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DISSERTATION

New strategies for quantifying trace elements in challenging liquid samples by means of inductively coupled plasma - mass spectrometry and - optical emission spectrometry.

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Avec la mer du Nord pour dernier terrain vague,
Et des vagues de dunes pour arrêter les vagues,
Et de vagues rochers que les marées dépassent,
Et qui ont à jamais le cœur à marée basse.
Avec infiniment de brumes à venir
Avec le vent d'est écoutez le tenir
Le plat pays qui est le mien.

Avec des cathédrales pour uniques montagnes,
Et de noirs clochers comme mats de cocagne
Ou des diables en pierres décrochent les nuages,
Avec le fil des jours pour unique voyage,
Et des chemins de pluie pour unique bonsoir,
Avec le vent de l'ouest écoutez le vouloir,
Le plat pays qui est le mien.

Avec un ciel si bas qu'un canal s'est perdu,
Avec un ciel si bas qu'il fait l'humilité
Avec un ciel si gris qu'un canal s'est pendu,
Avec un ciel si gris qu'il faut lui pardonner.

Jacques Brel "Le plat pays"

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ABSTRACT (ENGLISH)

Inductively coupled plasmas (ICPs) are widely and routinely used in the quantification of element concentrations in liquid samples and solutions. The combination of ICP with optical emission spectrometry (ICP-OES) is the older and more rugged technique, however, its sensitivity is often not sufficient in case of (ultra-)trace element quantification. Compared to ICP-OES, ICP-mass spectrometry (ICP-MS) offers superior sensitivity, but is inherently more prone to matrix effects.

Although ICP-OES and ICP-MS are well-established in routine-laboratories, the accuracy of analysis using a conventional instrumental set-up can be hampered due to the following reasons:

(1) *Matrix effects due to sample properties.* A liquid sample has to meet certain standards in terms of viscosity, concentration of dissolved organic substances and inorganic salts, as well as acidity to be suitable for direct measurement via the conventional instrumental set-up (with a nebulizer and spray chamber for sample introduction). Many types of samples, such as whole blood or fermentation media, do not meet those standards, which can result in different sample introduction efficiencies for samples and standards, or for different samples. This results in a different instrumental response for different samples, making it difficult to correctly quantify the analyte of interest. Such samples therefore require sample dilution or chemical digestion prior to analysis to reduce the influence of the concomitant matrix. Unfortunately, by doing so, the analyte is diluted as well, thus compromising the sensitivity of the method.

(2) *Selectivity.* The liquid sample can contain elements that cause spectral interferences in ICP-OES and ICP-MS, even after strong dilution. In ICP-OES, spectral interferences are possible (especially if elements are present in the sample which are very rich in emission lines, such as iron), but in most cases they can be circumvented by choosing alternative emission lines of the analyte. However, when using ICP-MS, spectral interferences are a bigger challenge, since many different polyatomic ions can potentially interfere with the analyte. For example, the correct quantification of ultra-trace amounts of palladium (as $^{105}Pd^{+}$) in the presence of copper is difficult, because a copper argide ion is produced ($^{40}Ar^{65}Cu^{+}$) with the same nominal mass-to-charge ratio as $^{105}Pd^{+}$. Using another palladium isotope does not solve this challenge, since a multitude of interfering ions can potentially occur, which also interfere with the measurement of the other Pd isotopes. Therefore, chemical separation of palladium from copper and other problematic elements has to be carried out prior to

the measurement, in order to guarantee a selective measurement, unless the spectral overlap can be overcome in another way, e.g., by using a higher mass resolution (sector field ICP-MS) or by exploiting chemical resolution or kinetic energy discrimination (quadrupole-based ICP-MS with a collision/reaction cell).

(3) *Sensitivity.* In case of very low analyte concentrations, conventional analysis does not offer the required sensitivity, and chemical pre-concentration of the analyte is required.

In the doctoral thesis presented hereafter, new strategies are presented which were developed to tackle those challenges. The thesis is structured in three parts:

The first part of this thesis describes experiments regarding the analysis of challenging liquid samples by means of dried droplet laser ablation in combination with ICP-based techniques. The advantage of laser ablation as a means of sample introduction over conventional nebulization is that sample viscosity, the concentration of organic substances, and acidity do not influence the sample introduction efficiency, and that therefore the problems mentioned in (1) can be overcome. Moreover, when drying a liquid sample, a certain pre-concentration is obtained, as the solvent is removed. This results in improved sensitivity, as mentioned in (3). Sensitivity is also improved when using LA, since LA systems typically have a better sample introduction efficiency than do nebulizers. However, as we found out, sample preparation for LA and quantification with LA-based techniques require meticulous optimisation to obtain reliable results. Part of one published review article describes these challenges into more detail. Two published articles describe improved sample preparation and measurement strategies for liquid samples by means of LA. The first article discusses the use of pre-cut filter paper disks as a substrate for depositing liquid samples prior to LA measurement. Using such pre-cut filter disks has been reported in the literature before, but the LA strategies applied previously did not yield straightforward quantitative data because the analyte distribution across a pre-cut filter paper disk is not homogeneous. Previous attempts to account for this inhomogeneity by applying internal standards were problematic, since this approach needs optimization on a case-to-case basis. The new approach presented here offers a universal and representative sampling strategy, which allows accounting for centro-symmetric analyte inhomogeneity in any kind of liquid sample deposited on circular filter paper disks. The applicability of the method was shown using the quantification of phosphorus in biochemical fermentation media as a case study. The second paper presented in this part of the thesis describes a novel sample preparation approach which allows for the rapid and straightforward splitting of a liquid sample into sub-aliquots which can then be analysed by means of LA. As opposed to the abovementioned approach which uses pre-cut filter paper disks, the sample

preparation strategy presented in this article does not require any manual pipetting steps. This is advantageous since sample collection can therefore be carried out outside of analytical laboratories. The applicability of the method is shown via the quantification of lead in human whole blood samples.

The second part of the thesis presents a novel extraction technique – dispersed particle extraction (DPE) – which allows to chemically separate (the) analyte(s) from matrix elements also present in the sample. The DPE-method uses sorbent particles which are suspended directly in the liquid sample. The analyte is selectively trapped on the particle surface, which allows its separation from the surrounding liquid sample, as well as its pre-concentration. After isolating the analyte(s) in this way, the sorbent particles are dissolved, and the solution diluted to be compatible with nebulizer sample introduction. Compared to established techniques which can achieve the same result (e.g., solid phase extraction), the DPE method presented avoids time-consuming conditioning of columns, problems related to incomplete analyte elution, as well as carry-over and memory effects, since new sorbent material is used every time. One published article describes the application of DPE to the determination of platinum and palladium in urban roadside dust. In this article, we show that concomitant matrix elements are effectively removed by the DPE-method, and apply the method to road-dust collected in downtown Vienna. With regard to the abovementioned three challenges with ICP-based techniques, the work presented in this second part of the thesis tackles selectivity (2) by removing concomitant matrix constituents. Although the obtained pre-concentration factors do not constitute a significant analyte enrichment, this is of minor concern in the context presented, since the road-dust samples are available in sufficient quantities, thus still allowing for satisfying method detection limits.

The third part of this thesis describes the combination of DPE with LA of dried liquid samples. By using DPE, concomitant elements are removed from the sample, as discussed in the second part of this thesis. By optimizing the final sample volume, also a significant pre-concentration of the analytes was possible. However, by increasing the pre-concentration factor, the solutions finally obtained contained high concentrations of dissolved sorbent material and were not suitable for sample introduction by means of a nebulizer. Also, the final volume was so small that nebulizer-based analysis would be difficult. Therefore, the solutions were introduced into the ICP by means of LA of dried droplets, as discussed in the first part of this thesis. The method was applied to the determination of Pt and Pd in airborne particulate matter. Owing to the selectivity of DPE, the increased sensitivity resulting from the improved pre-concentration factors, and the ability of LA to cope with samples containing a high matrix-load, it was possible to monitor the short-time variation of the platinum levels in airborne

particulate matter with sampling intervals of 4 hours only. The research presented in this third part of the thesis shows a way to overcome matrix effects (1), as well as issues regarding selectivity (2) and sensitivity (3).

KURZFASSUNG (DEUTSCH)

Induktiv gekoppelte Plasmas (ICPs) sind weit verbreitet in der routinemäßigen Quantifizierung von Elementkonzentrationen in flüssigen Proben. Die Kombination mit optischer Emissionsspektrometrie (ICP-OES) ist die ältere und robustere Technik, deren Empfindlichkeit jedoch in vielen Fällen nicht für die (Ultra-) Spurenanalytik ausreicht. Im Vergleich zu ICP-OES bietet ICP-Massen Spektrometrie (ICP-MS) eine weitaus bessere Empfindlichkeit, leidet aber methodenbedingt stärker unter Matrixeffekten.

Obwohl ICP-OES und ICP-MS weite Verbreitung in Routinelabors haben, können folgende Probleme eine korrekte Quantifizierung mittels konventionellem Geräteaufbau erschweren:

(1) *Matrixeffekte aufgrund von Probeneigenschaften.* Eine flüssige Probe muss bestimmte Bedingungen hinsichtlich Viskosität, Konzentration von gelösten organischen und anorganischen Bestandteilen, sowie Säuregehalt erfüllen, um mittels konventioneller Probeneintragssysteme (Zerstäuber und Sprühkammer) ins ICP gebracht werden zu können. Viele Probentypen, wie beispielsweise Vollblut oder Fermentationsmedien, erfüllen diese Bedingungen nicht. Das kann dazu führen, dass zwischen Proben und Standards oder zwischen verschiedenen Proben ein Unterschied im Probeneintrag entsteht. Das führt zu unterschiedlichem Feed-back des Messgerätes für unterschiedliche Proben, was eine korrekte Quantifizierung des Analyten erschwert. Solche Proben müssen deshalb vor der Messung stark verdünnt oder chemisch aufgeschlossen werden, um den Einfluss der Probenmatrix zu verringern. Dabei wird jedoch auch der Analyt verdünnt, was zu einem Verlust an Nachweisstärke führt.

(2) *Selektivität.* Eine flüssige Probe kann Elemente enthalten, die selbst nach starker Verdünnung zu spektralen Interferenzen in ICP-OES oder ICP-MS führen. Im Fall von ICP-OES sind solche spektralen Interferenzen möglich (zum Beispiel in Gegenwart von Elementen wie Eisen, die über sehr viele Emissionslinien verfügen), lassen sich aber in den meisten Fällen durch geeignete Wahl der Analyt-Linien vermeiden. Im Fall von ICP-MS sind spektrale Interferenzen jedoch eine größere Herausforderung, da viele verschiedene polyatomare Ionen mit dem Analyten interferieren können. Zum Beispiel ist die korrekte Quantifizierung von Palladium (als $^{105}Pd^{+}$) in der Gegenwart von Kupfer schwierig, da im ICP ein Kupfer-Argon Molekül-Ion entsteht ($^{40}Ar^{65}Cu^{+}$), das dasselbe nominelle Masse-zu-Ladungsverhältnis wie Palladium hat. Auch wenn andere Palladiumisotope für die Messung verwendet werden, kann dieses Problem nicht gelöst werden, da es eine Vielzahl an möglichen Interferenzen gibt, die auch die Messung dieser anderen Isotope stören können.

Deshalb ist es notwendig, Palladium und Kupfer vor der Messung chemisch voneinander zu trennen, um eine selektive Messung zu erlauben.

(3) *Sensitivität.* Im Fall von sehr niedrigen Analytkonzentrationen (zum Beispiel Platin in Feinstaub) bietet eine konventionelle Messung nicht die benötigte Empfindlichkeit, weshalb eine chemische Anreicherung und Aufkonzentrierung des Analyten notwendig ist.

In der vorliegenden Dissertationsschrift werden neue Strategien vorgestellt, die zur Vermeidung der genannten Probleme entwickelt wurden. Die Dissertation ist in drei Teile gegliedert:

Der erste Teil dieser Dissertation beschreibt Experimente, die darauf abzielen, herausfordernde flüssige Proben mittels "dried droplet Laser Ablation" mittels ICP-basierter Techniken zu messen. Der Vorteil von Laserablation gegenüber herkömmlichen Zerstäubern liegt darin, dass Viskosität, gelöste organische Bestandteile, oder Säuregehalt den Probeneintrag nicht beeinflussen. Die Probleme, die unter Punkt (1) angeführt wurden können daher mit dieser Methode überwunden werden. Darüber hinaus kann eine gewisse Anreicherung des Analyten erzielt werden, wenn ein Flüssigkeitstropfen eingetrocknet wird. Das führt zu einer verbesserten Empfindlichkeit, wie unter Punkt (3) erwähnt. Die generelle Sensitivität von Laserablation ist auch deshalb besser, weil diese Methode eine höhere Probeneintrags-Effizienz als herkömmliche Zerstäuber hat. Es stellte sich jedoch heraus, dass die Quantifizierung und die Probenvorbereitung einer gewissenhaften Optimierung bedürfen, um verlässliche Ergebnisse zu erhalten. Teil eines veröffentlichten Review-Artikels ist diesem Thema gewidmet. Zwei veröffentlichte Artikel beschreiben verbesserte Probenvorbereitungs- und Messmethoden für die Analyse von flüssigen Proben mittels Laserablation. Der erste Artikel beschreibt die Anwendung von Filterpapier Stanzen, die als Substrat für flüssige Proben verwendet werden. Die Verwendung solcher Stanzen wurde bereits in der Literatur erwähnt, aber die bisherige Laserablations-Strategie lieferte quantitative Daten nur über Umwege. Grund dafür ist eine inhomogene Analytverteilung auf den Filterstanzen. Bisher erfolgte Versuche, diese Inhomogenität durch Einsatz eines internen Standards auszugleichen waren problematisch, da sie einer Optimierung von Fall zu Fall bedurften. Die hier vorgestellte neue Laserablationsmethode bietet eine allgemeine und repräsentative Probenahme-Strategie, die zentrosymmetrische Inhomogenitäten auf scheibenförmigen Filterstanzen ausgleicht. Die Verwendbarkeit der Methode wurde durch die Quantifizierung von Phosphor in biochemischen Fermentationsmedien gezeigt. Der zweite Artikel in diesem Teil der Dissertation beschreibt eine neue Methode zur Probenvorbereitung, die es auf schnelle und einfache Weise erlaubt, eine flüssige Probe in kleinere Aliquote zu

teilen, um sie anschließend mittels Laserablation zu messen. Im Gegensatz zur oben erwähnten Methode mit Filterstanzen, entfallen in der hier vorgestellten Methode sämtliche Pipettierschritte. Das hat Vorteile hinsichtlich der Probenahme, da diese nun außerhalb des analytischen Labors durchgeführt werden kann. Die Anwendbarkeit der Methode wird mit der Quantifizierung von Blei in menschlichem Vollblut gezeigt.

Der zweite Teil der Dissertation stellt eine neue Extraktionsmethode vor, die so genannte "dispersed particle extraction" (DPE), die es ermöglicht, Analyten und Matrix-Elemente chemisch voneinander zu trennen. Die DPE-Methode verwendet Sorbenspartikel, die direkt in der flüssigen Probe suspendiert werden. Der Analyt wird auf der Partikeloberfläche selektiv eingefangen, was es erlaubt, ihn von der umgebenden Probenmatrix zu trennen, sowie ihn anzureichern. Nachdem der Analyt derart isoliert wurde, wurden die Sorbenspartikel aufgelöst, und die Lösung verdünnt, um mittels Zerstäuber ins ICP gebracht werden zu können. Verglichen mit etablierten Methoden, die für die selbe Aufgabe eingesetzt werden können (z.B. Solid Phase Extraction) vermeidet die hier vorgestellte DPE Methode das langwierige Konditionieren von Säulenmaterial, Probleme die aus unvollständiger Elution des Analyten resultieren, sowie Verschleppungen und Memory-Effekte, da für jede Extraktion frisches Sorbensmaterial verwendet wird. Ein veröffentlichter Artikel beschreibt die Anwendung von DPE für die Messung von Platin und Palladium in urbanem Straßenstaub. In diesem Artikel zeigen wir, dass Matrix-Elemente mit der DPE-Methode effektiv abgetrennt werden können, und wir wenden die Methode auf in Wien gesammelten Straßenstaub an. Von den drei oben erwähnten Problemen der ICP beschäftigt sich die in diesem Teil der Dissertation präsentierte Arbeit mit der Selektivität (2), da Matrixbestandteile abgetrennt werden. Die dabei ebenfalls erreichte Anreicherung ist nur sehr gering, was aber im vorliegenden Kontext wenig ausmacht, da die Straßenstaubproben in ausreichenden Mengen vorhanden sind, was noch immer für eine ausreichende Nachweisgrenze der Methode erlaubt.

Der dritte Teil dieser Dissertation beschreibt die Kombination von DPE mit dried droplet Laserablation. Durch den Einsatz von DPE werden Matrixelemente aus der Probe entfernt, wie bereits im zweiten Teil dieser Arbeit beschrieben. Durch Optimieren des End-Volumens wurde in diesem Fall eine deutliche Anreicherung des Analyten möglich. Durch diese stärkere Anreicherung enthielten die Lösungen am Ende der Probenvorbereitung jedoch hohe Konzentrationen an gelöstem Sorbensmaterial, was sie für eine Messung mittels Zerstäuber ungeeignet machte. Auch war das End-Volumen so klein, dass sich eine Analyse mittels Zerstäuber schwierig gestaltet hätte. Deshalb wurden die Lösungen mittels dried droplet Laserablation in ein ICP-MS gebracht, wie im ersten Teil dieser Arbeit bereits beschrieben.

Diese Methode wurde verwendet, um die Konzentration von Pt und Pd in Feinstaub zu messen. Dank der Selektivität der DPE Methode, der verbesserten Sensitivität, die sich aus der verbesserten Anreicherung ergab, sowie dem Umstand, dass Laserablation auch mit kleinen Probenvolumina und komplexer Matrix zurechtkommt, war es möglich, die kurzzeitige Variation der Platinkonzentration in Feinstaub mit einer Zeitauflösung von nur 4 Stunden zu bestimmen. Die im dritten Teil dieser Arbeit vorgestellten Ergebnisse bieten die Möglichkeit, Matrix-Effekte zu umgehen (1), und sowohl Selektivität (2) als auch Sensitivität (3) zu verbessern.

SAMENVATTING (VLAAMS/ NEDERLANDS)

Instrumentatie gebaseerd op een inductief gekoppeld plasma (ICP) wordt routinematig gebruikt voor de kwantificatie van metalen in vloeibare stalen en oplossingen. De combinatie van het ICP met optische emissie spectroscopie (ICP-OES) is een oudere en robuustere techniek, die echter vaak niet gevoelig genoeg is om (ultra-)spoorelementen te bepalen. ICP-massaspectrometrie (ICP-MS) – een nieuwere variant – is veel gevoeliger, maar is anderzijds wel meer onderhevig aan effecten gelinkt aan de matrix van de stalen (matrixeffecten). ICP-OES en ICP-MS instrumenten zijn gevestigde waarden binnen routine-labo's. De accuratesse van beide technieken kan echter bedreigd worden door de volgende fenomenen:

(1) **Matrixeffecten ten gevolge van de eigenschappen van de stalen.** Vloeibare stalen moeten aan verschillende voorwaarden voldoen inzake viscositeit, concentratie aan opgeloste organische stoffen en anorganische zouten en pH om succesvol geanalyseerd te kunnen worden met ICP-gebaseerde instrumentatie (typisch uitgerust is met een verstuiver en verstuiverkamer voor monsterintroductie). Veel types stalen, zoals volbloed of fermentatiemedia, voldoen niet aan deze voorwaarden; ten gevolge hiervan kan de monsterintroductie-efficiëntie voor stalen en kalibratiestandaarden en voor stalen onderling verschillen, wat resulteert in een verschillende instrumentrespons. In deze omstandigheden wordt een correcte kwantificatie moeilijk. Om dit probleem aan te pakken en het effect van de matrix te verminderen, zal een staal doorgaans een uitgebreide staalvoorbereiding ondergaan, die bijvoorbeeld een verdunning of chemische digestie zal omvatten. Helaas zal door de verdunning ook de concentratie van het element dalen, wat mogelijk problemen oplevert voor de bepaling van (ultra-)spoorelementen.

(2) **Selectiviteit.** Het vloeibaar staal of de oplossing kan, ondanks verdunning, ook significante hoeveelheden van elementen bevatten die aanleiding geven tot spectrale interferenties in ICP-OES en ICP-MS. Spectrale interferenties zijn mogelijk in ICP-OES (vooral elementen met veel emissielijnen zoals Fe vormen een probleem), maar in de meeste gevallen kunnen deze interferenties omzeild worden door andere emissielijnen van het analiet te gebruiken. Spectrale interferenties in ICP-MS zijn echter moeilijker te vermijden, gezien veel polyatomische ionen kunnen interfereren met het analiet. De kwantificatie van palladium-105 ($^{105}Pd^{+}$) wordt bij voorbeeld bemoeilijkt in de aanwezigheid van een hoge concentratie aan Cu, omdat het koper-argide ion ($^{40}Ar^{65}Cu^{+}$) dezelfde nominale massa heeft als $^{105}Pd^{+}$. Gebruik maken van een andere isotoop van palladium lost dit probleem niet op, gezien er vele interferenties mogelijk zijn. Palladium moet derhalve op voorhand chemisch van het

Cu geïsoleerd worden, tenzij een andere methode kan worden gebruikt om de spectrale overlap te vermijden, zoals gebruik van een hogere massaresolutie (sector veld ICP-MS) of van chemische resolutie of kinetische-energiediscriminatie (quadrupool ICP-MS met een botsings-/reactiecel).

(3) Gevoeligheid. In het geval van zeer lage analietconcentraties, biedt conventionele analyse niet de vereiste gevoeligheid, hierdoor is chemische aanrijking noodzakelijk.

In deze doctoraatsthesis worden nieuwe strategieën voorgesteld die ontwikkeld werden om deze problemen op te lossen. Deze thesis is onderverdeeld in drie delen:

Het eerste deel van deze thesis beschrijft de experimenten met betrekking tot de analyse van uitdagende vloeibare stalen met behulp van gedroogde druppel laser ablatie (LA) in combinatie met ICP-gebaseerde technieken. Het voordeel van LA ten opzichte van pneumatische verstuiving is dat de viscositeit van het staal, de concentratie aan organische stoffen en de zuurtegraad geen invloed hebben op de efficiëntie van de staalintroductie, waardoor de problemen vermeld onder punt (1), omzeild worden. Bovendien wordt er ook een analietaanrijking gerealiseerd bij het drogen van een vloeibaar staal, aangezien het solvent verwijderd wordt. Dit resulteert in een verbeterde gevoeligheid, zoals vermeld onder punt (3). Daarenboven wordt de gevoeligheid verbeterd door gebruik te maken van LA, aangezien LA-systemen een betere monsterintroductie-efficiëntie vertonen dan verstuivers. Er werd echter vastgesteld dat succesvolle LA-analyse een nauwgezette optimalisatie van de kwantificatiestrategie en staalvoorbereiding vereisen. Een deel van een gepubliceerd reviewartikel beschrijft deze uitdagingen in meer detail. Twee gepubliceerde artikels beschrijven een verbeterde staalvoorbereiding en meetstrategieën voor analyse vloeibare stalen met gebruik van LA voor monsterintroductie. In het eerste artikel wordt het gebruik van voorgesneden papieren filterschijven als substraat voor vloeibare stalen besproken. Over het gebruik van deze voorgesneden papieren filterschijven werd al eerder gerapporteerd in de wetenschappelijke literatuur, maar met de voordien toegepaste strategie was het verkrijgen van kwantitatieve informatie geen sinecure omdat de verdeling van het analiet over de filterschijf niet homogeen is. Vroegere pogingen om rekening te houden met deze heterogeniteit, gebruikmakend van een inwendige standard, waren problematisch, aangezien deze voor elke nieuwe toepassing opnieuw geoptimaliseerd moesten worden. De in dit werk ontwikkelde benadering met LA biedt een universele en representatieve strategie, die rekening houdt met de radiaal-symmetrische heterogeniteit van het analiet voor elk vloeibaar staal aangebracht op de papieren filterschijven. De toepasbaarheid van deze methode werd aangetoond aan de hand van de kwantificatie van fosfor in biochemische fermentatiemedia

als een case studie. Het tweede artikel in dit deel van de thesis beschrijft een nieuwe methode voor staalvoorbereiding die een snelle en rechtstreekse verdeling van het vloeibaar staal in meerdere kleinere hoeveelheden mogelijk maakt; deze kleine hoeveelheden kunnen vervolgens geanalyseerd worden gebruikmakend van LA voor monsterintroductie. In tegenstelling tot de bovenvermelde methode, gebruikmakend van voorgesneden papieren filterschijven, omvat deze staalvoorbereiding geen manuele stappen voor het pipetteren. Dit is voordelig aangezien staalname hierdoor mogelijk is buiten het laboratorium. De toepasbaarheid van deze methode werd aangetoond door de succesvolle kwantificatie van lood in humaan volbloed.

Het tweede deel van deze thesis stelt een nieuwe extractietechniek voor – gedispergeerde partikel extractie (DPE). Deze strategie maakt chemische scheiding van analiet(en) en matrixelementen aanwezig in het staal mogelijk. Deze DPE methode maakt gebruik van sorbentpartikels die in suspensie gebracht worden in het vloeibare staal. Het analiet wordt selectief gevangen op het partikeloppervlak; dit maakt zowel de afscheiding vanuit het omringende vloeibare staal, als aanrijking mogelijk. Na isolatie van het analiet / de analieten worden de sorbentpartikels opgelost en wordt de oplossing licht verdund om deze compatibel te maken voor monsterintroductie met pneumatische verstuiving. In vergelijking met gevestigde technieken die hetzelfde resultaat kunnen behalen (e.g., vaste fase extractie), vermijdt de voorgestelde DPE methode de tijdrovende conditionering van kolommen, problemen gerelateerd aan onvolledige elutie van het analiet, alsook cross-contaminatie en geheugeneffecten, aangezien telkens nieuw sorbent wordt gebruikt. Een gepubliceerd artikel beschrijft de toepassing van DPE voor de bepaling van platina en palladium in stedelijk straatstof. In dit artikel wordt aangetoond dat deze analietelementen efficiënt worden afgescheiden van de aanwezige matrixelementen door de DPE methode. Deze methode werd toegepast in de context van de bepaling van Pt en Pd in straatstof verzameld in Wenen. Het in dit tweede deel van de thesis voorgestelde werk pakt één van bovenvermelde uitdagingen van ICP aan, namelijk het verlies van gevoeligheid (2) door het verwijderen van de storende matrixcomponenten. De gebruikte methode bracht geen significante aanrijking van de analietelementen met zich mee, maar in deze context vormde dit geen probleem omdat er genoeg straatstof voorhanden was, waardoor de detectielimieten van de methode voldoende bleken.

Het derde deel van deze thesis beschrijft de combinatie van DPE met LA van gedroogde vloeibare stalen. Door gebruik te maken van DPE worden aanwezige matrixelementen verwijderd uit de analietfractie, zoals besproken in het tweede deel van deze thesis. Optimalisatie van het uiteindelijke staalvolume maakt het mogelijk om tegelijk een significante aanrijking van de analieten te realiseren. Echter, door toename van de aanrijkingsfactoren,

bevatten de oplossingen finaal ook hogere concentraties aan opgelost sorbent en waren deze niet geschikt voor monsterintroductie via een verstuiver. Bovendien was er finaal te weinig volume voor monsterintroductie via pneumatische verstuiving. Daarom werden de oplossingen geïntroduceerd in het ICP via ablatie van de gedroogde druppels, zoals besproken in het eerste deel van deze thesis. De methode werd toegepast om Pt en Pd concentraties te meten in luchtpartikels. Dankzij de selectiviteit van DPE en de daarmee gepaarde stijging van de gevoeligheid, de verhoogde aanrijkingsfactoren en de mogelijkheid van LA om te gaan met een hoge matrixlading, was het mogelijk om de korte-termijn variatie van het gehalte Pt in luchtpartikels met 4-uur intervallen te registreren. Het in dit derde deel van deze thesis voorgestelde onderzoek toont de mogelijkheid aan om matrixeffecten (1) te vermijden, evenals problemen met betrekking tot selectiviteit (2) en gevoeligheid (3) succesvol aan te pakken.

1. INTRODUCTION

A plasma can be seen as a partially ionized gas, containing free electrons and ions. Atoms that are brought into such a plasma can enter electronically excited states. When such electronic states decay, element-specific electromagnetic radiation is emitted, which can be used to obtain qualitative and quantitative information by means of optical emission spectrometry. Inside the plasma, atoms can also become ionized and those ions can be extracted from the plasma and separated according to their mass-to-charge ratio in a mass-spectrometer.

Bădărău and co-workers reported using an induction plasma as excitation source for optical emission spectrometry as early as the mid 1950ies [1]. Throughout the 1960ies, also inductively coupled plasmas (ICPs) were investigated as excitation sources, and the first commercial ICP optical emission spectrometer (ICP-OES) was available in the early 1970ies [2]. Combining the ICP source with a mass spectrometer was first reported by Houk and co-workers in 1980 [3], with first commercial ICP-MS instruments entering the market in the early 1980ies.

Nowadays, both ICP-OES and ICP-MS are available as rugged and user-friendly commercial instruments, delivered in combination with various types of sample introduction devices. The conventional set-up for the analysis of liquid samples consists of a nebulizer and a spray-chamber. Apart from conventional concentric nebulizers, specialized nebulizer designs allow for maximum sensitivity (e.g., total consumption nebulizers), they accommodate high concentrations of dissolved organics and inorganics (e.g., high solid nebulizers), or are tolerant towards suspensions (e.g., ultrasound-assisted nebulizers or V-groove nebulizers). Spray chambers (e.g., cyclonic, double-pass, heated, cooled) in combination with dedicated accessories (e.g., membrane de-solvation units) allow to minimize the amount of solvent entering the ICP, in order to reduce spectral interferences coming from the solvent, or to improve sensitivity. Automated sample introduction and dilution systems improve sample-throughput in routine laboratories, and improve repeatability of the measurement. A recent review article gives a comprehensive overview of the characteristics of sample introduction devices for liquid samples in ICP-based techniques [4].

Besides the mentioned accessories, also the ICP-OES/-MS instrumentation itself was improved over the years. For example, end-on (axial) plasma view was introduced in ICP-OES for better sensitivity, and collision/reaction cells were introduced to quadrupole ICP-MS to cope with polyatomic interferences. Lately, also triple-quad technology was introduced to quadrupole ICP-MS, to resolve especially challenging spectral interferences [5].

With the abovementioned developments in sample introduction devices and instrument design, many analytical challenges related to ICP-based techniques can be solved. However, some problems cannot be tackled solely from an instrumental point of view, but require an appropriate choice of sample preparation in combination with a suitable sample introduction strategy.

In this work, three specific challenges related to liquid samples are addressed, and new approaches are developed to overcome them. In order to better understand the improvements developed, the challenges shall be discussed in the following chapter.

2. Analytical Challenges

2.1. Challenge 1: Matrix effects due to properties of Liquid Samples

Liquid samples suitable for measurement with ICP-based techniques usually contain water as solvent which constitutes roughly 98% or more of the entire sample. Although organic solvents can be used in ICP-based techniques as well, and although most of the mentioned considerations regarding the composition and matrix-effects of a "typical" liquid sample hold true also for organic solvents, this discussion focusses on water as solvent, since only aqueous solutions were used throughout the research presented here. Apart from water, samples contain inorganic acids such as nitric acid or hydrochloric acid which are added to the sample to stabilize the analytes against sorption on vessel walls, fouling due to microbial activity, or precipitation which could occur under basic conditions due to, e.g., hydroxide formation. The samples may further contain major elements which are present at high concentrations and which typically are of no analytical interest. Such a major element could be sodium in the form of sodium chloride in seawater, or sodium in the form of acetate/phosphate/etc. arising from buffers added to the sample to obtain a certain pH necessary for chromatographic sample preparation. Lastly, the samples contain minor and trace elements. Only those are typically the target of ICP-based measurements.

A conventional approach to introduce a liquid sample into an ICP is to use a combination of nebulizer and spray-chamber. The nebulizer transforms the liquid into an aerosol, either by means of a pneumatic process (a stream of liquid is brought into a fast stream of gas, which results in breaking up the liquid into small droplets) or by means of an ultrasonic process (a stream of liquid is brought onto a vibrating platform which results in the release of small droplets) [4]. The primary aerosol generated from the nebulizer contains droplets with different sizes, and it is the task of the spray-chamber to cut the size-distribution of the droplets down by removing large droplets [6].

An ICP can usually support 20 µL min^{-1} of water being introduced, without becoming unstable [4]. With higher amounts of water entering the ICP, the plasma temperature drops, resulting in changes in the ionization/excitation processes in the ICP, which eventually results in changed response of the analytical instrument. Besides the total amount of solvent entering the ICP, also the size-distribution of the aerosol is of importance for the instrument's response [4, 6]. Small droplets can be quickly evaporated in the ICP, and the analytes contained in such droplets are readily atomized, excited and ionized. Larger droplets may require longer time for this, and analytes which are present in too large droplets might not be excited/ionized at all, as their residence time in the ICP is shorter than the time required for those processes.

The analytes present in large droplets therefore give a different instrumental response as those present in small droplets.

Changing the size-distribution of the aerosol can therefore change the excitation/ionization efficiency of the ICP, which in turn can lead to different instrumental response. Parameters that may influence the size-distribution of the aerosol are, e.g., sample viscosity, surface tension, and density, acid type and concentration, concentration of major and minor elements, as well as the presence of substances that change the rheological properties of the liquid, such as proteins which might occur in biological fluids like, e.g., whole blood. If viscosity and density change, the mass-flow of sample to the nebulizer changes, while gas flows stay constant, thus changing the operational parameters of the nebulizer, and therefore the size-distribution of the aerosol. Different concentrations of acids and elements can alter the way small particles coincide to form larger particles along the way from the nebulizer to the ICP [4, 6].

The sample matrix does not only influence the sample introduction efficiency, as just discussed, but it can also cause matrix effects within the ICP. For example, variations in the sample composition can result in different plasma temperatures and therefore electron number densities within the ICP. This causes different instrumental response for the analyte, both in ICP-MS and ICP-OES. Additionally, matrix-constituents can result in spectral interferences which will be discussed separately in chapter 2.2.

Summing up, there is a large number of factors that may influence the instrumental response for one specific sample. As each sample can contain different concentrations of substances causing these non-spectral matrix-effects, it can be difficult to obtain reliable quantitative data.

Different quantification approaches were developed in order to counteract the matrix-effects caused by the sample composition. The most straight-forward approach is to simply dilute the sample until the difference between samples or the difference between samples and standards becomes negligible. Too strong a dilution has of course to be avoided, since it compromises the sensitivity. Typically, this is done by analyte recovery studies as a function of dilution, which can be time-consuming. A recent approach presented by the Hieftje group shows how gradient pumps can be used to continuously dilute one sample during one single measurement, in order to determine this optimum dilution factor [7].

Instead of using dilution to bring the matrix of the liquid sample closer to the matrix of calibration standards, it is also possible to match the calibration standards to the samples by adding matrix-constituents to the calibration standards. This approach is called the matrix-

adjustment technique. Although useful in some cases, it does not account for variations between individual samples, thus limiting its universal applicability.

If matrix-effects still cannot be overcome either because very high dilution factors would be necessary or because variations in-between samples are large, the method of standard addition can be applied [8]. Each sample is split into several aliquots, which are mixed with increasing amounts of the analyte and measured. By extrapolation, it is possible to determine the analyte-concentration in the sample. Any matrix effect originating from the individual sample is therefore incorporated into the calibration approach.

Instrumental drift over long measurement periods can be tackled by the addition of an internal standard to the sample as well as to the calibration standards. Such drift can for example arise if the nebulizing efficiency gradually changes due to the deposition of solids on the nebulizer nozzle. The internal standard is not present in the native sample, behaves – ideally – very similar to the analyte in the ICP and therefore suffers the same change in instrument response over time as the analyte. By normalizing the analyte to the internal standard, instrumental drift (and to a certain extent also non-spectral matrix effects) can thus be mathematically corrected.

