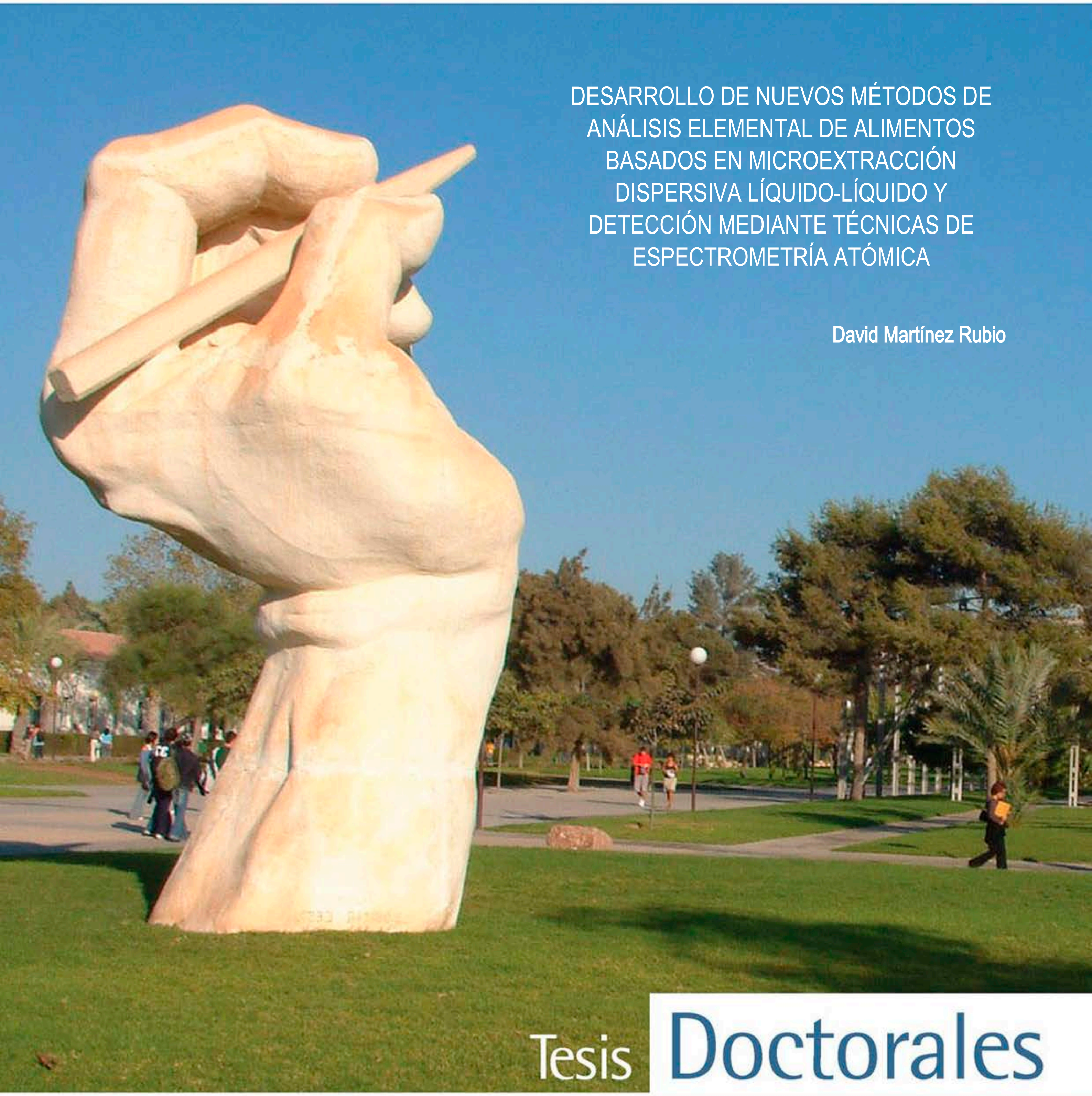

DESARROLLO DE NUEVOS MÉTODOS DE ANÁLISIS ELEMENTAL DE ALIMENTOS BASADOS EN MICROEXTRACCIÓN DISPERSIVA LÍQUIDO-LÍQUIDO Y DETECCIÓN MEDIANTE TÉCNICAS DE ESPECTROMETRÍA ATÓMICA

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David Martínez Rubio

Tesis presentada para la obtención de DOCTOR por la Universidad de Alicante con MENCIÓN DE DOCTOR INTERNACIONAL

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Dirigida por:

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CERTIFICAN QUE:

D. David Martínez Rubio, ha realizado bajo nuestra dirección, el trabajo que lleva por título:

"*Desarrollo de nuevos métodos de análisis elemental de alimentos basados en Microextracción Dispersiva Líquido-Líquido y detección mediante técnicas de Espectrometría Atómica*", que constituye su memoria para aspirar al Grado de Doctor, reuniendo, a nuestro juicio, las condiciones necesarias para ser presentado y defendido ante el tribunal correspondiente.

Y para que conste a los efectos oportunos, en cumplimiento de la legislación vigente, firmamos el presente certificado en Alicante a marzo de 2019.

Fdo. Dr. Juan Mora Pastor **Fdo. Dr. Guillermo Grindlay Lledó**

Analytical Atomic
Spectrometry Group

AGRADECIMIENTOS

Al terminar el Máster de Ciencia y Tecnología de los Alimentos, sólo tenía claro una cosa: quería dedicarme a la investigación y hacer la Tesis Doctoral. Al principio tuve muchas dudas ya que mi formación en Química era mínima. No obstante, con perseverancia, y con la ayuda de mis Directores de Tesis, he mejorado mis conocimientos y habilidades en Química. Gracias a mis dos directores, Juan y Guillermo, por vuestro apoyo e infinita paciencia. Mi agradecimiento también va dirigido a mis compañeros en el Grupo de Espectrometría Atómica Analítica por su ayuda, consejos y buenos momentos juntos. Finalmente, me gustaría agradecer a todos los miembros del Departamento de Química Analítica, Nutrición y Bromatología, tanto PDI como personal de administración, así como los técnicos por la amabilidad y los buenos momentos vividos durante esta etapa.

No todo ha sido fácil. A lo largo de mi doctorado, he sufrido varias operaciones que supusieron grandes cambios en mi vida y de los que, todavía, no estoy completamente recuperado. A lo largo de estas vicisitudes, mi familia me acompañó en todo momento. Por ello, me gustaría dar las gracias a mis hermanos y hermana, mis cuñadas y sobrinos/as. A mi madre Asunción y a mi padre José. Desde el principio mi madre se ha volcado en mí y me ha dado todo su cariño. Me siento muy orgulloso de ella. También me gustaría dar las gracias a mi abuela Asunción y mi abuelo Antonio que ojalá todavía estuvieran aquí para vivir este momento. Siempre les tendré en mi corazón. Por último, me gustaría dar las gracias a todas mis mascotas que me acompañan a todos sitios y forman una parte muy importante de mi vida.

A pesar de mis problemas de salud, decidí realizar una estancia en el extranjero y, más concretamente, en la Universidad Nacional de Cuyo (Argentina). Mi estancia allí fue muy satisfactoria tanto a nivel investigador como personal. Mi agradecimiento infinito al profesor Rodolfo Wuilloud y a todos los miembros del QUIANID por su ayuda y afecto durante mi estancia. En estos momentos finales de la Tesis, estoy muy contento por haber conseguido superar todos los obstáculos que han surgido estos años. Cierro este apartado con una frase de un gran genio:

“La mayoría de la gente dice que el intelecto es lo que hace a un gran científico. Están equivocados: es el carácter” (Albert Einstein).

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GLOSARIO DE TÉRMINOS Y ACRÓNIMOS

Termino	Descripción
4-NOPD	1,2-diamino-4-nitrobenceno
5-Br-PADAP	2-(5-bromo-2-pyridylazo)-5-dietillaminofenol
8-HQ	8-hidroxiquinoleina
APDC	Pirrilidinditiocarbamato de amonio
BmimPF$_6$	1-butil-3-metil-imidazolium hexafluorofosfato
C$_6$mim PF$_6$	1-hexil-3-metIllimidazolium hexafluorofosfato
C$_8$mimPF$_6$	1-octil-3-metIllimidazolium hexafluorofosfato
DDPA	Dietillditiofosfato
DDTC	Dietilditiocarbamato
DLLME	Microextracción Dispersiva Líquido Líquido
DMDC	Dimetil ditiocarbamato
ETAAS	Espectroscopía de Absorción Atómica Electrotérmica
ETV	Vaporización Electrotérmica
ETV-ICP-MS	Espectroscopía de Masas con Plasma de Acoplamiento Inductivo con Vaporización Electrotérmica
FAAS	Espectroscopía de Absorción Atómica en Llama
FIA	Análisis de Inyección en Flujo
HGAAS	Espectroscopía de Absorción Atómica por Generación de Hidruros
Hmim-Tf$_2$N	1-hexil-3-metIllimidazolium bis(trifluorometilsulfonil) imida
HPyPF$_6$	1-hexilpiridinio hexafluorofosfato
ICP	Plasma Acoplado Inductivamente
ICP-MS	Espectroscopía de Masas con Plasma Acoplado Inductivamente
ICP-AES	Espectroscopía de Emisión Atómica con Plasma Acoplado Inductivamente
IL	Líquido Iónico
KR	Reactor Anudado
LA	Ablación Laser
LoD	Límite de Detección
LLE	Extracción Líquido Líquido
MS	Espectrometría de Masas
OVAC	N-O-vanillidina-2-amino-p-cresol
PAN	1-(2-Piridilazo)-2-naftol

SPE	Extracción en Fase Sólida
SUPRAS	Disolventes Supramoleculares
TMK	4,4′-bis(dimetilamino)tiobenzofenona
TTA	Teoniltrifluoroacetona

RESUMEN/ABSTRACT

RESUMEN

Con objeto de garantizar la calidad de los alimentos tanto desde el punto de vista nutricional como toxicológico, existen diferentes normativas a nivel internacional para controlar el contenido de metales y metaloides en los alimentos. Este tipo de determinaciones se suele llevar a cabo mediante técnicas de Espectrometría Atómica por su sencillez y parámetros analíticos (p.ej. límite de detección, precisión, velocidad, etc.). No obstante, debido a la complejidad que presentan las matrices de los alimentos (i.e. grasas, proteínas, etc.) y a los bajos niveles de concentración de algunos elementos, se requiere de un tratamiento de extracción-preconcentración previo. Con este fin, algunos autores han utilizado la Microextracción Dispersiva Líquido-Líquido (DLLME). Sin embargo, el uso combinado de DLLME y técnicas de Espectrometría Atómica para la determinación de metales muestras de alimentos de matriz compleja (e.g., chocolate, vinos, etc.) apenas se ha abordado. Ello es debido que el acoplamiento DLLME a este tipo de técnicas sigue presentado serias limitaciones: (i) empleo de disolventes extractantes no adecuados a los principios de la Química Verde; (ii) escaso aprovechamiento del acoplamiento DLLME a Espectrometría de Emisión mediante Plasma de Acoplamiento Inductivo (ICP-AES) por los efectos de matriz originados por los extractantes orgánicos en el plasma; y, (iii) falta de criterio para seleccionar las condiciones de trabajo óptimas en ICP-AES para trabajar con extractos de DLLME.

En la presente Tesis Doctoral se han desarrollado nuevos métodos de análisis elemental en alimentos mediante el uso combinado de DLLME y técnicas de Espectroscopía Atómica. En primer lugar, se ha investigado la posibilidad de utilizar el líquido iónico hexafluorofosfato de 1-butil-3-metilimidazolio como medio de extracción en DLLME para la determinación de Cd y Pb en muestras de vino mediante Espectrometría de Absorción Atómica con Horno de Grafito. Los resultados demuestran que esta metodología se puede utilizar para controlar los metales anteriores en todo tipo de muestras de vino (tinto, rosado, blanco, espumoso y fortificado) según la normativa de la Organización Internacional de la Viña y el Viñedo (OIV). Por otro lado, se han investigado diferentes estrategias para analizar los extractos orgánicos (1-undecanol, disolventes supramoleculares, cloroformo) que se obtienen en DLLME mediante ICP-AES y así aprovechar la capacidad que ofrece esta técnica para determinar varios elementos de forma simultánea. Esta metodología se ha aplicado con éxito a la determinación de 6 elementos (Al, Cu, Fe, Mn, Ni y Zn) en aguas. Finalmente, se ha evaluado la posibilidad de acoplar DLLME e ICP-AES para la determinación simultanea de As, Cd y Pb en muestras de arroz, chocolate, mejillones y vino. utilizando como extractante un disolvente supramolecular (THF/1-undecanol). Los resultados obtenidos demuestran que la metodología desarrollada permite el control de dichos elementos según el Reglamento 1881/2006 de la Comisión.

ABSTRACT

Different international policies have been established to control the maximum metals and metalloids concentration levels in foods. Elemental analysis in food samples is usually carried out by means of Atomic Spectrometry techniques due to their simplicity and good analytical figures of merit (i.e., limit of detection, precision, sample throughput, etc). Nevertheless, a preliminary extraction-preconcentration step is sometimes required to mitigate matrix effects due to main food components (proteins, lipids, etc.) as well as to improve detection capabilities for some elements. To this end, dispersive liquid-liquid microextraction (DLLME) has been successfully reported in the literature. However, very few applications coupling DLLME to Atomic Spectrometry techniques for metal analysis in highly complex food samples (e.g., chocolate, wine, etc.) have been reported. Coupling DLLME to Atomic Spectrometry techniques still suffers from several limitations: (i) most of the solvents employed for metal extraction are not environmentally friendly; (ii) strong matrix effects originated by organics in the plasma makes difficult the use DLLME-ICP-AES coupling for simultaneous multielement analysis; and, (iii) lack of criteria for selecting the appropriate plasma operating conditions to operate with DLLME in ICP-AES. Consequently, very little applications for metal analysis in food samples with highly complex matrices (chocolate, wine, etc.)

In this work, new methodologies based on DLLME and atomic spectrometry detection have been developed for elemental analysis in food samples. First, the potential using 1-butyl-3-metyl-imizadolium hexafluorophosphate as extractant in DLLME for Cd and Pb determination in wine by means of electrothermal atomic absorption spectrometry has been evaluated. Results show that this methodology can be successfully applied for Cd and Pb determination in different types of wine (red, rosé, white, sparkling and fortified) according to the levels internationally established. Second, in order to benefit from simultaneous multielement capabilities afforded by ICP-AES, different analytical approaches have been explored and evaluated for analyzing DLLME extracts (1-undecanol, BmimPF$_6$ and chloroform). It has been observed that organic extracts can be directly introduced into the ICP-AES by means of flow injection analysis. Nevertheless, when operating highly viscous solvents, a preliminary dilution step with an appropriate organic solvent is mandatory. This strategy has been applied for the determination of six elements (Al, Cu, Fe, Mn, Ni y Zn) in water samples. Finally, a new methodology combining DLLME and ICP-AES has been developed for the simultaneous determination of As, Cd and Pb in several foods (chocolate, mussels, rice and wine) using a supramolecular solvent based on THF and 1-undecanol. This methodology allows As, Cd and Pb control in foods according to the Commission Regulation Nº 1881/2006 1881/2006.

OBJETIVOS Y ESTRUCTURA GENERAL DE LA TESIS DOCTORAL

1. Objetivos.

El objetivo principal de la presente Tesis Doctoral es el desarrollo de nuevos métodos analíticos de análisis elemental en alimentos mediante el uso combinado de la Microextracción Dispersiva Líquido-líquido (DLLME) y técnicas Espectroscópicas Atómicas. Con ello se pretende resolver algunas de las limitaciones que presentan este tipo de estrategias analíticas, entre otras: (i) empleo de disolventes extractantes no adecuados según los principios de la Química Verde; (ii) escaso aprovechamiento del acoplamiento de DLLME y la Espectrometría de Emisión mediante Plasma de Acoplamiento Inductivo (ICP-AES) por los efectos de matriz que producen los extractantes orgánicos en el plasma; (iii) falta de criterio para seleccionar las condiciones de trabajo óptimas en ICP-AES para trabajar con extractos de DLLME; y (iv) aplicación de DLLME a muestras de alimentos complejas (p.ej. vino, chocolate, etc.). Para ello, se plantean los siguientes objetivos específicos.

Objetivo específico 1: Desarrollo de una nueva metodología para la determinación de Cd y Pb en muestras de vino mediante DLLME y Espectrometría de Absorción Atómica con Horno de Grafito (ETAAS) utilizando hexafluorofosfato de 1-butil-3-metilimidazolio como medio de extracción.

Objetivo específico 2: Desarrollo y evaluación de diferentes estrategias para analizar los extractos obtenidos en DLLME mediante ICP-AES de forma rápida y sencilla.

Objetivo específico 3: Desarrollo de una nueva metodología para la determinación de As, Cd y Pb en alimentos (arroz, chocolate, mejillones y vino) mediante DLLME e ICP-AES utilizando disolventes supramoleculares como medio de extracción.

2. Estructura general del trabajo.

La presente Tesis Doctoral ha sido desarrollada en el Grupo de Investigación Espectroscopía Atómica Analítica del Departamento de Química Analítica, Nutrición y Bromatología de la Universidad de Alicante. Además, parte de la investigación ha sido desarrollada en colaboración con el Grupo de Investigación del Laboratorio de Química Analítica para Investigación y

Desarrollo (QUIANID) de la Universidad Nacional de Cuyo (Argentina) bajo la supervisión del Dr. Rodolfo G. Wuilloud. La Tesis Doctoral se estructura en cinco capítulos:

Capítulo 1. Introducción general.

Este capítulo introduce los conceptos básicos que son necesarios para entender las motivaciones que ha llevado a la realización de este trabajo. En primer lugar, se describe la importancia que tiene en la actualidad el análisis de metales y metaloides en alimentos. A continuación, se hace una breve revisión de las estrategias analíticas más relevantes para determinar estas especies, sobre todo las que se basan en el uso combinado de DLLME y técnicas de Espectrometría Atómica. Finalmente, se describen las limitaciones actuales de este tipo de estrategias a nivel práctico (p.ej. extractantes utilizados, sistemas de introducción de muestras, etc.)

Capítulo 2. Determination of cadmium and lead in wine samples by means of dispersive liquid-liquid microextraction coupled to electrothermal atomic absorption spectrometry.

En este trabajo se ha desarrollado un nuevo método para la determinación de Cd y Pb en muestras de vino. La determinación de estos elementos se realiza mediante ETAAS tras una etapa previa de extracción y preconcentración con DLLME y utilizando hexafluorofosfato de 1-butil-3-metilimidazolio ($BmimPF_6$) como medio de extracción. Los resultados demuestran que los Límites de Detección (LoD) obtenidos mediante esta estrategia para Cd (0.01 $\mu g\ L^{-1}$) y Pb (0.08 $\mu g\ L^{-1}$) son inferiores a los descritos con anterioridad en la bibliografía para ETAAS utilizando otro tipo de tratamientos de muestra (e.g. digestión ácida, análisis directo, etc.). No obstante, se ha observado que la determinación de Cd depende de forma significativa de los niveles de Zn en las muestras de vino ya que este concomitante compite con el Cd por el agente quelante. La metodología desarrollada se ha aplicado con éxito a diferentes tipos de vinos (p.ej. blanco, rosado, tinto, espumoso y fortificado) y permite el control de Cd y Pb según los niveles establecidos por la Organización Internacional de la Viña y el Viñedo.

Los resultados de este trabajo han dado lugar a las siguientes publicaciones (Apéndice):

Publicaciones:

1. Martínez D, Grindlay G, Gras L, Mora J. Determination of cadmium and lead in wine samples by means of dispersive liquid–liquid microextraction coupled to electrothermal atomic absorption spectrometry. J Food Compos Anal 2018; 67:178.

Póster:

2. Martínez D, Grindlay G, Gras L, Mora J. Determination of lead in wine samples by means of dispersive liquid liquid microextraction and graphite furnace atomic absortion. XXIV Reunión nacional de espectroscopia- VIII Congreso iberico de espectroscopia, celebrada en Logroño del 9 al 11 de julio de 2014.
3. Pérez E, Martínez D, Grindlay G, Gras L, Mora J. Determination of lead and cadmium in wine samples by means of dispersive microextraction and graphite furnace atomic absortion spectrometry. Euroanalysis 2015 Conference. Bordeaux (France), 6th-10th September 2015.

Capítulo 3. Coupling dispersive liquid-liquid microextraction to inductively coupled plasma atomic emission spectrometry: an oxymoron?

En este trabajo, se han evaluado diferentes estrategias analíticas para analizar los extractantes que se emplean de forma habitual en DLLME (p.ej. cloroformo, 1-undecanol y BmimPF$_6$) mediante ICP-AES. Los resultados demuestran que, mediante la selección adecuada del sistema de introducción de muestras (Sistema de Inyección en Flujo) y de las condiciones experimentales del plasma, se pueden analizar los extractos de DLLME de forma rápida y sencilla. Si se trabaja con un disolvente orgánico volátil (cloroformo), los extractos se pueden introducir de forma directa en el plasma mientras que para disolventes muy viscosos (1-undecanol y $BmimPF_6$) es necesaria una dilución previa. Las estrategias desarrolladas se han aplicado con éxito a la determinación simultánea de varios elementos (Al, Cu, Fe, Mn, Ni y Zn) en muestras de agua.

Los resultados de este trabajo han dado lugar a las siguientes publicaciones (Apéndice):

Publicaciones:

1. Martínez D, Torregrosa D, Grindlay G, Gras L, Mora J. Coupling dispersive liquid-liquid microextraction to inductively coupled plasma atomic emission spectrometry: An oxymoron? Talanta 2018;176:374.

Comunicaciones orales:

1. Grindlay G, Martínez D, Gras L, Mora J. Dispersive liquid-liquid microextraction with inductively coupled plasma atomic emission spectrometry: an oxymoron? 2^{nd} Caparica Christmas Conference on Sample Treatment held in capuchos-Portugal, the 5-7th, December of 2016.

Póster:

1. Pérez E, Martínez D, Torregrosa D, Grindlay G, Gras L, Mora J. Coupling dispersive liquid liquid microextraction with inductively coupled plasma techniques. EWCPS 2017 European Winter Conference on Plasma Spectrochemistry 2017. Sankt Anton (Austria), 19-24th February 2017.

Capítulo 4. Simultaneous determination of As, Cd and Pb in food samples by means of inductively coupled plasma atomic emission spectrometry: a comparison between knotted-reactor extraction and dispersive liquid-liquid microextraction.

En este trabajo, se ha desarrollado un nuevo método para la determinación de As, Cd y Pb en diferentes tipos de alimentos (arroz, chocolate, mejillones y vino). La determinación de estos elementos se ha realizado mediante ICP-AES tras una etapa previa de extracción y preconcentración con DLLME y utilizando como medio de extracción un disolvente supramolecular basado en THF y 1-undecanol. Los resultados muestran que se puede utilizar de forma combinada DLLME e ICP-AES para el control de As, Cd y Pb en arroz, chocolate, mejillones y vino según el Reglamento 1881/2006 de la Comisión. Esta estrategia es mucho más atractiva que los reactores anudados como sistema de extracción preconcentración ya que presenta una mayor velocidad de análisis y mejores LoD (2-3 veces). El principal inconveniente del reactor anudado es su baja eficiencia de extracción para As que lleva a la imposibilidad de controlar este metaloide en alimentos.

Capítulo 5. Conclusiones generales.

Aquí se presentan las conclusiones más importantes obtenidas a lo largo de todos los trabajos de investigación realizados.

Apéndice.

En esta sección se recogen todas las contribuciones científicas realizadas a lo largo del desarrollo de la presente Tesis Doctoral.

CAPÍTULO 1

INTRODUCCIÓN GENERAL

1.1. Elementos tóxicos en la industria alimentaria.

En la sociedad actual existe una gran preocupación por la Seguridad Alimentaria y el efecto de la alimentación sobre la salud. Una alimentación de calidad debe proporcionar todos los nutrientes que necesita el organismo para su correcto funcionamiento, pero, además, los alimentos han de ser inocuos y no contener ningún tóxico que suponga un perjuicio para el individuo. Esta preocupación, ha llevado a diferentes organismos internacionales y autoridades gubernamentales a establecer normativas muy estrictas dirigidas a garantizar la calidad de los alimentos tanto desde el punto de vista nutricional como del toxicológico [1]. Así, por ejemplo, el Reglamento (CEE) nº 315/93 del Consejo [2], regula los contaminantes en alimentos de forma genérica. En esta normativa, se plantean tres líneas principales de actuación: (i) mercado interior. Se prohíbe la comercialización de productos alimenticios que contengan tóxicos en proporciones inaceptables respecto de la salud pública y en particular desde el punto de vista toxicológico; (ii) buenas prácticas de fabricación. Los tóxicos deberán mantenerse al mínimo nivel posible mediante prácticas correctas en todas las fases de la cadena alimentaria, desde producción hasta el consumo; y (iii) salud pública. A fin de proteger la salud pública, se establecerán los límites máximos cuya tolerancia pudiese resultar necesaria por lo que respecta a determinados contaminantes. No obstante, pese a los avances logrados, los incidentes de Seguridad Alimentaria pueden ocurrir como, por ejemplo, los escándalos de melamina en China [3] y la carne de caballo en Europa [4].

Las especies tóxicas presentes en los alimentos suelen tener un origen antrópico (p.ej. contaminación ambiental, adulteraciones, etc.) pero, en ocasiones, estos también pueden encontrarse de forma natural en el propio alimento como consecuencia del metabolismo de ciertos organismos vivos presentes en él (p.ej. micotoxinas). Desde un punto de vista químico, los tóxicos se pueden clasificar en: (i) orgánicos (p.ej. melanina, dioxinas); (ii) inorgánicos (p.ej. Pb, Cd, etc.); y (ii) organometálicos, compuestos orgánicos que contienen un metal o metaloide en su estructura (p.ej. metilmercurio, tetrabutilestaño etc.).

Dentro del grupo de los tóxicos inorgánicos, destacan los metales y los metaloides (p.ej. As, Cd, Cr, Hg, etc.) [5]. Estas especies, aunque pueden tener un origen natural, llegan a los alimentos principalmente a través de la agricultura y de la industria y tienen efectos nocivos sobre la biota y los humanos [6,7][a]. El Comité permanente de la Cadena Alimentaria y Sanidad Animal conjuntamente con la Autoridad Europea de Seguridad Alimentaria (EFSA) establecen los límites máximos tolerados de metales tóxicos en alimentos. El Reglamento 1881/2006**,** de 19 de Diciembre de 2006 [8], de la Comisión Europea, fija el contenido máximo de As, Cd, Pb, Hg y Sn en los productos alimenticios. Recientemente, se han realizado algunas modificaciones en dicho Reglamento para algunos elementos (Cd [9], Pb [10] y As [11]) que, básicamente, añaden más alimentos a los que hay presentes en la normativa original. La Tabla 1.1. muestra los niveles máximos permitidos de As, Cd, Hg, Pb y Sn en función de los límites establecidos por las normativas vigentes en la Unión Europea. Como se puede observar, las fuentes principales para cada elemento tóxico, en general, se encuentran repartidas en distintos alimentos salvo para el caso del Sn que se encuentra específicamente en alimentos enlatados y el Hg que se encuentra en productos de origen marino. Los límites permitidos según el Reglamento [8], varían de un elemento a otro en función de su toxicidad e ingesta estimada total. Se observa que los niveles más restrictivos son para Cd y Pb seguido de As y Hg. Los niveles permitidos de Sn son más elevados (50mg/Kg de peso fresco) ya que se elimina del organismo con mucha más facilidad [12].

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[a] En este trabajo se utilizará el término “metal” de forma genérica para englobar tanto a los metales de transición como a los metaloides.

Tabla 1.1. Límite máximos permitidos de As, Cd, Sn, Pb y Hg en alimentos según establece la Unión Europea (Reglamento 1881/2006 [8], Reglamento 488/2015[9], Reglamento 2015/1005 [10] y Reglamento 2015/1006 [11]) y los efectos de dichos elementos sobre la salud.

Analito	**Alimentos**	**Efecto sobre salud**	**Límites máximos (mg/kg de peso fresco)**
As	Pescados, mariscos, carnes, aves de corral, productos lácteos y cereales. Agua contaminada y alimentos preparados con agua contaminada	Problemas relacionados con el desarrollo, neurotóxico, diabetes, enfermedades pulmonares y cardiovasculares (infarto de miocardio), efectos adversos en el embarazo y carcinógeno [13]	0,1-3
Cd	Moluscos, crustáceos, bivalvos, cefalópodos, vísceras, carnes, huevo, zanahoria, pescado, cacahuetes, soja, semillas de girasol, patatas, cereales y espinacas	Cáncer, afecta a los riñones, huesos y pulmones (edema pulmonar). Alteración de enzimas hepáticas, modificaciones cromosómicas, diarreas, dolor de estómago, vómitos severos, debilitamiento óseo, fallos en la reproducción y posibilidad incluso de infertilidad, daño al sistema nervioso central, al sistema inmunológico y desórdenes psicológicos [14]	0,05-1
Hg	Productos del mar	Dermatitis, rinitis, conjuntivitis, cáncer, hipersensibilidad, mercurialismo, insuficiencia renal. Síndrome digestivo: sabor metálico, mal aliento, náuseas y diarrea [15,16]	0,5-1
Pb	Pan, sopas, cereales, verduras, frutas, agua potable, bebidas, carne, azúcares y productros de confitería	Saturnismo, fatiga, disturbios al dormir, dolor de cabeza, irritabilidad, tartamudeo y convulsiones. También puede producir debilidad muscular, ataxia, mareos y parálisis, asimismo, la habilidad visual, el tacto fino y la noción del tiempo se pueden ver alterados, presentándose cuadros de ansiedad, alterándose el humor y la habilidad cognitiva, nefropatía, anemia, daño hepático, alteraciones genéticas y cáncer [17,18]	0,02-1,5
Sn	Alimentos enlatados	De manera general no causan efectos perjudiciales debido a que entran y abandonan el cuerpo rápidamente [12]	50-200

1.2. Análisis de metales tóxicos en alimentos.

Las técnicas que se emplean para determinar metales en alimentos son diversas y cabe destacar: (i) técnicas electroquímicas [19,20]; (ii) técnicas cromatográficas [21] ; y (iii) técnicas espectroscópicas [22]. Dentro de este último conjunto, las más empleadas son las de Espectrometría Atómica por su sencillez y parámetros analíticos (p.ej. selectividad, sensibilidad, precisión, exactitud, capacidad multielemental, etc.) (Tabla 1.2).

Tabla 1.2. Parámetros analíticos de diversas técnicas de Espectrometría Atómica.

Parámetro	FAAS	HGAAS	ETAAS	ICP-AES	ICP-MS
Límite de detección (LoD)	mg L^{-1}	mg L^{-1}	µg l^{-1}	mg L^{-1}- µg l^{-1}	ng L^{-1}-pg L^{-1}
Rapidez	Baja	Baja	Muy baja	Muy elevada	Elevada
Intervalo dinámico	10^3	10^3	10^2	10^5	10^5
Precisión	0,1-1%	0,4-7%	1-5%	0,3-2%	1-3%
Análisis isotópico	No	No	No	No	Sí
Coste	Bajo	Bajo	Media-alto	Alto	Muy alto
Elementos simultáneos determinados	1	1	1	Varios	Varios

Si el número de elementos a determinar en un alimento es inferior a 3 o 4, se emplean fundamentalmente técnicas Espectroscópicas de Absorción Atómica [23,24] basadas en la reducción de intensidad que experimenta la radiación de una determinada longitud de onda al pasar a través de un conjunto de átomos libres [25]. Dentro de las técnicas de Espectrometría Atómica analítica, se encuentran, la Espectroscopía de Absorción Atómica en Llama (FAAS), Espectroscopía de Absorción Atómica por Generación de Hidruros (HGAAS) y la Espectroscopía de Absorción Atómica Electrotérmica (ETAAS) [25]. En FAAS se emplea una llama para atomizar la muestra y se suele utilizar para la determinación de metales en gran concentración como Ca, Na, K, etc, así como de algunos metales a niveles traza [25]. Cuando se requiere determinar elementos tóxicos a niveles de ultratraza, se emplea HGAAS y ETAAS. La primera técnica se basa en la generación de hidruros volátiles (normalmente con $NaBH_4$) y su posterior introducción en la célula de atomización (llama). Esta forma de trabajo permite mejorar los LoD de forma significativa y resulta especialmente adecuada para metaloides (As, Sb, Se, etc.). En ETAAS, la muestra se atomiza en un horno de grafito [26]. Además de la mejora que proporciona en los LoD, el gran atractivo de esta técnica radica en la posibilidad de analizar alimentos en estado sólido de forma directa y sin ningún tratamiento de muestra previo. Sin embargo, es una técnica compleja y la matriz afecta de forma significativa a la exactitud y la precisión.

Cuando el objetivo es determinar un elevado número de elementos de forma simultánea, se emplean las técnicas espectroscópicas de plasma y, más específicamente, las de Plasma de Acoplamiento Inductivo (ICP) (Tabla 1.2). El ICP se genera por acoplamiento de la energía que proporciona un generador de radiofrecuencia a una corriente de Ar parcialmente ionizada mediante la generación de un campo electromagnético inducido por una espiral [27]. En la Espectroscopia de Emisión Atómica mediante Plasma de Acoplamiento Inductivo (ICP-AES), la especie de interés se determina a través de la radiación que emiten sus átomos/iones en el plasma [27]. Por otro lado, si se determinan los iones generados en función de su relación masa-carga mediante un espectrómetro de masas, se tiene la Espectroscopía de Masas con Plasma de Acoplamiento Inductivo (ICP-MS)[27]. En estas dos técnicas, la muestra a analizar se suele introducir en forma líquida mediante un nebulizador y una cámara de nebulización. No obstante, ambas técnicas permiten analizar solidos de forma directa mediante Vaporización Electrotérmica (ETV) o Ablación Laser (LA) [28,29]. Por regla general, ICP-MS se utiliza fundamentalmente para determinar metales tóxicos a niveles ultratraza debido a su elevada sensibilidad y bajos LoDs (Tabla 1.2). Un atractivo adicional de esta técnica es que proporciona información sobre los isótopos de un elemento y esto puede ser utilizado en estudios de autenticidad de alimentos [30]. El principal hándicap de ICP-MS para su uso en laboratorios de rutina es su elevado coste, complejidad y efectos de matriz [31]. En cuanto a ICP-AES, es una técnica más robusta y menos sensible a los efectos de matriz. Con esta técnica, la mayor parte de aplicaciones se centran en la determinación de elementos mayoritarios y traza. No obstante, si se emplea un tratamiento de muestra previo para extracción y preconcentración a los metales de interés, se pueden obtener LoDs próximos a los que proporciona ICP-MS.

1.3. Pretratamiento de muestra en Espectrometría Atómica.

Las matrices de los alimentos son notoriamente complejas ya que contienen componentes tan diversos como sales, carbohidratos, lípidos y proteínas dificultando el análisis elemental mediante técnicas de Espectrometría Atómica. Por ello, y con objeto de reducir los efectos de matriz, se suele realizar un pretratamiento de muestra previo. El tratamiento más habitual consiste en una digestión ácida a elevada temperatura para descomponer la muestra y, así, obtener una disolución homogénea que se pueda introducir en la célula de atomización. Inicialmente, los alimentos se descomponían con ayuda de un sistema a reflujo en una placa calefactora pero esta estrategia es muy lenta (>24 horas), da lugar a pérdidas de analitos volátiles y, debido a las elevadas cantidades de ácido que se necesitan, genera muchos residuos y tiene un coste elevado [32]. Para evitar estos inconvenientes, en la actualidad, la digestión se suele llevar a cabo en reactores de teflón cerrados donde el calentamiento se realiza mediante radiación de microondas. Esta estrategia permite calentar la disolución ácida de forma directa (no conducción-convección) mejorando la eficiencia del proceso y minimizando la cantidad de reactivos y de residuos generados [33]. Cuando se utiliza ETAAS, ICP-AES e ICP-MS, es posible realizar un análisis directo de las muestras sólidas, simplificando el procedimiento analítico [34]. No obstante, esta estrategia no es fácil de implementar en los laboratorios analíticos por la posible heterogeneidad de la muestra y la dificultad a la hora de calibrar [35]. Por lo que respecta a muestras líquidas, y a pesar de que pueden ser analizadas de forma directa, también son sometidas a un tratamiento de muestra previo para reducir interferencias como, por ejemplo: (i) filtración; (ii) dilución; y (iii) digestión ácida [32,33].

Independientemente del estado físico de la muestra, cuando se realiza la determinación de metales tóxicos es necesaria una etapa adicional de extracción y preconcentración para facilitar su cuantificación dados los bajos niveles en los que se encuentran. Estas estrategias, además, permiten reducir las interferencias producidas por la matriz.

1.4. Técnicas de extracción de metales.

Las técnicas de extracción de metales en alimentos son muy diversas y se clasifican en función de la naturaleza de las fases que se ponen en contacto [36]. En la extracción sólido-líquido (SPE), se pone en contacto un sólido adsorbente con la muestra líquida mientras que en la extracción líquido-líquido (LLE), la fase extractora es una disolvente orgánico inmiscible con la muestra.

En SPE, la fase sólida tiene mayor atracción por los analitos que el disolvente en el que se encuentran disueltos (normalmente en una disolución ácida). Conforme la muestra pasa a través del absorbente, los metales se concentran en su superficie, mientras que el resto de los componentes pasa a su través sin ser retenidos y son eliminados. Los adsorbentes utilizados para extraer y preconcentrar metales son muy variados e incluyen: sílice, carbón activado, celulosa, resinas quelantes y espumas de poliuretano [37]. No obstante, este tipo de extracción presenta diversos problemas operativos para su uso en el laboratorio de forma rutinaria como: (i) cambios de volumen en el material adsorbente; (ii) cambios en el empaquetamiento que afectan a la velocidad de elucción; (iii) desactivación y/o perdida a nivel superficial de los grupos funcionales responsables de la extracción de analito; y (iv) variabilidad en las propiedades adsorbentes entre lotes diferentes de producto [38]. El fundamento de la extracción líquido-líquido se basa en la distribución o reparto del analito entre dos fases inmiscibles. En el caso concreto de los metales, y dado que estas especies están mayoritariamente en forma iónica en agua y disoluciones ácidas, se requiere de un agente quelante que facilite la formación de un complejo neutro para su extracción en la fase orgánica. En este tipo de estrategia, la extracción eficiente de analito depende de diversos parámetros como, por ejemplo: (i) disolvente extractante; (ii) tipo de quelante y concentración; (iii) pH; y (iv) fuerza iónica [39].

El principal inconveniente de las técnicas de extracción convencionales radica en los elevados volúmenes de reactivos requeridos y los residuos que se generan, así como el coste e impacto ambiental que se deriva de ello [40]. Por ello, se han desarrollado nuevas estrategias analíticas que pretenden mitigar los inconvenientes anteriores. Por ejemplo, en el campo de la Espectrometría Atómica, se ha empleado los reactores anudados (KR) como forma de extraer metales [41]. En esta metodología, la muestra y la disolución del agente quelante se introduce en el interior de un capilar de teflón de un sistema FIA que ha sido previamente enrollado sobre sí mismo. Al mezclar las dos disoluciones, se forma el quelato de metal que queda retenido en las paredes internas del reactor. A continuación, se introduce en el sistema un eluyente (orgánico o disolución ácida de elevada concentración) para recuperar el analito. Este sistema permite trabajar de forma on-line y presenta una eficiencia en la retención de analito que se introduce en el sistema del 40-50% [42]. La Tabla 1.3. muestra algunas de las aplicaciones más relevante de esta técnica en Espectrometría Atómica. Tal y como se puede observar, la mayor parte de las

Tabla 1.3. Aplicaciones más relevantes del reactor anudado en Espectrometría Atómica.

Analito	Muestra	Eluyente	Quelante	Técnica	Ref.
Ag, Cd, Co, Cu, In, Mo, Ni, Pb y Sb	Aguas	Metanol	APDC	ICP-MS	[43]
As	Aguas	Etanol	PDC	ETAAS	[44]
Cd	Mejillones	Etanol	Quinina	FAAS	[45]
Cd	Miel	Etanol	5-Br-PADAP	FAAS	[46]
Cd	Vino	HNO_3	5-Br-PADAP	ICP-AES	[47]
Cd, Pb y Ni	Aguas	MIBK	DDTC	FAAS	[48]
Cu	Aguas y sedimentos	Etanol	DDTC	FAAS	[49]
Cu	Aguas y arroz	MIBK	DDTC	FAAS	[50]
Pb	Aguas	Etanol	DDTC	ETAAS	[51]
Sc	Aguas	30% w w^{-1} HNO_3	5-Br-PADAP	ICP-AES	[52]
Tierras raras	Aguas y rocas	1 M HNO_3	8-HQ	ICP-MS	[53]
V	Aguas	30% w w^{-1} HNO_3	5-Br-PADAP	ICP-AES	[54]

aplicaciones están relacionadas con el análisis de agua. Los trabajos con alimentos han sido más escasos por la complejidad que presenta la matriz de este tipo de muestras. Otra vía de acción que se ha seguido para resolver los inconvenientes de las técnicas clásicas de extracción ha sido su miniaturización. El principal atractivo de las técnicas de microextracción es que reducen el consumo de muestra y reactivos a unos pocos µL [40].

