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Fernando Maya Alejandro

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Desarrollo de nuevas metodologías analíticas de interés medioambiental y clínico mediante la técnica de análisis por inyección en flujo multijeringa

TESIS DOCTORAL

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Palma de Mallorca, Noviembre de 2010.

UNIVERSITY OF THE BALEARIC ISLANDS

PhD Programme in Experimental Sciences and Technology
(Chemical Sciences and Technology)

Development of new analytical methods of environmental and clinical interest exploiting the multisyringe flow injection analysis technique

PhD Thesis

Fernando Maya Alejandro

Palma de Mallorca, November 2010.

UNIVERSIDAD DE LAS ISLAS BALEARES

Departamento de Química

El Dr. Víctor Cerdà Martín, Catedrático de Química Analítica del Departamento de Química de la Universidad de las Islas Baleares y el Dr. José Manuel Estela Ripoll, Profesor Titular del Departamento de Química de la Universidad de las Islas Baleares, en carácter de directores de esta Tesis Doctoral,

CERTIFICAN:

Que el presente trabajo titulado: "Desarrollo de nuevas metodologías analíticas de interés medioambiental y clínico mediante la técnica de análisis por inyección en flujo multijeringa" ha sido realizado por Fernando Maya Alejandro en el Área de Química Analítica del Departamento de Química de la Universidad de las Islas Baleares, como requisito para optar al título de Doctor en Ciencias Experimentales y Tecnologías (Ciencia y Tecnología Química).

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Dr. Víctor Cerdà Martín

Dr. José Manuel Estela Ripoll

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RESUMEN

El objetivo de esta tesis doctoral es el de explorar el potencial de la técnica de análisis en flujo multijeringa (MSFIA), para el desarrollo de nuevas metodologías analíticas completamente automáticas, con el fin de obtener nuevos métodos con elevadas frecuencias de análisis, bajo coste, tamaño compacto y baja generación de residuos.

La técnica MSFIA ha sido desarrollada por el grupo de Química Analítica, Automatización y Medio Ambiente de la Universidad de las Islas Baleares, en el cual está siendo llevada a cabo esta tesis.

Aprovechando las nuevas posibilidades de análisis que ofrece dicha técnica, se ha conseguido tanto mejorar como proponer nuevas alternativas para la realización de análisis químicos, intentando solucionar problemas analíticos actuales, tales como:

- Desarrollo de metodologías multiconmutadas de inyección de reactivos y uso de detectores de fibras ópticas de núcleo líquido para el establecimiento de metodologías analíticas con detección espectrofotométrica, reduciendo drásticamente la generación de residuos químicos derivados del propio análisis. Siguiendo este principio, se desarrollaron métodos para la determinación espectrofotométrica de cloruros en aguas basada en el uso del $Hg(SCN)_2$ (método oficial de análisis) con una reducción muy importante del consumo de este reactivo, altamente tóxico.
- Desarrollo de una nueva metodología, que por primera vez automatiza completamente la determinación del parámetro AOX, reduciendo los riesgos inherentes a ésta y acortando el tiempo de análisis de horas a minutos. Este objetivo fue conseguido acoplando en-línea un dispositivo

de extracción en fase solida miniaturizado, seguido de un digestor UV y un sistema de reacción colorimétrico.

- Combinaciones de métodos de separación en línea con detección quimioluminiscente (QL), mejorando la selectividad de ésta y por lo tanto permitiendo el desarrollo de nuevas aplicaciones tales como: (i) detección rápida de sulfuro a niveles traza combinando en línea un dispositivo de separación por difusión gaseosa con detección QL. (ii) detección rápida y económica de diuréticos (tiazidas) en muestras de orina mediante la combinación de la extracción en fase sólida con cromatografía líquida y detección QL utilizando sistemas de baja presión. (iii) Detección rápida de oxalato en muestras de orina mediante el recubrimiento automático de columnas para cromatografía líquida de fase reversa con surfactantes, permitiendo la separación del oxalato de la matriz de la muestra y su posterior cuantificación a niveles traza mediante QL.

ABSTRACT

The main objective of this PhD Thesis is to explore the potential of the Multisyringe Flow Injection Analysis technique (MSFIA) for the development of new completely automated analytical methodologies with high analysis throughputs, low costs of acquisition and maintenance, compact size and a low production of waste products.

The MSFIA technique has been developed by the Analytical Chemistry, Automation and Environment research group of the University of the Balearic Islands, group where this PhD thesis has been performed.

Exploiting the new analytical features that the MSFIA technique provides, new analytical methodologies or improvements over existing ones have been accomplished in order to solve current analytical problems:

- Development of multicommutated methodologies for reagent injection and the use of long pathlength liquid waveguide capillary cells for the development of new analytical methodologies with spectrophotometric detection, obtaining a drastic reduction of the amount of waste products generated in this type of methods. According to this principle, methodologies for the determination of chloride in waters based on the use $Hg(SCN)_2$ (standard method) were developed, this methodologies required very low amounts of $Hg(SCN)_2$, a really toxic chemical.
- Development of a new analytical methodology for the completely automated determination of the Adsorbable Organic Halogens (AOX) parameter, shortening the analysis time from hours to minutes. This objective was achieved developing an in-line solid phase extraction, UV-chemical oxidation and spectrophotometric detection procedure.
- Development of novel in-line separation methods combined with chemiluminescence detection (CL), improving the selectivity of this type

of detection, thus enabling the implementation of CL as detection system for new applications, such as: (i) the fast determination of sulphide traces combining in-line gas diffusion with CL detection. (ii) Fast detection of thiazides with diuretic action in urine combining on-line solid phase extraction with monolithic column liquid chromatography and post-column CL detection. (iii) Fast determination of oxalate in urine using surfactant-coated short monolithic columns as a front end to CL detection.

ÍNDICE

CAPÍTULO 1

INTRODUCCIÓN GENERAL A LAS TÉCNICAS DE ANÁLISIS EN FLUJO

En este capítulo se expone una visión general de las técnicas de análisis en flujo, clasificándolas según su sistema de propulsión o manipulación de fluidos. Se destacan sus características más relevantes y se evalúa su estado actual. Además se presentan los antecedentes de esta tesis doctoral y sus objetivos.

1.1. Clasificación de las técnicas de análisis en flujo según su sistema de propulsión de fluidos

Pueden mencionarse las siguientes técnicas ordenadas de acuerdo a su antigüedad: análisis en flujo segmentado, análisis por inyección en flujo, análisis por inyección secuencial, análisis por inyección en flujo multiconmutado, análisis por inyección en flujo multijeringa y sistemas multibomba.

1.1.1. Análisis en flujo segmentado

La técnica de análisis en flujo segmentado (Segmented flow analysis, SFA) fue la primera técnica automática continua, la cual fue desarrollada por L. T. Skeggs[1] en 1957. Un sistema SFA está principalmente compuesto por una bomba peristáltica mediante la cual se realiza la aspiración continua de muestra y reactivos, un número variable de tuberías que conforman la red de flujo o "manifold" y un sistema de detección (Figura 1.1). Las muestras una vez aspiradas son segmentadas por burbujas de aire, de este modo se previene la contaminación entre ellas. Dicha segmentación permite el desarrollo de un flujo en régimen turbulento, facilitando de este modo la mezcla entre la muestra y los reactivos situados entre cada par de segmentos de aire.

Sin embargo, la simple segmentación por burbujas de aire no solucionó completamente el problema de la contaminación entre muestras por lo que se tuvo que recurrir al uso de disoluciones intermedias de lavado para eliminar los restos de muestras y reactivos adheridos a las paredes de las tuberías.

Las burbujas de aire son eliminadas previamente a la detección. Las señales obtenidas son de perfil rectangular, ya que las reacciones que se llevan

[1] L. T. Skeggs, Am. Chem. J. Clin. Pathol. 28 (1957) 311-322.

a cabo suelen estar en condiciones de equilibrio en el momento de la detección. La altura de la señal obtenida es proporcional a la concentración del analito, siempre que los reactivos estén en exceso. A pesar de su complejidad y modo de operación, este tipo de sistemas han sido comercializados exitosamente para aplicaciones clínicas, y posteriormente para aplicaciones ambientales.

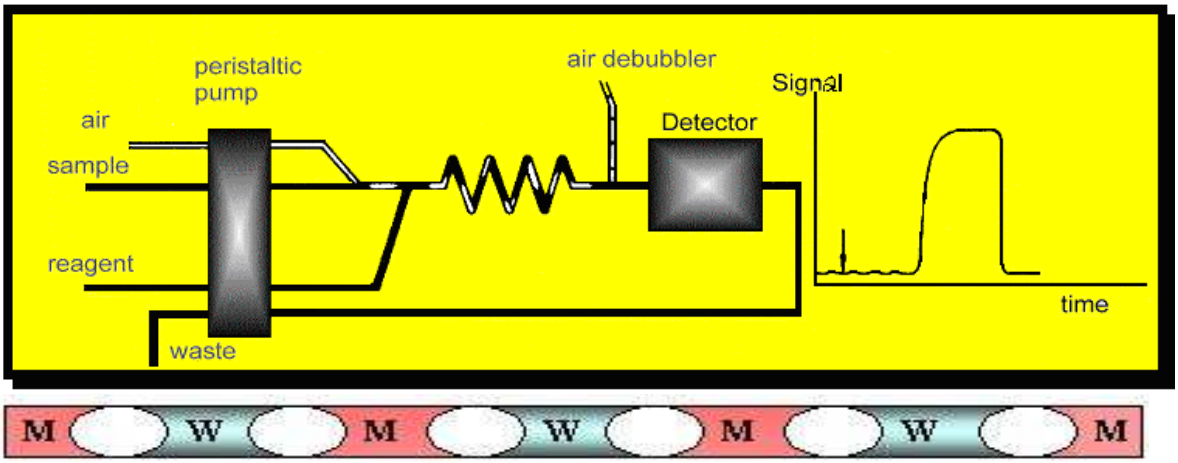

Figura 1.1. Representación esquemática de un sistema SFA

1.1.2. Análisis por inyección en flujo

La técnica de análisis por inyección en flujo (Flow Injection Analysis, FIA)[2] fue propuesta por J. Ruzicka y E.H. Hansen en 1975. A diferencia de la técnica SFA, un determinado volumen de muestra es insertado en un fluido portador bajo régimen continuo, mediante el uso de una válvula de inyección. Durante el proceso de transporte del segmento de muestra hacia el detector en un régimen de flujo laminar, esta sufre un proceso de dispersión controlada, la cual depende de una serie de parámetros tales como el volumen y la viscosidad

[2] J. Ruzicka, E. H. Hansen, Anal. Chim. Acta 78 (1975) 145-157.

de la muestra, el diámetro interno y la longitud de las tuberías, o el caudal utilizado.

Las señales obtenidas en los sistemas FIA, al contrario que en los sistemas SFA, tiene forma de pico de acuerdo a una distribución chi-cuadrada. Los sistemas FIA son de mayor simpleza instrumental y operacional que los sistemas SFA. Usando como fluido portador un reactivo, puede desarrollarse un sistema FIA mono-canal, desarrollándose el producto de reacción en la zona de dispersión del segmento de muestra en el reactivo (Figura 1.2). En los sistemas FIA se sustituye el uso de manifolds compuestos por tuberías de vidrio de ancho diámetro interno (2mm i.d.), por tuberías flexibles constituidas por polímeros químicamente inertes de menor diámetro (0.5-0.8mm i.d.). Dicha miniaturización conlleva una disminución en el gasto de reactivos y muestras.

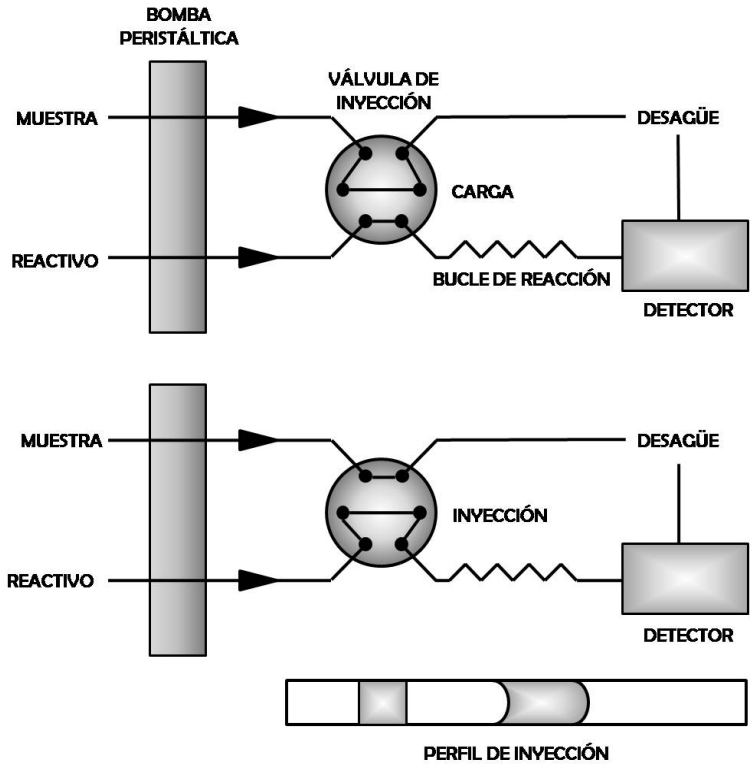

Figura 1.2. Representación esquemática de un sistema FIA, utilizando un reactivo como fluido portador.

La dispersión es controlada y reproducible, debido al estricto control del tiempo de residencia de la muestra en el sistema[3]. Estas características de la técnica FIA permiten la adquisición de señales sin la necesidad de que la reacción química llegue a una situación de equilibrio, aumentando la frecuencia de análisis, facilitando la detección de intermedios de reacción y evitando interferencias en algunos casos.

La técnica FIA debido a su bajo coste, simplicidad de operación y alta frecuencia de análisis sigue siendo actualmente la técnica de análisis en flujo más ampliamente utilizada.

1.1.3. Análisis por inyección secuencial

La técnica de análisis por inyección secuencial (Sequential Injection Analysis, SIA) fue propuesta por J. Ruzicka y G. D. Marshall en el año 1990[4] como una alternativa a los inconvenientes que posee la técnica FIA. La técnica SIA se basa en el uso de una bomba de pistón bi-direccional que acomoda una jeringa.

En la Figura 1.3 se detalla el modo de operación para el caso de una bomba de pistón con una jeringa la cual alberga una válvula solenoide en cabeza. Dicha jeringa puede realizar cuatro operaciones distintas en el manejo de fluidos, cargando o dispensando estos hacia sus reservorios o hacia el sistema de tuberías.

[3] J. Ruzicka, E. H. Hansen, Flow Injection Analysis. 2nd Ed., 1988, J. Wiley & Sons, New York.
[4] J. Ruzicka, G. D. Marshall, Anal. Chim. Acta 237 (1990) 329-343.

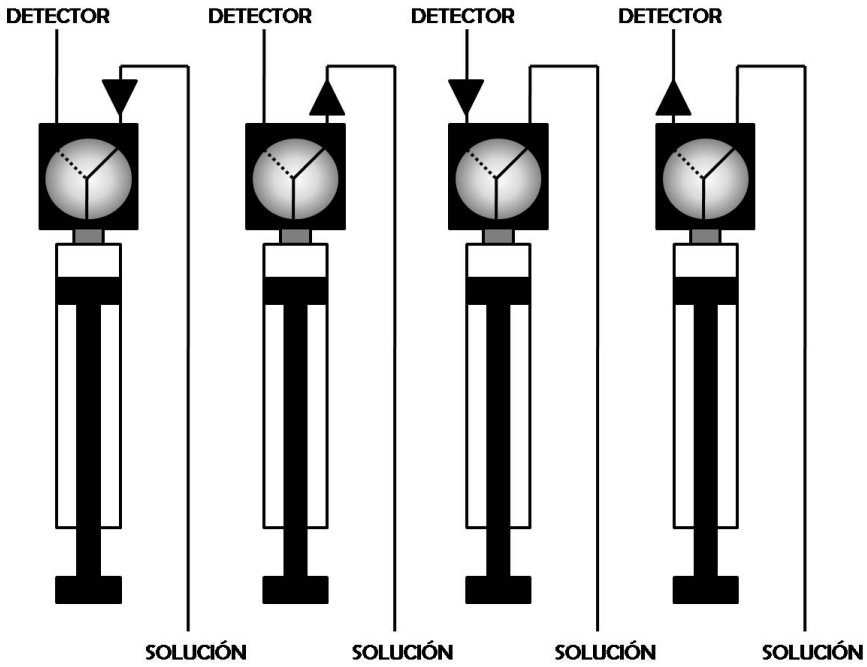

Figura 1.3. Diferentes operaciones de manejo de fluidos que pueden ser realizadas mediante la técnica SIA. De izquierda a derecha: Carga de fluido desde un depósito, descarga de fluido hacia un depósito, carga de fluido contenido en el sistema de tuberías, e inyección de solución hacia el sistema.

La configuración básica de un sistema SIA (Figura 1.4) consiste en la conexión de la bomba de jeringa previamente descrita, al puerto central de una válvula de selección utilizando un bucle de carga. Dicha jeringa contiene el fluido portador, mediante el cual la muestra será introducida en el sistema y posteriormente impulsada hacia el detector.

Uno de los puertos de la válvula de selección se usa para conectar esta con el sistema de detección. Los puertos restantes se usan para la introducción de muestra y reactivos en el sistema de flujo.

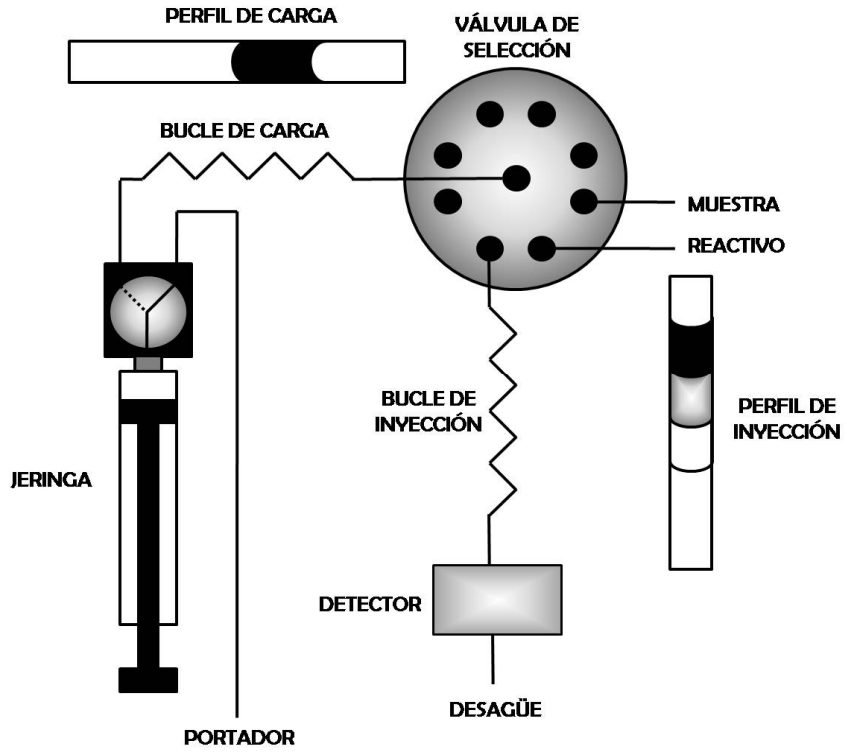

Figura 1.4. Representación esquemática de un sistema SIA. El reactivo y la muestra son secuencialmente aspirados en el bucle de carga. Posteriormente el flujo es invertido siendo estos inyectados hacia el detector. El producto de la reacción (color gris) se desarrolla en la zona de mezcla del reactivo con la muestra debido a la dispersión.

Al contrario que en las técnicas SFA y FIA, en la técnica SIA el flujo no es continuo, sino discreto. Dicha característica y la necesidad de la manipulación de los bolos de reactivos y muestra requieren el uso de ordenadores para su implementación. Ello provoca un incremento en costes y complejidad instrumental, pero a su vez proporciona una mayor versatilidad en la manipulación de fluidos.