The mentioned approaches to overcome matrix-effects all have in common that they require extensive sample handling and are time consuming and therefore expensive in the long run. One goal of the research carried out during this doctoral thesis was therefore to find an alternative way of sample introduction for challenging liquid samples which does not suffer from the abovementioned matrix-effects.

2.2. Challenge 2: Selectivity of ICP-MS and ICP-OES Measurements

Selectivity in terms of being able to distinguish between signals actually coming from the analyte and signals coming from spectral interferences is relevant both for ICP-OES and ICP-MS. In the doctoral thesis presented here, spectral interferences in ICP-OES are not discussed, since these are only of minor relevance with the applications investigated here. However, similar considerations regarding the removal of spectral interferences by means of chemical sample preparation are true in case of ICP-MS as well as in case of ICP-OES.

In the plasma of an ICP-MS, analytes are atomized and ionized. These analyte-ions are extracted from the plasma, and separated in a mass analyzer according to their mass-to-charge (m/z) ratio, in order to obtain qualitative and quantitative information. However, there are three sources of spectral interferences which can also produce a signal on the targeted m/z-ratio of the analyte. Firstly, it is possible that another element has an isotope at the same m/z-ratio as the analyte, resulting in isobaric interference. Secondly, it is possible that some ions

become multiply charged in the ICP, which causes signals at m/z-ratios lower than the actual mass of the ionized element (e.g., doubly charged $^{208}Pb^{++}$ gives a signal on m/z = 208/2 = 104 which coincides with $^{104}Pd^{+}$ and causes spectral overlap). Thirdly, molecular ions can be formed in cooler regions of the ICP, and some of these molecules can happen to have a m/z-ratio very close to the m/z-ratio of the analyte, causing spectral overlap (e.g., $^{40}Ar^{35}Cl^{+}$ causes spectral overlap at $^{75}As^{+}$).

Selecting isotopes of the analyte which are not influenced by spectral overlap would be the ideal way of tackling this challenge, but in many cases, this is not possible (e.g., in case of mono-isotopic analytes). Therefore, two approaches from the instrumental point of view can be applied to reduce the impact of spectral interferences on the analyte-signal.

The first approach is to increase the resolution power of the mass spectrometer. This allows to tackle the problem of spectral or isobaric overlap because spectral interferences always have a slightly different m/z-ratio than the analyte. However, when increasing the resolution, the transmission of a mass spectrometer deteriorates, resulting in a loss in sensitivity. In case of samples which contain higher concentrations of the target analyte, or in case where the sample intake can be easily increased, this is a suitable approach. However, when targeting trace-concentrations, compromising the sensitivity is no option.

The second approach aims at removing spectral interferences when operating an ICP-MS at low resolution. This can be achieved via the collision or reaction cell technology, which is based on the higher collisional cross-section of molecular (polyatomic) ions, compared to monoatomic ions. As the "bigger" polyatomic ions statistically collide more often with gas molecules introduced into the beam-path inside the ICP-MS than (monoatomic) analyte ions, their contribution to the signal is over-proportionally reduced due to reactions/collisions with the reaction gas. Interferences can be further cut down by also providing an electrostatic barrier ("kinetic energy discrimination", KED), which removes ions that still have their initial charge, but that lost part of their kinetic energy due to collisions. Lately, the "triple-quad" technique was implemented also in quadrupole ICP-MS analysis. Here, a set of three consecutive quadrupole systems allows to first select the analyte-mass (which can still be interfered by isobaric or spectral interferences), then to induce a selective chemical reaction between gas molecules and the analyte (or the interference) in a second quadrupole, and then to separate the newly produced ions in a third quadrupole. Thus, a separation of analyte and interferences due to chemical reactivity is achieved.

As in case of increasing the resolution of the spectrometer, the "traditional" approaches using collisional filtering are associated with a loss in sensitivity. The triple-quad technique can be

operated without major losses in sensitivity, if gases with sufficiently high reactivity are used [9].

The occurrence of isobaric and spectral interferences is linked to the presence of matrix-constituents that cause said interferences. Therefore, it is also possible to chemically remove critical matrix-elements from the sample before it is brought into the ICP. As the formation of interferences is now impaired due to the absence of interfering matrix elements, the ICP-MS can be operated at low resolution settings with maximal instrumental sensitivity. Traditionally, chemical analyte isolation is achieved by separation of analytes and problematic matrix elements using solid phase extraction. In this technique, the sample passes through a column filled with a resin that selectively traps the analyte while interfering elements are not retained. In a second step, the purified analyte-fraction is released from the sorbent and ready for analysis. The alternative approach is possible as well (i.e., retention of interfering elements, while the analyte is not retained).

The advantages of SPE are the availability of a large variety of resins with specific selectivity towards different analytes, as well as the availability of standardized procedures. Carrying out a SPE separation is however often related to work-intensive and time-consuming manual steps (e.g., [10]). Conditioning of the sorbent material and elution of the analyte require careful optimization. Since the sorbent material is often used several times, carry-over or memory-effects need to be monitored, and changes in sorbent properties due to ageing need to be considered (loss in capacity, increased back-pressure resulting in longer time required for the sample to pass through the resin). Although being well-established, alternatives to SPE are necessary in order to improve sample throughput and in order to reduce costs. In this thesis, one alternative to SPE was developed (see chapter 3.2).

2.3. Challenge 3: Sensitivity

The sensitivity of an analytical method (or the "method detection limit") is a measure for how much of the analyte can still be detected in the sample. As opposed to the "instrumental detection limit", the sensitivity of the method takes into account all steps including sample preparation as well as the actual measurement. The method detection limit is therefore an important figure of merit, as it shows the limitations of a given method at low concentrations, and as it allows to compare different methods.

The following factors influence the method detection limit. First, the sample intake determines, how much of the analyte ($m_{analyte}$) enters the analytical procedure (see figure 1). If the sample is a solid, it has to be converted into a liquid solution, e.g., by chemical sample digestion, resulting in a clear solution which is filled up to a final volume ("digest volume"). If

the sample is already liquid, sample digestion is still applied in many cases, in order to reduce the amount of organic sample constituents (e.g., UV-assisted microwave digestion). By doing so, the sample is diluted as well, thus also reducing unspecific matrix-effects as discussed in chapter 2.1. Dilution also allows to add internal standards or analyte-spikes for standard-addition. The resulting diluted sample solution then corresponds to the "digest volume" in figure 1. Although analyte-losses are possible during this first step, they are usually small in case of inorganic analytes. This is not the case for elements that might form volatile species, as for example arsenic or mercury, and minimizing analyte-losses in those cases contributes to a better (i.e., lower) method detection limit.

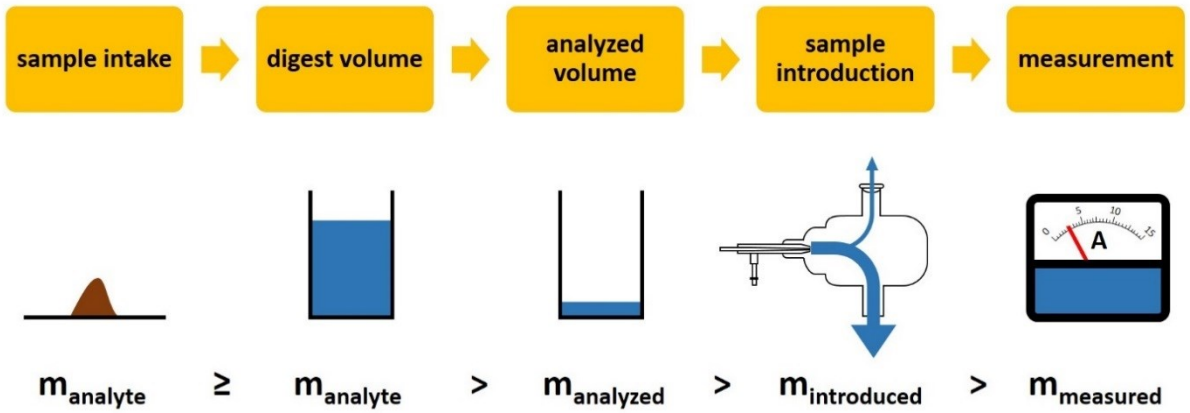

Figure 1 Schematic of processes that cause the method detection limit to deteriorate (m always corresponds to the analyte mass available at each stage of sample preparation/sample introduction).

The sample solution prepared in the first step is then introduced into the instrument for measurement. As digest volumes are usually in the range of several milliliters, only a fraction of the sample (and therefore the analyte) is actually brought to measurement. The analyzed mass of analyte ($m_{analyzed}$) is therefore often smaller than the initially applied mass ($m_{analyte}$). Also during sample introduction, only a part of the liquid is actually introduced into the instrument, thus further reducing the mass of analyte available for detection to $m_{introduced}$. When using conventional nebulizers for sample introduction, only a few percent of the analyzed sample volume is actually brought into the instrument, the rest of the aerosol is cut off in the spray-chamber and goes to the waste. Finally, the instrument has a certain detection efficiency, i.e., of all analyte atoms that enter the instrument, only a fraction causes a signal on the detector and is finally recorded as signal. For example, in radial ICP-OES measurements, only part of the plasma is observed during measurement, and radiation might additionally be absorbed by atmospheric gases surrounding the plasma, or by windows and optics in the spectrometer. In ICP-MS measurements, the ionization efficiency of the ICP, the extraction efficiency of the ICP interface, as well as the transmission efficiency of the mass

spectrometer contribute to losses of ions. When choosing high mass resolution, or when using collisional filtering for measurement, the transmission efficiency is generally worse than under standard measurement conditions.

Taking into account the abovementioned factors that worsen method detection limits, the following steps can be taken as countermeasures. Firstly, the sample intake could be increased, thus increasing the overall amount of analyte entering the analytical procedure. Secondly, the digest volume could be kept as small as possible by avoiding too strong dilution, or by evaporating solvents after digestion. This also helps to use the sample solution more efficiently (i.e., by increasing $m_{analyzed}$). Thirdly, sample introduction efficiency could be increased by using, e.g., alternative nebulizer designs such as "total consumption nebulizers". Lastly, instruments and instrumental conditions can be applied that provide maximum detection efficiency (e.g., axial plasma view in ICP-OES, high efficiency interfaces in ICP-MS).

Increasing the sample intake and/or reducing the digest volume is often not possible at the same time, as this often means that unspecific matrix-effects are enhanced (see chapter 2.1). Also in case of limited sample availability (e.g., forensic applications), increasing the sample intake is no option. Performing chemical sample pre-treatment (as described in chapter 2.2) can help during this step, as it allows to remove the sample matrix, while it also allows to pre-concentrate the analyte. Chemical sample pre-treatment therefore allows to minimize the "digest volume" (see figure 1), and thus improving method detection limits.

Increasing the detection efficiency is often not possible, as any analytical task requires a certain instrumental set-up to guarantee selectivity of the measurement (e.g., to remove analyte-specific spectral interferences as discussed in chapter 2.2).

3. Methods Applied to Tackle the Challenges

3.1. Method 1: Dried Droplet Laser Ablation

In 2005, Yang, Sturgeon and Mester [11] presented an innovative approach to introduce a liquid sample into an ICP-MS. They deposited a small droplet of the sample on a polymeric substrate, waited until the solvent was evaporated, and used a laser ablation system to obtain a laser-generated dry aerosol which was then brought into the ICP.

In order to discuss the advantages of this approach, the working principle and the limitations of laser ablation ICP-MS/-OES will be shortly summarized. When a solid is irradiated with laser light of high energy (i.e., short wavelength), this causes molecular bonds to break and material to be removed from the solid's surface. This process of laser ablation produces small solid particles suspended in the gas phase. Similar as in the case of nebulizers, there are different factors that influence the ablation and transport efficiency of this process [12]. The particle size distribution and the transport efficiency of a solid steal sample is for example different from that of a ceramic sample. Therefore, the instrumental response depends on the sample matrix - also in laser ablation.

What is now the difference between matrix-effects in nebulizers and in laser ablation, when it comes to the analysis of liquid samples? In the first case, the matrix-effects are directly related to the liquid's properties, as discussed in chapter 2.1. In the case of dried droplet laser ablation however, the sample-matrix consists to a large proportion of the solid substrate on which the liquid droplet is deposited. Since this solid substrate is the same for calibration standards and samples, the matrix which is relevant for laser ablation is more or less constant, even though the liquid matrices of the deposited droplets might vary.

Therefore, the preparation of dried droplets can be regarded as a way of matrix-adjustment. However, and in contrast to matrix-adjustment in the context of nebulizer-based sample introduction, this does not require any manipulations of the liquid sample. Therefore, dried droplet laser ablation is a quick and straight-forward way to achieve matrix-matching between challenging liquid samples and calibration standards.

3.2. Method 2: Dispersed particle Extraction

As mentioned in chapter 2.2, separating the analyte from the surrounding matrix can be achieved by means of different established techniques (solid phase extraction being the most popular one). As mentioned above, those techniques often suffer from low sample

throughput and high reagent consumption, as well as memory-effects and carry-over. Dispersed Particle Extraction (DPE) was therefore developed [13] as an alternative approach.

Instead of using a column filled with sorbent material and passing the liquid sample through this column, dispersed particle extraction uses sorbent particles which are freely suspended in the liquid sample. To provide sufficient capacity, the sorbent material used for dispersed particle extraction has a very high specific surface, providing many retention sites for the analyte. Once the analyte is immobilized on the particle's surface, it can be separated from the surrounding liquid by centrifuging the sample and collecting all particles on the bottom of the vial. The supernatant clear solution is decanted, the particles are washed with fresh solvent, and finally re-suspended in a small volume. Either the analyte-loaded particles are measured in the form of a suspension (which could potentially lead to nebulizer blockage), or the particles are dissolved by means of mineral acids prior to measurement.

Dispersed particle extraction allows to remove the sample matrix based on different chemical behavior of analytes and matrix constituents, as well as to pre-concentrate the sample, since the final volume obtained after dispersed particle extraction can be chosen to be smaller than the initial volume.

In the doctoral thesis presented here, platinum and palladium originating from automotive catalysts were analyzed in environmental samples by means of DPE. Due to their low concentrations, Pt and Pd were analyzed by means of ICP-MS, but, as discussed in section 2.2, spectral and isobaric interferences are problematic in ICP-MS detection. Table 1 gives an overview of typical interferences observed in ICP-MS measurements of Pt and Pd. They are separated into isobaric interferences (other elements with isotopes at the same m/z-ratio as the analyte), as well as into molecular ions (formed in the presence of oxygen or argon), and into doubly charged ions. As can be seen from table 1, many common elements are problematic in Pt and Pd measurements via ICP-MS. As these elements are present in the samples at much higher concentrations than the analytes, even low formation probabilities of the mentioned spectral interferences can cause significant impact on the measurement of (ultra-) trace concentrations of Pt and Pd.

Table 1. Some spectral interferences possible in ICP-MS measurements of Pt and Pd.						
	m/z	natural isotopic abundance (%)	Type of interference			
			isobaric	oxide	argide	doubly charged
Pd	102	1.02	^{102}Ru	$^{86}Sr^{16}O$	$^{40}Ar^{62}Ni$	^{204}Hg ^{204}Pb
	104	11.14	^{104}Ru	$^{88}Sr^{16}O$	$^{40}Ar^{64}Zn$	^{208}Pb
	105	22.33		$^{89}Y^{16}O$	$^{40}Ar^{65}Cu$	
	106	27.33	^{106}Cd	$^{90}Zr^{16}O$	$^{40}Ar^{66}Zn$	
	108	26.46	^{108}Cd	$^{92}Zr^{16}O$	$^{40}Ar^{68}Zn$	
	110	11.72	^{110}Cd	$^{94}Zr^{16}O$	$^{40}Ar^{70}Ge$	
Pt	190	0.01	^{190}Os	$^{174}Yb^{16}O$		
	192	0.78	^{192}Os	$^{174}Yb^{18}O$ $^{176}Hf^{16}O$		
	194	32.97		$^{178}Hf^{16}O$		
	195	33.83		$^{179}Hf^{16}O$		
	196	25.24	^{196}Hg	$^{180}Hf^{16}O$		
	198	7.16	^{198}Hg	$^{182}W^{16}O$		

Using higher resolution or collision-based filtering techniques (as discussed in chapter 2.2) would compromise the method detection limit, and in some cases extremely high resolution would be required to resolve the interference. Therefore, chemical isolation of the target analytes, combined with pre-concentration was carried out by means of dispersed particle extraction. The basis for the separation approach lies in the formation of anionic Pt and Pd chloro-complexes which can be separated from cationic interferences by means of strong anionic exchanger functionalities (quarternary amine groups) attached to the particle surface.

3.3. Combining Method 1 and Method 2

As outlined in chapter 3.1, dried droplet laser ablation is a sensitive and straight-forward method for the quantification of trace elements in challenging liquid samples. It has however one shortcoming: if the sample contains matrix-elements that cause spectral interferences (as outlined in chapter 2.2), dried droplet laser ablation provides no possibility to remove them prior to measurement.

Also, dispersed particle extraction (as discussed in chapter 3.2) has limitations, as a certain concentration of sorbent particles in the solution intended for ICP-MS analysis cannot be exceeded when using nebulizer-based sample introduction. This makes it impossible to pre-concentrate the sample to a very small final volume in order to obtain highest pre-concentration factors.

The combination of dispersed particle extraction with dried droplet laser ablation offers the possibility to overcome those two limitations. Interfering matrix elements are effectively

removed by dispersed particle extraction. Also, the samples can be pre-concentrated to a very small final volume (thus improving sensitivity, see chapter 2.3), as the dried droplet laser ablation method accommodates also high concentration of dissolved sorbent material.

Moreover, the dry plasma conditions of laser ablation (i.e., the absence of water) allows to further reduce the possibility of oxide-based spectral interferences as listed in table 1, and the superior sample introduction efficiency of laser ablation also contributes to an improved sensitivity.

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5. Scientific Publications

5.1. Research carried out during the PhD thesis

This thesis is composed of five publications, of which four are already published, whereas one publication is submitted to peer-review. The articles discuss different challenges as described in chapter 2, making use of the methods and techniques outlined in chapter 3. In addition, three poster contributions are included in this thesis. They describe another application of dispersed particle extraction – the selective extraction and pre-concentration of iron from seawater and digested whole blood. At the time of submitting this thesis, the publication describing this research is in preparation.

The first publication is entitled "Recent advances in quantitative LA-ICP-MS analysis: challenges and solutions in the life sciences and environmental chemistry" (Analytical and Bioanalytical Chemistry, 407 (2015) 6593 – 6617). It is a review article about different quantitative approaches using laser ablation in combination with ICP-MS. It has to be noted that only the chapter about dried droplet laser ablation is an integral part of this doctoral thesis (article pages 6605 – 6610). The chapter contains a (at the date of publication, and to the best of the author's knowledge) comprehensive list of all applications of dried droplet laser ablation. The publication history is outlined, and all published approaches of sample preparation are grouped into three methods. The first method makes use of a hydrophobic surface, onto which an aqueous sample droplet is deposited and dried. The second approach uses filter paper as substrate for droplet deposition, and the third approach relies upon a small circular disk of filter paper attached to a hydrophobic surface. All three approaches are compared in terms of method-inherent systematic errors, matrix-effects, sensitivity, reproducibility, as well as practicality. The first approach is superior in terms of sensitivity, although it suffers from more pronounced matrix-effects than the other two approaches (this is related to the morphology of the dried residue and its interaction with the laser), whereas the second approach has the best practicality, since the liquid is immediately consumed by the pores of the filter paper, thus allowing to collect samples "in the field" without the danger of spilling the samples before they dry. This approach however is the least sensitive one and can suffer (depending on the sample) from severe matrix-effects due to chromatographic fractionation within the filter paper. The third approach avoids chromatographic separation, and offers better sensitivity, but it requires a longer drying time and the production of the substrates intended for droplet deposition is cumbersome. Each approach has however advantages under certain conditions, and the aim of the chapter is to give a guideline as to which approach to select in which case.

The second publication enclosed in this thesis is entitled "Radial line-scans as representative sampling strategy in dried-droplet laser ablation of liquid samples deposited on pre-cut filter paper disks" (Spectrochimica Acta Part B, 101 (2014) 123-129). This article discusses in more detail the advantages and disadvantages of the third method described in the abovementioned review article. One general shortcoming of the method using pre-cut filter paper disks on hydrophobic surfaces is that the sample dries faster at the rim of the paper disk, thus resulting in a pre-concentration of all sample constituents on this rim. If the sample is analyzed by means of laser ablation in the center of the paper disk, the analyte-concentration is underestimated, whereas an analysis at the rim of the paper would lead to an over-estimation. As the degree of chromatographic separation across the substrate could depend on the liquid sample matrix (i.e., a difference between standards and samples is possible), quantification is not self-evident with this kind of sample-preparation. The use of an internal standard would require the analyte and the standard to behave similarly in terms of chromatographic separation on the paper disk, an assumption which does not have to be true in all cases of sample types. Therefore, an alternative laser ablation approach is presented in this publication: one line is ablated across the entire filter paper disk, from one rim to the other, passing through the center of the disk. The transient signal thus obtained is integrated. Any centro-symmetric variation of the analyte is thus effectively cancelled out. The applicability of the method is shown by analyzing phosphorus in biochemical fermentation media, and by comparing the method with conventional measurements using strong dilution and internal standardization.

The third article is entitled "Self-aliquoting micro-grooves in combination with laser ablation-ICP-mass spectrometry for the analysis of challenging liquids: quantification of lead in whole blood" (Analytical and Bioanalytical Chemistry, 408 (2016) 5671 – 5676). In this article, a new (fourth) method for dried droplet sample preparation is presented. This method allows for an automatic splitting of a liquid sample into very small sub-samples which dry quasi instantly and which are so small (with respect to the laser beam) that they do not cause any relevant matrix-effects. The method relies upon long grooves which are engraved into a polymeric substrate by means of the same laser-ablation system also used for measurement. These grooves are 1 cm long and 100 µm wide. A droplet of whole blood (or aqueous inorganic standard) was slid across the grooves by means of a rubber spatula. While passing the grooves, a small volume of blood was trapped in each of the groove. When performing a laser ablation measurement perpendicular to the grooves, transient signals in peak-shape are obtained which were then used for quantification. It was shown that external aqueous standards could be used for quantification, although it was necessary to rely upon iron as

internal standard. The reason for this is that the amount of liquid trapped within the grooves is matrix-dependent. The method allows however for straight-forward sample preparation also in "field conditions" and allows to screen a large number of samples with minimum effort. Besides the mentioned sample preparation technique, also the data treatment protocol was improved, compared to existing approaches. By using a calculation-approach so-far only used in context of isotope-ratio determinations, processing of the data was found to be more straight-forward and easy to accomplish. With the new approach, it is not necessary to set any integration boundaries of transient signals any more, as the calculation approach inherently corrects for blank values in the measurement.

The fourth article within this thesis is entitled "Extraction and pre-concentration of platinum and palladium from microwave-digested road dust via ion exchanging mesoporous silica microparticles prior to their quantification by quadrupole ICP-MS" (Microchimica Acta, 182 (2015) 2369 – 2379). It presents a dispersed particle extraction method which aims at the selective extraction of platinum and palladium from chemically digested road-dust samples. Details regarding the optimization of the method with respect to sample acidity, tolerance towards matrix constituents, amount of sorbent material required, as well as efficiency of the analyte isolation are given. As is shown in the article, co-existing sample elements which could cause spectral or isobaric overlap in ICP-MS detection are efficiently removed by the method. The method is applied to the analysis of digested road-dust samples collected in downtown Vienna. The trueness of the method is shown by measuring BCR CRM 723 reference material and by finding a good agreement with the certified values for Pt and Pd in this matrix.

The fifth article is entitled "Combining dispersed particle extraction with dried droplet laser ablation ICP-mass spectrometry for determining platinum and palladium in airborne particulate matter" and was submitted to the journal of "Applied Spectroscopy" for peer-review. This article discusses the combination of dispersed particle extraction with dried droplet laser ablation. The advantages of this combination lie in the fact that a strong pre-concentration is possible, as the laser ablation system is tolerant towards high concentrations of dissolved inorganic material in the liquid sample (which arises from dissolving the sorbent material previously used in dispersed particle extraction). Also the efficient sample introduction of the laser ablation system is advantageous in the context of trace-element quantification. Again, the method is validated by analyzing BCR CRM 723 reference material, and it is applied to airborne particulate matter samples (with an aerodynamic diameter ≤ 2.5 microns). Due to the good sensitivity of the method, it was possible to collect aerosol samples with intervals of 4 hours only. Although the instrumental detection limit would have allowed to quantify palladium as well, this was not possible due to high method and digestion blanks.

5.2. SELECTED PUBLICATIONS

5.2.1. PART I

This part of the thesis contains three articles that discuss the topic of dried droplet laser ablation.

ARTICLE 1 (REVIEW-ARTICLE)

Recent advances in quantitative LA-ICP-MS analysis: challenges and solutions in the life sciences and environmental chemistry. Review Article.

Andreas Limbeck, Patrick Galler, Maximilian Bonta, Gerald Bauer, **Winfried Nischkauer**, Frank Vanhaecke.

Analytical and Bioanalytical Chemistry, 407 (2015) 6593 – 6617.

http://dx.doi.org/10.1007/s00216-015-8858-0

One chapter of this review article was written by Winfried Nischkauer, and this part (pages 6605 – 6610 of the published article) should therefore be considered part of this doctoral thesis. The other chapters of the review article are provided to give the reader a full picture of the review article.

Anal Bioanal Chem (2015) 407:6593–6617
DOI 10.1007/s00216-015-8858-0

REVIEW

Recent advances in quantitative LA-ICP-MS analysis: challenges and solutions in the life sciences and environmental chemistry

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Abstract Laser ablation–inductively coupled plasma–mass spectrometry (LA-ICP-MS) is a widely accepted method for direct sampling of solid materials for trace elemental analysis. The number of reported applications is high and the application range is broad; besides geochemistry, LA-ICP-MS is mostly used in environmental chemistry and the life sciences. This review focuses on the application of LA-ICP-MS for quantification of trace elements in environmental, biological, and medical samples. The fundamental problems of LA-ICP-MS, such as sample-dependent ablation behavior and elemental fractionation, can be even more pronounced in environmental and life science applications as a result of the large variety of sample types and conditions. Besides variations in composition, the range of available sample states is highly diverse, including powders (e.g., soil samples, fly ash), hard tissues (e.g., bones, teeth), soft tissues (e.g., plants, tissue thin-cuts), or liquid samples (e.g., whole blood). Within this article, quantification approaches that have been proposed in the past are critically discussed and compared regarding the results obtained in the applications described. Although a large variety of sample types is discussed within this article, the quantification approaches used are similar for many analytical questions and have only been adapted to the specific questions. Nevertheless, none of them has proven to be a universally applicable method.

Keywords LA-ICP-MS · Quantitative analysis · Certified reference material · Matrix-matched standards · Internal standard correction · Liquid standards

ABC Highlights: authored by *Rising Stars and Top Experts.*

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Introduction

Laser ablation (LA) in combination with inductively coupled plasma–mass spectrometry (ICP-MS) is a powerful technique for the direct elemental analysis of solid samples. This technique provides major, minor, and trace element information with a wide elemental coverage, excellent limits of detection, and a linear dynamic range of up to 10 orders of magnitude, while also enabling microanalysis, depth profiling analysis, and 2-dimensional elemental mapping. Further advantages of LA-ICP-MS are minimal sample preparation, high sample throughput, access to isotopic information, and the possibility of analyzing both conductive and non-conductive and opaque and transparent materials [1–4].

However, two fundamental aspects of processes involved constrain the ability of LA-ICP-MS to act as a universal method for direct analysis of solid samples. The first major drawback of LA-ICP-MS is that the abundances of the ions detected after m/z separation are often not entirely representative of the composition of the original sample. In the literature, this problem is often referred to as "elemental fractionation" [5, 6], although this term is also used to describe time-dependent changes in the composition of the ion beam in the mass spectrometer. Besides the ablation process itself (e.g., non-stoichiometric effects due to the preferred ablation of more volatile compounds), the transport of the aerosol particles

from the ablation chamber into the ICP (e.g., differences in gravitational settling between smaller and larger particles) and vaporization, atomization, and ionization in the ICP (less efficient for larger particles) are also important contributors to fractionation effects. A detailed discussion of the individual contributions to elemental fractionation and the strategies developed for minimizing the influence exerted can be found in the literature [7–14].

The second major problem connected with the use of LA-ICP-MS for direct analysis of solid samples is the difference in the interaction between the laser beam and the sample surface observed for various matrices, causing changes in the mass of analyte ablated per pulse due to differences in the properties of the matrices investigated (e.g., absorptivity, reflectivity, and thermal conductivity). The aerosol particles produced during ablation of different matrices may vary in size and geometry, thus having an effect on the sample transport efficiency from the ablation cell to the plasma [15]. Both effects contribute to differences in the mass load of the plasma and give rise to matrix effects, since the vaporization, atomization, and ionization efficiencies of the analytes introduced into the plasma depend on the mass load [16]. Sample-related "matrix effects" therefore jeopardize the accuracy of LA-ICP-MS analysis and complicate quantification [2–4, 17–20].

As a result, elemental fractionation and matrix effects occur simultaneously, leading to LA-ICP-MS signals that are not representative of the elemental composition of the sample investigated. The sensitivity or absolute signal intensity can vary significantly for samples with the same analyte concentrations, but different matrix compositions and/or physical properties. At this point, it has to be mentioned that mass spectrometric separation and detection of the ions generated can also contribute to the bias in LA-ICP-MS results. However, an explanation of the corresponding sources of bias is beyond the scope of this work; details on these issues can be found in a recently published review article [21]. Figure 1 schematically summarizes the individual steps of LA-ICP-MS analysis prone to elemental fractionation and matrix effects.

As a consequence of the increasing interest in the use of LA-ICP-MS in various scientific fields, research has been devoted to overcoming the aforementioned drawbacks. In the few last years, attempts were made to address the limitations of LA-ICP-MS by improving the instrumental parameters relevant to aerosol formation. Most of this work focused on the influence of the wavelength of the laser radiation (especially important for transparent materials) and the pulse duration (especially important for metallic samples). With the use of shorter ultraviolet wavelengths and pulse durations in the femtosecond (fs) range, instead of the nanosecond range, a significant reduction of elemental fractionation and matrix effects is enabled. Furthermore, the laser beam profiles were changed from Gaussian to (pseudo) flat-top profiles, leading to optimized ablation performance. However, complete elimination of these effects is still not possible. Ongoing research is therefore dedicated to methodological developments that permit correct quantification with the currently available instrumentation for LA-ICP-MS analysis.

The purpose of this review is to summarize state of the art procedures and recent developments in quantitative LA-ICP-MS analysis of samples originating from the fields of life sciences and environmental chemistry. In addition to traditional approaches, novel concepts for the preparation of matrix-matched standards, such as the deposition of elemental coatings or thin polymeric films containing an internal standard on the sample surface, as well as quasi-simultaneous measurement of standard and sample using a spinning platform will be presented. Capabilities and limitations of the different approaches will be compared, critically examined, and evaluated on the basis of their suitability for general use.

Common concepts for quantification in LA-ICP-MS

Even though the application range of LA-ICP-MS is wide and the sample types analyzed are various, some approaches for quantification are applied to a large variety of sample types. The basic principles of the methods described below are the same, while modifications thereof will be presented in the sections dedicated to specific sample types.

External calibration utilizing certified reference materials (CRMs) which match the composition of the sample to be investigated to the largest possible extent—preferably exactly—is the most reliable method for accurate quantification in LA-ICP-MS [20, 22–24]. If this prerequisite is met, ablation, transport, atomization, and ionization of sample and standard can be considered to be (nearly) identical, enabling reliable quantification. For each CRM, a detailed certificate is available containing information regarding component concentrations. Additionally, in the literature, preferred concentration values are available for non-certified sample constituents [25]. However, the lack of appropriate CRMs for the majority of sample types (in particular for samples from environmental, biological, or medical origin) limits the applicability of this approach. Thus, alternative quantification strategies are mandatory.

A promising approach for quantification is the preparation of matrix-matched calibration standards, prepared from material with the same matrix as the sample [26–29]. Procedures for sample preparation preceding LA-ICP-MS analysis reported in the literature include fusion with borate, embedding in a polymer resin, or preparation of a pressed disk in the presence of a binder. Benefits of these sample preparation approaches are that they facilitate the addition of one or more internal standards, known amounts of the analyte(s) of interest (for standard addition purposes) or isotopically enriched spikes (for isotope dilution purposes), as well as the possibility to

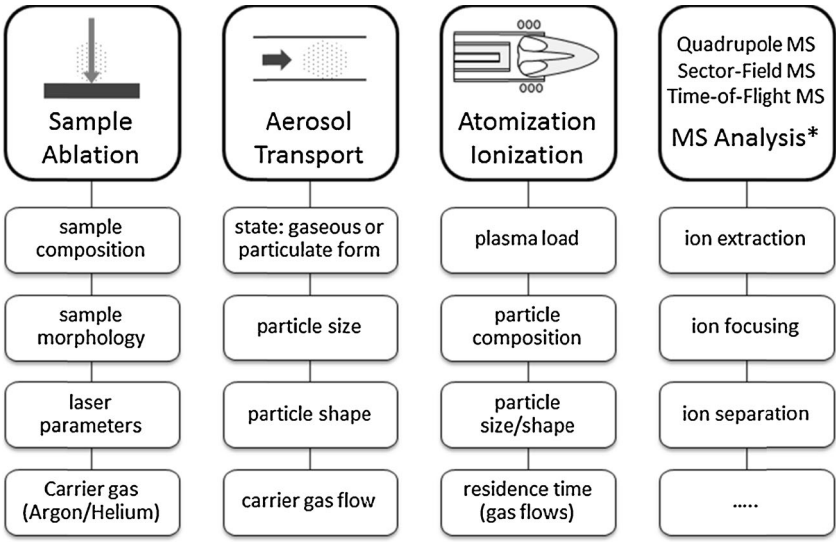

Fig. 1 Sources of error in LA-ICP-MS analysis, * not discussed within this review

adjust the analyte concentrations as required (dilution). A major drawback is the applicability to powdered samples only; samples that are compact in their native form require additional sample pretreatment (i.e., milling, grinding). Furthermore, it has to be considered that this type of adjustment of the sample matrix is automatically accompanied by analyte dilution, which decreases the detection power of the analysis approach.

A frequently applied method in combination with external calibration (i.e., CRMs and in-house standards) is signal normalization to an internal reference or internal standard [30–33]. This approach can be used to further improve the accuracy of the quantification results, since the influence of remaining differences between sample and standard can be minimized. Variations in sample ablation and transport as well as ICP-related alterations in signal intensity (e.g., changing plasma conditions) can be corrected for using an internal standard. A precondition for the successful application of this method is that the internal standard element and the analyte element are homogeneously distributed within the sample, and that their behavior during ablation, transport, and ionization is similar. In this course, crucial parameters are, e.g., the form in which the element is transported from the ablation cell into the ICP (gaseous or particulate) and its mass and ionization potential. The element being used as internal standard can either occur naturally in the sample or is added during the sample preparation process. Optimally, the concentration of the element used as internal standard in the sample is known. However, for successful application it is sufficient that the concentrations in the standard and sample are equal.

"Solid–liquid" calibration in which a dual flow system allows simultaneous introduction of a nebulized aqueous standard solution and laser ablated material is an attractive alternative to the use of matrix-matched solid standards [3, 34, 35]. In this procedure, the carrier gas flow coming from the ablation cell is mixed with an aerosol generated by nebulization of an aqueous standard solution. Besides the addition of aerosol to the gas lines leading to the ICP [36], the use of micronebulizers has also been proposed to add the aerosol to the gas flow directly at the site of material ablation [36, 37]. Not only standards with natural isotopic composition [36] but also isotopically enriched standards [37] have been used for such experiments. Standard and blank solution are alternately added to the sample stream, such that the accompanying difference in signal intensity can be used to quantify the analyte concentrations in the sample. A correction for the differences in ablation efficiency is required and when aiming to maintain the advantages of "dry plasma" conditions, such as a reduced level of oxide interferences, the wet aerosol must be desolvated prior to its mixing with the sample aerosol. However, for special applications, wet plasma conditions may also offer improved measurement conditions, e.g., in terms of signal stability [38]. This method enables quantification based on aqueous standards, and can compensate for matrix-related ionization differences. However, possible variations in ablation efficiency or altered transport efficiencies cannot be accounted for.