1.5. Microextracción Dispersiva Líquido-Líquido.

La Microextracción Dispersiva Líquido-Líquido (DLLME) es una técnica de microextracción desarrollada por Rezaee et al. en 2006 [55] con objeto de mitigar los inconvenientes que presenta la LLE. Desde su introducción, el número de aplicaciones de DLLME ha crecido de forma exponencial (Figura 1.1). Su principal atractivo radica en su sencillez, rapidez, altos factores de enriquecimiento, bajo consumo de disolvente extractante y mínima generación de residuos. De hecho, esta técnica cumple con muchos de los requisitos de la Química Analítica Verde [56].

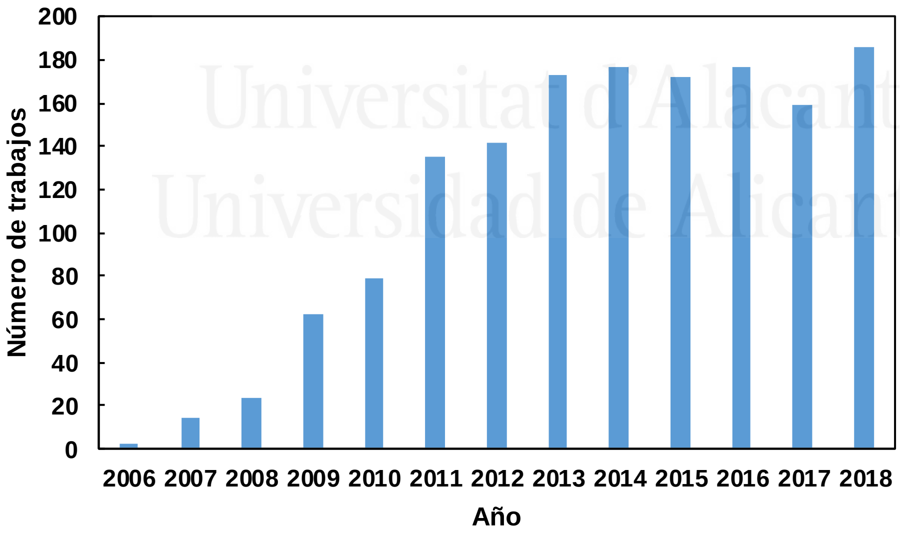

Figura.1.1. Número de trabajos sobre DLLME publicados entre 2006 y 2018. Base de datos Scopus; palabra clave: DLLME.

En esta técnica (Figura 1.2), el disolvente extractante se dispersa en la muestra (líquida) mediante el empleo de un tercer disolvente (dispersante) que es miscible tanto con la muestra como con el extractante. Para ello, y con ayuda de una jeringa, se inyecta la mezcla disolvente extractante-dispersante en la muestra de tal forma que se obtiene una suspensión de microgotas de extractante en la muestra. Las microgotas formadas tienen una elevada superficie específica, lo que resulta en una extracción enormemente eficiente. A continuación, se realiza un centrifugado para favorecer la coalescencia de las microgotas y se recupera el extractante con una jeringa. Si el disolvente extractante es más denso que el agua (p.ej. tetracloruro de carbono, cloroformo, etc.) [57], éste queda depositado en la parte inferior del recipiente que contiene a la muestra mientras que si es menos denso (p.ej. 1-undecanol) queda en la parte superior [15]. Como dispersante, se suelen utilizar alcoholes (metanol y etanol), acetona o acetonitrilo [15]. Para realizar con éxito la determinación de metales mediante DLLME, la muestra debe ser previamente acondicionada con un agente quelante (ditiocarbamatos, 8-hidroxiquinoleina, 1-(2-piridazilo)-2-naftol, etc.) y una disolución reguladora para que se formen los complejos de metal que serán extraídos en la fase orgánica [58]. En general, dentro del análisis inorgánico (metales), la mayor parte de aplicaciones de DLLME se ha centrado en muestras de agua debido a la baja complejidad que presenta la matriz de este tipo de muestras [59–62].

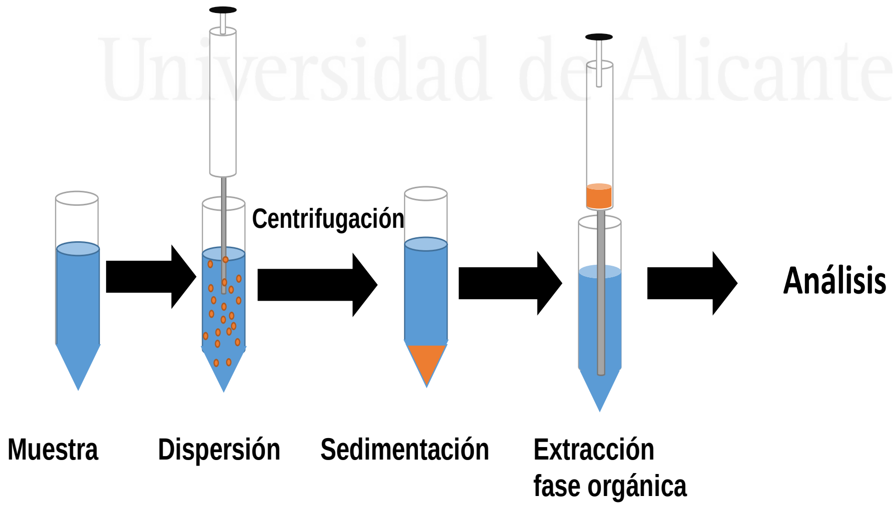

Figura.1.2. Esquema del procedimiento experimental en DLLME.

1.5.1 Tipos de extractantes empleados en DLLME.

Desde su invención, el número de disolventes extractantes que se ha utilizado en DLLME para la determinación de metales ha sido muy diverso. En un primer momento, y por motivos históricos, se utilizaron fundamentalmente disolventes orgánicos volátiles como tetracloruro de carbono, cloroformo o diclorobenceno (Tabla 1.4). Sin embargo, desde un punto de vista práctico y medio ambiental, este tipo de disolventes presenta diversos problemas como: (i) elevada volatilidad; (ii) toxicidad; y (iii) efectos nocivos sobre el medio ambiente (p.ej. capa de ozono). Para evitar los inconvenientes anteriores, se han buscado extractantes alternativos a los disolventes orgánicos volátiles. Entre ellos, cabe destacar alcoholes de cadena larga, líquidos iónicos y disolventes supramoleculares. Dentro de los alcoholes de cadena larga, se ha utilizado 1-undecanol y 1-dodecanol (Tabla 1.5). Estos alcoholes son menos densos que el agua y, por tanto, a diferencia de los disolventes orgánicos volátiles, se acumulan

Tabla 1.4. Aplicaciones más significativas de DLLME en Espectrometría Atómica utilizando como extractante disolventes orgánicos volátiles.

Analito	Muestra	Extractante	Dispersante	Quelante	Técnica	Ref.
As	Aguas	CCl_4	Metanol	APDC	ETAAS	[63]
Bi	Aguas	Diclorobenceno	Acetona	5-Br-PADAP	FAAS	[64]
Cd	Aguas	$CHCl_3$	Etanol	APDC	ICP-AES	[65]
Cd y Pb	Aguas	CCl_4	Metanol	APDC	ETAAS	[66]
Cd, Pb y Bi	Aguas	CCl_4	Metanol	DDTC	ICP-MS	[67]
Cr	Aguas	CCl_4	Etanol	APDC	FAAS	[68]
Cr, Cu, Ni y Zn	Aguas	CCl_4	Metanol	DDTC	ICP-AES	[69]
Pb	Aguas	CCl_4	Acetona	DDTP	ETAAS	[70]
Pb	Aguas	CCl_4	Metanol	DDTP	FAAS	[71]
Pd	Aguas	CCl_4	Etanol	TRH	ETAAS	[72]
Sb	Aguas	$CHCl_3$	Etanol	BPHA	ETAAS	[73]
Se	Aguas	CCl_4	Etanol	APDC	ETAAS	[74]

en la parte superior de la muestra [75]. A nivel práctico, recuperar este tipo de extractos no es fácil y, por ello, se suele someter a la mezcla extractante-muestra a una etapa de enfriamiento [76]. Dado que los puntos de fusión de estos alcoholes se encuentran ligeramente por debajo de los 25ºC, es posible recuperarlos en estado sólido una vez han sido congelados [76]. Los líquidos iónicos (ILs) son disolventes iónicos no moleculares con puntos de fusión por debajo de 100°C [77]. Las propiedades más notables incluyen su despreciable presión de vapor a temperatura ambiente, alta estabilidad térmica y viscosidad variable [77]. Su miscibilidad en agua y disolventes orgánicos puede controlarse seleccionando la combinación catión/anión o incorporando ciertos grupos funcionales en la molécula de IL[78]. Inicialmente, este tipo de disolventes se consideraron como inocuos para el medio ambiente. Sin embargo, algunos estudios cuestionan la validez de esta última afirmación por la complejidad de su síntesis y su efecto sobre la biota [79,80]. En general, este tipo de disolventes se han utilizado en aplicaciones medio ambientales pero no han sido aplicados de forma extensiva para la determinación de metales en alimentos (Tabla 1.6) [81]. Los disolventes supramoleculares (SUPRAS) consisten en agregados de micelas inversas de dimensiones nanométricas dispersas en una fase continua. Los SUPRAS se producen mediante un procedimiento de autoensamblaje al cambiar el pH [82], la temperatura [83] y/o la fuerza iónica [84] de una solución al tiempo que se agrega un disolvente anfílico [85]. Una forma muy sencilla de generar este tipo de estructuras se basa en combinar THF con un alcohol (1-undecanol) o un ácido (decanoico) de cadena larga. Al igual que los ILs, este tipo de disolventes apenas han sido utilizados para determinar metales en alimentos (Tabla 1.7).

Tabla 1.5. Aplicaciones más significativas de DLLME en Espectrometría Atómica utilizando como extractante alcoholes de cadena larga.

Analito	Muestra	Extractante	Dispersante	Quelante	Técnica	Ref.
Al	Aguas	1-undecanol	Acetona	Morin	ICP-AES	[86]
As	Arroz	1-undecanol	Metanol	DDTP	ETAAS	[87]
Cd	Agua, bebidas y arroz	1-dodecanol	Metanol	8-HQ	FAAS	[88]
Co Cr, Cu, y Mn	Aguas	1-undecanol	Acetona	TTA	CP-AES	[89]
Co, Cu y Ni	Aguas	1-undecanol	Acetona	DDTC	ETAAS	[90]
Co, Ni, Pb, U y Zn	Aguas	1-undecanol	Etanol	PAN	ICP-MS	[91]
Cu	Harina de Arroz y té	1-undecanol	Etanol	OVAC	FAAS	[92]
Cu	Arroz y mijo	1-dodecanol	Metanol	8-HQ	FAAS	[93]
Hg	Pescados y aguas	1-undecanol	Etanol	4-NOPD	ETAAS	[94]
Pb	Fórmulas infantiles	1-undecanol	Etanol	APDC	ETAAS	[95]

Tabla 1.6. Aplicaciones más significativas de DLLME en Espectrometría Atómica utilizando como extractante líquidos iónicos.

Analito	**Muestra**	**Extractante**	**Dispersante**	**Quelante**	**Técnica**	**Ref.**
As	Vinos	C_8mim-PF_6	Metanol	DDTC	ETAAS	[96]
As	Aguas salinas	C_6mim-PF_6	Etanol	TMK	ETAAS	[97]
Co	Agua, orina y saliva	C_6mim-PF_6	Metanol	PAN	ETAAS	[98]
Co	Suplementos nutricionales	C_6mim-PF_6	Metanol	DDTC	ETAAS	[99]
Cu	Agua	Hmim-Tf_2N	Acetona	TMK	FAAS	[100]
Cd y Pb	Aguas salinas	C_6mim-PF_6	Acetona	DDTC	FAAS	[101]
Pb	Agua	C_6mim-PF_6	Etanol	Ditizona	FAAS	[102]
Ni	Harina de arroz, té negro	BmimPF_6	Agitación magnética	PAN	FAAS	[103]
Zn	Agua y muestras de alimentos	C_6mim-PF_6	Etanol	8-HQ	FAAS	[104]
Zn	Leche en polvo humana	HPyPF_6	Acetonitrilo	8-HQ	FAAS	[105]

Tabla 1.7. Aplicaciones más significativas de DLLME en Espectrometría Atómica utilizando como extractante SUPRAS.

Analito	Muestra	Extractante	Dispersante	Quelante	Técnica	Ref.
As	Aguas y suelos	Ácido decanoico	THF	APDC	ETAAS	[106]
Cd	Agua y pelo	1-Decanol	THF	APDC	FAAS	[77]
Cd	Agua y vegetales	Ácido decanoico	THF	APDC	FAAS	[107]
Cu	Aguas, harina de arroz y de maíz	1-Decanol	THF	DMDC	FAAS	[108]
Cu y Pb	Aguas	Ácido nonaico	THF	Base de schiff	FAAS	[109]
Pb	Aguas, harina de arroz y de trigo	Ácido decanoico	THF	DDTC	ETAAS	[110]
Se	Agua, orina y cosméticos	1-Octanol	THF	APDC	ETAAS	[111]

1.5.2 Acoplamiento de DLLME a técnicas de Espectrometría Atómica.

Hasta la fecha, los estudios descritos en la bibliografía muestran que DLLME se ha utilizado de forma preferencial con técnicas de Espectrometría de Absorción Atómica, sobre todo ETAAS. El volumen de extracto obtenido tras un tratamiento de DLLME es de unos pocos microlitos (30-50 µL) y se adapta perfectamente a las condiciones de trabajo de un horno de grafito [112]. Además, con ayuda de un modificador y de la selección adecuada del programa de temperatura del horno es posible mitigar las interferencias que se derivan de trabajar con muestras de naturaleza orgánica [113]. A nivel práctico, resulta problemático trabajar con disolventes muy viscosos ya que el automuestrador del instrumento no es capaz de inyectar la muestra de forma reproducible en el interior del tubo de grafito. Por ello, y a pesar de que tiene un impacto negativo sobre la sensibilidad y los LoDs, se suele llevar a cabo una dilución previa del extractante con un disolvente orgánico (p.ej. metanol) para reducir la viscosidad [114]. Los estudios en FAAS han sido algo más limitados ya que el volumen de extracto no se adecúa a las características del sistema de introducción de muestras de esta técnica y requiere hacer ciertas modificaciones en el mismo [115]. A diferencia de ETAAS, el efecto de la matriz orgánica en el atomizador (llama) es inferior, dando lugar a menores interferencias espectrales.

Por lo que respecta a las técnicas de plasma, el uso de DLLME como técnica de preparación de muestra ha sido escaso. A priori, el uso combinado de DLLME con técnicas de ICP presenta un gran atractivo por la posibilidad de determinar varios analitos de forma simultánea, aumentado la velocidad de análisis y reduciendo la generación de residuos y coste. Sin embargo, el acoplamiento entre ambas técnicas presenta limitaciones. En primer lugar, el volumen de extracto obtenido en DLLME no se ajusta a las características del sistema de introducción de muestras líquidas estándar de un ICP. Por otro lado, los extractantes orgánicos que se utilizan en DLLME tienen un efectivo negativo sobre el plasma. Así, por ejemplo, los disolventes orgánicos volátiles (p.ej. tetracloruro de carbono, cloroforomo, etc.) disminuyen la temperatura del plasma y pueden llegar a extinguirlo. Los disolventes orgánicos que son altamente viscosos (1-undecanol, ILs, SUPRAS, etc.) afectan negativamente a la nebulización y al transporte de aerosol al plasma y, además, dan lugar a efectos de memoria. Con objeto de resolver las limitaciones anteriores, en la bibliografía, se han propuesto diversas estrategias como: (i) evaporación del disolvente y redisolución del extracto en una disolución ácida [116]; (ii) re-extracción en agua [117]; (iii) dilución con un disolvente adecuado [118]; y (iv) el uso de sistemas de introducción de muestras no convencionales como, por ejemplo, la atomización ETV o LA [119]. Si bien estas estrategias permiten el acoplamiento, introducen nuevos inconvenientes. Así, por ejemplo, algunas de las ventajas que ofrece DLLME (sencillez, elevada velocidad de análisis) se ven afectadas de manera negativa. Además, en algunos casos, requieren de modificaciones en la configuración del ICP que no son siempre posibles o que incurren en un gasto adicional elevado.

1.5.3 Análisis elemental en alimentos mediante DLLME y técnicas de Espectrometría Atómica: retos.

Atendiendo a la bibliografía, se detectan dos grandes retos a la hora de utilizar de forma combinada DLLME y técnicas de Espectrometría atómica para la determinación de metales en alimentos. En primer lugar, la mayor parte de los trabajos desarrollados hasta el momento emplean disolventes orgánicos volátiles como medio de extracción y, atendiendo a los principios de la Química Verde, sería conveniente substituir estos disolventes por otros menos peligrosos y tóxicos [120]. Por ello, y para mejorar la sostenibilidad de la extracción-preconcentración de metales mediante DLLME, se pretenden desarrollar nuevas metodologías analíticas basadas en el uso de líquidos iónicos (hexaflurofosfato de 1-butil-3-metilimizadolio) y disolventes supramoleculares (THF/1-undecanol) como extractantes. Hasta la fecha, este tipo de disolventes se han venido utilizando para la extracción de metales en aguas y no se conoce bien sus ventajas y limitaciones a la hora de trabajar con matrices más complejas como las que presentan los alimentos [121]. Por otro lado, se ha detectado que las técnicas de espectrometría de plasma (ICP-AES e ICP-MS) están infrautilizadas como sistema de detección con DLLME [122]. A diferencia de las técnicas de Absorción Atómica, las técnicas de plasma permitirían mejorar significativamente la velocidad de análisis por su capacidad para detectar más de un elemento de forma simultánea. Normalmente, la falta de trabajos en este campo se ha atribuido a los efectos negativos de los disolventes orgánicos en el plasma (interferencias espectrales y no espectrales). Sin embargo, existen evidencias significativas que cuestionan la validez de dichas afirmaciones [123]. La mayor parte de trabajos publicados se centran en la optimización de la extracción y apenas incluyen información sobre cómo se lleva la optimización de las condiciones experimentales del plasma y del sistema de introducción de muestras [124]. Este hecho es especialmente llamativo dada la gran influencia de las condiciones experimentales del plasma sobre los parámetros analíticos [125]. En este sentido, es importante señalar que ICP-AES e ICP-MS se han venido utilizando de forma extensiva como detector en técnicas cromatográficas o para el análisis de productos petroquímicos [126]. No existe ningún tipo de limitación para analizar moléculas orgánicas siempre que se optimice el instrumento de forma adecuada [123].

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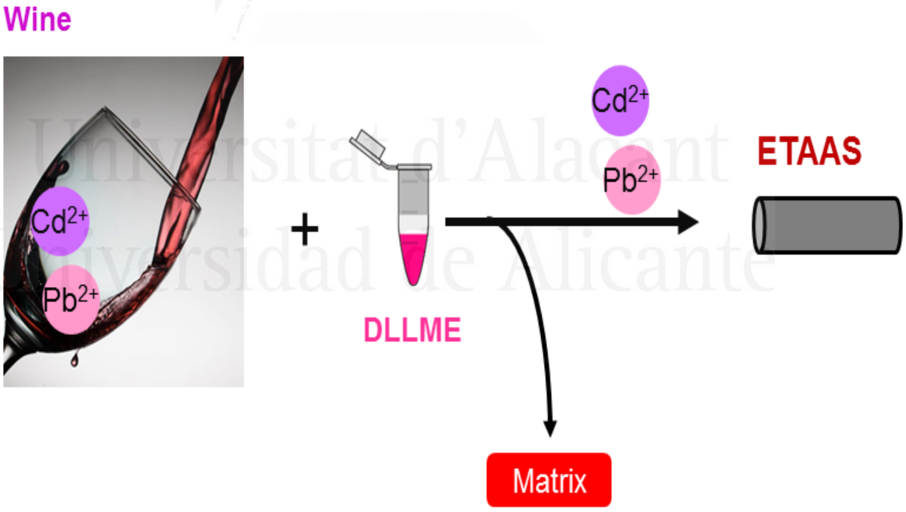
CAPÍTULO 2

DETERMINATION OF CADMIUM AND LEAD IN WINE SAMPLES BY MEANS OF DISPERSIVE LIQUID-LIQUID MICROEXTRACTION COUPLED TO ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY



1. Introduction.

Wine is one of the most widely consumed beverage around the world. Nevertheless, it exhibits significant Pb and Cd levels (i.e. low-medium µg L^{-1} range) that are mainly related to atmospheric contamination, pesticides and materials used in wine production, transport and storage [1,2]. Due to their hazardous nature, Cd and Pb maximum allowed levels in wine are regulated worldwide [3,4]. Thus, for instance, the Organisation Internationale de la Vigne et du Vin (OIV) limits Cd and Pb in wine to 10 µg L^{-1} and 150 µg L^{-1}, respectively [3].

Cadmium and Pb analysis in wine is usually carried out by means of spectrometric techniques [5]. Among them, mainly when no multi-element analysis is required, electrothermal atomic absorption spectrometry (ETAAS) is the most preferred choice due to its high selectivity and low limits of detection (LoDs). Direct wine analysis by ETAAS is difficult due to the occurrence of both spectral and non-spectral interferences originated from wine matrix components (organics and salts) [5]. For instance, non-volatile organic components of wine are a source of non-specific background absorption and affect long-term reproducibility because of the accumulation of carbonaceous residues after several graphite tube firings [6,7]. To deal with these interferences, some strategies can be applied: (i) a judicious optimization of ETAAS temperature program and modifier [6–10]; (ii) the use of transversally heated furnaces [6,10]; (iii) a Zeeman-based background correction [6,7,11]; and/or, (iv) the use of a sample pre-treatment step prior to the analysis. To this end, acid digestion [7,11,12] or dilution [6,13] have been employed but at the expense of the sample throughput and detection capabilities. Solid-phase extraction/preconcentration procedures using different type of columns have been also reported for the quantification of Cd and Pb in wines by flame atomic absorption spectrometry (FAAS) [14–17] at the concentration levels imposed by regulatory authorities. Up to date, the use of extraction/preconcentration procedures for Cd and Pb analysis in wine by means of ETAAS have not been investigated in detail though its potential advantages to reduce both spectral and non-spectral interferences derived from wine matrix.

Dispersive liquid-liquid micro-extraction (DLLME) has been stablished as a faster and greener sample preparation methodology than conventional liquid-liquid extraction and solid phase extraction approaches for elemental analysis [18,19]. DLLME improves LoDs and sample throughput as well as minimizes matrix effects, reagents requirements and waste generation. Different spectroscopic techniques have been employed to analyze DLLME extracts [20] Nevertheless, ETAAS is usually preferred due to its good capability to deal with micro-volume samples and organic solvents [21,22]. DLLME has been traditionally employed for metal analysis in water samples and, in a lesser extent, in [21]. Very little research has been performed evaluating the potential benefits (and drawbacks) of DLLME for inorganic analysis in wine samples. Seeger et al. [23] employed DLLME for Cu and Fe determination in wine by FAAS. Results afforded by DLLME-FAAS were comparable to those obtained by means inductively coupled plasma atomic emission spectrometry (ICP-AES) after an acid digestion treatment. However, sample throughput was partially compromised due to the mandatory use of standard addition calibration strategy due to the high matrix effects originated by the organic extracts in the flame. Escudero et al. [1,24] successfully employed a DLLME procedure for As speciation in wine by ETAAS. Experimental results show that As^{3+} extraction was highly selective and free from interferences originated by inorganic concomitants present in wine. To our best knowledge, no further works related on this topic have been reported.

The goal of this work is to develop a new analytical method for Cd and Pb determination in wine by means of DLLME-ETAAS. To this end, both DLLME (i.e. extractant mass, disperser type and volume, pH and ionic strength) and ETAAS (i.e. modifier and temperature program) experimental parameters have been optimized. Finally, the developed methodology has been applied to the analysis of different wine samples (white, rosé, red, sparkling and fortified). To evaluate the results obtained, wine samples have also been analyzed by means of inductively coupled plasma - mass spectrometry (ICP-MS) after a digestion procedure.

2. Experimental.

2.1. Reagents.

1-butyl-3-methyl-imidazolium hexafluorophosphate (BmimPF$_6$ ≥97%, sodium diethyldithiocarbamate trihydrate (DDTC, ≥99%), methanol (≥99.9%), ethanol (≥99.5%), sodium chloride (≥99.5%) and Pb, Cd and Pd mono-elemental standard solutions (1000 mg L^{-1}) were obtained from Sigma-Aldrich (Steinheim, Germany). Nitric acid (69% w w^{-1}), sodium dihydrogen phosphate (≥99.5%), disodium dihydrogen phosphate (≥99.5%), glacial acetic acid (99.7%), sodium acetate (99%) and potassium nitrate (99%) were purchased from Merck (Darmstadt, Germany). Calcium nitrate hexahydrated (99%), copper nitrate trihydrate (99%), iron (III) nitrate (98%), manganese nitrate (≥98.5%), magnesium nitrate (99%), strontium nitrate (99%) and zinc nitrate hexahydrate (98.5%) were obtained from Scharlab (Barcelona, Spain).

All solutions were prepared using high purity water (i.e. with conductivity lower than 18 MΩ cm^{-1}) obtained from a Milli-Q water system (Millipore Inc. Paris, France).

2.2. Wine samples.

Five Spanish wine samples, purchased in local markets, were analysed to cover different matrix characteristics and ethanol content: white (Fidencio, Spain, 11.5% w w^{-1}), rosé (Piccolo Giovanni, Italy, 9.0% w w^{-1}), sparkling (Cava Jaume Serra, Spain, 11.5% w w^{-1}), fortified (Mistela Murviedro, Spain, 15% w w^{-1}) and red wine (Fidencio crianza, Spain, 13.5% w w^{-1}).

2.3. Dispersive liquid-liquid microextraction.

The extraction/preconcentration procedure for Cd and Pb determination was performed as follows. First, 2 mL of wine sample or analyte standard were placed on a centrifuge tube with 100 µL of the suitable buffer solution (e.g. acetic acid/sodium acetate dyhidrogen/hydrogen phosphate). Then, 100 µL of NaCl and 100 µL of the DDTC solutions were added. Next, a mixture of BmimPF$_6$ (extractant) and methanol (disperser) was injected using a 1.00 mL syringe and the resulting system was shaken for 2 minutes using a vortex. To separate the two phases generated (water/ionic liquid), the mixture was centrifuged for 5 minutes at 3500 rpm. The lower layer of the ionic liquid was removed with a micropipete and then diluted with methanol (BmimPF_6/methanol 1:1 ratio) prior to ETAAS analysis.

2.4. Microwave digestion.

For comparison, wine samples were analyzed by ICP-MS (model 7700x, Agilent, Santa Clara, USA) after a microwave assisted digestion treatment in closed vessel. To this end, 5 g of the selected wine was weighed into a PTFA digestion vessel and 5 mL of concentrated nitric acid was added. The mixture was allowed to react and after clearance of fumes (20 min) the vessel was closed. Afterwards, samples were digested in a Star D microwave digestion system (Milestone, Shelton, CT, USA) using the program recommended by the manufacturer. Finally, the digested samples were made up to 20 g with water.

2.5. Instrumentation.

Cadmium and Pb in DLLME extracts were determined using an atomic absorption spectrometer (model 2100, Perkin Elmer, Norwalk, USA) equipped with a graphite furnace atomizer and a deuterium background correction system. Samples were introduced into the furnace by an auto-sampler (model AS-60, Perkin Elmer, Norwalk, USA). Pyrolytically coated graphite tubes without platform were used in this work. Cadmium and Pb signals were measured using the optimized temperature program given in Table 2.1. For comparison, wine samples were also analyzed by ICP-MS. Instrumental and experimental conditions employed in ICP-MS are described elsewhere [25].

Table 2.1. ETAAS operating conditions.

Parameter		Lead	Cadmium
Lamp current (Ma)		9	8
Wavelength (nm)		283.3	228.8
Spectral bandwith (nm)		0.7	
Inyected volume (µL)		20	
Furnace heating program			
Step	Tempaerature (ºC)		
Dry	100	20	10
Pyrolysis	350	10	20
	400-1300	10	20
Atomization	400-1300	1.0	5
Clean	2600	1.0	3

3. Results.

3.1. Optimization of graphite furnace experimental conditions.

Ionic liquids are considered safer and more environmentally friendly than the (volatile) organic solvent traditionally employed in DLLME procedures. In the present work, $BmimPF_6$ has been selected as extraction media. However, direct analysis of Cd and Pb in $BmimPF_6$ extracts by ETAAS is not straightforward due to the physical properties of both ionic liquid (low volatility and high viscosity) and analytes (high volatility). First, the high viscosity of $BmimPF_6$ (i.e. 381 cP at 25°C) makes difficult the sample handling. The direct introduction of ionic liquids into the furnace by means of a [26,27] did not afford reproducible results for Pb and Cd analysis even when using an autosampler. To solve this drawback, Li et al. [28] proposed to reduce the $BmimPF_6$ viscosity by dilution with an appropriate solvent (e.g. alcohols). In the present work, it was observed that irrespective of the dilution factor used (ranging from 1:0.5 to 1:3), $BmimPF_6$: methanol mixtures were easily introduced in the furnace by the autosampler with high reproducibility. Therefore, to reduce the analyte dilution, a 1:0.5 $BmimPF_6$ methanol dilution ratio was first selected after the DLLME procedure. Nonetheless, high background signals were registered irrespective of the modifier and temperature program employed and even using a deuterium correction system. Better results were obtained by employing a 1:1 $BmimPF_6$: methanol mixture.

3.1.1. Modifier.

In this work, palladium (as a nitrate salt) was selected as matrix modifier since it is usually employed for the analysis of Cd and Pb in carbon containing matrices [6,8,27,29] avoiding the structured background caused by other modifiers (i.e. phosphates) [7,8,30]. To check the influence of the palladium concentration on the absorbance of Cd and Pb, standard solutions of these elements (with a final concentration of 1 and 10 µg L^{-1}, respectively) with amounts of Pd ranging from 0.5 to 9 µg were prepared in a 1:1 $BmimPF_6$: methanol mixture. Experimental results shown that the use of Pd makes feasible to use higher pyrolysis temperatures thus reducing the background signal. Cadmium and Pb absorbance increased with Pd concentration up to 1 µg of Pd. For higher Pd concentrations, no noticeable effect was observed on the absorbance of both elements. Therefore, the amount of modifier was kept at 1 µg for further experiments.

3.1.2. Furnace temperature program.

Taking into account the high temperatures required to decompose $BmimPF_6$ (>350ºC), a two-step pyrolysis procedure was selected to reduce background signals [28,31,32]. The pyrolysis temperature was initially set at 350º C for 20 seconds and, then, it was increased up to values ranging from 400 to 1200ºC for 20 extra seconds. Figure 2.1 shows the pyrolysis and atomization curves for Cd and Pb. In this figure, absorbance of both elements were normalized against their respective maximum value. As it can be observed in Figure 2.1A, absorbance remains constant up to pyrolysis temperatures of 800-900ºC. Nevertheless, it is interesting to note that, when increasing the pyrolysis temperature from 400ºC up to the above-mentioned values, background signal was reduced approximately 3-fold. Due to these reasons, pyrolysis temperatures of 800ºC and 900ºC were selected for Cd and Pb, respectively. With regard the atomization temperature (Fig 1.B), the optimum value obtained for both elements was of 1600ºC. These temperatures are similar to those reported by other authors operating with different ionic liquids (i.e. 1-octyl-3-methylimidazolium bis (trifluoromethylsulfonyl) imide [27] and 1-hexyl-3-methylimizadolium hexafluorophosphate [28]. Finally, it is worth to mention that no significant memory effects were observed due to carbon deposition in the furnace after several graphite firings, thus ensuring the reproducibility of the signal measurements (5-10%).

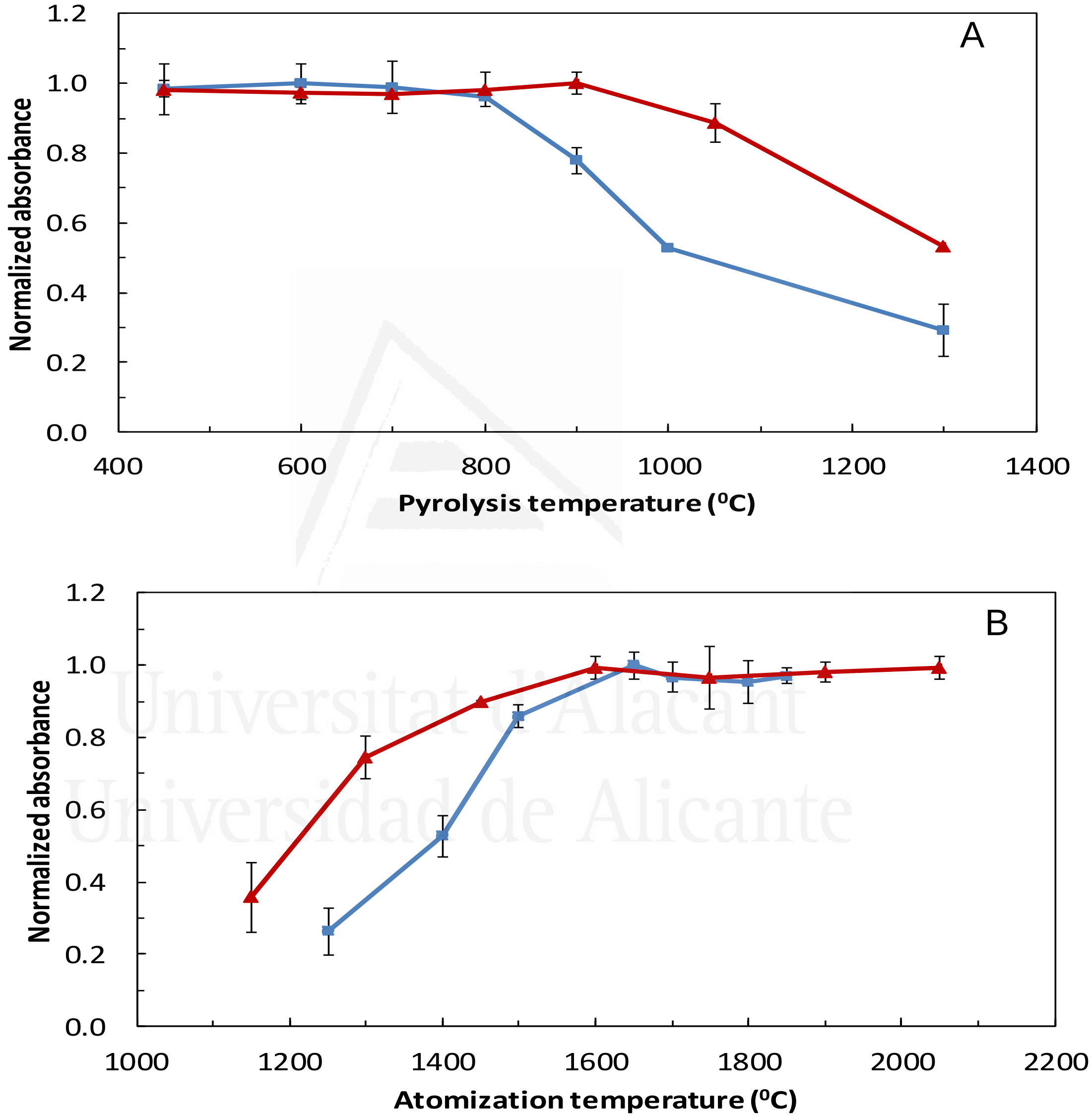


Figure. 2.1. Influence of the pyrolysis(A) and atomization(B) temperature on the normalized absorbance for Cd (■) and Pb (▲) in ETAAS. Modifier:1 µg Pd. Atomization temperature in (A): Cd and Pb: 1600ºC; Pyrolysis temperature in (B): Cd 800ºC, Pb 900ºC.

3.2. Optimization of DLLME experimental conditions.

Optimization of the DLLME procedure was carried out using 2 mL of a synthetic wine sample containing 12.5% v v^{-1} ethanol and potassium 1000 mg L^{-1} (i.e. the major organic and inorganic wine components). The variables tested were: (i) DDTC concentration (chelating agent); (iii) pH; (iii) ionic strength; (iv) $BmimPF_6$ mass (extractant solvent); and (v) methanol volume (disperser solvent).

3.2.1. Effect of chelating agent concentration and pH.

Metal extraction into the ionic liquid relies on the formation of a neutral chelate with DDTC. Figure 2.2 shows the influence of DDTC concentration on the absorbance of Cd and Pb. As it can be observed, absorbance for both elements increased with DDTC concentration up to 1.0 %. Above this value, no significant signal improvement was observed.

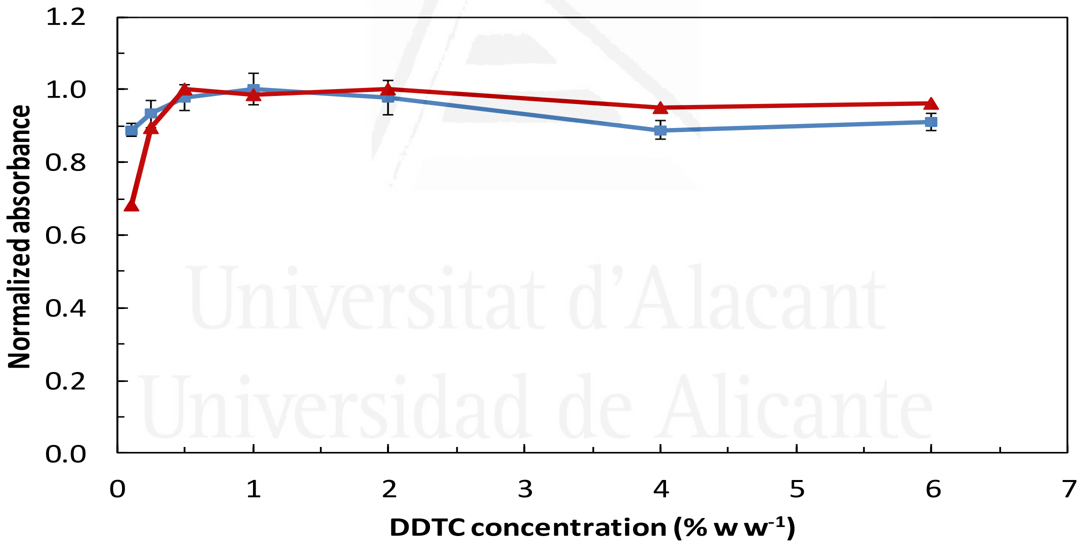


Figure. 2.2. Influence of the DDTC on the normalized absorbance for Cd (■) and Pb (▲) in ETAAS. $BmimPF_6$ mass: 150 µg; methanol volume: 600 µL; solution pH: 6; NaCl concentration: 5.0% w w^{-1}.

Solution pH also plays a significant role on the metal-chelate formation and, hence, on the Cd and Pb extraction. The influence of the pH on analyte extraction was investigated in the pH range from 3 to 9 (Figure 2.3). No significant influence of the solution pH on Cd absorbance was observed. Nevertheless, Pb signal shows a maximum at pH values around 6-7. From these findings, DDTC concentration and pH were set at 1% v v^{-1} and 6, respectively.