En comparación con la técnica FIA, la técnica SIA permite un ahorro considerable en el consumo de muestra y reactivos, una mayor flexibilidad en

el cambio de parámetros sin la necesidad de reconfiguraciones del manifold, y la posibilidad de desarrollar analizadores multi-paramétricos sin incrementar excesivamente la complejidad instrumental. La eliminación del uso de bombas peristálticas, y por lo tanto de los tubos de Tygon, los cuales son gravemente deteriorados por productos químicos agresivos tales como ácidos concentrados o solventes orgánicos, y que no afectan a las jeringas de vidrio usadas en esta técnica.

La técnica SIA debido a su mayor robustez instrumental que la técnica FIA, es capaz de soportar presiones más elevadas que esta, permitiendo el desarrollo de nuevas metodologías o tratamientos de muestra inicialmente no factibles mediante el uso de la técnica FIA.

Algunas desventajas de la técnica SIA en comparación con la técnica FIA residen en problemas en el desarrollo de metodologías que impliquen un gran número de reactivos, ante la dificultad de la obtención de un proceso de mezcla favorable. Asimismo, al no ser un método continuo, son necesarios constantes cambios en la válvula de selección, siendo por este motivo las frecuencias de análisis obtenidas más bajas que usando métodos FIA.

Desde su descubrimiento, la técnica SIA ha servido como plataforma instrumental para el desarrollo de un amplio abanico de aplicaciones[5].

[5] (a) G. D. Christian, Analyst 119 (1994) 2309-2314. (b) C. E. Lenehan, N. W. Barnett, S. W. Lewis, Analyst 127 (2002) 997-1020. (c) J. F. Van Staden, R. I. Stefan, Talanta 64 (2004) 1109-1113. (d) P. Solich, M. Polasek, J. Klimundova, J. Ruzicka, Trends Anal. Chem. 23 (2004) 116-126. (e) A. Economou, Trends Anal. Chem. 24 (2005) 416-425.

1.1.4. Análisis por inyección en flujo multiconmutado

La técnica de análisis por inyección en flujo multiconmutado (Multicommutated Flow Injection Analysis, MCFIA) fue desarrollada por B. F. Reis et al. en el año 1994[6]. Dicha técnica se basa en el uso de un manifold típico de la técnica FIA, al cual han sido incorporadas una serie de válvulas solenoides de tres vías (Figura 1.5). La acción de dichas válvulas es controlada mediante un ordenador, actuando éstas como conmutadores con dos posiciones distintas. El uso de dichas válvulas permite la recuperación de la muestra y los reactivos cuando la inyección de estos no es necesaria, obteniendo un importante ahorro de los mismos.

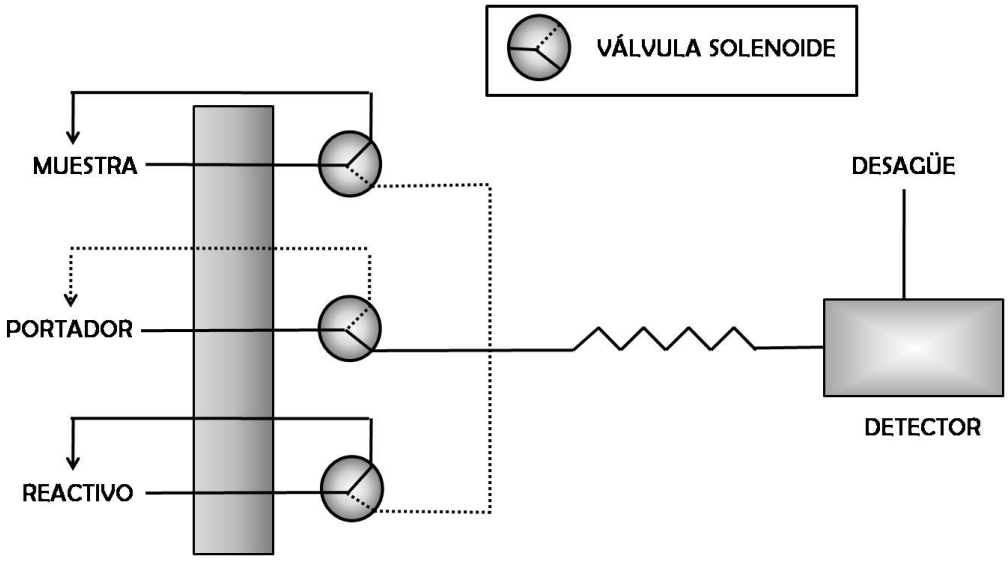

Figura 1.5. Representación esquemática de un sistema MCFIA.

Por lo tanto, los volúmenes de inserción usados en la técnica FIA, son sustituidos por tiempos de inserción, lo que nos permite desarrollar métodos

[6] B. F. Reis, M. F. Giné, E. A. G. Zagatto, J. L. F. C. Lima, R. A. S. Lapa, Anal. Chim. Acta 293 (1994) 129-138.

analíticos con el muestreo y la inyección de reactivos basada en el tiempo. Con ello se incrementa la versatilidad del sistema, puesto que no son necesarias realizar reconfiguraciones físicas al introducir cambios en el método que queremos aplicar.

La técnica MCFIA, al igual que la técnica SIA, es una plataforma analítica útil para el desarrollo de analizadores multi-paramétricos, sin embargo mediante la técnica MCFIA pueden desarrollarse metodologías con mayor frecuencia de análisis que con la técnica SIA, ya que no se tiene que invertir tiempo en cambios de posición de una válvula de selección.

Las desventajas de la técnica MCFIA residen en la imposibilidad del uso de reactivos agresivos debido al rápido deterioro de las tuberías flexibles utilizadas para impulsar los fluidos mediante una bomba peristáltica. Además de ser muy limitada la presión que pueden soportar dichos sistemas, imposibilitando el desarrollo de determinados pre-tratamientos de muestra que si pueden ser implementados fácilmente mediante la técnica SIA.

Las aplicaciones desarrolladas mediante la técnica MCFIA pueden verse recopiladas en varias revisiones bibliográficas[7].

[7] (a) M. Catalá-Icardo, J. V. Garcia-Mateo, J. Martinez-Calatayud, Trends. Anal. Chem. 21 (2002) 366-378. (b) F. R. P. Rocha, B. F. Reis, E. A. G. Zagatto, J. L. F. C. Lima, R. A. S. Lapa, J. L. M. Santos, Anal. Chim. Acta 468 (2002) 119-131. (c) M. A. Feres, P. R. Fortes, E. A. G. Zagatto, J. L. M. Santos, J. L. F. C. Lima, Anal. Chim. Acta 618 (2008) 1-17. (d) A. Morales-Rubio, B. F. dos Reis, M. de la Guardia, Trends Anal. Chem. 28 (2009) 903-913.

1.1.5. Análisis por inyección en flujo multijeringa

La técnica de análisis por inyección en flujo multijeringa (Multisyringe Flow Injection Analysis, MSFIA) fue introducida en el año 1999 por V. Cerdà et al.[8] en dicha técnica el uso de una bureta automática da lugar a un movimiento simultáneo y en paralelo de hasta cuatro jeringas las cuales se acomodan sobre una misma barra metálica impulsada por un único motor paso a paso. La técnica MSFIA es una estrategia que trata de combinar las ventajas de las técnicas FIA, SIA y MCFIA.

Por un lado, la técnica MSFIA nos permite la mezcla de muestra y reactivos por confluencia tal como en la técnica FIA, obteniendo elevadas frecuencias de análisis. Al tener cada jeringa una válvula solenoide de tres vías en su parte superior, la técnica MSFIA permite modos de operación similares a la técnica MCFIA disminuyendo el gasto de muestra y reactivos en comparación con los sistemas FIA clásicos, además de una mayor facilidad en el desarrollo de metodologías multi-paramétricas.

Por otro lado, la muestra y los reactivos siempre se encuentran en contacto con polímeros inertes o con jeringas de cristal, permitiéndonos al igual que en la técnica SIA el uso de reactivos químicos agresivos tales como ácidos concentrados (excepto el ácido fluorhídrico) o disolventes orgánicos. Además se logra un preciso manejo bi-direccional de los fluidos, una mayor estabilidad del flujo y trabajar a presiones más elevadas que usando las bombas peristálticas típicas de las técnicas SFA, FIA y MCFIA.

El control a través de ordenador permite la fácil modificación de las posiciones de las válvulas solenoides, así como los caudales, volúmenes y dirección de los flujos. El caudal relativo de una jeringa puede ajustarse seleccionando jeringas de distinto volumen (de 0.5 a 25mL). Una bureta

[8] (a) V. Cerdà, J. M. Estela, R. Forteza, A. Cladera, E. Becerra, P. Altimira, P. Sitjar, Talanta 50 (1999) 695-705. (b) F. Albertus, B. Horstkotte, A. Cladera, V. Cerdà, Analyst 15 (1999) 1373-1381.

multijeringa, aparte de operar con las cuatro válvulas de selección a las cuales están conectadas las jeringas, puede operar además con hasta cuatro válvulas solenoides adicionales.

En la Figura 1.6 se muestra un manifold MSFIA. Este sistema se basa en el uso de una bureta multijeringa, en la cual la primera jeringa, se utiliza para manipular el fluido portador que en combinación con un bucle de carga y una válvula solenoide adicional permite la introducción de la muestra. Una vez la muestra en el sistema, ésta es impulsada hacia el detector, siendo mezclada en ese trayecto con hasta tres reactivos distintos albergados en las restantes jeringas.

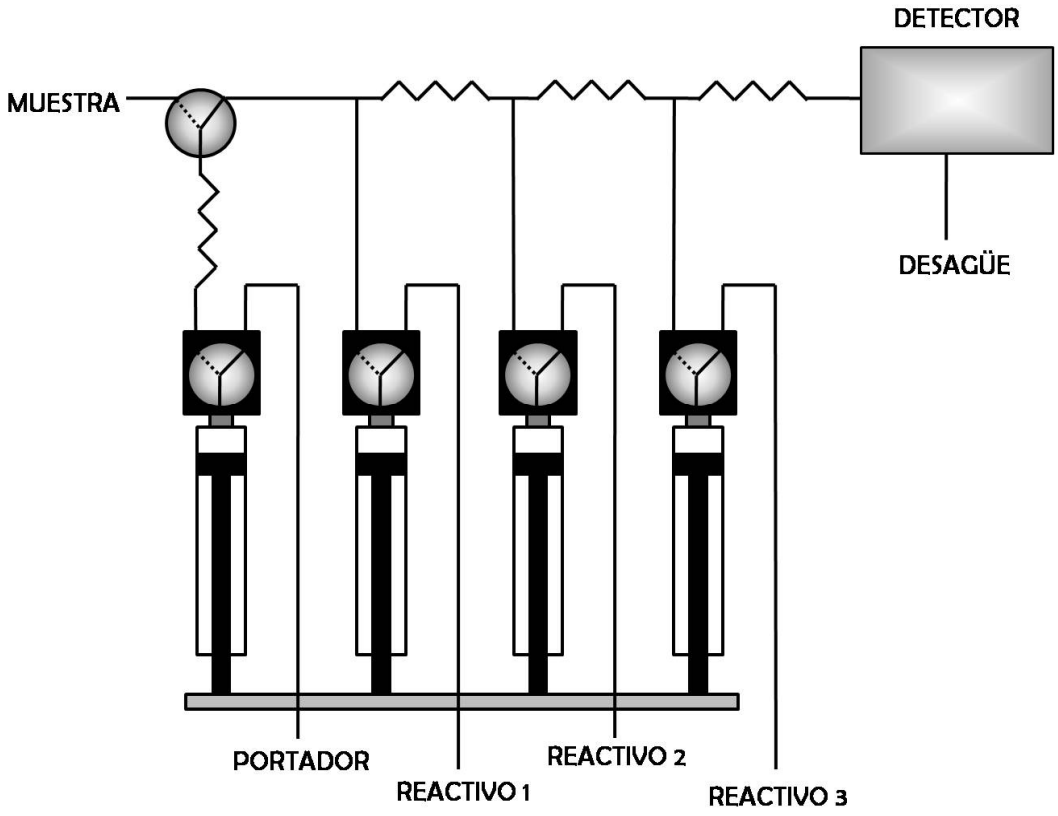

Figura 1.6. Representación esquemática de un sistema MSFIA

Las principales aplicaciones de la técnica MSFIA desde su descubrimiento hasta la actualidad pueden verse resumidas en una serie de revisiones bibliográficas[9].

1.1.6. Sistemas multibomba

La técnica de análisis en flujo multibomba (Multipumping flow systems, MPFS) fue descrita y caracterizada por R.A.S. Lapa et al. en el año 2002[10], basándose en la idea inicial de D. A. Weeks y K. S. Johnson del año 1996[11]. Dicha técnica se basa en el uso de microbombas solenoides de pistón independientes para la inyección y manejo de fluidos. En comparación con un sistema MCFIA, en los sistemas MPFS se sustituye la bomba peristáltica por las válvulas solenoides, una por canal impulsor.

Las microbombas solenoides pueden activarse y desactivarse tal como lo hacen las válvulas solenoides, permitiéndonos la inyección de muestra y reactivos sólo cuando estos son necesarios. Las microbombas funcionan mediante pulsos de voltaje, provocando cada pulso la adición de una cantidad predeterminada de líquido (3 – 50µL). De esta forma, el número de pulsos define el volumen inyectado, mientras que la frecuencia (hasta 250 min^{-1}) de los mismos define su caudal.

El flujo pulsado, es un flujo en régimen turbulento, lo que favorece la mezcla entre la muestra y los reactivos incrementando así la sensibilidad del

[9] (a) M. Miró, V. Cerdà, J. M. Estela, Trends Anal. Chem. 21 (2002) 199-210. (b) B. Horstkotte, O. Elsholz, V. Cerdà, J. Flow Injection Anal. 22 (2005) 99-109. (c) M. A. Segundo, L. M. Magalhaes, Anal. Sci. 22 (2006) 3-8. (d) V. Cerdà, R. Forteza, J. M. Estela, Anal. Chim. Acta 600 (2007) 35-45. (e) M. Fernández, H. M. González-San Miguel, J. M. Estela, V. Cerdà, Trends Anal. Chem. 28 (2009) 336-346. (f) L. M. Magalhaes, J. P. N. Ribeiro, M. A. Segundo, S. Reis, J. L. F. C. Lima, Trends Anal. Chem. 28 (2009) 952-960.
[10] R. A. S. Lapa, J. L. F. C. Lima, B. F. Reis, J. L. M. Santos, E. A. G. Zagatto, Anal. Chim. Acta 466 (2002) 125-132.
[11] D. A. Weeks, K. S. Johnson, Anal. Chem. 68 (1996) 2717-2719.

método. En la Figura 1.7 se representa un sistema MPFS básico, en el cual dos microbombas impulsan la muestra y el reactivo hacia el sistema de detección.

Algunas ventajas de estos sistemas son su resistencia frente a reactivos químicos agresivos, simplicidad, bajo precio y pequeño tamaño. Algunas desventajas son la susceptibilidad de las bombas de pistón a ser bloqueadas por la presencia de partículas, interrupciones del caudal por la presencia de burbujas de aire o la imposibilidad de realizar procedimientos que impliquen una determinada sobrepresión la cual si puede conseguirse mediante las técnicas basadas en bombas de jeringa.

Además de las microbombas solenoides de pistón, pueden también usarse bombas piezoeléctricas[12] para tales fines.

Las principales aplicaciones de la técnica MPFS desde su creación hasta la actualidad pueden verse resumidas en una serie de revisiones bibliográficas[13].

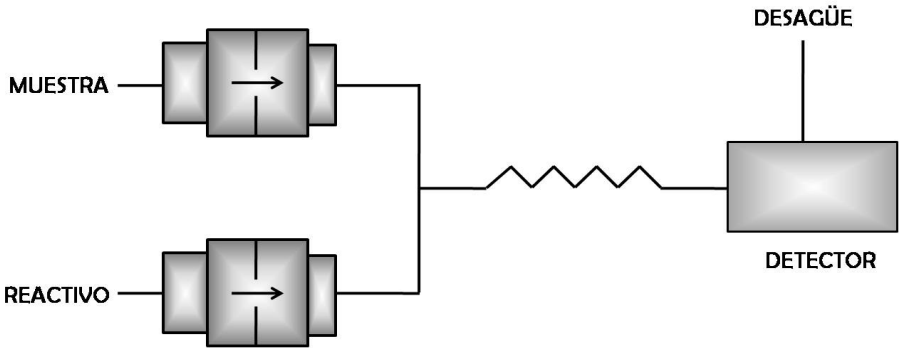

Figura 1.7. Representación esquemática de un sistema MPFS.

[12] M. F. T. Ribeiro, J. L. M. Santos, J. L. F. C. Lima, Anal. Chim. Acta 600 (2007) 14-20.
[13] (a) J. L. F. C. Lima, J. L. M. Santos, A. C. B. Dias, M. F. T. Ribeiro, E. A. G. Zagatto, Talanta 64 (2004) 1091-1098. (b) J. L. M. Santos, M. F. T. Ribeiro, J. L. F. C. Lima, A. C. B. Dias, E. A. G. Zagatto, Spectrosc. Lett. 40 (2007) 41-50. (c) J. L. M. Santos, M. F. T. Ribeiro, A. C. B. Dias, J. L. F. C. Lima, E. A. G. Zagatto, Anal. Chim. Acta 600 (2007) 21-28.

1.1.7. Laboratorio en válvula (Lab on valve)

El concepto del laboratorio en válvula (LOV, Lab on Valve) fue inicialmente propuesto por J. Ruzicka en el año 2000[14]. Sobre una válvula de selección rotatoria convencional de los sistemas SIA, se sustituye la culata por una placa de metacrilato con conductos integrados formándose una plataforma monolítica que permite efectuar en su interior los pretratamientos de muestra necesarios y la posterior detección de los analitos. De este modo se obtiene una considerable miniaturización del sistema en comparación con los sistemas SIA convencionales, siendo esta característica principalmente ventajosa en ciertos casos tales como el de los ensayos enzimáticos[15].

Una de las ventajas mas remarcables del LOV es su uso como plataforma para el manejo de materiales sólidos en suspensión, lo cual se conoce como "Análisis por Inyección de Cuentas" (BIA, Bead Injection Analysis)[16].

La técnica BIA se basa en el manejo de suspensiones de partículas para la creación de por ejemplo mini-columnas de extracción en fase sólida. Estas columnas una vez utilizadas, pueden ser retiradas del interior del sistema siguiendo el mecanismo contrario que para su formación, permitiendo la formación de una nueva columna.

En la literatura científica pueden encontrarse varias revisiones bibliográficas sobre el LOV[17].

[14] J. Ruzicka, Analyst 125 (2000) 1053-1060.
[15] C. H. Wu, L. Scampavia, J. Ruzicka, B. Zamost, Analyst (2001) 291-297.
[16] J. Ruzicka, L. Scampavia, Anal. Chem. 71 (2000) 257A-263A.
[17] (a) J. Wang, E. H. Hansen, Trends Anal. Chem. 22 (2003) 225-231. (b) J. H. Wang, E. H. Hansen, M. Miro, Anal. Chim. Acta 499 (2003) 139-147. (c) X. W. Chen, J. H. Wang, Anal. Chim. Acta 602 (2007) 173-180.

1.1.8. Combinación de las técnicas de análisis en flujo con técnicas de separación

Las técnicas de análisis en flujo también son utilizadas para automatizar y miniaturizar tratamientos de muestra previos a técnicas de separación como son la cromatografía líquida (HPLC), la cromatografía de gases (GC) o la electroforesis capilar (CE). En estos casos la técnica en flujo no se utiliza para desempeñar la metodología analítica completa sino sólo la parte basada en el tratamiento de la muestra previo a la inyección en el instrumento que se encargará de la separación y detección de los analitos, así como de la propia inyección de la muestra en dicho instrumento.