Although the quantification approaches mentioned above have been successfully applied in several research fields, including material sciences, geo- and cosmochemistry, environmental chemistry, biology, and medicine [3, 4, 14, 19, 22, 23, 39], their successful application to any kind of sample is not guaranteed. Thus, further improvements are required, which could be achieved either by reducing the extent of matrix effects by using optimized instrumental parameters (e.g., laser radiation wavelength, pulse duration, robustness of ICP) or by developing alternative strategies for quantification.

Analysis of hard tissues and compact samples

Naturally occurring compact materials, such as rocks and minerals, bones, teeth, claws, feathers, or nails, require no

pretreatment like milling or pressing prior to LA-ICP-MS analysis. However, often it might be necessary to flatten the exposed sample surface using a grinding and/or polishing step. Within this review, only samples of biological origin will be discussed. Samples of geological origin will not be described in detail here; for this kind of samples, detailed information can be found in journals devoted to geology and geochemistry.

While quantitative determination of major, minor, and trace elements in the samples mentioned above is the main field of application of LA-ICP-MS, the technique also offers the possibility of performing spatially resolved analysis, which is of interest for studying element distributions (mapping or imaging and depth profiling analysis) or inhomogeneities (solid or fluid inclusions) in many materials. Applications solely dedicated to visualizing elemental distributions are also beyond the scope of this paper, which focuses on those applications in which estimation of bulk or local concentrations forms at least part of the investigation and, possibly, some effort is made for cross-validation using an alternative analytical approach. Yet, calibration approaches discussed here can self-evidently also be deployed in mapping or imaging applications.

Biogenic carbonates: calibration

Although LA-ICP-MS lends itself specifically well to spatially resolved analysis, its application for bulk analysis is justified in cases where a dedicated area across a given set of samples has to be reproducibly analyzed in situ in order to enable comparison. This has been specifically exploited for the investigation of fish otoliths at their cores and edges for the purpose of origin determination of fish populations [31, 40–47], or for the investigation of changes in the prevailing conditions, reflected in the microchemistry of very narrow otolith bands [32, 48–50]. The daily accretion of calcium carbonate layers in otoliths and their permanent retention of chemical fingerprints in the form of various elemental impurities make them an ideal target for this type of investigation [51]. Similar incremental growth behavior and chemical matrix are found in mussel shells and corals. Combined with their immobility, it makes these objects valuable environmental monitors [26, 27, 52–54]. Also larvae tracking applications have been reported for mussels [55, 56]. This type of investigation can be summarized under the term *sclerochronology*.

The different NIST SRM glasses 610, 612, and 614 (National Institute of Standards and Technology, Gaithersburg, USA), with certified trace element concentrations over approximately three orders of magnitude, from the low microgram per gram level to hundreds of micrograms per gram, are by far the most frequently used materials for calibrating biogenic calcium carbonate measurements by LA-ICP-MS [31, 32, 40–43, 46, 48–50, 52–56]. Trying to improve the analytical results, Arkhipkin et al. compensated for the difference in matrix composition between the NIST SRM glass and biogenic calcium carbonate via the introduction of in-house correction factors [46]. However, according to Jochum et al., calibration using NIST SRM glasses as such already results in accurate values for the refractory elements, whereas a closer matrix-matching using calcium carbonate pellets has to be applied for low boiling point elements, such as Pb [53]. Another decisive parameter for measurement accuracy when using the NIST SRM glasses for calibration is the set of reference concentrations used for these materials [53, 57, 58]. Custom-made fused glasses are an alternative to the NIST SRM glasses for calibration purposes. Such glasses were prepared by Sinclair et al. by blending biogenic carbonate (coral powder) with silica in a ratio of 1:1, followed by fusion at 1650 °C and by Perkins et al. by blending $Li_2B_4O_7$ in excess with synthetic $CaCO_3$, MgO, and gravimetric additions of the analytes of interest, followed by fusion over a burner flame [26, 27]. Sinclair et al. obtained reference concentrations for their glasses using solution-based isotope dilution either by ICP-MS or thermal ionization mass spectrometry for all elements other than B, which was calibrated via LA-ICP-MS against NIST SRM 612 using B concentrations from the literature [26].

Besides NIST SRM glasses, carbonate pellets represent the second largest group of calibration materials in the field of biogenic carbonate analysis by LA-ICP-MS [27, 44, 45, 47, 52, 53, 59]. Different carbonate materials pressed into pellets have been used, including the commercially available synthetic calcium carbonates USGS MACS-1 and MACS-3 (United States Geological Survey, Reston, VA, USA) [44, 52, 53], fish otolith powder NRC FEBS-1 (National Research Council Canada, Ottawa, Canada) [47], synthetic in-house carbonates prepared by co-precipitation [45], or in-house standards prepared from gravimetric blends of the analytes of interest and either commercially available calcium carbonate or crushed biogenic carbonate [27]. In one case, the authors blended NIST SRM glasses and carbonate pellets to obtain calibration standards, without revealing whether all materials were used in the same calibration function [52]. However, despite the multitude of calibration materials obviously at hand, the fact that otoliths also contain a significant amount of organic matter is commonly not accounted for [27, 51].

Without exception, all authors use Ca as internal standard element for normalization, albeit with little agreement regarding their choice of the Ca nuclide used for this purpose. This is mainly a question of user experience, instrumental sensitivity, and the means available to overcome spectral interference. All Ca isotopes, apart from the most abundant (^{40}Ca) and the least abundant (^{46}Ca), have been reported in papers related to LA-ICP-MS analysis of biogenic calcium carbonates and referenced in this review. There is also a fair amount of disagreement with respect to the Ca concentration assumed or

measured for otoliths, which is critical for obtaining accurate data. Whereas some authors calculate a theoretical Ca concentration based on $CaCO_3$ stoichiometry [32, 40, 41, 45, 48, 50], others measure it in advance using conventional solution nebulization ICP-MS or ICP-OES [31, 47]. As a result, reported Ca concentrations range from 35 to 40 %. Even when estimates are based on $CaCO_3$ stoichiometry only, some disagreement is possible.

Biogenic hard tissues: claws, feathers, fish scales, and hair: calibration

LA-ICP-MS also becomes an asset when minimally invasive sampling and analysis are required. LA-ICP-MS has been used for the quantitative analysis of animal claws, feathers, fish scales, snake tail clippings, animal hair, human hair, and human finger nails [60–71].

Ethier et al. and Kaimal et al. used the NIST SRM 612 glass as a standard in the context of multi-element analysis of badger claws and bird feathers, respectively [60–62]. Both teams used concentration data obtained via LA-ICP-MS for statistical classification of their results. Whereas Ethier et al. used S as an internal standard as a consequence of the high cysteine content of the sample matrix keratin [60, 61], Kaimal et al., used ^{42}Ca, assuming a homogeneous Ca distribution [62]. For Ethier et al., the use of S as internal standard, quantified in advance using conventional solution nebulization ICP-MS, required the introduction of inter-element sensitivity factors, established from the ICP-MS mass response curve obtained upon ablation of NIST SRM glass. The authors indicated that this approach only yields semiquantitative data. Human and animal hair, as well as human finger nails or clippings thereof, all predominantly comprised of keratin, have been the subject of many studies owing to their capability as a biomonitor of past (trace) element exposure [66–70]. The application of in-house hair or nail material for calibration is quite commonplace in this context [66–70]. Rodushkin and Axelsson used in-house finger nail material, powdered and pressed into a pellet, for calibration of finger nail measurements [70]. Reference concentrations were obtained from conventional solution nebulization ICP-MS after sample digestion. For hair analysis, the certified GBW07601 hair reference material (Institute of Geophysical and Geochemical Exploration, Lanfang, China) was used for calibration [70]. Similarly, Stadlbauer et al. used BCR CRM 397 hair reference material (Institute for Reference Materials and Measurements, Geel, Belgium), pressed into a pellet with polyethylene as a binder [71]. Bartkus et al. and Arriaza et al. both used whole in-house hair standards, quantified for Pb and As by conventional solution nebulization and hydride generation ICP-MS, for calibration of LA-ICP-MS measurements [68, 69]. Dressler et al. pursued calibration of LA-ICP-MS measurements of mouse and human hair by simultaneous aspiration of multi-element solutions (at several concentration levels) via a conventional nebulizer [66]. The wet aerosol was mixed on-line with the dry aerosol coming from the ablation chamber in the injector tube of the ICP torch. Differences in aerosol generation and transport efficiencies between solution nebulization and LA were assessed by ablating in-house hair material with known analyte element concentrations. The in-house hair standard material was prepared by immersion of hair strands in a multi-solution, subsequent drying, and digestion of the material thus obtained for the determination of reference concentrations via conventional solution nebulization ICP-MS. Sela et al. used a similar approach, but one based on standard addition using an ultrasonic nebulizer equipped with a desolvation unit [67]. The dry aerosol intended for calibration was directed through the ablation cell for mixing with the LA aerosol before introduction into the ICP. Concentrations were determined for single hair strands and hair powder, both fixed on carbon tabs. As for the internal standard, both ^{32}S and ^{34}S have been used for hair and finger nail samples [66, 67, 70]. Rodushkin and Axelsson report S concentrations of 4.77± 0.41 % and 3.30±0.56 % for hair and finger nails, respectively [70]. S concentrations were obtained on the basis of hair and finger nail samples of approximately 100 Swedish individuals. Stadlbauer et al. used a quadrupole-based ICP-MS instrument equipped with a reaction cell and adopted sulfur in the form of $(^{32}S^{16}O)^+$ as internal standard to avoid spectral overlap of the $^{32}S^+$ peak with that from the oxygen dimer ion $^{16}O_2{}^+$ at m/z=32 [71]. Also ^{13}C has been reported as an internal standard for LA-ICP-MS analysis [68, 69]. However, the use of ^{13}C as internal standard is associated with some major drawbacks. Those will be described in the chapter ‘measurement of soft tissues and protein samples’ in the section ‘internal standards’ in more detail.

Holá et al. and Flem et al. both developed LA-ICP-MS methods for trace element quantification in fish scales as an alternative to otolith sampling [63, 64]. Since fish scales contain (Ca-deficient) hydroxyapatite, Holá et al. used NIST SRM 1486 bone meal for external calibration [63]. In contrast, Flem et al. used not less than six different glass reference materials for calibration, namely NIST SRMs 610, 612, 614, 616, NIST SRM 1830 soda limestone float glass, and USGS TB-1 basaltic glass [64]. TB-1 was only introduced for calibration of Sr. Flem et al. commented that for the purpose of their study, normalized data only would have sufficed, yet calibration against the different glasses was included in order to be able to at least provide concentration estimates for later use. Both groups used Ca as an internal standard, determined by electron microprobe analysis in both cases [63, 64]. Flem et al. quote an average Ca concentration of 37.4±0.4 % for a set of fish scales [64], whereas Holá indicate Ca concentrations for three line scans on one fish scale ranging from 23.5 to 26.5 % [63]. Holá et al. also gave some indication of the homogeneity of Ca in fish scales through a spatial distribution

map obtained by electron microprobe analysis [63]. Alternatively, calibration using spiked hydroxyapatite prepared as in-house calibration materials has been reported for LA-ICP-MS investigations of (human) bone and teeth [71].

The last example given describes the direct analysis of water snake tail clippings by LA-ICP-MS as an ecotoxicology tool [65]. Given the complexity of the sample material containing inorganic bone, calcium carbonate, muscle blood, and skin, Jackson et al. resorted to in-house preparation of matrix calibration standards from water snake tail sample material [42]. Reference concentrations were obtained from conventional solution nebulization ICP-MS and ^{13}C was used as internal standard for LA-ICP-MS.

Validation

It is not uncommon to omit validation from the analytical procedure entirely, which may be justified in cases where consistency of results is more important than accuracy, such as in statistical classification of the samples analyzed among different groups [40, 42, 43, 46, 62, 64]. In cases where analyte concentrations are obtained via LA-ICP-MS using non-matrix-matched standards, one should refrain from comparing results to other sets of data obtained for the same sample type by a different analytical approach without validating the quantitative results. Several approaches for this purpose were reported in the literature. Validation by re-measuring the calibration standard, in this case NIST SRM 610 glass, as a sample has also been described, but this is clearly a far from ideal assessment of measurement accuracy [32]. This is appreciated by some authors through the introduction of a reference material as an unknown in the analytical protocol. Different reference materials including USGS MACS-1 and MACS-3 synthetic calcium carbonate, NIES-022 fish otolith powder (National Institute for Environmental Studies, Tsukuba, Japan), and NRC FEBS-1 fish otolith powder have been used for this purpose, as have the limestone reference materials GSJ JLS-1 (Geological Survey of Japan, Tsukuba, Japan), GSR-6 (Ministry of Land and Resources, Beijing, China), and BAS CRM-393 (Bureau of Analysed Samples Ltd, Middlesbrough, UK) [27, 41, 44, 47, 53].

Some authors also validated their LA-ICP-MS results by conventional solution nebulization ICP-MS. Phung et al. performed LA-ICP-MS analysis in holes left by micro-drill sampling for solution nebulization ICP-MS analysis and subsequently compared results from both procedures involving two different LA-ICP-MS facilities [52]. Results agree generally within the quoted analytical errors, with a few exceptions depending on the hole analyzed. Sinclair et al. converted LA-ICP-MS line scans on corals into average concentrations for five elements and compared these to results from solution nebulization ICP-MS of a digest of the same sample [26]. Deviations ranged from approximately −3 to 30 % between the two methods. Dressler et al., Sela et al., and Rodushkin and Axelsson all compare their LA-ICP-MS results for human hair and nail samples to results from conventional solution nebulization ICP-MS [66, 67, 70]. Dressler et al. achieve agreement within analytical error [66], and the results of Rodushkin and Axelsson also showed a good correlation of LA-ICP-MS and solution nebulization ICP-MS results; generally, LA-ICP-MS results are within 30 % of solution nebulization ICP-MS results. Average LA-ICP-MS results obtained by Holá et al. for fish scales are generally higher than the corresponding solution nebulization ICP-MS data, which is also explained by the complexity of the sample matrix, i.e., analyte enrichment in the uppermost layer ablated from fish scales [63].

There is a general agreement between the authors regarding the use of gas blanks for baseline correction. Typically, limits of detection are calculated from three or ten times the standard deviation of the gas blank, divided by the slope of the calibration line or the instrumental sensitivity [31, 42, 44–46, 48]. However, as discussed by Rodushkin and Axelsson, detection limits in LA-ICP-MS depend on the volume of ablated material, the analyte mass, the ionization energy of the analyte, its isotopic abundance, and the ion transmission efficiency [70]. Aerosol size distribution and transport efficiency to the ICP-MS presumably play a role too. For the analysis of hair and nail, detection limits in LA-ICP-MS range from picograms per gram to nanograms per gram and are quoted as only marginally nferior to conventional solution nebulization ICP-MS as a result of sample dilution after digestion for the latter approach.

Measurement of soft tissues and protein samples

In recent years, LA-ICP-MS has also become a technique of growing interest in the life sciences. The effect of variations in trace elemental concentrations, and especially metal–protein interactions are increasingly studied in biological and biomedical investigations [72]. The sample types reported on vary from native samples, such as plant material [73–76], and thin sections of animal/human body tissues (e.g., liver [77], brain [78–80], eye tissue [81], kidney [82], and others), to electrophoretically separated metalloproteins [83, 84]. Typically, natural element distributions within the biological samples were investigated. In special cases, the use of isotopic analysis has been reported (e.g., as tracers for metal uptake in organisms). For example, Florez et al. exposed *Daphnia magna* to isotopically enriched Zn tracers and produced isotope ratio images with 30-μm spatial resolution [85]. A more detailed description of possibilities and limitations of isotopic analysis can be found elsewhere [86].

Like in every other field of LA-ICP-MS applications, quantification is a crucial aspect. Problems aggravating reliable quantification are the large variety of sample types and

properties, as well as a lack of suitable standard materials. However, especially when thin sections of sample material are used (preparation of thin sections is common practice in the medical sciences), some aspects of quantification are facilitated, and some new issues may arise. Those thin-cuts typically have thicknesses of 5–20 μm, thus providing the opportunity to ablate the sample material (the entire depth) completely with a few laser shots, i.e., during one cycle of analysis. The analysis of thin layers renders the analysis of tissue sample much easier, since differences in penetration depth of the laser beam into the sample material do not need to be considered. This gives the opportunity of applying thin layers containing a standard for signal normalization above or below the sample. Those also have to be completely ablated along with the sample. Independently from the quantification strategy used, reliable quantitative analysis is only possible when the thin sections of the samples have equal thicknesses. Some quantification approaches that will be described in later sections rely on this assumption as they cannot compensate for varying layer thickness. If the tissue thicknesses are varying within one sample or the whole tissue sample can not be ablated in a single run, i.e., for thicker samples or bulk material, internal standardization has to be used as for many other types of samples described in this article.

Quantitative imaging approaches

A large number of LA-ICP-MS applications involving soft tissue samples aim to unravel the 2-dimensional trace element distributions (bioimaging or mapping) [87–89]. To preserve information on the spatial analyte distribution, tissue samples are analyzed as thin slices without any prior homogenization step. Three major problems have to be addressed to ensure reliable analysis results: material ablation and aerosol transport are highly matrix-dependent, the efficiency and location of the ionization process in the ICP are a function of the particle size distribution of the aerosol produced via LA [24], which, similarly, is matrix-dependent, and during the measurement time, instrument instability and signal drift may occur because of changing experimental conditions (e.g., cone conditions, vacuum pressure). As a result of those factors, even the measurement of reliable qualitative distribution maps is not self-evident; ensuring reliable quantitative results is even more challenging. Not all approaches that have been presented in the past are capable of adequately addressing the complete range of limitations mentioned.

Solid standard materials

Another method for quantification is the use of solid standard materials. Thereby, a suitable standard material can be manufactured for almost every sample type. CRMs for tissues are only scarcely available, and are in most cases not compatible with the specific experimental conditions (e.g., tissue types, trace elements selected, and/or concentration range).

While quantitative elemental analysis of homogenous materials using LA-ICP-MS can often rely on matrix-matched standards, biological samples may significantly differ in their composition even within several sections of a single sample, and therefore different methods are needed to ensure accurate quantification. Various approaches have been proposed and used to facilitate and improve the quantification of trace elemental distributions in biological tissues and to overcome the problem of often pronounced sample inhomogeneity. Even though many alternative methods for quantification have been proposed, the classical and still most often used method is the use of matrix-matched standards [90]. Those have to be prepared in-house and tuned to the specific application. The preparation of matrix-matched tissue standards has been described by Hare et al. [90] in detail. In short, the selected tissue is homogenized and spiked with an aqueous standard containing the elements of interest; the spiking process is performed at different concentrations enabling the determination of calibration functions. An aliquot of the homogenized and spiked tissues is acid digested for determining the actual analyte element concentrations in the standards. After freezing of the standard, a cryo-cut of desired thickness is prepared for LA-ICP-MS measurement. Alternative approaches for quantification in LA-ICP-MS analysis of biological tissue using solid standards aim at rendering the process of standard preparation easier. Approaches to facilitate the manufacturing process of standard materials use gelatin [91], agarose gel [92], or sol-gel [93] standards, spiked with appropriate amounts of the elements of interest. The preparation of those materials is similar to that of matrix-matched tissue standards, but less tedious. The goal is to minimize the handling of biological materials and still end up with standards with a similar matrix composition (mostly in terms of carbon content and density). One approach uses a polymeric film, spiked with the elements of interest, applied to a glass slide before attaching a thin section of the sample [94]. Assuming simultaneous ablation of standard and sample material, this approach will lead to correction for matrix effects during the measurement—similar to a single standard addition approach. Another way to facilitate standard preparation for analysis of biological tissues is printing of standards onto paper using a commercially available office inkjet printer [95, 96]. Conventional paper can be used for standard preparation, and the inks may be spiked with elements of interest. This method has been successfully used to quantify trace elements in different biomaterials. Reifschneider et al. proposed a method to reduce matrix effects by embedding biological tissues into epoxy resins [97]. In the embedding method used, complete penetration of the resin into the tissue material was ensured. The standards are prepared from epoxy resins without embedded tissue; as the

main matrix material is the epoxy resin, no major difference in matrix composition exists between standards and samples. All methods discussed so far try to simulate the 'average' matrix conditions in the tissues presented. But, a biological sample can be very inhomogeneous and matrix compositions may vary significantly, even within a single sample. Local variations in the matrix composition can lead to inaccurate quantitative results because of alterations in material ablation, aerosol transport, and analyte ionization efficiencies. The approaches for quantification described can reduce such matrix-related effects on the ablation and analyte ionization. Still, those approaches offer no possibility for the correction of instrument instability and/or signal drift or for the reduction of signal variations originating from inhomogeneities in the sample matrix.

Internal standards

Similar to conventional solution ICP-MS measurements, an internal standard can help to correct for changes in the signal intensity originating from instrument instability and/or signal drift. During the long measurement times of imaging experiments (usually 4–30 h), gas flow rates, cone and vacuum conditions, and other experimental conditions may vary. Furthermore, the pronounced inhomogeneity of the samples investigated with profoundly changing matrix compositions require the use of (an) internal standard(s) for reliable quantification [98]. In most publications, carbon has been proposed as the internal standard, as it is abundant in every biological sample and is often uniformly distributed across the sample. However, it has been shown that carbon is not an optimal internal standard [99], as its ionization potential is significantly higher than those of commonly investigated elements, such as most transition metals, and an altered carbon load in the plasma may change the ionization efficiency of some of the analytes monitored substantially. Furthermore, the transport of carbon into the ICP can partly occur in the form of carbon dioxide, which will lead to transport properties and efficiencies that can markedly differ from those elements that are transported as particulate matter only [99]. Therefore, normalization to carbon as internal standard may lead to distortions of the actual analyte distribution, causing inaccurate quantification results. Sulfur has also been proposed as a sample-inherent internal standard [77]; however, it is not evenly distributed in most tissues and, because of its high first ionization energy, similar problems as mentioned for carbon may be expected. Therefore, alternative approaches for signal normalization have been developed to improve the existing quantification methods. Both online addition of wet aerosol [36, 37] and the method with the spiked polymer layers [94] or the epoxy resin [97] have used signal normalization as part of the quantification approach. However, in the first two approaches mentioned, no internal standard in the traditional meaning was used, as ablation of sample and internal standard take place subsequently, and not simultaneously. Another way to normalize the analyte signal which was proposed by Konz et al. [100] and shown to be feasible for quantification [81, 95] is the sputtering of the samples with a thin gold layer; however, this approach only provides a pseudo-internal standard for the same reason. Only the epoxy embedding method provides a true internal standard. The tissue samples are immersed in the epoxy resin containing the internal standard; the resin completely penetrates the tissue material. Therefore, the internal standard is ablated simultaneously with the sample material. Combining the preparation of external standards with matrices similar to the sample material with the use of an internal or pseudo-internal standard can counteract the matrix-related effects on the material ablation, aerosol transport, and analyte ionization, as well as that of instrument instability and/or signal drift.

The necessity of instrumental drift correction was described by Hare et al. [20] and shown by Bonta et al. [95] in later experiments. As mentioned earlier, the long measurement times of LA-ICP-MS imaging experiments may cause significant changes in the instrument sensitivity. This was illustrated in an imaging experiment of a printed pattern with blue ink [95]. Blue ink contains copper, which was investigated as the analyte of interest. The pattern has been coated with a thin gold layer for use as a pseudo-internal standard. Features with equal amounts of ink deposition (i.e., copper concentration) measured at different time points were compared regarding the signal intensity. Figure 2 shows the signal of ^{65}Cu with and without correction to the gold signal. During the 4.5-h measurement time, the absolute signal intensity for ^{65}Cu decreased by 25 %, indicating a strong sensitivity drift. Normalization to gold as pseudo-internal standard corrects for this drift and keeps the sensitivity constant throughout the measurement time. Thus, the necessity and feasibility of signal normalization is underlined as the results show that an

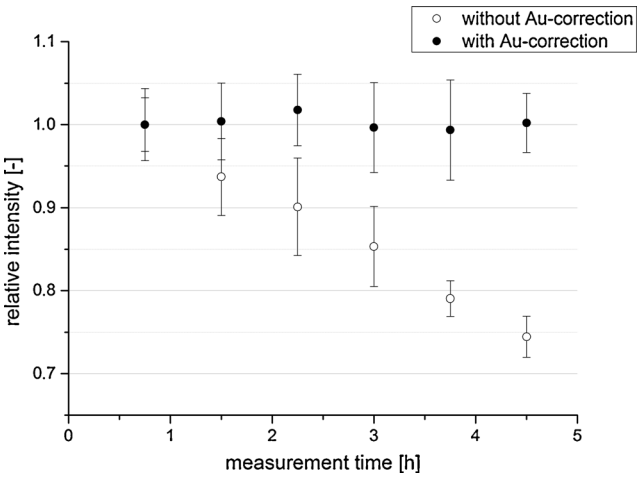

Fig. 2 Signal intensities at different time points with and without gold normalization; averages of 25 data points are displayed (n=25)

internal standard is necessary for reliable LA-ICP-MS imaging experiments.

In summary, quantitative determination of trace elements in biological tissues using LA-ICP-MS is still a challenging task, requiring extensive knowledge of sample composition and properties. Until now, no universal method has been established. Even though the preparation of matrix-matched standards seems to be the most straightforward method, some problems still remain. None of the other alternative methods are recognized as a reliable alternative to matrix-matched tissue standards because, as with every method, some limitations have to be considered.

Electrophoretically separated metalloproteins

Besides the analysis of native soft tissues, also other soft sample materials can be analyzed using LA-ICP-MS. A major group of samples are electrophoretic gels. Separation of proteins or peptides in porous gel matrices is a commonly used bioanalytical method [101, 102]. The biomolecules can be separated on the basis of their mobility in a gel matrix, and visualized using staining methods. Subsequent identification (e.g., using mass spectrometry) can be performed. With the increasing knowledge on metalloproteins, metal detection within the separated proteins has also gained importance. Sensitive elemental analytical techniques that allow for direct analysis of the metals from the gels, like LA-ICP-MS, are a powerful tool to obtain quantitative information on the metal content after electrophoretic separation of the proteins. Quantification of metal contents in electrophoretically separated gels is significantly different from trace element quantification in biological tissues, as a result of the homogeneous composition of the separation gel. However, some crucial aspects also have to be considered to ensure reliable results. Micronebulization of an aqueous standard at the ablation site using either standards with natural isotopic composition [36], or isotopically enriched solutions [37], has been proposed for quantification. In those approaches, changes in ablation and/or ionization behavior of the analytes are not taken into account and therefore an approach with species-specific isotope dilution was proposed by Konz et al. [103]. While in all other methods, the standard is added after material ablation, this method relies on direct addition of the standard to the sample and provides the possibility to compensate for all changes that affect the protein of interest. However, the disadvantage of the method is the fact that the protein of interest has to be available in pure form with an isotopically enriched metal cofactor.

Because the thickness of the gels is typically in the order of 1 mm, the entire thickness is not ablated during a few laser shots. Therefore, the use of carbon as internal standard has been proposed [104]. Still, transferring the proteins to a membrane after separation (blotting) is used far more often, as this allows one to avoid direct analysis of the gel. The investigation of so-called Western blot membranes using LA-ICP-MS has been described in detail elsewhere [105, 106]. In contrast to tissue analysis, the variety of proposed methods in the field of protein analysis is smaller. As with tissue samples, thus far, no optimal quantification method has been found.

Analysis of powdered samples

Sample preparation

Most LA-ICP-MS applications in the fields of environmental research and life sciences focus either on obtaining bulk information (with high precision and accuracy) or on obtaining spatially resolved information, sometimes only semiquantitatively. In contrast to the compact samples discussed in the previous sections, powdered samples require some kind of pretreatment prior to LA-ICP-MS measurement. The approaches most frequently applied for the preparation of compact samples from powders include milling/grinding/sieving for sample homogenization, combined with pelletization [107–112], fusion to sample disks [29, 107, 113–115], or mounting/embedding [110, 116–119] of the sample in a polymeric resin. In some cases, alternative approaches such as ablating standard and sample in quick succession by placing them on a rotary platform or electroplating have been reported [28, 33, 120–125].

During pelletization, the sample powder is compacted using a hydraulic press. Usually, the sample is mixed with a binder to improve powder grain adhesion and to produce stable pellets. Also additives (in liquid or solid form) can be introduced for internal standardization, quantification (e.g., for standard addition or isotope dilution purposes), or to affect laser–sample interaction (i.e., increased energy absorption at the applied laser wavelength). To achieve a better homogeneity, the components are often milled or ground prior to mixing. The pressing step is usually optimized in terms of press power and pressing time. Depending on the ratio of binder to sample, target analytes may be diluted by up to a factor of 10. Thereby, matrix differences between samples will be reduced, but the detection power is decreased. Pelletization is a very easy approach without the need of high-tech equipment. The homogeneity achieved in pressed pellets is sufficient for most applications. However, in some cases the reproducibility achievable is constrained by the sample homogeneity.

For fusion, the sample powder is usually combined with lithium tetraborate or lithium metaborate or a mixture of both. At high temperatures (over 1000 °C), the sample is dissolved in the molten flux and, after cooling, very homogeneous fusion disks are obtained. Like with pelletization, additives can be introduced for internal standardization or quantification. Also by diluting with flux, the matrix similarity increases, while the sensitivity decreases. Fully automated fusion generators are

available, reproducibly delivering homogenous samples and requiring only little analyst effort. Compared to pelletization, borate fusion offers a better homogeneity. However, fusion may be problematic for analytes with low boiling points (below 1000 °C), like As, Cd, or Zn. Analyte losses cannot be eliminated and therefore might give rise to systematic errors. Of course, with this type of sample preparation, information on Li and B as analyte elements is also lost.

The third approach that is frequently used is mounting/embedding. By mounting, the powdered samples are attached on an adhesive surface, like sticky tape or not completely dried epoxy resin. The surface can be coated before or after sample exposition to vary adhesive effects, or to introduce standards. Mounting is mostly used for qualitative and semiquantitative analysis. Adhesiveness is a limiting factor since the surface must be sticky enough to retain powder particles even after the nearby surface has been subjected to laser irradiation; total damage of the investigated particles or removal of particles next to the ablation site has to be prevented. The sample particulates can also be embedded completely in epoxy resin. After embedding, the resins usually need to be cut and polished prior to LA analysis. During preparation of the epoxy resins, standards and other supplements (e.g., surfactants for particle isolation) can also be added to improve the results. With both methods, information on individual particles can be obtained, which is not possible with pelletization or fusion. This aspect extends the possibilities of LA-ICP-MS (e.g., for 2-dimensional mapping), but the sample preparation is very tedious. However, as a result of the possibly widely different composition of the single particles, reliable quantification is practically impossible.

Quantification strategies

Application of CRMs and in-house standards

Signal quantification using solid standards could be accomplished using either CRMs or in-house prepared standards. Usually, sample and standard need to be converted into a compact sample pellet or disk by one of the methods described in the previous section. Regardless of the sample preparation technique used, in all cases an element initially present in the sample and standard or added during sample preparation is used as an internal standard to correct for differences in ablation, transport, and ionization efficiencies. Concentrations of the internal standard in the sample and standard must be determined by complementary techniques (e.g., SEM-EDX, energy dispersive x-ray analysis) or must be sufficiently well known on the basis of stoichiometry.

Hondrogiannis et al. [108] used LA-ICP-TOF-MS to successfully classify 25 vanilla samples according to their origin. Three grams of vanilla powder was directly pressed into a sample pellet. External calibration was achieved versus NIST SRM 1549 (non-fat milk powder), NIST SRM 1575a (trace elements in pine needles), NIST SRM 1515 (apple leaves), NIST SRM 1547 (peach leaves), and NIST SRM 1570a (trace elements in spinach leaves). The method was validated using NIST SRM 1573a (tomato leaves). Eze et al. used LA-ICP-MS to investigate the composition of coal fly ash [33]. Fusion disks of each sample were prepared according to an automatic gas fusion procedure (Claisse M4 gas fusion instrument) with Claisse Flux as binder material. Quantification of 18 elements was achieved via external calibration versus NIST SRM 612 and using ^{29}Si as an internal standard. USGS BCR-2 or BHVO 2G CRMs were used for method validation. Scarciglia et al. investigated soil and paleosoil samples [123]. Thin sections were prepared for LA-ICP-MS analysis and for calibration NIST SRM 612 was relied on because of the lack of soil CRMs. SiO_2, quantified with SEM-EDX, was used as an internal standard. Relative standard deviations (RSDs) were less than 8 % for all elements and less than 5 % for most of them. Further applications include the analysis of Sahara dust samples [118], desert varnish [121], soil samples [116], biomass ashes [29], fly ash samples [113], ash related deposits [120], coral skeletons [125], and forensic investigations [110]. Detailed information can be found in Table 1.

If no suitable CRMs are available, or the range of analytes cannot be covered, the preparation of in-house standards is another possibility for quantification. Coedo et al. determined six elements in electric arc furnace flue dust [111]. Samples were pressed into pellets using paraffin and cellulose/*N*-butylmethacrylate. Standards were prepared by spiking synthetic ZnO/Fe_2O_3 matrix (1:1) with multi-element solution standards and Rh as an internal standard. The approach was validated with four reference materials. Su et al. [112] determined the distribution of metals in single wood fibers. The fibers were fixed by pressing them onto pellets with graphite powder. For quantification, matrix-matched pellets were prepared with cellulose powder and softwood pulp, doped with multi-element standards. The difference in the amount of ablated material was compensated for by introducing a mass coefficient. Fitzpatrick et al. investigated sol-gel processes to establish in-house calibration standards [28]. They showed that S and Se can be added up to 3 % of the total xerogel concentration, while for transition metals the corresponding maximum level is 0.01 %. The xerogels thus obtained were used for calibration in the LA-ICP-MS analysis of NIST SRMs 610 and 612 (trace elements in glass), achieving satisfactory results with RSDs comparable to those achieved using glass CRMs. According to their work, xerogels seem to be a feasible alternative for glass CRMs. However, no accurate results could be produced for samples with high sulfide contents.

Improved standard approaches

Compared to the reported approaches using CRMs and in-house prepared matrix-matched standards, the quantification

Table 1 Pretreatment and quantification approaches for powdered samples

	Sample type	Sample preparation	Quantification	IS	Validation	Recovery	RSD	Ref.
Standard procedures	Vanilla samples	Pressed pellets	NIST SRM 1549, NIST SRM 1575a, NIST SRM 1515, NIST SRM 1547 and NIST SRM 1570a		NIST SRM 1573a	83–106 %	6.2–14.3 %	108
	Coal fly ash	Borate fusion	NIST SRM 612	Si	USGS BCR-2 or BHVO 2G			29
	Soil samples	Thin sections	NIST SRM 612	Si			<5–8 %	123
	Sahara dust samples	Adhesive tape	NIST SRMs 612 and GSD-1G	Si	USGS W-1 and BCR-2, MPI-DING T1-G and GSJ JG-1a	75–125 %	<15 %	118
	Desert varnish	Direct analysis	NIST 61X series		NIST SRM 612	80–120 %	<20 %	121
	Biomass ash	Borate fusion	NIST SRMs 610 and 612					33
	Fly ash samples	Borate fusion	NIST SRM 2691					113
	Ash related deposits	Embedded in epoxy resin	NIST SRM 2691					120
	Soil samples	Ashing, mounting in epoxy resin	NIST SRM 2691 and NIST SRM 1633b				15–40 %	116
	Coral skeletons	Glass-fused/cut into thin sections	NIST SRMs 610 and 612 and MPI-DING KL2-G	Ca	USGS MACS-1/NIST SRM 614	80–120 %	<4–15 %	125
	Forensic applications	Tape mounting and pelletization	USGS PACS-2, NIST SRM 2704, NIST SRM 2710 and NIST SRM 2710a	Sc, Lu			<15 %	110
	Furnace flue dust	Pressed pellets	Synthetic ZnO/Fe_2O_3 matrix	Rh	CRM 876-1, AG-6203, AG-6201, and AG-SX3705	85–115 %	<7 %	111
	Wood fibers	Pressed pellets	Pellets prepared with cellulose powder, softwood pulp					112
	Glass, silicate	Xerogel disks	NIST SRMs 610 and 612					32
	Aerosol samples	Direct analysis	Standard addition				<10–18 %	124
	Sunflower leaves	Direct analysis	Standard addition					73
	Compost samples	Pressed pellets	BCR-144R and CRM029-050 and standard addition				<10 %	109
Improved procedures	Various CRMs	Borate fusion	Isotope dilution		NIST SRM 1944, 2586, 2702, 2710a, 2711a, and 2780	95–120 %	<3 %	114
	Various CRMs	Pressed pellets, borate fusion	On-line isotope dilution		NIST SRM 610, 612, and 614, MESS-2 and PACS-2 and NIST SRM 2710a, 2711a, 1944, 2702, and 2780	85–110 %	3–21 %	107
	Oxide grains	Mounted on epoxy resin	On-line isotope dilution, ratio analysis		SRM U950a and U010 as well as natural uraninite grains		0.4–2.7 %	117
Specific approaches	Environmental samples	Borate fusion	Standard addition		NIST SRM 612	85–115 %	~10 %	115
	Carbonate	Adhesive tape	MPI-DING reference glasses		NIST SRM 610	90–110 %	1.6–24 %	119
	Soil & dust samples	Electroplating	Ratio analysis		NIST SRM 4353			122

strategies can be even further improved by exploiting the concepts of single standard addition, multiple standard additions, or isotope dilution. Thus, remaining differences between sample and standard can be further compensated for, enhancing the quality of LA-ICP-MS analysis. Okuda et al. used LA-ICP-MS to analyze aerosol samples collected on cellulose nitrate filters [124]. The filters were directly ablated and 15 elements were investigated. Calibration was achieved by spiking the filters with standard solutions. Precisions better than 10 % RSD could be achieved, except for Al (11 %) and Cu (18 %).

da Silva and Arruda [73] prepared sample pellets for the measurement of Se and S in sunflower leaves, which were spiked with different amounts of the elements of interest. Different certified materials were used for validation of the method. Jiménez et al. [109] investigated compost samples for the presence of toxic metals. The samples were homogenized, ground, sieved several times, and pressed into pellets (200 mg). Quantification was achieved by external calibration versus matrix-matched standards (BCR-144R and CRM029-050) and standard addition with aqueous standards. The RSDs for quantification were better than 10 % for most elements. Particle size and therefore milling time were identified as factors having a high impact on the method precision.