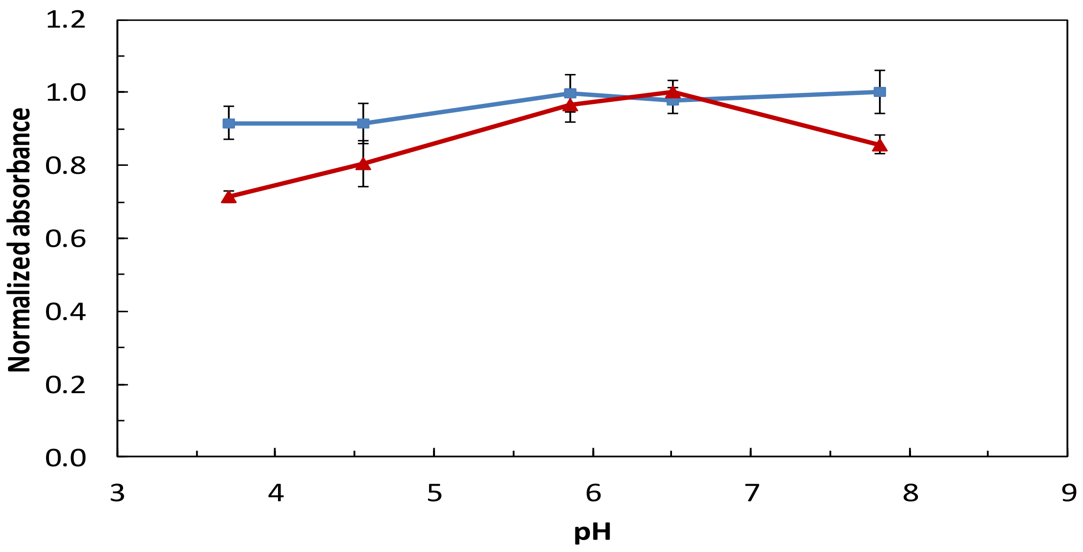


Figure. 2.3. Influence of the solution pH on the normalized absorbance for Cd (■) and Pb (▲) in ETAAS. BmimPF$_6$ mass: 150 µg; methanol volume: 600 µL; DDTC concentration: 1.0 w w-1; NaCl concentration: 5.0% w w^{-1}.

3.2.2. Effect of solution ionic strength.

The influence of the ionic strength on Cd and Pb extraction from the aqueous phase to the organic phase was investigated using synthetic wine samples spiked with variable amounts of NaCl (0-15% w v^{-1}). Cadmium and Pb absorbance showed a maximum for the solution containing 5.0 % w v^{-1} NaCl. These results suggest that analyte extraction is partially favoured by increasing the media ionic strength. Therefore, this optimum NaCl concentration was employed in further experiments.

3.2.3. Effect of the amount of extractant.

To evaluate the influence of BmimPF$_6$ amount on Cd and Pb extraction, ionic liquid masses ranging from 150 to 300 mg were tested. BmimPF$_6$ masses lower than 150 mg were not explored due to the lack of reproducibility in drop formation and the low masses of ionic liquid recovered after the DLLME treatment (<15 µg). It was observed that Cd and Pb absorbance decreased when increasing the amount of BmimPF$_6$. These findings are easily explained taking into account that analyte preconcentration factor is reduced when increasing the mass of extractant [28,33,34]. Therefore, a BmimPF$_6$ mass of 150 mg was selected for further studies.

3.2.4 Effect of the disperser solvent nature and volume.

Two organic solvents (methanol and acetone) were tested as potential dispersers for $BmimPF_6$. Results shown that Cd and Pb signals were on average 1.2-fold higher for methanol than for acetone. Similar findings were obtained by Khan et al., [34] operating $BmimPF_6$ for Cd extraction with 1-(2-pyridylazo)-2-naphthol in environmental and biological samples. Figure 2.4 shows the influence of methanol volume on the normalized Cd and Pb absorbance in ETAAS. As it can be observed, absorbance for these elements increases with methanol volume up to 600 µL due to a higher extraction efficiency of the metal-chelate complex from the water to the organic phase. Volumes higher than 600 µL were not employed due to the lack of $BmimPF_6$ droplet formation after the DLLME treatment. These results suggest that the $BmimPF_6$ solubility in the synthetic wine standards is favored by the disperser solvent. In fact, this issue was not found operating ethanol-free wine standards. Therefore, methanol volume was set at 600 µL.

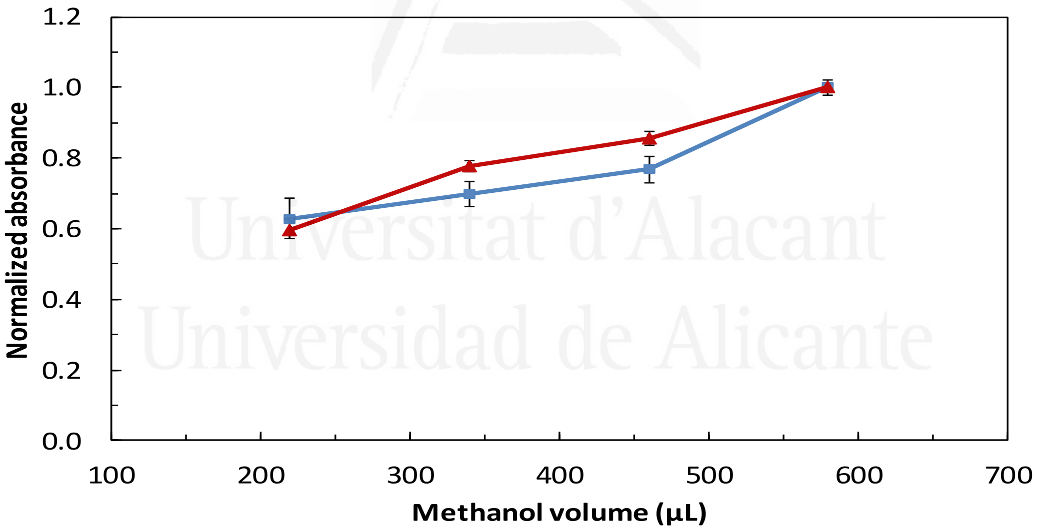


Figure. 2.4. Influence of the disperser volume on the normalized absorbance for Cd (■) and Pb (▲) in ETAAS. $BmimPF_6$ mass: 150 µg; DDTC concentration: 1.0 w w^{-1}; solution pH: 6; NaCl concentration: 5.0% w w^{-1}.

3.3. Analytical figures of merit.

Due to the lack of a certified wine reference material for Cd and Pb analysis, method accuracy was evaluated by means of a recovery test using different wine samples. Taking into account the levels usually found for these elements in wines, samples were spiked with 1 µg L^{-1} of Cd and 10 µg L^{-1} of Pb.

The assay was performed using the optimum ETAAS and DLLME operating conditions described in the previous sections. Results for the recovery test are reported in Table 2.2. As it can be observed, Pb recovery was almost quantitative (96-100%), regardless the wine sample selected. However, results obtained for Cd were systematically lower (i.e., 20-79% lower) than the expected ones. These results suggest that other elements present in wine are affecting the Cd extraction. According to the literature [5], along with potassium, wines contain significant amounts of: (i) Ca (100 mg L^{-1}); (ii) Mg (100 mg L^{-1}); (iii) Fe (4 mg L^{-1}); (iv) Cu (1 mg L^{-1}); and (v) Zn (1 mg L^{-1}). Thus, to insight on the origin of the poor Cd recoveries obtained, different synthetic wine (12% v v^{-1} ethanol and 1000 mg L^{-1} potassium) matrices containing each of the above-mentioned concomitants were prepared. Cadmium recovery was quantitative for all the matrices tested except for the Zn containing one. Thus, when operating matrices containing Zn levels below 0.5 mg L^{-1}, Cd recoveries were quantitative. From this concentration on, Cd recoveries always decrease when increasing the concentration of Zn present in the matrix. At concentrations of 1 mg Zn L^{-1}, Cd recovery was found of 76±4%. Taking into account these findings, Zn concentration was determined in all the wine samples tested. As expected, a direct correlation between Zn levels and Cd recovery was found (Table 2.2). So the highest Cd recoveries were obtained for those samples containing the lowest Zn concentration levels. As regards the precision of Cd and Pb measurements, the relative standard deviation obtained after the analysis of five independent replicates range between 4-9% (values typically obtained in ETAAS).

Table 2.2. Recovery values for Cd and Pb by DLLME-ETAAS and concentration of Zn present in the different wine samples.

Type of wine	**Recovery (%)***		**Zn (μg L^{-1})***
	Pb	**Cd**	
White	98±4	79±5	800±30
Rosé	99±5	52±4	1400±90
Sparkling	98±4	51±6	1300±100
Fortified	100±3	36±4	2100±180
Red	96±4	20±5	2800±130

*Mean ± standard deviation, 5 replicates

Cadmium and Pb LoDs were calculated using the calibration graph according to the method described by Hubuax et al. (i.e. based on 5 standards, 95% confidence level) [35]. LoDs obtained for Cd and Pb were 0.01 and 0.08 µg L^{-1}, respectively. As reported in Table 2.3, these values were about 8 and 9-fold lower than those obtained in ETAAS using a direct analysis method (i.e., without DLLME). These improvement factors are in agreement with the experimental enrichment factors obtained for Cd and Pb when operating with DLLME. These results suggest that LoDs could be further improved by increasing the analyte enrichment factor. In fact, LoDs obtained in this work are higher than those reported in the literature using other DLLME procedures for Cd and Pb analysis by ETAAS [21]. It can be explained taking into account that in the method proposed, the extraction with BmimPF$_6$ (i) requires a dilution with methanol prior to the sample introduction into the graphite furnace, thus reducing the analyte enrichment factor. In addition, also the sample volume used in this work was at least between 2.5 and 5-fold lower than those usually reported due to the limited volume of the centrifuge tubes employed; and, (ii) is negatively affected by the presence of ethanol in the wine matrix. In spite of these facts, data in Table 2.3

Table 2.3. Comparison of the LoD obtained using the diverse methodologies reported for Cd and Pb determination in wine by ETAAS.

Element	Sample preparation	Background correction	LoD (µg L^{-1})	Ref.
Cd	Direct analysis	Deuterium	0.08	This work
	DLLME	Deuterium	0.01	
	Direct analysis	Zeeman	0.03	[7]
	Digestion	Zeeman	0.1	[11]
	Dilution	Zeeman	0.03	[6]
Pb	Direct analysis	Deuterium	0.16	This work
	DLLME	Deuterium	0.08	
	Direct analysis	Zeeman	0.8	[7]
	Digestion	Zeeman	1.0	[11]
	Dilution	Zeeman	0.8	[6]

clearly demonstrate that the LoDs obtained with the method proposed in this work are lower than those previously reported for Cd and Pb in wines by ETAAS using alternative sample preparation methodologies. In addition, the proposed method reduces both instrumental analysis complexity and costs since it does not require neither time consuming digestion treatments nor complex background correction systems to deal with spectral interferences.

3.4. Analysis of wine samples.

Five wine samples have been analyzed to quantify Cd and Pb levels using the methodology developed. Lead analysis was performed by means of calibration with standards containing ethanol 12% v v^{-1} and 1000 mg K L^{-1}. Given the variability of the Zn content in the wine samples, standard addition was chosen as the calibration strategy for Cd analysis. Other approaches (e.g. Zn matched standards, additional extraction treatments) were not investigated due to their higher complexity as well as their negative impact on the benefits derived of using DLLME for sample preparation (e.g. sample throughput, simplicity, etc.). Results obtained were compared to those obtained using a microwave acid sample digestion procedure and ICP-MS detection. Wine digestion decompose organic components mitigating carbon matrix effects. As it can be derived from data shown in Table 2.4, no significant difference was found in the Pb concentration values obtained by DLLME-ETAAS and ICP-MS. Lead

Table 2.4. Lead and cadmium concentration values in the different wine samples by means of DLLME-ETAAS and ICP-MS.

Type of wine	**Pb (µg L^{-1})***		**Cd (µg L^{-1})***	
	DLLME-ETAAS	**ICP-MS**	**DLLME-ETAAS**	**ICP-MS**
White	2.0±0.2	1.8±0.1	< 0.01	
Rosé	2.17±0.16	2.14±0.07	< 0.01	
Sparkling	1.75±0.15	2.0±0.1	< 0.01	
Fortified	1.8±0.3	1.7±0.1	< 0.01	
Red	2.7±0.2	2.7±0.2	< 0.01	

*Mean ± standard deviation, 5 replicates concentration levels in the tested wine samples agreed with those previously reported [28]. Cadmium concentration levels in all the samples tested were below the LoD of both DLLME-ETAAS and ICP-MS (Table 2.4).

4. Conclusions.

Results reported in this work demonstrate that DLLME using ionic liquids is a very simple and useful sample-pre-treatment methodology for accurate determination of Cd and Pb in wine samples by ETAAS. Cadmium extraction in $BmimPF_6$ is strongly affected by the presence of Zn in wine samples. As a consequence, the use of a standard addition calibration strategy is mandatory for this element. Under optimum conditions, the LoDs obtained for Cd and Pb are lower than those previously reported in ETAAS using conventional sample preparation strategies (i.e., sample dilution, digestion and direct analysis). These results highlight the great potential of DLLME for metal analysis in wine but further research is still required mainly to improve the method selectivity (especially for Cd) and LoDs.

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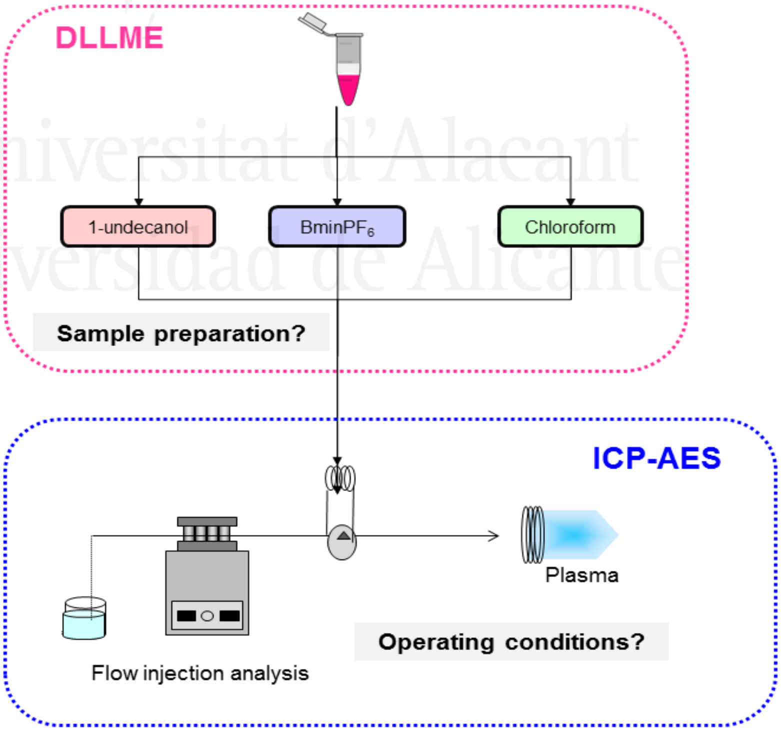
CAPÍTULO 3

COUPLING DISPERSIVE LIQUID-LIQUID MICROEXTRACTION TO INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION SPECTROMETRY: AN OXYMORON?



1. Introduction.

Dispersive liquid-liquid micro-extraction (DLLME) has been stablished as a fast and green sample preparation methodology to separate and preconcentrate analytes from samples with complex matrices [1]. Though most of the analytical applications reported in the literature have been focused on organic analytes, DLLME has also been employed for metal and non-metal analysis by means of spectroscopic techniques [2]. In these cases, detection is usually accomplished by means atomic absorption spectrometry (AAS) due to the robustness of the atomization sources (i.e. flame or furnace) and to the reasonably good analytical figures of merit [3,4]. Among the AAS techniques, electrothermal atomic absorption spectrometry (ETAAS) is often preferred because of the limited sample volume available after DLLME procedures. Nevertheless, its low sample throughput limits the application of the technique.

Inductively coupled plasma (ICP)-based techniques (i.e., ICP- atomic emission, ICP-AES, and ICP- mass spectrometry, ICP-MS) have a greater analytical potential than AAS techniques. ICP-based techniques allow simultaneous multi-element analysis, thus increasing sample throughput with minimum sample and reagent consumption as well as waste generation. However, due to the limited volume available after the microextraction procedure and low plasma tolerance to the organic solvents usually employed for analyte extraction [2,5], DLLME is not usually employed coupled to ICP-based techniques [6,7]. Thus, high volatile solvents (such as carbon tetrachloride, chloroform, etc.) have a deleterious effect on plasma conditions that negatively affects analytical figures of merit and could even lead to plasma extinction. Also, high viscous solvents (e.g. 1-undecanol or ionic liquids), cause additional problems arising from a poor nebulization efficiency and strong memory effects. To address these shortcomings, several strategies have been suggested in the literature: (i) solvent evaporation and subsequent acid reconstitution before the analysis [8,9]; (ii) water back-extraction [10,11]; (iii) dilution with an appropriate solvent [12–15]; and (vi) the use of alternative sample introduction systems such as flow injection analysis (FIA) [16], electrothermal vaporization [17] or laser ablation [18]. Nevertheless, despite the above-mentioned approaches make feasible DLLME coupling to ICP-based techniques, some severe drawbacks still remain. In fact, the main inherent benefits of DLLME (e.g. simplicity, high sample throughput, etc.) are mostly counterbalanced due to the additional steps required to perform the analysis. Moreover, some approaches may require complex modifications in the ICP configuration that are not always available in most of the analytical laboratories.

From the above-mentioned considerations, it can be derived that DLLME-ICP-based techniques is a rather complex coupling and, hence, usually discarded from a practical point of view. However, a comprehensive review of the works reported in this field reveals that most of the previous studies have been mainly focused on the optimization of the extraction procedure. Nevertheless, no studies including the optimization of the experimental and instrumental conditions of the plasma source have been performed up to date. This is a very surprisingly fact taking into account the strong influence of the ICP parameters (e.g. plasma power, sample uptake rate, nebulizer gas flow rate, etc.) and the sample introduction system on the analytical figures of merit [5,19,20]. In our opinion, to couple DLLME-ICP-based techniques, the optimization of the full variables (experimental and instrumental) of both DLLME and ICP is mandatory. The extensive number of applications based on the use of plasma-based techniques for elemental analysis in organic matrices [19] justify the interest of this coupling.

The goal of this work is to explore and evaluate different analytical approaches for coupling DLLME to ICP-AES. To this end, several organic solvents usually employed in DLLME procedures and covering different range of the main physical properties affecting the signal response in ICP-AES (i.e., viscosity and volatility) have been selected: 1-undecanol, 1-butyl-3-methyl-imidazolium hexafluorophosphate and chloroform. These solvents have been introduced (either directly or after a dilution step with alcohol or acid solutions) into the plasma source by means of a flow injection device. Main ICP-AES experimental variables (plasma r.f. power, nebulizer gas flow rate and carrier flow rate) have been also optimized to make feasible the analysis of these organic solvents by ICP-AES and to achieve the best analytical figures of merit. Finally, the proposed DLLME-ICP-AES approaches have been compared and evaluated by analyzing several water samples (i.e. marine, tap and river).

2. Experimental.

2.1. Chemicals.

Organic solvents (i.e., 1-undecanol, chloroform, 1-butyl-3-methylimidazolium hexafluorophosphate, acetone, methanol, absolute ethanol and 1-propanol) and chelating agents (i.e. diethyldithiocarbamate (DDTC), 2-theonyltrifluoroacetone (TTA), ammonium pyrrolidine dithiocarbamate (APDC) and a multi-element 200 mg L^{-1} organometallic solution were purchased from Sigma-Aldrich (Steinheim, Germany). Sodium chloride, 69% w w^{-1} nitric acid, 36% w w^{-1} hydrochloric acid, 85% w w^{-1} phosphoric acid, sodium dihydrogen phosphate, acetic acid and sodium acetate were obtained from Panreac (Barcelona, Spain). An ICP-IV multi-element 1000 mg L^{-1} solution was provided by Merck (Darmstadt, Germany).

2.2. Solutions.

Three different extractant solvents, namely: (i) 1-undecanol; (ii) 1-butyl-3-methyl-imidazolium hexafluorophosphate (BmimPF$_6$); and (iii) chloroform, have been evaluated. These solvents were selected, among the most common extractants in DLLME, to face most of the main problems arising from DLLME-ICP-AES coupling (e.g. solvent viscosity and volatility) (see Table 3.1) [21–23].

Table 3.1. Physical properties of the organic solvents tested in this work at 25ºC.

	Surface tension (mN m^{-1})	**Viscosity (mPa s^{-1})**	**Density (g mL^{-1})$^{\&}$**	**Vapor pressure (mmHg)**
1-undecanol	26.5	17.2	0.83	0.0004
BmimPF$_6$	47.7	381	1.38	-
Chloroform	26.7	0.56	1.49	26.2
Methanol	22.7	0.54	0.79	128
Ethanol	22.0	1.07	0.79	59
1-propanol	20.9	1.95	0.80	21
HCl (36% w w^{-1})	65.8	1.84	1.6	35

$^{\&}$20ºC

When operating with viscous solvents (1-undecanol or BmimPF$_6$), a dilution step prior to the analysis by ICP-AES was mandatory. Thus, 1-undecanol was diluted in different alcohols, namely, methanol, ethanol and 1-propanol. This approach was also employed for $BmimPF_6$ but, in this case, 36% w w^{-1} hydrochloric acid solution was additionally tested as a dilution solvent. Physical properties of the different dilution solvents employed for 1-undecanol and $BmimPF_6$ are also gathered in Table 3.1. Dilution ratios ranging from 1:0.5 to 1:3 for both 1-undecanol and $BmimPF_6$ were tested. Analyte standard solution in each media was prepared in two-steps. First, an aliquot of the aqueous 1000 mg L^{-1} ICP-IV multi-elemental reference solution was spiked into the proper dilution solvent (i.e. methanol, ethanol, 1-propanol or 36% w w^{-1} hydrochloric acid solution). Next, this solution was mixed in the right proportion with 1-undecanol or $BmimPF_6$ for a final analyte concentration of 1 mg L^{-1}. Chloroform-based samples were directly analyzed by ICP-AES. Therefore, analyte chloroform standard (1 mg L^{-1}) was prepared by diluting the appropriate aliquots of a 200 mg L^{-1} multi-elemental organometallic solution in this solvent.

2.3. Instrumentation.

ICP-AES measurements were performed using an Agilent 720 ICP-AES (Agilent, Santa Clara, USA) with axial viewing using the operating conditions reported in Table 3.2. Different sample introduction systems were tested depending on the characteristics of the extractant solvent used. Thus, when operating with both 1-undecanol and chloroform, a standard sample introduction system made of a concentric pneumatic nebulizer (Seaspray, Glass Expansion, Australia) and a cyclonic spray chamber (Cinnabar, Glass Expansion, Australia) was used. As regards $BmimPF_6$, a micronebulizer (OneNeb, Ingeniatrics, Sevilla, Spain) coupled to a single-pass with impact bead PTFE spray chamber (Thermo Scientific, Germany) was employed. All the organic solvents were drived to the nebulizer by means of a V-451 flow injection manifold (Upchurch Scientific, Silsden, United Kingdom) equipped with a 25 µL loop valve. DLLME extracts were injected using a home-made 300 µL plastic syringe with PEEK coated quartz capillary needle (200 µm i.d., PEEKSIL, Upchurch, Oak Harbor, Washington, USA). Samples were introduced into a carrier stream controlled by a peristaltic pump (Model Minipuls 3, Gilson, France). Different carriers were evaluated through this work: (i) 1% w w^{-1} HNO_3; (ii) 1% w w^{-1} HCl; and (iii) air.

Table 3.2. ICP-AES operating conditions.

	Solvent		
Agilent 720 ICP-AES	**1-undecanol**	**$BmimPF_6$**	**Chloroform**
Plasma forward power (W)	1400		
Argon flow rate (L min^{-1})			
Plasma	15.0		
Auxiliary	1.25		2.50
Nebulizer	0.6-0.9		0.5-0.8
Carrier flow rate (mL min^{-1})	0.4-1.5		0.4-1.0
Sample introduction system			
Nebulizer	Seaspray®	Seaspray®/Oneneb®	Seaspray®
Spray chamber (material)	Cyclonic (Glass)	Cyclonic (Glass)/Single pass with impact bead (PFA)	Cyclonic (Glass)
Flow injection loop volume (µL)	25		
Acquisition time (s)/replicates	30/3		

Signal acquisition was performed by means of the transient signal (TRS) software of Agilent's ICP-AES. Microsoft Excel® software was employed for manually signal integration. The wavelengths of the emission lines monitored in this work are listed in Table 3.S1 (Appendix).

2.4. Samples.

Three water samples covering a wide range of matrix characteristics were tested: (i) tap water (University of Alicante); (ii) river water (Vinalopó river, N 38º28´15.0096", W 0º48'15.0336"); and (iii) marine water (Mediterranean Sea, N 38º22´31.7424", W 0º24'32.5224"). All samples were collected in polyethylene terephthalate bottles and, after a filtration step with a 0.45 µm syringe filter, acidified and stored at 4ºC until the analysis.

2.5. DLLME procedures.

Three DLLME procedures for water analysis were employed to evaluate the different coupling strategies developed in the present work. Next, these methodologies are briefly described.

<u>1-undecanol-based extraction.</u>

Metal extraction with 1-undecanol was carried out using the DLLME procedure described by Yamini et al. [12] with some minor modifications. A sample solution of 5 mL was placed into a 10 mL screw-cap and pH was adjusted using an acetic acid-acetate buffer solution (pH: 6). Next, it was spiked with 600 µL of a 30 w v^{-1} NaCl solution and 25 µL of a 1 g L^{-1} TTA solution in methanol. Simultaneously, 50 µL of 1-undecanol were mixed with 500 µL of acetone and the mixture quickly injected into the sample solution by using a 5.0 mL of syringe. A cloudy solution was formed and, after a centrifugation step (5 min 4000 rpm), the sample solution was transferred into an ice bath where the 1-undecanol (≈45 µL) was solidified at the top of the top of the test tube. Finally, 1-undecanol was transferred into an Eppendorf tube where it was melted and diluted with methanol (1:0.5 ratio) before ICP-AES analysis.

<u>BmimPF$_6$-based extraction.</u>

In this procedure, based on that reported by Wen et al. [24], 5 mL of the sample were spiked with a phosphoric/dihydrogen phosphate buffer solution to adjust the pH (4). Next, 70 mg of NaCl and 100 µL of a 9% APDC solution added to the sample. A mixture of BmimPF$_6$ (150 mg) and methanol (600 µL) was injected into the sample to form a cloudy solution. The sample was then centrifuged (5 min 4000 rpm) and BmimPF$_6$ (settled at the bottom of the glass test tube) finally transferred into an Eppendorf tube, where it was diluted with methanol (1:0.5 proportion) before ICP-AES analysis.

<u>Chloroform-based extraction.</u>

In this procedure, based on that previously described by Hemmatkhah et al. [25], 5 mL of water containing 3.5% w w^{-1} NaCl and 0.010 g DDTC were placed in a 10 mL screw cap glass tube with conical bottom. The solution pH was adjusted to 6.0 with an acetic acid/acetate buffer solution. Next, 100 mg of chloroform were dissolved in 400 µL of ethanol and the mixture was injected into the glass tube containing the sample. A cloudy solution is formed and, after centrifugation (4000 rpm, 2 minutes), chloroform was sedimented at the bottom of the conical test tube. Chloroform was then transferred into an Eppendorf tube and directly analyzed by ICP-AES.

3. Results.

3.1. Coupling 1-undecanol-based DLLME procedures to ICP-AES.

3.1.1. Strategies for 1-undecanol introduction into the ICP.

Direct analysis of 1-undecanol by ICP-AES is not a feasible task due to its high viscosity (17.2 mPa s^{-1}, see Table 3.1). As a consequence, this solvent cannot be properly displaced by the peristaltic pump, thus giving rise to poor signal reproducibility and high wash-out times. In addition, when operating with pneumatic nebulizers, high viscosity solvents generate coarser aerosols than the low viscous ones thus negatively affecting the aerosol transport into the plasma and, then, the analytical signal [5,26]. To solve these problems and make feasible the introduction of 1-undecanol solutions in ICP-AES, several strategies were evaluated. First, since solvent viscosity decreases with temperature, 1-undecanol was heated (from 40-70ºC) before being injected into the FIA system. Though sample pumping improved increasing the temperature, memory effects were still significant. Alternatively, PTFE tubing from the peristaltic pump to the nebulizer was also heated but no improvement was observed. A second approach reported in the literature to deal with 1-undecanol matrix samples by ICP-AES is its dilution with alcohols [12,14]. Up to date, no systematic study about the influence of the alcohol nature and concentration used for 1-undecanol dilution have been carried out, in spite of the strong influence of the sample matrix on the analytical figures of merit in ICP-AES [5,27]. In the present work, methanol, ethanol and 1-propanol were investigated as dilution solvents for 1-undecanol. Different 1-undecanol: alcohol mixtures (ranging from 1:0.5 to 1:3) containing 1 mg L^{-1} analyte were prepared and measured by ICP-AES (Figure 3.1). In general, mixture composition did not have a significant influence on the analytical signal. Signals for the 1:0.5 and the 1:1 mixtures were very similar but their peak areas were around 1.2-fold lower than those obtained for the 1:2 and 1:3 ones. Similar findings were observed when using ethanol and 1-propanol. These results suggest that alcohol dilution slightly improves the aerosol generation due to the reduction of the solution viscosity. Nevertheless, since the 1-undecanol dilution factor is not too high, no significant differences are expected between the different mixtures tested. From these experiments, the 1:0.5 mixture was selected for further analysis since it provides the minimum sample dilution after the DLLME treatment.

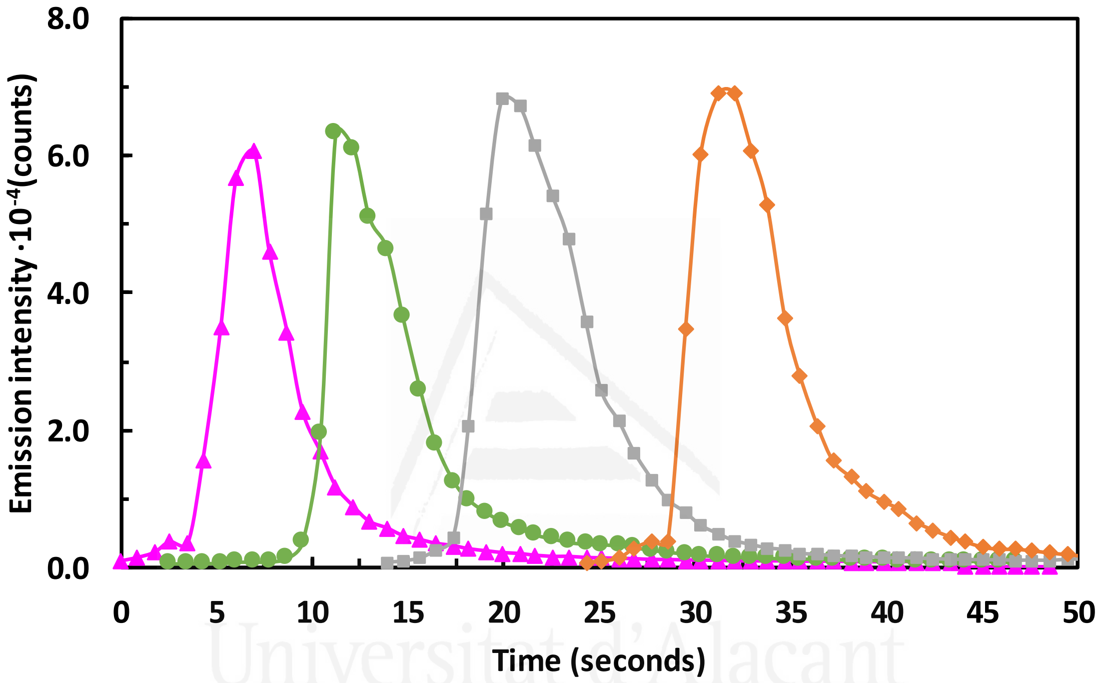


Figure 3.1. Cd II 214.439 nm emission signal profile for different 1-undecanol:methanol mixtures. (▲) 1:0.5; (●) 1:1; (■) 1:2; and (◆) 1:3. Q_g: 0.7 L min^{-1}; Q_l: 0.6 mL min^{-1}. FIA carrier: 1% w w^{-1} HNO_3.

The proposed dilution factor is lower than that previously reported in the literature (i.e., usually ≥ 1:1) [12,14]. In general, the type of alcohol employed to dilute 1-undecanol had a limited influence on the analyte emission signal (Figure 3.2). Emission signals obtained for the methanol mixture were about 15% higher, on average, than those obtained with ethanol or 1-propanol. This behavior was the expected considering the highest volatility of methanol (Table 3.1). Plasma robustness was checked by means of the Mg II (280.271 nm)/Mg I (285.213 nm) line intensity ratio for the different 1-undecanol/alcohol mixtures [28]. In all cases, Mg II/Mg I ratio were similar to that obtained for water, thus suggesting that the presence of 1-undecanol/alcohol mixtures did not affect plasma temperature.

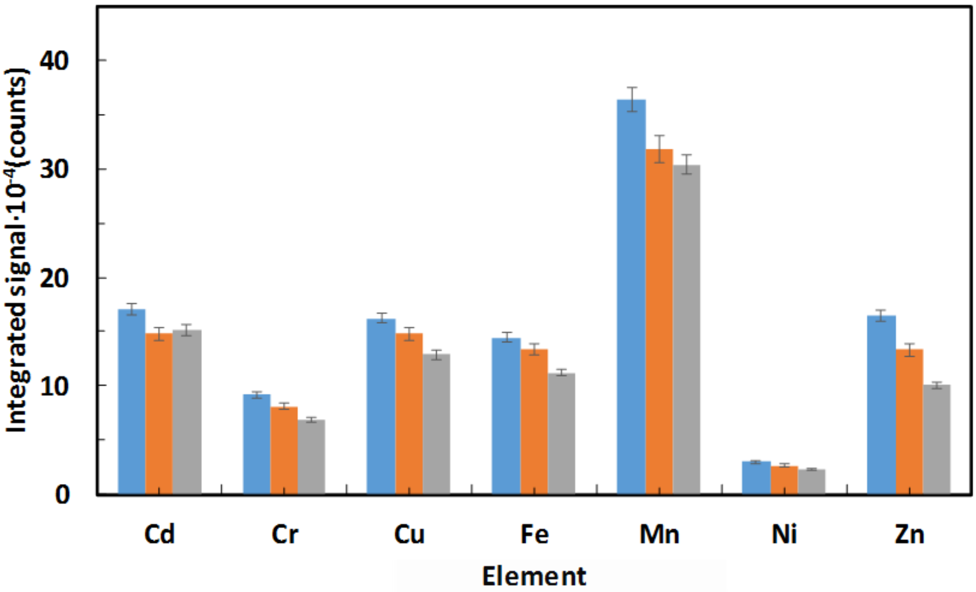


Figure 3.2. Influence of the alcohol employed for 1-undecanol dilution on the integrated emission signal of several elements. Q_g: 0.7 L min^{-1}; Q_l: 0.6 mL min^{-1}; 1-undecanol/ alcohol ratio: 1:0.5; FIA carrier: 1% w w^{-1} HNO_3. (🟦) Methanol; (🟧) ethanol;(⬜) 1-propanol.

Finally, the influence of the carrier nature on the signal obtained in DLLME-ICP-AES was also evaluated. Up to date, poor attention has been paid in the literature to optimize the FIA experimental conditions required for 1-undecanol analysis by ICP-AES, mainly regarding the nature of the carrier solution. In fact, with the exception of Yamini et al. [12] that report the use of 80% 1-propanol v v^{-1} as a carrier, no significant details about carrier characteristics are found in the literature [11]. Despite its potential interferences [29], acids are preferred over organic solvents in ICP-AES since they can be directly introduced into the plasma avoiding the use of complex instrumental arrangements (e.g. oxygen addition, desolvation, etc.) [19,27]. Nevertheless, no study reporting the use of acids as carrier solutions have been found for the analysis of DLLME extracts by ICP-AES. In the present work, 1% w w^{-1} nitric acid and 1% w w^{-1} hydrochloric acid solutions were tested as carriers instead of organic solvents for the first time. Results demonstrated that acid solutions can be successfully used to introduce 1-undecanol/alcohol mixtures into the plasma with high reproducibility and no memory effect. No differences between the signals afforded with both acids were registered.

3.1.2. Optimization of ICP-AES experimental conditions.

Analytical figures of merit in ICP-based techniques strongly depend on plasma experimental conditions. For this reason, the influence of the nebulizer gas (Q_g) and carrier flow rate (Q_l) on analyte signal was carried out. Plasma r.f. power was kept closed to maximum nominal value available with the instrument (1400 W) to favor analyte atomization and ionization. Figure 3.3 shows the influence of Q_g on Cd II 214.439 nm integrated emission signal for the 1:0.5 1-undecanol: methanol mixture at different Q_l values. Results indicate that the highest emission signals were obtained at Q_g of 0.7 L min^{-1}, irrespective of the Q_l tested. Moreover, results in this figure also indicate that the highest emission signals were obtained at the lowest Q_l employed. Thus, Cd II 214.439 nm integrated emission signal raises 2.3-fold when decreasing Q_l from 1.5 to 0.6 mL min^{-1} at the optimum Q_g. Similar results were observed for the different alcohol mixtures and analytes tested. This behavior can be explained in terms of aerosol generation and transport [26] and plasma characteristics [27]. Thus, the Mg II/Mg I ratios measured at 0.6 and 1.5 mL min^{-1} were of 6.0 and 5.0, respectively.

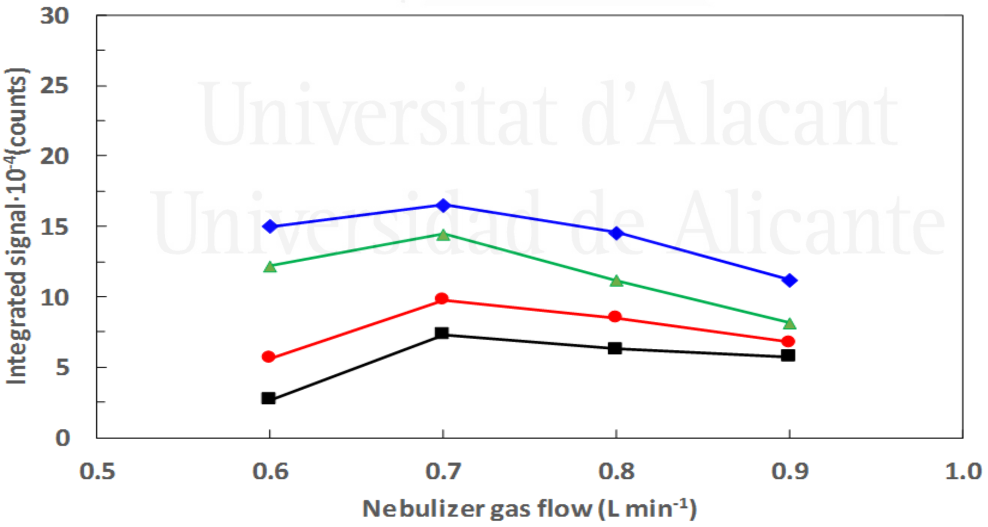


Figure 3.3. Influence of the nebulizer gas flow rate on Cd II 214.439 nm integrated emission signal operating 1:0.5 1-undecanol: methanol mixture at different Q_l. (◆) 0.6 mL min^{-1}; (▲) 0.9 mL min^{-1}; (●) 1.2 mL min^{-1}; and (■) 1.5 mL min^{-1}. FIA carrier: 1% w w^{-1} HNO_3.

3.2. Coupling BminPF$_6$-based DLLME procedures to ICP-AES.

3.2.1. Strategies for BminPF$_6$ introduction into the ICP.

When operating with BmimPF$_6$, similar (or even worse) experimental drawbacks than those described for 1-undecanol are observed. In fact, the viscosity of BmimPF$_6$ is higher (about 22-fold higher) than that of 1-undecanol (Table 3.1). Therefore, a dilution step with an appropriate solvent previous to the BmimPF$_6$ solutions into the plasma is also mandatory. Following a similar approach used with 1-undecanol, BmimPF$_6$ could be diluted with methanol, ethanol and 1-propanol in different proportions (ranging from 1:0.5 to 1:3). Different BmimPF$_6$ methanol mixtures (1:0.5 to 1:3) containing 1 mg L^{-1} analyte were measured to evaluate matrix influence on the signal. It was observed that when operating the 1:1 mixture, signal was about 60% higher than that obtained with the 1:0.5 one. Signals for the highest diluted BmimPF$_6$ ratios (1:2 or 1:3) were similar to those for the 1:1 ratio. These findings suggest the beneficial effects of the BmimPF$_6$ dilution on the aerosol generation due to the reduction of solution viscosity. This behavior is in agreement with the previous findings obtained with 1-undecanol but the influence of dilution on analyte emission signal for BminPF$_6$ was more significant due to its higher viscosity. The signal improvement registered for the 1:1 mixture did not compensate the analyte dilution factor expected when operating the 1:1 dilution instead of the 1:0.5 one (2-fold). Therefore, the 1:0.5 dilution was selected for further studies. As expected from the low dilution factors employed for BmimPF$_6$, no significant differences on analyte signals were registered operating the different BmimPF$_6$ mixtures (Figure 3.S1, Appendix). Thus, for instance, the use of methanol as a diluent solvent provided the highest signals but signal improvement when compared to ethanol and 1-propanol was only 1.2 fold. This value is similar to that previously found with 1-undecanol.