Algunos ejemplos de este tipo de sistemas son: (i) La automatización mediante la técnica SIA de un procedimiento de extracción en fase sólida de cafeína, teofilina y teobromina mediante el uso de polímeros de impresión molecular, para su consecutiva separación y detección por HPLC con detección UV[18]. (ii) La combinación de la técnica MSFIA con un LOV para la extracción en fase sólida renovable de 7 bifenilos policlorados previa a su separación y cuantificación mediante GC[19]. (iii) La extracción en fase sólida de orto-, meta- y para-nitrofenol mediante la técnica MSFIA previa a su inyección en un sistema de CE de construcción propia[20].

En el caso concreto de la cromatografía líquida, nuevos materiales con aplicación como fase reversa en este tipo de técnica han aparecido recientemente, como es el caso de las columnas monolíticas, las cuales se componen de un solo bloque con pequeños poros (en vez de las columnas clásicas formadas por partículas de pequeño diámetro). Columnas monolíticas de pequeña longitud permiten la realización de separaciones de baja

[18] G. Theodoridis, C. K. Zacharis, P. D. Tzanavaras, D. G. Themelis, A. Economou, J. Chromatogr. A 1030 (2004) 69-76.
[19] J. B. Quintana, W. Boonjob, M. Miró, V. Cerdà, Anal. Chem. 81 (2009) 4822-4830.
[20] B. Horstkotte, O. Elsholz, V. Cerdà-Martín, Talanta 76 (2008) 72-79.

complejidad a presiones menores que las columnas clásicas, permitiéndonos la realización de separaciones cromatográficas simples mediante técnicas de análisis en flujo, y no solo utilizar estas en una parte concreta de metodologías en las que participen varios analitos. Más información sobre este tópico se encuentra en el Capítulo 5 de esta tesis.

1.2. Estado actual de las técnicas de análisis en flujo y antecedentes de los objetivos de esta tesis doctoral

Actualmente las técnicas de análisis en flujo han alcanzado una determinada madurez en ciertos aspectos. Desde su creación en los años sesenta hasta la actualidad se han publicado más de 20.000 artículos científicos basados en este tipo de técnicas. Muchos de ellos contienen una determinada aplicación de una técnica en flujo concreta, siendo estas aplicaciones enfocadas a la determinación de uno o varios compuestos de interés en un determinado campo del análisis químico. Algunos de los campos en los que las técnicas de análisis en flujo han sido aplicados son, entre otros: Bio-análisis, Análisis medioambiental, Análisis farmacéutico, Análisis de alimentos,…

A pesar de su amplio número de aplicaciones y el desarrollo de nuevas técnicas de análisis en flujo más avanzadas, tales como las técnicas MPFS, MSFIA o LOV. Dichas técnicas siguen poseyendo una serie de limitaciones, o no han sido explotadas suficientemente para el desarrollo de algunos conceptos en los cuales potencialmente jugarían un papel importante.

Algunos de estos puntos de actual relevancia son los siguientes:

1- Química verde[21] o el desarrollo de métodos de análisis más respetuosos con el medio ambiente[22]. No sólo las técnicas en flujo nos permiten miniaturizar las diversas etapas que componen un método analítico, disminuyendo la cantidad de residuos generados, sino que también nos permiten explotar alternativas que nos posibilitan la reducción o reutilización de estos.
2- El desarrollo de índices totales o parámetros suma, para la rápida obtención de información analítica sobre una muestra concreta. Ya que si bien el desarrollo de este tipo parámetros permite el ahorro de trabajo, tiempo y dinero, son métodos lentos y poco reproducibles, pudiendo ser estos inconvenientes solventados mediante su implementación en sistemas de análisis en flujo.
3- Existen sistemas de detección altamente eficaces como la detección quimioluminiscente. Dichos sistemas precisan ser implementados en técnicas en flujo para su uso, ya que es difícil trabajar con ellos de modo manual. Sin embargo, sus aplicaciones se reducen a muestras sencillas debido a su limitada selectividad, o simplemente como sistema de detección post-columna en técnicas de separación tales como la cromatografía líquida o la electroforesis capilar. Actualmente, las posibilidades que nos brindan las técnicas de análisis en flujo para la automatización y miniaturización de tratamientos de muestra para incrementar la selectividad en métodos de detección quimioluminiscente no han sido plenamente explotadas. Esto permitiría el desarrollo de métodos de análisis rápidos y de elevada sensibilidad para la determinación de compuestos de interés en muestras de matriz compleja.

[21] (a) M. Poliakoff, J. M. Fitzpatrick, T. R. Farren, P. T. Anastas, Science 297 (2002) 807-810. (b) E. S. Beach, Z. Cui, P. T. Anastas, Energy Environ. Sci. 2 (2009) 1038-1049.
[22] (a) P. T. Anastas, Crit. Rev. Anal. Chem. 29 (1999) 167-175. (b) L. H. Keith, L. U. Gron, J. L. Young, Chem. Rev. 107 (2007) 2695-2708.

1.3. Objetivos

El objetivo de esta tesis doctoral consiste en el desarrollo de nuevos avances en los tres puntos comentados en el apartado anterior. Para realizar esta tarea se ha seleccionado la técnica MSFIA como una herramienta altamente versátil y potencialmente eficaz para alcanzar dichos objetivos.

Las metodologías analíticas desarrolladas a lo largo de esta tesis se aplicaron principalmente al análisis de compuestos de actual interés medioambiental. Sin embargo, algunos de los métodos desarrollados también se aplicaron a otros tipos de muestras más complejas, tales como la orina o la cerveza.

El trabajo que se pretende desarrollar puede dividirse en tres bloques:

1- Desarrollo de nuevas estrategias mediante la técnica MSFIA para la minimización de residuos derivados de los análisis químicos. Para ello se pretenden desarrollar nuevos métodos multiconmutados, además del uso de fibras ópticas de núcleo líquido para detección espectrofotométrica. Como sistema modelo se ha tomado el método para la determinación colorimétrica de cloruros, el cual se basa en su reacción con $Hg(SCN)_2$ y Fe^{3+}. Este es un método eficaz para la determinación de cloruros, pero que presenta el gran inconveniente de la elevada toxicidad del $Hg(SCN)_2$. Los métodos a desarrollar estarán enfocados a la obtención de alternativas menos contaminantes para la ejecución de este clásico método de análisis.
2- En este punto, se pretende aprovechar la versatilidad de la técnica MSFIA para desarrollar el primer sistema de análisis en flujo capaz de realizar la determinación del total de compuestos orgánicos halogenados presentes en una muestra. De este modo, se obtendría una alternativa

más rápida y automatizada para la determinación de este parámetro, en comparación con los analizadores comerciales disponibles actualmente.

3- Se pretenden desarrollar nuevos métodos de análisis en los cuales se aplique la detección quimioluminiscente para la determinación de compuestos o especies de interés en muestras complejas de diversa procedencia. Para ello se pondrán a punto reacciones de quimioluminiscencia basadas en los sistemas del luminol y del Tris(2,2´-bipiridil)rutenio(II), combinando estos con unidades de separación por difusión gaseosa con la finalidad de obtener un método selectivo para el sulfuro.
4- Se pretende también combinar el uso del MSFIA con el de las columnas monolíticas de corta longitud para la determinación simultánea de trazas de varios diuréticos.
5- Mediante la modificación de las características de dichas columnas monolíticas por adición de un tensioactivo, se quiere desarrollar una aplicación cromatográfica basada en el intercambio de aniones, desarrollando un método eficaz para la determinación de oxalato.

CAPÍTULO 2

PARTE EXPERIMENTAL. INSTRUMENTACIÓN Y SOFTWARE

En este capítulo se describe la instrumentación y software utilizados en los trabajos presentados en esta tesis[1]. Se utiliza la técnica MSFIA, conjuntamente con sistemas de válvulas adicionales y la detección espectrofotométrica o quimioluminiscente. El control de la instrumentación, tanto de bombas, como válvulas y detectores ha sido llevada a cabo mediante el programa AutoAnalysis 5.0.

Puede hallarse información detallada sobre la instrumentación utilizada en cada trabajo en su correspondiente capítulo o artículo de investigación anexo.

[1] A. Cerdà, V. Cerdà, An introduction to flow analysis. Sciware S.L. 2009. Palma de Mallorca

2.1. Sistema de análisis en flujo

En todos los trabajos presentados en esta tesis la manipulación automática de fluidos fue realizada mediante el uso de buretas automáticas multijeringa (Crison Instruments, Alella, Spain) (Figura 2.1).

Figura 2.1. Bureta multijeringa equipada con jeringas de volumen igual a 10, 5, 2.5 y 1mL.

Este módulo puede ser equipado con hasta cuatro jeringas, tal como ya se ha detallado en el punto 1.1.5 de esta tesis. En su parte superior o cabeza, las jeringas están conectadas directamente mediante una conexión "Luer bayonet" a válvulas solenoides de multiconmutación de tres vías (N-Research, Caldwell,

NJ, USA), las cuales están integradas en la parte frontal del módulo multijeringa.

El motor paso a paso de la multijeringa está conectado a una barra metálica externa sobre la cual se acomodan los émbolos de las jeringas mediante un tornillo. Por lo tanto, todas las jeringas que se usen para un determinada aplicación se moverán simultáneamente en la misma dirección, siendo los caudales relativos inyectados (dispense) o aspirados (pickup) proporcionales a los volúmenes de las jeringas utilizadas.

Cada válvula solenoide de tres vías tiene un puerto común (COM) siempre conectado a la cabeza de la jeringa. Un puerto que está conectado al puerto COM, cuando la válvula se encuentra desactivada, este puerto se denomina "puerto normalmente abierto" (normally open port, NO) y un puerto conectado con el COM cuando la válvula es activada, este puerto se denomina "puerto normalmente cerrado" (normally closed port, NC). Por este motivo cuando una válvula está desconectada decimos que está en posición OFF (el puerto COM conecta con el puerto NO), y cuando está conectada decimos que está en posición ON (el puerto COM conecta con el puerto NC).

Las válvulas solenoides de serie pueden operar a presiones de hasta 2 bar, y al tener unos tiempos rápidos de conmutación (35 ms para activarse o desactivarse) permiten la conmutación sin la necesidad de detener el flujo.

Las buretas multijeringa empleadas en estas tesis utilizan motores de 5.000, 16.000 o 40.000 pasos, mientras que las jeringas usadas fueron de entre 1 y 10mL. Esto permite que con el mismo módulo multijeringa se pueda trabajar en un rango de caudales de varios órdenes de magnitud, desde decenas de $\mu L\ min^{-1}$ hasta decenas de $mL\ min^{-1}$. Estas jeringas (Hamilton, Bonaduz, Switzerland) son de vidrio y su émbolo es metálico con un

revestimiento de politetrafluoroetileno en su cabeza que permite el uso de reactivos agresivos.

Las jeringas suelen estar conectadas en su posición OFF a los depósitos que contienen las diferentes disoluciones que se requieren para la ejecución de un determinado método. En su posición ON conectan hacia la red de tuberías que une la bureta multijeringa con el sistema de detección.

Además de las cuatro válvulas solenoides acomodadas en la multijeringa, esta tiene en su parte posterior cuatro puertos (Figura 2.2) cuyo voltaje de salida puede regularse hasta un máximo de unos 13 voltios. Esto permite el control de hasta cuatro válvulas adicionales. El uso de estas válvulas adicionales amplía el abanico de posibilidades en el manejo de fluidos inicialmente permitidas por un módulo multijeringa. Por otra parte, estas cuatro salidas permiten el control de otros dispositivos, tales como la puesta en marcha de motores, ventiladores, disparo de relés,…

Figura 2.2. Conexiones situadas en la parte posterior de una bureta multijeringa. De izquierda a derecha: Salida para la conexión de hasta cuatro válvulas solenoides adicionales, conexiones RS232C (controlador y periférico) y conexión a la fuente de alimentación.

El funcionamiento de las válvulas solenoides adicionales es idéntico al de las válvulas que están incorporadas a la multijeringa. Existen varios modelos de estas válvulas, como las de la empresa Takasago, las cuales soportan presiones de entre 2 y 6 bar. En la Figura 2.3 pueden apreciarse los distintos tipos de válvulas solenoides adicionales utilizadas.

Figura 2.3. Diferentes tipos de válvulas solenoides utilizadas.

Además de este tipo de válvulas, en varios experimentos se ha utilizado un módulo de válvulas VA1+1 (Crison) (Figura 2.4). El módulo de válvulas se compone de una válvula de selección rotatoria de 8 puertos típica de los sistemas SIA en su parte superior, y una válvula de inyección de 6 puertos y dos posiciones, clásica de los sistemas FIA, en su parte inferior.

Figura 2.4. Módulo de válvulas mixto. Válvula de selección de 8 puertos en su parte superior. Válvula de inyección de 6 puertos y dos posiciones en su parte inferior.

Estos instrumentos se conectan a través del puerto serie RS232C a un ordenador personal. Cada instrumento tiene un puerto principal que se conecta al controlador y un puerto periférico que se conecta al elemento siguiente de una cadena (Figura 2.2), de modo que el controlador del primer instrumento de la cadena se conecta al ordenador. El segundo instrumento de la cadena se conecta mediante su controlador al puerto periférico del primero, y así sucesivamente.

La red de tuberías o manifold se fabricó totalmente de PTFE, principalmente de 0.8mm i.d., incluyendo los bucles de carga de muestra y los reactores anudados para el desarrollo de la reacción (Figura 2.5).

Los reactores anudados se utilizaron para incrementar la dispersión radial del bolo de reacción, y simultáneamente disminuir la dispersión axial. Como resultado se obtienen señales en forma de pico más estrechas y más altas en comparación con los bucles de reacción convencionales.

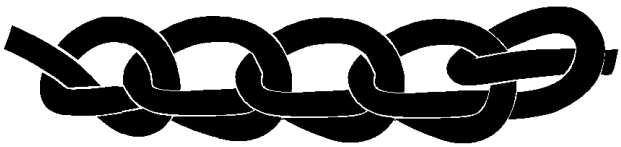

Figura 2.5. Reactor anudado.

2.2. Detección espectrofotométrica

En varios trabajos presentados en esta tesis se utiliza la detección espectrofotométrica en la zona de radiación del visible. Para ello se realizan medidas de intensidad de radiación con el fin de estudiar la absorción de esta por las sustancias de nuestro interés.

Generalmente realizan tres medidas con el espectrofotómetro, una con la fuente de radiación apagada (corriente oscura, I_{osc}), las otras dos con la fuente encendida, una a través del blanco (I_0), y la tercera en presencia de la muestra (I). La absorbancia se obtiene a partir de estas tres medidas mediante la ecuación:

$$Abs = -\log_{10} T = \log_{10} \frac{I_0 - I_{osc}}{I - I_{osc}}$$

Los valores de absorbancia obtenidos pueden relacionarse con la concentración de la sustancia de interés mediante la Ley de Lambert-Beer. La absorbancia medida es proporcional al producto de la concentración de la sustancia de interés, la longitud del paso óptico de la celda de detección y el coeficiente de absortividad molar, siendo este último una constante para cada tipo de sustancia.

Para efectuar este tipo de medidas se utilizaron lámparas de tungsteno como fuente de radiación. Una cubeta de 1 cm de paso óptico diseñada para la realización de medidas en sistemas en flujo fue utilizada como celda de detección. Sin embargo, en uno de los trabajos presentados se utilizó una celda capilar de paso óptico grande cuyo funcionamiento viene detallado en un apartado posterior (punto 3.5).

Como detector de la radiación se ha utilizado un espectrofotómetro CCD (Ocean Optics, Dunedin, USA). La fuente de radiación, celda de detección y

espectrofotómetro se conectan directamente mediante conectores dispuestos para tal efecto, o mediante fibras ópticas.

Las medidas de absorbancia de todos los experimentos presentados se realizaron a dos longitudes de onda diferentes con el objeto de minimizar el Efecto Schlieren[2]. Este efecto provoca distorsiones en las señales debido a cambios en la composición del fluido en la celda de detección. Estos cambios son causados por variaciones del índice de refracción de los líquidos que pasan a través de la cubeta. La compensación de este efecto se realiza restando a la absorbancia medida a la longitud de onda analítica la absorbancia medida al mismo tiempo, a una longitud de onda en la cual la absorbancia de la especie de interés sea despreciable.

2.3. Detección quimioluminiscente

La quimioluminiscencia (QL) se define como la producción de radiación electromagnética como resultado de una reacción química. Esto ocurre en algunas reacciones en las cuales alguno de los estados intermedios o productos de reacción obtenidos se encuentra en un estado excitado, produciéndose una emisión de radiación (ultravioleta, visible o infrarroja cercana) al volver este a un estado de menor energía.

Las primeras reacciones QL fueron observadas en la antigüedad en seres vivos tales como las luciérnagas o algunos gusanos, dicha emisión de luz es conocida como bioluminiscencia. Las reacciones QL propiamente dichas fueron desarrolladas posteriormente.

Algunos de los compuestos quimioluminiscentes más conocidos fueron descritos hace ya cerca de 80 años. Por ejemplo, las propiedades del luminol

[2] E. A. G. Zagatto, M. A. Z. Arruda, A. O. Jacintho, I. L. Mattos, Anal. Chim. Acta 234 (1990) 153-160.

fueron descritas en 1928[3], y las del Tris(2,2´-bipiridil)rutenio(II) en 1936[4]. Sin embargo este tipo de reacciones empezaron a tener una aplicabilidad analítica hace unos 30 años. La QL puede darse en estado sólido, líquido o gaseoso.

El uso de un sistema de detección QL ofrece una serie de ventajas tales como la obtención de una elevada sensibilidad, rangos lineales de trabajo de varios órdenes de magnitud, así como la simplicidad de los detectores ya que no se requiere el uso de ninguna fuente de radiación.

Por estos motivos la detección QL, y más concretamente la QL en fase líquida (que es la que ha sido utilizada en esta tesis) se ha convertido en una herramienta de detección bien establecida y con un amplio campo de aplicaciones. Estas aplicaciones pueden encontrarse en varios libros, capítulos de libros y revisiones bibliográficas publicadas a lo largo de los últimos años[5]. Este modo de detección es ampliamente utilizado en sistemas de análisis en flujo[6], cromatografía líquida[7] y electroforesis capilar[8].

[3] H. O. Albrecht, Z. Phys. Chem. 136 (1928) 321.

[4] F. H. Burstall, J. Chem. Soc. (1936) 41-45.

[5] (a) A. M. García-Campaña, W. R. G. Baeyens, Editors, Chemiluminescence in Analytical Chemistry, Marcel Dekker, New York (2001). (b) N. W. Barnett, P. S. Francis, Chemiluminescence: liquid-phase chemiluminescence. In: P. J. Worsfold, A. Townshend, C. F. Poole, Editors, Encyclopedia of Analytical Science (second ed.), Elsevier, Oxford (2005), 511-520. (c) Y. Su, H. Chen, Z. Wang, Y. Lv, Appl. Spectrosc. Rev. 42 (2007) 139-176. (d) L. Gámiz-Gracia, A. M. García-Campaña, J.J. Soto-Chinchilla, J. F. Huertas-Pérez, A. González-Casado, Trends Anal. Chem. 24 (2005) 927-942. (e) L. J. Kricka, Anal. Chim. Acta 500 (2003) 279-286. (f) A. M. García-Campaña, W. R. G. Baeyens, X. Zhang, E. Smet, G. Van Der Weken, K. Nakashima, A. C. Calokerinos, Biomed. Chromatogr. 14 (2000) 166-172.

[6] P. Fletcher, K. N. Andrew, A. C. Calokerinos, S. Forbes, P. J. Worsfold, Luminescence 16 (2001) 1-23.

[7] L. Gámiz-Gracia, A. M. García-Campaña, J. F. Huertas-Pérez, F. J. Lara, Anal. Chim. Acta 640 (2009) 7-28.

[8] X. J. Huang, Z. L. Fang, Anal. Chim. Acta 414 (2000) 1-14.

Cada reacción QL tiene un determinado rendimiento cuántico y velocidad de reacción, que se afectan por factores como la temperatura, la fuerza iónica, la presencia de aceptores de la energía transferida, la presencia de interferentes, la polaridad del medio y el pH. Asimismo, cada reacción QL tiene un determinado tiempo de reacción durante el cual se produce la emisión. Por lo general, inmediatamente después de la mezcla de todos los componentes de la reacción se produce el máximo de emisión, la cual decae rápidamente en un tiempo menor de un segundo, y en algunos casos se mantiene varios minutos, e incluso horas. En la Figura 2.6 puede apreciarse un ejemplo de comportamiento de la emisión QL en función del tiempo.