If applicable for the target analyte(s), the addition of isotope-enriched spikes is also feasible, enabling analyte quantification using isotope dilution. This approach offers best results in terms of precision and accuracy, but is also expensive and limited to elements for which at least two isotopes can be measured interference-free. Malherbe et al. investigated the potential of this approach for the analysis of different CRMs. Prior to LA-ICP-MS measurement, sample disks were prepared by borate fusion [114]. NIST SRM 1944 (New York/New Jersey waterway sediment), NIST SRM 2586 (trace elements in soil containing lead from paint), NIST SRM 2702 (inorganics in marine sediment), NIST SRM 2710a (Montana I soil), NIST SRM 2711a (Montana II soil), and NIST SRM 2780 (hard rock minewaste) and a meteorite sample were analysed. The results obtained were in good agreement with the corresponding certified values and the precision was better than 3 % RSD for all elements investigated. For the analysis of powdered samples, the previously described concept of liquid standard nebulization is also used for quantification of LA-generated aerosols. Fernández et al. [107] proposed a quantification method with on-line double isotope dilution for a wide range of matrices. Samples were either analyzed directly (NIST SRMs 610, 612, and 614), pressed into pellets (USGS MESS-2 and PACS-2), or fused with lithium borate (NIST SRMs 2710a, 2711a, 1944, 2702, and 2780). The ablated aerosol of either standard or sample is mixed with the nebulized isotope-enriched spike solution or blank solution. The RSDs ranged from 6 to 21 % for pressed pellets and from 3 to 21 % for borate fusion. Lloyd et al. analyzed uranium oxide grains, retrieved from soil and dust samples [117]. The grains were mounted on epoxy resin; the latter was then ground and polished to access the interior of the grains. As a result of the lack of a suitable standard reference material containing ^{236}U, quantification was achieved by introducing liquid reference materials NBL U950a (uranium particles) and NBL U010 (uranium isotopic standard) via a desolvating nebulizer. Natural uraninite grains were used as tertiary reference material to correct for mass bias. U isotope ratio analysis was implemented successfully with precisions of 0.4 % and 2.7 % RSD for $^{235}U/^{238}U$ and $^{236}U/^{238}U$, respectively.

Specific approaches

Claverie et al. [115] proposed a new approach for quantification of six elements of environmental concern, e.g., in soil or sediment samples, using pellets fixed on a spinning platform. Samples as well as standards were prepared by lithium borate fusion. By placing standard and sample next to each other on a platform, which is spinning at a high speed during laser ablation, quasi-simultaneous ablation of sample and standard is achieved. The mixed ablation aerosols are analyzed and quantification is based on standard addition or isotope dilution. For five standard reference materials and meteorite rock, an average precision of 10 % RSD could be achieved. The experimental results obtained compared well with the corresponding certified values with maximum deviations of 15 %. Lu et al. [119] tried to overcome the need for an internal standard for LA-ICP-MS analysis of carbonate materials. Using an equation-based approach, the so-called MRM-NoIS calibration strategy, a successful quantification of different carbonate minerals was achieved by using four reference materials (NIST SRM 610, USGS MACS-3, USGS GP-4, MPI-DING). For trace elements RSDs of less than 10 % and for rare earth elements (REEs) and major compounds RSDs of less than 5 % were observed. Cizdziel et al. investigated plutonium in US soil and dust samples [122]. Pu was spiked with a tracer, leached from the sample, and extracted from the leachate by anion exchange chromatography. Afterwards, the recovered analyte was electroplated on a stainless steel planchette disk for LA-ICP-MS analysis. LA-ICP-MS results were compared with liquid ICP-MS results, which were validated using NIST SRM 4350b (river sediment, radioactivity), NIST SRM 4353 (rocky flats soil number 2), and IAEA 385 (radionuclides in Irish Sea sediment). The authors reported a successful fingerprinting of Pu in soil and dust samples with LA-ICP-MS.

Figures of merit

Since detection limits and data for the level of reproducibility achieved have not been published in any of the reviewed contributions, this section aims at providing a comparison between the methods, accounting for all special applications.

Besides the approach used for preparation of compact samples, the quantification strategy also has to be taken into consideration. Furthermore, sample homogeneity is another limiting factor (especially for pressed pellets). Also, the concentration ranges of the target analytes as well as the instrumentation applied influence the quality of the results obtained.

In general, detection limits (LOD) were found to vary between several micrograms per kilogram and some milligrams per kilogram, depending on the instrumentation used and the analyte of interest. With pelletization, LODs around 0.035 mg kg^{-1} were obtained for different elements in vanilla samples [108]; for compost samples [109], values ranging from 0.01 to 0.8 mg kg^{-1} were reported. For fusion, the LODs varied from 0.02 to 4 mg kg^{-1} [114] with SF-ICP-MS and Q-ICP-MS. For tape mounting analysis and subsequent LA-ICP-MS analysis using SF-ICP-MS, detection limits from 0.3 μg kg^{-1} to 10 mg kg^{-1} were observed [118]. LODs varying from 0.001 to 0.5 mg kg^{-1} were reported for direct analysis of the sample without pretreatment [121].

In contrast to sensitivity, the reproducibility of a measurement is less dependent on the MS instrumentation used. Overall, reported RSDs were in the order of less than 3 to 50 %. The pelletization approach resulted in measurement reproducibilities varying between 6 and 21 % for the elements Pb, Rb, and Sr [107], and 6 to 14 % for 11 elements in vanilla [108] and less than 15 % for 12 elements in soil [110]. Fusion approaches showed comparably lower RSDs, a result which could be attributed to the improved sample homogeneity obtained with this approach. Published results vary between less than 3 % [114], 3 and 21 % for Pb, Rb, and Sr [107], and 10 % for six elements in environmental matrices [115], depending on target element and calibration strategy. For applications using the mounting/embedding approach for sample preparation, RSDs ranged from 15 to 50 % for the halogens Cl, Br, and I in ashed soil samples [116], and 15 to 25 % for 60 elements in dust samples using fs-LA-ICP-MS [118]. Poorer RSDs often result from the low analyte signals observed when analyzing single particles, which give rise to very small amounts of ablated material only.

Dried droplet analysis of liquid samples

As demonstrated in the previous sections, LA-ICP-MS is a versatile tool for solid sampling, suited for both bulk analysis and for mapping analyte distributions, as well as for combinations thereof. Consequently, the vast majority of samples being analyzed by LA-ICP-MS today are solids, especially since LA-ICP-MS circumvents the sometimes cumbersome digestion procedures otherwise required. For liquid samples and sample solutions, sample introduction in ICP-MS analysis is traditionally accomplished using a nebulizer. Pneumatic nebulizers are available in numerous modifications to suit practically any kind of liquid matrix [126]. However, heavily matrix-loaded liquid samples, such as urine or blood, present a challenge even for the most matrix-tolerant nebulizers. Such demanding matrices require at least dilution or partial digestion, which results in an increased workload. If high sample throughput is required, alternative sample introduction methods are therefore necessary.

As an alternative to pneumatic nebulization, laser ablation of dried liquids offers the aforementioned matrix-tolerance and sample throughput. In this section, the concept and some practical aspects of dried droplet laser ablation will be discussed. The performance of the method, as well as instrumental limitations will be highlighted, and a comprehensive overview of the related literature, including application examples, will be given.

The concept of dried droplet laser ablation

The basic concept of dried droplet laser ablation consists of depositing a well-defined volume of liquid sample on a solid support, evaporating the solvent, and examining the remaining dried residue by means of LA-ICP-MS. To unmistakably state that only the dried residue of a liquid sample is being analyzed, the term “dried droplet laser ablation” will be used throughout. To the best of our knowledge, Yang et al. introduced this method to ICP-MS in 2005 [127]. The present review will focus exclusively on the ablation of dried liquid samples, although it has been demonstrated that direct liquid ablation is also possible [128, 129].

As simple as the concept of dried droplet laser ablation may seem, its implementation can hold some pitfalls. One risk is to compromise the natural homogeneity inherent to the liquid sample. Method development in dried droplet laser ablation should therefore aim at preserving the sample’s original elemental composition throughout the analytical process, or at providing a sound strategy to compensate for any inhomogeneities introduced artificially during sample preparation. There are some methods reported in the literature that appear to be similar to dried droplet laser ablation in the sense that some part of a liquid sample is dried and analyzed by laser ablation. However, with those methods, the homogeneity of the liquid sample is abandoned by design. Hence, it is difficult or impossible to obtain quantitative information. Such methods are, for example, the combination of thin-layer chromatography with laser ablation [130–134] or the analysis of substrates which are immersed in a sample, removed from the liquid, and subsequently dried [135, 136].

The motivation for using dried droplet laser ablation instead of more established sample introduction techniques is in all cases reported to be (a combination of) the following four features: coverage of (sub-)microliter sample volumes, while offering (sub-)microgram per liter detection limits in case of ICP-MS detection, removal of solvent to allow

coupling ICP-MS as an element-specific detector to chromatographic systems and to obtain less polyatomic interferences arising from the solvent, simplification of sample logistics, as well as direct sampling of challenging liquid matrices. Although some of these features could also be achieved with alternative solid sampling methods, such as solid sampling graphite furnace AAS or electrothermal vaporization (ETV) ICP-MS, dried droplet laser ablation offers two significant advantages over graphite furnace techniques. First, it is a genuine multi-element technique as opposed to conventional AAS, or compared to high-resolution continuum source AAS with limited multi-element capabilities. Secondly, LA allows complete desorption of the sample, whereas in ETV carbide formation may hamper correct quantification (e.g., [137, 138]). Such problems are not observed with LA [139]. In some cases, sample throughput was found to be higher with dried droplet laser ablation than with ETV-ICP-MS [139] but this certainly depends on the measurement protocols deployed and cannot be generalized.

Preparation of dried droplets

As stated above, the key aspect of dried droplet laser ablation is to preserve the inherent homogeneity of the liquid sample throughout sample preparation and measurement. If an artificial inhomogeneity is newly introduced, this should be done in a reproducible way, in order to be able to fully compensate for it. From everyday experience, it is well known that dried residues, e.g., coffee stains in the kitchen, are usually far from being homogeneously shaped. It is the scope of this section to provide some very basic physical insights into the processes involved in droplet-drying, although the literature on this topic is vast and the interested reader is referred to the numerous specialized reviews. Three parameters play a major role in terms of dried droplet shape: (a) choice of the surface used for droplet deposition, (b) drying conditions, and (c) the matrix of the liquid sample:

(a) When drying droplets on hydrophilic surfaces, ring-shaped residues are frequently obtained. This "coffee stain effect" was described by Deegan et al. [140, 141] to be caused by a radial flow, which transports liquid from the core of the droplet to its perimeter, where the solvent evaporates more easily. This radial flow is the consequence of one precondition inherent to this physical model: the contact line (the perimeter of the droplet) does not shrink during the drying process. Several authors provided experimental data to support this model (e.g., [142–144]). Contrarily, in the case of a hydrophobic surface, deposition of concentric rings or small spots in the center of the droplet can be observed [145–147], as the contact line continuously or periodically shrinks while drying.

(b) In addition to the flow patterns that lead to ring deposition, the interaction of dissolved or colloidal matter with the contact area, as well as convective currents can contribute to the dried pattern [144, 148]. Such convective currents are related to the drying rate, which in turn depends on temperature, relative humidity, and heat conductivity of the substrate [144, 149, 150]. Hence, the experimental setup might also influence the shape of the dried residue.

(c) Finally, the matrix of the sample (e.g., salt or protein concentration) also influences the shape of the dried residue [151, 152]. In the first publications that describe dried droplet laser ablation, small sample aliquots of 20 μL were pipetted onto hydrophobic polystyrene plates and dried under ambient conditions [127, 139, 153]. Owing to differences in the matrix (purely inorganic salts in the case of standards, with organic constituents in case of samples), the shape and size of the dried residue depended strongly on the sample matrix [127].

In view of the aforementioned three parameters, a drying droplet is a complex system and it is easy to understand that the morphology of the final dried residue is difficult to predict. Although the references provided show that it is indeed possible to control the morphology of the dried residue, such experiments are most likely beyond the scope of analytical laboratories. However, it is possible to minimize the influence of those factors, which have the most pronounced effect. As the choice of the solid substrate plays a major role in terms of droplet morphology, this factor was considered and optimized in most reported cases of dried droplet laser ablation. The following three types of solid substrates were applied: 1. hydrophobic surfaces, 2. filter paper (paper diameter much greater than droplet diameter), and 3. confined, circular, and hydrophilic areas (diameter of circular area no greater than droplet diameter). For visualization, examples of these approaches are provided in Fig. 3.

Method I Using a hydrophobic surface for droplet deposition results in a small dried residue, as described above. Typical diameters of dried residues are around 100–1600 μm, depending on droplet volume and sample matrix (Table 2). As the droplet shrinks continuously, the coffee stain effect will be observed only at a very late stage of the drying process, or not at all. Hence, it is straightforward to ablate the entire dried residue with only a few laser pulses. Hsieh et al. [154, 155] demonstrated that this approach allows for external calibration when quantifying metals in whole blood. Yet, as other authors have found, the extreme preconcentration on a small spot has the disadvantage of pronounced matrix effects by co-existing sample constituents. For example, Yang et al. [127] showed significant signal suppression by sodium present in the sample. On the other hand, deposition of droplets on a substrate

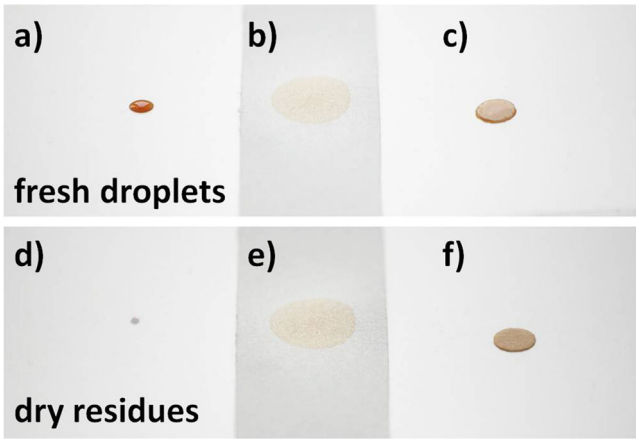

Fig. 3 Strategies for sample application in dried droplet LA analysis. Deposition of a defined sample volume on hydrophobic surfaces (**a**), filter paper with dimensions much greater than droplet diameter (**b**), and confined, circular, hydrophilic areas with diameter of circular area no greater than droplet diameter (**c**). Dried residues after evaporation of the solvent on a hydrophobic surface (**d**), filter paper (**e**), and precut filter disks of filter paper (**f**)

that enhances sample ablation as a result of strong interaction with the laser light has been demonstrated [156, 157], in combination with automated deposition of liquids. Table 2 summarizes the literature that applied the approach of hydrophobic surfaces.

Method II Applying a droplet on a large piece of filter paper makes sample preparation very easy, as the liquid is immediately absorbed by capillary action [158, 159]. Once dried, the residues can be easily transported and stored as the analyte is incorporated in the paper fibers [160]. Consequently, blotting cards are widely used in clinical settings [161], e.g., in collection of blood samples. Yet, even if the dispensed volume of sample is well defined, differences in viscosity can lead to a different migration behavior on the paper, combined with chromatographic effects [162, 163]. Careful design of laser ablation patterns is therefore required. Table 3 gives an overview of papers that report on analysis of "freely migrated" droplets on filter paper, via LA ICP-MS. Also, typical diameters of dried residues and applied sample volumes are given.

Method III By providing a hydrophilic area with a clearly defined border, samples can only migrate within this area. Therefore, samples with low viscosity or challenging matrix are confined on the same area as purely aqueous samples. This approach was used by Choi et al. [164] to analyze photo-resist, deposited on micro-machined polymer pillars. A somewhat different approach was presented by Aramendía et al. [160] who applied precut disks of filter paper on a hydrophobic surface. By doing so, large volumes of sample could be applied on a small area, thus enhancing sensitivity 10-fold [160]. However, the coffee stain effect is very pronounced with this type of sample preparation, since the precut filter disk is the ideal model of a fixed contact line (as described above, a fixed contact line results in a constant droplet area during the drying process. As the evaporation on the rim of the droplet is faster than in the center of the droplet, a liquid flow is created which transports material to the rim of the droplet which is the origin of the coffee stain effect). Crater-shaped analyte distributions were therefore obtained [18, 160, 165]), as also observed in MALDI-MS [166]. Table 4 gives an overview of the related literature and typical sample loadings (microliters per millimeter squared).

Quantification approaches, sensitivity, and reproducibility

The quantification process also needs to be adapted to the approach used for sample preparation. The sensitivity is influenced to a large extent by the analyte loading, i.e., the amount of sample per unit area (e.g., microliters per millimeter squared). In Tables 2–4, this value can be compared for the three application modes (methods I–III), and in general, highest analyte loading is observed with method I. However, as discussed above, matrix effects are most pronounced with this approach; therefore, lower analyte loadings (method II or method III) can be beneficial, depending on the individual analytical setup and sample type.

In the case of sample preparation on a hydrophobic surface (method I), the small residue can be ablated completely using spot, grid, or line patterns. Thus, the entire signal is collected in a short time, giving rise to a sensitivity which is comparable to that achievable with conventional nebulizer systems. Yang et al. [127] even demonstrated a 2–7-fold improved absolute sensitivity (counts per nanogram) compared to pneumatic nebulization when analyzing aqueous standard solutions. This finding is also due to the fact that the transport efficiency of laser ablation systems is superior to that of pneumatic nebulizer systems [127]. The general sensitivity of dried droplet laser ablation in combination with hydrophobic surfaces obtained in practice can therefore be expected to be similar to that with pneumatic nebulization (see Table 2).

Partial ablation of freely deposited droplets (method II) might result in erroneous results, as demonstrated in [160], unless closely matrix-matched standards are used [158]. The reason for this is that sample-to-sample variations in terms of viscosity result in different sample spread across the filter. The complete consumption of the dried residue [159] is a feasible way to avoid this problem, but requires specialized laser equipment. If the deposited volumes are very small [167, 168], quantitative ablation from filter paper becomes easy, especially in the presence of substances that improve the laser ablation yield, such as black ink deposited prior to the droplet [167, 168].

Table 2 Method I: droplet deposition on hydrophobic surface

Sample matrix	Droplet volume	Size of dried residue	Sample loading[a]	Surface material	LOD	Reproducibility	Additional information	Ref.
Drinking water, yeast extract	20 μL	150–500 μm	10^{-4}–10^{-3} μL μm^{-2}	PS	0.08–0.12 ng mL^{-1} (aqueous), 0.06–0.09 ng mL^{-1} (standard addition), 0.05–0.08 ng mL^{-1} (isotope dilution)	4.6–23 % RSD (aqueous with I.S., n=4), 4.7–8.2 % RSD (standard addition with I.S., n=4), 3.5–3.9 % RSD (isotope dilution, n=6); 0.5 % RSD (Se in yeast, isotope dilution, n=5)	NaAc matrix was added to water samples	[127]
HPLC fractions of yeast extract	20 μL	600 μm	7×10^{-5} μL μm^{-2}	PS	36–110 μg g^{-1} (Se)	0.55–0.77 % RSD (species-specific isotope dilution)	No matrix required due to high salt load of samples	[153]
Digested biological tissue, nearshore seawater, and river water	20 μL	100 μm 1.6 mm	10^{-5}–3×10^{-3} μL μm^{-2}	PS	0.033 pg mL^{-1} (Pu), 0.051 pg mL^{-1} (Th), 0.072 pg mL^{-1} (U).	8 % RSD (with I.S., n=10)	Chromogenic matrix investigated	[137]
Cr species via capillary electrophoresis	100 nL	100–500 μm	5×10^{-7}–10^{-5} μL μm^{-2}	PETG	0.2–6.5 μg L^{-1}	Below 3 % RSD	α-Cyano-4-hydroxycinnamic acid	[156]
Blood reference materials	0.5 μL	700–900 μm	8×10^{-7}–10^{-6} μL μm^{-2}	Other	0.1 ng mL^{-1}	Below 10 % RSD for all samples	Methylene blue as indicator and to improve ablation yield	[154]
Cell cultivation medium and cell lysate	<20 nL	<300 μm	3×10^{-7} μL μm^{-2}	PETG	26 fg Cu (100 nL droplet, therefore 26 ng L^{-1})	5 % RSD under optimized conditions for samples	Rhodamine B added for visibility	[157]
Cr species via liquid-liquid micro extraction, synthetic seawater	7 μL	5 mm	4×10^{-7} μL μm^{-2}	PS	0.11 μg L^{-1}	4–8 % RSD	Organic matrix, internal standard	[169]
Various "meat" reference materials (oyster tissue, etc.)	50–100 μL	1 cm	6×10^{-7}–10^{-6} μL μm^{-2}	PTFE, PS	0.05–6 μg kg^{-1} dry mass (corresponds to 1.25–240 ng L^{-1})	5–10 % RSD	No additive, organic digest	[170]
Mineral water, tap water, swimming pool water, and water from two artificial lakes	1 μL	600 μm	4×10^{-6} μL μm^{-2}	PTFE	0.05–0.81 ng mL^{-1}	~5 % RSD (for n=3, recovery experiment)	Methylene blue added for visibility	[171]
Seronorm blood reference material	0.5 μL	–	–	PTFE	0.14–29 ng mL^{-1} (Be–Mg)	6 % RSD within-run precision, 4–8 % RSD between-run precision	Methylene blue added for visibility	[155]
SLRS-4 river water reference material, lake water, and synthetic seawater	1 μL	480–850 μm	2×10^{-6}–6×10^{-6} μL μm^{-2}	PTFE	0.03–0.2 pg mL^{-1} (enrichment factor 32)	2–5 % RSD	Methylene blue added for visibility	[172]
Human urine from Fabry disease patient and control	1 μL	–	–	PTFE[)]	0.003–0.58 μg g^{-1}	<20 % RSD	Spiked samples for calibration	[173]

PS polystyrene, *PETG* poly(ethyleneterephthalate)glycol, *PTFE* polytetrafluoroethylene, *Other* "hydrophobic filter membrane"

[a] Sample volume/area of dried residue (assuming a circular spot)

Table 3 Method II: droplet deposition on large filter paper sheets

Sample matrix	Droplet volume	Size of dried residue	Sample loading[a]	Surface material	LOD	Reproducibility	Additional information	Ref.
Blood spotted on paper, from a lab proficiency test	–	–	–	Whatmann filter paper	0.9 μg dL^{-1} (Pb)	7 % RSD (in-between droplets and also within droplet)	Sample directly spotted without any other treatment	[158]
Co in a drug preparation, Pb in whole blood, and Sn in food samples	500 nL	–	–	Filter paper with additive	1–60 ng L^{-1}	10 % RSD (spot-to-spot)	Different additives to improve laser yield	[168]
Pb and Cd in BCR-634 whole blood reference material	200 pL	–	–	Filter paper with additive	0.5 pg Pb 0.02 pg Cd (equal to 2.5 and 0.1 ng L^{-1} with 200 nL of sample)	25 % for Pb and 8 % for Cd with standard solutions using ^{13}C as internal standard, for samples: 5 % for Pb and 35 % for Cd	Repeated deposition of 65 pL droplets, ablation of several droplets at the same time	[167]
Blood (reference materials and real samples)	5 μL	5–6 mm	3×10^{-7} μL m^{-2}	Filter paper	0.040–0.054 μg L^{-1}	3–9 % RSD (quantitative) 1500 ppm (isotope ratios)	Analysis vial split aerosol-flow (single-collector/multi-collector ICP-MS)	[159]

[a] Sample volume/area of dried residue (assuming a circular spot)

Table 4 Method III: droplet deposition on confined, circular, and hydrophilic areas

Sample Matrix	Droplet volume	Size of dried residue	Sample loading	Surface material	LOD	Reproducibility	Additional information	Ref.
Photo-resistant used in photolithography	64.7 pL	150 μm	4×10^{-6} μL $μm^{-2}$	PDMS-columns (micro-machined)	2.33, 15.4, 5.72 ng mL^{-1} (Al, Cu, Pb)	17.1–46.9 % RSD (due to extremely low sample volume)	No matrix added, photo resist	[164]
Human urine from supposedly healthy patients	300 μL	16 mm	10^{-6} μL $μm^{-2}$	Filter paper (precut saturated filter disks)	0.1–13 μg L^{-1}	2–5 % RSD	No additive	[160]
Cu isotopes in urine of Wilson's disease patients, treated patients and one control patient	300 μL	16 mm	10^{-6} μL $μm^{-2}$	Filter paper (precut saturated filter disks)	–	200–500 ppm RSD intra-spot, and 540 ppm RSD inter-spot	No additive, corona ablation with 10 kHz	[18]
Phosphorus in fermentation media	10 μL	5 mm	5×10^{-7} μL $μm^{-2}$	Filter paper (precut saturated filter disks)	10 μg mL^{-1} (ICP-OES detection)	10 % RSD	Analysis via laser ablation ICP-OES	[165]

[a] Sample volume / area of dried residue (assuming a circular spot)

Table 5 Selection of frequently applied procedures for signal quantification in LA-ICP-MS analysis

Quantification approach	Biogenic carbonates	Hard tissues	Soft tissue	Powdered samples	Liquid samples
CRM/SRM	27, 38–48, 50–54	58–62		108, 118, 121, 123	154
In-house prepared standards					
Non matrix-matched standards					
Use of well-characterized materials	43	64–68			158
Thin films on sample or substrate			94		
Gelatin, agarose gel, sol-gel standards			91, 92, 93	32	
Printed pattern			95, 96		167, 168
Dried droplets (aqueous standards)					127, 138, 154, 157
Matrix-matched standards					
Preparation of pellets	69			71, 109, 111, 112	
Fusion to disks	31, 32			29, 33, 113–115	
Embedding into polymer resin			97	116, 120	
Homogenized tissues			90		
Matrix-adjusted dried droplets					155, 164, 169–173
Specific approaches		63, 66, 67			
Nebulized liquid standards					
Calibration/standard addition	64, 65		88, 89	117	
IDMS			89, 103	107	
Internal standard correction					
Sample-inherent element	27, 28, 38, 39, 43, 46	58–67	75, 104	29, 118, 123, 125	
Homogeneously spiked to the sample			90, 97	110, 114	127, 139, 160, 169
Applied as thin layer on/below sample			79, 94, 95, 100		160
On-line addition of dried aerosol			88, 89		

In the case of uniform sample geometry due to circular hydrophobic areas (method III), the coffee stain effect is very pronounced, as discussed above. Although a quite homogeneous analyte distribution is obtained in the center of the droplet, the extent of ring formation will depend on the sample matrix. Therefore, standard addition or isotope dilution is required if the laser is focused onto the center of the filter disk. In a recent publication, Nischkauer et al. [165] showed that the bias resulting from this centrosymmetric distribution of analytes can be easily compensated for. Instead of performing laser ablation only in the center [160] or only in the rim [18] of the precut filter disk, it was proposed to perform radial line scans that pass across the entire sample, including the center. The resulting U-shaped signal was then integrated and was found to be proportional to the concentration in the initially liquid sample, without the need to ablate the entire filter disk, and without the need for matrix-matching or the use of an internal standard [165].

The reproducibility of dried droplet laser ablation is intrinsically compromised, compared to pneumatic nebulization, as a result of the additional error introduced by repeated droplet deposition and by the transient signal mode. Interdroplet reproducibilities range between 3 % and 23 % RSD for samples measured directly, with typical values ranging between 3 % and 10 % RSD [127, 137, 154, 155, 157, 158, 160, 165, 167–173]. When automated, droplet deposition can be achieved with greater precision [174, 175], but the higher uncertainty inherent to solid sampling techniques will most likely persist. In case of isotope dilution and isotope ratio measurements, better reproducibility was reported (540 ppm RSD interspot [18], 0.55–0.77 % RSD for species-specific isotope dilution [153]) than in the case of pure quantitative measurements.

Conclusions

Although geoscience is still the main field of application of LA-ICP-MS, its use in the fields of environmental research and life sciences increased continuously during the last few years. High sensitivity combined with excellent spatial resolution is the main reason for using LA-ICP-MS in the analysis of hard and soft tissues, as well as of powdered environmental samples. The capabilities for performing imaging studies or isotope ratio measurements are additional advantages of LA-ICP-MS. The applications published so far cover a wide range of sample matrices, target analytes, and concentration ranges. Nevertheless, they have one common problem—reliable

quantification. The strategies used to circumvent the influence of elemental fractionation and matrix effects in LA-ICP-MS analysis, which are considered as the main problems hampering reliable quantification, are rather similar, although the resulting interferences differ between individual applications. Table 5 presents a compilation of the most frequently applied approaches for quantification, indicating that the use of in-house prepared standards in combination with an internal standard is the dominating strategy. Improved concepts for sample preparation as well as for application of matrix-matched standards will further enhance the potential of LA-ICP-MS for the analysis of environmental, biological, and biomedical samples. The choice of an appropriate internal standard is still of major concern in many applications, especially when no sample-inherent element is available. Thus, further methodological developments are required, e.g., in the case of tissue analysis the application of polymeric layers or thin metal coatings has been shown to be promising. Special attention should be paid to the application of fs-laser systems, which offer distinct improvements in terms of matrix effects and elemental fractionation. Another prerequisite for the acceptance of LA-ICP-MS as an alternative to traditional procedures for the quantitative determination of trace elements is the availability of appropriate reference materials. Especially for life science applications, the development and production of a larger range of CRMs is highly desirable. In contrast, for environmental samples (soil, fly ash, dust, etc.) a wide variety of CRMs is available. However, as those materials have been designed for liquid analysis after mineralization, none of them is applicable for direct LA-ICP-MS analysis because of their inhomogeneity on the microscale. Particularly considering the comparability of measurement results, the availability of at least a couple of compact standard materials with sufficient homogeneity should be aspired to.

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ARTICLE 2

Radial line-scans as representative sampling strategy in dried-droplet laser ablation of liquid samples deposited on pre-cut filter paper disks.

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Radial line-scans as representative sampling strategy in dried-droplet laser ablation of liquid samples deposited on pre-cut filter paper disks ☆

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ABSTRACT

Nebulising liquid samples and using the aerosol thus obtained for further analysis is the standard method in many current analytical techniques, also with inductively coupled plasma (ICP)-based devices. With such a set-up, quantification via external calibration is usually straightforward for samples with aqueous or close-to-aqueous matrix composition. However, there is a variety of more complex samples. Such samples can be found in medical, biological, technological and industrial contexts and can range from body fluids, like blood or urine, to fuel additives or fermentation broths. Specialized nebulizer systems or careful digestion and dilution are required to tackle such demanding sample matrices. One alternative approach is to convert the liquid into a dried solid and to use laser ablation for sample introduction. Up to now, this approach required the application of internal standards or matrix-adjusted calibration due to matrix effects. In this contribution, we show a way to circumvent these matrix effects while using simple external calibration for quantification. The principle of representative sampling that we propose uses radial line-scans across the dried residue. This compensates for centro-symmetric inhomogeneities typically observed in dried spots. The effectiveness of the proposed sampling strategy is exemplified via the determination of phosphorus in biochemical fermentation media. However, the universal viability of the presented measurement protocol is postulated. Detection limits using laser ablation-ICP-optical emission spectrometry were in the order of 40 μg mL^{-1} with a reproducibility of 10 % relative standard deviation (n = 4, concentration = 10 times the quantification limit). The reported sensitivity is fit-for-purpose in the biochemical context described here, but could be improved using ICP-mass spectrometry, if future analytical tasks would require it. Trueness of the proposed method was investigated by cross-validation with conventional liquid measurements, and by analyzing IAEA-153 reference material (Trace Elements in Milk Powder); a good agreement with the certified value for phosphorus was obtained.

1. Introduction

Controlling nutrients in fermentation media has become a key aspect in process monitoring as biochemical fermentations gain more and more importance in industry and research. Limitations in biomass production caused by a lack of phosphorus are a current focus in biochemical engineering. With a better understanding of this phosphorus-limitation, optimum productivity can be achieved while applying just the required amount of this element. Moreover, the growth-rate of biomass can be controlled via the availability of phosphorus.

Studying phosphorus-limitation requires quantification of total phosphorus, as well as total dissolved phosphorus, during the biochemical reaction (TP and TDP, respectively, as defined in [1]). While conventional approaches using UV–vis spectrometry of the phosphomolybdenum blue complex are highly sensitive for phosphate, they are difficult to implement with the biochemical fermentation matrix at hand because the formation of complexes is interfered by the co-presence of silicate, nitrate, nitrite, sulphide, and several metal ions [1]. Another drawback is that the phosphomolybdenum blue complex is formed exclusively from the phosphate anion. Therefore, UV–vis spectrometry requires full chemical digestion of the samples in order to also detect phosphorus contained in species different from phosphate [1,2].

A powerful alternative for the established, but cumbersome photometric approach is atomic (emission, absorption or mass) spectrometry, as well as X-ray fluorescence spectrometry (XRF). With these methods, TP and TDP quantification can be achieved without (complete) digestion since these methods are generally not influenced by speciation. Inductively coupled plasma-optical emission spectroscopy (ICP-OES) and mass spectrometry (ICP-MS), as well as atomic absorption

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spectrometry (AAS) are suitable techniques for this task. We will not expand on AAS further because the method presented in this contribution was designed with the aim of future extension to multi-element quantification. Nevertheless, AAS is a powerful technique for TP single-element quantification [3], also with the option of (limited) multi-element capabilities using continuum sources. XRF and especially total reflection XRF are also possible methods for phosphorus determination. However, sensitivity for elements with low mass number is low and direct analysis of samples without pre-treatment is a difficult task [4,5].

ICP-OES and ICP-MS are rugged techniques, but analyzing fermentation media without any sample preparation at all remains challenging. One reason for this is the presence of high concentrations of organic compounds and inorganic salts. Such compounds can block sample introduction interfaces and therefore, sample dilution is common practice. In order to facilitate sample preparation and to enhance sample throughput, alternatives for sample dilution are required. Ideally, sample preparation should be straightforward (or automated) with the option of being carried out completely on-site. Moreover, samples should be stabilized to allow for simplified transport, and for archiving. In addition, the method should be tolerant towards suspended particulates (e.g., agglomerates of proteins, cell membranes), especially in the case of TP quantification.