Attempting to find alternatives to alcohols for BmimPF$_6$ dilution, the possibility of using hydrochloric acid was explored in this work for the first time. Preliminary experiments demonstrated that when mixing 1:1 BmimPF$_6$: concentrated hydrochloric acid (36 % w w^{-1}), a single phase was obtained after 4-5 hours at room temperature. Interestingly, the mixture viscosity was clearly lower than that of the pure BmimPF$_6$. Several experimental evidences suggest that the structure of the ionic liquid is modified in the presence of hydrochloric acid. The ionic liquid/acid mixture has a brownish color similar to that of the hexafluorophosphoric acid solutions [30]. This compound is not stable in aqueous media and it is found in equilibrium with phosphoric acid, phosphoric conjugate forms and hydrofluoric acid [31]. Thus, assuming the formation of hexafluorophosphoric acid from the reaction between BmimPF$_6$ and hydrochloric acid, hydrofluoric acid must be formed. This hypothesis was clearly confirmed after checking a glass surface that was in contact with a 1:1 BmimPF$_6$:HCl mixture for 15 minutes. Finally, it was also observed that blank signals for DLLME extracts operating glass vials were systematically higher than those obtained operating plastic ones. The miscibility of BmimPF$_6$ with hydrochloric acid was further investigated by modifying acid concentration and BmimPF$_6$: acid ratio. Results indicate that BmimPF$_6$ was only miscible with concentrated hydrochloric acid, regardless the acid proportion employed (from 1:0.5 to 1:3). Finally, several strategies were tested to improve the miscibility kinetics between BmimPF$_6$ and hydrochloric acid. First, the different ionic liquid – hydrochloric acid mixtures were shaken either with a vortex or ultrasounds but unsuccessfully. Better results were obtained when heating the BmimPF$_6$/HCl mixture in Eppendorf tubes at temperatures between 40-70ºC. Operating this way, a single phase was obtained after heating the mixture at 70ºC for 5 min (fast enough for practical purposes). It is worth to point out that concentrated nitric acid is also useful for BmimPF$_6$ dilution but it was discarded due to the flammability and oxidizing capabilities of BmimNO$_3$. Because of HF presence in the BmimPF$_6$: acid mixture, the standard ICP-AES sample introduction system was replaced by a nebulizer and a spray made of PTFE. For the sake of comparison, this configuration was also employed with the experiments performed with BmimPF$_6$ alcohol solutions. Also the plasma torch is susceptible to be affected by the presence of HF. Nevertheless, after several hours operating BmimPF$_6$: hydrochloric acid mixtures no sign of HF attack was observed. Presumably, it was due to the low sample volume introduced and the system wash-out with the carrier solution. Although at first glance, physical properties of BmimPF$_6$: hydrochloric acid mixtures are expected to be less favorable for aerosol generation and transport, analyte signals for the BmimPF$_6$: acid mixtures were almost identical to those afforded by ethanol and 1-propanol (Figure 3.S1, appendix). Plasma robustness was examined for the different BmimPF$_6$ mixtures but no significant differences in the Mg II/Mg I intensity ratio were observed. In fact, the value obtained for this parameter was similar to that found when operating with water (and, hence, 1-undecanol: alcohol mixtures). Considering the above-discussed results, it seems to be clear that the use of hydrochloric acid for $BmimPF_6$ dilution does not afford any advantage against the use of alcohols. Moreover, the standard glass-made sample introduction system could be used instead of the PTFE since no HF is formed when diluting $BmimPF_6$ with alcohols.

As regards the nature of the carrier solutions, similar to that observed with 1-undecanol, nitric acid and hydrochloric acid solutions can be employed as carriers for $BmimPF_6$ analysis. The use of inorganic acid solutions as carrier is clearly simpler and less prone to interferences than that previously proposed by Ranjbar et al. [16] for metal analysis with 1-hexyl-3-methyl imidazolium bis(trifluoromethylsulfonyl)imide (i.e. 80% v v^{-1} 1-propanol solution). In fact, no significant memory effects were registered for the different diluted $BmimPF_6$ mixtures operating the acid carriers. Analyte wash out was similar to that obtained with 1-undecanol (25-30 s).

3.2.2. Optimization of ICP-AES experimental conditions.

The influence of ICP experimental conditions (Q_g and Q_l) on the analyte signal (and plasma properties) obtained when operating with $BmimPF_6$ were analogous to those already shown for 1-undecanol (Fig. 4.2). Thus, despite of the use of a different sample introduction system, the optimum Q_g for $BmimPF_6$ was also found at 0.7 L min^{-1}. Interestingly, it was observed that the influence of Q_l on analyte signal was less significant than with 1-undecanol. Regardless the solvent employed for $BmimPF_6$ dilution, analyte signal rose approximately 1.6-fold when decreasing Q_l from 1.5 to 0.6 mL min^{-1}. This behavior can be attributed to the higher viscosity of $BmimPF_6$ mixtures regarding to 1-undecanol ones.

3.3. Coupling chloroform-based DLLME procedures to ICP-AES.

3.3.1. Strategies for chloroform introduction into the ICP.

Opposite to that occurring with 1-undecanol and $BmimPF_6$, the low viscosity of chloroform permits it to generate pneumatic aerosols with no additional dilution treatment. Nevertheless, the direct analysis of chloroform DLLME extracts by ICP-

AES has been previously avoided in the literature due to the undesirable effects caused by this solvent in ICP-AES (mainly signal instability and negative effects on the plasma excitation characteristics) [16]. Instead, additional pretreatments to remove chloroform have been recommended before metal analysis by ICP (e.g. back extraction, evaporation, etc.) [8–10,15]. In this work, however, it was noted that chloroform could be directly introduced in the ICP with the FIA manifold. The volume of chloroform introduced into the instrument (25 µL) was low enough to avoid carbon deposits and plasma shutdown. Nevertheless, it is important to note that plasma appearance was affected by the high solvent load consequence of the high chloroform volatility (e.g. green light emission from the C_2 band in the aerosol channel) [5]. With the goal of improving the analytical response, some minor changes were performed on the ICP-AES operating conditions employed with viscous solvents [19]. First, auxiliary gas flow was increased from 1.25 to 2.25 L min^{-1} thus improving plasma tolerance to organics [5]. On the other hand, Q_l higher than 1.0 mL min^{-1} were not employed to avoid plasma flickering. Operating on this way, there is not any experimental limitation to operate chloroform directly in ICP-AES thus taking advantage all the benefits of DLLME (e.g. sample throughput, simplicity, analyte enrichment factors, etc.).

3.3.2. Optimization of ICP-AES experimental conditions.

In line with the strategy used with the viscous solvent, either 1% w w^{-1} nitric and hydrochloric acids were employed as carriers for chloroform. In addition, air was also tested since it could be advantageous for volatile solvent introduction into the ICP [32,33]. Figure 3.4 shows the results obtained for Cd II 214.439 nm integrated emission signal using chloroform and both 1% w w^{-1} nitric acid and air as FIA carriers. Results for 1% w w^{-1} hydrochloric acid are not shown since they were similar to those obtained with the nitric acid solution. From results in Figure.3.4 it can be derived that emission signal strongly depends on the carrier employed. Thus, when operating air as carrier, Cd signals (Figure 3.4.B) were higher than those obtained for 1 % w w^{-1} HNO_3 (Figure 3.4.A), regardless the Q_l tested. These findings could be explained considering that when using air: (i) the analyte is not dispersed in the liquid stream, (ii) the spray chamber is kept dry between injection which in turns favor solvent evaporation and aerosol transport to the plasma; and (iii) analyte losses due to coalescence and aerosol turbulence are reduced. Nevertheless, from a practical point of view, the benefits of using air as a carrier were counterbalanced by the higher memory effects due to the lack of a solution to wash-out the system between samples during the analysis [32].

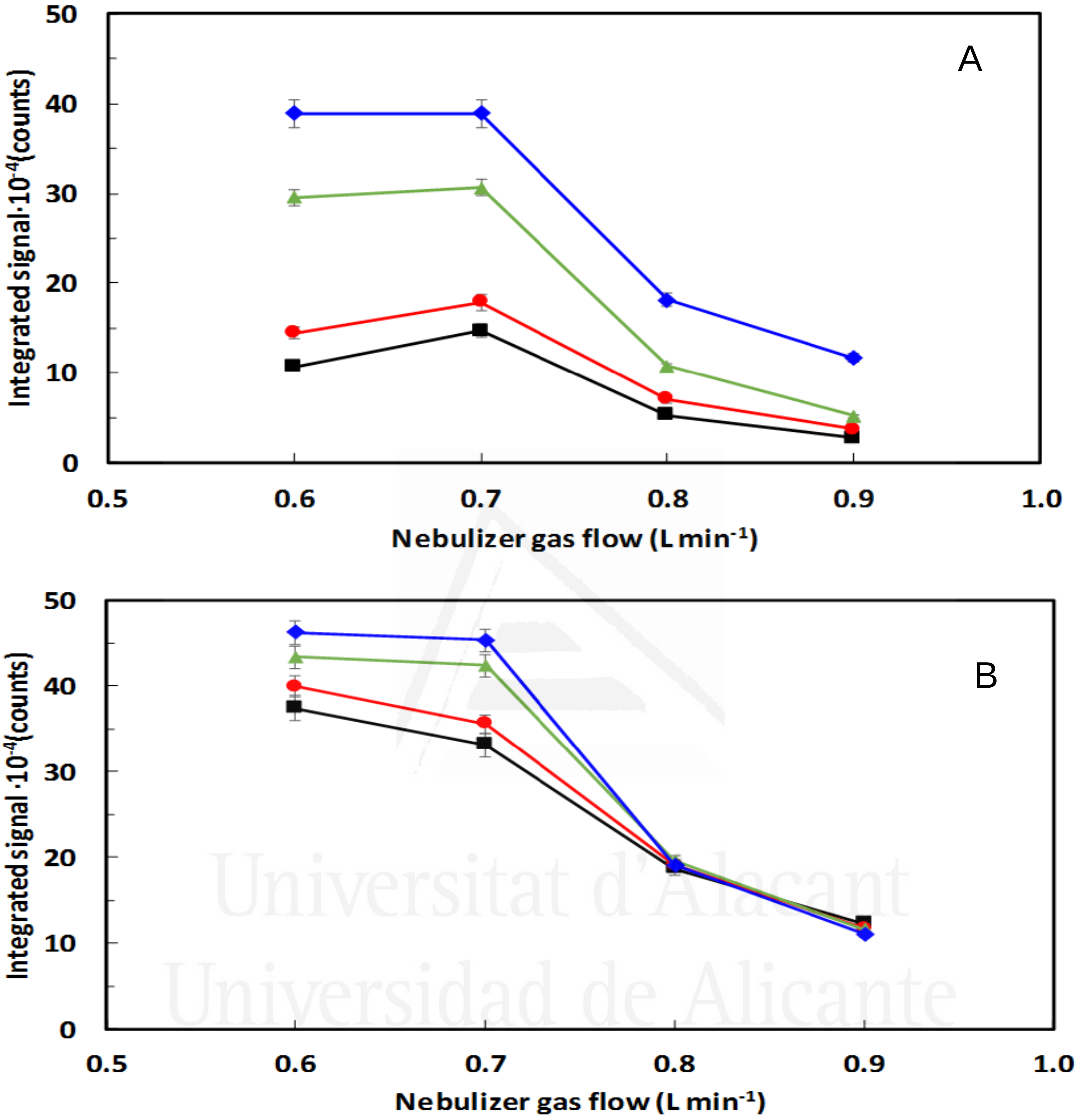


Figure. 3.4. Influence of the nebulizer gas flow rate on Cd II 214.439 nm integrated emission signal with chloroform at different sample uptake rate using 1% w w^{-1} nitric acid (A) and air (B) as FIA carriers. (◆) 0.4 mL min^{-1}; (▲) 0.6 mL min^{-1}; (●) 0.8 mL min^{-1}; and (■) 1.0 mL min^{-1}. FIA carrier: 1% w w^{-1} HNO_3.

Figure 3.4 also shown that, in general, irrespective of the carrier used, the optimum Q_g for Cd signal was also found at 0.7 L min^{-1} but this optimum value was more diffuse than that observed with viscous solvents (Figure 3.3). In fact, as it can be seen in Figure 3.4, a signal plateau was obtained between 0.6 and 0.7 L min^{-1} for some Q_l values, especially when operating with air carrier. Finally, as expected, signal improved when decreasing Q_l due to a better aerosol generation and transport and plasma characteristics [27]. It is interesting to note that differences between both carriers were reduced when decreasing Q_l [33]. Thus, when Q_l is decreased from 1.0 to 0.4 mL min^{-1}, analyte signal ratio between air and nitric acid passed from 1.84 to 1.17-fold. To explain this behavior, it must be considered that liquid evaporation is favored at low Q_l values, thus improving analyte transport (i.e. less aerosol losses) and, hence, differences between air and liquid carriers are reduced. Though the use of air as a carrier afforded higher signals than the acid solutions, this approach was unattractive from a practical point of view due to memory effects. Opposite to that observed when operating viscous solvents, and despite the experimental changes made on the ICP setup, plasma characteristics were strongly deteriorated by the presence of chloroform. Thus, for a given set of experimental conditions, the MgII/MgI ratio was half of that obtained with water, 1-undecanol or BmimPF$_6$.

3.4. Analysis of real samples.

The multi-element analysis (i.e., Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) of different water samples was performed to validate the strategies developed for coupling DLLME to ICP-AES. To this end, previously described DLLME procedures for 1-undecanol [12], BmimPF$_6$ [24] and chloroform [25] were employed for metal extraction in waters. Next, each organic extract was analyzed by ICP-AES under the optimum conditions obtained for each solvent (Table 3.3). Q_l was set at 0.4 mL min^{-1} for all the solvents tested to favor aerosol generation and plasma characteristics. Though better results are theoretically expected decreasing further this parameter, nebulization process becomes less stable [20] thus distorting the emission signal profile and reducing signal precision. Moreover, higher wash-out times were required compromising sample throughput.

Table 3.3. Optimum coupling strategies for the analysis of 1-undecanol, BmimPF$_6$ and chloroform extracts by ICP-AES.

Parameter	**1-undecanol**	**BmimPF$_6$**	**Chloroform**
Dilution Solvent	Methanol		-
Dilution ratio	1:0.5		-
Carrier	1 % w w^{-1} HNO$_3$		
Q_g (L min^{-1})	0.7		
Q_l (mL min^{-1})	0.4		

Preliminary experiments showed that analyte extraction efficiency was strongly dependent on the water salt content and, hence, some changes in the extraction procedures were required. To study the influence of water salts (ionic strength) on analyte extraction for each DLLME procedure, 100 µg L^{-1} analyte standard solution containing variable amounts of NaCl from 0.1 to 7.5 % w w^{-1} were used. Results shown that each DLLME methodology is differently affected by NaCl. Thus, analyte extraction efficiency for the 1-undecanol-based DLLME procedure decreased with NaCl concentration. On the other hand, extraction efficiency showed a maximum at 3.5 % w w^{-1} NaCl when operating with chloroform. Interestingly, no influence of NaCl concentration on the analyte extraction efficiency was observed when using BmimPF$_6$. These results suggest that NaCl content (i.e. solution ionic strength) exerts a great influence on metal extraction since it affects both the solubility of the metal-chelate complex in the sample as well as the miscibility between the organics and water. From these experiments, it was clear the significance of controlling salt content to avoid interferences. Taking into account these findings, both standards and samples were spiked with NaCl 3.5% w w^{-1} for all the DLLME procedures to perform calibration using a single set of standards.

First, a recovery test was performed to evaluate the accuracy. To this end, all the samples were spiked with a multi-element standard solution for a final concentration of 100 µg L^{-1} and, then, they were analyzed by ICP-AES after the appropriate DLLME treatment. Results obtained are shown in Table 3.4. As it can be observed, recoveries for all the elements with 1-undecanol and chloroform were almost quantitative (i.e., recoveries ranging from 96 to 109%). However, analyte recoveries for BmimPF$_6$ were only quantitative for Pb. It must be considered that the BmimPF$_6$-based DLLME procedure used in the present work was initially developed for Pb determination and, hence, results for this element were totally expected. The origin of the poor recoveries for the remaining elements could be partially related to pH influence on APDC chelating capabilities [34]. This topic, however, was not further investigated since it was beyond the scope of this work.

Table 3.5, shows the results of the elemental analysis of water samples obtained using the 1-undecanol and chloroform-based DLLME procedures. For the sake of comparison, the results obtained with a direct water analysis by ICP-AES are also included. Data for the BmimPF$_6$-based DLLME procedure are not included since it only worked for Pb and the concentration of this element was below LoD (<5 µg L^{-1}). In fact, none of the methodologies tested could detect Pb as well as Cd and Cr due to their low concentration levels in the samples analyzed. In general, results for the elemental analysis using DLLME procedures agree with those obtained using a direct analysis procedure. Nevertheless, the use of DLLME methodologies allowed the analysis of a higher number of elements (e.g. Al, Fe, etc.) in water samples due to their lower limits of detection (Table 3.6). In comparison with a direct water analysis, DLLME methodologies afford, on average, a LoD improvement of 8 and 13-fold when operating with 1-undecanol and chloroform, respectively. These results confirm the usefulness of the coupling strategies evaluated for the analysis of DLLME extracts by ICP-AES. It is important to remark that the improvement in the analytical figures of merit reported for DLLME-ICP-AES was related to two different factors: (i) the preconcentrating process itself; and, (ii) the higher analyte transport efficiency afforded when using organic solvents in ICP-AES. To evaluate the contribution of aerosol generation and transport with organics on the analytical figures of merit (sensitivity and LoD), the corresponding calibration curve for organics and water were compared (Table 3.S2, Appendix). The use of 1-undecanol and BmimPF$_6$ improved sensitivity and LoD 2.2-fold on average for the different elements tested. LoD improvement for chloroform was also similar (2.8-fold) but less than expected according to signal enhancement factors (6.5-fold on average) due to high blank signals originated by the chloroform impurities. Therefore, a higher improvement in LoDs for chloroform is still feasible improving reagent quality. Nonetheless, different commercial chloroform providers were tested but similar backgrounds were observed in all cases.

Table 3.4. Recoveries obtained for analyte spiked water samples using different DLLME procedures based on 1-undecanol, $BmimPF_6$ and chloroform extraction. ICP-AES: Q_g: 0.7 L min^{-1}, Q_l:0.4 mL min^{-1}. FIA carrier: 1% w w^{-1} HNO_3.

	Recoveries values (%)								
	1-undecanol			**$BmimPF_6$**			**Chloroform**		
Element	**Sea**	**Tap**	**River**	**Sea**	**Tap**	**River**	**Sea**	**Tap**	**River**
Al	102±4	99±2	100±4	<5	<5	<5	99±4	100±2	99±5
Cd	99±3	101±3	98±3	<5	14±10	14±10	103±3	102±3	109±3
Cr	97±5	97±4	98±3	<5	23±13	<5	100±1	103±3	99±3
Cu	100±3	98±4	98±3	<5	19±13	<5	98±3	99±3	98±2
Fe	99±4	98±4	98±4	<5	<5	<5	100±4	99±5	100±3
Mn	101±4	100±3	101±3	70±10	80±20	60±10	99±2	97±3	95±5
Ni	100±3	100±4	98±3	<5	15±9	14±7	99±4	98±4	102±3
Pb	99±5	96±5	98±4	99±5	96±6	96±7	98±4	97±3	99±2
Zn	100±2	98±4	100±4	<5	<5	<5	99±4	98±4	102±4

Table 3.5. Results of the analysis of water samples in ICP-AES using 1-undecanol and chloroform-based DLLME procedures as well as direct sample analysis (no preconcentration).

	Concentration (μgL^{-1})								
	1-undecanol			BmimPF$_6$			Chloroform		
Element	**Sea**	**Tap**	**River**	**Sea**	**Tap**	**River**	**Sea**	**Tap**	**River**
Al	<150	215±14	<150	75±2	226±5	135±4	71±4	220±5	135±4
Cu	230±20	145±6	350±9	207±4	150±4	331±4	208±3	140±4	335±3
Fe	<90	<90	<LoD	61±4	59±5	23±3	64±2	57±1	24±5
Mn	240±20	<LoD	<LoD	238±3	<LoD	<LoD	229±3	<LoD	<LoD
Ni	400±5	130±7	140±12	407±2	111±5	140±2	410±5	110±3	136±3
Zn	100±15	85±7	<60	98±2	73±4	56±4	97±4	72±4	53±4

Precision is presented in form of confidence intervals obtained as *ts* where *t* is the Student's *t* (4.3 for a 95% confidence level) and *s* is the standard deviation of three replicates of the analysis

Table 3.6. Limits of detection (LoD) in ICP-AES for direct sample analysis and 1-undecanol and chloroform-based DLLME procedures. ICP-AES: Q_g: 0.7 L min^{-1}, Q_l:0.4 mL min^{-1}; 1-undecanol dilution: methanol; 1:0.5 ratio; FIA carrier: 1% w w^{-1} HNO_3.

	Limit of detection (μg L^{-1})		
Element	**Direct analysis**	**1-undecanol-DLLME**	**Chloroform-DLLME**
Al	50	6	35
Cd	30	6	1.4
Cr	40	6	6
Cu	30	2	7
Fe	30	4	4
Mn	40	22	6
Ni	30	8	0,7
Pb	20	4	1.1
Zn	20	3	2

4. Conclusions.

Results in this work clearly demonstrate that there is not any limitation for coupling DLLME to ICP-AES when experimental conditions are wisely selected. In fact, despite the different physical properties shown by the organic solvents usually employed in DLLME, a single set of experimental conditions can be employed for metal analysis. In addition, it should be taking into account that analytical figures of merit in ICP-AES are not only improved by the DLLME treatment process but also to aerosol generation and transport afforded by the organics regarding to water. When compared to FAAS and ETAAS detection, the use of ICP-AES makes feasible the simultaneous analysis of different metals thus improving sample throughput. In addition, internal standardization calibration could be implemented to improve accuracy and precision as well as to mitigate potential matrix effected derived by the organics in the plasma.

It is expected that the strategies developed in this work could also be applied for ICP-MS. Nonetheless, special attention should be paid in this case to the spectral and non-spectral interferences due to carbon since ICP-MS is more sensitive to matrix effects. In fact, the use of organics could be beneficial to further improve the analytical figures of merit since the ionization of some hard-to-ionize elements (e.g. As, Se, etc.) is improved by carbon presence in the plasma [35]. These experiments are currently being carried out in our laboratories.

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6. Appendix.

Table 3.S1. Spectroscopic data values for the selected lines tested

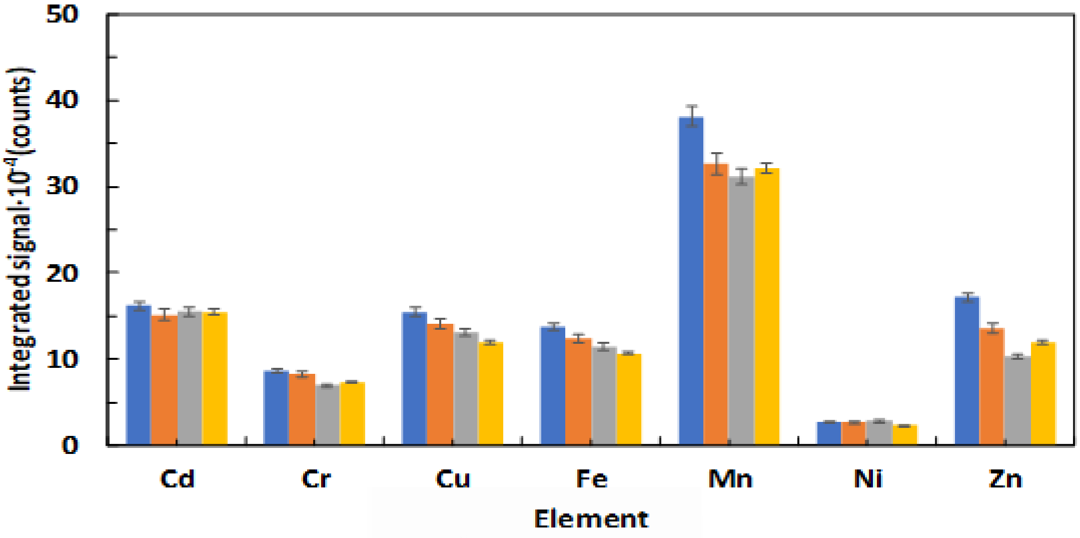
Element	Wavelenght/ nm (line type)	E_{exc}/ eV	E_{ion}/ eV	E_{sum}/ eV
Al	396.152 (I)	3.14	-	3.14
Cd	214.439 (II)	5.78	8.99	14.77
Cr	357.868 (I)	3.46	-	3.46
Cu	213.598 (II)	8.52	7.73	16.25
Fe	238.204 (II)	5.20	7.87	13.07
Mg	280.271 (II)	4.42	7.72	12.07
	285.213 (I)	4.35	-	4.35
Mn	257.610 (II)	4.81	7.44	12.25
Ni	231.604 (II)	6.39	7.64	14.03
Pb	220.353 (II)	7.37	7.42	14.79
Zn	213.857 (I)	5.80	-	5.80

E_{sum} = ionization energy (E_{ion}) + excitation energy (E_{exc})

Table 3.S2. Sensitivity and LoD enhancement factors operating organic solvent when compared to water. ICP-AES: Q_g: 0.7 L min^{-1}, Q_l:0.4 mL min^{-1}.

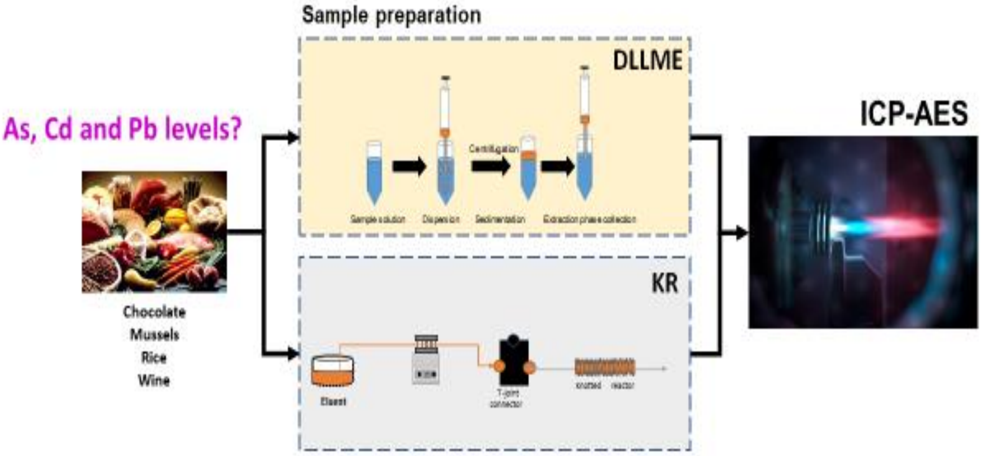
	Sensitive enhancement factor			LoD enhancement factor		
Element	**1-undecanol**	**$BmimPF_6$**	**Chloroform**	**1-undecanol**	**$BmimPF_6$**	**Chloroform**
Al	1.9	2.6	8.2	1.9	2.0	1.4
Cd	1.4	1.7	6.3	1.8	1.8	3.5
Cr	3.4	2.6	7.5	3.2	2.2	3.6
Cu	2.5	2.6	8.4	2.0	2.3	4.0
Fe	2.5	2.5	8.3	2.0	1.8	2.7
Mn	2.0	2.5	5.1	1.8	2.3	2.3
Ni	1.8	2.9	4.0	1.7	2.0	3.2
Pb	2.7	2.4	5.0	2.3	1.8	2.5
Zn	2.1	1.9	6.0	1.9	2.2	2.0

Figure 3.S1. Influence of the solvent employed for $BmimPF_6$ dilution on the integrated emission signal of several elements. Q_g: 0.7 L min^{-1}; Q_l: 0.6 mL min^{-1}; 1-undecanol/ solvent ratio: 1:0.5; FIA carrier: 1% w w^{-1} HCl. (🟨) HCl; (◼) 1-propanol; (🟧) ethanol; (🟦) methanol.



CAPÍTULO 4

SIMULTANEOUS DETERMINATION OF As, Cd AND Pb IN FOOD SAMPLES BY MEANS OF INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION SPECTROMETRY: A COMPARISON BETWEEN KNOTTED-REACTOR EXTRACTION AND DISPERSIVE LIQUID-LIQUID MICROEXTRACTION.



1. Introduction.

Inductively coupled plasma atomic emission spectrometry (ICP-AES) is widely employed in food sciences for major, minor and trace elemental analysis due to its outstanding figures of merit such as: (i) accuracy and precision; (ii) limits of detection in the order of µg L^{-1}; (iii) dynamic range; and (iv) multi-element capabilities [1]. Since the conventional sample introduction system in ICP-AES operates with liquid samples, a preliminary preparation step is required to analyze foods. Solid samples are usually analyzed after solubilization by an acid digestion treatment. Though beverages can be directly introduced into the plasma, they also require a preliminary sample pretreatment (i.e. filtration, dilution and even acid digestion) to mitigate both spectral and non-spectral interferences [1]. Determination of toxic elements in foods, such as: As, Cd, Pb, etc. by means of ICP-AES is more troublesome since detection capabilities achieved by commercial instrumental techniques are mostly close to (or even above) the maximum allowed levels established by current international food security policies, particularly those from EU [2]. Consequently, after sample decomposition, an additional extraction-preconcentration treatment is mandatory for accurate quantitation of toxic elements in foods.

However, coupling extraction-preconcentration techniques to ICP-AES for metal analysis is challenging [3]. Organic solvents or organic-water mixtures are usually employed in solid-liquid and liquid-liquid extraction procedures, but they could negatively affect the plasma discharge giving rise to both spectral and non-spectral interferences and could even lead to plasma extinction [4,5]. Depending on the extraction-preconcentration methodology, several strategies have been proposed to deal with the interferences caused by organic compounds in ICP-AES such as: (i) solvent evaporation followed by acid reconstitution [6]; (ii) water back-extraction [7]; (iii) dilution with an appropriate solvent [8]; and (iv) the use of non-conventional sample introduction systems (e.g. electrothermal vaporization) [9]. Nevertheless, these strategies increase the complexity of analysis, rise costs and reduce sample throughput. Moreover, they do not benefit of physicochemical properties of organics for aerosol generation and transport. Due to their lower surface tension and higher volatility, organic solvents generate finer aerosol and give rise to higher aerosol transport than aqueous solutions [10,11].

Consequently, an improvement on sensitivity and limits of detection (LoD) is expected. Martinez et al. [12] have recently demonstrated that some organic extracts used for dispersive liquid-liquid microextraction (DLLME) (e.g. chloroform, 1-undecanol and 1-butyl-3-methylimidazolium hexafluorophosphate) can be directly analyzed by means of ICP-AES. These authors observed that LoD were indeed enhanced by the preconcentration itself, but also, by a higher sample introduction efficiency observed due to the injection of organic solvents into the plasma.

The goal of this work was to evaluate the analytical capabilities of ICP-AES for the simultaneous determination of As, Cd and Pb in food samples according to current EU policies. To this end, knotted reactor (KR) and DLLME were selected for analyte extraction-preconcentration, since these techniques have been scarcely applied for toxic metal determination in foods with ICP-AES detection. The study included a throughout optimization of ICP-AES experimental conditions for direct analysis of organic extracts. Next, KR and DLLME extraction conditions were optimized for the simultaneous determination of As, Cd and Pb in a single run, taking advantage of ICP-AES multi-element capabilities. Finally, KR-ICP-AES and DLLME-ICP-AES methods were validated and applied to several food samples (i.e. chocolate, mussels, rice and wine) representative of the commodities regulated by EU food policies.

2. Experimental.

2.1. Chemicals.

Tetrahydrofuran (THF, 99%), acetone (≥99.5%) and methanol (≥99.9%) were obtained from Honeywell (New Jersey, USA). 1-decanol (99%), 1-undecanol (99%), decanoic acid (98%), acetonitrile (99%), sodium citrate tribasic dihydrate (99%), ammonium pyrrolidine dithiocarbamate (APDC) (99.9%) and elemental standard solutions (1000 mg L^{-1}) were purchased from Sigma-Aldrich (Steinheim, Germany). Absolute ethanol (99.9%), nitric acid (69% w w^{-1}), sodium dihydrogen phosphate (≥99.5%), sodium chloride (≥99.5%), disodium hydrogen phosphate (≥99.5%), glacial acetic acid (99.7%), sodium acetate (99%) and 1-propanol (≥99.5%) were obtained from Panreac (Barcelona, Spain). Finally, citric acid (≥99.5%) was obtained from VWR (Radnor, USA).

2.2. Samples.

Four different food samples covering different type of matrices were analyzed in this work: (i) mussels (*Mytilus edulis chilensis,* Chile); (ii) rice (La Fallera, Spain); (iii) red wine (Caño Viejo, Spain, alcoholic content: 10% w w^{-1}) and (iv) chocolate (Nestle, Spain, cocoa content: 44% w w^{-1}). These foods are representative of the commodities regulated for toxic metals by the EU [2].

2.3. Sample preparation.

In this work, except for wine, all the samples were acid digested prior the extraction-preconcentration step by means either KR or DLLME. Mussels, rice and chocolate samples were digested in a microwave oven model Start D (Milestone, Italy) using the program recommended by the manufacturer (Table 4.S1). Before the digestion treatment, mussels were dried at 60ºC for 48h and all the samples were grinded (Ultracomb, China). After sample decomposition, digests were quantitatively transferred into a 20mL volumetric flask and neutralized with a NaOH solution. Finally, samples (digest and untreated wine) were handled with the corresponding sample extraction/preconcentration treatment. Experimental conditions for both KR and DLLME were optimized by means of experimental design [13]. Data analysis was carried out using Statgraphics® centurion 16.1.11 32-bit software (Statpoint Technologies, USA).

2.3.1. Knotted reactor extraction.

Contrary to previous works in ICP-AES [14], the KR was operated off-line. An overview of KR operation is shown in Figure 4.1. First, a sample solution of 4 mL was placed into a 10 mL vial and pH was adjusted with the corresponding buffer solution (i.e. acetic acid/sodium acetate, dihydrogenphosphate/ hydrogenphosphate, etc.). Next, the sample (flow rate 2.0 mL min^{-1}) and APDC solution (flow rate 0.5 mL min^{-1}) were mixed with the aid of a T-joint and the mixture was loaded on an PTFE knotted reactor (i.d. 500 µm, 334 cm length) in which the analyte-APDC complex is retained. Next, the complex was eluted with 150 µL of an appropriate organic solvent (i.e. methanol, ethanol, 1-propanol, acetic acid and acetonitrile) and transferred to an Eppendorf tube. Finally, a cleaning step was carried out using a 3% w w^{-1} nitric acid solution, which was circulated for 2 minutes at a rate of 3 mL min^{-1}.

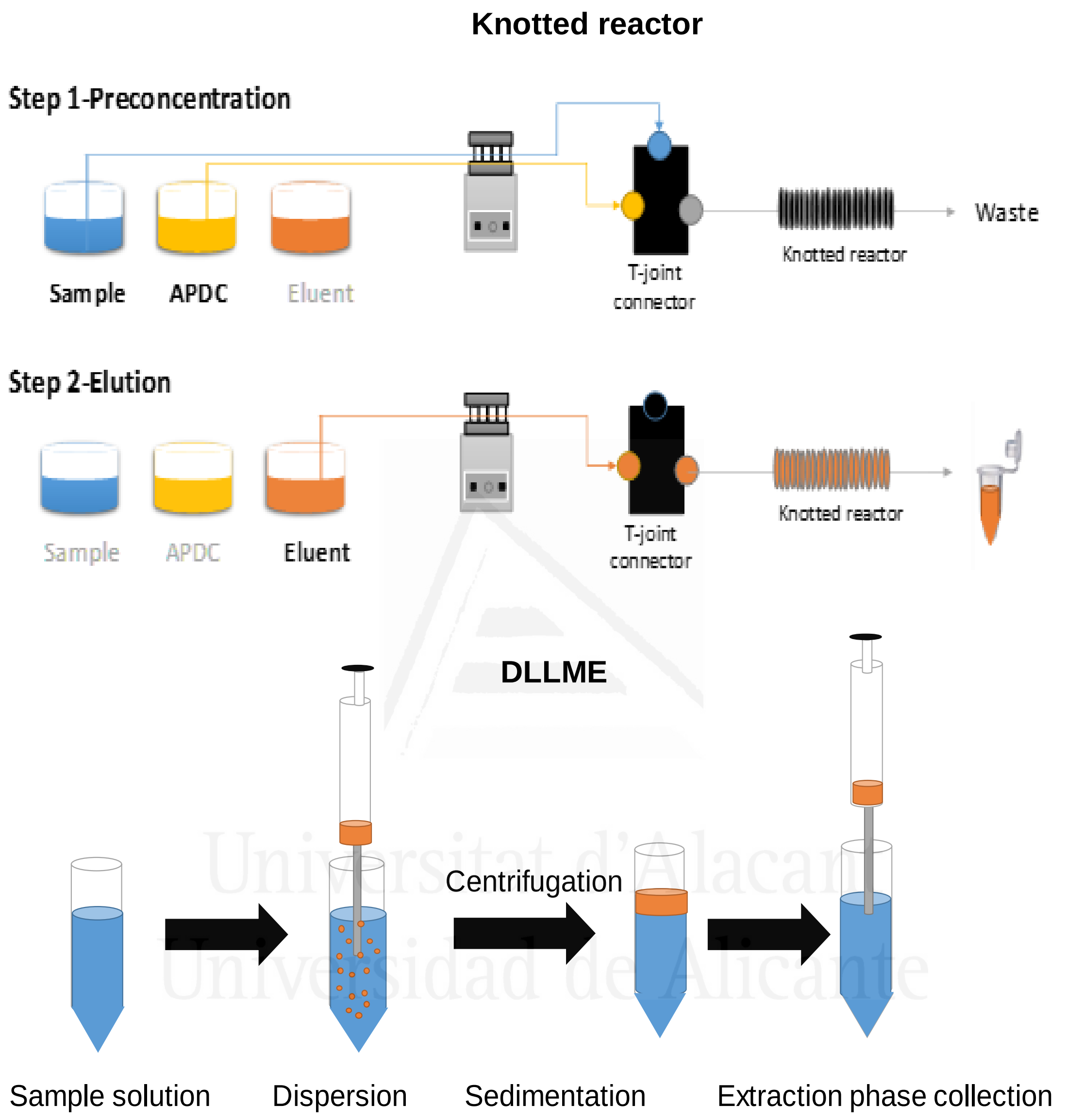


Figure. 4.1. Schematic diagram of the experimental procedure employed with KR and DLLME.

2.3.2. Dispersive liquid–liquid microextraction.

Supramolecular solvents have been selected as the extraction media since they are more environmentally friendly than the traditional volatile organic solvents (e.g. chloroform, etc.) employed in DLLME. These solvents are nanostructured liquids spontaneously generated from aqueous or hydro-organic solutions of amphiphiles through a self-assembly process known as coacervation [15,16]. In this work, three supramolecular solvents have been generated by combining THF with different surfactants (i.e. 1-decanol, 1-undecanol and decanoic acid). Figure 4.1 shows a scheme of the DLLME experimental procedure. Sample aliquots of 4 mL were spiked with a buffer solution to adjust the pH and 100 µL of APDC (2%) were added for complex formation. Next, a mixture of THF and the corresponding surfactant was added to the sample with a 2.5 mL glass syringe (Hamilton s/1000, USA). A cloudy solution was formed and, after centrifugation (90 s at 3500 rpm), the micelle upper layer was transferred to an Eppendorf tube, where it was diluted prior to ICP-AES analysis due to its high viscosity. To this end, different dilution solvents (i.e. methanol, ethanol, 1-propanol, acetonitrile and acetic acid) and supramolecular:dilution solvent ratios (1:0.5 to 1:3) were investigated.

2.4. Instrumentation.

ICP-AES measurements were performed using an Agilent 720 ICP-AES instrument (Santa Clara, USA) with axial viewing, under the operating conditions reported in Table 1. Sample introduction was achieved using a concentric pneumatic nebulizer (Seaspray, Glass Expansion, Australia) and a cyclonic spray chamber Cinnabar, Glass Expansion, Australia). Samples were introduced into the system with the aid of a flow injection manifold (Model V-451, Upchurch Scientific, Silsden, United Kingdom) equipped with a 25 µL loop valve and a homemade 300 µL plastic syringe with a PEEK coated quartz capillary needle (200 µm i.d., PEEKSIL, Upchurch, Oak Harbor, Washington, USA). Samples were introduced into a 1% w w^{-1} HNO_3 carrier stream controlled by a peristaltic pump (Model Minipuls 3, Gilson, France). As I 193.696 nm, Cd II 214.439 nm and Pb II 220.353 nm were the monitored wavelengths for each analyte. Signal acquisition was performed by means of the transient signal (TRS) software of the ICP-AES instrument. Microsoft Excel® software was employed for manual signal integration.