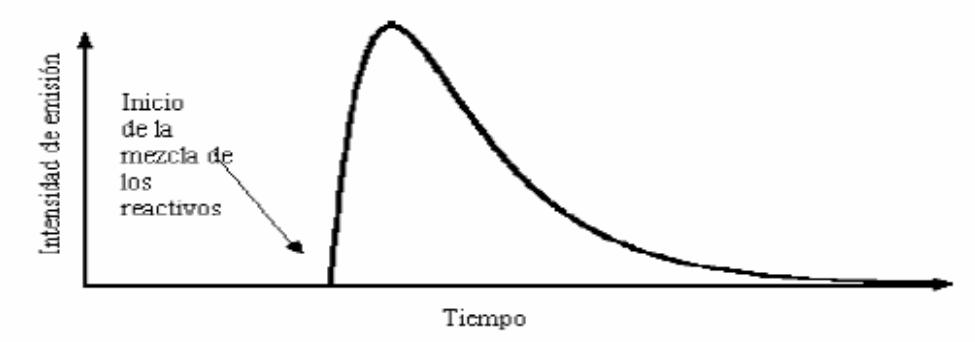

Figura 2.6. Comportamiento de la intensidad de emisión quimioluminiscente con tiempo

El proceso por el cual se emite la quimioluminiscencia es el mismo que para los otros fenómenos luminiscentes (fluorescencia y fosforescencia), excepto que no se necesita ninguna fuente de excitación luminosa. El proceso de generación de la QL se puede esquematizar en los dos siguientes pasos (Figura 2.7):

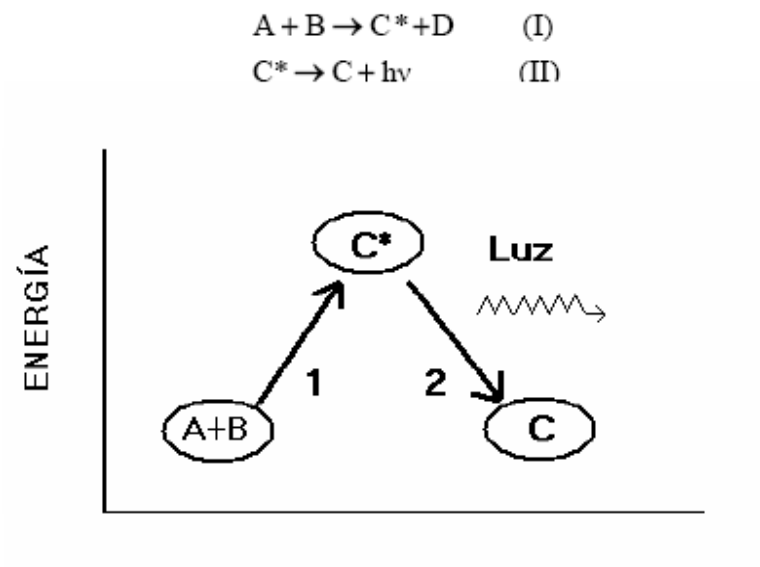

Figura 2.7. Esquema del fenómeno de la quimioluminiscencia. 1) Excitación, 2) Relajación y emisión de luz.

Por lo tanto, este tipo de reacciones serán útiles para la determinación de cualquier especie que pueda participar en ella como reactivo (A ó B). También pueden ser útiles en la determinación de especies que si bien no participan en la reacción, tienen un efecto indirecto cuantitativo sobre ella (catalizadores, inhibidores,…).

Muy pocas reacciones químicas producen QL, ya que para que esto ocurra tienen que cumplirse las tres condiciones siguientes:

1- Liberar la suficiente energía para que la radiación emitida se encuentre dentro de la región del espectro del visible. Para ello la energía de los fotones emitidos en el proceso de relajación tiene que ser de entre 44 y 71 Kcal mol^{-1}.

2- Generalmente son reacciones en las que se forma alguna especie intermedia en estado excitado.
3- Dicho estado excitado es luminiscente o transferirá su energía a otra molécula luminiscente.

Debido a la rapidez de las reacciones QL se tienen dificultades a la hora de implementarlas manualmente y en obtener unos resultados reproducibles. Por este motivo la automatización juega un papel importante en la detección QL.

En el caso de las técnicas de análisis en flujo, la detección QL consiste en la mezcla de los reactivos de la reacción a elevados caudales, intentando siempre obtener que la máxima emisión de luz se produzca justo frente al detector. Es decir, el volumen entre el punto de mezcla y el punto de observación y el caudal determinarán el tiempo de reacción transcurrido y por tanto la intensidad QL observada (Figura 2.8).

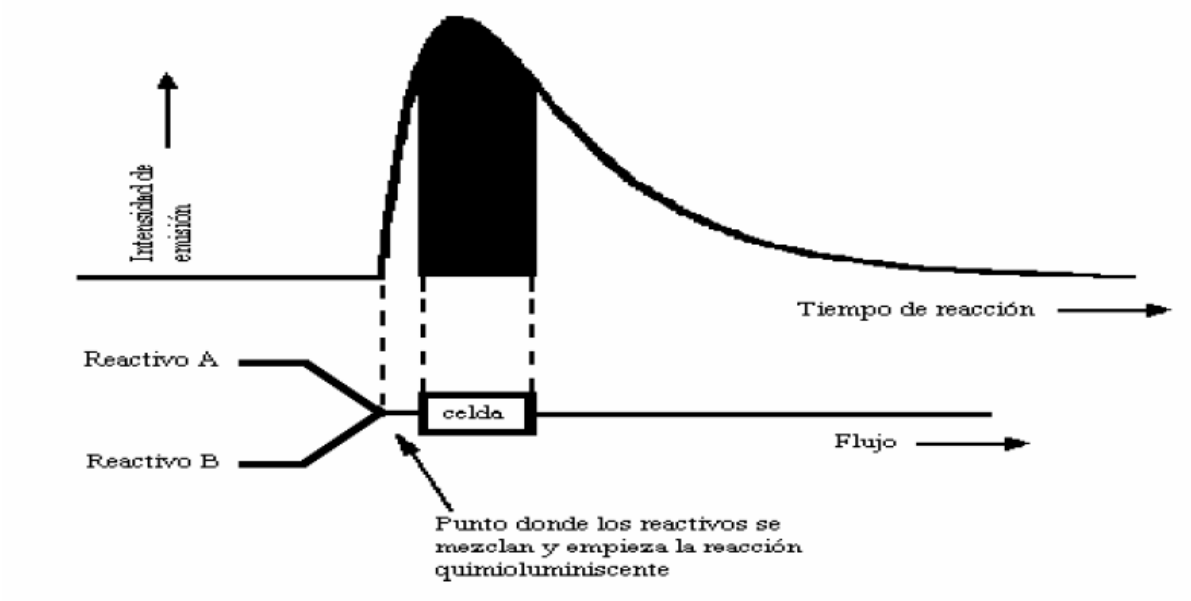

Figura 2.8. Importancia de la situación del detector en el "manifold".

El sistema de detección QL está formado por una celda de flujo por la cual circularán los fluidos y (desde la que) se emitirá la QL. Esta celda se coloca justo frente al detector de radiación el cual será un módulo fotosensor conectado a una serie de dispositivos electrónicos externos (Figura 2.9). Dicho módulo fotosensor está compuesto por un tubo fotomultiplicador[9], un circuito de alimentación de alto voltaje y un circuito amplificador de bajo ruido. En nuestro caso, el dispositivo contiene toda la electrónica esencial para la conversión de bajo a alto voltaje para la alimentación de los electrodos del fotomultiplicador, la división del potencial para crear diferencias de potencial constantes entre los diversos electrodos y un voltaje de referencia de 1.2V para el ajuste de la ganancia y la conversión de la intensidad de luz emitida a un voltaje de 0 a 10V.

El detector quimioluminiscente viene montado en una caja de fabricación casera y puede ser fácilmente manejado mediante la fuente de ± 12 V, que en nuestro caso proviene de un conversor analógico-digital (A/D) de 10 bit (Ibercomp, Palma de Mallorca, http://www.ibercomp.es) equipado con ocho canales analógicos de entrada.

No es necesaria una fuente eléctrica adicional en el detector para su apropiado funcionamiento. El único requisito es la construcción de una fuente auxiliar e independiente de 9 V a causa de la imposibilidad de usar las pantallas con la misma fuente que el modulo fotosensor. Otro componente fundamental es el circuito de realimentación, que permite el control de la ganancia del fotomultiplicador (G) por medio de un potenciómetro externo de 10 kΩ. La posición del potenciómetro regula el voltaje de realimentación que puede ser cambiado en el rango entre 0.3 V (Ganancia = 1) y 0.8 V (Ganancia =

[9] Los tubos fotomultiplicadores, en los cuales los fotones incidentes causan la emisión de electrones por parte del fotocátodo y los electrones emitidos son amplificados en cascada, son los sensores más utilizados en las aplicaciones quimioluminiscentes. Pero debido a que su respuesta no es igual en todas las longitudes de onda, el tubo fotomultiplicador usado debe poseer una respuesta apropiada a la longitud de onda o al intervalo de longitudes de onda de emisión. http://www.hamamatsu.com/.

1000). Un resistor limitador de voltaje de 2 kΩ es usado para impedir voltajes de realimentación por encima de 0.8 V que podrían dañar el fotomultiplicador.

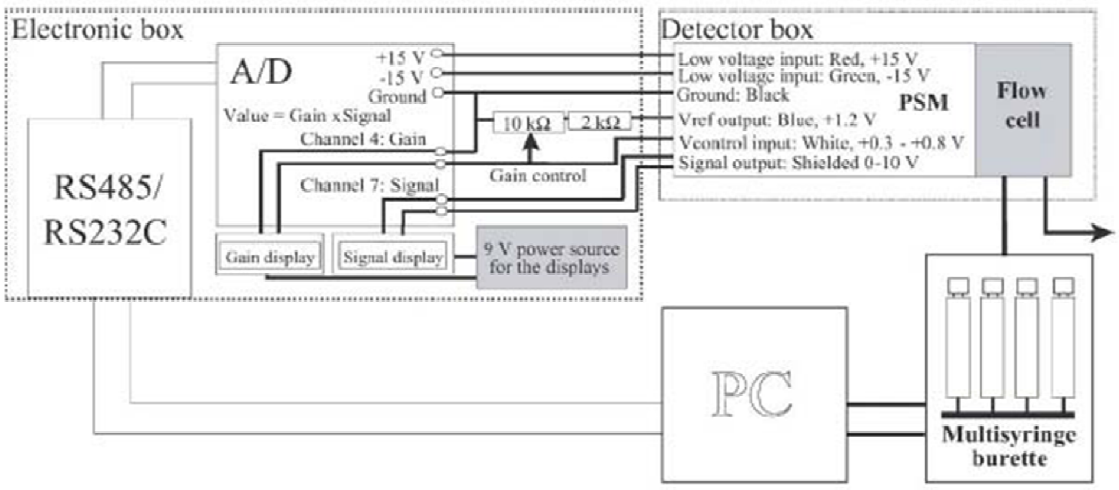

Figura 2.9. Esquema del detector quimioluminiscente de la empresa SCIWARE.

Las celdas de flujo utilizadas se basan en el grabado en una placa de teflón de un canal en forma de espiral con una entrada y una salida del flujo, este canal es sellado con una ventana de cuarzo que se coloca delante del fotomultiplicador.

La celda de flujo y el fotomultiplicador se colocan en el interior de una caja negra de modo que se evite totalmente la entrada de luz externa.

2.4. Software

Algunos instrumentos tales como buretas, válvulas y detectores utilizados esta tesis, requieren para su control y para el posterior desarrollo de los métodos automatizados de análisis un ordenador y software adecuados.

El programa AutoAnalysis 5.0[10] nos permite la integración del control de todos los instrumentos detallados en el párrafo anterior, y a su vez la adquisición y el procesamiento de los datos obtenidos. Dicho programa fue desarrollado por el Grupo de Química Analítica, Automatización y Medio Ambiente[11] de la Universidad de las Islas Baleares.

El programa AutoAnalysis 5.0 ha sido realizado en Delphi5.0 y Visual C++ 6.0 y está basado en el uso de librerías de enlace dinámico (DLL´s) de 32 bits. Este programa es multitarea y permite simultáneamente el desarrollo de metodologías analíticas controlando la totalidad de la instrumentación, realizar el tratamiento de datos, y la edición de documentos con otros programas comerciales.

El programa incluye un menú de configuración que permite incorporar los diferentes instrumentos que componen un determinado analizador en flujo. En dicho menú pueden instalarse las DLL´s correspondientes a los diferentes canales e instrumentos que se requieran para el desarrollo de una determinada aplicación. En la Figura 2.10 puede apreciarse dicho menú, el cual tiene en su parte derecha superior e inferior las diversas conexiones e instrumentación disponibles, respectivamente. En su parte central puede verse la configuración actual. Como ejemplo, se encuentra detallada la conexión de una bureta

[10]Programa AutoAnalysis 5.0 suministrado por SCIWARE: http://www.sciware-sl.com, E-mail:info@sciware-sl.es.

[11] E. Becerra, A. Cladera, V. Cerdà, Lab. Rob. Autom. 11 (1999) 131-140.

multijeringa y un módulo de válvulas a través de una canal serie CRISON, además de un detector de quimioluminiscencia (Auto Hg) y un espectrofotómetro (Ocean Optics), cada uno a través de sus respectivos canales.

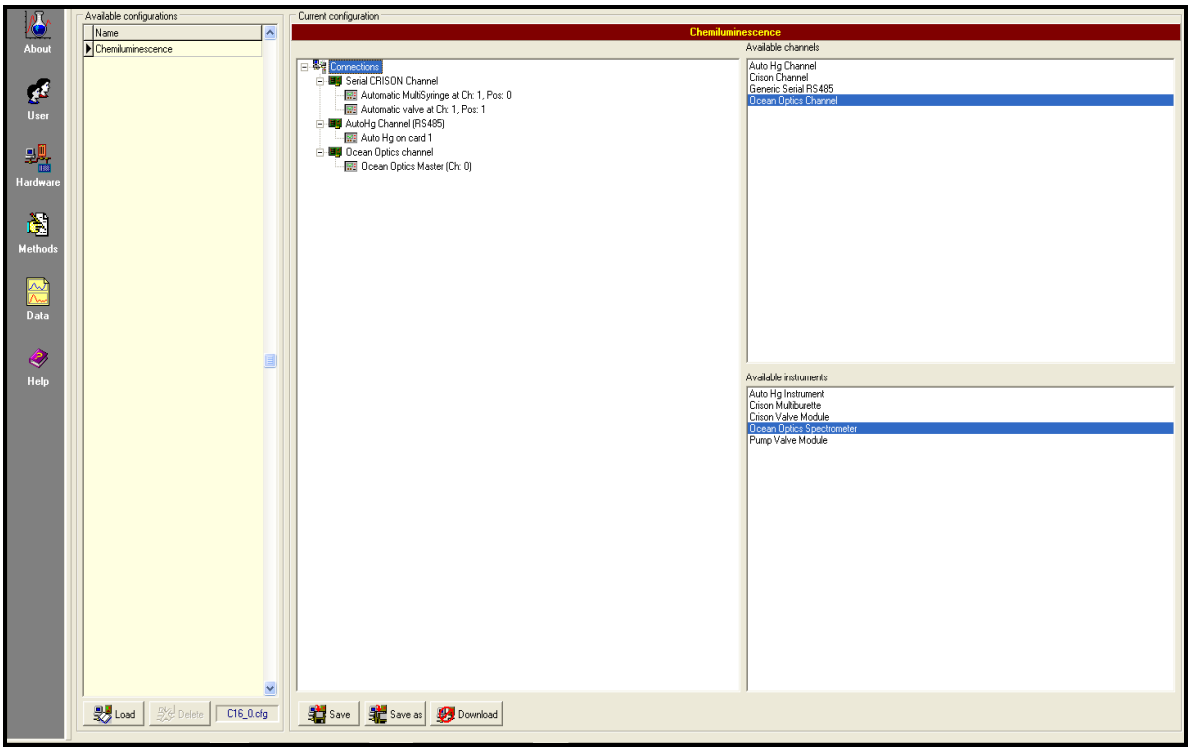

Figura 2.10. Panel de control de conexiones del programa AutoAnalysis 5.0.

Una vez controlados los instrumentos, estos pueden ser manejados manual ó automáticamente mediante la creación de secuencias de instrucciones cuyo conjunto constituye el método analítico. Cada paso de la secuencia representa una operación ejecutada por uno de los instrumentos controlados por el programa. Ejemplos de operaciones pueden ser el desplazamiento de un volumen, la conmutación de una válvula o la activación de un detector, entre otros.

Además, el programa permite la ejecución de una serie de comandos adicionales típicos de programación, que si bien no afectan a los instrumentos, son esenciales para el desarrollo de metodologías analíticas. Algunos ejemplos son la creación de ciclos para la repetición de procedimientos, la creación de marcas, el control del tiempo para realizar cinéticas o métodos en flujo detenido, decisiones condicionales, entre otros. Una vez compiladas todas las órdenes en forma de un método, estas pueden ejecutarse una tras otra, o simultáneamente, empezando la siguiente orden de la lista sin esperar a que acabe la ejecución de la orden anterior.

Un ejemplo de un método desarrollado mediante el programa AutoAnalysis 5.0 puede verse detallado en la Figura 2.11. Las órdenes escritas ejecutan el cambio de muestra y la posterior toma de esta para ser inyectada en un manifold con detección quimioluminiscente. La muestra es mezclada con los reactivos necesarios para la producción de la emisión de radiación.

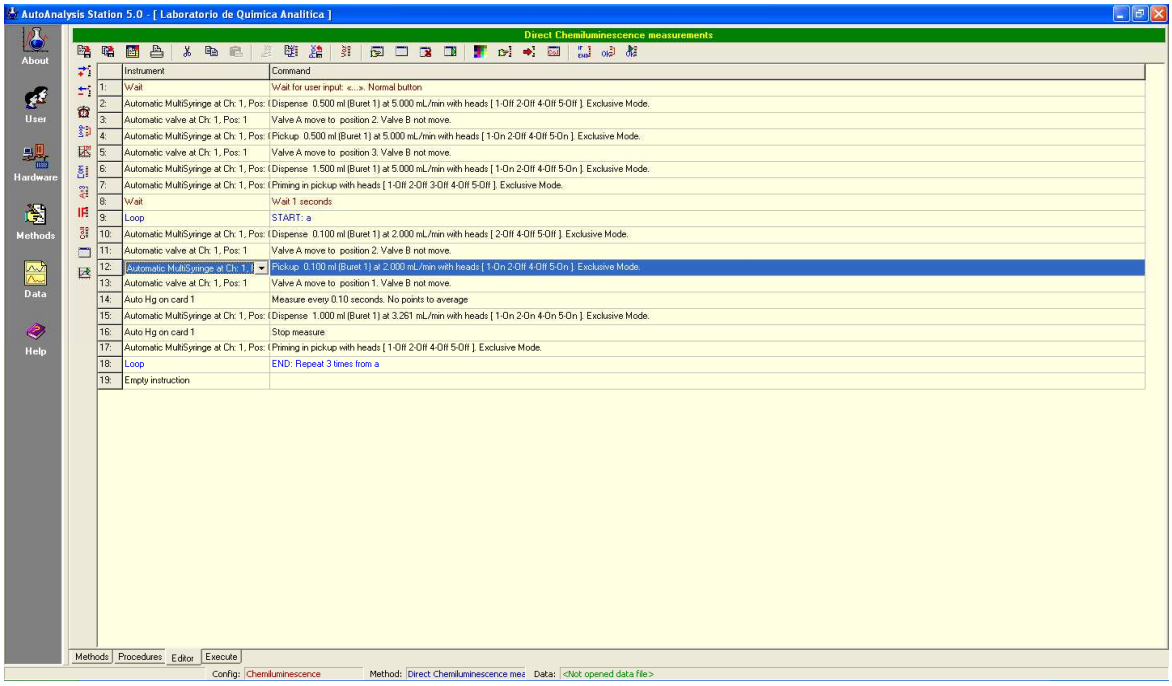

Figura 2.11. Panel de edición de métodos del programa AutoAnalysis 5.0.