One way to meet these conditions is to prepare dried droplets of the samples. This approach can be performed under field conditions and results in minimum work-load. Elemental analysis of such samples can then be performed later on, using suitable solid-sampling techniques (e.g., laser ablation combined with ICP-MS [6] or ICP-OES, as well as solid sampling graphite furnace AAS [7]).

In this article, we present a measurement protocol for laser ablation, which allows analyzing representative sub-samples of a dried sample droplet. Thus, systematic errors commonly observed with dried-droplet sample preparation caused by inhomogeneous sample distribution on the supporting substrate are effectively overcome. The approach presented here allows for external aqueous calibration, reduces sample-handling to a minimum (no dilution, no addition of internal standard), and thus offers a simplified quantitative method for TDP-quantification in biochemical fermentation media.

2. Materials and methods

1. Reagents and consumables

High-purity water was prepared in-lab using an Easypure water system (Thermo, USA, resistivity 18.2 MΩ cm). All reagents used were of analytical grade or higher, unless stated otherwise. Concentrated nitric acid, sodium chloride, saccharose and L-tartaric acid were purchased from Merck, Germany. A phosphate stock solution of 10 g L^{-1} P was prepared by dissolving $NaH_2PO_4{}^*2H_2O$ in high-purity water. This stock solution was quantified versus a P-containing multi-element standard solution (ARISTAR, VWR Germany) using solution-nebulisation ICP-OES analysis to determine its exact concentration. For method validation, IAEA-153 reference material (Trace Elements in Milk Powder) was used. The dried milk-powder was reconstituted by adding high-purity water, shaking and ultrasonication for 30 minutes. Although not identical in sample matrix, this reference material has a similar composition as the samples investigated (i.e., high concentration of organic compounds, high load of Na, Mg, and Ca).

2. Instrumentation

Measurements were performed using an iCAP 6500 ICP-OES spectrometer in radial view mode, using a quartz torch and a quartz injector tube of 2 mm inner diameter (Thermo Scientific, USA). Laser ablation (LA) was carried out with a NWR 213 nm solid state Nd:YAG laser (New Wave Research, USA). The ablation chamber was equipped with an ablation cup, which allows for fast wash-out, while offering a large volume ablation chamber. During LA, a gas flow of 0.9 L min^{-1} of helium was flushed through the cell. After the ablation chamber, a gas flow of 0.4 L min^{-1} Ar was added via a Y-connector. The LA-system was connected directly to the ICP-torch via 1 m of PTFE tubing of 4 mm inner diameter.

While continuously ablating a spiked filter disk (10 μL of spiked fermentation medium dispensed and dried on pre-cut paper filters), plasma conditions and gas flow rates were initially optimized. The pixel position for all emission lines was checked prior to each measurement session and adjusted to obtain best results in terms of signal-to-noise ratio and to ensure accurate background correction. A summary of the final ICP-OES parameters used for LA, as well as for conventional pneumatic nebulisation (PN, see also Section 5) can be found in Table 1.

3. Elemental mapping and quantification via LA

For LA-ICP-OES mapping of the P-distribution, the dry filters were coated with a thin layer of gold using an Agar B7340 sputter coater (Agar Scientific Limited, Essex, UK). Samples were placed 5 cm away from the gold target, and a sputtering current of 10 mA was applied for 60 s (chamber pressure approx. 0.1 mbar). Later, when ablating these Au-coated samples with the LA system, the omnipresent gold signal (corresponding to all ablation passes) was used to automatically detect individual ablation patterns via ImageLab software (developed by H. Lohninger [8]). With this software, reconstructing the elemental map from the individual laser patterns is significantly facilitated and fully automated. Sample-inherent elements could not be used as tracers for the ablation patterns, as their concentrations either varied laterally (e.g., P, Na), or resulted in detector saturation (carbon). In the context of mapping, only qualitative information was required. Hence, the gold signal was used exclusively for automatic data evaluation, and to identify signal drift.

LA parameters were initially optimized for best signal-to-noise ratio and for fast data acquisition (high laser scan speed to reduce measurement time) by ablating a spiked fermentation sample applied on a pre-cut paper disk. LA maps were recorded by performing adjacent and parallel line-scans (conditions see Table 2). For mapping purposes, signals were recorded in time-resolved mode using readout intervals of 2.5 s.

For quantitative measurements of the filter disks, samples were prepared as described in Section 4. Only one line-scan (conditions see Table 2) across each filter was performed in a way that the centre of the disk was met. As opposed to the mapping protocol described above, no gold-layer was applied on the samples. Readout intervals in the iTEVA software (Thermo Scientific, USA) were set to 4 s for better signal-to-noise ratio (in the mapping context, a time-resolution of 2.5 s was required to provide a sufficient number of data points per ablation pass to allow for automated data treatment). Integration of the time-resolved data was done via the corresponding tool in the iTEVA software.

Table 1
Operating parameters of the ICP-OES Spectrometer.

		LA	PN
Plasma power	W	1450	1400
Radial observation height	mm	15	11
Plasma gas flow rate	L min^{-1}	12	12
Nebulizer gas flow rate	L min^{-1}	0.4[a]	0.7
Auxiliary gas flow rate	L min^{-1}	0.6	0.6
Analytical wavelengths (nm)			
P	177.495[b]		178.284[c]
Au	208.209		-

[a] make-up gas for LA carrier gas, [b] used for quantification, [c] used for quality control.

Table 2
Operating parameters of the laser ablation system.

System	NWR 213 nm Nd:YAG nano-second laser
Scan pattern	Line scan
Spot diameter	250 μm
Laser power	90%
Scan speed	50 μm s^{-1}
Laser Fluence	2.6 J cm^{-2}
Repetition rate	20 Hz
Carrier gas flow	0.9 L min^{-1} He

4. Dried-droplet sample preparation

Circular disks of 5 mm diameter were cut from ash-free filter paper (Whatman 589/1, black ribbon, ashless, 110 mm filter diameter) using a steel punching tool. These pre-cut disks were applied on glass microscopic slides using double-sided tape, as proposed in [9]. On these targets, liquid samples were dispensed using an Eppendorf pipet and slowly dried. Samples prepared in the analytical laboratory (i.e., standards, reference material) were dried under ambient conditions in a VFT 1525 ultraclean laminar flow hood (WEISS Technik, Austria). Samples prepared under realistic conditions (method cross-validation, see Section 3) were dried directly in the laboratory, only covered with a tilted glass petri-dish to avoid apparent contamination. The procedural blank of both approaches was monitored and no significant contribution to the analyte signal was observed.

5. Conventional ICP-OES procedure

For conventional analysis via pneumatic nebulisation (PN), the samples were diluted 20-fold with 1% (v/v) HNO_3. Indium was added as internal standard (final concentration: 1.5 μg mL^{-1}) and a conventional Meinhard-type glass nebulizer mounted onto a glass cyclonic spray chamber (both: Thermo, USA) was used for sample introduction. A CETAC ASX-520 autosampler (CETAC Technologies, USA) was used for automated sample uptake, while a peristaltic pump assured long-term stability. Initial method development was achieved by optimizing signals in terms of signal-to-noise ratio. Signals were recorded during PN of a diluted and spiked sample solution (see Table 1). Performance of the system (peak position) was checked prior to each measurement session with a spiked and diluted sample solution. Background-corrected emission signals (integration time: 6 s, n = 4) were recorded and processed using iTEVA software (Thermo Scientific, USA). Quantification of phosphorus was done via external aqueous calibration and normalization to the respective indium signal.

6. Fermentation process

The fermentation samples analyzed in this contribution were taken from a chemostat fermentation process (continuous feed of fresh nutrient medium and continuous harvest of the culture broth). The objective of the biochemical investigation was to optimize conditions for biological methanogenesis. This process aims at biochemically reducing carbon dioxide to methane gas in the presence of hydrogen. Similar to established chemical methane synthesis following the Sabatier process [10], biological methanogenesis will allow converting hydrogen or a mixture of hydrogen and carbon monoxide (syngas) into methane gas. Excess energy produced, e.g., in power plants using alternative/renewable energy can thus be effectively converted to methane gas. As opposed to hydrogen, methane can be safely stored and easily distributed via existing gas networks. Compared to the Sabatier process, biological methanogenesis can be achieved at lower temperatures and is more tolerant towards primary gas purity, resulting in a better process efficiency. Based on previous reports of biological methanogenesis [11,12], limitations in phosphorus concentration were studied here, also with the aim of optimizing future media composition. The process medium contained typical sources of nitrogen, minor concentrations of inorganic salts and typical trace elements. Carbon dioxide was used as exclusive carbon source for the process.

The harvested process medium was initially stored at −18 °C. Upon arrival in the analytical lab, the samples were unfrozen (at 2 °C), centrifuged (3000 rpm, 5 min, Hettich-EBA 20, Germany) and the supernatant clear solution was stored at 2 °C until further analysis. Hence, the total dissolved phosphorus fraction (TDP) was considered for quantification. As any suspended matter was removed during the centrifugation step, acidification of samples was sufficient for conventional nebulizer-based ICP-OES measurements.

3. Results & discussion

1. Reproducible sample preparation and representative sampling

In most reports on dried-droplet sample preparation in combination with laser ablation, the smallest possible dried sample residues were sought for [13–22]. Small spots can be ablated completely with only a few laser shots. This is advantageous, since it provides good sensitivity, comparable to conventional sample introduction systems [15]. This way of droplet preparation requires the use of a hydrophobic surface which allows the droplet to continuously shrink while drying (in all reported cases, a polymeric surface).

Following these reports, we applied biochemical fermentation media onto polyethylene disks. Different dilutions (1:1, 1:2, 1:5, 1:10, all diluted with water) were prepared. The obtained dry residues were different in shape and size. Especially, dried residues of aqueous standard solutions had a significantly different geometry than those of the fermentation broth (see Fig. 1). This might be due to foam-suppressing agents used during fermentation or due to differences in salt concentration. In any case, these differences require the addition of an internal standard. As a consequence of adding an internal standard, sample handling would become cumbersome and would not be possible on location as outlined earlier.

Recently, Aramendía et al. showed an alternative way for dried-droplet sample preparation [9]. They prepared pre-cut disks of filter paper, which were attached to a hydrophobic surface. When applying liquid samples onto these pre-cut filter-disks, the liquid is trapped on the confined area which is defined by the paper disk. After drying, each of these spots has exactly the same geometry which facilitates sample localisation and measurement. To compensate for the loss in sensitivity (the sample does not contract to a small spot any more), it is possible to apply comparatively large volumes on the filter disks (300 μL on a 16 mm diameter disk [9]).

The procedure described in [9] yields dried spots of uniform and highly reproducible geometry. Although the macroscopic sample geometry is controlled when using this approach, the lateral distribution within the filter disk is not homogeneous. To exemplify this effect, Fig. 2 a) shows the lateral distribution of phosphorus on a pre-cut filter disk, prepared and measured as described in Sections 3 and 4 (liquid sample: spiked fermentation broth). The phosphorus signal changes substantially within the dried filter disk. The extent of this local pre-concentration depends on the matrix of the liquid sample; for example, when analyzing the area in the centre of the paper filter disk, Aramendía et al. report 20-30% lower signals in purely aqueous solutions compared to spiked sample solutions [9]. This situation is not satisfactory as it calls for the use of an internal standard, standard addition, isotope dilution, or matrix-adjusted calibration.

Taking a closer look at existing measurement strategies for pre-cut filter disks in the context of dried-droplet analysis, only two approaches have been reported so far. Either, a square sub-sample was ablated in the centre of the disk [9] or a ring-shaped sub-sample was analyzed [23]. Obviously, the first approach would under-estimate the concentration in the case depicted in Fig. 2 b) because phosphorus is

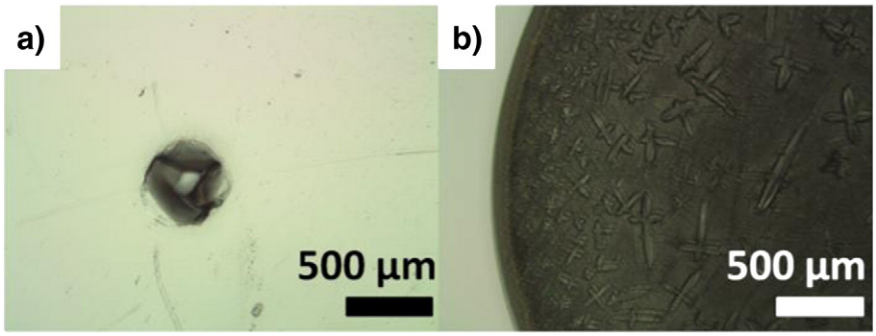

Fig. 1. a) 5 µL of aqueous standard (200 µg mL^{-1} P), and b) 5 µL of fermentation sample, both dried on polyethylene substrate (dried residue in b) only partially visible).

predominantly present in the rim of the paper disk and thus not included in the measurement. Contrarily, the second approach would overestimate the concentration in the present samples (see Fig. 2 c)). To summarize, both approaches are systematically biased since non-representative sub-samples of the paper disks are analyzed. One possible way to completely circumvent problems related to the centro-symmetrical inhomogeneity would be to ablate the entire sample as in the case of preparing samples on hydrophobic surfaces. However, considering the size of the filter disks (several mm) and the usually available laser-spot diameters (of a few hundred µm at maximum) in combination with typical repetition rates in the Hz-range, complete ablation of the filter disk with nanosecond laser systems would result in a very time-consuming approach.

Therefore, we suggest applying an alternative measurement protocol, which combines the advantages of preparing samples on pre-cut filter disks with the advantages of ablating only a fraction of the entire filter area. However, and in contrast to existing approaches, this fraction is selected such that it forms a representative sub-sample of the initially

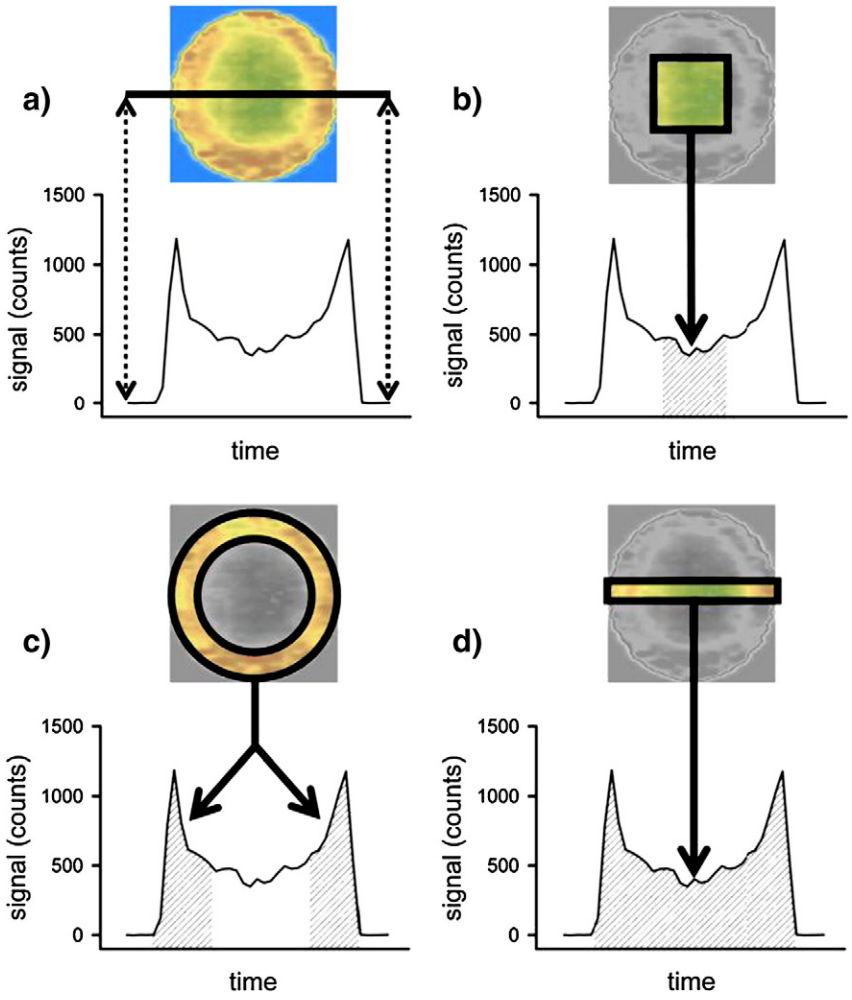

Fig. 2. a) The phosphorus distribution within the droplet is not laterally homogeneous (blue: low signal, brown: high signal). A radial line-scan yields a U-shaped response. Results according to previously reported quantification approaches b) [9] and c) [23], as well as according to the proposed representative approach d) (this publication) are schematically shown. The highlighted (projected) area is used for quantification in the respective quantification approaches.

liquid droplet. We propose performing line-scans that pass over the entire circular disk. The signal obtained is then integrated which results in a representative sub-sample (see Fig. 2 d)). This hypothesis is based on the assumption that any chromatographic migration effects on a circular area will lead to centro-symmetrical patterns. Hence, the radial sampling approach should be representative for the deposited liquid droplet. However, only the integrated signal will be representative for the liquid sample as the signal will fluctuate along the ablation path due to chromatographic patterns (see the U-shaped response in Fig. 2). The viability of this hypothesis will be further exemplified in the following paragraphs. For the sake of completeness, it has to be noted that radial line-scans have been previously reported for the analysis of dried sample droplets [24]. However, the approach reported by Kumtabtim et al. still required the application of matrix-matched calibration because sample geometry was not controlled (freely dried droplets were investigated). Combining paper filter disks with the proposed radial measurement protocol therefore provides new insights in dried-droplet quantification.

2. Figures of merit

In order to verify the hypothesis that integrated radial line-scans indeed yield representative information, the slopes of aqueous calibration and standard addition were compared. To this end, a representative sample of biochemical medium was spiked with increasing concentrations of phosphorus, corresponding to the concentration levels applied in aqueous calibration. As can be seen in Fig. 3, a good agreement between the slopes of the two curves was obtained. The similarity in slope is a first indication for the absence of differences in signal generation related to sample matrix.

In a second step, the phosphorus signal in the presence of three model substances which are likely to be co-present in the fermentation samples was studied. For that purpose, aqueous solutions containing 500 μg mL^{-1} P were prepared with incrementally increasing concentrations of sodium chloride, saccharose and tartaric acid (0.3, 1, 3, 10, 30, and 100 g L^{-1}). The latter two compounds are model substances for organic matrix in general. The blank signal of all three substances was checked and found to be ≤ blank level. The results obtained were compared with a purely aqueous solution of the same phosphorus concentration to obtain recoveries in presence of the model matrix. Recoveries within 90%-110% were considered as tolerable (given the reproducibility of laser measurements, see Table 3). Concentrations of up to 100 g L^{-1} NaCl, 30 g L^{-1} saccharose, and 10 g L^{-1} tartaric acid were found to be tolerable. In real fermentation broth samples, Na concentrations in the range of 11 g L^{-1} – 16 g L^{-1} were found (determined via dilution followed by PN sample introduction to ICP-OES).

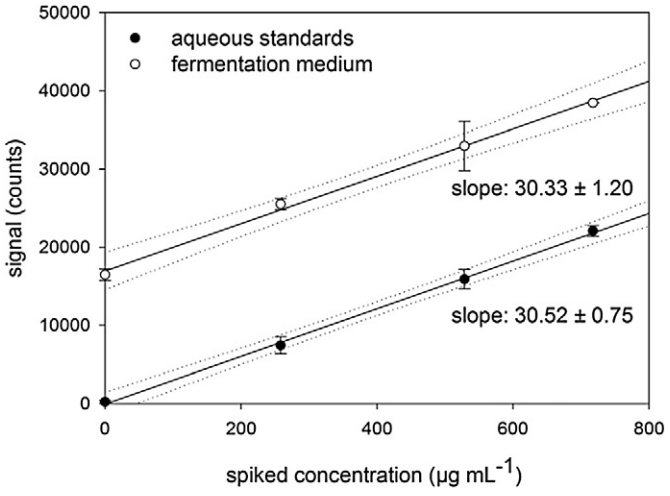

Fig. 3. Slopes of standard addition (spiked fermentation medium) and aqueous calibration lines (data points: n = 4, error bar = 1 standard deviation, uncertainties in slope: standard error of the linear regression).

Table 3
Figures of merit.

	Laser	Nebulizer
Detection limit (LOD)[a]	10 μg mL^{-1}	0.3 μg mL^{-1}
Quantification limit (LOQ)[a]	40 μg mL^{-1}	1 μg mL^{-1}
Linearity tested until	3,500 μg mL^{-1}	24 μg mL^{-1}
Reproducibility (n = 4)	10% RSD (5.3% - 14%)[b]	1.5% RSD (1.1% - 2.4%)[b]

[a] in the undiluted sample.
[b] at different concentration levels, ranging from 10xLOQ to the highest standard level used for linearity check (average, in brackets: minimum and maximum).

To further investigate the effect of sample load, the sample volume applied on the pre-cut filter paper was incrementally increased. The ratio signal/volume is constant up to 17.5 μL of applied sample (see Fig. S1, Appendix A). Also in this investigation, increased sample load had no observable effect on the analytical performance.

The applied sample volume directly affects the achievable sensitivity, and therefore, larger sample volumes would be beneficial in terms of detection limits. With aqueous standards, a maximum capacity of 1.3 μL mm^{-2} could be applied (25 μL sample dispensed on a disk of 5 mm diameter and 19 mm^2 area which is comparable to the result reported by Aramendía et al. [9]). However, only 0.9 μL mm^{-2} of the fermentation broth could be applied in a straightforward way (i.e., 17.5 μL dispensed on a filter of 5 mm diameter). Volumes above 17.5 μL often tended to leave the pre-cut filter disks, probably due to foam-suppressing agents added during fermentation. Hence, to avoid any spilling, a volume of 10 μL (corresponding to 0.5 μL mm^{-2}) was used for all samples and standards.

The linearity of the phosphorus response was checked by analyzing spiked fermentation medium samples up to 3.5 g L^{-1} P (expected concentrations in the samples are below 2.5 g L^{-1} P).

From aqueous calibration curves, the detection (3 s) and quantification (10s) limits presented in Table 3 were obtained. In comparison to conventional ICP-OES techniques, the sensitivity is 40 times lower. However, considering the minimum sample preparation required, the absence of an internal standard, as well as the possibility for sample storage, we consider the method as fit-for-purpose in the given analytical context.

To further test the trueness of the method proposed, a certified reference material (IAEA-153, Trace Elements in Milk Powder) was analyzed by LA of dried droplets prepared as described above. The reference material was quantified via external calibration using aqueous P-standard solutions. The experimental result for the phosphorus concentration of 10.1 ± 0.7 mg g^{-1} (± 1 standard deviation) agrees well with the certified value of 10.1 mg g^{-1} (95% confidence interval between 9.0 mg g^{-1} and 11.0 mg g^{-1}). The milk powder was reconstituted with water to obtain a solution of 250 μg mL^{-1} phosphorus, which is similar in concentration to the biochemical media.

Although the matrix of the reference material is different from that of the samples investigated, we consider the correct quantification of the reference material as additional validation, accompanying the abovementioned experiments regarding standard addition and matrix tolerance. To our knowledge, there is no certified reference material with a suitable matrix composition for the analytical question at hand.

3. Application example

In order to assess the trueness of the method, a set of 20 different fermentation samples with P-concentrations ranging from 200 μg mL^{-1} to 1,800 μg mL^{-1} was analyzed by the laser ablation method. To this end, 10 μL of the samples were applied on pre-cut paper filter disks, dried and ablated using the proposed radial line scan. Signals were integrated

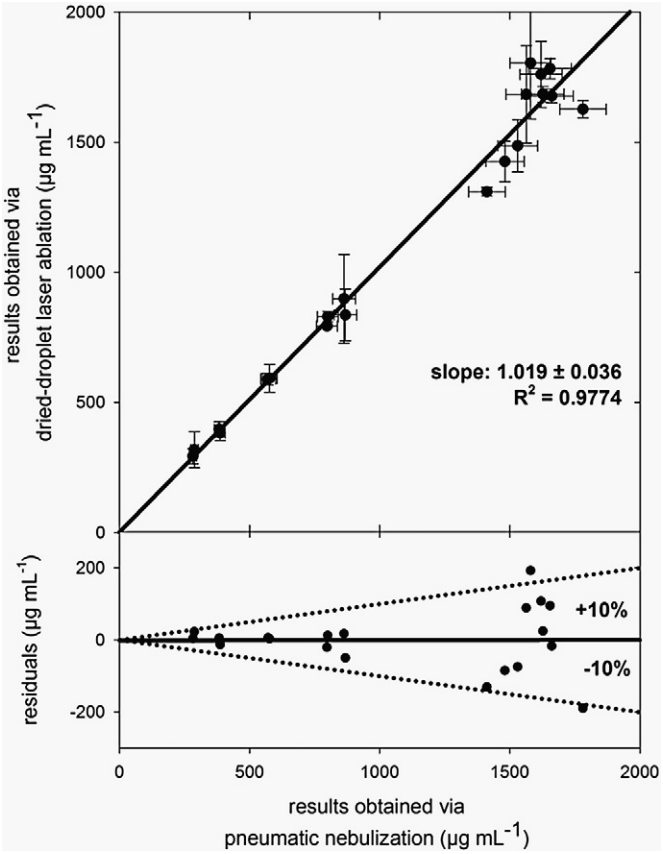

Fig. 4. Correlation between results obtained via laser ablation-ICP-OES analysis of dried droplets (n = 2) and via conventional liquid pneumatic nebulisation ICP-OES analysis (n = 4), respectively. Error bars correspond to 1 standard deviation, uncertainty of slope: standard error of the linear regression.

over the entire filter (n = 2 filters per sample) and quantified versus aqueous calibration standards also applied on filter paper disks.

The same set of 20 samples was also diluted as described in Section 5 and analyzed using the conventional nebulizer/spray-chamber set-up in combination with ICP-OES. Between the results obtained with both methods, a good correlation was obtained and residuals from the interpolated line are in most cases below 10%, across the entire range of concentrations (see Fig. 4). Considering the potentially high biochemical variability (reproducibility of sampling and sub-sampling), the measurement variability observed is satisfactory in the context investigated.

4. Conclusion

In this contribution, we present a method for analyzing challenging liquids, based on laser ablation of dried sample droplets deposited on pre-cut paper filter disks. Our method solves the problem of non-representative sampling, which so far necessitated internal standardisation, matrix-adjusted calibration, standard addition, or isotope dilution. With the approach presented, straightforward external aqueous calibration becomes possible. Key feature of our method is to perform radial line-scans across the entire dried droplet and to integrate the resulting transient signal. Reproducibility and detection limits of the approach presented are higher (10-fold and 40-fold, respectively) than those of existing routine techniques relying on nebulisation of diluted samples. However, the present method offers significant improvements in terms of facilitated sample preparation, storage and calibration, which outweigh the limitations mentioned. Moreover, issues related to sensitivity could be easily overcome with an alternative detection set-up such as LA-ICP-MS or laser-induced breakdown spectroscopy (LIBS). For upcoming research, the method will be extended to allow for simultaneous multi-element quantification in biochemical media, also in combination with automated sample collection and preparation. Thus, it will be possible to follow the fermentation process with superior time-resolution. The general viability of the proposed measurement protocol will be investigated, also with other matrices, such as process additives (silicon oil, anti-foam agents) and blood.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.sab.2014.07.023.

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ARTICLE 3

Self-aliquoting micro-grooves in combination with laser ablation-ICP-mass spectrometry for the analysis of challenging liquids: quantification of lead in whole blood.

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RAPID COMMUNICATION

Self-aliquoting micro-grooves in combination with laser ablation-ICP-mass spectrometry for the analysis of challenging liquids: quantification of lead in whole blood

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Abstract We present a technique for the fast screening of the lead concentration in whole blood samples using laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS). The whole blood sample is deposited on a polymeric surface and wiped across a set of micro-grooves previously engraved into the surface. The engraving of the micro-grooves was accomplished with the same laser system used for LA-ICP-MS analysis. In each groove, a part of the liquid blood is trapped, and thus, the sample is divided into sub-aliquots. These aliquots dry quasi instantly and are then investigated by means of LA-ICP-MS. For quantification, external calibration against aqueous standard solutions was relied on, with iron as an internal standard to account for varying volumes of the sample aliquots. The $^{208}Pb/^{57}Fe$ nuclide ratio used for quantification was obtained via a data treatment protocol so far only used in the context of isotope ratio determination involving transient signals. The method presented here was shown to provide reliable results for Recipe ClinChek® Whole Blood Control levels I–III (nos. 8840–8842), with a repeatability of typically 3 % relative standard deviation ($n = 6$, for Pb at 442 μg L^{-1}). Spiked and non-spiked real whole blood was analysed as well, and the results were compared with those obtained via dilution and sectorfield ICP-MS. A good agreement between both methods was observed. The detection limit (3 s) for lead in whole blood was established to be 10 μg L^{-1} for the laser ablation method presented here.

Keywords Laser ablation-ICP-MS · Whole blood analysis · Lead quantification · Dried-droplet laser ablation · Transient signals

Introduction

The metal lead is known for its toxicity, both acute and chronic. By substituting tetra-ethyl lead with purely organic compounds as anti-knocking agents in petrol, by exchanging water pipes made from lead with pipes made from polymeric material and by replacing the once widely used white pigment lead white $2PbCO_3 \cdot Pb(OH)_2$ with TiO_2, the population is less exposed to this metal than in the past. However, in certain work-related contexts, exposure can still be high. According to a directive of the council of the European Union [1], "medical surveillance is carried out if […] a blood-lead level greater than 400 μg Pb L^{-1} blood is measured in individual workers." To determine the lead concentration in whole blood of potentially exposed workers, suitable analytical techniques are therefore required.

Conventional approaches for analysis of lead in whole blood usually require dilution or even digestion of the blood matrix prior to measurement. After digestion by means of concentrated mineral acids and oxidizers, the sample is also diluted. This clear solution is then suitable for analysis via graphite furnace-atomic absorption spectrometry (GF-AAS) or inductively coupled plasma-mass spectrometry (ICP-MS). For digested and diluted samples, quantification can often be accomplished via external aqueous calibration or matrix-

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adjusted calibration. However, chemical digestion of the blood matrix comes at the cost of low sample throughput, as well as high reagent consumption, which in turn can lead to increased blank values and costs. When simply diluting the sample, matrix effects have to be taken into consideration by using standard addition. Moreover, coagulation of the blood has to be prevented, by diluting the blood as soon as possible after collection.

Alternative approaches that avoid mineralization or dilution of blood can be found in the field of solid-sampling techniques. Among others, Resano et al. have shown that it is possible to apply blood droplets onto filter paper, to cut out the dry spot obtained and to analyse it via GF-AAS for its lead content [2]. Instead of AAS, which is essentially a single-element method, Cizdziel [3] proposed to use laser ablation (LA)-ICP-time-of-flight MS for analysis of blood dried on filter paper.

However, and in contrast to solid-sampling AAS, an accurate and quantitative assessment of dried blood spots by means of LA is a challenging task. The diameter of the laser beam is in the micrometre range, whereas the diameter of a dried blood spot is typically in the millimetre range. Therefore, only a part of the sample is consumed during single-shot or spot-drilling analysis. For obtaining correct quantitative results, this small part of the sample used for analysis has to be representative of the liquid blood sample before deposition. When depositing liquids on filter paper, chromatographic splitting into different sample fractions is possible and thus might compromise the sample's homogeneity. As such effects can vary from sample to sample, or between samples and standards, correct quantification is severely hampered. An extended discussion of the problems related with dried-droplet LA can be found elsewhere [4].

One way to solve the problem of chromatographic separation is to ablate the entire dried blood spot. Any lateral inhomogeneity is thus obviated. However, with commercial LA systems operating with repetition rates in the hertz range, ablation of such large areas can take a very long time. There are femtosecond LA systems with repetition rates in the kilohertz range and with scan speeds of several millimetres per second. Such systems can ablate one entire dried blood spot within reasonable time [5], yet they are to date not available as routine instruments. Also, recently available ultra-fast cells allow higher laser repetition rates (up to several hundred Hz) using the more traditional nanosecond LA systems [6].

Another possibility to solve the problem of non-representative sub-samples is to apply a radial scanning approach. In a recent publication, we discussed this in more detail [7]. The idea behind this concept is that any chromatographic separation of a deposited sample droplet should always be symmetrical to the centre of the dried spot. If the laser is scanned from one side to the other across the dried spot, passing through the centre of the spot, all possible chromatographic variations are recorded. However, it is necessary to produce strictly circular droplets, and the shortcoming of this approach is that sample application requires a skilled operator.

To compensate for chromatographic effects by ablating the entire droplet, while maintaining reasonable sample throughput and straightforward sample preparation, the size of the blood spot has therefore to be reduced [8]. However, pipetting volumes in the nanolitre range using pipettes or syringes is a challenging task, and localizing such small dried droplets with the microscope of the LA system is difficult. Therefore, a straightforward way for producing nanolitre sub-samples of a liquid blood sample was developed in this work.

Splitting a sample droplet into many identical sub-samples in the nanolitre range can be achieved by means of self-aliquoting micro-array plates, as proposed by Pabst et al. in the context of matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) [9]. Such self-aliquoting arrays consist of a smooth polymeric slide, which contains small cavities of several micrometres in diameter. Such cavities can be readily produced with a LA system. If a liquid droplet is swiped over such cavities, small amounts of the liquid are trapped in each cavity.

In this contribution, the initial concept described in Pabst et al. [9] was successfully transferred from MALDI-MS to LA-ICP-MS and further optimized to allow for more straightforward sample application and analysis. This approach was combined with a data treatment scheme so far only used in the context of isotope ratio determination with transient signals. The method developed was applied to the determination of lead in Recipe ClinChek® whole blood reference material and a freshly collected and spiked whole blood sample. External calibration against aqueous standard solutions and iron as an internal standard was possible.

Materials and methods

Reagents and standard solutions

For standard dilution, water with a resistivity of 18 MΩ cm obtained from an Easypure system (Thermo, Germany) was used throughout. Single-element standard solutions at 1000 mg L^{-1} were obtained from Merck, Germany. Four standard solutions were prepared from those stock solutions, with final lead concentrations of 26, 76, 316, and 614 ng mL^{-1}. Each of these solutions contained iron at a concentration of 376 μg mL^{-1}.

Recipe ClinChek® Whole Blood Control levels I–III (nos. 8840, 8841 and 8842, containing 59.1, 228 and 446 ng mL^{-1} lead as well as 379, 380 and 377 μg mL^{-1} iron, respectively) were reconstituted using Easypure water according to the manufacturer's instructions. Reconstituted blood reference material was used immediately for analysis and not stored longer than 24 h.

A fresh whole blood sample was investigated as well. As the concentration of lead in the sample was relatively low, two levels of lead were spiked to the sample. First, the sample was split into three aliquots of 1 mL, and to each aliquot, 0.1 mL of 1 % HNO_3 containing adequate concentrations of lead was added. The concentrations of the spike were chosen such that the resulting whole blood contained spiked concentrations of 0, 150, and 250 ng mL^{-1}. The spiked volume was equal to 10 % of the initial sample volume, and should result only in minimal changes of sample matrix, compared to the original, non-spiked blood.

Instrumental

The LA system used for production of the micro-array plates and the micro-grooves was a New Wave Research frequency-quintupled Nd:YAG solid-state laser operating at 213 nm. The same system was used for sample analysis via LA-ICP-MS. Ablation was carried out under helium atmosphere (0.8 L min^{-1}). After the ablation chamber, argon was admixed to the helium stream as make-up gas at a flow rate of 0.8 L min^{-1}.

The dry aerosol produced upon laser ablation was transported into a Thermo iCAP Qc quadrupole ICP-MS unit operating under standard conditions (1550 W plasma power, nickel cones, cool gas at 14 L min^{-1}, auxiliary gas at 0.8 L min^{-1}, 5 ms dwell time, monitoring of the ^{57}Fe and ^{208}Pb ion signals, total cycle time 13 ms). Data collection was accomplished using the instrument software (QTegra™) in time-resolved mode.

For comparing the laser approach presented here with a traditional nebulizer-based method, spiked and non-spiked real whole blood samples were diluted and analysed using standard addition and a Thermo Element XR sectorfield instrument. Details on this method can be found in the Electronic Supplementary Material (ESM).