Table 4.1. ICP-AES operating conditions

Plasma forward power (W)	**1400**
Argon flow rate (L min^{-1})	
Plasma	15.0
Auxiliary	1.5
Nebulizer	0.6-0.9
Carrier flow rate (mL min^{-1})	0.4-1.3
Sample introduction system	
Nebulizer	Sea Spray
Spray chamber	Cyclonic
Flow injection loop volume (µL)	25
Acquisition time (s)	50
Replicates	4

For comparison, digested samples and untreated wine were also analyzed by means of ICP-MS. Instrumental and experimental details for ICP-MS measurements are described elsewhere [17].

3. Results.

3.1. Knotted-reactor extraction.

3.1.1. Coupling KR to ICP-AES.

When KR-based systems are used coupled to atomic absorption spectrometry, organic solvents are traditionally employed as the elution solvent to favor analyte-complex solubility [12]. In ICP-based techniques, however, inorganic eluents are preferred instead to avoid the negative effects of organic compounds on the plasma [18,19]. In this work, the possibility of applying organic solvents with KR in ICP-AES was investigated since these solvents favor aerosol generation and analyte transport, thus improving analytical figures of merit in comparison to inorganic acid solutions [12]. To this end, several modifications were introduced on the experimental arrangement traditionally employed in ICP-AES [14]. First, the KR was operated off-line, resulting in a higher flexibility regarding experimental conditions (e.g. carrier flows, organic solvents, etc.). On the other hand, to improve plasma stability operating with organic solvents, eluates from the KR were introduced into the ICP-AES by means of a FIA-manifold using a 1% w w^{-1} nitric acid solution as the carrier medium [12].

First, the influence of the elution solvent on analyte signal in ICP-AES was evaluated. To this end, a 2 mg L^{-1} analyte standard solution was preconcentrated within the KR according to the experimental procedure described in Section 2.4. Next, the analyte-complex was eluted with 150 µL of different organic solvents (i.e. methanol, ethanol, 1-propanol, acetonitrile and acetic acid) and the extracts were analyzed directly by means of ICP-AES. For the sake of comparison, a standard sample in 10% w w^{-1} nitric acid solution was also registered. Figure 4.2 shows Cd II 214.439 nm signal profiles with the different organic eluents tested. It was observed that, when operating with 1-propanol, acetic acid and 10% w w^{-1} nitric acid solution, the plasma was highly robust, and the analyte signal profile was well-defined. For the remaining matrices (i.e. methanol, ethanol and acetonitrile), however, plasma was unstable and strong memory effects were observed. These results are easily explained considering that plasma energy operating with volatile solvents is significantly reduced due to the higher solvent plasma load [20,21]. As expected by physicochemical properties (i.e. surface tension) [10,11,21], analyte signals for 1-propanol and acetic acid solutions were significantly higher than those obtained with 10% w w^{-1} nitric acid. Signal improvements operating with 1-propanol and acetic acid were, on average, 1.7 and 1.8-fold, respectively. Based on these experiments, acetic acid was selected as the analyte elution solvent with the KR.

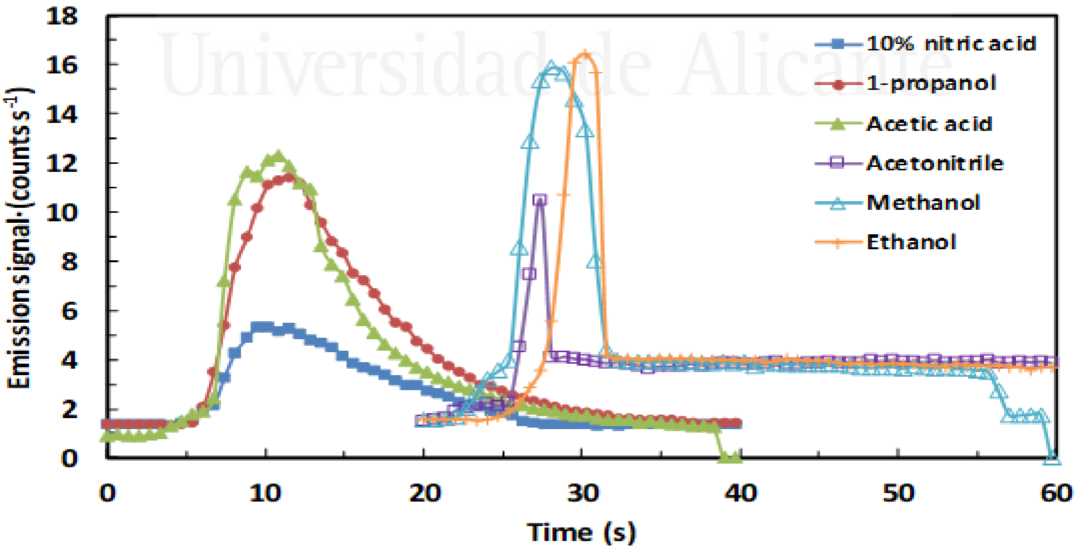


Figure 4.2. Influence of the elution solvent on the Cd II 214.439 nm emission signal profile with KR. ICP-AES operating conditions: Q_g: 0.7 L min^{-1}; Q_l: 0.4 mL min^{-1}. Analyte concentration: 2 mg L^{-1}. (▲) Acetic acid; (●) 1-propanol; (■) nitric acid; (—) ethanol; (△) methanol; (□)

3.1.2. Optimization of ICP-AES experimental conditions.

Inductively coupled plasma atomic emission experimental conditions were optimized to operate with acetic acid extracts. Plasma r.f. power was kept close to the maximum available instrumental nominal value (i.e. 1400 W) to favor matrix decomposition as well as analyte atomization, ionization and excitation within the plasma. To evaluate the influence of the nebulizer gas flow rate (Q_g) and sample uptake rate (Q_l) on signal emission, a 1 mg L^{-1} analyte standard solution in acetic acid was prepared. Figure 4.3 shows the influence of Q_g on Cd II 214.439 nm integrated signal for acetic acid at different Q_l values. Results indicate that analyte emission is favored when decreasing both Q_g and Q_l. This behavior can be explained in terms of aerosol generation and plasma robustness [14]. Similar findings were observed for As I 193.696 nm and Pb II 220.353 nm. From data gathered in Figure 4.3, an optimum Q_g of 0.6 L min^{-1} and a Q_l of 0.4 mL min^{-1} were selected to analyze acetic acid extracts. These conditions allowed the long-term ICP-AES operation with acetic acid eluates without formation of carbon deposits on the torch.

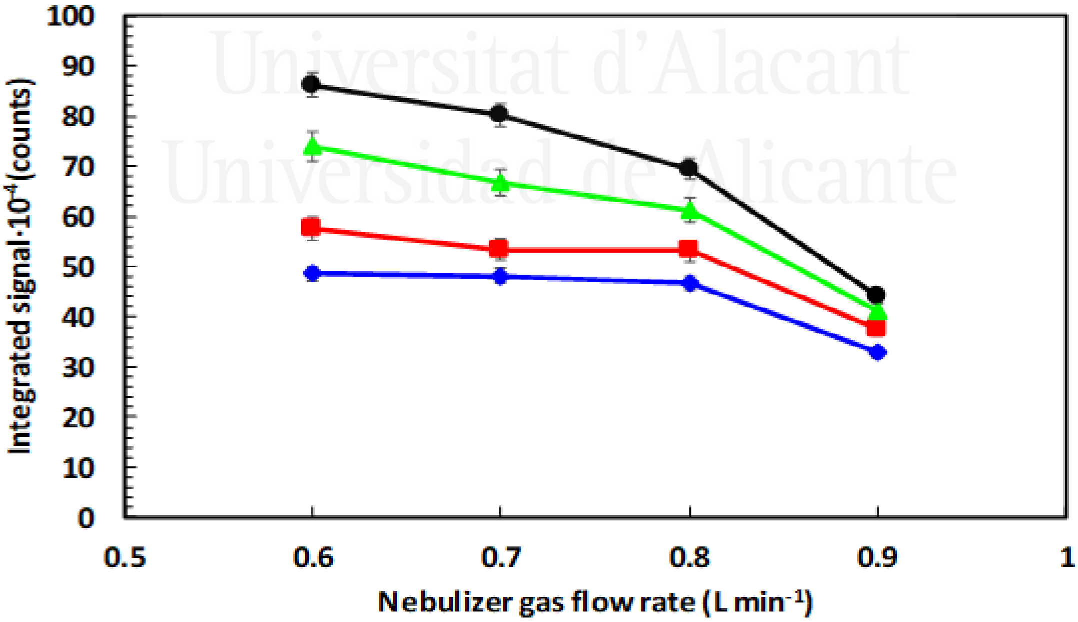


Figure 4.3. Influence of the nebulizer gas flow rate on Cd II 214.439 nm integrated emission signal operating acetic acid at different Q_l with KR: (●) 0.4 mL min^{-1}; (▲) 0.7 mL min^{-1}; (■) 1 mL min^{-1}; and (◆) 1.3 mL min^{-1}. Analyte concentration: 1 mg L^{-1}.

3.1.3. Optimization extraction conditions with KR.

The experimental variables controlling metal extraction in the KR were optimized by means of a central composite design (CCD) [14]. After checking previous studies in the literature [14,18,19] and some preliminary experiments, pH, APDC concentration, KR length and sample elution flow were identified as the most significant variables controlling As, Cd and Pb extraction. Each variable was investigated in five levels: (i) pH. 9 (++), 7 (+), 5 (0), 3 (-) and 1 (--); (ii) APDC concentration: 1.65 % w w^{-1} (++), 0.8 % w w^{-1} (+), 0.53 % w w^{-1} (0), 0.27 % w w^{-1} (-) and 0.005 % w w^{-1} (--); (iii) reactor length: 334 cm (++), 256 cm (+), 178 cm (0), 100cm (-) and 22 cm (--); and (iv) elution flow rate: 0.56 mL min^{-1} (++), 0.45 mL min^{-1} (+), 0.34 mL min^{-1} (0), 0.23 mL min^{-1} (-) and 0.11 mL min^{-1} (--). A total of 25 experiments were performed by triplicate using a standard 1 mg L^{-1} analyte solution (Table 4.S2).

No conclusive results were obtained during the optimization of As extraction since ICP-AES signals were not significantly affected by changes in the KR extraction conditions. Alternatively, As concentration was increased up to 15 mg L^{-1} to improve optimization, but no significant improvement was achieved. This behavior is explained considering that As-APDC complex is poorly retained within the KR (i.e. 18%) [23] and that the sensitivity of As in ICP-AES measurements is low. Therefore, and taking into account legal requirements for this element in foods [2], the analysis of As by means of KR-ICP-AES was discarded. To examine the significance of each variable on Cd and Pb extraction, data was analyzed by ANOVA and the effects were summarized by means of the corresponding Pareto charts (Figure 4.4). The ANOVA data analysis revealed that pH, APDC concentration and reactor length have a (positive) significant effect on Cd and Pb extraction. These results are logical, considering that metal-APDC complex formation is favored by increasing chelate concentration and decreasing solution pH [24]. Similarly, a higher reactor length favors metal-chelate complex retention in the KR.

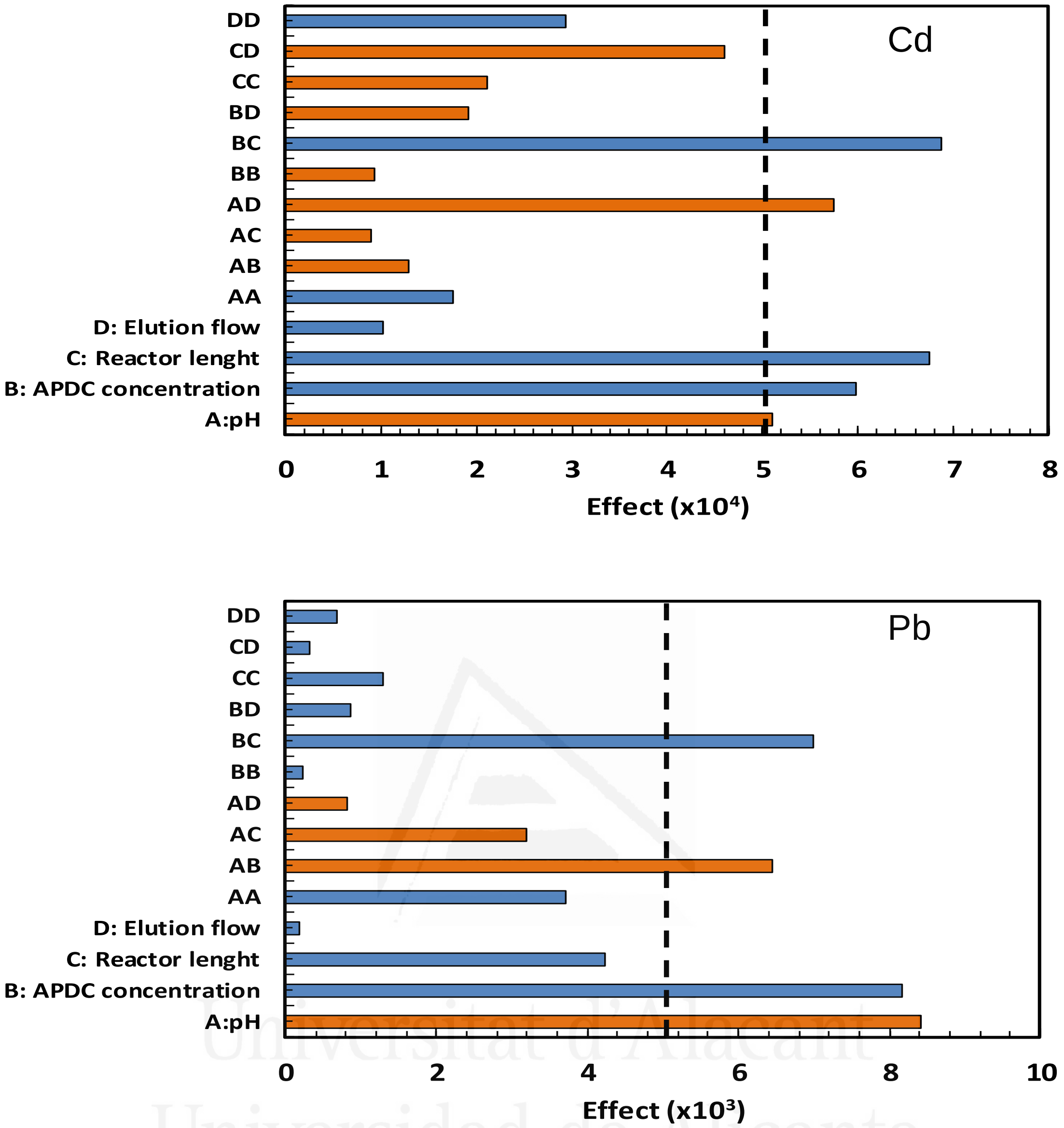


Figure 4.4. Pareto charts obtained in the optimization study of the main variables affecting Cd and Pb extraction with KR. (🟦) positive effect; (🟧) negative effect. Dotted vertical line corresponds to 95% confidence level. ICP-AES operating conditions: Q_g: 0.7 L min^{-1}; Q_l: 0.4 mL min^{-1}. Analyte concentration: 1 mg L^{-1}.

Experimental data also revealed that there are some interactions among the investigated variables. Thus, Cd extraction significantly depended on two-factor interactions effects, pH/elution flow rate and APDC concentration/reactor length. On the other hand, Pb extraction significantly depended on pH/APDC concentration and APDC concentration/reactor length. Table 4.2 shows the optimum experimental conditions derived from the CCD model for Cd and Pb extraction. In general, optimal experimental conditions for both elements were rather similar, although some differences were noticed on the pH and the elution flow rate. Because of the simultaneous multi-elemental capabilities of ICP-AES, a compromise pH and elution flow rate should be selected for the simultaneous determination of both elements in a single run. From the CCD model, it was predicted that Cd and Pb extraction would be reduced in 5% when operating at pH 1.2 and with an elution flow rate of 0.3 mL^{-1}. This data was experimentally verified and, consequently, the above-mentioned pH and elution flow rate were selected accordingly for further studies.

Table 4.2. Compromise experimental conditions for the simultaneous analysis of Cd and Pb by means of KR.

	Cd	Pb	Compromise
pH	1	1.4	1.2
APDC concentration (w w^{-1})	1.06	1.06	1.06
Elution flow rate (mL min^{-1})	0.4	0.11	0.32
Reactor length (cm)	333	334	334

3.2. Dispersive liquid-liquid microextraction.

3.2.1. Coupling DLLME to ICP-AES.

Supramolecular solvents based on the use of THF with either alcohols [25,26,27] or organic acids [28] have been employed in the literature for metal extraction by means of DLLME and atomic absorption spectrometry detection. To date, however, no previous attempt to apply this type of solvents in ICP-based techniques has been reported. Therefore, some experiments were initially performed to evaluate the best strategy to make use of them in ICP-AES. It should be considered that supramolecular solvents are highly viscous substances [28] and, consequently, analytical figures of merit could be compromised due to its negative influence on aerosol generation [4].

In this work, supramolecular solvents were prepared by combining THF with different surfactants, namely: (i) 1-decanol [25,26]; (ii) 1-undecanol [27]; and (iii) decanoic acid [28]. Initially, supramolecular solvents were directly introduced into the ICP-AES instrument by the FIA manifold, but emission signals from As, Cd and Pb were highly irreproducible due to poor nebulization and memory effects. Therefore, they were diluted with methanol (1:1 proportion) to decrease their viscosity. Supramolecular solvents based on 1-decanol and 1-undecanol could be introduced into the plasma in a highly reproducible manner, but the nebulizer and FIA system was quickly blocked after injections with the decanoic acid-based micelles. A closed inspection of the nebulizer and system tubing revealed the appearance of a white solid deposit which corresponded to decanoic acid. Solubility of decanoic acid in the carrier solution (1% w w^{-1} nitric acid) is low (approximately 0.15 g L^{-1}) and, consequently, this substance precipitates inside the sample introduction system. In fact, decanoic acid precipitate was also visible inside the spray chamber. To improve decanoic acid solubility, water-organic mixtures could be used as a carrier solution instead of the nitric acid solution, but this approach compromises both short and long-term instrument performance due to the continuous organic solvent load to the plasma [4]. Consequently, decanoic acid-based supramolecular solvents were discarded for further studies. Supramolecular solvents based on 1-decanol and 1-undecanol were more attractive since these alcohols are in liquid form at room temperature and no precipitate could form in the presence of the carrier solution.

Because the analytical figures of merit in ICP-based techniques strongly depend on the physicochemical properties of the sample matrix [5], the influence of the solvent employed to dilute the supramolecular solvent on analyte signal in ICP-AES was examined. In addition to methanol, four additional solvents were tested: (i) ethanol; (ii) 1-propanol; (iii) acetonitrile; and (iv) acetic acid. For each of them, 1:1 supramolecular:dilution solvent mixtures were prepared containing 1 mg L^{-1} of As, Cd and Pb. Figure 4.5 shows the influence of the dilution solvent on the integrated net analyte signals for the 1:1 diluted 1-decanol-based micelles. The highest signal for all the analytes was obtained operating with acetic acid, followed by the alcohols and acetonitrile. Differences among the tested solvents were mainly related to blank corrections, since all the mixtures yielded similar raw signals. Comparable findings were also observed using 1-undecanol as surfactant. A priori, higher signals would have been expected for alcohols and acetonitrile due to their higher volatility, which theoretically favors aerosol transport efficiency [10,11]. Experimental data suggest, however, that volatility is not critical for the selection of the dilution solvent due to the low dilution factors employed in this work. Based on the results presented in Fig. 5, acetic acid was selected to dilute the supramolecular solvent. Additionally, the influence of the supramolecular solvent:acetic acid ratio on emission signals was also examined. To this end, supramolecular:acetic acid ratios ranging from 1:0.25 to 1:3 were investigated. All the assayed mixtures had a fixed amount of analyte (1 mg L^{-1}), thus allowing the evaluation of the influence of the matrix physicochemical properties on aerosol generation and transport. For all the analytes, emission signals improved with dilution up to a 1:1 supramolecular:acetic acid ratio and remain constant with further acetic acid additions (Figure 4.S1). This data suggests that dilution is beneficial to improve aerosol generation, probably due to a reduction of sample viscosity. Nevertheless, from a practical point of view, high dilution of DLLME extracts is useless on practical analysis due to its negative effect on sensitivity and limits of detection (LoD). When increasing DLLME extract dilution from 1:0.5 to 1:1 analyte concentration is decreased 50% but signal improvement is just 43%. In this work, a 1:0.5 dilution ratio was selected as a compromise between analyte figures of merit and sample handling.

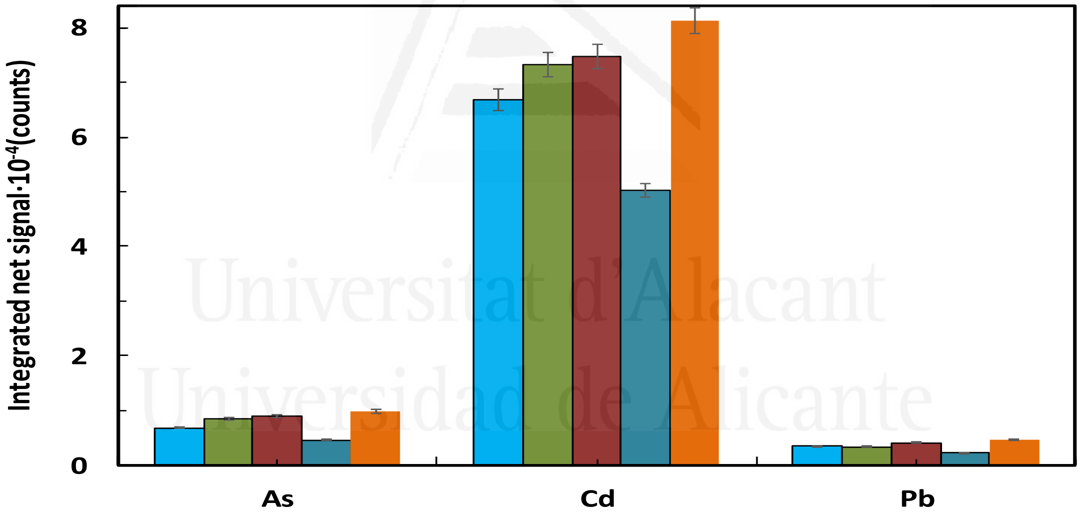


Figure 4.5. Influence of the dilution solvent employed for supramolecular solvent dilution on the integrated emission signal of As I 193.696 nm, Cd II 214.439 nm and Pb II 220.353 nm. ICP-AES operating conditions: Q_g: 0.7 L min^{-1}; Q_l: 0.4 mL min^{-1}; supramolecular/dilution solvent ratio: 1:1; analyte concentration: 1 mg L^{-1}. (🟦) Methanol;(🟩); ethanol (🟥); 1-propanol; (🟦) acetonitrile;(🟧) acetic acid.

3.2.2. Optimization of ICP-AES experimental conditions.

Next, plasma experimental conditions were optimized to operate with the 1:0.5 supramolecular solvent:acetic acid mixtures. The influence of both Q_g and Q_l on analyte signal for DLLME was similar to that previously observed for pure acetic acid extracts with the KR (Figure 4.S2). These results are not unexpected considering that organic extracts are introduced into the plasma with the same sample introduction system. Consequently, Q_g and Q_l were respectively fixed at 0.6 L min^{-1} and 0.4 mL min^{-1}.

3.2.3. Optimization extraction conditions with DLLME.

A CCD design was employed for a detailed optimization of extraction conditions with DLLME. Some preliminary experiments were performed to evaluate the influence of the surfactant nature (1-decanol and 1-undecanol) on analyte extraction. It was observed that metal extraction was almost independent of the selected surfactant and, hence, 1-decanol was selected for further studies. According to this preliminary experiments and previous works [29], pH, APDC concentration, THF volume and surfactant mass metal extraction were identified as the main relevant variables controlling metal extraction. Each of the four variables selected were investigated in five levels: (ii) pH. 12 (++), 9 (+), 6 (0), 3 (-) and 0 (--); (ii) APDC concentration: 0.55 % w w^{-1} (++), 0.40 % w w^{-1} (+), 0.25 % w w^{-1} (0), 0.10 % w w^{-1} (-) and 0.00 % w w^{-1} (--); (iii) THF volume: 0.53 mL (++), 0.40 mL (+), 0.28 mL (0), 0.15 mL (-) and 0.03 mL (--); and (iv) surfactant mass: 320 mg (++), 240 mg (+), 160 mg (0), 80 mg (-) and 0 (--). A total of 25 experiments were performed by triplicate using a 1 mg L^{-1} standard analyte solution (Table 4.S3). To examine the significance of each variable on metal extraction, data were analyzed by ANOVA and effects were summarized by means Pareto charts (Figure 4.6). It was observed that the most significant variables depended on the studied analyte. For instance, Cd and Pb extraction was significantly affected by pH, APDC concentration, THF volume and surfactant mass. Extraction recovery for both metals was favored by increasing pH, APDC concentration and THF volume as well as by decreasing surfactant mass. On the other hand, only THF exerts a (positive) significant effect on As extraction. In either case, it was noticed that the investigated variables were not orthogonal, since analyte extraction was also dependent on two-factor interaction effects. Table 4.3 shows the optimum experimental conditions derived from the CCD model for As, Cd and Pb extraction by means of DLLME. As it shown, there are significant differences on the optimum pH and surfactant mass values for each element. For instance, Cd and Pb extraction is maximum at pH values around 6, whereas As requires highly acidic conditions (pH = 1.8). As was

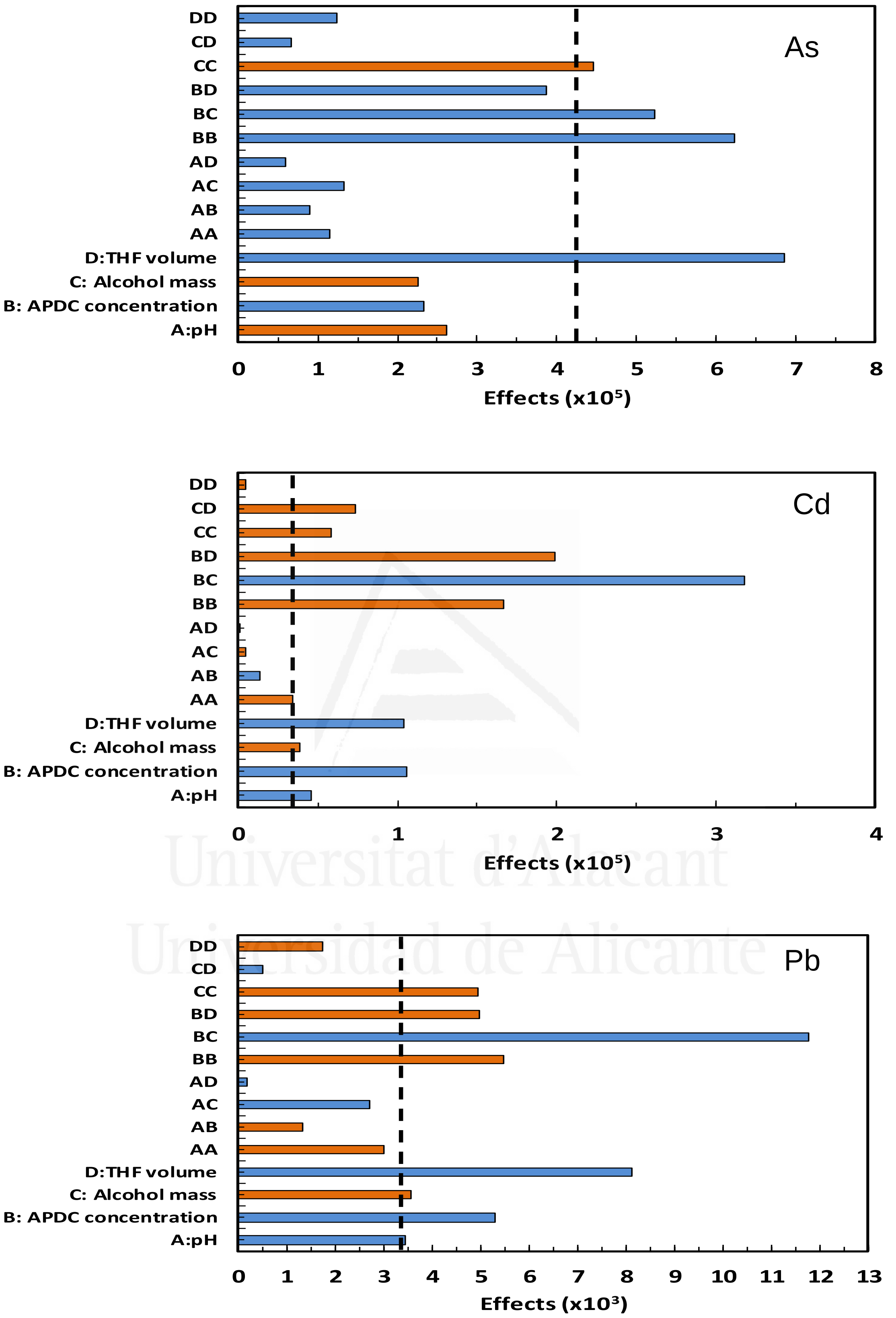


Figure 4.6. Pareto charts obtained in the optimization study of the main variables affecting Cd and Pb extraction with DLLME. (🟦) positive effect; (🟧) negative effect. Dotted vertical line corresponds to 95% confidence level. ICP-AES operating conditions: Q_g: 0.7 L min^{-1}; Q_l: 0.4 mL min^{-1}; supramolecular/acetic acid dilution ratio: 1:0.5; analyte concentration: 1 mg L^{-1}.

previously necessary with KR extraction, the CCD model was examined to select a compromise set of experimental conditions for the simultaneous analysis of all the analytes in a single run. Table 4.3 gathers the compromise DLLME experimental conditions selected for As, Cd and Pb extraction. It was observed that, under those conditions, extraction efficiency for all the analytes was reduced 10% on average in comparison with the optimum conditions for each element.

Table 4.3. Compromise experimental conditions for the simultaneous analysis of As, Cd and Pb by means of DLLME.

	As	Cd	Pb	Compromise
pH	1.8	6.6	5.6	4.0
APDC (% w w^{-1})	0.2	0.08	0.3	0.2
THF (mL)	0.5	0.5	0.5	0.5
Alcohol (mg)	135	48	158	100

3.3. Comparison between KR and DLLME.

Analytical figures of merit achieved by KR and DLLME for As, Cd and Pb determination by means of ICP-AES are summarized in Table 4.4. This table shows the number of elements quantifiable by each approach, LoD, enrichment factor (EF), dynamic range, sample throughput and consumptive index. The LoD was calculated from the analyte calibration graph according to IUPAC's recommendation as 3 times the standard deviation of the blank signal divided by the calibration curve slope. Enrichment or preconcentration factor is defined as the ratio of analyte concentration in the organic phase (i.e. pure acetic acid with KR and 1:0.5 supramolecular:acetic acid mixture with DLLME) to the initial concentration in the aqueous phase [30]. Finally, the consumptive index (CI) is defined as the ratio between the sample volume and EF [31]. From data gathered in Table 4.4, DLLME is clearly a more attractive sample preparation strategy than KR for metal analysis by means of ICP-AES. First, it allows the simultaneous determination of As, Cd and Pb whereas KR is just limited to the last two elements. On the other hand, DLLME is more efficient preconcentrating metals since, despite DLLME organic extracts require a dilution step for ICP-AES analysis, it still affords a higher EF. Consequently, DLLME presents improved LoD (on average 3-fold) and CI in comparison with the KR. An additional benefit of DLLME regarding KR is the higher sample throughput, due to its simpler experimental arrangement. Finally, no significant differences were observed on the dynamic ranges between both strategies.

When compared to conventional ICP-AES, both approaches significantly improve LoDs for As, Cd and Pb determination (Table 4.S4). Thus, LoD improvement afforded by the KR was on average 12.5-fold whereas for DLLME was 38-fold. These improvements on the LoD achieved by both pretreatments could be attributed to: (i) the preconcentration itself (i.e. EF) (Table 4.4); (ii) the beneficial effect of organic on aerosol generation and transport regarding conventional aqueous standards [12]; and (iii), particularly for As, carbon influence on analyte excitation-ionization mechanism [31].

Table 4.4. Analytical figures of merit afforded by KR and DLLME for As, Cd and Pb determination by means of ICP-AES.

	As		**Cd**		**Pb**	
	KR	**DLLME**	**KR**	**DLLME**	**KR**	**DLLME**
LoD (µg L^{-1})	-	2.4	2	0.6	4	1.6
EF	-	27	4	18	6	24
Dynamic range (µg L^{-1})	-	2.4-1200	2-4000	0.6-900	4-1000	1.6-1000
Sample throughput (samples h^{-1})	-	12	2	12	2	12
Consumption index	-	6.8	1	4.5	1.5	6

Analytical figures of merit afforded by both KR and DLLME have also been compared with those previously reported in the literature operating these strategies with ICP-AES detection (Table 4.5). First, no comparison was feasible for As, since this element has not been previously studied with ICP-AES. Regarding KR preconcentration, analytical figures of merit for Cd and Pb are worse than those reported by Lara et al. [18] and Olsina et al. [19] but it should be considered that a ultrasonic nebulizer was employed to improve aerosol generation and transport in those works. On the other hand, Cd and Pb data for DLLME was similar to those found in previous works but using less sample volume (4 mL). However, one of the main advantages presented by the herein proposed method is its robustness for the analysis of food samples, which represent far more complex matrices than water and liquid matrices studied in previous works.

Table 4.5. Comparison of different KR and DLLME methodologies proposed in the literature for As, Cd and Pb determination.

Analyte	**Strategy**	**Extractant**	**LoD ($\mu g/ L^{-1}$)**	**EF**	**DR ($\mu g/L^{-1}$)**	**V_{sample} (ml)**	**Sample**	**Ref.**
Cd	KR	3 M nitric acid	0.005	-	-	-	Water	[18]
Pb	KR	4 M hydrochloric	0.2	-	-	-	Water	[19]
Cd	DLLME	Carbon tetrachloride	0.19	17	1-1000	10	Water	[12]
Cd	DLLME	Chloroform	1.4	13	-	5	Water	[33]
Cd	DLLME	Trichloroethylene	0.2	13	-	40	Water	[34]
Cd	DLLME	1-undecanoll	6	8	-	5	Water	[12]
Cd	DLLME	1-undecanoll	0.8	0.8	0-100	9	Water, soft-drinks and spirits	[29]
Pb	DLLME	Carbon tetrachloride	0.5	17	1-1000	10	Water	[33]
Pb	DLLME	Chloroform	1.1	13	-	5	Water	[12]
Pb	DLLME	1-undecanol	4	8	-	5	Water	[12]
Pb	DLLME	1-undecanol	2	-	0-300	9	Water and drinks	[29]

3.4. Methods validation.

European conformity guidelines for analytical methods of food contaminants were employed to validate both KR and DLLME methodologies [32]. To this end, four food samples were analyzed, namely: (i) chocolate; (ii) mussels; (iii) rice; and (iv) wine. These samples were selected to cover different matrices, thus allowing the evaluation of selectivity and robustness of each sample preparation strategy under different experimental conditions. All the samples were subjected to an acid digestion procedure in a MW oven before the corresponding extraction-preconcentration treatment, except for wine, which was directly analyzed. Calibration was performed by means of matrix-matched standards. Thus, standards for digested sample analysis were prepared simulating the acid content present after the digestion treatment (nitric acid 10% w w^{-1}), whereas standards for wine analysis were prepared containing the most relevant organic and inorganic components (ethanol 12% v v^{-1} and 1000 mg K L^{-1}) in wine.

The accuracy of the methods was evaluated by means of a recovery test (Table 4.6). Food samples were spiked with known amounts of As, Cd and Pb for a final concentration of 600 µg kg^{-1}. European union guidelines establish that trueness of the measurements for analyte concentration levels above 10 µg Kg^{-1} is successfully assessed when the recovery values are within -20% to 10% [27].

Table 4.6. Metals recovery assay for different commodities with KR and DLLME.

	KR		**DLLME**		
Sample	**Cd**	**Pb**	**As**	**Cd**	**Pb**
Mussel	86±4	89±5	98±4	98±3	97±5
Chocolate	97±7	87±5	97±7	101±4	98±5
Rice	90±4	79±6	97±5	98±6	102±5
Wine	93±5	97±2	96±5	98±3	99±5

According to this criterion, and with independence of the considered sample, quantitative recoveries for all the elements were obtained operating both with a KR and DLLME. The repeatability was determined by analyzing six replicates of each food sample on the same day for each methodology. Relative standard deviation (RSD) values for Cd and Pb in KR treatment were in the range of 3-5%. Similar values were found for As, Cd and Pb with DLLME. The reproducibility (inter-assay precision) of each methodology was evaluated as the RSD of the measurements obtained for six replicates on three different days. In this case, RSD values for both strategies ranged from 5 to 10%. Alternatively, the analyzed food samples were simultaneously analyzed by ICP-MS without preconcentration (Table 4.S5). Calibration was also carried out with matrix-matched standards. For mussels, results afforded by KR- and DLLME-ICP-AES agreed with those obtained in ICP-MS. No comparison was feasible for the remaining samples since the LoDs achieved by KR- and DLLME-ICP-AES were not low enough to quantify the As, Cd and Pb levels present. Nevertheless, with the exception of As with KR, LoDs obtained with both methods allow to control these elements in chocolate, mussels, rice and wine samples according to the policy stablished by the EU [1] (Table 4.S6). The potential use of KR and DLLME for As, Cd and Pb analysis in other foods regulated by the EU 1881/2006 directive was also evaluated. To this end, a theoretical LoD was calculated for each method assuming an acid digestion treatment of 0.5 g sample and dilution up to 25 mL (i.e. similar experimental conditions to those employed in this work). In general, except for children destined commodities, LoDs obtained with DLLME-ICP-AES would be low enough to quantify As, Cd and Pb. Regarding KR-ICP-AES, this strategy is more limited for metal control in foods, since it does not allow As quantification and Cd and Pb detection capabilities are lower than those afforded by DLLME (Table 4.S7).

4. Conclusión.

Results in this work demonstrate that, after an extraction-preconcentration procedure, ICP-AES could be successfully employed for As, Cd and Pb control in food samples according to Commission regulation Noº1881/2006. Among the two extraction procedures evaluated, DLLME is rather a more attractive approach than KR. The former strategy allows to quantify all the elements simultaneously in a single run whereas the later is just limited to Cd and Pb. Moreover, DLLME improves LoD (2-3fold) and sample throughput regarding KR. When compared to conventional ICP-AES analysis (no extraction-preconcentration), both approaches improve LoD more than 10-fold thanks to the preconcentration procedure itself but also due to the beneficial effect of organics on aerosol generation and transport regarding aqueous standards.

5. References.

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6. Appendix.

Table 4.S1. Experimental conditions for the acid digestion treatment of mussels, rice and chocolate samples.

Sample	Sample(g)	Digestion solution	Step	Time (min)	Temperature (°C)	Pressure (bar)
Mussels	0.4	5 mL of HNO_3 + 3 mL water	1	15	200	45
Chocolate	0.5		2	15		
Rice	1.0		1	10	180	45
			2	15		

Table 4.S2. Cadmium and Pb signal response for CCD design operating KR-ICP-AES. ICP-AES operating conditions: Q_g: 0.7 L min^{-1}; Q_l: 0.4 mL min^{-1}. Analyte concentration: 1 mg L^{-1}.

