mechanisms of tumour radioresistance requires complementary, reliable, and well characterised methods to spatially quantify manganese and its transporter proteins. Laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) provides a single technological platform to construct quantified images of elements and may be extended to measure biomolecules via incorporation of immunoassays. However, high quality and reproducible analyses require quality assurance across all steps of the workflow including the characterisation of antibodies, nanoparticles and antibody tagging protocols. Accordingly, this thesis introduces a portfolio of methods of hyphenated ICP-MS for quality assurance of elemental and biomolecule analyses.

Chapter 2 introduces novel and universal workflows for the analysis of intact proteins via capillary electrophoresis (CE) and presents guidelines for the targeted selection of appropriate background electrolytes via consideration of the target proteins’ isoelectric point. Neutral dimethyl polysiloxane capillaries with dynamic coatings of cationic cetyltrimethylammonium bromide or anionic sodium dodecyl sulfate, and bare fused silica capillaries were systematically evaluated for the analysis of seven model proteins over a wide pH range. Multiple capillary and background electrolyte combinations were suitable for the analysis of each protein. The concept was demonstrated by the analysis of caseins and whey proteins in milk which separated the most abundant proteins, including the isoforms of A1 and A2 β-casein and β-lactoglobulin A and B.

Chapter 3 presents the development of a simple, robust, and cost-effective interface to hyphenate CE and ICP-MS to enhance the sensitivity and specificity for the analysis of limited volume and complex biological samples. The interface components were thoroughly investigated to highlight crucial aspects that need to be considered when developing and assembling a CE-ICP-MS interface. The interface’s functionality, linearity and robustness were evaluated by separation and quantification of gadolinium-based contrast agents in urine samples collected after magnetic resonance imaging (MRI) examination.

Chapter 4 combined these advancements to determine labelling efficiencies of metal conjugated antibodies by CE-ICP-MS, which are widely used in cytometry and imaging for the identification and examination of protein expression. The number of lanthanide ions per protein was measured in seven MAXPAR™ polymer conjugated antibodies. Variable numbers of lanthanides were observed between different antibodies, as well as antibodies of the same kind, highlighting the importance of quality control workflows. The CE-ICP-MS method was also applied to 15 nm gold nanoparticles to demonstrate feasibility to distinguish un-conjugated and antibody conjugated nanoparticles.

Chapter 5 details novel methods of single-particle ICP-MS to characterise the composition, size distribution and particle-particle interactions of (upconversion)

nanoparticles. The optimization of ion extraction, ion transport, and the operation of the quadrupole with increased mass bandwidth improved the signal-to-noise ratios significantly and decreased the size detection limits for all nanoparticle dispersions investigated. Gold nanoparticles were analysed as a model system to demonstrate the effects of increasing ion transmission, subsequently the methods were applied to determine stoichiometries and size distributions of three types of lanthanide-doped upconversion nanoparticles. A Poisson model was further applied to assess particle−particle interactions in the nanoparticle dispersions.

Chapter 6 deployed these advanced techniques to demonstrate immuno-mass spectrometry imaging and elemental bioimaging of manganese transporters and transition metals in human melanomas. The transporter protein ZIP8 was visualised with an ^{153}Eu polymer labelled anti-ZIP8 antibody, and the expression levels of the ZIP14 transporter protein were localised with an immunoassay of an unlabelled primary antibody with a secondary antibody-nanoparticle conjugate. Manganese, copper, zinc, and iron distributions were imaged on consecutive sections of the microarray and co-localised with the ZIP8 and ZIP14 expressions. The results show a variable correlation of transition elements and proteins, demonstrating the complex interplay between metals and their respective transporters.