Micro-array plates and linear grooves

Two different types of arrays were investigated for blood deposition. First, micro-cavity arrays as described in Pabst et al. [9] were prepared by ablating circular craters into poly (methyl methacrylate) (PMMA) microscope slides (2.5 × 7.2 cm, Betzold, Austria) using the NWR 213 LA system. The laser parameters used for production of micro-array plates were 7.5 J cm^{-2} laser fluence, 20 Hz repetition rate, 4 s dwell time and 100 μm beam diameter. The distance between craters was 300 μm and the craters were arranged in a 4 × 4 pattern.

The second, newly developed design consists of three sets of ten parallel grooves. Each of the grooves was 100 μm wide and 1 cm long (see Fig. 1a). The laser parameters used for production of the micro-grooves were 10 J cm^{-2} laser fluence, 20 Hz repetition rate, 100 μm spot diameter and 100 μm s^{-1} scan speed. To fabricate one set of ten micro grooves, roughly 15 min is required. This, and the fact that the slides are not expensive, allows for single use of the slides.

Deposition of blood and analysis of dry residues

One small droplet (approximately 5 μL) of blood, reference material or aqueous standard solution was deposited on the polymeric slide, just next to one set of linear micro-grooves. The liquid was then spread across the slide using a rubber spatula. After passing the micro-grooves, the remaining solution was removed from the microscopic slide in one continuous motion (see Fig. 1b). Due to the small liquid volume trapped in each groove or micro-array, the samples dried quasi instantly. This approach was used for both types of slides containing micro-cavities following the design of Pabst et al. [9] as well as for the newly developed design using micro-grooves.

The dried samples were analysed by means of LA-ICP-MS. Laser parameters used were 3.1 J cm^{-2} laser fluence, 20 Hz repetition rate, 200 μm spot diameter and 100 μm s^{-1} scan speed. In the case of the micro-cavities following the design by Pabst et al. [9], the laser traces used for analysis were adjusted such that each cavity was consecutively ablated. In the case of the newly designed micro-grooves, the LA pattern was arranged in a way that each line scan crosses the grooves perpendicularly (see Fig. 1c).

Results

Optimizing the design of the cavities

In a first approach, micro-array plates with circular cavities were prepared and filled with aqueous standard solutions which were dried and analysed by LA-ICP-MS. The design of the cavities corresponds to the one described in [9] with minor variations. When analysing an aqueous standard with 400 ng mL^{-1} lead, typical values for the relative standard deviation were around 5 % ($n = 16$, 16 individual integrated peak areas, one for each cavity, using iron as an internal standard to compensate for varying filling of individual cavities).

However, when filling the circular micro-cavities with whole blood reference material, the results obtained were not satisfying. Firstly, when wiping the blood swiftly over the

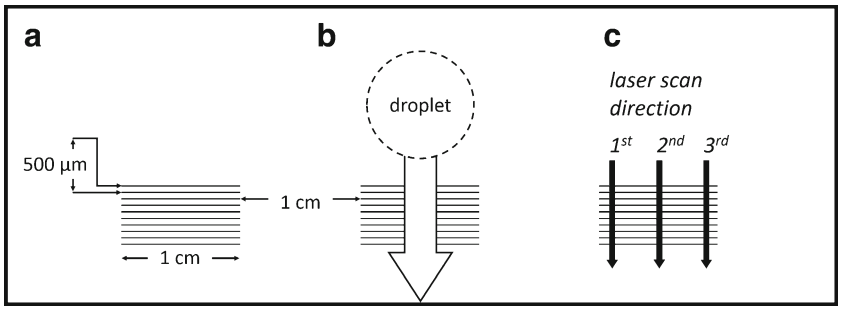

Fig. 1 **a**–**c** Schematic of the proposed LA-ICP-MS method using micro-grooves. Each groove is 1 cm long and 100 µm wide. For analysis, the laser is scanned according to a line perpendicular to the grooves

cavities, also the area between the cavities was found to produce significant signals upon LA-ICP-MS analysis. To circumvent this problem, the rubber spatula was wiped over the cavities applying higher pressure. This in turn resulted in very low peak areas, since a large part of the blood is removed again from the cavities during the swiping step.

To overcome this problem, the design of the micro-cavities was changed from circular cavities to long micro-grooves. Such grooves have the advantage that they are filled with a sufficient quantity of blood while having only negligible contamination in between the individual grooves. Additionally, it is possible to see the micro-grooves with the naked eye, facilitating sample deposition. Figure 1 shows the final design of the optimized micro-groove cavities. To the best of the authors' knowledge, this is the first time such a design is proposed for quantitative analysis of liquid samples.

Figures of merit and evaluation of internal standard

Four sets of the newly designed micro-groove cavities were filled with aqueous standard solutions and a blank solution (containing only iron), and analysed by LA-ICP-MS. The obtained transient signals for ^{208}Pb and ^{57}Fe were integrated (left boundary: first steep increase of the iron signal, right boundary: lead signal drops back to instrumental background level) and the ratio ^{208}Pb/^{57}Fe was calculated. From the standard deviation of six blanks, the detection limit was found to be 10 µg L^{-1} lead (3 s).

The repeatability of the lead signal at a concentration of 600 ng mL^{-1} was found to be approximately 5 % for $n = 3$ repetitions from one set of micro-grooves. The linearity of the calibration curve improved upon using iron as an internal standard, indicating that slightly different volumes of sample are trapped in each set of micro-grooves. As explained in [9], the amount of liquid trapped within one circular micro-cavity depends on the depth of the cavity and the surface structure and roughness at the edge of the cavity. Also, the amount of liquid trapped within a cavity depends on the liquid's properties. Therefore, a difference in matrix can lead to a difference in sample volume retained in each cavity, making it necessary to use iron as an internal standard.

Data treatment

Integration of transient signals is a commonly applied way for data treatment in laser ablation and was also used here to determine the detection limit. However, integration requires manual setting of integration boundaries, and all data (background and actual signal) contribute equally to the result [10, 11]. An alternative data treatment approach for obtaining isotope ratios of transient signals was developed by Fietzke et al. [10, 11]. In the past, this approach has been used in the context of multi-collector ICP-MS, also in combination with LA, but to the best of our knowledge, this is the first time it was evaluated using LA single-collector ICP-MS.

For each sample, all data points recorded in one measurement (i.e. ten transient peaks including gas blank in between; see Fig. 2a) were plotted in a ^{208}Pb/^{57}Fe plot. Then, the best-fitting straight line was traced through the data points, its slope representing the ^{208}Pb/^{57}Fe ratio (see Fig. 2b). Dronov and Schram [12] have recently used the described method in combination with single-collector quadrupole ICP-MS and liquid samples. The authors suggested to use orthogonal distance regression (ODR) instead of conventional least-squares regression [12], to account for the fact that values on the abscissa as well as values on the ordinate are influenced by uncertainties [13]. Therefore, the ODR package of OriginPro 2016G was used for data treatment throughout this work.

The advantage of the presented type of data treatment is that data points with a high signal intensity obtain more statistical weight than do data points close to the instrumental background, which is due to the leverage effect. Hence, the blank values basically do not contribute to the slope of the curve, and the entire dataset can be used directly, obviating the necessity to manually set integration boundaries. This allows for a very straightforward data treatment protocol. However, as the lead concentration decreases, the slope does not reach zero, but rather the correlation coefficient of the interpolated line deteriorates. This means that with this method it is not possible to determine detection limits in the conventional way. However, if there is significant signal on both

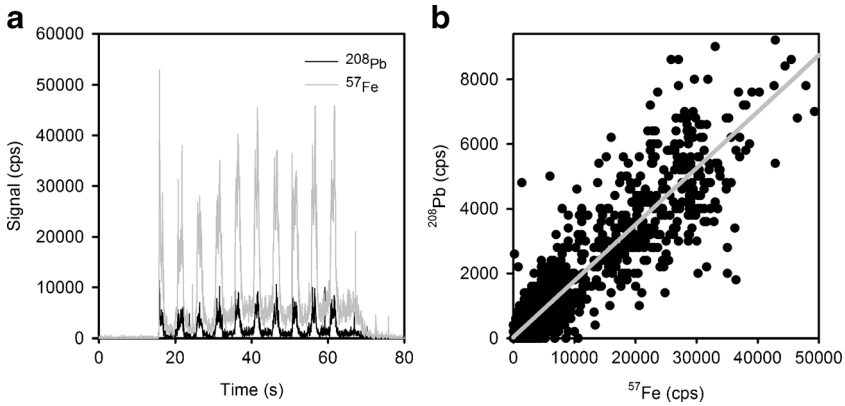

Fig. 2 Example of data treatment. The transient signals are recorded (**a**); the entire data of one such scan are then plotted in a Pb/Fe plot (**b**); the slope of the interpolated straight line corresponds to the Pb/Fe ratio

isotopes, this method allows for a very straightforward quantitative data evaluation.

Analysis of whole blood samples

For analysis, the aqueous standard solutions, the three reference material levels, as well as the three levels of spiked real whole blood were applied onto micro-grooves. LA-ICP-MS analysis of the samples and standards was performed, resulting in a set of transient signals similar to the one depicted in Fig. 2a. The time-resolved ICP-MS signal thus consisted of ten transient lead and iron signals, each one a result of passing the laser beam over each of the ten micro-grooves in perpendicular direction. Slight variations in signal height are due to differences in sample volume trapped in each micro-groove. Since the volume of sample trapped within the micro-grooves is not known, iron was used as an internal standard. This also compensated for different retention efficiencies in the micro-grooves due to different matrix compositions (differences in between blood samples or in between blood and aqueous standards). The $^{208}Pb/^{57}Fe$ ratio was calculated from the slope of an interpolated straight line, as described above. All samples were quantified using external, aqueous standards.

With the method developed, Recipe ClinChek® whole blood control sample levels I, II and III (order nos. 8840, 8841 and 8842, respectively) were analysed for their lead concentration. For quantification, aqueous standards with Fe and Pb concentrations close to the reference material were used. For each sample or standard, two sets of micro-grooves were analysed three times each. One line scan was performed in the centre of the micro-grooves; the other two were performed on each side (see Fig. 1c). There was no significant difference in terms of Pb/Fe ratio between the three positions. The results were found to be in very good agreement with the certified values: level I (reference value 59.1 ± 11.8 ng mL^{-1}, found 58 ± 12 ng mL^{-1}), level II (reference value 228 ± 46 ng mL^{-1}, found 228 ± 6 ng mL^{-1}) and level III (reference value 446 ± 89 ng mL^{-1}, found 442 ± 10 ng mL^{-1}; all data: $n = 6$).

To further investigate the capabilities of the method, a fresh whole blood sample was analysed, which was spiked with two increasing concentrations of lead. From each of these real whole blood samples, one aliquot was analysed by the abovementioned laser ablation method. The samples were also analysed by conventional ICP-MS analysis, by diluting the blood 100-fold and performing standard addition quantification using indium as an internal standard. Details regarding this conventional method can be found in the ESM. Both methods are in good agreement with regard to the found lead concentration (LA method 137 ± 10 and 286 ± 22 μg L^{-1}, dilution method 135 ± 8 and 240 ± 17 μg L^{-1}, for level 1 and level 2, respectively). However, in the non-spiked whole blood sample (level 0), concentrations were below the detection limit of the LA method.

Discussion

It has to be considered that the iron concentration in the whole blood reference material is known and constant in all three levels of the certified reference material. This makes internal standardization very straightforward. In the real sample, the iron concentration was approximately 20 % higher than in the reference material and in the aqueous standards. This could lead to an underestimation of the lead concentration in the present case. Nevertheless, it was possible to distinguish between different levels of lead concentration and to obtain a reasonable agreement with the reference method.

In the future, when analysing a wide range of real blood samples, natural variation of the iron concentration is expected and has to be taken into consideration. For healthy individuals, the concentration of iron in whole blood lies typically around 450 mg L^{-1} (309–521 mg L^{-1} [14], 425–500 mg L^{-1} [15], 445–521 mg L^{-1} [16]). Variation in the iron concentration can therefore also affect the quantification of lead. However, the aim of the method presented here is to spot samples with anomalously high lead concentrations (400 μg L^{-1} or higher, as compared to the typical lead concentration of 40 μg L^{-1} [17] in the non-occupationally exposed population).

Variations in iron concentration should therefore not lead to false-negative results for the lead concentration. In other words, even with natural variations in the iron concentration, anomalously high lead concentrations will still be detected. Samples thus identified can then be investigated with more accurate but more time-consuming conventional methods. However, monitoring the iron level in whole blood would improve the accuracy of the analysis if needed in a different context. Along the same lines, accuracy can also be further improved by monitoring all four, or at least the three most abundant, isotopes of lead to take into account natural variation in the isotopic composition of the element.

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Compliance with ethical standards The authors declare that they have no conflict of interest.

Human whole blood was used for method validation. Ethical approval was obtained for using these samples by an independent commission connected to the Ghent University Hospital.

Blood donors signed an informed consent.

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5.2.2. PART II

This part of the thesis contains one article that discuss the topic of dispersed particle extraction. Three posters are included in this section as well. These posters show an alternative application of dispersed particle extraction that was developed in the course of this thesis. Publication of this data is being prepared at the moment of thesis submission.

ARTICLE 4

Extraction and pre-concentration of platinum and palladium from microwave-digested road dust via ion exchanging mesoporous silica microparticles prior to their quantification by quadrupole ICP-MS.

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ORIGINAL PAPER

Extraction and pre-concentration of platinum and palladium from microwave-digested road dust via ion exchanging mesoporous silica microparticles prior to their quantification by quadrupole ICP-MS

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Abstract We report on the use of mesoporous silica microparticles (μPs) functionalized with quarternary amino groups for the isolation of platinum and palladium tetrachloro complexes from aqueous road dust digests. The μPs have a size ranging from 450 to 850 nm and are suspended directly in the aqueous digests, upon which the anionic Pt and Pd complexes are retained on the cationic surface. Subsequently, the μPs are separated by centrifugation. Elements that cause spectral interferences in ICP-MS determination of Pt and Pd can be quantitatively removed by adding fresh 0.240 mol L^{-1} HCl to the μPs and by repeating the centrifugation step. The analyte-loaded μPs are then dissolved in 0.1 mL of 2 mol L^{-1} HF, diluted to 2 mL, and the solutions thus obtained are analyzed by quadrupole ICP-MS. This method avoids analyte elution from the sorbent. This "dispersed particle extraction" approach yielded a run-to-run relative standard deviation≤ 5 % for Pt and≤4 % for Pd (at 0.1 ng mL^{-1}, n=4 road dust digests). Method detection limits (expressed as concentrations in the dust samples) are 2 and 1 ng g^{-1} for Pt and Pd, respectively. The method was validated by analysis of a reference material (BCR CRM 723) and applied to the analysis of road dust samples collected in downtown Vienna. Pt and Pd concentrations in samples collected in summer and in winter were compared, with concentrations ranging from 205 to 1445 ng g^{-1} for Pt and from 201 to 1230 ng g^{-1} for Pd.

Keywords Dispersed particle extraction · Functionalized mesoporous silica particles · Strong anionic exchanger · Inductively coupled plasma mass spectrometry · Platinum group elements · Environmental analysis

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Introduction

In order to reduce the emission of harmful by-products from the combustion of petrol in engines, automotive catalysts have been increasingly implemented during the last decades [1]. Although modern catalysts are effective in reducing CO, NO_x and residual hydrocarbons, they release platinum group elements (PGEs) during operation (e.g., [2, 3]). As a consequence of aerosol deposition, concentrations of platinum, palladium and rhodium in roadside soils increased over the years [4]. Studies have revealed that catalyst-borne PGEs can be taken up by plants and other species (e.g., [1, 5]). The toxicological potential of traffic-related PGEs is still not completely understood, making it necessary to study their effect on various species in exposure studies, as well as to improve analytical methods for reliable environmental monitoring of those elements.

Due to their relatively low sensitivity, inductively coupled plasma-optical emission spectrometry (ICP-OES) or atomic absorption spectrometry (AAS) are restricted to exposure

studies with higher-than-natural PGE concentrations (among others [6, 7]). To conduct exposure studies with realistic concentration levels, or to analyze native environmental samples, significant analyte pre-concentration is necessary when applying ICP-OES or AAS (e.g., [8–13]).

ICP-mass spectrometry (ICP-MS) offers the required sensitivity for PGE quantification in native environmental samples, as well as multi-element capability and ruggedness [14, 15]. Nevertheless, the determination of PGEs by means of ICP-MS is a challenging task due to spectral interferences arising from ubiquitous matrix elements. In ICP-MS, the occurrence of polyatomic ions jeopardizes the accuracy of ultra-trace level PGE concentration data. The signals from $^{40}Ar^{65}Cu^{+}$ and $^{40}Ar^{66}Zn^{+}$, e.g., overlap with those from $^{105}Pd^{+}$ and $^{106}Pd^{+}$, respectively. The number of such polyatomic interferences, formed from elements present in the sample matrix, the solvent and/or entrained air, is long [14]. Some of these interferences cannot be avoided even when measuring at high mass resolution in sector-field ICP-MS instrumentation [16]. Given the complexity of the situation, interference-free conditions can also not readily be obtained via chemical resolution in a multipole collision/reaction cell ICP-MS [17, 18]. Considering the difficulties in overcoming spectral interferences solely by instrumental means in this context, there is a wide consensus that PGEs have to be chemically isolated prior to analysis.

Conventionally, chemical isolation of PGEs is achieved by means of co-precipitation (e.g., [19, 20]) or solid-phase extraction (SPE) [14], preceded by adequate digestion procedures [21]. Although co-precipitation is an established technique, it is cumbersome and requires costly high-purity chemicals. In contrast, SPE has the potential for higher sample throughput and requires fewer chemicals. Key principle in PGE isolation by SPE is the formation of anionic PGE chloro-complexes. Their anionic character allows separation from the predominantly cationic interferences using two approaches: i) retention of matrix cations, while PGE-containing anions pass through a strong cationic exchanger resin (SCX, e.g., [16, 22]), and ii) selective retention of PGE-containing anionic complexes, while the cationic matrix ions pass through a strong anionic exchanger resin (SAX, e.g., [20, 23, 24]), followed by the elution of the retained analytes. Due to the repeated use of SPE-columns, memory-effects are a major problem, especially in case of SAX functionalities which strongly retain PGE chloro-complexes. To minimize memory-effects, the elution is typically performed with concentrated mineral acids [23, 24] or noxious complexing agents, such as thiourea [14]. Consequently, elution of the PGEs from SAX resin is problematic in terms of safety, waste management and ICP-load.

Also in this work, strong anionic exchanger (SAX) functionalities are applied to selectively retain PGE chloro-complexes. However, to circumvent the challenges and disadvantages associated with PGE elution, the recently developed approach of "dispersed particle extraction" (DPE) [25, 26] was used. In DPE, a mesoporous sorbent-resin is suspended directly in the liquid sample. After analyte sorption, the micro-particles are separated from the surrounding liquid matrix. Due to the high surface area of the micro-particles, small amounts of the resin are sufficient for PGE retention. As a consequence, in a subsequent step, the analyte-loaded sorbent material can be dissolved directly, thus circumventing the elution step. The analytes are then introduced to an ICP-MS together with the disintegrated micro-particles.

So far, the DPE-approach achieved good results in extracting cationic metals from environmental aqueous samples [25], as well as in extracting cationic rare earth elements from saline waters [26]. Here, we have deployed the DPE-approach for the first time to the isolation of anionic platinum and palladium chloro-complexes from chemically digested road dust samples. This new approach was validated by successful analysis of reference material BCR CRM 723 (road dust) and used for analysis of road dust samples collected in downtown Vienna (Austria).

Experimental

Reagents and materials

In all experiments, the reagents used were of analytical grade or higher purity. The chemicals used for the synthesis of the SAX sorbent material were of synthetic grade or higher purity. Concentrated nitric acid, hydrochloric acid, hydrofluoric acid and hydrogen peroxide were purchased from Merck, Germany (www.merckmillipore.com). 1000 mg L^{-1} stock solutions of platinum, palladium, and indium in 5 % (*v/v*) HCl were obtained from Fluka, Germany (www.sigmaaldrich.com), and used for the preparation of calibration standards by dilution with 2 % (*v/v*) HCl or as internal standard. High purity water was prepared using an Easypure water system (Thermo, USA, resistivity ≥18 MΩ cm, www.thermofisher.com). BCR CRM 723 road dust reference material was obtained from IRMM (Geel, Belgium, https://ec.europa.eu/jrc/en/reference-materials).

Instrumentation

Measurements were performed using an iCAP Qc quadrupole ICP-MS instrument (Thermo, Bremen, Germany, www.thermofisher.com), equipped with a concentric nebulizer and a quartz cyclonic spray chamber connected to the ICP-torch for sample introduction (quartz injector tube of 1.5 mm inner diameter). Sample uptake was accomplished via an ESI (Omaha, NE, USA, www.icpms.com) SC2-DX autosampler in

combination with an ESI FAST sample introduction system (1 mL sample loop). Prior to each measurement session, the ICP-MS instrument settings were optimized using a solution containing 1 μg L^{-1} of indium, barium, uranium and cerium to achieve satisfying sensitivity, oxide ratios (CeO^+/Ce^+<2 %) and doubly charged ion levels (Ba^{++}/Ba^+<3 %). Typical operation conditions are given in Table 1. All measurements of solutions after dispersed particle extraction were performed in standard ICP-MS mode. Three isotopes of sufficient abundance were selected for each analyte. ^{115}Indium was used as an internal standard. For monitoring the matrix elements, nuclides of sufficient abundance were measured in kinetic energy discrimination (KED) mode using a mixture of 7 % hydrogen in helium as collision gas at 3 mL min^{-1} and an energy barrier of –3 V.

Road dust samples were digested using a microwave-assisted closed vessel treatment (Multiwave 3000, HF 100 vessels, Anton Paar, Austria, www.anton-paar.com). Separation of SAX sorbent particles from the surrounding liquid sample was done in a Heraeus Megafuge 16 centrifuge (Thermo Scientific, www.thermofisher.com), equipped with a FIBERLite F15-6x100 angular rotor (pitch angle 25°, acceleration: 17,000×*g*) using 15 mL metal-free polypropylene vials (maximum tolerable acceleration: 17,000×*g*, VWR collection, VWR, Germany, www.vwr.com).

Synthesis and characterization of SAX micro-particles

The ion exchanging resin with mesoporous structure was synthesized in-house according to methods described previously [25, 26] and modified with quarternary amine functionalities according to [27]. To summarize, the following synthetic steps were performed: first, sub-micron silica particles of nanometre porosity were obtained by hydrolysis of tetraethoxysilane under basic pH conditions (NH_3) in the presence of cetyltrimethylammonium bromide (CTAB) surfactant. The micelles formed by the CTAB surfactant act as template for nano-pores. The particles were removed from the solution by centrifugation, washed with water and ethanol and calcinated at 550 °C for 5 h to remove the surfactant. In a second step, the silica surface was activated using concentrated HCl. Then, aminopropyltrimethoxysilane was added to introduce amine groups onto the surface of the particles. In a third step, the amines were quarternized with methyl iodide. The particles were characterized using nitrogen sorption at 77 K (ASAP 2000, micromeritics, USA, www.micrometrics.com) and Scanning Electron Microscopy (Quanta 200 MK2, FEI, USA, www.fei.com). The physical properties of the silica particles are: 1084 m^2 g^{-1} specific surface area, 2.5 nm average pore diameter, and particle diameters ranging from 450 to 850 nm. The loading capacity was found to be 0.0024 milliequivalent g^{-1}. This value was determined by saturating the particles with $[PdCl_4]^{2-}$ in 0.240 mol L^{-1} HCl and quantifying the adsorbed palladium after three washing steps. Under those conditions, analyte-concentrations of up to 100 ng mL^{-1} can therefore be extracted with constant extraction efficiency, which is far above the here investigated concentrations which are below ng mL^{-1}. Saturation of the particles should therefore not be observed for the concentrations expected for environmental samples. Figure 1 shows a SEM micrograph of the mesoporous material finally obtained.

Table 1 Instrumental settings of the iCAP Qc (Thermo, Bremen)

Nebulizer gas flow rate	0.95	L min^{-1}
Cool gas flow rate	14	L min^{-1}
Auxiliary gas flow rate	0.8	L min^{-1}
Plasma power	1550	W
cone material	Nickel	
dwell time per isotope	0.01 s (4 main runs, 80 sweeps each)	
sample flow rate	0.5	mL min^{-1}
Nuclides monitored	^{105}Pd, $^{106}Pd^a$, ^{108}Pd, ^{194}Pt, $^{195}Pt^a$, ^{196}Pt, ^{115}In[b]	

[a] nuclide used for quantification

[b] internal standard

Sample collection and digestion

Dust samples were collected in March 2011 and July 2011 in downtown Vienna, Austria (location "Museumsplatz": 48.20370°N, 16.35923°E, 180 m above sea level). Sampling was done 1 week after the last rain or snow event from dry ground. At both sampling events, one sample was collected in a subterranean parking garage (location 1) and one sample

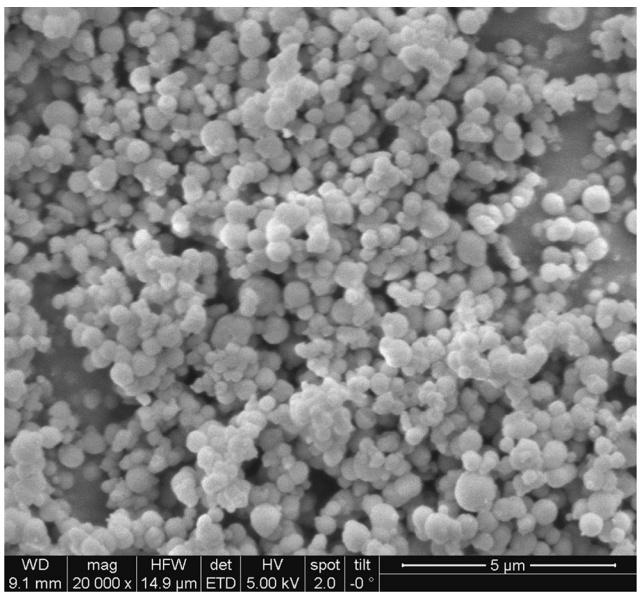

Fig. 1 SEM micrograph of mesoporous silica particles functionalized with SAX functionalities (bar=5 μm, acceleration voltage: 5 kV)

was collected next to the street at 1 m distance from the curbstone (location 2). Sampling was achieved using a PE brush and shovel. At least 200 g of dust were collected at each location. The collected material was dried at 70 °C until constant weight, then sieved to obtain the particle fraction< 0.5 mm and thoroughly homogenized. Until further use, the samples were stored in PE plastic bags in an exsiccator over silica gel.

Solid samples (30–100 mg) were digested using a three-step microwave-assisted closed-vessel treatment, following the method suggested for BCR CRM 723 reference material in [28] with minor adaptations. A Multiwave 3000 microwave system (Anton Paar, Austria, www.anton-paar.com) in combination with high-pressure Teflon vessels was used for this purpose (heating ramp: 20 min, hold-time: 35 min, maximum power: 900 W, maximum internal temperature: 240 °C, maximum pressure: 40 bar). In the first step, a mixture of 4 mL of concentrated HNO_3 and 2 mL of H_2O_2 (30 %) were added to the solid sample and the mixture thus obtained was submitted to the microwave program mentioned above. After cooling down, an additional 0.5 mL of concentrated HNO_3 was added and the mixture was submitted to a second microwave treatment (same program as mentioned above). Finally, 3 mL of concentrated HCl and 1 mL of concentrated HF were added, and once again, the microwave digestion was carried out. This intense digestion ensured complete oxidation of the elemental carbon present in the samples and the reference material and it also allowed digestion of silicates. This microwave-program was relied on for digestion of both the reference material and the collected dust samples.

Upon completion of the microwave-assisted digestion, the clear solutions were quantitatively transferred into PTFE-beakers and the acids were evaporated at 85 °C to near-dryness. Subsequently, 5 mL of *aqua regia* were added and the samples were again evaporated to near-dryness. This procedure was repeated three times, each time with addition of 5 mL of concentrated HCl. This repeated boiling in HCl ensured conversion of Pt and Pd into their chloro-complexes [16]. Moreover, remaining hydrofluoric acid is removed by this procedure, thus avoiding possible precipitation of fluorides and destruction of silica sorbent particles in the subsequent sample pre-treatment procedure. Finally, the samples were taken up in 21 mL of 0.240 mol L^{-1} HCl and – if not analyzed immediately – stored refrigerated (4 °C) until further use. Digested samples were not stored longer than 36 h to avoid losses by sorption to plastic containers.

After each digestion run, the microwave system was cleaned by applying the same chemicals as required for one sample digestion run, whereas the PTFE-beakers were cleaned by immersion in boiling fresh *aqua regia* overnight (2 times). After cleaning, all vessels were thoroughly rinsed with high-purity water and dried under ambient conditions.

General description of the DPE procedure

Ten milliliters of sample digest (0.240 mol L^{-1} HCl) were transferred into a metal-free centrifugation tube. The optimum amount of SAX sorbent material (2 mg) was added in the form of an aqueous suspension and the sample was homogenized in an ultrasonic bath. A first centrifugation step was performed at 17,000×*g* for 10 min. The particles were found to be strongly compressed onto the walls and the bottom of the centrifuge tube and therefore, it was possible to swiftly decant the supernatant solution (see Fig. 2(1)). For further removal of matrix constituents, the precipitate was re-suspended in 10 mL of 0.240 mol L^{-1} HCl. After manual shaking and ultrasonic agitation, the centrifugation step was repeated and the supernatant solution discarded (Fig. 2(2)). Thereby, matrix components that remained on the particles during the first decantation-step can be removed. This washing cycle was repeated two more times (Fig. 2(3) and (4)). Finally, the mesoporous particles were destroyed by adding 0.1 mL of a mixture containing 2 mol L^{-1} HF and 1.2 mol L^{-1} HCl. After adding indium as internal standard, the solutions were diluted to 2 mL using high purity water (Fig. 2(5)). As schematically depicted in Fig. 2, this procedure yields a stepwise removal/dilution of any matrix constituents that do not bind to the SAX-sorbent material. Contrarily, analytes that are adsorbed onto the sorbent resin remain at constant concentration and finally become pre-concentrated. The theoretical enrichment factor depends on the ratio of starting volume to final volume and was 5.

Results and discussion

Optimization of dispersed particle extraction

During initial experiments, it was found that the recovery of rhodium was very low, compared to Pt and Pd. When performing a reduction with $SnCl_2$, recoveries for Pt, Pd, and Rh improved. However, it was found that the high concentrations of Sn that were added to the samples resulted in major spectral interferences. On the one hand, the internal

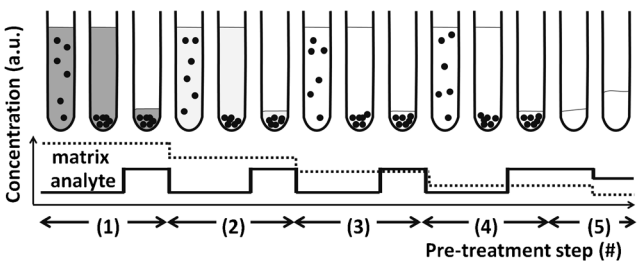

Fig. 2 Schematic of the DPE sample pre-treatment process (black spots: SAX micro-particles). This figure is based on quantitative analyte-recoveries

standard was influenced ($^{115}In^+$ is affected by the isobaric interference of $^{115}Sn^+$). This problem may be solved by using an other element for internal standard. Yet, on the other hand, the solutions treated with Sn resulted in a significantly enhanced Pt-blank. This may be caused by the formation of $SnArCl^+$ in the presence of hydrochloric acid medium resulting in polyatomic ions of masses 194, 195, 196. Therefore, as the addition of Sn produced severe problems, it was opted for not analyzing Rh and for optimizing the method for Pt and Pd only, using hydrochloric acid medium.

Three factors affect the retention of Pt and Pd on SAX micro-particles: (i) the pH, (ii) the amount of micro-particles, and (iii) the time allowed for interaction of the analytes with the sorbent material ("interaction time"). To a certain extent, these factors are related to one another, and therefore, an iterative optimization process was done. First, the optimum pH value was found by providing 10 mL of a solution containing 0.1 ng (Pt, Pd) mL^{-1} with varying concentrations of HCl (0.012 to 0.360 mol L^{-1}) and using a fixed amount of 2 mg sorbent material. An optimum was found at 0.240 mol L^{-1} of hydrochloric acid. Then, the amount of sorbent material was varied between 0.5 and 5 mg using the same experimental setup as mentioned above, and using 0.240 mol L^{-1} HCl medium. At 2 mg of SAX sorbent material, a plateau was reached, i.e., no further improvement of the recovery was observed at higher amounts of sorbent material, and therefore, 2 mg were used in the further experiments. The interaction time in the ultrasonic bath (time between the addition of sorbent and the start of the first centrifugation step) was found to have no observable effect on the recovery (times of 1, 2, 5, 10, and 15 min were investigated), however, without ultrasonication, slightly lower recoveries were observed. Therefore, 5 min of ultrasonication were used in all further experiments. With the optimum interaction time and particle amount, a final investigation of the influence of sample acidity on recovery was carried out to ascertain that optimum conditions were indeed used (optimum: 0.240 mol L^{-1} HCl).

Evaluation of spectral interferences

As discussed in the introduction, the removal of spectral interferences is a very important prerequisite for obtaining correct quantitative results. In the road dust CRM (BCR 723), many parent nuclides can be found which potentially cause such spectral interferences (see [14] for an extensive list). Therefore, the effectiveness of removing Cu, Zn, Sr, Cd, Hf, Mo, Y, and Zr from the samples when applying the proposed DPE-method was investigated in more detail. To this end, solutions containing those elements in concentrations expected after digesting BCR 723 road dust were prepared from single-element standards (concentrations ranging from 10,000 to 100 ng mL^{-1}, depending on the element). To simulate the digestion, the solutions were mixed with aqua regia and HF and evaporated repeatedly to near-dryness as described above (see section "sample collection and digestion"). Finally, the samples were taken up in 0.240 mol L^{-1} HCl and the proposed DPE sample pre-treatment process was carried out three times. The supernatants resulting from each of the three pre-treatment steps were analyzed for their respective element concentrations. As can be seen in Fig. 3, the concentrations of all elements decrease with every washing step, namely to 2.3–7.5 % of the initial concentration after the first washing step (signals obtained from the first supernatant correspond to the digest without any pre-treatment and are normalized to 100 %), to 0.01–0.2 % after the second washing step, and to levels not significantly different from the blank after the third

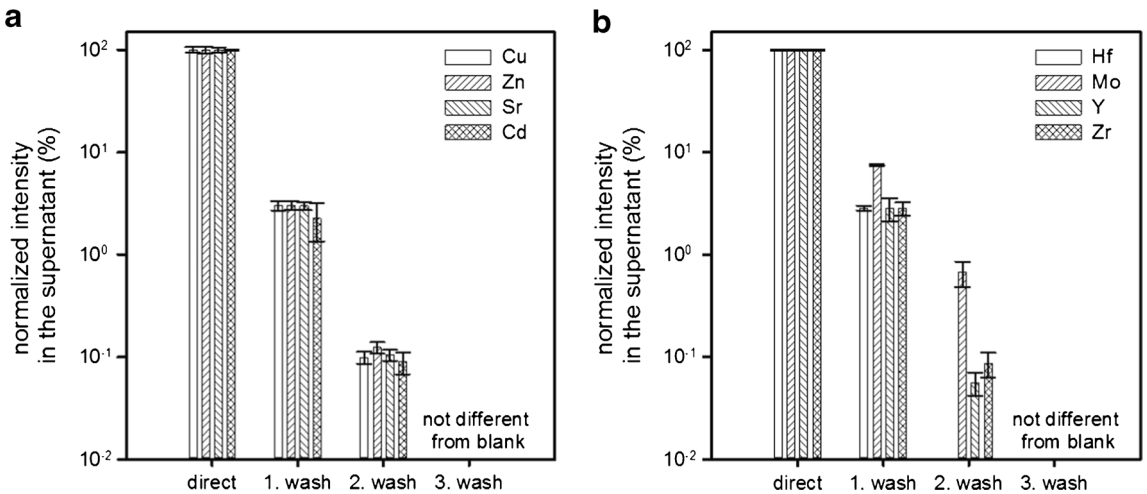

Fig. 3 Three successive washing steps result in an effective removal of potentially interfering elements (concentrations at the beginning: 10,000 ng mL^{-1} for Cu, Zn, Sr, 1000 ng mL^{-1} for Y, Zr, and 100 ng mL^{-1} for Cd, Hf, Mo, *error bars* represent the standard deviation of n=3 replicates)

washing step. Therefore, three washing steps were used in all further experiments.