				Integrated signal (counts))·10^{-3}	
pH	**APDC concentration (w w^{-1})**	**Reactor lenght (cm)**	**Elution flow (mL min^{-1})**	**Cd**	**Pb**
3	0.27	100	0.23	55±2	20.0±0.5
7	0.8	256	0.23	268±2	25.0±0.5
5	0.54	178	0.11	257±2	20.0±0.7
5	0.54	178	0.34	113±1	17.0±0.6
5	0.54	178	0.56	126±2	13.0±0.5
3	0.27	100	0.45	165±1.7	20.0±0.8
5	0.54	334	0.34	149±1.7	19.0±0.7
3	0.8	100	0.45	165±2	25.0±1.2
3	0.8	100	0.23	45±1.3	22.0±0.8
5	0.54	22	0.34	31±1.0	17.0±0.7
7	0.8	100	0.45	46±1.3	19.0±0.7
3	0.27	256	0.23	103±2	14.0±0.6
5	1.07	178	0.34	145±2	19.0±0.6
5	0.54	178	0.34	113±3	17.0±0.7
9	0.54	178	0.34	160±2	17.0±0.7
5	0.005	178	0.34	83±1.5	12.0±0.5
7	0.27	100	0.23	30.0±1.4	11.8±0.5
7	0.8	256	0.45	63±3	20.0±0.7
7	0.27	256	0.23	47±2	16.0±0.8
3	0.8	256	0.23	25±6	42.0±0.2
3	0.27	256	0.45	95±2	17.0±0.5
1	0.54	178	0.34	170±6	28.0±0.8
7	0.27	256	0.45	60±2	15.0±0.5
7	0.27	100	0.45	38±1.8	14.8±0.3
7	0.8	100	0.11	56±2	15.5±0.6

Table 4.S3. Arsenic, Cd and Pb signal response for a CCD design operating DLLME. Q_g: 0.7 L min^{-1}; Q_l: 0.4 mL min^{-1}; supramolecular/acetic acid dilution ratio: 1:0.5; analyte concentration: 1 mg L^{-1}.

				Integrated signal (counts))·10^{-3}		
pH	**APDC concentration (w w^{-1})**	**Alcohol mass (mg)**	**THF volume (mL)**	**As**	**Cd**	**Pb**
3	0.1	80	0.15	23.0±0.5	50.0±1.3	7.9±0.3
6	0.25	160	0.025	13.0±0.3	190±7	8.7±0.5
9	0.4	80	0.15	14.0±0.3	93.0±5	6.7±0.2
9	0.4	80	0.4	27.0±0.6	170±9	10.5±0.2
3	0.4	80	0.15	21.0±0.4	100±5	11.0±0.4
6	0.55	160	0.275	20.0±0.5	300.0±1.0	22.0±0.8
12	0.25	160	0.275	23.0±0.4	130±6	3.0±0.2
9	0.1	240	0.4	25.0±0.6	110±4	14.0±0.2
9	0.4	240	0.4	30.0±0.8	190±6	21.7±0.4
6	0.25	160	0.275	23.0±0.7	260±9	20.2±0.2
0	0.25	160	0.275	27.0±0.9	72±4	3.0±0.2
6	0.25	160	0.275	20.0±0.7	260±9	18.0±0.6
3	0.4	240	0.4	27.0±0.8	120±5	10.0±0.4
3	0.4	80	0.4	31.0±0.9	160±5	20.0±0.7
6	0	160	0.275	21.0±0.8	56±2	4.6±0.2
6	0.25	160	0.525	30.0±1.0	300±10	18.0±0.5
9	0.1	80	0.15	17.0±0.7	139±3	8.0±0.3
6	0.25	0	0.275	0	0	0
9	0.1	80	0.4	29.0±0.8	289±9	16.5±0.7
3	0.1	80	0.4	25.0±0.8	275±9	17.9±0.4
9	0.1	240	0.15	18.0±0.5	131±6	5.2±0.2
9	0.4	240	0.15	20.0±0.6	184±6	8.4±0.5
6	0.25	320	0.275	22.0±0.7	280±9	13.2±0.2
3	0.4	240	0.15	18.0±0.7	170±4	6.0±0.4
3	0.1	240	0.4	30.0±1.0	127±2	12.2±0.5
3	0.1	240	0.15	16.0±0.9	150±9	3.20±0.05

Table 4.S4. LoD improvement afforded by Kr and DLLME regarding conventional ICP-AES analysis (no preconcentration)

Element	KR	DLLME
As	-	40
Cd	15	50
Pb	10	25

Table 4.S5. Elemental analysis of the mussel sample by means of ICP-based techniques. ICP-AES experimental conditions: Q_g: 0.7 L min^{-1}; Q_l: 0.4 mL min^{-1}.

	Concentration (mg Kg^{-1})		
Element	**KR-ICP-AES**	**DLLME-ICP-AES**	**ICP-MS**
As	-	10.3±0.3	10.5±0.4
Cd	3.8±0.8	3.4±0.3	3.2±0.3
Pb	<LoD	0.40±0.05	0.30±0.01

Table 4.S6. Arsenic, Cd and Pb maximum allowed levels in chocolate, mussels, rice and wine according to EU 1881/2006 policy and LoDs afforded by KR- and DLLME-ICP-AES.

	Maximum allowed levels (µg Kg^{-1})			**LoD KR-ICP-AES (µg Kg^{-1})**			**LoD DLLME-ICP-AES (µg Kg^{-1})**		
Sample	**As**	**Cd**	**Pb**	**As**	**Cd**	**Pb**	**As**	**Cd**	**Pb**
Chocolate	-	300	-	-	100	200	130	30	80
Mussels	-	1000	1500	-	130	250	150	40	100
Rice	200	200	200	-	50	100	25	15	40
Wine	200	10	150	-	1	1.5	2.4	0.6	1.6

Table 4.S7. Arsenic, Cd and Pb maximum allowed levels in several foods according to EU 1881/2006 policy and theoretical LoDs for KR- and DLLME-ICP-AES considering an acid digestion treatment of 0.5 g sample and dilution up to 25 mL.

Element	**Food**	**Maximun allowed level (µg Kg^{-1})**	**LoD (µg Kg^{-1})**	
			KR	**DLLME**
As	Pancakes, wafers, cookies and rice cakes	300	------	150
	Rice destined for the production of food for infants and young children	100		
Pb	Honey	100	200	80
	Food complement	3000		
	Fats and oils including milk fat	100		
	Meat (excluding offal) of bovine animals, sheep, pigs and poultry	100		
	offal of cattle, sheep, pigs and poultry	500		
	Crustaceans	500		
	cephalopods	300		
	Cereals and dried vegetables	200		
	Sweet corn and fruits excluding cranberries, strawberry trees, currants and elderberries	100		
	blueberries, strawberry trees, currants and elderberries	200		
	Vegetables	100-300		
	Raw milk, thermally treated milk and milk for the manufacture of dairy products	20		
	Preparations for infants and young children: powder and liquid	50, 10		
	Foods for special medical purposes intended specifically for infants and young children: powder and liquid form	50, 30		
	Drinks for children and young children sold. Marketed liquid or to be reconstituted following the manufacturer's instructions, including fruit juices	30		

Continuation table 4.s7

Cd	Cereals excluding wheat and rice	100	100	30
	Grains of wheat and rice Wheat bran and wheat germ for direct consumption Soybeans	200		
	Vegetables and fruits excluding root and tuber vegetables, leafy vegetables, fresh herbs, brassica vegetables, young stems, mushrooms and seaweed	50		
	Root and tuber vegetables (excluding celerios, chirivias, salsifies and rustica radishes), young stems (celery excluded).	100		
	Leafy vegetables, free herbs, vegetables of the Brassica genus, celery, celeriac, chirivias, salsifies, rustic radishes and the following mushrooms: mushrooms, oyster mushroom and shiitake mushroom	200		
	Milk chocolate with a total dry matter content of cocoa> 30	100		
	Chocolate with a total cocoa dry matter content <50; milk chocolate with a total dry matter content of cocoa ≥30%	300		
	Chocolate with a total dry matter content of cocoa≥50%	500		
	Bivalve molluscs and cephalopods	1000		
	anchovy, swordfish and sardine fish	250		
	Melva fish	150		
	Food supplements composed exclusively of dried seaweed, products made from seaweed or dried bivalve molluscs	3000		
	Preparations for infants and continuation preparations: Powdered preparations for infant formulated from proteins obtained from cow's milk Prepared in liquid for lactating persons based on proteins obtained from cow's milk Prepared powders for lactated elaborated from isolated soy proteins alone or mixed with milk protein Prepared in liquid for lactating children prepared from isolated soy proteins alone or mixed with milk protein	10 5 20 10		

Figure 4.S1. Influence of the supramolecular:acetic acid mixture ratio on the integrated emission signal of Cd II 214.439 nm. ICP-AES operating conditions: Q_g: 0.7 L min^{-1}; Q_l: 0.4 mL min^{-1}. Analyte concentration: 1 mg L^{-1}. (🟦) As; (🟩); Cd (🟥) Pb.

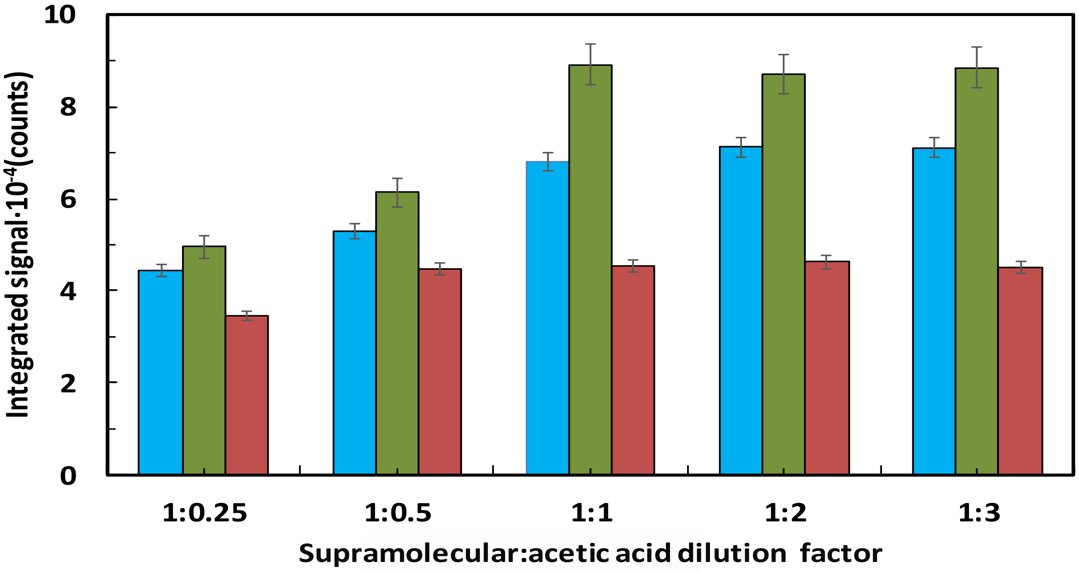
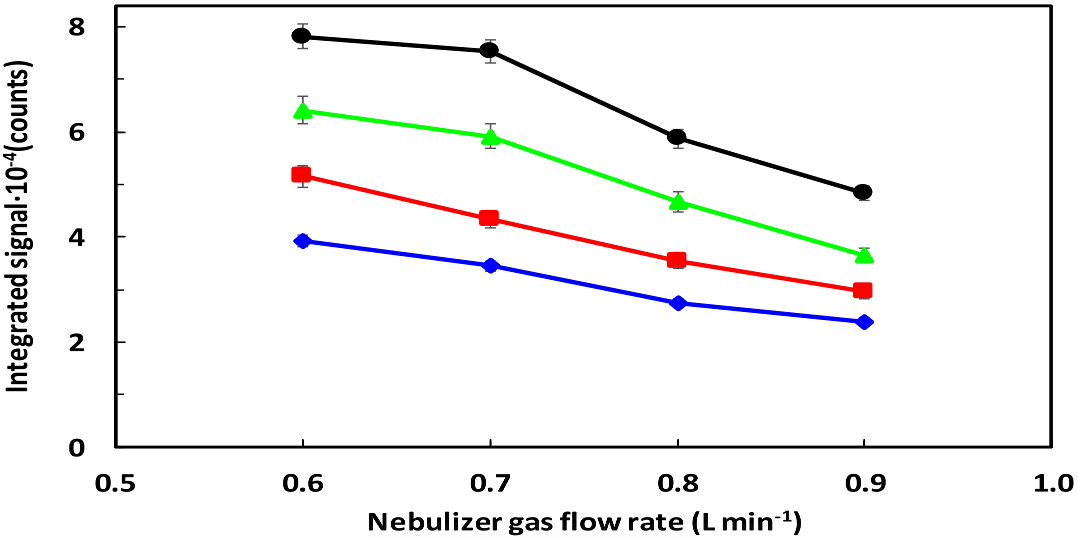


Figure 4.S2. Influence of the nebulizer gas flow rate on Cd II 214.439 nm integrated emission signal operating 1:1 supramolecular:acetic acid mixture at different Q_l with DLLME. (●) 0.4 mL min⁻¹; (▲) 0.7 mL min⁻¹; (■) 1.0 mL min⁻¹; and (◆) 1.3 mL min⁻¹.



CAPÍTULO 5

CONCLUSIONES

Los resultados obtenidos en la presente Tesis Doctoral demuestran el gran potencial analítico que resulta de combinar DLLME con técnicas de Espectrometría Atómica para el análisis elemental de alimentos de diferente naturaleza y complejidad. Así:

1. Es posible llevar a cabo la determinación de Cd y Pb en muestras de vino mediante DLLME-ETAAS utilizando como extractante hexafluorofosfato de 1-butil-3-metilimizadolio. La metodología desarrollada permite mejorar los límites de detección (hasta 8 y 9 veces para Cd y Pb, respectivamente) con respecto a los métodos convencionales empleados para el análisis de vinos. (i.e., dilución, digestión ácida, etc.). Además, la metodología desarrollada es válida para todo tipo de vinos (tinto, rosado, blanco, espumoso y fortificado) y permite el control de Cd y Pb en los niveles máximos establecidos por la Organización Internacional de la Vid.
2. Mediante la selección adecuada de las condiciones de trabajo y del sistema de introducción de muestras, es posible utilizar de forma combinada DLLME e ICP-AES para la determinación simultánea de varios elementos en aguas y alimentos. De hecho, es posible utilizar un único conjunto de condiciones experimentales en el plasma para analizar directamente los extractantes que se emplean en DLLME (cloroformo, 1-undecanol y $BmimPF_6$) independientemente de sus propiedades físicoquímicas. No obstante, en el caso de disolventes muy viscosos, es obligatorio recurrir a una etapa de dilución (1:0.5) para reducir el efecto negativo de esta propiedad física.
3. Es posible determinar As, Cd y Pb en alimentos de forma simultánea mediante DLLME-ICP-AES utilizando como extractante un disolvente supramolecular basado en THF y 1-undecanol. Los resultados muestran que esta metodología presenta ventajas significativas respecto a la de reactor anudado en términos de número de elementos que se pueden determinar y parámetros analíticos. La metodología desarrollada permite controlar los niveles de As, Cd y Pb en alimentos según los criterios establecidos según el Reglamento 1881/2006 de la Comisión.

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Original research article

Determination of cadmium and lead in wine samples by means of dispersive liquid–liquid microextraction coupled to electrothermal atomic absorption spectrometry

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ABSTRACT

Wine contains significant amounts of Cd and Pb coming from different contamination sources. The analysis of these elements still means an enormous challenge due to its low concentration levels and the complexity of the wine matrix. Therefore, new highly sensitive analytical methods are currently required. The present work reports a new method based on the use of dispersive liquid–liquid microextraction (DLLME) and electrothermal atomic absorption spectrometry (ETAAS) determination. Results demonstrate that, under optimum DLLME experimental conditions, accurate Pb determination can be easily performed using external calibration. Cadmium determination requires a standard addition calibration strategy since its accuracy is strongly affected by the presence of Zn in the wine matrix. The limits of detection afforded by the method proposed were 0.01 and 0.08 μg L^{-1} for Cd and Pb, respectively. These values were around one order of magnitude lower than those previously reported for wine analysis by ETAAS. This simple methodology has been successfully employed for Cd and Pb analysis in different types of wines (i.e., white, red, rosé and sparkling wines). The results are in agreement with those obtained by Inductively Coupled Plasma – Mass Spectrometry after a time-consuming microwave-assisted acid digestion treatment.

1. Introduction

Wine is one of the most widely consumed beverage around the world. Nevertheless, it exhibits significant Pb and Cd levels (i.e. low-medium μg L^{-1} range) that are mainly related to atmospheric contamination, pesticides and materials used in wine production, transport and storage (Pyrzynska, 2004; Pohl, 2007). Due to their hazardous nature, Cd and Pb maximum allowed levels in wine are regulated worldwide (EC, 2006; OIV, 2016). Thus, for instance, the Organisation Internationale de la Vigne et du Vin (OIV) limits Cd and Pb in wine to 10 μg L^{-1} and 150 μg L^{-1}, respectively (OIV, 2016).

Cadmium and Pb analysis in wine is usually carried out by means of spectrometric techniques (Grindlay et al., 2011). Among them, mainly when no multi-element analysis is required, electrothermal atomic absorption spectrometry (ETAAS) is the most preferred choice due to its high selectivity and low limits of detection (LODs). Direct wine analysis by ETAAS is difficult due to the occurrence of both spectral and non-spectral interferences originated from wine matrix components (organics and salts) (Grindlay et al., 2011). For instance, non-volatile organic components of wine are a source of non-specific background absorption and affect long-term reproducibility because of the accumulation of carbonaceous residues after several graphite tube firings (Freschi et al., 2001; Ajtony et al., 2008). To deal with these interferences, some strategies can be applied: (i) a judicious optimization of ETAAS temperature program and modifier selection (Freschi et al., 2001; Kristl et al., 2002; Karadjova et al., 2004; Dessuy et al., 2008; Ajtony et al., 2008); (ii) the use of transversally heated furnaces (Freschi et al., 2001; Dessuy et al., 2008); (iii) a Zeeman-based background correction (Freschi et al., 2001; Kim, 2004; Ajtony et al., 2008); and/or, (iv) the use of a sample pre-treatment step prior to the analysis. To this end, acid digestion (Kim, 2004; Capelo et al., 2005; Ajtony et al., 2008) or dilution (Freschi et al., 2001; Karadjova et al., 2007) have been employed but at the expense of the sample throughput and detection capabilities. Solid-phase extraction/preconcentration procedures using different type of columns have been also reported for the quantification of Cd and Pb in wines by flame atomic absorption spectrometry (FAAS) (Lemos et al., 2002; Bakircioglu et al., 2003; Tuzen and Soylak, 2004; Tuzen et al., 2005) at the concentration levels imposed by regulatory authorities. Up to date, the use of extraction/preconcentration procedures for Cd and Pb analysis in wine by means of

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ETAAS have not been investigated in detail though its potential advantages to reduce both spectral and non-spectral interferences derived from wine matrix.

Dispersive liquid–liquid micro-extraction (DLLME) has been stablished as a faster and greener sample preparation methodology than conventional liquid–liquid extraction and solid phase extraction approaches for elemental analysis (Zgoła-Grzeskowiak and Grzeskowiak, 2011; El-Shahawi and Al-Saidi, 2013). DLLME improves LODs and sample throughput as well as minimizes matrix effects, reagents requirements and waste generation. Different spectroscopic techniques have been employed to analyze DLLME extracts (Kocot et al., 2016). Nevertheless, ETAAS is usually preferred due to its good capability to deal with micro-volume samples and organic solvents (Andruch et al., 2013; de la Calle et al., 2016). DLLME has been traditionally employed for metal analysis in water samples and, in a lesser extent, in foods (Andruch et al., 2013). Very little research has been performed evaluating the potential benefits (and drawbacks) of DLLME for inorganic analysis in wine samples. Seeger et al. (2015) employed DLLME for Cu and Fe determination in wine by FAAS. Results afforded by DLLME-FAAS were comparable to those obtained by means inductively coupled plasma atomic emission spectrometry (ICP-AES) after an acid digestion treatment. However, sample throughput was partially compromised due to the mandatory use of standard addition calibration strategy due to the high matrix effects originated by the organic extracts in the flame. Escudero et al. (2013), successfully employed a DLLME procedure for As speciation in wine by ETAAS. Experimental results show that As^{3+} extraction was highly selective and free from interferences originated by inorganic concomitants present in wine. To our best knowledge, no further works related on this topic have been reported.

The goal of this work is to develop a new analytical method for Cd and Pb determination in wine by means of DLLME-ETAAS. To this end, both DLLME (i.e. extractant mass, disperser type and volume, pH and ionic strength) and ETAAS (i.e. modifier and temperature program) experimental parameters have been optimized. Finally, the developed methodology has been applied to the analysis of different wine samples (white, rosé, red, sparkling and fortified). To evaluate the results obtained, wine samples have also been analyzed by means of inductively coupled plasma – mass spectrometry (ICP-MS) after a digestion procedure.

2. Experimental

2.1. Reagents

1-Butyl-3-methyl-imidazolium hexafluorophosphate ($BmimPF_6$, ≥97%), sodium diethyldithiocarbamate trihydrate (DDTC, ≥99%), methanol (≥99.9%), ethanol (≥99.5%), sodium chloride (≥99.5%) and Pb, Cd and Pd mono-elemental standard solutions (1000 mg L^{-1}) were obtained from Sigma-Aldrich (Steinheim, Germany). Nitric acid (69% w w^{-1}), sodium dihydrogen phosphate (≥99.5%), disodium dihydrogen phosphate (≥99.5%), glacial acetic acid (99.7%), sodium acetate (99%) and potassium nitrate (99%) were purchased from Merck (Darmstadt, Germany). Calcium nitrate hexahydrated (99%), copper nitrate trihydrate (99%), iron (III) nitrate (98%), manganese nitrate (≥98.5%), magnesium nitrate (99%), strontium nitrate (99%) and zinc nitrate hexahydrate (98.5%) were obtained from Scharlab (Barcelona, Spain).

All solutions were prepared using high purity water (i.e. with conductivity lower than 18 MΩ cm-1) obtained from a Milli-Q water system (Millipore Inc. Paris, France).

2.2. Wine samples

Five Spanish wine samples, purchased in local markets, were analyzed to cover different matrix characteristics and ethanol content: white (Fidencio, Spain, 11.5% w w^{-1}), rosé (Piccolo Giovanni, Italy, 9.0% w w^{-1}), sparkling (Cava Jaume Serra, Spain, 11.5% w w^{-1}), fortified (Mistela Murviedro, Spain, 15% w w^{-1}) and red wine (Fidencio crianza, Spain, 13.5% w w^{-1}).

2.3. Dispersive liquid–liquid microextraction

The extraction/preconcentration procedure for Cd and Pb determination was performed as follows. First, 2 mL of wine sample or analyte standard were placed on a centrifuge tube with 100 µL of the suitable buffer solution (e.g. acetic acid/sodium acetate dihydrogen/hydrogen phosphate). Then, 100 µL of NaCl and 100 µL of the DDTC solutions were added. Next, a mixture of $BmimPF_6$ (extractant) and methanol (disperser) was injected using a 1.00 mL syringe and the resulting system was shaken for 2 min using a vortex. To separate the two phases generated (water/ionic liquid), the mixture was centrifuged for 5 min at 3500 rpm. The lower layer of the ionic liquid was removed with a micropipette and then diluted with methanol ($BmimPF_6$/methanol 1:1 ratio) prior to ETAAS analysis.

2.4. Microwave digestion

For comparison, wine samples were analyzed by ICP-MS (model 7700 ×, Agilent, Santa Clara, USA) after a microwave assisted digestion treatment in closed vessel. To this end, 5 g of the selected wine was weighed into a PTFA digestion vessel and 5 mL of concentrated nitric acid was added. The mixture was allowed to react and after clearance of fumes (20 min) the vessel was closed. Afterwards, samples were digested in a Star D microwave digestion system (Milestone, Shelton, CT, USA) using the program recommended by the manufacturer. Finally, the digested samples were made up to 20 g with water.

2.5. Instrumentation

Cadmium and Pb in DLLME extracts were determined using an atomic absorption spectrometer (model 2100, Perkin Elmer, Norwalk, USA) equipped with a graphite furnace atomizer and a deuterium background correction system. Samples were introduced into the furnace by an auto-sampler (model AS-60, Perkin Elmer, Norwalk, USA). Pyrolytically coated graphite tubes without platform were used in this work. Cadmium and Pb signals were measured using the optimized temperature program given in Table 1. For comparison, wine samples were also analyzed by ICP-MS. Instrumental and experimental conditions employed in ICP-MS are described elsewhere (Abdrabo et al., 2015).

Table 1
ETAAS operating conditions.

Parameter	Lead	Cadmium
Lamp current (mA)	9	8
Wavelenght (nm)	283.3	228.8
Spectral bandwith (nm)	0.7	
Injected volume (µL)	20	
Furnace heating program		

Step	Temperature (°C)	Ramp (s)	Hold (s)
Dry	100	20	10
Pyrolysis	350	10	20
	400–1300	10	20
Atomization	1000–2100	1.0	5
Clean	2600	1.0	3

3. Results

3.1. Optimization of graphite furnace experimental conditions

Ionic liquids are considered safer and more environmentally friendly than the (volatile) organic solvent traditionally employed in DLLME procedures. In the present work, $BmimPF_6$ has been selected as extraction media. However, direct analysis of Cd and Pb in $BmimPF_6$ extracts by ETAAS is not straightforward due to the physical properties of both ionic liquid (low volatility and high viscosity) and analytes (high volatility). First, the high viscosity of $BmimPF_6$ (i.e. 381 cP at 25 °C) makes difficult the sample handling. The direct introduction of ionic liquids into the furnace by means of a syringe (Manzoori et al., 2009; López-García et al., 2013) did not afford reproducible results for Pb and Cd analysis even when using an autosampler. To solve this drawback, Li et al. (2009) proposed to reduce the $BmimPF_6$ viscosity by dilution with an appropriate solvent (e.g. alcohols). In the present work, it was observed that irrespective of the dilution factor used (ranging from 1:0.5 to 1:3), $BmimPF_6$:methanol mixtures were easily introduced in the furnace by the autosampler with high reproducibility. Therefore, to reduce the analyte dilution, a 1:0.5 $BmimPF_6$:methanol dilution ratio was first selected after the DLLME procedure. Nonetheless, high background signals were registered irrespective of the modifier and temperature program employed and even using a deuterium correction system. Better results were obtained by employing a 1:1 $BmimPF_6$:methanol mixture.

3.1.1. Modifier

In this work, palladium (as a nitrate salt) was selected as matrix modifier since it is usually employed for the analysis of Cd and Pb in carbon containing matrices (Freschi et al., 2001; Kristl et al., 2002; Dessuy et al., 2008; Ajtony et al., 2008; Grindlay et al., 2009; López-García et al., 2013) avoiding the structured background caused by other modifiers (i.e. phosphates) (Welz and Sperling, 1999; Kristl et al., 2002; Ajtony et al., 2008). To check the influence of the palladium concentration on the absorbance of Cd and Pb, standard solutions of these elements (with a final concentration of 1 and 10 $\mu g\,L^{-1}$, respectively) with amounts of Pd ranging from 0.5 to 9 µg were prepared in a 1:1 $BmimPF_6$:methanol mixture. Experimental results shown that the use of Pd makes feasible to use higher pyrolysis temperatures thus reducing the background signal. Cadmium and Pb absorbance increased with Pd concentration up to 1 µg of Pd. For higher Pd concentrations, no noticeable effect was observed on the absorbance of both elements. Therefore, the amount of modifier was kept at 1 µg for further experiments.

3.1.2. Furnace temperature program

Taking into account the high temperatures required to decompose $BmimPF_6$ (> 350 °C), a two-step pyrolysis procedure was selected to reduce background signals (Li et al., 2009; Berton and Wuilloud, 2010; Stanisz and Zgoła-Grześkowiak, 2013). The pyrolysis temperature was initially set at 350 °C for 20 s and, then, it was increased up to values ranging from 400 to 1200 °C for 20 extra seconds. Fig. 1 shows the pyrolysis and atomization curves for Cd and Pb. In this figure, absorbance of both elements were normalized against their respective maximum value. As it can be observed in Fig. 1A, absorbance remains constant up to pyrolysis temperatures of 800–900 °C. Nevertheless, it is interesting to note that, when increasing the pyrolysis temperature from 400 °C up to the above-mentioned values, background signal was reduced approximately 3-fold. Due to these reasons, pyrolysis temperatures of 800 °C and 900 °C were selected for Cd and Pb, respectively. With regard the atomization temperature (Fig. 1B), the optimum value obtained for both elements was of 1600 °C. These temperatures are similar to those reported by other authors operating with different ionic liquids (i.e., 1-octyl-3-methylimidazolium bis (trifluoromethylsulfonyl) imide) (López-García et al., 2013) and 1-hexyl-3-methylimizadolium hexafluorophosphate (Li et al., 2009). Finally, it is worth to mention that no significant memory effects were observed due to carbon deposition in the furnace after several graphite firings, thus ensuring the reproducibility of the signal measurements (5–10%).

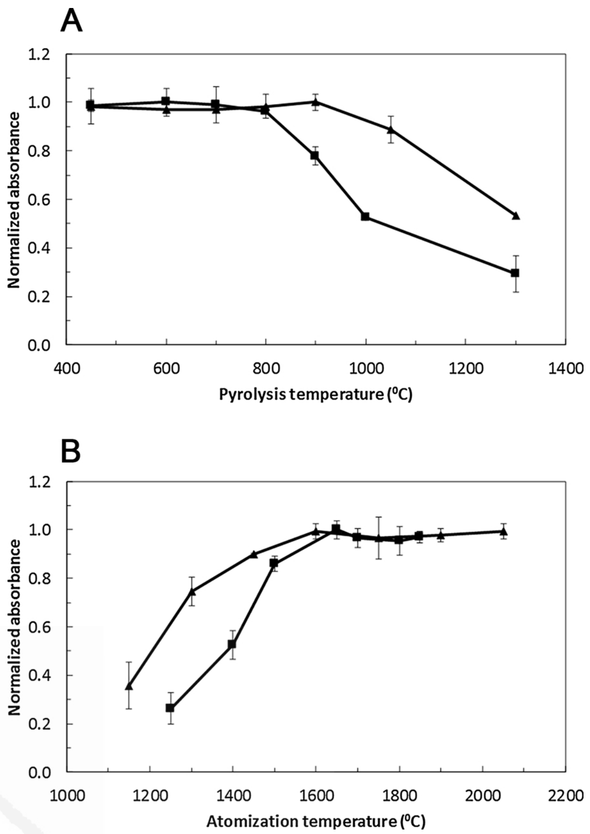

Fig. 1. Influence of the pyrolysis (A) and atomization (B) temperature on the normalized absorbance for Cd (-■-) and Pb (-▲-) in ETAAS. Modifier:1 µg Pd. Atomization temperature in (A): Cd and Pb: 1600 °C; Pyrolysis temperature in (B): Cd 800 °C, Pb 900 °C.

3.2. Optimization of DLLME experimental conditions

Optimization of the DLLME procedure was carried out using 2 mL of a synthetic wine sample containing 12.5% $v\,v^{-1}$ ethanol and potassium 1000 $mg\,L^{-1}$ (i.e. the major organic and inorganic wine components). The variables tested were: (i) DDTC concentration (chelating agent); (iii) pH; (iii) ionic strength; (iv) $BmimPF_6$ mass (extractant solvent); and (v) methanol volume (disperser solvent).

3.2.1. Effect of chelating agent concentration and pH

Metal extraction into the ionic liquid relies on the formation of a neutral chelate with DDTC. Fig. 2 shows the influence of DDTC concentration on the absorbance of Cd and Pb. As it can be observed, absorbance for both elements increased with DDTC concentration up to 1.0%. Above this value, no significant signal improvement was observed.

Solution pH also plays a significant role on the metal-chelate formation and, hence, on the Cd and Pb extraction. The influence of the pH on analyte extraction was investigated in the pH range from 3 to 9 (Fig. 3). No significant influence of the solution pH on Cd absorbance was observed. Nevertheless, Pb signal shows a maximum at pH values around 6–7. From these findings, DDTC concentration and pH were set at 1% $v\,v^{-1}$ and 6, respectively.

3.2.2. Effect of solution ionic strength

The influence of the ionic strength on Cd and Pb extraction from the aqueous phase to the organic phase was investigated using synthetic

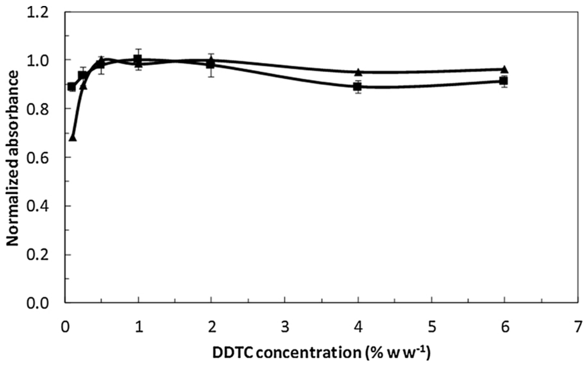

Fig. 2. Influence of the DDTC on the normalized absorbance for Cd (-■-) and Pb (-▲-) in ETAAS. $BmimPF_6$ mass: 150 µg; Methanol volume: 600 µL; solution pH: 6; NaCl concentration: 5.0% w w^{-1}.

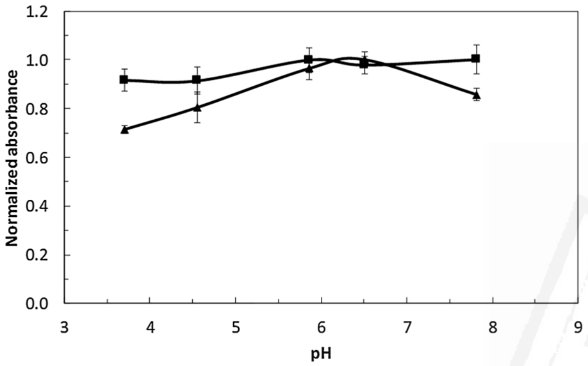

Fig. 3. Influence of the solution pH on the normalized absorbance for Cd (-■-) and Pb (-▲-) in ETAAS. $BmimPF_6$ mass: 150 µg; Methanol volume: 600 µL; DDTC concentration: 1.0 w w^{-1}; NaCl concentration: 5.0% w w^{-1}.

wine samples spiked with variable amounts of NaCl (0–15% w v^{-1}). Cadmium and Pb absorbance showed a maximum for the solution containing 5.0% w v^{-1} NaCl. These results suggest that analyte extraction is partially favoured by increasing the media ionic strength. Therefore, this optimum NaCl concentration was employed in further experiments.

3.2.3. *Effect of the amount of extractant*

To evaluate the influence of $BmimPF_6$ amount on Cd and Pb extraction, ionic liquid masses ranging from 150 to 300 mg were tested. $BmimPF_6$ masses lower than 150 mg were not explored due to the lack of reproducibility in drop formation and the low masses of ionic liquid recovered after the DLLME treatment (< 15 µg). It was observed that Cd and Pb absorbance decreased when increasing the amount of $BmimPF_6$. These findings are easily explained taking into account that analyte preconcentration factor is reduced when increasing the mass of extractant (Li et al., 2009; Yousefi and Shemirani, 2010; Khan et al., 2013). Therefore, a $BmimPF_6$ mass of 150 mg was selected for further studies.

3.2.4. *Effect of the disperser solvent nature and volume*

Two organic solvents (methanol and acetone) were tested as potential dispersers for $BmimPF_6$. Results shown that Cd and Pb signals were on average 1.2-fold higher for methanol than for acetone. Similar findings were obtained by Khan et al. (2013) operating $BmimPF_6$ for Cd extraction with 1-(2-pyridylazo)-2-naphthol in environmental and biological samples. Fig. 4 shows the influence of methanol volume on the normalized Cd and Pb absorbance in ETAAS. As it can be observed, absorbance for these elements increases with methanol volume up to 600 µL due to a higher extraction efficiency of the metal-chelate complex from the water to the organic phase. Volumes higher than 600 µL were not employed due to the lack of $BmimPF_6$ droplet formation after the DLLME treatment. These results suggest that the $BmimPF_6$ solubility in the synthetic wine standards is favoured by the disperser solvent. In fact, this issue was not found operating ethanol-free wine standards. Therefore, methanol volume was set at 600 µL.

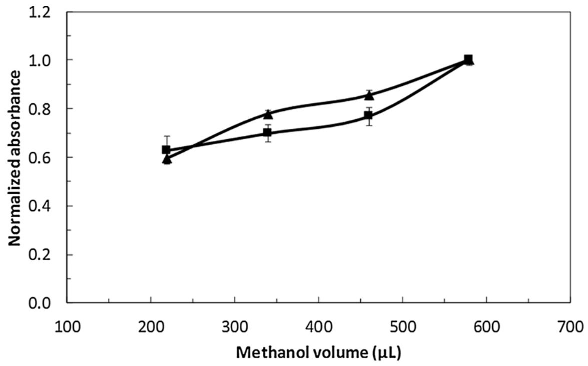

Fig. 4. Influence of the disperser volume on the normalized absorbance for Cd (-■-) and Pb (-▲-) in ETAAS. $BmimPF_6$ mass: 150 µg; DDTC concentration: 1.0 w w^{-1}; solution pH: 6; NaCl concentration: 5.0% w w^{-1}.

3.3. *Analytical figures of merit*

Due to the lack of a certified wine reference material for Cd and Pb analysis, method accuracy was evaluated by means of a recovery test using different wine samples. Taking into account the levels usually found for these elements in wines, samples were spiked with 1 µg L^{-1} of Cd and 10 µg L^{-1} of Pb. The assay was performed using the optimum ETAAS and DLLME operating conditions described in the previous sections. Results for the recovery test are reported in Table 2. As it can be observed, Pb recovery was almost quantitative (96–100%), regardless the wine sample selected. However, results obtained for Cd were systematically lower (i.e., 20–79% lower) than the expected ones. These results suggest that other elements present in wine are affecting the Cd extraction. According to the literature (Grindlay et al., 2011), along with potassium, wines contain significant amounts of: (i) Ca (100 mg L^{-1}); (ii) Mg (100 mg L^{-1}); (iii) Fe (4 mg L^{-1}); (iv) Cu (1 mg L^{-1}); and (v) Zn (1 mg L^{-1}). Thus, to insight on the origin of the poor Cd recoveries obtained, different synthetic wine (12% v v^{-1} ethanol and 1000 mg L^{-1} potassium) matrices containing each of the above-mentioned concomitants were prepared. Cadmium recovery was quantitative for all the matrices tested except for the Zn containing one. Thus, when operating matrices containing Zn levels below 0.5 mg L^{-1}, Cd recoveries were quantitative. From this concentration on, Cd recoveries always decrease when increasing the concentration of Zn present in the matrix. At concentrations of 1 mg Zn L^{-1}, Cd recovery was found of 76 ± 4%. Taking into account these findings, Zn concentration was determined in all the wine samples tested. As expected, a direct correlation between Zn levels and Cd recovery was found (Table 2). So the highest Cd recoveries were obtained for those samples

Table 2
Recovery values for Cd and Pb by DLLME-ETAAS and concentration of Zn present in the different wine samples.

Type of wine	Recovery (%)[a]		Zn (µg L^{-1})[a]
	Pb	Cd	
White	98 ± 4	79 ± 5	800 ± 30
Rosé	99 ± 5	52 ± 4	1400 ± 90
Sparkling	98 ± 4	51 ± 6	1300 ± 100
Fortified	100 ± 3	36 ± 4	2100 ± 180
Red	96 ± 4	20 ± 5	2800 ± 130

[a] Mean ± standard deviation, 5 replicates.

Table 3
Comparison of the LOD obtained using the diverse methodologies reported for Cd and Pb determination in wine by electrothermal-based spectroscopic techniques.

Element	Sample preparation	Background correction	LOD ($\mu g\ L^{-1}$)	References
Cd	Direct analysis	Deuterium	0.08	This work
	DLLME	Deuterium	0.01	
	Direct analysis	Zeeman	0.03	Ajtony et al., 2008
	Digestion	Zeeman	0.1	Kim, 2004
	Dilution	Zeeman	0.03	Freschi et al., 2001
Pb	Direct analysis	Deuterium	0.16	This work
	DLLME	Deuterium	0.08	
	Direct analysis	Zeeman	0.8	Ajtony et al., 2008
	Digestion	Zeeman	1.0	Kim, 2004
	Dilution	Zeeman	0.8	Freschi et al., 2001

containing the lowest Zn concentration levels.

As regards the precision of Cd and Pb measurements, the relative standard deviation obtained after the analysis of five independent replicates range between 4 and 9% (values typically obtained in ETAAS).