Una vez un método es ejecutado, aparece una pantalla en la que se pueden observar los datos adquiridos, los cuales posteriormente pueden ser procesados y guardados (Figura 2.12).

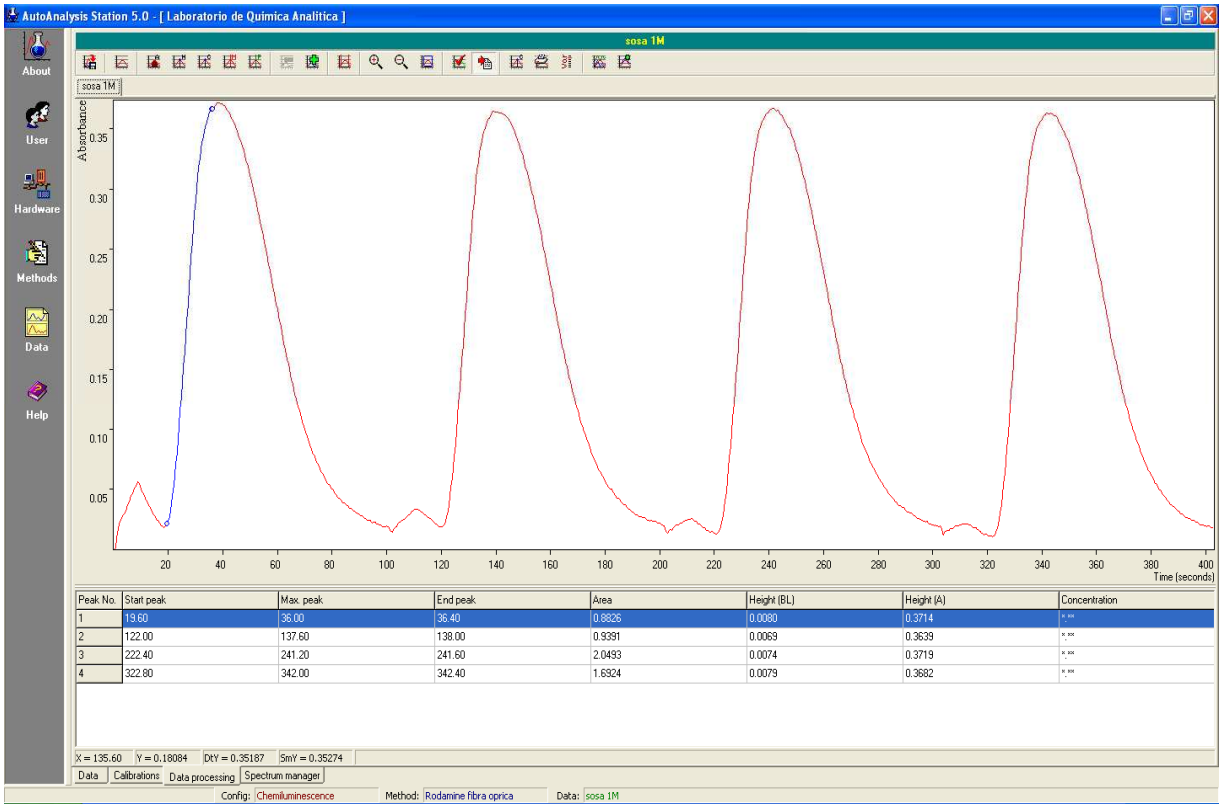

Figura 2.12. Panel de tratamiento de datos del programa AutoAnalysis 5.0.

Dentro del procesamiento de datos, el programa AutoAnalysis 5.0 nos permite el suavizado de los picos obtenidos, así como calcular su primera y segunda derivada, la visualización de la línea base utilizada en el cálculo de áreas y alturas de las señales obtenidas, corregir su integración en el caso de que estas no sean correctas, eliminar señales no deseadas, realizar y trabajar con curvas de calibración integradas, o realizar el procesamiento en línea de los datos mediante el uso de condicionales.

CAPÍTULO 3

CONTRIBUCIÓN A LA MINIMIZACIÓN DE RESIDUOS EN LOS ANÁLISIS QUÍMICOS. APLICACIÓN A LA DETERMINACIÓN ESPECTROFOTOMÉTRICA DE CLORUROS EN AGUAS.

Este capítulo trata sobre el potencial de la técnica MSFIA para el desarrollo de nuevas metodologías analíticas automáticas con un mayor grado de cumplimiento de los principios de la llamada química verde.

Se utiliza la técnica MSFIA para la minimización de los residuos producidos al aplicar metodologías analíticas con detección espectrofotométrica. Como ejemplo se ha seleccionado el método oficial de análisis de cloruros en aguas basado en la reacción del Cl^- con $HgSCN_2$ y Fe^{3+}. Se estudian las ventajas que se pueden obtener al implementar dicho método mediante la técnica MSFIA, y posteriormente las ventajas adicionales que ofrece dicha técnica en combinación con fibras ópticas de núcleo líquido para el mismo propósito. Además se presenta una revisión bibliográfica sobre las ventajas de la técnica MSFIA en el desarrollo de metodologías analíticas más respetuosas con el medio ambiente.

3.1. Química Verde (Green Chemistry)

En los últimos años el movimiento de la Química Verde (Green Chemistry) ha promovido nuevas estrategias dirigidas a la reducción de los riesgos que comporta el uso de productos químicos tanto para los humanos, como para el medio ambiente[1].

La química verde utiliza técnicas y metodologías para reducir o eliminar el uso de materias primas, reactivos, disolventes y subproductos en un determinado proceso que puedan entrañar un peligro para la salud humana o el medio ambiente.

Por lo tanto, un objetivo importante es la reducción de los peligros asociados a los productos y procesos que son esenciales en la sociedad actual, tanto para la economía mundial como para mantener el nivel de calidad de vida al que estamos acostumbrados gracias a la química.

El concepto de la química verde se basa en doce principios los cuales proporcionan un marco o guía de actuación para el diseño de nuevos materiales, productos, procesos y sistemas. Dichos principios están orientados hacia la búsqueda de soluciones innovadoras que nos proporcionen un desarrollo más sostenible, solucionando problemas actuales, mejorando productos o procesos muy utilizados actualmente.

Los doce principios de la química verde son los siguientes:

1- Prevención. Es mejor evitar la producción de residuos, que su tratamiento una vez estos han sido generados.
2- Economía atómica. Los métodos de síntesis deben diseñarse para aprovechar al máximo las materias primas que participan en el proceso.

[1] (a) P. T. Anastas, J. C. Warner, Green Chemistry: Theory and Practice; Oxford University Press: New York, 1998.

3- Diseño de métodos sintéticos menos peligrosos, en los cuales se disminuya o elimine la toxicidad de las sustancias generadas en ese proceso.
4- Diseño de productos químicos más seguros. Sustitución de un producto químico diseñado para una determinada función por otro de menor toxicidad capaz de desempeñar la misma función.
5- Reactivos y disolventes más seguros. Evitar el uso de productos auxiliares en un proceso, o por lo menos sustituirlos por otros de menor toxicidad.
6- Diseño dirigido a la eficiencia energética. En un determinado proceso, buscar alternativas para obtener un ahorro energético en su ejecución.
7- Uso de materias primas renovables, para poder ser reutilizadas cuando el producto fabricado deje de ejercer su función.
8- Simplificación de procesos. Reducir procedimientos de derivatización, principalmente cuando estos se ejecutan a escala industrial.
9- Catálisis. Usar reacciones catalíticas, en vez de reactivos en proporciones estequiométricas.
10- Degradación. Sustituir productos persistentes en el medio ambiente, por productos biodegradables capaces de desempeñar la misma función.
11- Análisis en tiempo real para la prevención de la generación de productos no deseados. Cuanto más rápidamente sea obtenida una información química en un determinado proceso, un mayor control se tendrá sobre este.
12- Química más segura para la prevención de accidentes. Elección de las sustancias utilizadas en los procesos químicos para minimizar los riesgos potenciales tales como explosiones o fuegos.

3.1.1. Química verde y el desarrollo de nuevas metodologías de análisis más respetuosas con el medio ambiente

Algunos de los principios generales de la química verde detallados en el punto anterior, se pueden aplicar en el desarrollo de nuevos métodos analíticos[2].

Dichos principios aplicables son sobre todo los siguientes:

1- Prevención.
2- Uso de reactivos y disolventes más seguros.
3- Diseño dirigido a la eficiencia energética.
4- Simplificación de procesos.
5- Análisis en tiempo real para la prevención de la generación de productos no deseados.
6- Química más segura para la prevención de accidentes.

Al hablar de química analítica ambiental, es irónico observar que para obtener información química sobre el medio ambiente es prácticamente seguro que se produzcan una serie de residuos, los cuales a su vez pueden contribuir a crear un problema de contaminación medio ambiental. Esto se debe al uso de productos químicos para la preservación de muestras, preparación, control de calidad, calibración y/o limpieza del equipamiento, que dan lugar a una cantidad de residuos de toxicidad que pueden ser incluso mayor que el de la propia muestra. Por todo esto, la química analítica verde se está convirtiendo en una nueva e importante sub-área de la química verde[3].

El objetivo de la química analítica verde es el desarrollo de nuevas metodologías analíticas que generen menos residuos, de menor peligrosidad y más respetuosos con el medio ambiente.

[2] P. T. Anastas, Crit. Rev. Anal. Chem. 29 (1999) 167-175.
[3] (a) L. H. Keith, L. U. Gron, J. L. Young. Chem. Rev. 107 (2007) 2695-2708. (b) S. Armenta, S. Garrigues, M. de la Guardia, Trends Anal. Chem. 27 (2008) 497-511.

Se puede establecer un orden de prioridad para el desarrollo de metodologías analíticas más respetuosas con el medio ambiente:

1- Sustituir una metodología analítica por otra en la cual no se genere ningún residuo tóxico.
2- En el caso de que la producción de residuos tóxicos sea inevitable, intentar que la toxicidad de estos sea la menor posible.
3- Si se produce un residuo tóxico, intentar minimizar la cantidad producida.
4- Cuando no puede minimizarse la generación de un residuo, dicho residuo debería ser reciclado y si es posible reutilizado.
5- En caso de no poder aplicarse ninguno de los cuatro puntos anteriores, el propio método debería contemplar un procedimiento para la eliminación o tratamiento de los residuos producidos.

Una herramienta eficaz para la implementación de estos principios son las técnicas de análisis en flujo[4], las cuales son sobre todo efectivas en el tercer de los anteriores puntos, es decir; en la minimización de los residuos producidos al implementar una metodología analítica.

3.1.2. Aportaciones de la técnica de análisis por inyección en flujo multijeringa al campo de la química verde y la minimización de residuos

Entre las técnicas de análisis en flujo introducidas en el primer capítulo, la técnica MSFIA ha demostrado ser una herramienta eficaz para la minimización de los residuos producidos en el desarrollo metodologías analíticas. Dicha eficacia reside en general en la miniaturización del procedimiento analítico y en particular en el desarrollo de procedimientos multiconmutados para la

[4] F. R. P. Rocha, J. A. Nobrega, O. Fatibello-Filho, Green Chem. 3 (2001) 216-220.

inyección de reactivos, la miniaturización de procedimientos de extracción en fase sólida, la inmovilización de reactivos, la miniaturización de métodos basados en la cromatografía líquida o de pretratamiento de muestra acoplados a sistemas de electroforesis capilar. Dichas ventajas pueden verse recopiladas en el punto 3.7.

3.2. Consideraciones sobre el cloruro

El ion cloruro es uno de los aniones mayoritarios en las aguas naturales, su concentración dependerá de los terrenos que estas hayan atravesado a lo largo de su recorrido. Esta cantidad suele ser mayor en las aguas residuales.

El aumento de la concentración de cloruros en las aguas puede tener un origen diverso. En las zonas costeras este incremento puede deberse a intrusión salina del agua del mar. En el caso de zonas áridas, puede deberse al lavado de los suelos debido a fuertes lluvias. Otro caso es el de contaminación de aguas naturales por aguas residuales.

La concentración de cloruro en aguas naturales no suele sobrepasar los 50-60 mg L^{-1}, y por tanto dicha concentración no suele ser un problema de potabilidad en las aguas de consumo. Sin embargo, niveles altos de cloruro pueden dañar conducciones y estructuras metálicas, aparte de perjudicar el crecimiento vegetal. La reglamentación española establece como valor orientativo una concentración de 250 mg L^{-1} de cloruro. Los efectos negativos provocados por un exceso de cloruros se ven acentuados a partir de concentraciones mayores de 750 mg L^{-1}.

La determinación de cloruros se realiza en los planes de vigilancia ambiental que se llevan a cabo en las Islas Baleares, y sirve como indicador de intrusión salina de agua marina o residual.

Existen varios métodos reconocidos oficialmente para la determinación de cloruros en aguas, estos se basan en valoraciones con nitrato de plata o nitrato mercúrico, potenciometría, cromatografía iónica o en un método automático con detección espectrofotométrica.

3.3. Detección espectrofotométrica de cloruros en aguas

Los aniones cloruro (Cl^-), bromuro (Br^-) y ioduro (I^-), pero no el fluoruro (F^-), reaccionan con mercurio tiocianato ($HgSCN_2$) produciéndose un equilibrio de desplazamiento en el que estos se enlazan al mercurio con la consiguiente liberación de tiocianato (SCN^-) al medio. El SCN^- liberado reacciona con hierro (III) (Fe^{3+}) generándose un complejo de coordinación de fórmula $[Fe(SCN)(H_2O)_5]^{2+}$, el cual puede ser detectado espectrofotométricamente a una longitud de onda de 470-480nm.

Al ser el cloruro el halógeno en mayor proporción en las aguas, este método presenta una buena selectividad, sensibilidad y velocidad de análisis cuando es implementado en analizadores automáticos. Sin embargo, su principal desventaja reside en la generación de residuos con altas concentraciones de Hg^{2+}, lo cual va totalmente en contra de la tendencia actual de la química analítica verde.

3.4. Determinación de cloruros en aguas mediante la técnica de análisis por inyección en flujo multijeringa

Desde los primeros analizadores automáticos para la determinación espectrofotométrica de cloruros hasta la actualidad, se han desarrollado diversas implementaciones de dicho método en diferentes plataformas de análisis en flujo, viéndose disminuida la generación de residuos al ir

incrementando el grado de automatización y miniaturización de dichas técnicas.

Por este motivo, se ha implementado por primera vez dicho método espectrofotométrico mediante la técnica MSFIA, orientando la metodología hacía la minimización de residuos, haciendo énfasis en las ventajas que proporciona el uso de esta técnica en comparación con otras técnicas de análisis en flujo. El trabajo se muestra completo en el punto 3.8.

3.5. Detectores espectrofotométricos de largo paso óptico basados en fibras ópticas de núcleo líquido

Las celdas de detección de largo paso óptico constituidas por capilares de fibras ópticas de núcleo líquido, se basan en el uso de un capilar compuesto por un material con un índice de refracción menor que el índice de refracción del material que está contenido en ella. Este hecho permite que la luz introducida en el núcleo de dicho capilar sea totalmente reflectada en su interior, a lo largo de su camino desde la fuente de radiación hacia el detector. Basándose en este principio, las celdas de flujo son útiles para detectores de espectrofotometría, espectroscopia Raman, así como para técnicas luminiscentes (fluorescencia, quimioluminiscencia,…). Estas celdas se caracterizan por su pequeño volumen interno y largo paso óptico.

En una medición espectrofotométrica la concentración del analito en la muestra es directamente proporcional a la absorbancia, e indirectamente proporcional al coeficiente de absortividad molar y al paso óptico de la celda de detección. Al incrementar el paso óptico de la celda se incrementa proporcionalmente la absorbancia medida, y por tanto mejora la sensibilidad del método.

A finales de la década de los años 80, la empresa DuPont introdujo un nuevo material denominado Teflon AF, el cual es un co-polímero amorfo formado por tetrafluoroetileno y 2,2-bis(trifluorometil)-4,5-difluoro-1,3-dioxol. La creación de dicho material se debe a la necesidad de disponer de un material con un índice de refracción menor que el del agua (<1.33).

Existen dos tipos de tuberías de Teflón AF: Tipo I y Tipo II. El Teflón AF Tipo I es simplemente un capilar formado por dicho material. El Teflón AF Tipo II consiste en un capilar de sílice recubierto de Teflón (Figura 3.1). La luz es totalmente reflejada internamente en la interfase Teflón-Sílice, con un ángulo de incidencia de la radiación mayor que el ángulo crítico.

Estos capilares, han demostrado ser útiles como celdas de flujo para mediciones espectrofotométricas, llegándose a utilizar con longitudes de hasta 4-5m[5].

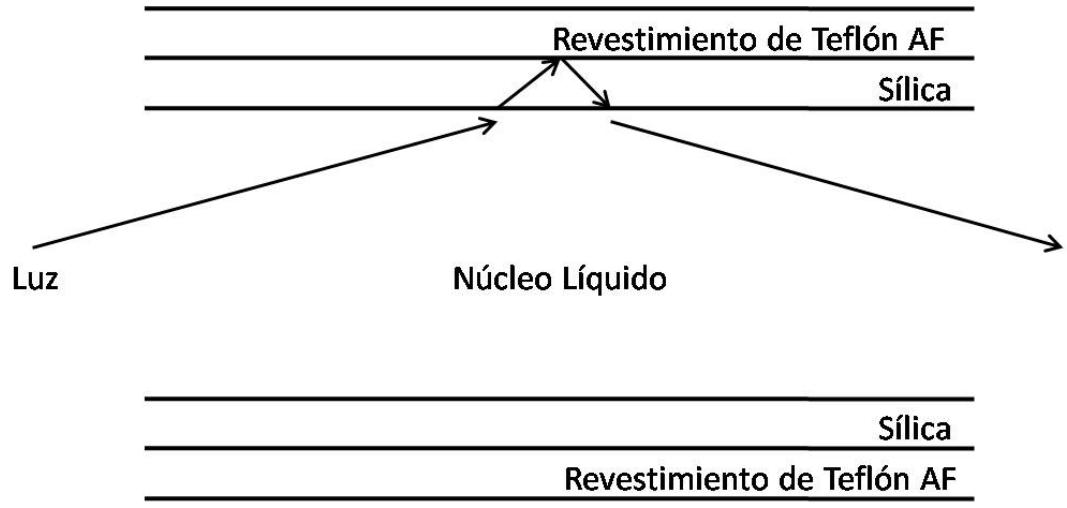

Figura 3.1. Estructura de un capilar de Teflón AF de Tipo II.

[5] (a) R. D. Waterbury, W. Yao, R. H. Byme, Anal. Chim. Acta 357 (1997) 99-102. (b) J.-Z. Zhang, C. Kelble, F. J. Millero, Anal. Chim. Acta 438 (2001) 49-57. (c) J. M. Santana-Casiano, M. González-Dávila. F. J. Millero, Environ. Sci. Technol. 39 (2005) 2073-2079.

3.6. Combinación de la técnica de análisis por inyección en flujo multijeringa con el uso de fibras ópticas de núcleo líquido para el desarrollo de metodologías analíticas con detección espectrofotométrica más limpias: Avances en la determinación de cloruros en aguas

Existen actualmente varias aplicaciones de las celdas de detección basadas en fibras ópticas de núcleo líquido para la determinación de diversos analitos, tanto realizando la inyección del producto de reacción manualmente como mediante técnicas de análisis en flujo. Dichas aplicaciones pueden verse recopiladas en la revisión bibliográfica realizada por L. J. Gimbert y P. J. Worsfold[6].

Las aplicaciones están normalmente dirigidas a la obtención de una mejor sensibilidad y límite de detección que los obtenidos para la misma aplicación mediante el uso de celdas de detección convencionales. Hasta el momento el uso de este tipo de celdas capilares no ha sido orientado hacia la minimización de los residuos producidos en los análisis químicos. Por este motivo se ha desarrollado un sistema que combina el potencial de la técnica MSFIA y de las celdas capilares de núcleo líquido, para establecer metodologías analíticas con detección espectrofotométrica más respetuosas con el medio ambiente.