Analyte recovery and figures of merit

Typical recoveries for aqueous standard solutions, as well as for road dust digests and different dilutions thereof were in the range of 64–80 % for Pd and 21–35 % for Pt. These values represent the variability of the method under different matrix conditions (aqueous standards, digested samples) and different analyte-concentrations, using a three-step sample pre-treatment scheme. It should be noted that the goal of the approach presented here was rather to remove interfering matrix elements than to obtain quantitative recoveries for Pt and Pd.

From the analyte-recovery and the theoretical pre-concentration factor of 5 (10 mL starting volume, 2 mL final volume), the pre-concentration factors for Pd and Pt were determined to range between 3.2–4 and 1.05–1.75, respectively.

For quantification purposes, four-point standard addition was used for every sample. Adequate amounts of Pt and Pd were spiked to the digests prior to performing the DPE pre-treatment.

The process of digestion, analyte sorption, and step-wise matrix removal typically yielded a reproducibility of≤5 % relative standard deviation (RSD) for Pt and≤4 % RSD for Pd (at 0.1 ng mL^{-1}, n=4 road dust digests). Internal standardization with indium was carried out to compensate for potential instrument instability and/or signal drift. Detection limits were comparable to typical values obtained with quadrupole ICP-MS instrumentation (2 pg mL^{-1} for Pt and 1 pg mL^{-1} for Pd, calculated from 8 blank solutions pre-treated independently with the proposed DPE-procedure, 3 s-criterion). The method quantification limits in the native dust samples were 2 and 1 ng g^{-1} for Pt and Pd, respectively.

The step-wise removal of interfering elements, as shown in Fig. 3, allowed for a successful quantification of palladium and platinum in BCR CRM 723. Found concentrations were 80.2±0.7 ng g^{-1} for Pt and 6.2±2.6 ng g^{-1} for Pd (n=4, results given as average and standard deviation of 4 sample digests). These values are in good agreement with the certified values of 81.3±2.5 ng g^{-1} Pt and 6.1±1.9 ng g^{-1} Pd.

Analysis of road dust samples

The dust samples collected in March and July 2011 were digested, the optimized DPE-procedure was applied, and Pt and Pd were quantified by means of quadrupole ICP-MS in standard mode. The results are summarized in Fig. 4. All concentrations were above the method quantification limit. Found concentrations for platinum ranged from 205 to 1445 ng g^{-1}, which is in the same order of magnitude as reported in the literature [29–32]. Concentrations of palladium ranged from 201 to 1230 ng g^{-1}, which is also in agreement with reported data [31, 32].

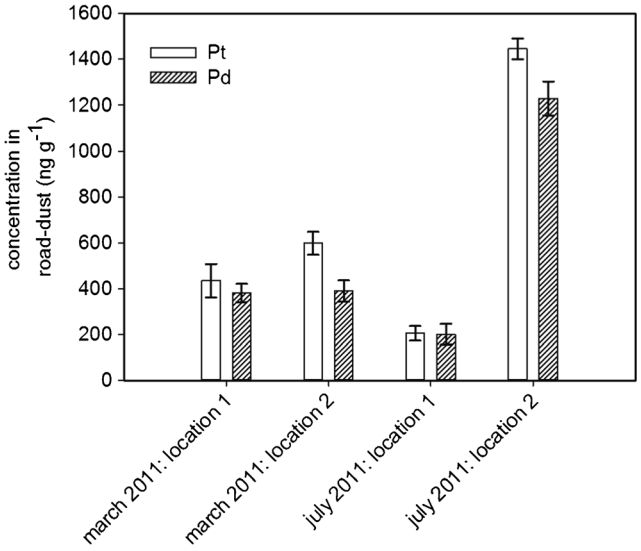

Fig. 4 Found concentrations in roadside-dust (location 1: subterranean parking garage, location 2: road-side, all concentrations above method quantification limit, *error bars* represent the standard deviation of n=2 replicates)

The roadside concentrations (location 2) in July were found to be higher than those observed in March. Dust sources, such as gravel used for winter service and dust caused by abrasion of the road-surface, are more prominent during winter and result in a higher overall dust load. Provided that the amount of PGEs emitted by the traffic is constant over the year, the higher dust load in winter could therefore result in a dilution of Pt and Pd.

The elemental Pt/Pd ratio was found to be 1.2±0.2 (average and standard deviation for the four dust samples). Kanitsar et al. [33] have found a Pt/Pd ratio of 2.7±0.6 in total suspended matter (airborne dust), whereas Petrucci et al. [34] have found Pt/Pd ratios ranging from 0.1 to 2.4 in PM_{10} airborne particulate matter. Earlier investigations, which are summarized in [29], reported on much higher Pt/Pd ratios of up to 37.5. Changes in the composition of the catalyst material (also reflecting changes in raw material prices) also contribute to this trend.

Conclusions

We have investigated a novel isolation procedure for platinum and palladium chlorocomplexes from aqueous road dust sample digests. The method of dispersed particle extraction was successfully adapted to allow for the retention of anionic Pt and Pd chlorocomplexes. Method quantification limits of 2 and 1 ng g^{-1} for Pt and Pd, respectively, are comparable to those of existing procedures [14, 15, 35]. By fully exploiting the potential of dispersed particle extraction

in terms of pre-concentration, higher pre-concentration factors would be feasible.

The advantages over conventional solid-phase extraction are that (i) no conditioning of the sorbent material is necessary, that (ii) the often troublesome elution step is avoided, and that (iii) memory effects are fully circumvented by using new sorbent material for every experiment. Moreover, the approach can be adjusted to the analytical problem at hand to increase the enrichment factor or to improve the separation efficiency, either by adjusting the start and end volumes, or by changing the number of washing steps. The amount of sorbent material required for one analysis (2 mg) rationalizes the in-house synthesis of the material, and certainly undercuts the costs for single-use solid-phase extraction columns. The method presented here facilitates sample pre-treatment and improves sample throughput. Importantly, no significant blank issues were observed and the lifetime of cones and nebulizer were not affected, even though rather high amounts of silicon in the form of dissolved silica particles were introduced into the ICP-MS instrument over longer time. If the presence of Si forms a problem in future applications, one may use organic mesoporous particles. These will not cause a background of mineral elements.

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POSTER 1

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Selective extraction and pre-concentration of iron in the presence of zinc and copper using customized ionic liquids

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IRON – why we need to isolate it prior to analysis

The isotopic composition of iron in human blood has been shown to be an indicator for the iron-status of an individual [1]. This phenomenon ***could be used for early diagnosis of aberrant iron metabolism***, observed in patients suffering from diseases such as hemochromatosis type I or anemia of chronic disease.

Iron-status-induced deviations from the natural iron-isotope ratio are found to be very small. ***For adequate correction of instrumental mass discrimination*** in isotopic analysis via multi-collector ICP-MS, ***it is mandatory to isolate the iron fraction*** and to remove the sample-matrix.

IRON SEPARATION – state of the art

Traditional column-based chromatographic methods (e.g., [2]) are based on different distribution coefficients of the various matrix and analyte elements and the resin. These differences allow a sequential elution of the elements from the column. Although they are fit for purpose, such column-based approaches ***are laborious and time-consuming***.

OBJECTIVE

By using customized ionic liquids which provide task-specific functional groups with a good selectivity towards iron, only one separation step should be sufficient and therefore ***it should be possible to increase the sample-throughput***. The aim of this research was to a) screen potential ionic liquids that retain iron and to b) find conditions that allow for a selective extraction of iron, also in the presence of copper, zinc, as well as alkaline and earth-alkaline metals present in whole blood.

INSTRUMENTAL

Measurements were performed on an iCAP 6500 ICP-OES spectrometer (Thermo, Bremen), equipped with a radial optic system, a cyclonic spray chamber, as well as a Babington-type nebulizer. Plasma parameters were: 1300 W RF-power, 1.50 L min^{-1} auxiliary gas (Ar), 12 L min^{-1} cooling gas (Ar), 0.4 L min^{-1} nebulizer gas, 0.4 mL min^{-1} sample uptake, 10 mm observation height, as well as 8 s integration time. Samples were taken up via the peristaltic pump of the spectrometer; all tubing was of solvent-resistent material. High-performance centrifuge vials (15 mL, VWR Brand, 17000 x g) were used for sample preparation. Aqueous samples were prepared from nitrate salts of iron, copper, and zinc. An organic indium solution (Conostan) was added to the organic phase as internal standard after pre-treatment, calibration was achieved also via organic multi element standard solutions (Conostan). Phase-separation was achieved via Eppendorf pipettes.

SAMPLE PREPARATION

Aqueous solutions containing iron, buffer, and concomitant elements (Cu, Zn, Na, K, Ca, Mg, depending upon the experiment, see below) were mixed with 2 mL of organic phase (ionic liquid dissolved in n-Octanol), shaken vigorously and separated via centrifugation (2 min @ 2000 rpm). The organic phase was diluted 1:1 with Conostan Premisolv. At the same time, indium was added as internal standard. Quantification of iron was achieved via external, matrix-adjusted calibration.

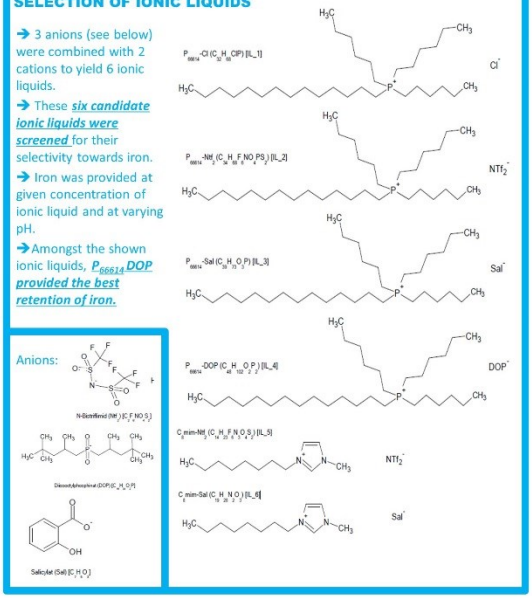
SELECTION OF IONIC LIQUIDS

➔ 3 anions (see below) were combined with 2 cations to yield 6 ionic liquids.

➔ These ***six candidate ionic liquids were screened*** for their selectivity towards iron.

➔ Iron was provided at given concentration of ionic liquid and at varying pH.

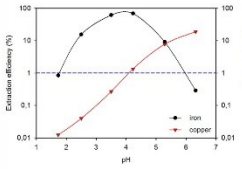
➔ Amongst the shown ionic liquids, ***P_{66614} DOP provided the best retention of iron.***



METHOD OPTIMIZATION

Two parameters were optimized: ***1. maximum Fe recovery*** ***while keeping 2. Cu and Zn below 3% recovery***

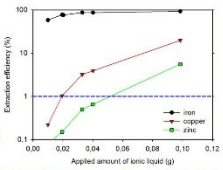
a) **Sample pH** in the presence of copper and zinc (Fe : Cu : Zn = 1 : 1 : 1)



➔ At **pH 4**, iron is extracted with ***69% recovery***, whereas copper is still below 1% and zinc is not extracted (always below 0.05% recovery).

b) **Amount of ionic liquid**

➔ With an amount of 0.02 g of ionic liquid, the recovery of ***iron increases to 81%,*** whereas Cu and Zn remain below 1%.



c) **Extraction time**

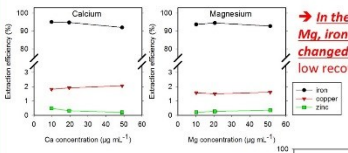
➔ When increasing the extraction time to 4.0 min, the recovery of iron increases to 90%, optimized phase-separation ***results in > 97% iron recovery.***

d) **Working range**

The iron-extraction is quantitative (≥ 95%) from 0.1 µg (Fe, Cu, Zn) mL^{-1} untill 1.1 µg (Fe, Cu, Zn) mL^{-1}, while Cu and Zn are not extracted (≤ 3%).

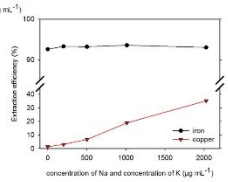
MATRIX TOLERANCE

Native blood contains high amounts of Ca, Mg, Na, and K (14.3, 16.0, 1440, 1025 µg mL^{-1}, respectively [3]). First, the effect of the group II elements Ca and Mg on iron-recovery in the presence of Cu and Zn was investigated.



➔ ***In the presence of Ca and Mg, iron-recovery is not changed*** and Cu and Zn have low recoveries.

➔ ***In the presence of a mixture of Na and K***, copper starts to be co-extracted alongside iron. But still, the ***iron-recovery is not deteriorated***, even at total ion-concentrations of 4000 µg (Na, K) mL^{-1}.



SUMMARY & OUTLOOK

- ✓ The ionic liquid P_{66614}^+ DOP- allows ***selective separation of Fe*** from Cu and Zn.
- ✓ Under optimized conditions, ***Fe-recovery is nearly quantitative*** (> 95%)
- ✓ Group I elements (***Na, K) have no influence on Fe-recovery***
- ✓ Group II elements ***(Ca, Mg) have no influence on Fe-recovery***
- ✓ However: Group I elements (Na, K) lead to a co-extraction of Cu

➢ Fe-recovery under realistic conditions (digested whole-blood CRM) will be carried out to investigate effects of a) the complete inorganic matrix (pooled interferences) and b) the effect of remaining organic matrix (non-digested carbon)

➢ Effect of possible co-extraction of Cu on the result of Fe-isotope-ratios will be investigated

➢ Alternatives to liquid-liquid extraction will be implemented

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POSTER 2

Presented at "ANAKON", Graz, Austria, 2015.

Customized dispersed-particle extraction for the improved quantification of iron in seawater

W Nischkauer[1,2], K Bica[3], F Vanhaecke[2], A Limbeck[1]

IRON – essential trace-nutrient

The abundance of iron in the earth crust is high, and for many species, iron is an essential trace-nutrient. However, its solubility in sea-water is low, thus limiting (phytoplankton-) growth [1]. To better understand this iron-deficiency in oceans, reliable quantification is necessary. ICP-MS offers the required sensitivity, but the saline matrix is problematic. Contrarily, ICP-OES is less affected by the matrix, but it lacks the detection power to quantify iron-concentrations of typically 0.5 µg L^{-1} [2]. Chemical pre-concentration or matrix-removal are therefore required prior to analysis.

IRON SEPARATION – state of the art

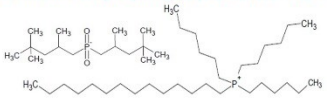
Traditional column-based chromatographic methods (e.g., [3]) are based on different distribution coefficients of the various matrix and analyte elements and the resin. These differences allow a sequential elution of the elements from the column. Although they are fit for purpose, such column-based approaches are laborious and time-consuming.

OBJECTIVE

Instead of conventional column-based methods, we used a ***customized ionic liquid*** which provides ***task-specific*** functional groups with a good selectivity towards iron. First, this ionic liquid was used to optimize conditions by liquid-liquid extraction. Once optimum conditions were established, the ionic liquid was immobilized on a solid support (micron-sized particles) to facilitate phase separation by dispersed particle extraction.

INSTRUMENTAL

Measurements of ***organic solvents*** were performed on an iCAP 6500 ***ICP-OES spectrometer*** (Thermo, Bremen), equipped with a radial optic system, a cyclonic spray chamber, as well as a Babington-type nebulizer. Plasma parameters were: 1300 W RF-power, 1.5 L min^{-1} auxiliary gas (Ar), 12 L min^{-1} cooling gas (Ar), 0.4 L min^{-1} nebulizer gas, 0.4 mL min^{-1} sample uptake, 10 mm observation height, as well as 8 s integration time. Samples were taken up via the peristaltic pump of the spectrometer; all tubing was of solvent-resistent material. Measurements of ionic-liquid impregnated ***particles*** were performed on an iCAP Q ***ICP-MS spectrometer*** (Thermo, Bremen). Sample introduction was achieved using a Babington-type nebulizer. Instrumental parameters were: 1250 W RF-power, 1.5 L min^{-1} auxiliary gas (Ar), 12 L min^{-1} cooling gas (Ar), 1.1 L min^{-1} nebulizer gas (Ar), 0.4 mL min^{-1} sample uptake, analyzed nuclides: ^{56}Fe, ^{57}Fe, 80 sweeps, 4 main runs.



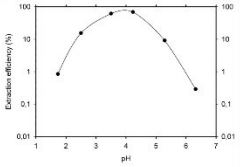
[P_{66614}][DOP] ionic liquid (Trishexyl-Tetradecyl-Phosphonium cation in comibantion with Diisooctylphosphinate anion, *structure see above*) was synthesized in-lab. High-performance centrifuge vials (15 mL, VWR Brand, 17000 x g) were used for sample preparation. Aqueous samples were prepared from iron(III)nitrate*6 H_2O. An organic indium solution (Conostan) was added to the organic phase as internal standard after pre-treatment, calibration was achieved also via organic multi element standard solutions (Conostan). Phase-separation was achieved via Eppendorf pipettes.

LIQUID-LIQUID EXTRACTION

Aqueous solutions containing iron, buffer, and concomitant elements (Na, K, Ca, Mg, depending upon the experiment, see below) were mixed with 2 mL of organic phase (ionic liquid dissolved in n-Octanol), shaken vigorously and separated via centrifugation (2 min @ 2000 rpm). The organic phase was diluted 1:5 with n-Butanol and indium was added as internal standard. Qua external, matrix-adjusted calibration.

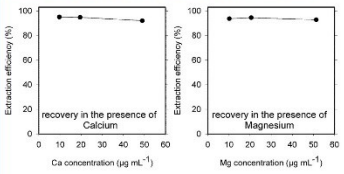
RESULTS OF FIRST OPTIMIZATION

After careful optimization of sample pH via addition of buffer, sample volume, extraction time, amount of ionic liquid, as well as handling during phase separation, a recovery of > 95% was obtained for iron in aqueous solutions.

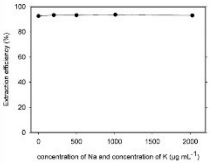


MATRIX TOLERANCE I: individual matrix constituents

Sea-water contains high amounts of Ca, Mg, Na, and K. The effect of the group II elements Ca and Mg on iron-recovery was investigated.



➔ Calcium and Magnesium have no detrimental effect on the iron-recovery.

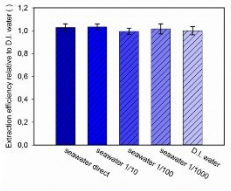


In a next step, the cumulative effect of Na and K on the iron-recovery was tested.
➔Also here, no negative effects were observed.

➔Individually, Na, K, Ca, and Mg do not affect the iron-extraction.

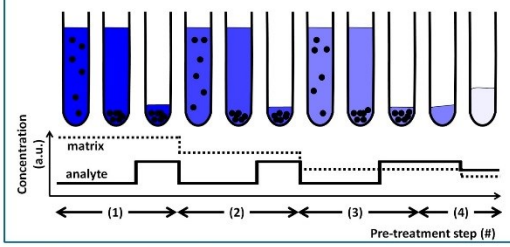
MATRIX TOLERANCE II: artificial sea

Artificial sea-water was prepared by adding 11.95 g NaCl, 4.78 g $MgCl_2$*$6H_2O$, 2.08 g Na_2SO_4, 0.50 g $CaCl_2$, as well as 0.36 g KCl to 500 mL distilled water. This water was used directly or diluted 10, 100, and 1000-fold and spiked with iron. Recoveries were compared with a purely aqueous solution ➔ No detrimental effects were observed, even when applying the artificial sea-water directly.



DISPERSED-PARTICLE EXTRACTION

An aqueous solution containing iron was adjusted to correct pH, ionic-liquid-impregnated micro-particles were added to the solution, brought to reaction in an ultrasonic bath, and centrifuged. The supernatant solution was decanted and the iron-fraction eluted from the precipitate via 0.5 mL of concentrated HNO_3. This solution was filled up to 5 mL, indium was added as internal standard and the samples were analyzed via ICP-MS. ➔ An iron-recovery of 96.1+/- 2.2 % was achieved.



SUMMARY & OUTLOOK

✓ The ionic liquid [P_{66614}] [DOP] allows ***separation of Fe***

✓ Under optimized conditions, ***Fe-recovery is nearly quantitative***

✓ Group I elements (***Na, K) have no influence on Fe-recovery***

✓ Group II elements ***(Ca, Mg) have no influence on Fe-recovery***

✓ ***Synthetic sea-water has no influence on Fe-recovery***

✓ The results obtained with liquid-liquid extraction can be adopted for dispersed-particle-extraction

✓ Dispersed-particle-extraction yields satisfying Fe-recovery (96%) while avoiding problematic organic solvents and tedious analysis of organic phases.

➢ Fe-recovery in synthetic sea-water in combination with dispersed-particle-extraction will be carried out.

➢ To investigate effects of a) the complete inorganic matrix (pooled interferences) and b) the effect of remaining organic matrix, real sea-water samples will be used for recovery experiments.

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POSTER 3

Presented at "Euroanalysis", Bordeaux, France, 2015.

DISPERSED PARTICLE EXTRACTION FOR THE QUANTIFICATION OF IRON IN SEA WATER

W Nischkauer[1,2], K Bica[3], F Vanhaecke[2], A Limbeck[1]

IRON – essential trace-nutrient

The abundance of iron in the earth crust is high, and for many species, iron is an essential trace-nutrient. However, its **solubility in sea-water is low**, thus limiting (phytoplankton-) growth [1]. To better understand this iron-deficiency in oceans, **reliable quantification** is necessary. ICP-MS offers the required sensitivity, but the saline matrix is problematic. Contrarily, ICP-OES is less affected by the matrix, but it lacks the detection power to quantify iron-concentrations of typically 0.5 µg L^{-1} [2]. **Chemical pre-concentration or matrix-removal are therefore required** prior to analysis.

IRON EXTRACTION– state of the art

Traditional column-based chromatographic methods (e.g., [3]) are based on different distribution coefficients between the various matrix and analyte elements and the resin. These differences allow a sequential elution of the elements from the column. Although they are fit for purpose, such **column-based approaches are laborious** and time-consuming.

OBJECTIVE

As an alternative approach, we used a ***customized ionic liquid*** which provides ***task-specific*** functional groups with a good selectivity towards iron. This ionic liquid was immobilized on micron-sized particles. By suspending those particles directly in the liquid sample, it was possible to perform "dispersed particle extraction" (*see below*).

The aim was to provide a method which has a high sample throughput and which allows for a satisfying removal of the saline matrix.

INSTRUMENTAL

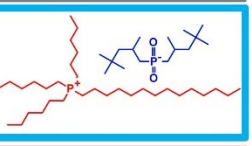
Measurements were performed on an Element XR ***ICP-MS spectrometer*** (Thermo, Bremen). Sample introduction was achieved by using a concentric glass nebulizer. Instrumental parameters were: 1050 W RF-power, 1 L min^{-1} auxiliary gas (Ar), 15 L min^{-1} cooling gas (Ar), 0.9 L min^{-1} nebulizer gas (Ar), 0.2 mL min^{-1} sample uptake, analyzed nuclides in medium mass-resolution: ^{54}Fe, ^{56}Fe, ^{115}In (internal standard), mass window of 100, a sample time of 0.01 s, 20 samples per peak, a search window of 50, an integration window of 50, using E-Scan mode, the detector fixed in counting mode, and calculating the peak-average for quantification.

SAMPLE PREPARATION

Aqueous solutions containing iron and potential matrix-elements, as well as artificial sea-water and real seawater samples were diluted 1/10 with 0.1% (v/v) HNO_3, mixed with buffer and thoroughly homogenized. Then, 2 mg of TSIL-impregnated micro-particles (details see below) were added in the form of an aqueous suspension. The samples were shaken manually for 5 min and finally centrifuged at 4400 rpm for 5 min (Eppendorf centrifuge 5702, equipped with a swing-bucket rotor A-8-17, resulting in 2800 x g acceleration). The supernatant was discarded by means of a pipette, and fresh solvent was added. The particles were re-suspended and centrifuged once more. The precipitate was eluted two times with 20% (v/v) HNO_3. The united eluted fractions were spiked with indium and quantified versus an external aqueous calibration.

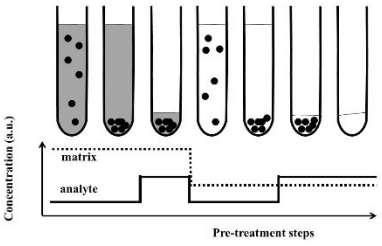
TSIL-impregnated micro particles

In preliminary experiments, potential task-specific ionic liquids (TSIL) were screened. P_{66614}-DOP was found to be selective for iron. This TSIL was immobilized on cellulose micro-particles (25% loading) and used in the following experiments.



DISPERSED PARTICLE EXTRACTION

This method which was recently presented by our group [4-6] makes use of **freely suspended sorbent particles** which selectively extract the analyte of interest in one step. After sorption, the particles are separated from the solution by means of centrifugation, the supernatant is discarded and fresh solvent is added. Thus, non-extracted matrix elements are diluted step-wise whereas analyte-ions are retained in the vessels. After the analyte-isolation, remaining particles are removed in a final elution step. In the here presented context, one washing step was found to be sufficient for removal of the saline matrix.

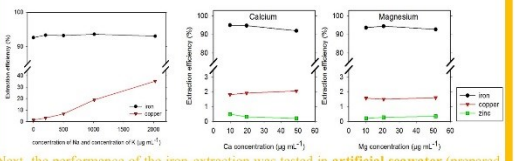


Collection and pre-treatment of seawater

Seawater was collected at the beach of Oostende, Belgium, using a PE bottle which was pre-washed twice with 1% HNO_3. The bottle was rinsed twice with seawater, and then 0.5 L sample were drawn. The sample was centrifuged to remove particulates, acidified to 1% HNO_3 and stored refrigerated (4°C) until further use.

RESULTS

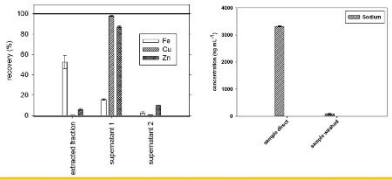
First, the **selectivity** of the TSIL towards iron was tested, especially in comparison to Cu and Zn which might also be present in seawater and have a similar chemistry. The selectivity towards iron is good and remained unchanged, also after the addition of significant amounts of Na, K, Ca, and Mg which are major components of seawater.



Next, the performance of the iron-extraction was tested in **artificial seawater** (prepared according to [7]), as well as in **spiked real seawater**. As can be seen, the recovery slightly decreased when providing more harsh matrix conditions. Nevertheless, extraction of iron was found to be possible even in real seawater using minimum sample dilution and preparation (only dilution 1/10 and pH-adjustment by adding a buffer).

sample matrix	spike recovery
aqueous standard	72% ± 2%
artificial seawater	67% ± 4%
real seawater	62% ± 3%

The effectiveness of **removing the saline matrix** was shown by quantifying Na, Cu and Zn in every step of the pre-treatment process. As can be seen, the Na-matrix is satisfyingly removed after one washing step. Moreover, after this one washing step, practically no Cu and Zn are found in the sample solution which shows the selectivity of the method even under difficult conditions.



CONCLUSION and OUTLOOK

- Extracting iron can be achieved in a selective way (no co-extraction of Cu and Zn)
- The saline matrix is removed to a great extent
- Sample preparation of seawater is kept to a minimum (centrifugation, acidification)
- The extraction procedure is fast (5-6 min per sample)
- No memory-effects since fresh sorbent material is used for every extraction
- minimum use of reagents (20 mL 0.1% HNO_3, 1 mL 20% HNO_3)
- low consumption of sorbent (2 mg per analysis)

➔ Show trueness of method by analyzing CRM
➔ Apply to larger number of seawater samples
➔ Adapt method to non-saline waters (drinking water)

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5.2.3. PART III

This chapter contains one article which discusses the combination of dispersed particle extraction with dried droplet laser ablation.

ARTICLE 5 (SUBMITTED)

COMBINING DRIED DROPLET LASER ABLATION ICP-MS WITH DISPERSED PARTICLE EXTRACTION FOR DETERMINING PLATINUM AND PALLADIUM IN AIRBORNE PARTICULATE MATTER.

This article was submitted for peer-review to the journal of Applied Spectroscopy.

Combining dispersed particle extraction with dried droplet laser ablation ICP-mass spectrometry for determining platinum and palladium in airborne particulate matter

Winfried Nischkauer[a,b], Andrei Izmer[b], Marie-Alexandra Neouze[c], Frank Vanhaecke[b] and Andreas Limbeck[a]*

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Abstract

A combination of analyte pre-concentration using dispersed particle extraction (DPE) and dried droplet laser ablation-ICP-mass spectrometry (LA-ICP-MS) was developed with the aim to quantify Pt and Pd in urban particulate matter with an aerodynamic diameter ≤ 2.5 micrometres ($PM_{2.5}$). The $PM_{2.5}$ aerosol was collected on cellulose ester filters during a sampling period of three days, with sampling intervals of 4 hours only. Each of the filters was chemically digested, and the resulting solution was pre-concentrated using DPE. Droplets taken from the pre-concentrated sample were deposited on polymeric disks and dried. These dry spots were then analysed by means of LA-ICP-MS. This approach allowed ICP-MS analysis of solutions with high content of dissolved sorbent particles coming from the DPE procedure. Furthermore, spectral interferences arising from sample-inherent matrix elements as well as solvent-related interferences could be removed by the proposed approach. The method was validated by determining the Pt-concentration in BCR CRM 723 road dust certified reference material and a good agreement with the certified value was obtained. The temporal variation of Pt during the three-day sampling period is discussed, with

respect to automotive traffic. The daily average of Pt measured in the air corresponds to typical values observed in urban areas in Central Europe.

Introduction

Airborne particulate matter (APM) contains various toxic elements such as As, Cd, Cr, and Pb, which have the potential to become bio-available when inhaled or ingested [1]. The presence of those toxic elements in APM is well-documented, but APM also contains platinum group elements (PGEs) at a pg m^{-3} level [2–6]. These PGEs are predominantly emitted from automotive catalysts [7] although other sources have to be considered as well (e.g., hospital effluents, jewellery, electronic industry). Since environmental studies indicate that PGEs are taken up by plants and animals [8–10], the toxicological relevance of PGEs in APM is under discussion [5]. As one step on the way to understanding the toxicity of PGEs contained in APM, it is therefore necessary to determine their total concentration in APM, and especially in the $PM_{2.5}$ size-fraction, in order to assess the amounts of PGEs available for potential uptake via the respiratory tract.

The two major challenges in PGE quantification in APM are their low concentrations and the complex matrix of this type of sample. As a result of the low concentrations of Pt and Pd, dilution or an inefficient use of the sample during sample preparation and analysis must be avoided. Solid-sampling techniques such as laser ablation (LA) [11] or electrothermal vaporisation (ETV) [12] use the sample and the analyte efficiently, and are therefore ideal for the task at hand. However, direct LA of an APM sample is problematic as it requires suitable standard materials for quantification. Moreover, the dust is spread over a large filter area, thus compromising sensitivity, and the so-called nugget-effect makes it difficult to assess average concentrations in APM reliably: when scanning across environmental samples, it is often observed that PGEs are not evenly distributed, but appear in small but highly concentrated agglomerates [11]. These are most likely particles that break off from car exhaust catalysts and remain intact during transport and sampling. Correct quantification of such APM samples is therefore difficult. When using ETV for sample introduction, such sample inhomogeneity plays a minor role, but PGEs are prone to react with the graphite furnace of an ETV-system,

which results in analyte losses and carry-over effects [12]. In order to obtain representative results for PGEs in APM samples, inductively coupled plasma-mass spectrometry is therefore often used in combination with sample digestion. Digested samples can be chemically pre-treated prior to ICP-MS measurement, thus removing potentially interfering sample constituents. For a detailed list of such spectral interferences in ICP-MS detection of PGEs, we refer to the literature [13]. Moreover, sample pre-treatment also allows for a pre-concentration of the analytes, an important feature in ultra-trace element quantification.

Many methods are suitable for such a chemical sample clean-up prior to PGE determination in combination with ICP-MS (e.g., tellurium co-precipitation, nickel or lead fire-assays), but solid-phase extraction is a widely accepted and practical method (e.g., [6,13]). Recently, we have presented "dispersed particle extraction" (DPE) as an advanced method which is based on solid-phase extraction, but circumvents some of the latter method's shortcomings (i.e., no ageing of sorbent material, no carry-over, no analyte elution required [14]). DPE proved to be a suitable method for quantifying PGEs in digested urban road-dust, a sample available in large quantities.

Aim of the present article was to monitor the temporal variation of Pt and Pd in $PM_{2.5}$ samples with a time-resolution of 4 hours. This amounts to less than 100 µg of aerosol collected per sample. Thus, in contrast to, e.g., soil or road-dust analysis, the available sample material is limited in the present case. Conventional DPE sample preparation with nebulization as sample introduction strategy would therefore not be sensitive enough. Application of a higher pre-concentration factor and the use of, e.g., a total-consumption nebulizer to accommodate the small sample volume thus obtained would not be possible as well, due to expected frequent nebulizer failure caused by the DPE sorbent material. An alternative sample introduction method was therefore required.

The dried-droplet approach is tolerant towards sample matrix constituents and allows introducing challenging liquid samples into an ICP in a straight-forward way. In short, a small aliquot of the liquid sample is deposited on a solid surface, dried, and the residue is then investigated by means of LA-ICP-MS. Besides the good sample introduction efficiency associated with LA, and the capability of accommodating small sample volumes, dried droplet LA also provides dry plasma conditions, since no water

is introduced into the ICP. This lack of oxygen helps in reducing oxide-based polyatomic interferences (e.g., $^{179}Hf^{16}O^{+}$ interfering with $^{195}Pt^{+}$) [13]. The various aspects of dried droplet LA are summarized in more detail in a recent review article [15].

To sum up, the method presented here combines DPE sample pre-concentration with dried-droplet LA-ICP-MS for the following reasons: 1. removal of elements giving rise to interferences (DPE), 2. efficient sample enrichment (DPE), 3. improved tolerance towards dissolved solids (dried droplet LA-ICP-MS), 4. Better sample introduction efficiency associated with LA, and 5. the benefit of further reducing solvent-based interferences by using dry plasma conditions (dried droplet LA-ICP-MS). By using this combined analytical approach, it was possible to determine short-time variations in the Pt concentrations in urban APM with a time-resolution of only four hours and for absolute masses of collected dust ranging between 33 µg and 98 µg per sample.

Experimental

Sampling of $PM_{2.5}$ aerosol

Urban particulate matter with an aerodynamic particle diameter ≤ 2.5 µm was collected on March 20th, 21st, 22nd, 2014 in downtown Vienna, Austria (location 48° 12′ 4.9′′ N 16° 21′ 48.3′′ E, 185 m above sea-level, at 4 m distance from and 1.5 m above a 5-lane road). The aerosol was size-classified using a $PM_{2.5}$ sampling head (Digitel, Switzerland) and collected on cellulose ester filters (GN-4, diameter: 47 mm, Pall Life Sciences, Michigan, USA). The average sampled air-volume collected during each 4 h sampling interval was 7.8 m^3.

Procedural blanks were obtained by inserting a blank filter into the collecting device and removing it after aspiring air for 10 s. All handling of the filters was carried out with polyethylene tweezers, filters were stored in sealed polyethylene petri-dishes until chemical digestion.

Alongside the $PM_{2.5}$ sampling line, a second line was operated ($PM_{2.5}$ sampling head, nominal capacity: 1.3 m^3 h^{-1}, Digitel, Switzerland). This line was connected to a continuous aerosol counter (dust monitor FH 62 I-R, Thermo ESM Andersen), which provided the mass of $PM_{2.5}$ in the air as average concentration every 30 min. For checking the accuracy of this instrument, the feed-back of the aerosol counter was

compared with the total mass collected using the other sampling head during an initial test-run of 48 h. A good agreement between the two methods was found (dust monitor: 1300 µg, weighing of filter: 1600 µg, both values obtained during the same 48 h sampling period).