Cadmium and Pb LODs were calculated using the calibration graph according to the method described by Hubuax et al. (i.e. based on 5 standards, 95% confidence level) (Hubaux and Vos, 1970). LODs obtained for Cd and Pb were 0.01 and 0.08 $\mu g\ L^{-1}$, respectively. As reported in Table 3, these values were about 8 and 9-fold lower than those obtained in ETAAS using a direct analysis method (i.e., without DLLME). These improvement factors are in agreement with the experimental enrichment factors obtained for Cd and Pb when operating with DLLME. These results suggest that LODs could be further improved by increasing the analyte enrichment factor. In fact, LoDs obtained in this work are higher than those reported in the literature using other DLLME procedures for Cd and Pb analysis by ETAAS (de la Calle et al., 2016). It can be explained taking into account that in the method proposed, the extraction with $BmimPF_6$: (i) requires a dilution with methanol prior to the sample introduction into the graphite furnace, thus reducing the analyte enrichment factor. In addition, also the sample volume used in this work was at least between 2.5 and 5-fold lower than those usually reported due to the limited volume of the centrifuge tubes employed; and, (ii) is negatively affected by the presence of ethanol in the wine matrix. In spite of these facts, data in Table 3 clearly demonstrate that the LODs obtained with the method proposed in this work are lower than those previously reported for Cd and Pb in wines by ETAAS using alternative sample preparation methodologies. In addition, the proposed method reduces both instrumental analysis complexity and costs since it does not require neither time consuming digestion treatments nor complex background correction systems to deal with spectral interferences.

3.4. Analysis of wine samples

Five wine samples have been analyzed to quantify Cd and Pb levels using the methodology developed. Lead analysis was performed by means of calibration with standards containing ethanol 12% $v\ v^{-1}$ and 1000 $mg\ K\ L^{-1}$. Given the variability of the Zn content in the wine samples, standard addition was chosen as the calibration strategy for Cd analysis. Other approaches (e.g. Zn matched standards, additional extraction treatments) were not investigated due to their higher complexity as well as their negative impact on the benefits derived of using DLLME for sample preparation (e.g. sample throughput, simplicity, etc.). Results obtained were compared to those obtained using a microwave acid sample digestion procedure and ICP-MS detection. Wine digestion decompose organic components mitigating carbon matrix effects. As it can be derived from data shown in Table 4, no significant difference was found in the Pb concentration values obtained by DLLME-ETAAS and ICP-MS. Lead concentration levels in the tested wine samples agreed with those previously reported (Grindlay et al., 2009). Cadmium concentration levels in all the samples tested were below the LoD of both DLLME-ETAAS and ICP-MS (Table 4).

Table 4
Lead and cadmium concentration values in the different wine samples by means of DLLME-ETAAS and ICP-MS.

Type of wine	Pb ($\mu g\ L^{-1}$)[a]		Cd ($\mu g\ L^{-1}$)[a]	
	DLLME-ETAAS	ICP-MS	DLLME-ETAAS	ICP-MS
White	2.0 ± 0.2	1.8 ± 0.1	< 0.01	
Rosé	2.17 ± 0.16	2.14 ± 0.07	< 0.01	
Sparkling	1.75 ± 0.15	2.0 ± 0.1	< 0.01	
Fortified	1.8 ± 0.3	1.7 ± 0.1	< 0.01	
Red	2.7 ± 0.2	2.7 ± 0.2	< 0.01	

[a] Mean ± standard deviation, 5 replicates.

4. Conclusions

Results reported in this work demonstrate that DLLME using ionic liquids is a very simple and useful sample-pre-treatment methodology for accurate determination of Cd and Pb in wine samples by ETAAS. Cadmium extraction in $BmimPF_6$ is strongly affected by the presence of Zn in wine samples. As a consequence, the use of a standard addition calibration strategy is mandatory for this element. Under optimum conditions, the LoDs obtained for Cd and Pb are lower than those previously reported in ETAAS using conventional sample preparation strategies (i.e., sample dilution, digestion and direct analysis). These results highlight the great potential of DLLME for metal analysis in wine but further research is still required mainly to improve the method selectivity (especially for Cd) and LoDs.

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Determination of lead in wine samples by means of Dispersive Liquid-Liquid Microextraction and Graphite Furnace Atomic Absorption Detection

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Universitat d'Alacant
Universidad de Alicante
Analytical Atomic Spectrometry Group
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Introduction

Wine is a widely consumed beverage around the world with significant Pb levels. Lead is a toxic metal with numerous adverse effects such as hemoglobin synthesis and nervous system(1). The source of Pb in wine is mainly related to atmospheric precipitation, pesticides and materials used in production, transport and storage. The commission Europe(CE) set maximum values lead in wines as 200 µg/l (2). Lead analysis is usually performed by means of spectroscopic techniques such as Graphite Atomic Absorption Spectrometry (GFAAS) and Inductively Coupled Plasma Mass Spectrometry due to its lower limits of detection (3). However, results are strongly affected by the occurrence of both spectral and non-spectral interferences. To mitigate wine matrix effects, several sample pre-treatment strategies such as dilution or acid digestion has been employed.

The goal of this work is to develop a new methodology for Pb determination in wine by means GFAAS after a extraction/preconcentration procedure based on the use of dispersive liquid-liquid microextraction (DLLME) and ionic liquids (1-butyl-3-methylimidazolium hexafluorophosphate, BmimPF$_6$). To this end, the influence of chelating agent, extractant and dispersant volume, pH and ionic strength on Pb detection has been investigated.

Experimental conditions

Instrumentation: Perkin Elmer 2100 Atomic Absorption spectrometer with Perkin Elmer HGA 700

Autosampler: Perkin Elmer AS-70

Furnace temperatures:

Pyrolysis: 800ºC

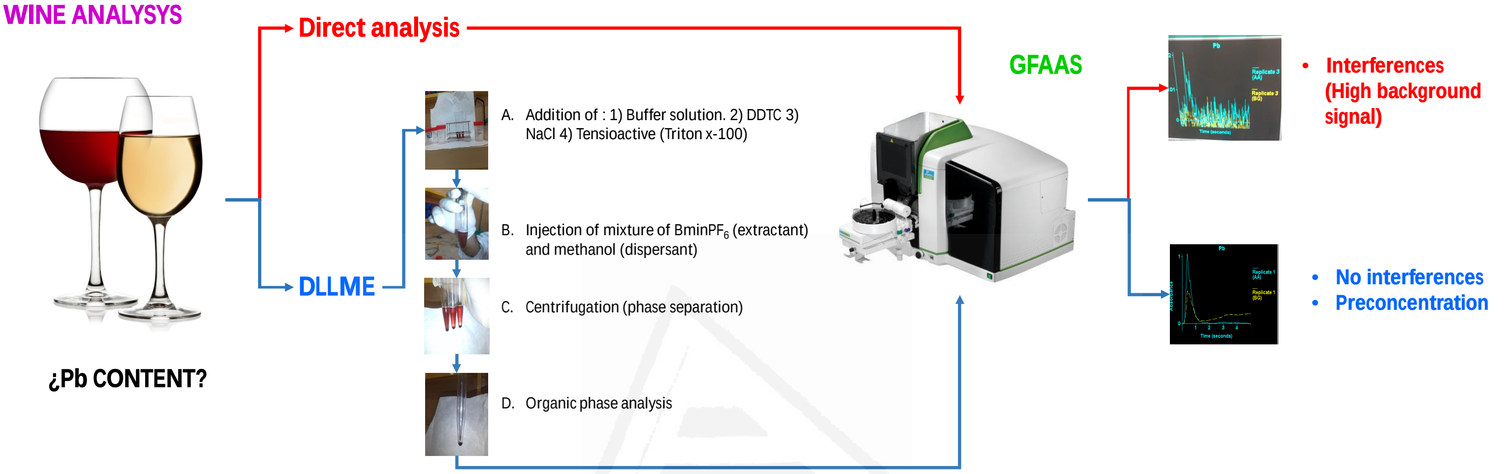
Atomization: 1800ºC

Modifier: Pd (100 mg/L; 8 µL)

Sample volume: 20 µL

Table 1. DLLME experimental variables studied

Parameter	Range
DDTC	0,1-8% w/w
pH	3,34-7,41
NaCl	0-20% w/w
BmimPF$_6$	120-290 mg
Methanol	300-1000 µl
Triton x-100	10-110 mg



WINE ANALYSYS

¿Pb CONTENT?

Direct analysis

DLLME

A. Addition of : 1) Buffer solution. 2) DDTC 3) NaCl 4) Tensioactive (Triton x-100)
B. Injection of mixture of BminPF$_6$ (extractant) and methanol (dispersant)
C. Centrifugation (phase separation)
D. Organic phase analysis

GFAAS

- **Interferences (High background signal)**

- **No interferences**
- **Preconcentration**

DLLME procedure optimization

Influence of DDTC

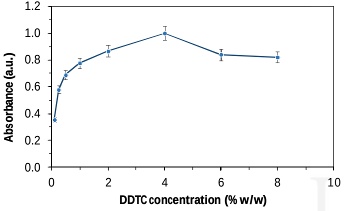


Figure 1. Influence of the DDTC concentration on the lead absorbance. pH: 5,03; BminPF$_6$: 140mg; methanol: 500 µl; NaCl: 20 % w/w; tensioactive: 25mg.

Influence of pH

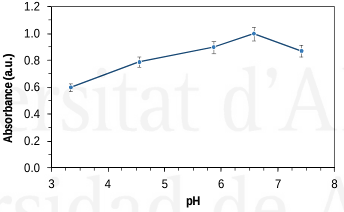


Figure 2. Influence of the pH on the lead absorbance. DDTC: 2% w/w; BminPF$_6$: 140 mg; methanol: 500 µl; NaCl: 20% w/w; tensioactive: 25mg.

Influence of extractant

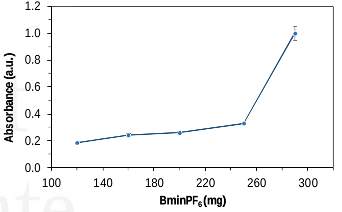


Figure 3. Influence of the extractant on the lead absorbance. DDTC: 2% w/w; pH: 5,03; methanol: 500 µl; NaCl: 20% w/w; tensioactive: 25mg.

Influence of methanol

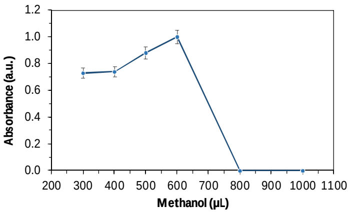


Figure 4. Influence of the methanol on the lead absorbance. DDTC: 2% w/w; pH: 5,03; BminPF$_6$: 140mg; NaCl: 20 % w/w; tensioactive: 25mg.

Influence of NaCl

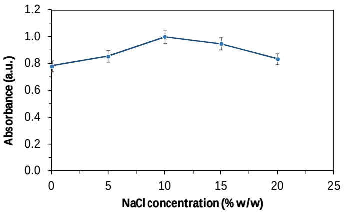


Figure 5. Influence of the NaCl concentration on the lead absorbance. DDTC: 2% w/w; pH: 5.03; BminPF$_6$: 140 mg ; methanol: 500 µl; tensioactive: 25mg.

Influence of tensioactive

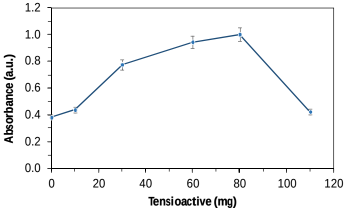
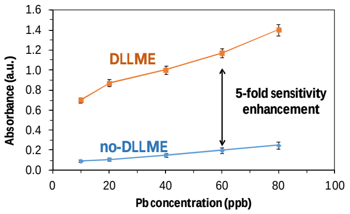


Figure 6. Influence of the tensioactive on the lead absorbance. DDTC : 2% w/w; pH: 5,03; BminPF$_6$: 140 mg; methanol: 500 µl; NaCl: 20% w/w.

Analytical figures of merit



Pb recovery test: 103±9%

Repetibility: 2-7%

Limits of detection: 0.3 ppb

Figure 7. Pb calibration curve with and without DLLME sample pretreatment. DLLME experimental conditions: DDTC: 4% w/w; pH: 6,71; BminPF$_6$: 140 mg; methanol: 600 µl; NaCl: 10 % w/w; tensioactive: 50 mg. Calibration with matrix-matching standards (ethanol 12% v v^{-1} + K 1000 mg L^{-1})

Conclusions

- Lead determination in wine is feasible using DLLME coupled to GFAAS
- DLLME improves limits of detection and reduces matrix effects when compared to direct wine analysis by GFAAS
- Pb extraction efficiency in wine by means of DDLME depends on the chelating agent , extractant, dispersant and tensioactive concentration as well as the solution pH and ionic strength.

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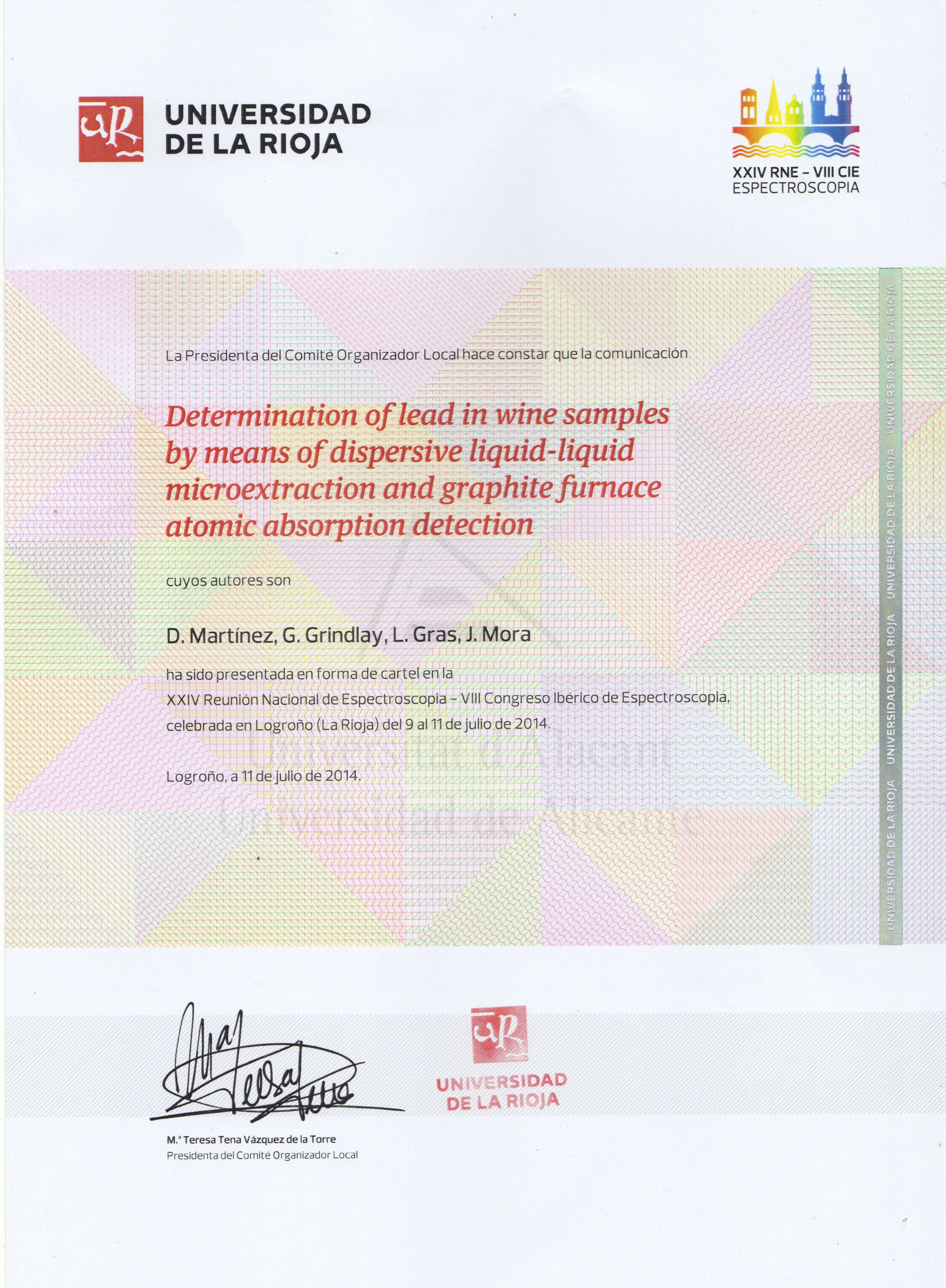
3. G. Grindlay, J. Mora, L. Gras, M.T.C. de Loos-Vollebregt. Atomic spectrometry methods for wine analysis: A critical evaluation and discussion of recent applications, Analytica Chimica Acta 691 (2011) 18–32.



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La Presidenta del Comité Organizador Local hace constar que la comunicación

Determination of lead in wine samples by means of dispersive liquid-liquid microextraction and graphite furnace atomic absorption detection

cuyos autores son

D. Martínez, G. Grindlay, L. Gras, J. Mora

ha sido presentada en forma de cartel en la
XXIV Reunión Nacional de Espectroscopia – VIII Congreso Ibérico de Espectroscopia,
celebrada en Logroño (La Rioja) del 9 al 11 de julio de 2014.

Logroño, a 11 de julio de 2014.

M.ª Teresa Tena Vázquez de la Torre
Presidenta del Comité Organizador Local

The European Conference on Analytical Chemistry

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Certificate for poster presentation

to whom it may concern

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Tel +33 5 40 00 62 82
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Bordeaux, Sept. 10th 2015

Dear Sir/Madam,

this is to confirm that :

Emma Perez Hernandez
University of Alicante, Spain

attended the EUROANALYSIS 2015 Conference held in Bordeaux, France, 6th-10th September 2015. She presented the following poster:

P560. “Determination of lead and cadmium in wine samples by means of dispersive microextraction and graphite furnace atomic absorption spectrometry”
Emma Pérez, David Martínez, Guillermo Grindlay, Luis Gras and Juan Mora

Should you require more information, please do not hesitate to contact me at philippe.garrigues@u-bordeaux.fr.

Many thanks for your collaboration.

Best regards.

Dr. Philippe Garrigues
Chairman of the EUROANALYSIS2015 Conference

Determination of lead and cadmium in wine samples by means of Dispersive Liquid-Liquid Microextraction and Graphite Furnace Atomic Absorption Detection

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Analytical Atomic Spectrometry Group

18th edition of EuroAnalysis, The European Conference on Analytical Chemistry

EUROANALYSIS XVIII
Bordeaux, France 6th- 10th September 2015
EuCheMS European Chemical Sciences Division of Analytical Chemistry
www.euroanalysis2015.com

Introduction

Lead and cadmium levels in wines are regulated due to their negative impact on human health. The analysis of these elements is usually performed by means of spectroscopic techniques such as Graphite Atomic Absorption Spectrometry (GFAAS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) due to its lower limits of detection. However, results are strongly affected by the occurrence of both spectral and non-spectral interferences. To mitigate wine matrix effects, several sample pre-treatment strategies such as dilution, acid digestion or sample extraction could be employed. The goal of this work is to develop a new methodology for Pb and Cd determination in wine by means GFAAS after an extraction/preconcentration procedure based on the simultaneous use of dispersive liquid-liquid microextraction (DLLME) and ionic liquids (1-butyl-3-methylimidazolium hexafluorophosphate). To this end, the influence of chelating agent, extractant and dispersant volume, pH and ionic strength on both Pb and Cd detection has been investigated.

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Autosampler: Perkin Elmer AS-70

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Atomization: 1800ºC

Modifier: Pd (100 mg/L; 8 µL)

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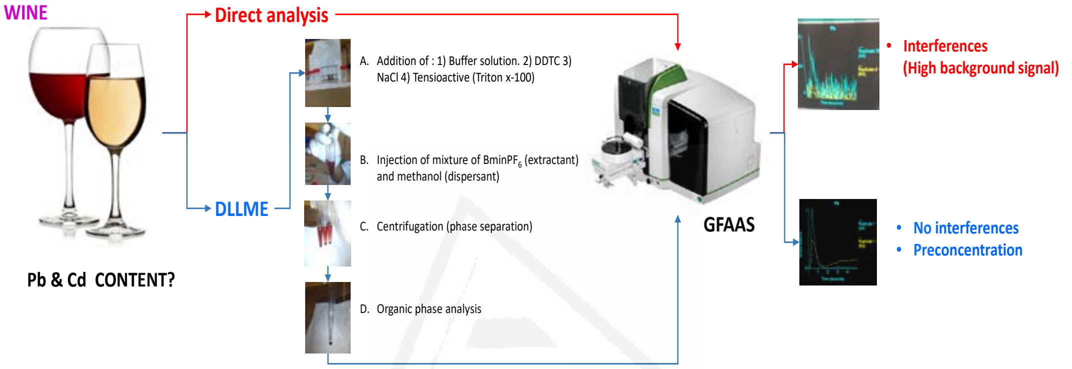

WINE

Pb & Cd CONTENT?

Direct analysis

DLLME

A. Addition of : 1) Buffer solution. 2) DDTC 3) NaCl 4) Tensioactive (Triton x-100)

B. Injection of mixture of $BminPF_6$ (extractant) and methanol (dispersant)

C. Centrifugation (phase separation)

D. Organic phase analysis

GFAAS

- Interferences (High background signal)

- No interferences
- Preconcentration

DLLME procedure optimization

Influence of DDTC

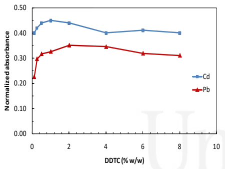

Figure 1. Influence of the DDTC concentration on the lead and cadmium absorbance. pH: 5,03; $BminPF_6$: 140mg; methanol: 500 µl; NaCl: 20 % w/w; tensioactive: 25mg.

Influence of pH

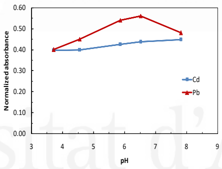

Figure 2. Influence of the pH on the lead and cadmium absorbance. DDTC: 2% w/w; $BminPF_6$: 140 mg; methanol: 500 µl; NaCl: 20% w/w; tensioactive: 25mg.

Influence of extractant

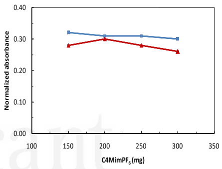

Figure 3. Influence of the extractant ($BminPF_6$) volume on the lead and cadmium absorbance. DDTC: 2% w/w; pH: 5,03; methanol: 500 µl; NaCl: 20% w/w; tensioactive: 25mg.

Influence of dispersant

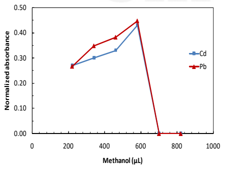

Figure 4. Influence of the dispersant (methanol) volume on the lead and cadmium absorbance. DDTC: 2% w/w; pH: 5,03; $BminPF_6$: 140mg; NaCl: 20 % w/w; tensioactive: 25mg.

Influence of ionic strength

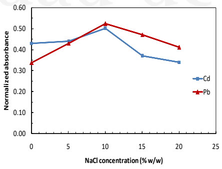

Figure 5. Influence of the NaCl concentration on the lead and cadmium absorbance. DDTC: 2% w/w; pH: 5.03; $BminPF_6$: 140 mg ; methanol: 500 µl; tensioactive: 25mg.

Influence of tensioactive

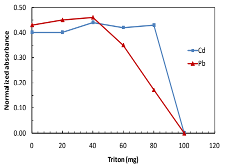

Figure 6. Influence of the tensioactive amount on the lead and cadmium absorbance. DDTC : 2% w/w; pH: 5,03; $BminPF_6$: 140 mg; methanol: 500 µl; NaCl: 20% w/w.

Analytical figures of merit

Table 2. Analytical figures of merit for the DLLME-GFAAS methodology. Experimental conditions: DDTC: 4% w/w; pH 6.51; $BminPF_6$: 140 mg ; methanol: 500 µl; tensioactive: 25mg.

Vine sample	Pb	Cd
ıcentration factor	8	7
ɔf detection	0.7	0.1
₹epetatibility	5%	7%

Table 3. Analytical recovery test using optimum extraction conditions.

	Pb	Cd
White	98±4	79±5
Rosé	99±5	52±4
Fortified	100±3	36±4
Cava	98±4	51±6
Red	96±4	20±5

Table 4. Influence of wine matrix concomitants on analyte recovery using optimum extraction conditions.

	Cd
Ca (100)	±
Mg (100)	±
Fe (4)	98±5
Mn (3)	±
Cu (1)	95±3
Zn (1)	76±4

Wine analysis

Table 5. Determination of Cd and Pb levels in different wine samples.

White	2.0±0.2	<0.1
Rosé	±	<0.1
Fortified	1.8±0.3	<0.1
Cava	±	<0.1
Red	2.7±0.2	<0.1

Conclusions

- Cd and Pb determination in wine is feasible using DLLME coupled to GFAAS
- DLLME improves limits of detection and reduces matrix effects when compared to direct wine analysis by GFAAS
- Cd and Pb extraction efficiency in wine by means of DDLME depends on the chelating agent , extractant, dispersant and tensioactive concentration as well as the solution pH and ionic strength.

Acknowledgments

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Coupling dispersive liquid-liquid microextraction to inductively coupled plasma atomic emission spectrometry: An oxymoron?

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ARTICLE INFO

Keywords:
Metals
Sample preparation
Dispersive liquid-liquid microextraction
Flow-injection
Atomic emission spectrometry
Inductively coupled plasma

ABSTRACT

Coupling dispersive liquid-liquid micro-extraction (DLLME) to inductively coupled plasma atomic emission spectrometry (ICP-AES) is usually troublesome due to the limited plasma tolerance to the organic solvents usually employed for metal extraction. This work explores different coupling strategies allowing the multi-element determination by ICP-AES of the solutions obtained after DLLME procedures. To this end, three of the most common extractant solvents in DLLME procedures (1-undecanol, 1-butyl-3-methyl-imidazolium hexafluorophosphate and chloroform) have been selected to face most of the main problems reported in DLLME-ICP-AES coupling (i.e., those arising from the high solvent viscosity and volatility). Results demonstrate that DLLME can be successfully coupled to ICP-AES after a careful optimization of the experimental conditions. Thus, elemental analysis in 1-undecanol and 1-butyl-3-methyl-imidazolium hexafluorophosphate extracts can be achieved by ICP-AES after a simple dilution step with methanol (1:0.5). Chloroform can be directly introduced into the plasma with minimum changes in the ICP-AES configuration usually employed when operating with aqueous solutions. Diluted inorganic acid solutions (1% w w^{-1} either nitric or hydrochloric acids) have been successfully tested for the first time as a carrier for the introduction of organic extractants in ICP-AES. The coupling strategies proposed have been successfully applied to the multi-element analysis (Al, Cu, Fe, Mn, Ni and Zn) of different water samples (i.e. marine, tap and river) by DLLME-ICP-AES.

1. Introduction

Dispersive liquid-liquid micro-extraction (DLLME) has been stablished as a fast and green sample preparation methodology to separate and preconcentrate analytes from samples with complex matrices [1]. Though most of the analytical applications reported in the literature have been focused on organic analytes, DLLME has also been employed for metal and non-metal analysis by means of spectroscopic techniques [2]. In these cases, detection is usually accomplished by means atomic absorption spectrometry (AAS) due to the robustness of the atomization sources (i.e. flame or furnace) and to the reasonably good analytical figures of merit [3,4]. Among the AAS techniques, electrothermal atomic absorption spectrometry (ETAAS) is often preferred because of the limited sample volume available after DLLME procedures. Nevertheless, its low sample throughput limits the application of the technique.

Inductively coupled plasma (ICP)-based techniques (i.e., ICP-atomic emission, ICP-AES, and ICP-mass spectrometry, ICP-MS) have a greater analytical potential than AAS techniques. ICP-based techniques allow simultaneous multi-element analysis, thus increasing sample throughput with minimum sample and reagent consumption as well as waste generation. However, due to the limited volume available after the microextraction procedure and low plasma tolerance to the organic solvents usually employed for analyte extraction [2,5], DLLME is not usually employed coupled to ICP-based techniques [6,7]. Thus, high volatile solvents (such as carbon tetrachloride, chloroform, etc.) have a deleterious effect on plasma conditions that negatively affects analytical figures of merit and could even lead to plasma extinction. Also, high viscous solvents (e.g. 1-undecanol or ionic liquids), cause additional problems arising from a poor nebulization efficiency and strong memory effects. To address these shortcomings, several strategies have been suggested in the literature: (i) solvent evaporation and subsequent acid reconstitution before the analysis [8,9]; (ii) water back-extraction [10,11]; (iii) dilution with an appropriate solvent [12–15]; and (vi) the use of alternative sample introduction systems such as flow injection analysis (FIA) [16], electrothermal vaporization [17] or laser ablation [18]. Nevertheless, despite the above-mentioned approaches make feasible DLLME coupling to ICP-based techniques, some severe drawbacks still remain. In fact, the main inherent benefits of DLLME (e.g. simplicity, high sample throughput, etc.) are mostly counterbalanced

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due to the additional steps required to perform the analysis. Moreover, some approaches may require complex modifications in the ICP configuration that are not always available in most of the analytical laboratories.

From the above-mentioned considerations, it can be derived that DLLME-ICP-based techniques is a rather complex coupling and, hence, usually discarded from a practical point of view. However, a comprehensive review of the works reported in this field reveals that most of the previous studies have been mainly focused on the optimization of the extraction procedure. Nevertheless, no studies including the optimization of the experimental and instrumental conditions of the plasma source have been performed up to date. This is a very surprisingly fact taking into account the strong influence of the ICP parameters (e.g. plasma power, sample uptake rate, nebulizer gas flow rate, etc.) and the sample introduction system on the analytical figures of merit [5,19,20]. In our opinion, to couple DLLME-ICP-based techniques, the optimization of the full variables (experimental and instrumental) of both DLLME and ICP is mandatory. The extensive number of applications based on the use of plasma-based techniques for elemental analysis in organic matrices [19] justify the interest of this coupling.

The goal of this work is to explore and evaluate different analytical approaches for coupling DLLME to ICP-AES. To this end, several organic solvents usually employed in DLLME procedures and covering different range of the main physical properties affecting the signal response in ICP-AES (i.e., viscosity and volatility) have been selected: 1-undecanol, 1-butyl-3-methyl-imidazolium hexafluorophosphate and chloroform. These solvents have been introduced (either directly or after a dilution step with alcohol or acid solutions) into the plasma source by means of a flow injection device. Main ICP-AES experimental variables (plasma r.f. power, nebulizer gas flow rate and carrier flow rate) have been also optimized to make feasible the analysis of these organic solvents by ICP-AES and to achieve the best analytical figures of merit. Finally, the proposed DLLME-ICP-AES approaches have been compared and evaluated by analyzing several water samples (i.e. marine, tap and river).

2. Experimental

2.1. Chemicals

Organic solvents (i.e., 1-undecanol, chloroform, 1-butyl-3-methylimidazolium hexafluorophosphate, acetone, methanol, absolute ethanol and 1-propanol) and chelating agents (i.e. diethyldithiocarbamate (DDTC)), 2-theonyltrifluoroacetone (TTA), ammonium pyrrolidine dithiocarbamate (APDC) and a multi-element 200 mg L^{-1} organometallic solution were purchased from Sigma-Aldrich (Steinheim, Germany). Sodium chloride, 69% w w^{-1} nitric acid, 36% w w^{-1} hydrochloric acid, 85% w w^{-1} phosphoric acid, sodium dihydrogen phosphate, acetic acid and sodium acetate were obtained from Panreac (Barcelona, Spain). An ICP-IV multi-element 1000 mg L^{-1} solution was provided by Merck (Darmstadt, Germany).

2.2. Solutions

Three different extractant solvents, namely: (i) 1-undecanol; (ii) 1-butyl-3-methyl-imidazolium hexafluorophosphate ($BmimPF_6$); and (iii) chloroform, have been evaluated. These solvents were selected, among the most common extractants in DLLME, to face most of the main problems arising from DLLME-ICP-AES coupling (e.g. solvent viscosity and volatility) (see Table 1) [21–23].

When operating with viscous solvents (1-undecanol or $BmimPF_6$), a dilution step prior to the analysis by ICP-AES was mandatory. Thus, 1-undecanol was diluted in different alcohols, namely, methanol, ethanol and 1-propanol. This approach was also employed for $BmimPF_6$ but, in this case, 36% w w^{-1} hydrochloric acid solution was additionally tested as a dilution solvent. Physical properties of the different dilution solvents employed for 1-undecanol and $BmimPF_6$ are also gathered in Table 1. Dilution ratios ranging from 1:0.5 to 1:3 for both 1-undecanol and $BmimPF_6$ were tested. Analyte standard solution in each media was prepared in two-steps. First, an aliquot of the aqueous 1000 mg L^{-1} ICP-IV multi-elemental reference solution was spiked into the proper dilution solvent (i.e. methanol, ethanol, 1-propanol or 36% w w^{-1} hydrochloric acid solution). Next, this solution was mixed in the right proportion with 1-undecanol or $BmimPF_6$ for a final analyte concentration of 1 mg L^{-1}.

Table 1
Physical properties of the organic solvents tested in this work at 25 °C.

	Surface tension (mN m^{-1})	Viscosity (mPa s^{-1})	Density (g mL^{-1})[a]	Vapor pressure (mmHg)
1-undecanol	26.5	17.2	0.83	0.0004
$BmimPF_6$	47.7	381	1.38	–
Chloroform	26.7	0.56	1.49	26.2
Methanol	22.7	0.54	0.79	128
Ethanol	22.0	1.07	0.79	59
1-propanol	20.9	1.95	0.80	21
HCl (36% w w^{-1})	65.8	1.84	1.6	35

[a] 20 °C.

Chloroform-based samples were directly analyzed by ICP-AES. Therefore, analyte chloroform standard (1 mg L^{-1}) was prepared by diluting the appropriate aliquots of a 200 mg L^{-1} multi-elemental organometallic solution in this solvent.

2.3. Instrumentation

ICP-AES measurements were performed using an Agilent 720 ICP-AES (Agilent, Santa Clara, USA) with axial viewing using the operating conditions reported in Table 2. Different sample introduction systems were tested depending on the characteristics of the extractant solvent used. Thus, when operating with both 1-undecanol and chloroform, a standard sample introduction system made of a concentric pneumatic nebulizer (Seaspray, Glass Expansion, Australia) and a cyclonic spray chamber (Cinnabar, Glass Expansion, Australia) was used. As regards $BmimPF_6$, a micronebulizer (OneNeb, Ingeniatrics, Sevilla, Spain) coupled to a single-pass with impact bead PTFE spray chamber (Thermo Scientific, Germany) was employed. All the organic solvents were drived to the nebulizer by means of a V-451 flow injection manifold (Upchurch Scientific, Silsden, United Kingdom) equipped with a 25 µL loop valve. DLLME extracts were injected using a home-made 300 µL plastic syringe with PEEK coated quartz capillary needle (200 µm i.d., PEEKSIL, Upchurch, Oak Harbor, Washington, USA). Samples were introduced into a carrier stream controlled by a peristaltic pump (Model Minipuls 3, Gilson, France). Different carriers were evaluated through this work: (i) 1% w w^{-1} HNO_3; (ii) 1% w w^{-1} HCl; and (iii) air.

Signal acquisition was performed by means of the transient signal (TRS) software of Agilent's ICP-AES. Microsoft Excel® software was employed for manually signal integration. The wavelengths of the emission lines monitored in this work are listed in Table S1 (Appendix).

2.4. Samples

Three water samples covering a wide range of matrix characteristics were tested: (i) tap water (University of Alicante); (ii) river water (Vinalopó river, N 38°28′15.0096″, W 0°48′15.0336″); and (iii) marine water (Mediterranean Sea, N 38°22′31.7424″, W 0°24′32.5224″). All samples were collected in polyethylene terephtha-

Table 2
ICP-AES operating conditions.

	Solvent		
Agilent 720 ICP-AES	1-undecanol	BmimPF$_6$	Chloroform
Plasma forward power (W)	1400		
Argon flow rate (L min^{-1})			
Plasma	15.0		
Auxiliary	1.25		2.50
Nebulizer	0.6–0.9		0.5–0.8
Carrier flow rate (mL min^{-1})	0.4–1.5		0.4–1.0
Sample introduction system			
Nebulizer	Seaspray®	Seaspray®/Oneneb®	Seaspray®
Spray chamber (material)	Cyclonic (Glass)	Cyclonic (Glass)/Single pass with impact bead (PFA)	Cyclonic (Glass)
Flow injection loop volume (µL)	25		
Acquisition time (s)/replicates	30/3		

late bottles and, after a filtration step with a 0.45 µm syringe filter, acidified and stored at 4 °C until the analysis.

2.5. DLLME procedures

Three DLLME procedures for water analysis were employed to evaluate the different coupling strategies developed in the present work. Next, these methodologies are briefly described.

2.5.1. 1-undecanol-based extraction

Metal extraction with 1-undecanol was carried out using the DLLME procedure described by Yamini et al. [12] with some minor modifications. A sample solution of 5 mL was placed into a 10 mL screw-cap and pH was adjusted using an acetic acid-acetate buffer solution (pH: 6). Next, it was spiked with 600 µL of a 30 w v^{-1} NaCl solution and 25 µL of a 1 g L^{-1} TTA solution in methanol. Simultaneously, 50 µL of 1-undecanol were mixed with 500 µL of acetone and the mixture quickly injected into the sample solution by using a 5.0 mL of syringe. A cloudy solution was formed and, after a centrifugation step (5 min 4000 rpm), the sample solution was transferred into an ice bath where the 1-undecanol (≈ 45 µL) was solidified at the top of the top of the test tube. Finally, 1-undecanol was transferred into an Eppendorf tube where it was melted and diluted with methanol (1:0.5 ratio) before ICP-AES analysis.

2.5.2. BmimPF$_6$-based extraction

In this procedure, based on that reported by Wen et al. [24], 5 mL of the sample were spiked with a phosphoric/dihydrogen phosphate buffer solution to adjust the pH (4). Next, 70 mg of NaCl and 100 µL of a 9% APDC solution added to the sample. A mixture of BmimPF$_6$ (150 mg) and methanol (650 µL) was injected into the sample to form a cloudy solution. The sample was then centrifuged (5 min 4000 rpm) and BmimPF$_6$ (settled at the bottom of the glass test tube) finally transferred into an Eppendorf tube, where it was diluted with methanol (1:0.5 proportion) before ICP-AES analysis.

2.5.3. Chloroform-based extraction

In this procedure, based on that previously described by Hemmatkhah et al. [25], 5 mL of water containing 3.5% w w^{-1} NaCl and 0.010 g DDTC were placed in a 10 mL screw cap glass tube with conical bottom. The solution pH was adjusted to 6.0 with an acetic acid/acetate buffer solution. Next, 100 mg of chloroform were dissolved in 400 µL of ethanol and the mixture was injected into the glass tube containing the sample. A cloudy solution is formed and, after centrifugation (4000 rpm, 2 min), chloroform was sedimented at the bottom of the conical test tube. Chloroform was then transferred into an Eppendorf tube and directly analyzed by ICP-AES.

3. Results

3.1. Coupling 1-undecanol-based DLLME procedures to ICP-AES

3.1.1. Strategies for 1-undecanol introduction into the ICP

Direct analysis of 1-undecanol by ICP-AES is not a feasible task due to its high viscosity (17.2 mPa s^{-1}, see Table 1). As a consequence, this solvent cannot be properly displaced by the peristaltic pump, thus giving rise to poor signal reproducibility and high wash-out times. In addition, when operating with pneumatic nebulizers, high viscosity solvents generate coarser aerosols than the low viscous ones thus negatively affecting the aerosol transport into the plasma and, then, the analytical signal [5,26]. To solve these problems and make feasible the introduction of 1-undecanol solutions in ICP-AES, several strategies were evaluated. First, since solvent viscosity decreases with temperature, 1-undecanol was heated (from 40 to 70 °C) before being injected into the FIA system. Though sample pumping improved increasing the temperature, memory effects were still significant. Alternatively, PTFE tubing from the peristaltic pump to the nebulizer was also heated but no improvement was observed. A second approach reported in the literature to deal with 1-undecanol matrix samples by ICP-AES is its dilution with alcohols [12,14]. Up to date, no systematic study about the influence of the alcohol nature and concentration used for 1-undecanol dilution have been carried out, in spite of the strong influence of the sample matrix on the analytical figures of merit in ICP-AES [5,27]. In the present work, methanol, ethanol and 1-propanol were investigated as dilution solvents for 1-undecanol. Different 1-undecanol:alcohol mixtures (ranging from 1:0.5 to 1:3) containing 1 mg L^{-1} analyte were prepared and measured by ICP-AES (Fig. 1). In general, mixture composition did not have a significant

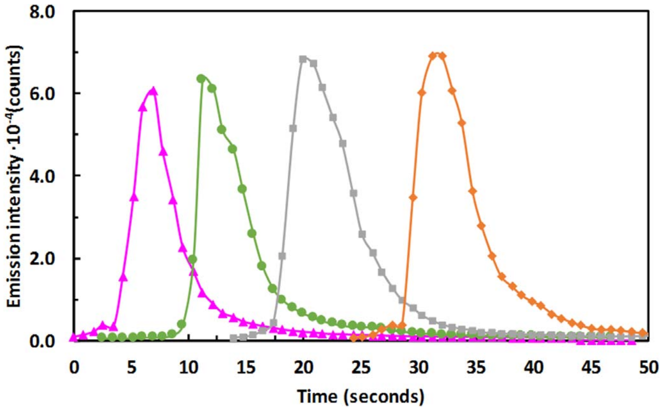

Fig. 1. Cd II 214.439 nm emission signal profile for different 1-undecanol:methanol mixtures. (▲) 1:0.5; (●) 1:1; (■) 1:2; and (◆) 1:3. Q$_g$: 0.7 L min^{-1}; Q$_l$: 0.6 mL min^{-1}. FIA carrier: 1% w w^{-1} HNO$_3$.