Como primera aplicación de dicho sistema se ha elegido la determinación espectrofotométrica de cloruros en aguas detallada en el punto 3.3. La nueva aplicación de las celdas capilares de largo paso óptico se presenta íntegramente en el punto 3.9.

[6] L. J. Gimbert, P. J. Worsfold, Trends Anal. Chem. 26 (2007) 914-930.

3.7. Artículo original I

Multisyringe Flow Injection Technique for Development of Green Spectroscopic Analytical Methodologies

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Multisyringe Flow Injection Technique for Development of Green Spectroscopic Analytical Methodologies

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ABSTRACT Since its creation, the Multisyringe Flow Injection Analysis (MSFIA) technique has proved to be a highly useful tool for the automation of sample pretreatment procedures prior to spectroscopic detection (UV-vis spectrophotometry, fluorescence, or chemiluminescence). This article reviews the recent applications of the MSFIA technique and how they have contributed to the Green Analytical Chemistry field. These contributions are mainly based on the development of multicommutated analytical methodologies for the minimization of the consumption of reagents, automation and miniaturization of solid phase extraction, reagent immobilization, or in the automation and miniaturization of the required pretreatments prior to separation techniques.

KEYWORDS automated sample pretreatment, Green Chemistry, Multisyringe Flow Injection Analysis, spectroscopic detection

INTRODUCTION

It is a well-known fact that the production of any chemical activity produces some impact just by itself, while a huge number of chemical substances or chemical processes are widely used in increasing the quality of life, but also producing an environmental impact, in some cases of world-wide extension. Concerning these problems in 1990s emerged a new movement called Green Chemistry (GC),[1] promoting the use of chemistry with the aim of the minimization of the feedstocks and by-products produced in the use of any chemical activity. Green Analytical Chemistry (GAC) is a sub-area of GC, recently reviewed by Armenta et al.[2] GAC is based on the improvement of analytical methodologies for the determination of target compounds in any area of interest, turning on the classical methodologies in a more environmentally friendly ones.[3,4] Here it should be pointed out that flow injection (FI)[5] techniques are a powerful tool for greening classical analytical methodologies. Since their creation (in the 1970s) until now, the first Flow Injection Analysis (FIA) systems[6] have been improved gradually by the development of the Sequential Injection Analysis (SIA) systems,[7] the Multicommutated Flow Injection Analysis (MCFIA) systems,[8] or the

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Multisyringe Flow Injection Analysis (MSFIA) systems.[9–12] The evolution of flow techniques produces an improvement in the automated handling of fluids, providing an increase in efficiency, accuracy, economy, and quickness, besides other relevant features. The use of these techniques allows the approximation of the classic analytical methodologies, to the principles stated by the GC movement.

This revision is focused in the recent contributions of the MSFIA technique in the automation of analytical methodologies with spectroscopic detection, and how these improved methodologies have contributed to the GC or GAC field.

GREEN ANALYTICAL CHEMISTRY (GAC)

It is a well-known fact that the amounts of waste products generated in a laboratory-scale process are lower than those generated in an industrial-scale process. Nevertheless, it is an important fact that requires consideration due to the high number of hazardous chemicals used, starting for the sample preservation procedures and finishing in the cleaning procedure of the instrumentation used once the analysis is completed. As a representative example, in the use of analytical methodologies for the determination of hazardous substances for the environment, ironically the chemicals used along the analytical methodology often contribute to further environmental problems.

The Green Chemistry movement is based in 12 principles.[1] Between them, the most applicable to analytical chemistry are the following: (a) waste prevention; (b) use of safer chemicals; (c) design for energy efficiency; (d) reduce derivatives; (e) real-time analysis for pollution prevention; and (f) inherent safer chemistry for accident prevention. If we are talking about the development of analytical methodologies, the expression "Green Chemistry" is usually related to the design of improved methods in which the use or generation of hazardous substances is reduced or completely removed.

The different contributions of the flow techniques to the development of greener analytical methodologies can be established in a priority order[13]: An ideal analytical methodology from the point of view of GC should be a reagentless procedure. If it is not possible, the next step should be the replacement of hazardous reagents for other more benign ones. Advantageous characteristics of these two greener alternatives can be increased with the use of FI techniques owing to their intrinsic advantages, such as the downscaling of the different analytical operations that make up an analytical methodology. But when the use of reagents is indispensable and the substitution of these is not a feasible option, the best alternative is the minimization of its consumption. At this point is when the automation of analytical methodologies by means of FI techniques plays its main role in the GC context.

Lastly, if any of the previous options is applicable in a concrete analytical methodology, the use of FI techniques could facilitate the implementation of other alternatives such as the waste recycling (with concomitant reutilization) or the automated treatment of the generated waste products.

MULTISYRINGE FLOW INJECTION ANALYSIS (MSFIA) TECHNIQUE

MSFIA technique is based on the use of a multisyringe burette for the automated handling of fluids into a flow network (FN). The FN is specially designed for the implementation of each concrete analytical methodology. As can be seen in Fig. 1, the multisyringe is composed of a maximum number of 4 syringes of different volumes (usually between 1–10 mL). Syringes are set between a metal bar common to all of them, and a three-way solenoid valve for each one of them. The four syringes are displaced simultaneously and at the same flow rate (only if all of them have the same volume). Each syringe, as can be seen in Fig. 1, achieves four different movements: (a) Pickup in "Off" position (Load solution from a reservoir); (b) Pickup in "On" position (Load solution from the FN); (c) Dispense in "Off" position (Unload solution toward a reservoir); (d) Dispense in "On" position (Unload or inject solution toward the FN). MSFIA technique presents a high flexibility in the automated handling of fluids due to its 32 different combinations for flow management (if four syringes are used). The current versions of the commercialized multisyringe modules have a piston pump of 40.000 steps, which allows a perfectly reproducible flow management at the μL range (1 motor step = 25 nL of flow displacement, using a 1 mL syringe).

This flexible and precise flow management provided by the MSFIA technique allows the

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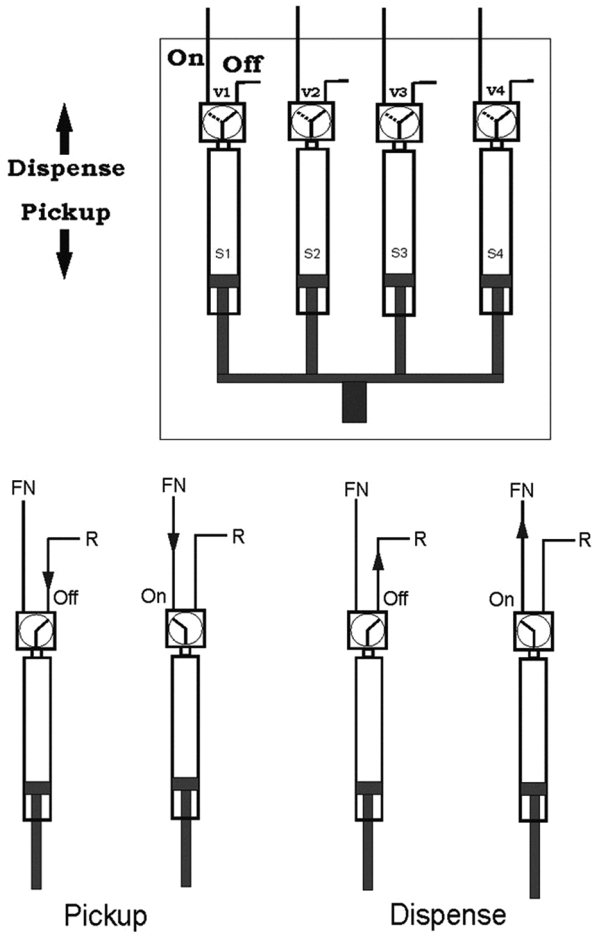

FIGURE 1 **(Up) Schematic representation of a typical multisyringe burette used in MSFIA systems. (Down) Different possibilities of fluid handling allowed by each syringe of a multisyringe burette.**

implementation of acknowledged analytical methodologies in a more environmentally benign way. It is produced due to the efficient downscaling of the different pretreatment steps required in each concrete analytical method, such as: sampling, development of chemical reactions, solid- or liquid-phase extraction, membrane isolation (gas diffusion, pervaporation, dialysis), mineralization (UV, chemical, microwave), or separation procedures (capillary electrophoresis, liquid chromatography). Furthermore, the selection of the MSFIA technique for the automation of analytical methods provides other advantageous features in comparison with the other existent flow techniques, such as: (a) robustness similar to SIA technique, avoiding the use of the Tygon® tubes commonly used in FIA and MCFIA systems, thus allowing the use of aggressive reagents (concentrated acids or organic solvents); (b) as in a multicommutated technique, the volumes are perfectly controlled, obtaining a saving of reagents in comparison with classical FIA systems; (c) the required volumes of sample and reagents can be injected toward the detector following a forward flow scheme as in FIA technique, thus avoiding the usual problems in the development of the reaction product when two or more reagents are used in SIA technique. By this way the injection throughput is increased, and thus the efficiency of the system.

CONTRIBUTIONS OF THE MULTISYRINGE FLOW INJECTION TECHNIQUE AS A TOOL FOR GREENING SPECTROSCOPIC ANALYTICAL METHODOLOGIES

Simple Multicommutated Procedures with Spectroscopic Detection

Several analytical methodologies have been implemented in the MSFIA technique through the use of multicommutated procedures, minimizing by this way the consumption of reagents. These methodologies are based in the precise injection of the minimum amounts of the required reagents to carry out a concrete determination. Some examples that can be found in the recent literature are: (a) the spectrophotometric determination of S^{2-} in waters by the methylene blue method, diminishing the consumption of N,N-dimethyl-p-phenylenediamine (DMPD), $NH_4Fe(SO_4)_2$ and HCl[14]; (b) the time-based determination using fluorimetric detection of Al^{3+} in drinking waters using 8-hydroxyquinoline-5-sulphonic acid,[15] or the (c) multicommutated procedure for the spectrophotometric determination of Cl^- in waters[16] using Fe^{3+} and $Hg(SCN)_2$. In all the previous examples the consumption of reagents is diminished in comparison with FIA systems and in similar levels than in SIA systems but achieving higher injection throughputs than these last ones.

If we study more deeply the concrete case of the spectrophotometric determination of Cl^- in waters based on the $Cl^-/Hg(SCN)_2/Fe^{3+}$ reaction system where the reaction product $[Fe(NCS)(H_2O)_5]^{2+}$ is followed at 470–480 nm, we can appreciate that it is a very useful methodology (easy, cost-effective,

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sensitive, reproducible, and quick—overcoat if it is automated). By this reason, the automated version of this methodology is an acknowledged reference method.[17,18] However, this method presents a serious drawback, that is, the use of considerable amounts of $Hg(SCN)_2$, a highly toxic reagent. Since the first direct FIA system for this purpose[19] in the year 1976, the reagent consumption was improved following alternative strategies based on automation such as membrane reagent introduction in 1998,[20] the $Hg(SCN)_2$ immobilization in an epoxy resin in 2005,[21] or the multicommutated MSFIA procedure in 2008.[16] The consumption of Fe^{3+} and Hg(II) for the previously commented systems can be seen in Table 1. We can appreciate a progressive reduction in the consumption of Hg(II) with the concomitant increase of the automation degree. With the MSFIA method[16] the lowest reagent consumption levels are achieved. The manifold correspondent to this MSFIA system for the spectrophotometric determination of chloride in waters in schematically depicted in Fig. 2.

Another improvement is the combination of MSFIA technique with cold vapor atomic absorption spectrometry (CVAAS)[22] and cold vapor atomic fluorescence spectrometry (CVAFS),[23] both for the determination of mercury in water and fish samples (after manual mineralization of the solid sample). In this line must be added the proposed MSFIA systems with hydride generation atomic fluorescence spectrometry (HGAFS) for the determination of inorganic arsenic,[24] inorganic antimony,[25] and for the speciation of inorganic selenium[26] and inorganic arsenic.[27] On the one hand, as seen in Table 2, the implementation of these methodologies in the MSFIA technique provides a lower consumption of reagents than in FIA systems and a concomitant high efficiency as can be seen in the injection throughput values. On the other hand, the reagent consumption is similar or higher than in SIA systems, but still maintaining a higher efficiency in terms of injection throughput.

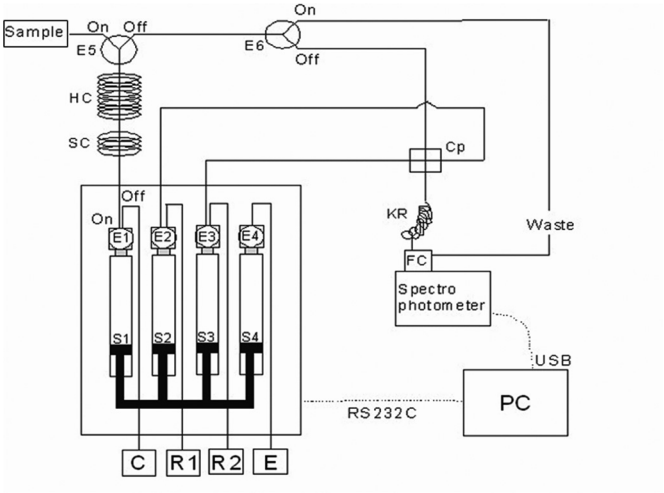

FIGURE 2 **Schematic representation of the MSFIA manifold for chloride determination. C: carrier (distilled water); R1: $Hg(SCN)_2$ reagent; R2: Fe^{3+} reagent; E: empty (not used); S1–S4: syringes; E1–E6: solenoid valves; On–Off: solenoid valves positions; SC: security coil; HC: holding coil; Cp: confluence point; KR: knotted reactor; FC: flow cell.**

Solid Phase Extraction

Another relevant alternative in the implementation of analytical methodologies in a cleaner way, is the use of solid-phase extraction (SPE) instead of the classic liquid–liquid extraction (LLE). An improved and greener way to perform SPE is based on its miniaturization and consequent exploitation in

TABLE 1 **Summarized Analytical Performance of Several Flow Systems for the Spectrophotometric Cl^- Determination Based on the $Cl^-/Hg(SCN)_2/Fe^{3+}$ Reaction System**

	Ref. 19	Ref. 20	Ref. 21	Ref. 16
Linear range ($mg\,L^{-1}$)	0–40	0.3–25	2–8	1–40
LOD ($mg\,L^{-1}$)	–	0.3	0.5	0.2
R.S.D. (%)	–	–	2.2	0.8
Injection throughput (h^{-1})	300	18	100	130
Type of water sample	River	River-pond	Natural	Mineral-tap-well
Hg(II) (μmg per determination)	680	600	120	45
Hg(II) consumption	–	1.1 fold lower	5.7 fold lower	15.1 fold lower
Fe(III) (μg per determination)	7210	11100	50000	335
Fe(III) consumption	–	1.5 fold higher	6.9 fold higher	21.5 fold lower

*Hg(II) and Fe(III) consumptions are referred to ref. [19]

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TABLE 2 Comparison of MSFIA Systems with AFS or AAS Detection with Analogous FIA and SIA Systems

Technique analyte	CVAAS mercury 22		HGAFS arsenic 24		HGAFS antimony 25		HGAFS selenium 26	
Reference	$SnCl_2$ (mg inj^{-1})	IT (h^{-1})	$NaBH_4$ (mg inj^{-1})	IT (h^{-1})	$NaBH_4$ (mg inj^{-1})	IT (h^{-1})	$NaBH_4$ (mg inj^{-1})	IT (h^{-1})
FIA	27.5	90	56	45	26–60	40–51	36	48
SIA	0.5	30	0.6	33	—	—	—	—
MSFIA	8	44	0.72	113	0.9	80	0.63	84

IT—Injection throughput.

automated FI systems, besides the direct sensing of the analytical signal in the same surface of the solid support.

The use of the MSFIA technique for the in-line SPE procedures for analyte isolation and preconcentration provides a reduction of the required amounts of solid sorbents and eluents. Some examples of SPE–MSFIA systems presented in the literature are: the preconcentration and fluorimetric determination of warfarin in waters using a packed column with a C_{18} resin[28]; the determination of arsenic in waters by HGAFS using a packed column with an anion exchange resin,[29] the smart analyzer for the determination and speciation of iron in waters using chelating disks,[30] the spectrophotometric determination of the total phenolic index based in the 4-aminoantipirine method using a packed column with Amberlite XAD-4 resin[31]—in which LLE with chloroform is substituted by an in-line SPE procedure; or the spectrophotometric determination of nitro-substituted phenol isomers based on the use of sorbent disks made from co-polymeric poly(styrenedivinylbenzene) modified with benzenesulphonic groups.[32]

Furthermore, if we are talking about efficiency in analytical methodologies, we have the requirement to talk about the MSFIA methods and its combination with multipumping flow systems (MPFS),[33] implemented for the sample pretreatment of radioactive species. The required pretreatment for matrix isolation is accomplished in a completely automated fashion, saving reagents and time per determination. Once the sample pretreatment has been completed, the target analytes are determined off-line with a low background proportional counter. In the recent literature appear two MSFIA systems for the pretreatment of the determination in waters of radioactive strontium[34] and radioactive yttrium.[35] Furthermore can be found two hybrid MSFIA—MPFS systems for the pretreatment of the determination of radium[36] in waters and for the separation and preconcentration of americium and plutonium in waters[37] using a transuranide (TRU) resin.

Finally, a relevant fact in the contribution of SPE–MSFIA procedures in the sample pretreatment prior to spectroscopic measurements is the accomplishment of reflectometric measurements based on optical fiber flow-through optical sensors (optrodes).[38] By this way is attained a considerable reduction in the consumption of reagents in comparison with reflectometric batch or classic FIA systems. Some examples of this are: the flow-through optical fiber sensor for sulphide determination based on the in-line generation of the methylene blue dye and its subsequent preconcentration in octadecyl-chemically modified (C_{18}) disks[39]; or the flow-through solid-phase reflectometric method for the simultaneous multiresidues determination of nitrophenol derivatives, which is based on the in-line anion exchange sorptive preconcentration of the target species followed by direct detection onto the sorbent material by means of chemometric deconvolution of the overlapped spectra.[40]

Reagent Immobilization

The versatility of the MSFIA technique can be exploited for the development of analytical methodologies using immobilized reagents. Reagents are packed in a flow-through mini-column allowing an increased efficiency of the procedure from the point of view of GAC.

Soto et al.[41] developed a MSFIA system with spectroscopic UV detection for the determination of hypochlorite in commercial products. This system is based on the selective decomposition of hypochlorite by means of an immobilized cobalt oxide

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catalyst. The resultant analytical methodology is almost an ideal Green Analytical Method.

Other MSFIA approaches based on reagent immobilization are: the inclusion of a flow-through solid-phase chemiluminescent sensor with immobilized 3-aminophtalhydrazide (luminol), for the chemiluminometric determination of orthophosphate in waters[42]; or the use of a glucose-oxidase packed-bed reactor for the conversion of the substrate followed by post-column chemiluminescent detection of the generated oxidizing species after reaction with luminol.[43]

MSFIA Hyphenation with Separation Techniques Prior to UV-vis Spectrophotometric Detection

Two of the most recent applications of MSFIA technique are based on its hyphenation with separation techniques. Concretely, two new hyphenated flow techniques have been recently developed, named multisyringe chromatography (MSC) and multisyringe flow injection analysis-capillary electrophoresis (MSFIA-CE).

The MSC technique was developed by González-San Miguel et al.,[44] reporting a low pressure completely automated alternative to liquid chromatographic separations, following an alternative quick and smart way. This technique is based on the use of short monolithic columns allowing the accomplishment of liquid chromatographic separations at lower pressures than in HPLC classic systems. With the miniaturization of the chromatographic system the amount of the required reagents and solvents is also minimized, allowing us to work in a more environmentally friendly way. The MSC technique has been applied in the determination of β-lactamic antibiotics in pharmaceuticals,[45] or in the determination of B_1, B_6, and B_{12} vitamins also in pharmaceuticals.[46] The MSC technique was also combined with in-line SPE for the determination of losartan potassium and hydrochlorothiazide in waters.[47] The general trend of the MSC systems, which is shown in Table 3 for the case of the determination of B_1, B_6, and B_{12} vitamins, is the reduction of reagent consumption in comparison to conventional HPLC systems and concomitantly achieving higher injection throughputs. So, the MSC technique can be defined as a low cost, quick, versatile, and greener alternative way to perform liquid chromatographic separations.