Reagents and materials

Reagents were of analytical grade or higher purity. Concentrated HNO_3, HCl and HF were purchased from Merck (Germany). High purity water was obtained using an Easypure water system (Thermo, USA, 18.2 MΩ cm). 1000 mg L^{-1} single-element standard solutions for Pt (PlasmaCAL, SCP Science, France) and Pd (Inorganic Ventures, USA) were used for the preparation of calibration standards, or for standard addition purposes. All dilutions were done with 0.240 mol L^{-1} HCl. Indium was added as internal standard after dispersed particle extraction (1000 mg L^{-1} stock solution from Specpure, Alfa Aesar, USA).

A sub-micron mesoporous silica sorbent material with high specific surface was synthesized in-house following a sol-gel procedure described in detail elsewhere [14]. Reagents used for synthesis were of synthesis grade, and the blank signal of the sorbent particles synthesized with these reagents resulted in a signal not distinguishable from one of an empty target used for dried droplet deposition.

Instrumentation

Measurements were accomplished using a GeoLas 200M ArF* excimer laser (MicroLas laser systems, Germany) coupled to an Element XR sectorfield ICP-MS (Thermo Scientific, Germany, instrumental parameters are given in table 1). Prior to each measurement session, the ICP-MS instrument was tuned for best sensitivity and for low oxide ratios, as well as for low signals on double-charged ions (Ba^{++}) using a Meinhard-type concentric glass nebulizer connected to a cyclonic spray chamber for sample introduction. Next, the sample introduction system was switched to the LA unit without extinguishing the plasma and further system optimization was performed via ablation of the NIST SRM 612 glass reference material (NIST, MD, USA) for best sensitivity, flat-topped peak shapes and a U/Th ratio close to unity.

Table 1. Thermo Scientific Element XR sector field ICP-MS Spectrometer: Operating parameters.

Plasma power	900 W
Cool gas flow rate	15 L min^{-1}
Auxiliary gas flow rate	0.9 L min^{-1}
Carrier gas flow rate	0.6 L min^{-1} Helium
Make-up gas flow rate	0.6 L min^{-1} Argon
Mass resolution m/Δm	300
Scanning-mode	E-scan
Mass window	20%
Samples per peak	5
Detection mode	Triple
Runs/passes	150/1
Time per run	706 ms
Nuclides monitored	^{105}Pd, ^{106}Pd, ^{108}Pd, ^{115}In, ^{194}Pt, ^{195}Pt, ^{196}Pt

Sample digestion

A Multiwave 3000 microwave system (Anton Paar, Austria) with high-pressure Teflon vessels was used for the digestion of solid samples (heating ramp: 20 min, hold-time: 35 min, maximum power: 900 W, maximum internal temperature: 240°C, maximum pressure: 40 bar). Reagents added for digestion were selected according to a procedure described previously [14]. In short, the microwave program was run three times. During the first two runs, concentrated HNO_3 was used in combination with H_2O_2 to oxidize any organic material (filter material, organic sample constituents, and

elemental carbon). Then, in a third step, HCl and HF were added to digest PGEs and silicates. After the microwave digestion, the concentrated acids were removed by slow evaporation at 85°C. A subsequent addition of concentrated HCl removed any remaining fluoride and converted PGEs in their chloride form. Finally, the samples were taken up in 14.5 mL of 0.240 mol L^{-1} HCl and stored refrigerated until further use (4°C, maximum storage 36 h).

BCR CRM 723 road dust certified reference material (Institute for Reference Materials and Measurements, Belgium) and filter samples were digested according to the procedure described above. A sample intake of 30 mg was chosen for the CRM, and the material was weighed on a Sartorius MC-210 P microbalance (Sartorius, Germany, readability 0.01mg) after equilibration in an air-conditioned room (24h, 20±1°C and 50±5% relative humidity). Each cellulose-ester filter contained an average of 60 µg dust with values ranging between 33 µg and 98 µg. Each filter was digested entirely, resulting in one digested solution of 14.5 mL per filter.

Dispersed Particle Extraction (DPE)

Due to the low amount of analyte, each digested filter-sample was used for DPE entirely, whereas the digested reference material was first divided into four aliquots, and spiked with increasing amounts of Pt and Pd for quantification via standard additions. For the CRM, this resulted in an additional 4-fold dilution after digestion, corresponding to an effective sample intake of 7.5 mg solid CRM per replicate. To perform external matrix-adjusted calibration (for quantification of filter-digests, as well as for the CRM), digestion blanks were spiked with increasing amounts of Pt and Pd and pre-treated with the DPE method.

A detailed description of the method development and the optimum conditions for pre-concentrating Pt and Pd via DPE were reported previously [14]. To perform DPE, 40 µL of a 1 g L^{-1} suspension of mesoporous sorbent particles were added to the sample digests. The solution was thoroughly mixed (30 s Vortex and 2 min ultrasonication). Subsequently, the analyte-loaded sorbent particles were separated from the solution via centrifugation (10 min, 70,000 x g, Heraeus Megafuge 16, Thermo Scientific, FIBERLite F15-6x100 angular rotor), the supernatant was carefully decanted, and the remaining particles re-suspended in 14.5 mL of fresh 0.240 mol L^{-1} HCl. This was

repeated one more time for efficient matrix-removal [14]. Finally, the particles were re-suspended in 150 µL of a solution containing 2 mol L^{-1} HF, and 1.2 mol L^{-1} HCl. This last step resulted in the swift disintegration of the sorbent-particles. High-purity water and indium (as an internal standard) were added to the clear solutions to obtain a final volume of 200 µL. This finally obtained solution contained an indium concentration of 1 µg L^{-1}, as well as a silicon concentration of 100 mg L^{-1} (resulting from the dissolution of the sorbent material, ignoring the formation of volatile Si-species in the presence of HF).

Dried droplet sample preparation and laser ablation

Sample preparation of dried droplets was optimized as described below, and the optimized procedure was carried out as follows: 5 µL of the sample solutions obtained after DPE were positioned on polyethylene petri-dishes (VWR, Germany) using a micro-pipette (0.1 – 10 µL, Eppendorf, Germany) and dried under ambient conditions in a VFT 1525 ultraclean laminar flow hood (WEISS Technik, Austria). After complete drying, the samples were introduced into the ablation chamber. Each sample spot was quantitatively ablated during 60 s using a laser beam diameter of 120 µm, a repetition rate of 5 Hz and a laser fluence of 8 J cm^{-2}. The laser-aerosol was transported to the ICP-MS via 1.5 m of tygon tubing of 4 mm inner diameter.

Data treatment

For quantifying Pt and Pd in the digested and pre-concentrated dust samples, the following strategy was applied: transient signals of indium and the analytes were recorded during ablation of one dried droplet. The whole dataset thus obtained (signals as function of time) was split into datasets containing only data of the internal standard and one analyte. For each of these new datasets, the two columns of data were plotted in an x/y graph (x: internal standard, y: analyte). The signals were thus found along a straight line, with its slope corresponding to the ratio of analyte to internal standard. To determine this slope, a straight line was interpolated using "orthogonal distance regression" (to account for uncertainties in the x as well as in the y values). Due to the leverage effect, individual blank values do contribute little to the slope, and signals with high intensity contribute more. Therefore, each complete data set arising from ablation of one dried droplet (60 s LA, with 20 s gas

blank before and 20 s gas blank after ablation) could be used without manually setting any integration borders. Details regarding this data treatment procedure can be found elsewhere [16–19]. This data treatment resulted in slopes (i.e., analyte signals normalized to the internal standard) for all samples, as well as for calibration standards. A calibration curve was established from matrix-adjusted standards (spiked digestion blanks) in order to quantify the analyte concentrations in the digested filter samples.

To determine detection limits, it was necessary to use the conventional approach of manually setting integration borders for each sample individually, and to integrate the time-resolved data. The reason for this is that if analyte-signals are low, the linear correlation of the x/y graph disappears. Slopes for actual blank measurements are therefore not obtained by this method [19]. The conventional, integration-based approach yielded integrated counts also for the blanks, which were then used to establish detection limits according to the 3s criterion. Since this integration approach is labour-intensive, it was done only for establishing the detection limit (see below). The digested dust samples were quantified with the alternative approach described above.

Results and Discussion

Method optimization

Parameters for optimum extraction of PGEs using DPE – such as sample acidity, amount of sorbent particles, extraction time, parameters of centrifugation and decantation step – were used as reported previously [14]. The initial sample volume was however changed from 10 mL to 14.5 mL, and the final volume after DPE was changed from 2 mL to 200 µL. The final volume was chosen because this volume can still be handled easily, allows for a sufficient number of replicate dried droplets, and provides a theoretical pre-concentration factor of 72.5.

Parameters for dried droplet LA were optimized as follows: volumes ranging between 1 and 10 µL of the sample solutions obtained after DPE-extraction were deposited on polyethylene petri-dishes and dried. The dry residues were investigated with the light microscope of the LA-unit, with regard to size and morphology of the dry residue. It was found that in all cases, the coffee stain effect (formation of ring-shaped dry residues) was not pronounced, and disk-like dry residues were obtained rather than

ring-shaped residues. This is probably due to the high concentration of dissolved solids (silica material from the DPE sorbent material). A droplet volume of 5 µL was chosen, since the dry residues thus obtained were approximately 500 – 700 µm in diameter, allowing for a comprehensive LA during reasonable time. Figure 1 shows typical dried droplets obtained for spiked digestion blanks and for digested reference material after dispersed particle extraction. Although each droplet had a slightly different morphology, the results obtained for matrix-adjusted calibration and for standard addition calibration agreed well (see below). This indicates that no significant differences in terms of laser ablation, transport efficiency and ionization in the ICP were observed. By repeatedly ablating the area of a dried residue, it was initially checked whether the material is removed completely already during the first ablation pass. It was found that the signals obtained upon a second and third measurement of the same location were equal to the gas blank. The signal obtained when ablating a blank petri dish did not result in significant analyte signal intensities either. Hence, ablation was performed during 60 s and the laser beam scanned laterally across the dry residues in order to ablate each residue completely.

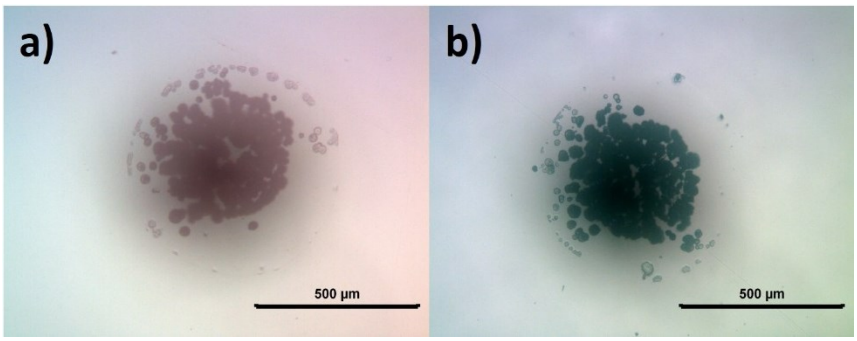

Figure 2 Dried droplets of pre-concentrated method blank (a), and BCR CRM 723 (b) (deposited sample volume in both cases: 5 µL, scale-bar corresponds to 500 µm).

Figures of merit

The instrumental limit of detection (LOD) for Pt and Pd was determined from the standard deviation of 4 reagent blanks which underwent the DPE pre-treatment and LA measurement (3s-criterion). Instrumental LODs calculated for the liquid solution prior to DPE enrichment were found to be 0.2 ng L^{-1} for Pt, and 0.4 ng L^{-1} for Pd. These LODs would allow for the quantification of Pt and Pd in the BCR CRM 723 reference material. This takes into account a concentration of 81.3 ng g^{-1} of Pt and a

concentration of 6.1 ng g^{-1} Pd in the solid CRM, a sample intake of 30 mg, a digestion volume of 14.5 mL, and a 4-fold dilution which was necessary for performing standard additions, as discussed below. The resulting solution would therefore contain 42 ng L^{-1} Pt and 3 ng L^{-1} Pd. Both concentrations would be above the instrumental detection limit.

However, when considering the method detection limit which also takes into account the contribution of the microwave digestion, acid evaporation and the blank filter material, LODs were found to be higher for both elements. Method detection limits were found to be 1 ng L^{-1} for Pt, and 4 ng L^{-1} for Pd. This indicates that the limitation in terms of detection limit comes from the blank value of the filter material, as well as the digestion procedure.

The Pt concentration measured in the BCR CRM 723 was found to be in good agreement with the certified value when using standard additions (found: 82 ± 5 ng g^{-1}, n = 2, ± 1 standard deviation, certified: 81.3 ± 2.5 ng g^{-1}) and when using external matrix-adjusted calibration (found: 84 ± 8 ng g^{-1}, n = 2). Therefore, no matrix effects arising from the digested and pre-concentrated aerosol and/or the digested sorbent particles was observed, allowing for the use of external matrix-adjusted calibration for quantification of Pt on the filters. Elements initially present in the particulate sample do not hamper the quantification, as they are removed during the pre-treatment process (for a detailed study regarding removal of potentially interfering elements, see [14]). Matrix-adjusted calibration was necessary, to provide identical conditions in terms of dissolved silica-material (arising from dissolving the DPE sorbent particles). Pd could not be quantified in the CRM digests, as the solution contained concentrations below the method LOD for this element. Reducing the method blank, would however allow the quantification of this element, as the instrumental sensitivity is sufficient.

The repeatability of the dried droplet LA measurements was determined from repeatedly measuring matrix-adjusted standard solutions (200 ng L^{-1}) and was found to be typically 5% relative standard deviation (n = 3). The repeatability of the entire sample preparation process of sample digestion, DPE, LA, and ICP-MS measurement was established from replicate digestions and measurements of BCR CRM 723 certified reference material. The Pt concentration of the CRM digest prior to the DPE

procedure was 40 ng L^{-1}. For Pt, the repeatability of this entire procedure was found to be 14 % relative standard deviation (RSD, n = 4). This increase in RSD shows the additional variation introduced by sample digestion and pre-treatment, as well as the influence of the sample concentration on repeatability.

Measurement of PM $_{2.5}$ filter samples

The aerosol concentration in air was monitored with the continuous particle monitor during the entire sampling period. The PM $_{2.5}$ aerosol-concentration with data points every 30 min is shown in figure 2 a, indicating a substantial variation during each day and along the three-day sampling period. The temperatures during the sampling period were ranging between 1°C and 10°C, with no precipitation. The measured daily average PM $_{2.5}$ concentrations (11 µg m^{-3}, 18 µg m^{-3}, and 19 µg m^{-3} for the three days of the sampling period) were below the limit value of 25 µg m^{-3}, but values on Friday and Saturday were above the upper and the lower assessment thresholds of 17 µg m^{-3} and 12 µg m^{-3}, respectively [20]. The traffic situation on the sampled road is generally high during weekdays, and especially during the morning hours. During the week-end, there is less traffic. Thus, the increased PM $_{2.5}$ concentration in the air during the sampling period can therefore not be attributed to traffic alone. Contributions from domestic heating, long-range transport, as well as general build-up due to a possible inversion weather situation are likely.

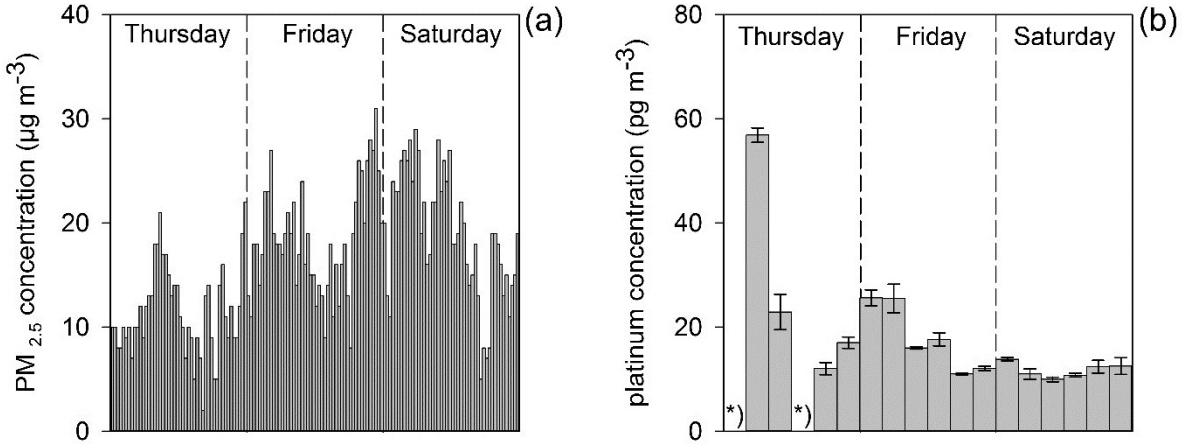

*Figure 2 (a) PM 2.5 aerosol concentration (µg m^{-3}) at the sampling location, (b) Pt concentration in the air (pg m^{-3}), reported as average of three replicate measurements (error bars represent one standard deviation of the mean, all data with error bars were above LOQ, data marked with *) were below the LOD).*

The digested and pre-concentrated filter samples were analysed using the proposed dried-droplet LA-ICP-MS approach, as described above. Quantification was achieved via normalization to the internal standard (In) and matrix-adjusted calibration (a spiked digestion blank which was pre-treated with the abovementioned DPE procedure) in order to obtain identical dried-droplets for standards and samples. The calibration yielded analyte mass per filter (ng analyte / filter). This value was converted to concentrations in air using the air-volume sampled during each sampling interval. The results thus obtained are shown in figure 2 b. Taking into account the sampled air-volume, method LODs in air were established to be 2 pg m^{-3} and 7 pg m^{-3} for Pt and Pd, respectively. Two Pt-values and all Pd-values were below the LOD. The repeatability of the Pt measurements ranged between 2% RSD and 21%RSD. The Pt concentration in the air was generally low, with higher concentrations on Thursday between 4:00 a.m. and 12:00 a.m. We assume that this increase is caused by automotive traffic during this time. Also on Friday, a slight increase of the Pt concentration can be seen between those hours, however, not as pronounced. The daily average for Pt in air was found to be 27 pg m^{-3}, 18 pg m^{-3}, and 12 pg m^{-3} on the three consecutive days, which is in general agreement with concentrations reported by others for urban locations in Central Europe [2,21,22]. The Pd concentration was always found to be below the LOD of 7 pg m^{-3}, which is also in agreement with typical Pd concentrations in urban $PM_{2.5}$ [2,6,21,22].

Conclusion and Outlook

In this paper, we present an approach relying on a combination of dispersed particle extraction and dried droplet laser ablation ICP-MS, which allows for the sensitive quantification of Pt in urban airborne particulate matter. The collected particulate matter is used efficiently, which allowed for detection limits low enough to allow for a sampling interval of 4 h only. By isolating and pre-concentrating the analytes present in the aerosol into a digest volume of 200 µL only and by using the dry plasma conditions offered by dried-droplet LA, potentially interfering matrix elements were removed, and due to low oxide ratios in the plasma, any remaining spectral interferences were further reduced. High sensitivity was obtained owing to the abovementioned efficient sample use, as well as the high sample introduction efficiency associated with LA. Only by opting for LA, the high pre-concentration

factors were possible, as the presence of dissolved sorbent material is expected to be detrimental in the case of conventional nebulizer-based sample introduction.

With the method presented, it was possible to observe the time-dependent variation of the Pt concentration in urban air. Further improvements of the method are necessary, in order to also determine Pd concentrations. In particular, digestion blanks need to be reduced, as they caused a distinct increase in the method-LOD when compared to the instrumental LOD.

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6. List of Publications

6.1. Articles

Determination of Pt, Pd and Rh in *Brassica Napus* using solid sampling electrothermal vaporization inductively coupled plasma optical emission spectrometry.

Winfried Nischkauer, Esther Herincs, Markus Puschenreiter, Walter Wenzel, Andreas Limbeck.

Spectrochimica Acta B, 89 (2013) 60 – 65.

http://dx.doi.org/10.1016/j.sab.2013.08.013

Radial line-scans as representative sampling strategy in dried-droplet laser ablation of liquid samples deposited on pre-cut filter paper disks.

Winfried Nischkauer, Frank Vanhaecke, Sébastien Bernacchi, Christoph Herwig, Andreas Limbeck

Spectrochimica Acta B, 101 (2014) 123-129.

http://dx.doi.org/10.1016/j.sab.2014.07.023

Recent advances in quantitative LA-ICP-MS analysis: challenges and solutions in the life sciences and environmental chemistry. Review Article.

Andreas Limbeck, Patrick Galler, Maximilian Bonta, Gerald Bauer, **Winfried Nischkauer**, Frank Vanhaecke.

Analytical and Bioanalytical Chemistry, 407 (2015) 6593 – 6617.

http://dx.doi.org/10.1007/s00216-015-8858-0

Extraction and pre-concentration of platinum and palladium from microwave-digested road dust via ion exchanging mesoporous silica microparticles prior to their quantification by quadrupole ICP-MS.

Winfried Nischkauer, Marie-Alexandra Neouze, Frank Vanhaecke, Andreas Limbeck.

Microchimica Acta, 182 (2015) 2369 – 2376.

http://dx.doi.org/10.1007/s00604-015-1643-0

Self-aliquoting micro-grooves in combination with laser ablation-ICP-mass spectrometry for the analysis of challenging liquids: quantification of lead in whole blood.

Winfried Nischkauer, Frank Vanhaecke, Andreas Limbeck.

Analytical and Bioanalytical Chemistry, 408 (2016) 5671 – 5676.

http://dx.doi.org/10.1007/s00216-016-9717-3

Improvements in the direct analysis of advanced materials using ICP-based measurement techniques. Review Article.

Andreas Limbeck, Maximilian Bonta, **Winfried Nischkauer**.

Revised manuscript is under review.

Bioparticles coated with ionic liquid for the pre-concentration of rare earth elements from microwave-digested tea samples and the subsequent quantification by ETV-ICP-OES.

Sara Hosseinzadegan, **Winfried Nischkauer**, Katharina Bica, Andreas Limbeck.

Revised manuscript is under review.

Combining dispersed particle extraction with dried droplet laser ablation ICP-mass spectrometry for determining platinum and palladium in airborne particulate matter.

Winfried Nischkauer, Andrei Izmer, Marie-Alexandra Neouze, Frank Vanhaecke, Andreas Limbeck.

Manuscript is submitted to Journal.

Quantification of iron in seawater by means of dispersed particle extraction using bioparticles coated with ionic liquid and detection by means of sectorfield ICP-MS. Working title.

Winfried Nischkauer, Katharina Bica, Frank Vanhaecke, Andreas Limbeck.

Manuscript is being prepared.

6.2. KEYNOTE LECTURE

Presenting author is marked in bold.

W. Nischkauer, F. Vanhaecke, A. Limbeck:
"Self-Aliquoting Micro-Array Plates in combination with Laser Ablation ICP-MS for the direct quantification of challenging liquid samples";
Euroanalysis XVIII, Bordeaux, France; 06.09.2015 - 10.09.2015; in: "Book of Abstracts", (2015), 1 S.

6.3. ORAL PRESENTATIONS AT CONFERENCES

W. Nischkauer, A. Limbeck, F. Vanhaecke:
„Self-aliquoting micro-array plates in combination with dried-droplet laser ablation for the quantification of trace elements in whole blood";
ESAS, Eger, Hungary, 31.3.2016 – 2.4.2016

W. Nischkauer, F. Vanhaecke, A. Limbeck:
Lead-quantification in blood via LA-ICP-MS
27th Mass Spec Forum, 23. – 24. February 2016, Vienna.

S. Hossein-Zadegan, W. Nischkauer, K. Bica, A. Limbeck:
"ETV-ICP-OES measurement of rare earth elements retained on natural bio-particles covered with ionic liquid";
Euroanalysis XVIII, Bordeaux, France; 06.09.2015 - 10.09.2015; in: "Book of Abstracts", (2015), 1 S.

W. Nischkauer, F. Vanhaecke, C. Herwig, **A. Limbeck**:
"Quantification of micro nutrients in fermentation media using LA-ICP-OES/MS analysis of dried sample droplets";
European Symposium on Atomic Spectrometry 2014, Prag, Czech Republic; 16.03.2014 - 21.03.2014; in: "Book of Abstracts", (2014), ISBN: 978-80-905704-1-2; 104 S.

W. Nischkauer, M. Bonta, A. Limbeck:
"Dried-droplet laser ablation in connection to ICP-MS and ICP-OES for the quantification of major and minor elements in cow milk";
2015 Euopean Winter Conference on Plasma Spectrochemistry, Münster, Germany; 22.02.2015 - 26.02.2015; in: "Book of Abstracts", (2015), 1 S.

M. Bonta, W. Nischkauer, A. Limbeck:
"Analysis of trace and bulk elements in biological samples using a split stream laser-ablation system with simultaneous ICP-OES and ICP-MS detection";
SCIX 2014, Reno, Nevada, US; 28.09.2014 - 03.10.2014; in: "Final Program Book of Abstracts", (2014), S. 68.

S. Hossein-Zadegan, W. Nischkauer, M.-A. Néouze, A. Limbeck:
"FI-ICP-OES Determination of Rare Earth Elements in Environmental Samples Using Dispersed Particle Extraction with Surface Functionalized Magnetic Nanoparticles";
5th EuCheMS Chemistry Congress, Istanbul, Turkey; 31.08.2014 - 04.09.2014; in: "5th EuCheMS Chemical Congress - Abstracts", (2014), 512 S.

S. Hossein-Zadegan, W. Nischkauer, M.-A. Néouze, A. Limbeck:
"Rare earth elements determination in urban dust samples by ICP-OES; enrichment by functionalized magnetic nanoparticles";
10. ASAC Junganalytikerinnenforum, Tulln; 13.06.2014 - 14.06.2014; in: "Book of Abstracts", (2014), S. 32.

M. Kistler, J. Ofner, J. Firmkranz, A Kadnar, E. Eitenberger, G. Friedbacher, J. Lohninger, W. Nischkauer, A. Limbeck, B. Lendl, A. Kasper-Giebl:
"Chemical and physical characterization of size resolved particles collected in a chemical engineering laboratory.";
3rd Workplace and Indoor Aerosol Conference AEROSOLS 2014, Wroclaw, Poland; 13.05.2014 - 16.05.2014; in: "AEROSOLS 2014", (2014), S. 27 - 28.

W. Nischkauer, A. Izmer, M.-A. Néouze, F. Vanhaecke, A. Limbeck:
"Determination of Platinum group elements (Pt, Pd, Rh) in urban PM10 samples via dispersed particle extraction followed by dried-droplet Laser-Abaltion ICP-MS";
2014 Winter Conference on Plasma Spectrochemistry, Amelia Island, Florida, US; 06.01.2014 - 11.01.2014; in: "2014 Winter Conference Program and Abstracts", (2014), ISSN: 0161-6951; 360 S.

W. Nischkauer, M.-A. Néouze, A. Limbeck:
"Dispersed particle extraction - unkomplizierte Abtrennung von störender Matrix für die verbesserte quantitative Analyse von Pt und Pd in Straßenstaub";
14. Edelmetallforum, Ulm, Germany; 07.04.2014 - 08.04.2014; in: "Book of Abstracts", (2014), S. 6.

W. Nischkauer, F. Vanhaecke, M.-A. Néouze, A. Limbeck:
"Quantitative Bestimmung von Pt und Pd in urbanem Feinstaub mittels Dispersed Particle Extraction gefolgt von Dried-Droplet Laser Ablation ICP-MS";
24. ICP-MS Anwendertreffen, Geesthacht, Germany; 15.09.2014 - 18.09.2014; in: "Abstractband", (2014), S. 29.

W. Nischkauer, A. Tchaikovsky, R. Janski, M.-A. Néouze, A. Limbeck:
"Dispersed particle extraction - a novel approach for analyte enrichment and matrix removal";
European Winter Conference on Plasma Spectrochemistry 2013, Krakow; 10.02.2013 - 15.02.2013; in: "European Winter Conference on Plasma Spectrochemistry", (2013).

W. Nischkauer, M.-A. Néouze, C. Puls, A. Limbeck:
"Platinum Group Elements in Urban Roadside Dust: Analyte Enrichment and Matrix Removal via Dispersed Particle Extraction";
10. Symposium Massenspektrometrische Verfahren Elementspurenanalyse, Tulln, Austria; 10.09.2012 - 12.09.2012; in: "ABSTRACTBAND", (2012), S. 29.

W. Nischkauer, C. Puls, M.-A. Néouze, A. Limbeck:
"Platinum Group Elements In Roadside Dust - Enrichment And Matrix Removal By Means Of Functionalized Nano-Particles";
ISEAC 37 (37th International Symposium on Environmental Analytical Chemistry, Antwerp, Belgium; 22.05.2012 - 25.05.2012; in: "Book of Abstracts", (2012), S. 99.

W. Nischkauer, M.-A. Néouze, A. Limbeck:
"Enrichment Of Palladium From Aqueous Sample Solutions Using Functionalized Nano- Particles";
Euroanalysis 16, Belgrad; 11.09.2011 - 15.09.2011; in: "Compendium of all Abstracts", (2011), S. 129.

C. Puls, E. Herincs, W. Nischkauer, A. Limbeck:
"Bioavailability of anthropogenic Platinum group emissions";
9th Chemistry conference Plovdiv, Plovdiv, Bulgaria (eingeladen); 14.10.2011 - 16.10.2011; in: "Book of Abstracts", (2011), S. 13.

6.4. POSTER PRESENTATIONS AT CONFERENCES

Rovelli S., A. Limbeck, W. Nischkauer, M. Bonta, F. Borghi, A. Cattaneo and D. M. Cavallo;
"Characterization of toxic trace metals in size-segregated fine and ultrafine particles within an urban environment";
DUST 2016 – 2nd International Conference on atmospheric dust. Castellaneta Marina, Italy, June 12-17, 2016

Hossein-Zadegan S., Nischkauer W., Bica K. and A. Limbeck.
Quantification of Rare Earth Elements Retained on Natural Bio-Particles Covered with Ionic Liquid in Combination with ETV-ICP-OES. Eger, Hungary, March 31st to April 2nd, 2016.

Nischkauer W., **Bonta M.**, Vanhaecke F. and A. Limbeck.
Quantifying Challenging Liquid Samples Using Self-Aliquoting Micro-Array Plates and Dried-Droplet Laser Ablation. 2016 Winter conference on plasma spectroscopy, Tuscon, Arizona, US, January 11-16, 2016.

W. Nischkauer, F. Berzsenyi, K. Bica, F. Vanhaecke, A. Limbeck:
"Selective extraction of iron in the presence of zinc and copper using customized ionic liquids: feasibility and application to digested whole blood";
2015 Euopean Winter Conference on Plasma Spectrochemistry, Münster, Germany; 22.02.2015 - 26.02.2015; in: "Book of Abstracts", (2015), 1 S.

W. Nischkauer, K. Bica, F. Vanhaecke, A. Limbeck:
"Analyte-pre-concentration using a customized dispersed-particle extraction protocol for the improved quantification of iron in seawater via ICP-OES";
ANAKON 2015, Graz; 23.03.2015 - 26.03.2015; in: "Book of Abstracts", (2015), S. 234.

W. Nischkauer, K. Bica, F. Vanhaecke, A. Limbeck:
"Dispersed Particle Extraction For The Straight-Forward Quantification Of Iron In Sea Water";
Euroanalysis XVIII, Bordeaux, France; 06.09.2015 - 10.09.2015; in: "Book of Abstracts", (2015), 1 S.

S. Hossein-Zadegan, W. Nischkauer, K. Bica, A. Limbeck:
"Dispersed Particle Extraction using ionic liquid-coated magnetic nanoparticles in combination with a FI-ICP-OES procedure for sensitive analysis of REE";
Euroanalysis XVIII, Bordeaux, France; 06.09.2015 - 10.09.2015; in: "Book of Abstracts", (2015), 1 S.

S. Hossein-Zadegan, W. Nischkauer, M.-A. Néouze, A. Limbeck:
"Dispersed particle extraction using magnetic nano-particles for the quantification of rare earth elements from road-dust digests";
European Symposium on Atomic Spectrometry 2014, Prag, Czech Republic; 16.03.2014 - 21.03.2014; in: "Book of Abstracts", (2014), ISBN: 978-80-905704-1-2; 193 S.

S. Hossein-Zadegan, W. Nischkauer, M.-A. Néouze, A. Limbeck:
"Improved Analysis of Trace Elements in Environmental Samples using Sulfonated Polystyrene Microparticles in Combination with ETV-ICP-OES Analysis";
5th EuCheMS Chemistry Congress, Istanbul, Turkey; 31.08.2014 - 04.09.2014; in: "5th EuCheMS Chemical Congress - Abstracts", (2014), 559 S.

W. Nischkauer, C. Herwig, F. Vanhaecke, A. Limbeck:
"Dried-droplet Laser-Ablation in combination with ICP-OES and ICP-MS for the determination of major and minor elements in heavily matrix-loaded fermentation media";
2014 Winter Conference on Plasma Spectrochemistry, Amelia Island, Florida, US; 06.01.2014 - 11.01.2014; in: "2014 Winter Conference Program and Abstracts", (2014), ISSN: 0161-6951; 145 S.

W. Nischkauer, F. Vanhaecke, J. Lohninger, A. Limbeck:
"Analysis of liquid foodstuffs and beverages by means of dried droplet laser ablation icp oes";
European Winter Conference on Plasma Spectrochemistry 2013, Krakow; 10.02.2013 - 15.02.2013; in: "European Winter Conference on Plasma Spectrochemistry", (2013).

W. Nischkauer, E. Herincs, A. Limbeck:
"Development Of An Electro-Thermal Vaporisation Inducitively Coupled Plasma Atomic Emission Spectroscopy - Procedure For The Determination Of Platinum Group Elements In Plants";
Euroanalysis 16, Belgrad; 11.09.2011 - 15.09.2011; in: "Compendium of all Abstracts", (2011), S. 504.

7. Curriculum Vitae

Personal data

Name	Winfried NISCHKAUER
Date of birth	January 26th 1986
Nationality	Austrian
E- Mail	winfried.nischkauer@tuwien.ac.at winfried.n@gmx.at
Phone (mobile)	+43 664 784 01 57

Education

Since 03/2012	PhD-studies at Vienna University of Technology (TU Wien, Austria) and Ghent University (UGent, Belgium). Promotor: Andreas LIMBECK (TU Wien), Co-promotor: Frank VANHAECKE (UGent). The doctorate is carried out in the framework of a Joint PhD agreement, the research is financed by the Austrian Science Fund (FWF) and UGent (BOF grant).
10/ 2005 – 11/2011	Diploma studies at TU Wien with a specialization in „Materials Technology and Analytics“, Diploma thesis „Development of advanced methods for the determination of Platinum Group Elements in plant material“ (supervised by A. Limbeck und M. Néouze), Final academic degree: Dipl.-Ing. (equivalent to a MSc.), „with distinction“.
06/ 2004	general qualification for university entrance („with distinction“).

Work experience

since 03/ 2013	PhD at the Institute of Chemical Technologies and Analytics (CTA), TU Wien.
03/ 2012-02/2013	Project-assistant at the CTA, TU Wien.
07/ 2011- 02/2012	Tutor in the “Quantitative Analytical Practicum“, CTA, TU Wien.
10/ 2010- 01/ 2011	Tutor in the “Quantitative Analytical Practicum“, CTA, TU Wien.
07/ 2010 – 08/2010	PLANSEE SE, 6600 Reutte, Austria: qualitative and quantitative analysis of refractory metals.
02/ 2010	Institute of applied synthesis chemistry, TU Wien, department of Macromolecular Chemistry: synthesis of ionic liquids.
09/ 2008	OMV, 2320 Schwechat, Austria: quality control and analysis of fuels.

Languages

German:	mother tongue
English:	fluently spoken and written
French:	fluently spoken and written (DELF, Level B2)
Other:	Romanian (A1.2), Dutch (basic knowledge), Italian (basic knowledge)

Other

Since 2006	lecturer at the „young magicians club“, Magischer Klub Wien.
Since 2005	regular member of the „Stadtkapelle Purkersdorf“, marching music band (clarinet)
Since 2004	board member of the Viennese Magic Klub („Magischer Klub Wien”, 2004-2008: 1st treasurer, since 2009: vice-treasurer)

Vienna, 26th of September 2016 signature (Winfried Nischkauer)