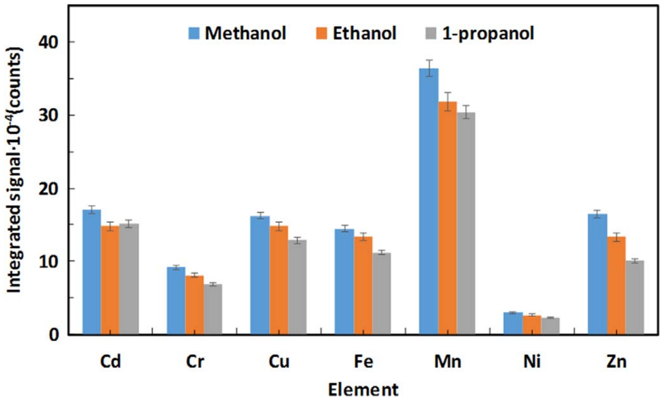

Fig. 2. Influence of the alcohol employed for 1-undecanol dilution on the integrated emission signal of several elements. Q_g: 0.7 L min^{-1}; Q_l: 0.6 mL min^{-1}; 1-undecanol/alcohol ratio: 1:0.5; FIA carrier: 1% w w^{-1} HNO_3.

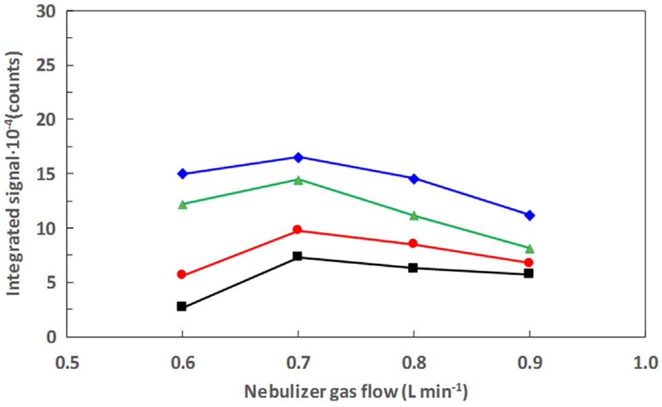

Fig. 3. Influence of the nebulizer gas flow rate on Cd II 214.439 nm integrated emission signal operating 1:0.5 1-undecanol:methanol mixture at different Q_l. (◆) 0.6 mL min^{-1}; (▲) 0.9 mL min^{-1}; (●) 1.2 mL min^{-1}; and (■) 1.5 mL min^{-1}. FIA carrier: 1% w w^{-1} HNO_3.

influence on the analytical signal. Signals for the 1:0.5 and the 1:1 mixtures were very similar but their peak areas were around 1.2-fold lower than those obtained for the 1:2 and 1:3 ones. Similar findings were observed when using ethanol and 1-propanol. These results suggest that alcohol dilution slightly improves the aerosol generation due to the reduction of the solution viscosity. Nevertheless, since the 1-undecanol dilution factor is not too high, no significant differences are expected between the different mixtures tested. From these experiments, the 1:0.5 mixture was selected for further analysis since it provides the minimum sample dilution after the DLLME treatment. The proposed dilution factor is lower than that previously reported in the literature (i.e., usually ≥ 1:1) [12,14]. In general, the type of alcohol employed to dilute 1-undecanol had a limited influence on the analyte emission signal (Fig. 2). Emission signals obtained for the methanol mixture were about 15% higher, on average, than those obtained with ethanol or 1-propanol. This behavior was the expected considering the highest volatility of methanol (Table 1). Plasma robustness was checked by means of the Mg II (280.271 nm)/Mg I (285.213 nm) line intensity ratio for the different 1-undecanol/alcohol mixtures [28]. In all cases, Mg II/Mg I ratio were similar to that obtained for water, thus suggesting that the presence of 1-undecanol/alcohol mixtures did not affect plasma temperature.

Finally, the influence of the carrier nature on the signal obtained in DLLME-ICP-AES was also evaluated. Up to date, poor attention has been paid in the literature to optimize the FIA experimental conditions required for 1-undecanol analysis by ICP-AES, mainly regarding the nature of the carrier solution. In fact, with the exception of Yamini et al. [12] that report the use of 80% 1-propanol v v^{-1} as a carrier, no significant details about carrier characteristics are found in the literature [10]. Despite its potential interferences [29], acids are preferred over organic solvents in ICP-AES since they can be directly introduced into the plasma avoiding the use of complex instrumental arrangements (e.g. oxygen addition, desolvation, etc.) [19,27]. Nevertheless, no study reporting the use of acids as carrier solutions have been found for the analysis of DLLME extracts by ICP-AES. In the present work, 1% w w^{-1} nitric acid and 1% w w^{-1} hydrochloric acid solutions were tested as carriers instead of organic solvents for the first time. Results demonstrated that acid solutions can be successfully used to introduce 1-undecanol/alcohol mixtures into the plasma with high reproducibility and no memory effect. No differences between the signals afforded with both acids were registered.

3.1.2. Optimization of ICP-AES experimental conditions

Analytical figures of merit in ICP-based techniques strongly depend on plasma experimental conditions. For this reason, the influence of the nebulizer gas (Q_g) and carrier flow rate (Q_l) on analyte signal was carried out. Plasma r.f. power was kept closed to maximum nominal value available with the instrument (1400 W) to favor analyte atomization and ionization. Fig. 3 shows the influence of Q_g on Cd II 214.439 nm integrated emission signal for the 1:0.5 1-undecanol:methanol mixture at different Q_l values. Results indicate that the highest emission signals were obtained at Q_g of 0.7 L min^{-1}, irrespective of the Q_l tested. Moreover, results in this figure also indicate that the highest emission signals were obtained at the lowest Q_l employed. Thus, Cd II 214.439 nm integrated emission signal raises 2.3-fold when decreasing Q_l from 1.5 to 0.6 mL min^{-1} at the optimum Q_g. Similar results were observed for the different alcohol mixtures and analytes tested. This behavior can be explained in terms of aerosol generation and transport [26] and plasma characteristics [27]. Thus, the Mg II/Mg I ratios measured at 0.6 and 1.5 mL min^{-1} were of 6.0 and 5.0, respectively.

3.2. Coupling BmimPF$_6$-based DLLME procedures to ICP-AES

3.2.1. Strategies for BmimPF$_6$ introduction into the ICP

When operating with BmimPF$_6$, similar (or even worse) experimental drawbacks than those described for 1-undecanol are observed. In fact, the viscosity of BmimPF$_6$ is higher (about 22-fold higher) than that of 1-undecanol (Table 1). Therefore, a dilution step with an appropriate solvent previous to the BmimPF$_6$ solutions into the plasma is also mandatory. Following a similar approach used with 1-undecanol, BmimPF$_6$ could be diluted with methanol, ethanol and 1-propanol in different proportions (ranging from 1:0.5 to 1:3). Different BmimPF$_6$:methanol mixtures (1:0.5 to 1:3) containing 1 mg L^{-1} analyte were measured to evaluate matrix influence on the signal. It was observed that when operating the 1:1 mixture, signal was about 60% higher than that obtained with the 1:0.5 one. Signals for the highest diluted BmimPF$_6$ ratios (1:2 or 1:3) were similar to those for the 1:1 ratio. These findings suggest the beneficial effects of the BmimPF$_6$ dilution on the aerosol generation due to the reduction of solution viscosity. This behavior is in agreement with the previous findings obtained with 1-undecanol but the influence of dilution on analyte emission signal for BmimPF$_6$ was more significant due to its higher viscosity. The signal improvement registered for the 1:1 mixture did not compensate the analyte dilution factor expected when operating the 1:1 dilution instead of the 1:0.5 one (2-fold). Therefore, the 1:0.5 dilution was selected for further studies. As expected from the low dilution factors employed for BmimPF$_6$, no significant differences on analyte signals were registered operating the different BmimPF$_6$ mixtures (Fig. S1, Appendix). Thus, for instance, the use of methanol as a diluent solvent provided the highest signals but signal improvement when compared to ethanol and 1-propanol was only 1.2 fold. This value is similar to that previously found with 1-undecanol.

Attempting to find alternatives to alcohols for $BmimPF_6$ dilution, the possibility of using hydrochloric acid was explored in this work for the first time. Preliminary experiments demonstrated that when mixing 1:1 $BmimPF_6$:concentrated hydrochloric acid (36% w w^{-1}), a single phase was obtained after 4–5 h at room temperature. Interestingly, the mixture viscosity was clearly lower than that of the pure $BmimPF_6$. Several experimental evidences suggest that the structure of the ionic liquid is modified in the presence of hydrochloric acid. The ionic liquid/acid mixture has a brownish color similar to that of the hexafluorophosphoric acid solutions [30]. This compound is not stable in aqueous media and it is found in equilibrium with phosphoric acid, phosphoric conjugate forms and hydrofluoric acid [31]. Thus, assuming the formation of hexafluorophosphoric acid from the reaction between $BmimPF_6$ and hydrochloric acid, hydrofluoric acid must be formed. This hypothesis was clearly confirmed after checking a glass surface that was in contact with a 1:1 $BmimPF_6$:HCl mixture for 15 min. Finally, it was also observed that blank signals for DLLME extracts operating glass vials were systematically higher than those obtained operating plastic ones. The miscibility of $BmimPF_6$ with hydrochloric acid was further investigated by modifying acid concentration and $BmimPF_6$:acid ratio. Results indicate that $BmimPF_6$ was only miscible with concentrated hydrochloric acid, regardless the acid proportion employed (from 1:0.5 to 1:3). Finally, several strategies were tested to improve the miscibility kinetics between $BmimPF_6$ and hydrochloric acid. First, the different ionic liquid – hydrochloric acid mixtures were shaken either with a vortex or ultrasounds but unsuccessfully. Better results were obtained when heating the $BmimPF_6$/HCl mixture in Eppendorf tubes at temperatures between 40–70 °C. Operating this way, a single phase was obtained after heating the mixture at 70 °C for 5 min (fast enough for practical purposes). It is worth to point out that concentrated nitric acid is also useful for $BmimPF_6$ dilution but it was discarded due to the flammability and oxidizing capabilities of $BmimNO_3$. Because of HF presence in the $BmimPF_6$:acid mixture, the standard ICP-AES sample introduction system was replaced by a nebulizer and a spray made of PTFE. For the sake of comparison, this configuration was also employed with the experiments performed with $BmimPF_6$:alcohol solutions. Also the plasma torch is susceptible to be affected by the presence of HF. Nevertheless, after several hours operating $BmimPF_6$:hydrochloric acid mixtures no sign of HF attack was observed. Presumably, it was due to the low sample volume introduced and the system wash-out with the carrier solution. Although at first glance, physical properties of $BmimPF_6$:hydrochloric acid mixtures are expected to be less favorable for aerosol generation and transport, analyte signals for the $BmimPF_6$:acid mixtures were almost identical to those afforded by ethanol and 1-propanol (Fig. S1, Appendix). Plasma robustness was examined for the different $BmimPF_6$ mixtures but no significant differences in the Mg II/Mg I intensity ratio were observed. In fact, the value obtained for this parameter was similar to that found when operating with water (and, hence, 1-undecanol:alcohol mixtures). Considering the above-discussed results, it seems to be clear that the use of hydrochloric acid for $BmimPF_6$ dilution does not afford any advantage against the use of alcohols. Moreover, the standard glass-made sample introduction system could be used instead of the PTFE since no HF is formed when diluting $BmimPF_6$ with alcohols.

As regards the nature of the carrier solutions, similar to that observed with 1-undecanol, nitric acid and hydrochloric acid solutions can be employed as carriers for $BmimPF_6$ analysis. The use of inorganic acid solutions as carrier is clearly simpler and less prone to interferences than that previously proposed by Ranjbar et al. [16] for metal analysis with 1-hexyl-3-methyl imidazolium bis(trifluoromethylsulfonyl)imide (i.e. 80% v v^{-1} 1-propanol solution). In fact, no significant memory effects were registered for the different diluted $BmimPF_6$ mixtures operating the acid carriers. Analyte wash out was similar to that obtained with 1-undecanol (25–30 s)

3.2.2. Optimization of ICP-AES experimental conditions

The influence of ICP experimental conditions (Q_g and Q_l) on the analyte signal (and plasma properties) obtained when operating with $BmimPF_6$ were analogous to those already shown for 1-undecanol (Fig. 2). Thus, despite of the use of a different sample introduction system, the optimum Q_g for $BmimPF_6$ was also found at 0.7 L min^{-1}. Interestingly, it was observed that the influence of Q_l on analyte signal was less significant than with 1-undecanol. Regardless the solvent employed for $BmimPF_6$ dilution, analyte signal rose approximately 1.6-fold when decreasing Q_l from 1.5 to 0.6 mL min^{-1}. This behavior can be attributed to the higher viscosity of $BmimPF_6$ mixtures regarding to 1-undecanol ones.

3.3. Coupling chloroform-based DLLME procedures to ICP-AES

3.3.1. Strategies for chloroform introduction into the ICP

Opposite to that occurring with 1-undecanol and $BmimPF_6$, the low viscosity of chloroform permits it to generate pneumatic aerosols with no additional dilution treatment. Nevertheless, the direct analysis of chloroform DLLME extracts by ICP-AES has been previously avoided in the literature due to the undesirable effects caused by this solvent in ICP-AES (mainly signal instability and negative effects on the plasma excitation characteristics) [16]. Instead, additional pretreatments to remove chloroform have been recommended before metal analysis by ICP (e.g. back extraction, evaporation, etc.) [8–10,15]. In this work, however, it was noted that chloroform could be directly introduced in the ICP with the FIA manifold. The volume of chloroform introduced into the instrument (25 µL) was low enough to avoid carbon deposits and plasma shutdown. Nevertheless, it is important to note that plasma appearance was affected by the high solvent load consequence of the high chloroform volatility (e.g. green light emission from the C_2 band in the aerosol channel) [5]. With the goal of improving the analytical response, some minor changes were performed on the ICP-AES operating conditions employed with viscous solvents [19]. First, auxiliary gas flow was increased from 1.25 to 2.25 L min^{-1} thus improving plasma tolerance to organics [5]. On the other hand, Q_l higher than 1.0 mL min^{-1} were not employed to avoid plasma flickering. Operating on this way, there is not any experimental limitation to operate chloroform directly in ICP-AES thus taking advantage all the benefits of DLLME (e.g. sample throughput, simplicity, analyte enrichment factors, etc.).

3.3.2. Optimization of ICP-AES experimental conditions

In line with the strategy used with the viscous solvent, either 1% w w^{-1} nitric and hydrochloric acids were employed as carriers for chloroform. In addition, air was also tested since it could be advantageous for volatile solvent introduction into the ICP [32,33]. Fig. 4 shows the results obtained for Cd II 214.439 nm integrated emission signal using chloroform and both 1% w w^{-1} nitric acid and air as FIA carriers. Results for 1% w w^{-1} hydrochloric acid are not shown since they were similar to those obtained with the nitric acid solution. From results in Fig. 4 it can be derived that emission signal strongly depends on the carrier employed. Thus, when operating air as carrier, Cd signals (Fig. 4 B) were higher than those obtained for 1% w w^{-1} HNO_3 (Fig. 4A), regardless the Q_l tested. These findings could be explained considering that when using air: (i) the analyte is not dispersed in the liquid stream, (ii) the spray chamber is kept dry between injection which in turns favor solvent evaporation and aerosol transport to the plasma; and (iii) analyte losses due to coalescence and aerosol turbulence are reduced. Nevertheless, from a practical point of view, the benefits of using air as a carrier were counterbalanced by the higher memory effects due to the lack of a solution to wash-out the system between samples during the analysis [32].

Fig. 4 also shown that, in general, irrespective of the carrier used, the optimum Q_g for Cd signal was also found at 0.7 L min^{-1} but this

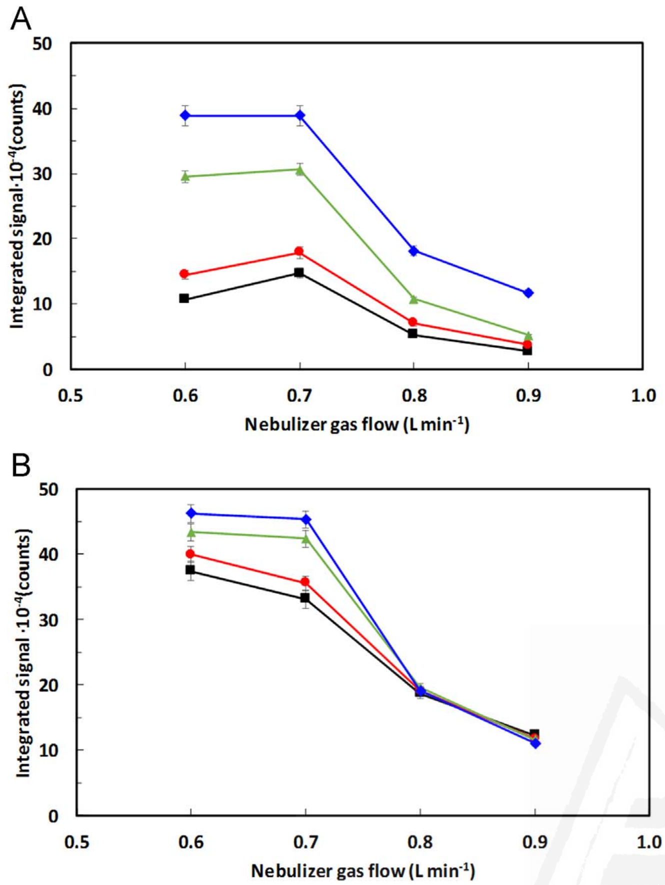

Fig. 4. Influence of the nebulizer gas flow rate on Cd II 214.439 nm integrated emission signal with chloroform at different sample uptake rate using 1% w w^{-1} nitric acid (A) and air (B) as FIA carriers. (◆) 0.4 mL min^{-1}; (▲) 0.6 mL min^{-1}; (●) 0.8 mL min^{-1}; and (■) 1.0 mL min^{-1}. FIA carrier: 1% w w^{-1} HNO_3.

optimum value was more diffuse than that observed with viscous solvents (Fig. 3). In fact, as it can be seen in Fig. 4, a signal plateau was obtained between 0.6 and 0.7 L min^{-1} for some Q_l values, especially when operating with air carrier. Finally, as expected, signal improved when decreasing Q_l due to a better aerosol generation and transport and plasma characteristics [27]. It is interesting to note that differences between both carriers were reduced when decreasing Ql [33]. Thus, when Q_l is decreased from 1.0 to 0.4 mL min^{-1}, analyte signal ratio between air and nitric acid passed from 1.84 to 1.17-fold. To explain this behavior, it must be considered that liquid evaporation is favored at low Q_l values, thus improving analyte transport (i.e. less aerosol losses) and, hence, differences between air and liquid carriers are reduced. Though the use of air as a carrier afforded higher signals than the acid solutions, this approach was unattractive from a practical point of view due to memory effects. Opposite to that observed when operating viscous solvents, and despite the experimental changes made on the ICP setup, plasma characteristics were strongly deteriorated by the presence of chloroform. Thus, for a given set of experimental conditions, the MgII/MgI ratio was half of that obtained with water, 1-undecanol or $BmimPF_6$.

3.4. Analysis of real samples

The multi-element analysis (i.e., Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) of different water samples was performed to validate the strategies developed for coupling DLLME to ICP-AES. To this end, previously described DLLME procedures for 1-undecanol [12], $BmimPF_6$ [24] and chloroform [25] were employed for metal extraction in waters. Next, each organic extract was analyzed by ICP-AES under the optimum conditions obtained for each solvent (Table 3). Q_l was set at 0.4 mL min^{-1} for all the solvents tested to favor aerosol generation and plasma characteristics. Though better results are theoretically expected decreasing further this parameter, nebulization process becomes less stable [20] thus distorting the emission signal profile and reducing signal precision. Moreover, higher wash-out times were required compromising sample throughput.

Table 3
Optimum coupling strategies for the analysis of 1-undecanol, $BmimPF_6$ and chloroform extracts by ICP-AES.

Parameter	1-undecanol	$BmimPF_6$	Chloroform
Dilution solvent	Methanol		–
Dilution ratio	1:0.5		–
Carrier	1% w w^{-1} HNO_3		
Q_g (L min^{-1})	0.7		
Q_l (mL min^{-1})	0.4		

Preliminary experiments showed that analyte extraction efficiency was strongly dependent on the water salt content and, hence, some changes in the extraction procedures were required. To study the influence of water salts (ionic strength) on analyte extraction for each DLLME procedure, 100 µg L^{-1} analyte standard solution containing variable amounts of NaCl from 0.1% to 7.5% w w^{-1} were used. Results shown that each DLLME methodology is differently affected by NaCl. Thus, analyte extraction efficiency for the 1-undecanol-based DLLME procedure decreased with NaCl concentration. On the other hand, extraction efficiency showed a maximum at 3.5% w w^{-1} NaCl when operating with chloroform. Interestingly, no influence of NaCl concentration on the analyte extraction efficiency was observed when using $BmimPF_6$. These results suggest that NaCl content (i.e. solution ionic strength) exerts a great influence on metal extraction since it affects both the solubility of the metal-chelate complex in the sample as well as the miscibility between the organics and water. From these experiments, it was clear the significance of controlling salt content to avoid interferences. Taking into account these findings, both standards and samples were spiked with NaCl 3.5% w w^{-1} for all the DLLME procedures to perform calibration using a single set of standards.

First, a recovery test was performed to evaluate the accuracy. To this end, all the samples were spiked with a multi-element standard solution for a final concentration of 100 µg L^{-1} and, then, they were analyzed by ICP-AES after the appropriate DLLME treatment. Results obtained are shown in Table 4. As it can be observed, recoveries for all the elements with 1-undecanol and chloroform were almost quantitative (i.e., recoveries ranging from 96% to 109%). However, analyte recoveries for $BmimPF_6$ were only quantitative for Pb. It must be considered that the $BmimPF_6$-based DLLME procedure used in the present work was initially developed for Pb determination and, hence, results for this element were totally expected. The origin of the poor recoveries for the remaining elements could be partially related to pH influence on APDC chelating capabilities [34]. This topic, however, was not further investigated since it was beyond the scope of this work.

Table 5 shows the results of the elemental analysis of water samples obtained using the 1-undecanol and chloroform-based DLLME procedures. For the sake of comparison, the results obtained with a direct water analysis by ICP-AES are also included. Data for the $BmimPF_6$-based DLLME procedure are not included since it only worked for Pb and the concentration of this element was below the limit of detection (LoD) (< 5 µg L^{-1}). In fact, none of the methodologies tested could detect Pb as well as Cd and Cr due to their low concentration levels in the samples analyzed. In general, results for the elemental analysis using DLLME procedures agree with those obtained using a direct analysis procedure. Nevertheless, the use of DLLME methodologies allowed the analysis of a higher number of elements (e.g. Al, Fe, etc.) in water samples due to their lower limits of detection (Table 6). In comparison with a direct water analysis, DLLME methodologies afford,

Table 4
Recoveries obtained for analyte spiked water samples using different DLLME procedures based on 1-undecanol, BmimPF$_6$ and chloroform extraction. ICP-AES: Q$_g$: 0.7 L min^{-1}, Q$_l$:0.4 mL min^{-1}. FIA carrier: 1% w w^{-1} HNO$_3$.

	Recovery values (%)								
	1-undecanol			BmimPF$_6$			Chloroform		
Element	**Sea**	**Tap**	**River**	**Sea**	**Tap**	**River**	**Sea**	**Tap**	**River**
Al	102 ± 4	99 ± 2	100 ± 4	< 5	< 5	< 5	99 ± 4	100 ± 2	99 ± 5
Cd	99 ± 3	101 ± 3	98 ± 3	< 5	14 ± 10	14 ± 10	103 ± 3	102 ± 3	109 ± 3
Cr	97 ± 5	97 ± 4	98 ± 3	< 5	23 ± 13	< 5	100 ± 1	103 ± 3	99 ± 3
Cu	100 ± 3	98 ± 4	98 ± 3	< 5	19 ± 13	< 5	98 ± 3	99 ± 3	98 ± 2
Fe	99 ± 4	98 ± 4	98 ± 4	< 5	< 5	< 5	100 ± 4	99 ± 5	100 ± 3
Mn	101 ± 4	100 ± 3	101 ± 3	70 ± 10	80 ± 20	60 ± 10	99 ± 2	97 ± 3	95 ± 5
Ni	100 ± 3	100 ± 4	98 ± 3	< 5	15 ± 9	14 ± 7	99 ± 4	98 ± 4	102 ± 3
Pb	99 ± 5	96 ± 5	98 ± 4	99 ± 5	96 ± 6	96 ± 7	98 ± 4	97 ± 3	99 ± 2
Zn	100 ± 2	98 ± 4	100 ± 4	< 5	< 5	< 5	99 ± 4	98 ± 4	102 ± 4

on average, a LoD improvement of 8 and 13-fold when operating with 1-undecanol and chloroform, respectively. These results confirm the usefulness of the coupling strategies evaluated for the analysis of DLLME extracts by ICP-AES. It is important to remark that the improvement in the analytical figures of merit reported for DLLME-ICP-AES was related to two different factors: *(i)* the preconcentrating process itself; and, *(ii)* the higher analyte transport efficiency afforded when using organic solvents in ICP-AES. To evaluate the contribution of aerosol generation and transport with organics on the analytical figures of merit (sensitivity and LoD), the corresponding calibration curve for organics and water were compared (Table S2, Appendix). The use of 1-undecanol and BmimPF$_6$ improved sensitivity and LoD 2.2-fold on average for the different elements tested. LoD improvement for chloroform was also similar (2.8-fold) but less than expected according to signal enhancement factors (6.5-fold on average) due to high blank signals originated by the chloroform impurities. Therefore, a higher improvement in LoDs for chloroform is still feasible improving reagent quality. Nonetheless, different commercial chloroform providers were tested but similar backgrounds were observed in all cases.

Table 6
Limits of detection in ICP-AES for direct sample analysis and 1-undecanol and chloroform-based DLLME procedures. ICP-AES: Q$_g$: 0.7 L min^{-1}, Q$_l$:0.4 mL min^{-1}; 1-undecanol dilution: methanol; 1:0.5 ratio; FIA carrier: 1% w w^{-1} HNO$_3$.

	Limit of detection (µg L^{-1})		
Element	**Direct analysis**	**1-undecanol-DLLME**	**Chloroform-DLLME**
Al	50	6	35
Cd	30	6	1,4
Cr	40	6	6
Cu	30	2	7
Fe	30	4	4
Mn	40	22	6
Ni	30	8	0,7
Pb	20	4	1,1
Zn	20	3	2

4. Conclusions

Results in this work clearly demonstrate that there is not any limitation for coupling DLLME to ICP-AES when experimental conditions are wisely selected. In fact, despite the different physical properties shown by the organic solvents usually employed in DLLME, a single set of experimental conditions can be employed for metal analysis. In addition, it should be taking into account that analytical figures of merit in ICP-AES are not only improved by the DLLME treatment process but also to aerosol generation and transport afforded by the organics regarding to water. When compared to FAAS and ETAAS detection, the use of ICP-AES makes feasible the simultaneous analysis of different metals thus improving sample throughput. In addition, internal standardization calibration could be implemented to improve accuracy and precision as well as to mitigate potential matrix effected derived by the organics in the plasma.

It is expected that the strategies developed in this work could also be applied for ICP-MS. Nonetheless, special attention should be paid in this case to the spectral and non-spectral interferences due to carbon since ICP-MS is more sensitive to matrix effects. In fact, the use of organics could be beneficial to further improve the analytical figures of merit since the ionization of some hard-to-ionize elements (e.g. As, Se, etc.) is improved by carbon presence in the plasma [35]. These experiments are currently being carried out in our laboratories.

Table 5
Results of the analysis of water samples in ICP-AES using 1-undecanol and chloroform-based DLLME procedures as well as direct sample analysis (no preconcentration). ICP-AES: Q$_g$: 0.7 L min^{-1}, Q$_l$:0.4 mL min^{-1}. FIA carrier: 1% w w^{-1} HNO$_3$.

	Concentration (µg L^{-1})								
	Direct analysis			1-undecanol-DLLME			Chloroform-DLLME		
Element	**Sea**	**Tap**	**River**	**Sea**	**Tap**	**River**	**Sea**	**Tap**	**River**
Al	< 150	215 ± 14	< 150	75 ± 2	226 ± 5	135 ± 4	71 ± 4	220 ± 5	135 ± 4
Cu	230 ± 20	145 ± 6	350 ± 9	207 ± 4	150 ± 4	331 ± 4	208 ± 3	140 ± 4	335 ± 3
Fe	< 90	< 90	< LoD	61 ± 4	59 ± 5	23 ± 3	64 ± 2	57 ± 1	24 ± 5
Mn	240 ± 20	< LoD	< LoD	238 ± 3	< LoD	< LoD	229 ± 3	< LoD	< LoD
Ni	400 ± 5	130 ± 7	140 ± 12	407 ± 2	111 ± 5	140 ± 2	410 ± 5	110 ± 3	136 ± 3
Zn	100 ± 15	85 ± 7	< 60	98 ± 2	73 ± 4	56 ± 4	97 ± 4	72 ± 4	53 ± 4

Precision is presented in form of confidence intervals obtained as *ts* where *t* is the Student's *t* (4.3 for a 95% confidence level) and *s* is the standard deviation of three replicates of the analysis.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2017.08.036.

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Coupling dispersive liquid-liquid microextraction with inductively coupled plasma based techniques

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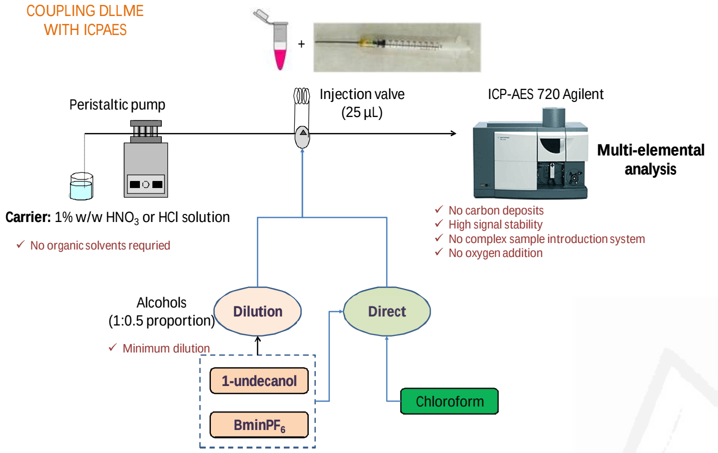
INTRODUCTION

Dispersive liquid-liquid microextraction (DLLME) has been stablished as a fast and green sample preparation methodology to extract and preconcentrate different kind of species from complex matrices.DLLME improves limits of detection and minimizes interferences with minimum reagents requirements and waste generation. Up to date, the coupling DLLME with inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS) has been limited mainly due to the properties of the organic solvents usually employed (i.e. high viscosity and/or volatility) To avoid these drawbacks, different approaches have been suggested (e.g. solvent evaporation, water back-extraction, etc.) but they counterbalance main DLLME benefits and may require complex modifications in the ICP configuration. The goal of this work is to evaluate different strategies for the analysis of DLLME extracts with ICP-based techniques. To this end, the influence of the sample introduction system and ICP experimental conditions on analytical figures of merit have been evaluated for several solvents usually employed in DLLME procedures (chloroform, 1-undecanol and 1-Butyl-3-methylimidazolium hexafluorophosphate).

EXPERIMENTAL

COUPLING DLLME WITH ICPAES



Carrier: 1% w/w HNO_3 or HCl solution

- ✓ No organic solvents requried

- ✓ No carbon deposits
- ✓ High signal stability
- ✓ No complex sample introduction system
- ✓ No oxygen addition

- ✓ Minimum dilution

Table 1. ICP-AES operating conditions

	SOLVENT		
Agilent 720 ICP-AES	Chloroform	1-undecanol	BminPF$_6$
Plasma forward power (W)	1400		
Argon flow rate (L/min)			
Plasma	16.5	15	
Auxiliary	2.5	1.25	
Nebulizer	0.5-0.8	0.6-0.9	
Carrier flow rate (mL/min)	0.4-1.2	0.6-1.5	
Sample introduction system			
Flow injection loop volume (µL)	25		
Acquisition time (s)/replicates	40/3		

Table 2. DLLME extraction procedures tested

Parameter	Method A	Method B	Method C
Type of solvent (volume µL)	1-undecanol (40)	BminPF$_6$ (110)	Chloroform (60)
Disperser (volume µL)	Acetone (600)	Acetone (400)	Ethanol (400)
pH	6	2.2	6
Chelating agent (concentration %)	TTA (0.3)	DDTC (0.3)	APDC (0.5)
Reference	Y. Yamini et al., J. Chromatogr. 1217 (2010) 2358	M. Dehghani et al., Asian J. Chem. 25 (2013) 3984	P. Hemmatkhah et al., Microchim Acta 166 (2009) 69

ICP-AES optimization

HIGH VISCOUS SOLVENTS

↓ Q_l: Improves aerosol generation and transport

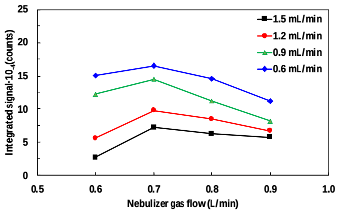


Figure 1. Influence of the nebulizer gas flow rate on the Cd II 214.439 nm intengrated emission signal operating 1-undecanol at different Q_l. Dilution solvent: methanol; 1-undecanol-alcohol proportion: 1:0.5.

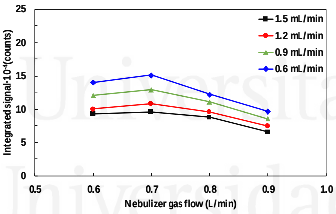


Figure 2. Influence of the nebulizer gas flow rate on the Cd II 214.439 nm integrated emission signal operating BMIMPF$_6$ at different Q_l Dilution solvent: methanol; 1-undecanol-alcohol proportion: 1:0.5.

Dilution solvent: Little influence on aerosol generation and transport.

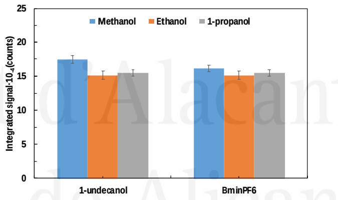


Figure 3. Influence of the dilution solvent on the Cd II 214.439 nm integrated emission signal operating 1-undecanol and BminPF$_6$. Q_g: 0.7 L/min; Q_l: 0.4 mL/min

HIGH VOLATILE SOLVENTS

↓ Q_l: Improves aerosol generation and transport
No carbón deposits and reproducibility issues

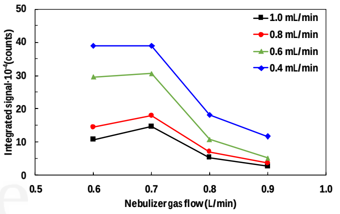


Figure 4. Influence of the nebulizer gas flow rate on the Cd II 214.439 nm integrated emission signal operating chloroform at different Q_l

Real sample analysis

Table 3. Analyte enrichment factor (EF) and limits of detection (LoD) for several elements operating the different DLLME-ICP-AES procedures. ICP-AES: Q_g: 0.7 L/min, Q_l: 0.6 mL/min. 1-undecanol and BminPF$_6$ dilution solvent: methanol; Organic-alcohol proportion: 1:0.5.

	Signal enhancement factor			Limit of detection enhancement factor		
Element	**1-undecanol**	**BminPF$_6$**	**Chloroform**	**1-undecanol**	**BminPF$_6$**	**Chloroform**
Cd	1.4	1.7	6.3	1.5	2.0	3.5
Cr	2.4	1.6	7.5	1.3	1.8	3.6
Cu	1.5	2.6	8.4	1.3	2.2	4.0
Fe	1.5	2.5	8.3	2.1	2.3	2.7
Ni	1.3	1.9	4.0	1.5	1.8	3.2
Pb	2.7	2.4	8.0	1.6	2.3	1.0

- Limit of detection improvement in agreement with signal enhancement with the exception of chloroform (high blank signals, metal contamination
- Signal and limit detection enhancement in ICP-AES by organics due to higher aerosol generation and transport efficiency.

Table 4. Recoveries obtained for analyte spiked water samples using the different DLLME procedures based on 1-undecanol, BminPF6 and chloroform. Analyte spike 100 µg/L. ICP-AES: Q_g: 0.7 L/min, Q_l:0.6 mL/min.

	Recovery values (%)					
	1-undecanol		BminPF6		Chloroform	
Element	**Sea**	**River**	**Sea**	**River**	**Sea**	**River**
Cd	96±3	99±3	<5	<5	100±3	98±3
Cr	92±5	90±3	<5	<5	99±1	96±3
Cu	97±3	95±3	<5	<5	95±3	96±2
Fe	95±4	94±3	<5	<5	96±4	97±3
Ni	97±3	94±3	<5	<5	95±4	99±3
Pb	94±5	95±4	99±5	96±6	97±4	94±4

Multi-elemental analysis depends on the selectivity of the extraction procedure

Table 5. Results of the analysis of water samples in ICP-AES using the 1-undecanol and chloroform-based DLLME procedures. ICP-AES: Qg: 0.7 L/min, Ql:0.6 mL/min.

	Concentration (µg/L)			
	1-undecanol		Chloroform	
Element	Sea	River	Sea	River
Cd	<6	<6	<1.4	<1.4
Cr	<6	<6	<5	<5
Cu	207±4	150±4	140±4	335±3
Fe	61±4	23±3	64±2	24±5
Ni	407±2	140±2	410±5	136±3
Pb	<4	<4	<1.1	<1.1

Good agreement between the different DLLME methodologies

CONCLUSIONS

- ✓ **Coupling DLLME to ICP-AES is totally feasible allowing multi-elemental analysis**
- ✓ **Plasma short and long-term stability not compromised using flow injection analysis and the conventional sample introduction system**
- ✓ **Analysis of DLLME organic solvents beneficial for ICP-AES sensitivity and limits of detection**

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O 43A - Dispersive liquid-liquid microextraction with inductively coupled plasma atomic emission spectrometry– an oxymoron?

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Abstract

Inductively coupled plasma atomic emission spectrometry (ICP-AES) is usually employed for the analysis of metal, metalloids and some non-metals in different kind of samples (i.e. environmental, pharmaceutics, foods, etc.). The main advantages of these techniques are: (i) accuracy and precision; (ii) low limits of detection (µg L^{-1}-ng L^{-1} range); (iii) high dynamic range; and (iv) multi-element capabilities. However, sample throughput is often limited by manual and labor-intensive sample preparation methodologies (e.g. acid digestion, liquid-liquid extraction, etc.)

Dispersive liquid-liquid micro-extraction (DLLME) has been stablished as a faster and greener sample preparation methodology to separate and preconcentrate analytes from complex matrices. DLLME improves limits of detection and minimizes interferences with minimum reagents requirements and waste generation. Up to date, the use of DLLME with ICP-AES has been very limited due to the physicochemical properties of the extractants employed. High volatile solvents (e.g. carbon tetrachloride) usually have a deleterious effect on plasma conditions that could even lead to plasma extinction. On the other hand, high viscous solvents (e.g. undecanol or most of the common ionic liquids) could not be directly introduced into the plasma with the conventional liquid sample introduction system. To address these issues, different approaches have been suggested (e.g. solvent evaporation, water back-extraction, etc.) but they counterbalance main DLLME benefits and may require complex modifications in the ICP configuration (e.g. desolvation system or oxygen addition)

The goal of this work is to explore different strategies to analyze DLLME extracts with ICP-AES. To this end, three extractants (chloroform, undecanol, 1-butyl-3-methyl-imidazolium hexafluorophospahte, $Bmin_4PF_6$) showing different physicochemical properties have been selected. Experimental results shown that chloroform could be directly introduced in ICP-AES using flow injection analysis (FIA) without any previous pre-treatment. The analysis of undecanol and $Bmin_4PF_6$ is also feasible by FIA-ICP-AES but a dilution step with a less viscous solvent is required. These strategies have been applied for trace and ultra-trace analysis of different environmental and food samples.

Key Words: dispersive liquid-liquid microextraction, inductively coupled plasma atomic emission spectrometry, flow injection analysis

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