TABLE 3 **Comparison of the Analytical Features of the MSC System for the Determination of Vitamins B1, B6, and B12 in Pharmaceuticals with Those of Earlier HPLC Methods for the Same Purpose**

Parameter	MSC	HPLC[a]
Chromatographic separation time (min)	8	13–26
Flow rate (mL min^{-1})	0.5	0.8–1.5
Mobile phase consumption (mL analysis^{-1})	4.0	13.6–20.8
Sample throughput (h^{-1})	7	2–4
Stationary phase	C_{18}	C_{18}
Mean particle size (μm)	Monolith	3–10
Length $\times$ i.d. (mm)	25 $\times$ 4.6	150–300 $\times$ 3.9–4.6
Elution mode	Isocratic	Isocratic or Gradient
Pressure	Low	High

[a]Results obtained from references [50–55].

The MSFIA-CE technique was initially characterized as a SIA-CE[48] technique for the direct determination of mono-substituted nitrophenolic isomers. It was followed by the incorporation of two additional syringes, developing the first hyphenated MSFIA-CE[49] system. This novel system took profit of the increased versatility allowed by the two additional syringes, carrying out the efficient and miniaturized in-line SPE of mono-substituted nitrophenolic isomers, followed by their subsequent separation and spectrophotometric detection at a wavelength of 401 nm. The automation and miniaturization of the required pretreatments prior to the CE separation can be accomplished effectively in an in-line perspective by means of MSFIA technique, increasing the efficiency of the analytical method from the point of view of GAC.

CONCLUSIONS

In this article has been carried out a revision of the recent literature about the newest applications of the MSFIA technique. It has been focused to the minimization of the consumption of reagents and other different strategies inherent to MSFIA technique and their contribution to the Green Analytical Chemistry field.

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Finally, we would like to propose the use of the MSFIA technique combined with spectroscopic detectors as an efficient tool for the automation of sample pretreatment, becoming analytical methodologies in a cleaner way.

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3.8. Artículo original II

Spectrophotometric Determination of Chloride in Waters Using a Multisyringe Flow Injection System

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Spectrophotometric determination of chloride in waters using a multisyringe flow injection system

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Abstract

A multisyringe flow injection system (MSFIA) with spectrophotometric detection is proposed as a fast, robust and low-reagent consumption system for the determination of chloride (Cl^-) in waters. The system is based in the classic reaction of Cl^- with Fe^{3+} and $Hg(SCN)_2$, but due to the hazardous properties of this last reagent, the proposed methodology has been developed with the aim to minimize the consumption of this one, consuming less than 0.05 mg of Hg for a Cl^- determination, being the system of this type with the lowest Hg consumption. The linear working range was between 1 and 40 mg L^{-1} Cl^- and the detection limit was 0.2 mg L^{-1} Cl^-. The repeatability (RSD) was 0.8% for a 10 mg L^{-1} Cl^- solution, and the injection throughput was 130 h^{-1}. The proposed system is compared with other chloride monitoring flow systems, this comparison is realized with a point of view of the equilibrium between the obtained analytical features and produced residues toxicity. The proposed system was applied to the determination of Cl^- in mineral, tap and well water.
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Keywords: Multisyringe flow injection analysis (MSFIA); Chloride; Mercury-thiocyanate; Water samples

1. Introduction

Chloride (Cl^-) is one of the typical anions which its determination in waters has a great interest, overcoat in places with water salinity problems, being Cl^- one of the most widespread analysed anions in analytical laboratories. To obtain a maximum efficiency in a routinely analysis of Cl^- in a great number of water samples, a low-cost and automated analytical methodology is required. The principal route to obtain a simple and very fast, robust, sensitive, selective, completely automated and very high-autonomic system for Cl^- monitoring in waters is a flow injection system (FI) with spectrophotometric detection [1–6]. Usually these systems are based in the reaction between Cl^- and $Hg(SCN)_2$, being the liberated SCN^- quantity in previous reaction, proportional to the Cl^- concentration in the reaction medium. This free SCN^- reacts with Fe^{3+} [7], obtaining an intensely coloured coordination iron(III)-thiocyanate complex measured at 480 nm.

On the other hand, these advantages are eclipsed by the use of mercury in the form of $Hg(SCN)_2$, due to the high toxicity of this reagent, the proposed method does not seem to agree initially with the "Green Chemistry" analytical current trend. By this reason other spectrophotometric methods, in agreement with clean chemistry has been proposed [8–10], in these methods the measured property is usually the turbidity produced by the AgCl formation from the reaction between Cl^- and Ag^+. These turbidimetric methods have the advantage to produce less hazardous wastes than the $Hg(SCN)_2$-based methods for Cl^- monitoring, but also they have some disadvantages as the obtention of worse analytical features.

The Cl^-–$Hg(SCN)_2$–Fe^{3+} reaction system is also used actually in various commercial tests for Cl^- determination in waters (Merck, Macherey-Nagel), and for the sum parameter adsorbable organic halogens (AOX) determination in waters (Macherey-Nagel), prior preconcentration and digestion of the analyte.

The proposed method takes the advantages of working with MSFIA systems [11–14], in order to develop a direct colorimetric determination of Cl^- in waters, being this methodology similar to the used in first proposed FI systems [1,2], but with

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a reduction of the produced waste until better levels than in the most recently proposed flow systems [5,6]. Then we intend with this work, show that MSFIA technique is adapted easily in a routinely analysis procedure, obtaining very good features in all points of view.

2. Experimental

2.1. Reagents and solutions

All reagents were of analytical grade and solutions were prepared with distilled water. Fe^{3+} stock solution was prepared dissolving 20.2 g $Fe(NO_3)_3{\cdot}9H_2O$ (Riedel-de Haën) in 0.1 L of a 1 mol L^{-1} HNO_3 solution. $Hg(SCN)_2$ working solution was prepared dissolving in a steam bath 0.12 g of $Hg(SCN)_2$ (Fluka) in 0.1 L of distilled water. Cl^- stock solution (1000 mg L^{-1}) was prepared dissolving 1.649 g of previously dried NaCl (Scharlau) in distilled water and filling to a final volume of 1 L. Working solutions were prepared by suitable dilutions from the stock solutions.

2.2. Flow system, optical detector and software

The correspondent manifold to the proposed method is shown in Fig. 1. A multisyringe piston pump (Crison, Barcelona, Spain) is used as a liquid driver, this system is characterized for having four syringes (S1–S4) which are moved by a single motor. In this work only three syringes (Hamilton, Switzerland) were used, S1 = 10 mL, S2 = 5 mL and S3 = 5 mL. Each syringe has a three-way solenoid valve (N-Research, Caldwell, NJ) at the head (E1–E4), these valves allow us the application of multicommutation schemes. The connection between the multisyringe module and the PC is a RS232C interface. Also, in our system, two additional solenoid valves (MTV-3-N 1/4 UKG, Takasago, Japan) were used (E5–E6).

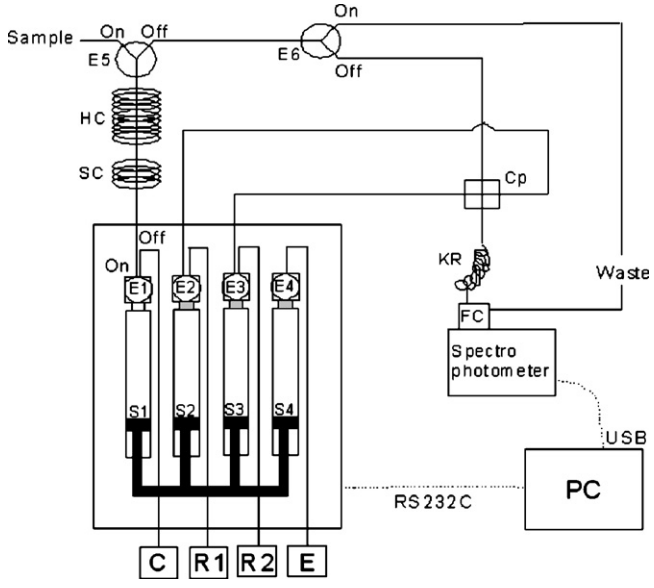

Fig. 1. Schematic representation of the MSFIA manifold for chloride determination. C: carrier (distilled water); R1: $Hg(SCN)_2$ reagent; R2: Fe^{3+} reagent; E: empty (not used); S1–S4: syringes; E1–E6: solenoid valves; On–Off: solenoid valves positions; SC: security coil; HC: holding coil; Cp: confluence point; KR: knotted reactor; FC: flow cell.

The E5 solenoid valve, let us the loading of the sample into the holding coil (HC), also the system has a security coil (SC) to prevent the introduction of the sample into the S1. The E6 solenoid valve has the function to prevent the carryover between two consecutive samples, and allow us to deposit the dead volume of the previous sample directly to the waste reservoir. All coils and connections were made from polytetrafluoroethylene (PTFE) 0.8 mm i.d. tubing.

The reaction coil (KR), was a knotted reactor made also from PTFE 0.8 mm i.d. With the use of these type reactors, we obtained a better mix between sample and reagents, and less dispersion than with a conventional reaction coil. The four-way confluence point (Cp) was made from polymethylmethacrylate (PMMA).

The detection system was composed by a light source (Sciware, Palma de Mallorca, Spain) with a wolfram lamp, a 18 μL inner volume and 1 cm optical path flow cell (FC) (Hellma, Müllheim, Germany), and a USB 2000 miniature spectrophotometer (Ocean Optics, Dunedin, FL, USA). Dual-wavelength spectrophotometry (480 and 720 nm) was used to minimize Schlieren effect.

Instrumental control and data acquisition were performed through the Autoanalysis 5.0 software package, developed by our research group, it allow us the automated implementation of MSFIA analytical methodologies. Autoanalysis 5.0 has a single protocol, and depending which are the instruments needed for a determined experiment, the correspondent dynamic link libraries (DLL's) are installed [15]. In the proposed experiment we need the suitable DLL's for the multisyringe module (it includes the four syringes and the two additional solenoid valves) and the spectrophotometer.

2.3. MSFIA procedure for chloride monitoring

The principal steps on the MSFIA used protocol are the following ones:

1. The syringes are filled everyone with their correspondent solution; all valves E1–E6 are in Off position. *Note*: the depicted flow rates and volumes are referred to S1, being the flow rates and volumes of S2–S3 the half than the S1 ones.
2. A volume of 3.5 mL is dispensed at 15 mL min^{-1} with all valves in Off position.
3. Sample loading. A volume of 3.5 mL of sample is filled up at 8 mL min^{-1} with the valves E1 = E5 = On, with other valves in Off position.
4. Injection protocol. Multicommuted reaction procedure, which is depicted in Table 1. This procedure consists on the accomplishment of three injections of the sample from the volume loaded in the previous step and posterior cleaning of the possible sample excess. In all steps, the volumes depicted are dispensed into the system almost continuously, with little intervals of 50 ms, the enough time for valves commutation.

Table 1
Multicommuted dispensing protocol for sample reaction

Step	Valves			Injected volume (mL)		Flow rate (mL min^{-1})	
	E1	E2	E3	S1	Total	S1	Total
1	On	Off	Off	0.90	0.90	6	6
2	On	On	On	0.12	0.24	3	6
3	On	Off	Off	0.90	0.90	6	6
4	On	On	On	0.12	0.24	3	6
5	On	Off	Off	0.90	0.90	6	6
6	On	On	On	0.12	0.24	3	6
7	On	Off	Off	2.50	2.50	6	6

Valves E5 and E6 are in Off position in all steps, valve E4 is not used.

The total flow rate remains constant in all the sub-steps. Then we can consider this step as a unique emptying movement of the syringes, but depending on the time, sample and reagents are mixed obtaining a signal (E1 = E2 = E3 = On), or only is dispensed the sample (E1 = On, E2 = E3 = Off), cleaning the FC.

5. The syringes are filled again with their correspondent solutions at 15 mL min^{-1}, with all valves in Off position, remaining prepared for next injection.

3. Results and discussion

3.1. Investigation of chemical variables

The concentrations of $Hg(SCN)_2$ and Fe^{3+} reagents were studied (Fig. 2), to obtain good conditions for the coloured reaction product development.

Fe^{3+} concentration was studied in a 4×10^{-3} to 5×10^{-1} mol L^{-1} range. The selected concentration was 10^{-1} mol L^{-1}.

$Hg(SCN)_2$ concentration was studied in a 8×10^{-3} to 2×10^{-5} mol L^{-1} range. The selected concentration was 3×10^{-3} mol L^{-1}.

Lower reagent concentrations than the selected ones, causes a signal decrease. Higher reagent concentration does not cause a significant net absorbance increase, due to the blank signal increasing.

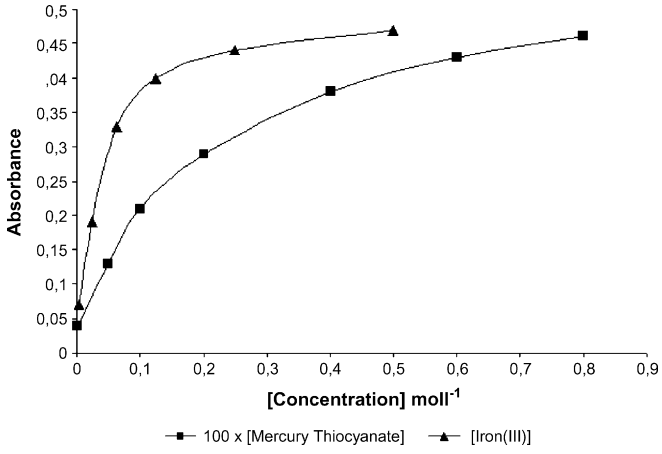

Fig. 2. Influence of reagent concentrations on the absorbance (analytical signal peak height–blank peak height), obtained analyzing a 30 mg L^{-1} Cl^- solution.

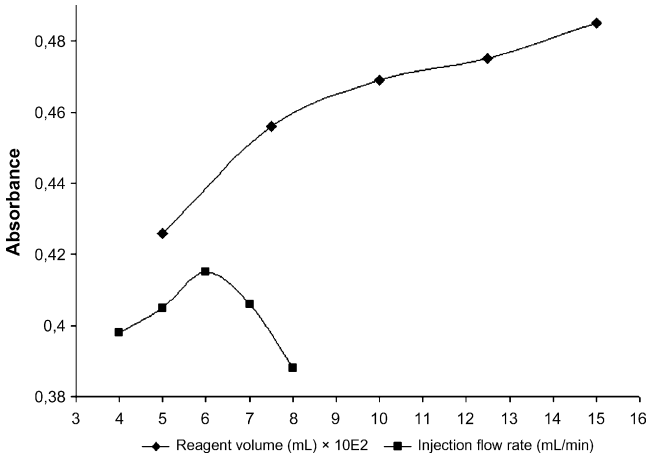

Fig. 3. Influence of injected reagent volumes and reaction plug injection flow rate on the absorbance (analytical signal peak height–blank peak height), obtained analyzing a 30 mg L^{-1} Cl^- solution.

3.2. Investigation of MSFIA parameters

In this work, the investigated parameters were injection flow rate; multicommutation procedure injected reagent volumes, sample volume and knotted reaction coil length.

As is shown in Fig. 3, reaction plug injection flow rate was studied between 4 and 8 mL min^{-1}, being the best signal height obtained with a 6 mL min^{-1} flow rate.

In the multicommutation injection step, the injected volumes of Fe^{3+} and $Hg(SCN)_2$ reagents were studied in the range between 0.05 and 0.15 mL, obtaining the best relation between net absorbance peak height and the lowest amount of spent $Hg(SCN)_2$, with the injection of 0.06 mL of each reagent solution. Increasing these reagent volumes the net absorbance does not increase significantly (Fig. 3).

Also was studied sample volume needed between two consecutive injections to obtain two completely separated signal peaks, this volume was studied in a range between 0.5 and 1.5 mL, and the selected volume was 0.9 mL.

The total sample volume loaded in the holding coil for the consecutive three injections were studied in a range between 3 and 4 mL, the selected volume was 3.5 mL. Lower volumes produce a height decrease of the third analytical peak.

The knotted reaction coil length was studied in a range between 20 and 150 cm. A 40 cm knotted reaction coil is enough to obtain the maximum analytical peak height. Reactors of less than 40 cm did not allow a good sample–reagents mixing, and longer reactors caused an increase of the reaction plug dispersion.

3.3. Interference studies

In order to investigate the selectivity of the proposed method, the effect produced by diverse anions usually presented in water samples, were studied. The tested anions were: HCO_3^-, CO_3^{2-}, NO_2^-, NO_3^-, PO_4^{3-} and SO_4^{2-}.

The effect of each interferences was examined by measuring the net absorbance obtained from 10 mg L^{-1} Cl^- solutions

Table 2
Analytical performance of the MSFIA proposed system for chloride monitoring, and the FI method with immobilized $Hg(SCN)_2$ [6]

Analytical parameter	MSFIA	FIA [6]
Detection limit ($mg\,L^{-1}$)	0.2	0.5
Linear dynamic range ($mg\,L^{-1}$)	1–40	2–8
Repeatability (%) (10 $mg\,L^{-1}$) ($n = 10$)	0.8	2.2
Injection frequency (h^{-1})	130	100

containing one of the previously stated potential interferences. Later, the previous study was repeated, but increasing 10 times the added amount of the potential interfering anions. An error in the analytical signal lower than a ±5% was obtained in all cases.

3.4. Analytical features of the proposed system

The analytical figures of merit of the proposed MSFIA system are reported in Table 2. Furthermore the proposed system is also compared with another low-reagent consumption spectrophotometric FI system for Cl^- monitoring, which is also based in the Cl^-–$Hg(SCN)_2$–Fe^{3+} reaction system [6], obtaining a moderate improvement of these figures using the MSFIA system.

The obtained linear working range was similar to the obtained with other flow systems based in the same reaction [1–6], furthermore $0.0132 \pm 0.0005\,L\,mg^{-1}$ sensitivity and a 0.9973 ± 0.0009 regression coefficient were obtained. The detection limit [16] was calculated as three times the standard deviation of the absorbance for 10 injections of the blank (distilled water), following the MSFIA procedure for Cl^- monitoring proposed in point 2.3. The peak height repeatability is reported as the RSD (%) from 10 consecutive injections of a 10 $mg\,L^{-1}$ Cl^- standard solution.

3.5. Comparison of the proposed system with other chloride monitoring systems

Nowadays, several FI systems for Cl^- determination had been proposed.

On one hand, various spectrophotometric Cl^- monitoring flow systems based in colorimetric reactions had been reported. These methods are based in the Cl^-–$Hg(SCN)_2$–Fe^{3+} reaction system, being very simple, direct, fast and robust methods [1–6], but with the disadvantage of a waste generation which contains high amounts of Hg. A comparison between these methods was made in [6], reporting in it the $Hg(SCN)_2$ consumption of each system.

Actually the lowest $Hg(SCN)_2$ consumption in a FI system for Cl^- monitoring is 0.12 mg of Hg(II) for a Cl^- determination [6], and it is due to the immobilization of the reagent in a solid phase reactor (SPR), being the autonomy of this system limited to 1000 Cl^- determinations. The proposed MSFIA system only requires 0.045 mg of Hg(II) per Cl^- determination, and does not require the use of a SPR, obtaining with only 1 g of $Hg(SCN)_2$ the realization of approximately 15,000 Cl^- determinations. Then we can affirm that the proposed system has a higher autonomy level, combined with the lowest reagent consumption.

On the other hand, can not be discussed that the spectrophotometric FI systems focused to the Cl^- determination, which are based in the turbidimetry produced by AgCl [8–10], are cleaner systems than the $Hg(SCN)_2$-based ones. Zenki and Iwadou [8] proposed a closed-loop FIA system with repetitive Cl^- determination, while Mesquita et al. [9] proposed a SIA system-based in the same reaction, which are simple, direct and environmentally friendly systems. In counterpoint, these systems have worse analytical features (i.e. higher detection limits) than the previously commented colorimetric systems.

These analytical features has been improved recently by Bonifácio et al. [10], exploiting the immobilization of silver chloranilate in a SPR, in this SPR the Cl^- ions are retained producing AgCl, and releasing free chloranilate ions which are measured spectrophotometrically at 530 nm, obtaining good analytical features due to the use of a 100 cm pathlength spectrophotometer, being the robustness of the system determined by the limited duration of this SPR.

Nowadays exist another MSFIA system for Cl^- monitoring, but with potentiometric detection [17], we agree that it works with a more environmentally friendly methodology, but the proposed spectrophotometric MSFIA system has other better analytical features, as a higher sample throughput or a lower detection limit. Then depending of a concrete application, potentiometric or spectrophotometric MSFIA systems will be the most adapted system in each case, or also would be feasible the development of a potentiometric/spectrophotometric multidetection FI system as the recent FIA system developed by Nascimento Moreira et al. [18].

Several commercialised tests for to the routine analysis of Cl^- in water samples, are based in the same reaction than the proposed methodology, but due to the automation of the MSFIA technique, we obtain apart of a higher sample throughput, a more than 100-fold decrease in used $Hg(SCN)_2$ amount [19].

3.6. Analytical application

Once adopted the final system configuration, it was applied to the determination of Cl^- in real samples. Mineral water, tap water and well water samples were analyzed with the proposed MSFIA method, and compared with the official APHA–AWWA–WPFC method [20]. The samples were previously diluted if it was necessary. The obtained results were

Table 3
Determination of chloride in environmental samples using the MSFIA proposed method, and the official APHA–AWWA–WPFC [20] method, three repetitions per sample were realized

Sample	Chloride ($mg\,L^{-1}$)		Relative error[a] (%)
	MSFIA method	Official method	
Mineral water	41.6 ± 0.3	41.1 ± 0.3	1
Tap water	51.1 ± 0.1	49.5 ± 0.2	3
Well water	102.4 ± 0.1	99.4 ± 0.2	3

[a] Relative error between MSFIA method and official method value.

compared applying *t*-test for paired data, obtaining no significant differences at a 95% confidence level. The results are presented in Table 3.

4. Conclusions

In the present work, a direct MSFIA system with spectrophotometric detection for Cl^- monitoring has been proposed. The developed system has a high robustness and the absence of elements with a determined limited duration, as solid phase reactors, unstable reagents, or the previously manual preparation of complex reagents. Then, the obtained system has a high autonomy degree and the absence of fragile parts, and in consequence of this, the system can run for a very long time with only a small quantity of reagents.

The analytical performance of the proposed methodology has not significant differences when is compared to other similar proposed systems [1–6], but with the advantage to work with a very autonomous system, with the lowest reagent consumption and a very high sample throughput.

In conclusion, we encourage the use of multisyringe flow injection technique not only as a useful tool in the development of new analytical methodologies, but also in the automation of standard methods of analysis, which are used generally following a manual methodology. Obtaining with this change a long list of advantages, with the unique requirement of basic laboratory automation knowledge acquisition on the part of the analyst.

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3.9. Artículo original III

Multisyringe Flow Injection Analysis Hyphenated With Liquid Core Waveguides for the Development of Cleaner Spectroscopic Analytical Methods: Improved Determination of Chloride in Waters

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TECHNICAL NOTE

Multisyringe flow injection analysis hyphenated with liquid core waveguides for the development of cleaner spectroscopic analytical methods: improved determination of chloride in waters

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Abstract In this work, the hyphenation of the multisyringe flow injection analysis technique with a 100-cm-long pathlength liquid core waveguide has been accomplished. The $Cl^-/Hg(SCN)_2/Fe^{3+}$ reaction system for the spectrophotometric determination of chloride (Cl^-) in waters was used as chemical model. As a result, this classic analytical methodology has been improved, minimizing dramatically the consumption of reagents, in particular, that of the highly biotoxic chemical $Hg(SCN)_2$. The proposed method features a linear dynamic range composed of two steps between (1) 0.2–2 and (2) 2–8 mg Cl^- L^{-1}, thus extended applicability due to on-line sample dilution (up to 400 mg Cl^- L^{-1}). It also presents improved limits of detection and quantification of 0.06 and 0.20 mg Cl^- L^{-1}, respectively. The coefficient of variation and the injection throughput were 1.3% (n=10, 2 mg Cl^- L^{-1}) and 21 h^{-1}. Furthermore, a very low consumption of reagents per Cl^- determination of 0.2 μg Hg(II) and 28 μg Fe^{3+} has been achieved. The method was successfully applied to the determination of Cl^- in different types of water samples. Finally, the proposed system is critically compared from a green analytical chemistry point of view against other flow systems for the same purpose.

Keywords Green analytical chemistry · Multisyringe flow injection analysis · Liquid core waveguide · Long pathlength spectrophotometry · Chloride determination · Mercury thiocyanate

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Introduction

A wide variety of analytical methodologies are used in the present-day society, getting information about environmentally relevant target compounds or parameters. To carry out a concrete analytical methodology often requires the use of chemicals, and a concomitant environmental impact is produced, even higher than that of the target compound. By this reason among others, the Green Chemistry Movement [1] promotes the search of alternative ways to minimize the potential risks of chemicals, both for humans and the environment. In this regard it should be pointed out that analytical chemistry is a relatively underexamined area from the point of view of green chemistry [2, 3], but a high number analytical methodologies are massively used generating big amounts of highly toxic chemical wastes.

A useful alternative for the development of analytical methodologies in a more environmentally benign way is their automation through the use of flow injection (FI) techniques [4]; among them, multisyringe flow injection analysis (MSFIA) [5, 6] demonstrates good capabilities for contribution to the green analytical chemistry (GAC) field. With the automation based on FI techniques, the human exposure to chemicals and the required amounts of reagents/solvents are both diminished.

An acknowledged FI method for the assessment of water quality is the FIA method for the spectrophotometric (SPM) determination of Cl^- in waters, which is based on the $Cl^-/Hg(SCN)_2/Fe^{3+}$ reaction system [7, 8]. Furthermore, this methodology is also satisfactorily used as front-end detection system for the quantification of halogenated organic compounds once an in-line preconcentration/isolation and oxidation of the target com-

pounds has been carried out [9]. The $Cl^-/Hg(SCN)_2/Fe^{3+}$ reaction system is based on the reaction produced between Cl^- and $Hg(SCN)_2$ releasing free SCN^- ions, which concomitantly react with an excess of Fe^{3+} developing an intensely colored blood-red $[Fe(SCN)(H_2O)_5]^{2+}$ aqua complex [10], which is followed spectrophotometrically at 470–480 nm. The main drawback of this method is the generation of hazardous waste products containing high loads of Hg(II).

Recent approaches toward a cleaner way to implement this method have been proposed; these are based on the immobilization of $Hg(SCN)_2$ using a FIA system [11] or developing a multicommutated procedure implemented on a MSFIA system [12].

Liquid core waveguides (LCWs) are a powerful weapon for the enhancement of the analytical features in methodologies with SPM detection [13]. LCWs are based on the use of a capillary with a lower refractive index (n_D=1.31) than the liquid core contained in it, so the light introduced into the liquid core of the capillary is totally internally reflected down the capillary towards the detector. If we focus our attention to the FI methods with SPM detection in the visible range, we can notice that the potential of LCWs has been mainly applied on the improvement of the sensitivity of analytical methods. A few examples of these are the determination of (1) nitrite and nitrate in natural waters [14], (2) phenols in natural and wastewaters [15], (3) iron(II) using a 4.5-m liquid core waveguide [16], or (4) phosphate in natural waters [17]. In counterpoint of this, in chemical reactions with high blank signals, the implementation of a 100 cm LCW as flow cell is not feasible in the same conditions as using a flow cell of 1 cm optical pathlength. It is caused by the high strong absorbances of the blank signals (~100 times higher). But if our aims are focused to the GAC point of view [3], in methodologies where hazardous chemicals are used (e.g. $Hg(SCN)_2$-Fe^{3+} method for Cl^- determination), the potential of LCWs for SPM detection opens new ways in the improvement of analytical methods, reducing drastically the required amounts of reagents.

The aim of this work is the accomplishment of a cleaner way to perform SPM analytical determinations. This is achieved by the use of a long pathlength capillary flow cell (liquid core waveguide) hyphenated with the simultaneous and precise microfluidic handling provided by the MSFIA technique. As chemical model, the SPM determination of Cl^- in waters using $Hg(SCN)_2$ and Fe^{3+} was chosen, with the chemical and hydrodynamic parameters selected in order to attempt a low consumption of reagents (focused overcoat to the reduction of $Hg(SCN)_2$ consumption).

Experimental

Reagents and solutions

All reagents were of analytical grade, and solutions were prepared with distilled water. Distilled water was used as carrier. A 0.63-mmol $Hg(SCN)_2$ L^{-1} stock solution was prepared by dissolving in a gentle steam bath 0.050 g $Hg(SCN)_2$ (Fluka) in 0.25 L of distilled water. A 0.5-mol Fe^{3+} L^{-1} stock solution was prepared dissolving 20.2 g $Fe(NO_3)_3 \cdot 9H_2O$ (Riedel-de Haën) in 0.1 L of a 1-mol HNO_3 L^{-1} solution. A 1,000-mg Cl^- L^{-1} stock solution was prepared by dissolving 1.649 g of previously dried NaCl (Scharlau) in distilled water and filling up to a final volume of 1 L. Working solutions were prepared by suitable dilutions from the stock solutions.

Multisyringe flow injection setup and software

As can be seen in Fig. 1, the proposed MSFIA setup is based on the automated management of fluids through the use of a multisyringe burette (MS) from Crison (Barcelona,

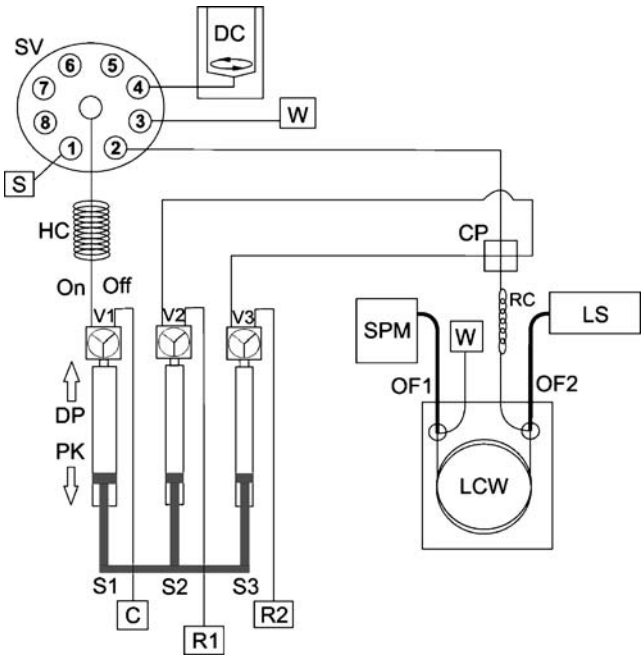

Fig. 1 Schematic depiction of the proposed MSFIA-LCW for the spectrophotometric Cl^- determination; *S1–S3* syringes; *C* carrier reservoir; *R1* $Hg(SCN)_2$ solution reservoir; *R2* Fe^{3+} solution reservoir; *PK* pickup; *DP* dispense; *V1–V3* solenoid valves; *On* solenoid valve position used for the connection of syringes to the flow network; *Off* solenoid valve position for the connection of syringes with reservoirs; *HC* holding coil; *SV* selection valve; *S* sample reservoir; *W*, waste; *DC* dilution chamber; *CP* confluence point; *RC* knotted reaction coil; *LS* light source; *SPM* spectrophotometer; *OF1–OF2* optical fibers; *LCW* liquid core waveguide

Spain). The MS module allows working simultaneously with four syringes (Hamilton, Bonaduz, Switzerland). Each syringe is fixed between a common metallic bar and its own three-way solenoid valve (N-Research, West Caldwell, NJ, USA). In this work were used three syringes (S1–S3). S1 (5 mL) is used for carrier. S2 (1 mL) contains the Hg $(SCN)_2$ reagent. S3 (1 mL) contains the Fe^{3+} reagent. Depending on the position of the solenoid valves, fluids contained in syringes are loaded or dispensed towards the flow network (On), or towards their corresponding reservoir (Off).

An eight-port selection valve (SV) is used for sample introduction into the flow network. Furthermore, the SV was used for the on-line sample dilution and as bypass to the waste reservoir. The flow network was made from polytetrafluoroethylene tubing (0.8 mm i.d.), including a 6-m holding coil (HC) and a 0.8-m knotted reaction coil (RC). Also used was a four-way confluence point made from polymethylmethacrylate (PMMA). For on-line sample dilution, a lab-made PMMA open air dilution chamber (2.5 mL internal volume) was used. This chamber contains a small magnetic stirring bar, achieving by this way a homogeneous sample dilution.

The detection system is composed of a deuterium–halogen light source (Mikropack, Germany), two optical fibers 400 μm in diameter (Ocean Optics, USA), a flow cell made from a 100-cm Type II Teflon AF liquid core waveguide (World Precision Instruments, Florida, USA; internal diameter 550 μm, effective pathlength 100.0± 0.5 cm, internal volume 240 μl), and a USB2000 miniaturized fiber-optic spectrometer (Ocean Optics), connected to a computer via a USB interface. Dual-wavelength spectrophotometry (470 and 700 nm) was used to compensate possible errors caused by changes of the refractive index.

The instrumental control and data acquisition were performed by the AutoAnalysis 5.0. software package commercialized by Sciware (Palma de Mallorca, Spain).

Analytical procedure for the determination of chloride in waters using the proposed MSFIA-LCW system

The basic steps of the proposed procedure are the following:

- A sample volume is loaded into the HC; this volume will be selected in relation to the dilution factor that the analyst wants to achieve. Immediately after, it is dispensed towards the dilution chamber, being diluted with carrier up to a final maximum volume of 2.5 mL (Vsample+ Vcarrier=2.5 mL). For calibration or diluted samples, a sample/standard volume of 0.6 mL is directly loaded into HC, and on-line dilution is not used.
- One aliquot of 0.6 mL of the diluted sample is loaded again into the HC. A volume of 0.5 mL of this aliquot is injected towards the reaction coil (V1 = On, V2 = Off, V3 = Off). At this moment, when the sample/aliquot is flowing through the confluence point, the flow is stopped, and volumes of 50 μl of each reagent are injected (V1 = Off, V2 = On, V3 = On) producing a sample–mixed reagents–sample segment, this being injected through the RC and towards the LCW (V1 = On, V2 = Off, V3 = Off), developing the reaction product by means of dispersion of the reagents plug into the sample stream.
- The spectrophotometer starts data acquisition, and the signal is registered. Immediately after, from the rest of the sample contained in the dilution chamber, two additional repetitions of the determination can be achieved. Finally, the dilution chamber is rinsed, and the system is prepared for the determination of Cl^- in the next sample.

Results and discussion

Selection of chemical and hydrodynamic parameters

A selection of the major chemical and hydrodynamic parameters of the proposed system was accomplished. This selection was focused to the minimization of the consumption of reagents without sacrificing other relevant analytical features of the system.

A preliminary assessment for the selection of the useful ranges of reagent concentrations was accomplished. These were found between 1–30 μmol $Hg(SCN)_2$ L^{-1} and 5–30 mmol Fe^{3+} L^{-1}. Narrower ranges obtained for reagent concentrations were re-studied in a more accurate way selecting the lower concentrations of $Hg(SCN)_2$ and Fe^{3+}, which provide the higher increase of the analytical signal in relation to the blank signal. As can be seen in Fig. 2, the selected concentrations were 20 μmol $Hg(SCN)_2$ L^{-1} and 10 mmol Fe^{3+} L^{-1} (for a Cl^- concentration of 10 mg L^{-1}).

The sample/aliquot volume was studied between 0.4 and 0.8 mL selecting a volume of 0.6 mL for proper experiments. When a volume of 0.5 mL of the previous sample/aliquot is injected towards the detector, the concentration of the sample will be the maximum in the confluence point, and subsequently the signal will be the maximum (this was studied with volumes in the range of 0.2–0.8 mL), in that moment, the sample stream is stopped, and the reagents are injected in it.

The volumes of each reagent were studied between 10 and 100 μl, selecting as can be seen in Fig. 2 a volume of

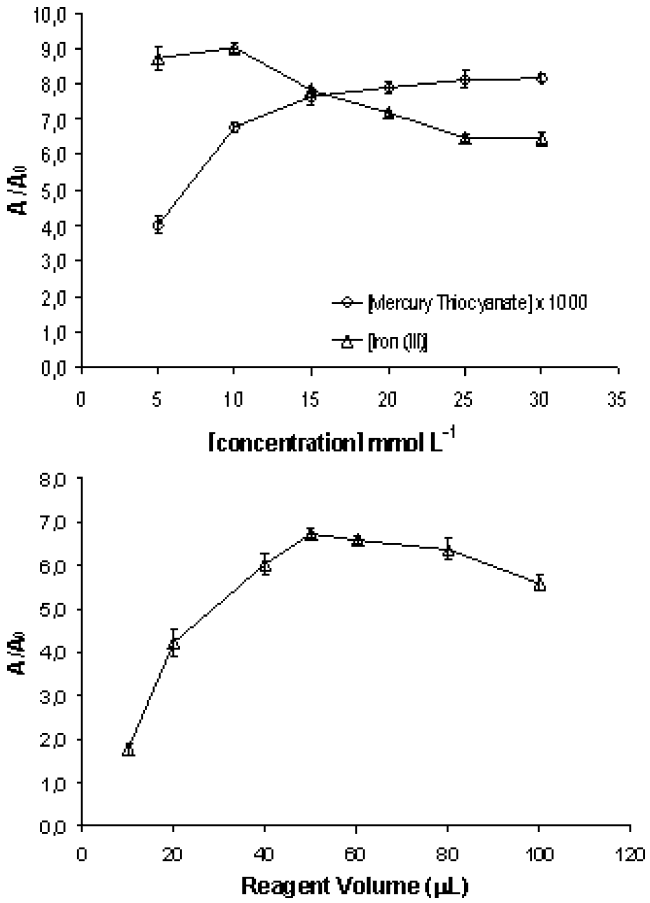

Fig. 2 Influence of the concentration and the volume of reagents (each reagent Fe^{3+} and $Hg(SCN)_2$ are always injected in the same relation of volume) on the relative absorbance intensity (Absorbance of the analytical signal (*A*)/Absorbance of the blank signal (A0))

50 μl $Hg(SCN)_2$ and 50 μl Fe^{3+} per Cl^- determination. The reagent plug inserted into the sample stream has a total volume of 100 μl. The reaction product is developed by dispersion of the small plug of reagents into the sample stream during its injection through the knotted reaction coil.

In order to ensure a suitable reaction product development thus avoiding its excessive dispersion, the length of the knotted reaction coil was studied in the range of 40–120 cm. A knotted reaction coil of 80 cm was selected. The injection flow rate of the reaction product into the LCW was studied in the range between 1 and 4 mL min^{-1}, selecting a flow rate of 2.5 mL min^{-1}.

On-line dilution is an essential feature in this experimental setup, in order to attain a more extended applicability. The maximum capacity of the dilution chamber is 2.5 mL, and the satisfactorily used lower sample volume is 50 μl, resulting a maximum dilution ratio of 1:50 (sample/carrier). The use of lower sample volumes than 50 μl causes differences higher than 5% between on-line diluted and non-diluted (directly injected) standards, for the same final concentrations. By this way, on-line dilution provides us some advantages such as (1) a higher automation degree, (2) a versatile and larger linear dynamic range, and (3) the minimization of potential interferences from the matrix of the sample.

Interference study

It is a well-known fact that the displacement of SCN^- ions from the $Hg(SCN)_2$ is not selective to the presence of chloride. Bromide and iodide also produce this SCN^- displacement, causing a signal peak height increase. This lack of method selectivity is relevant in wastewaters, but it is negligible in nonpolluted waters due to the low concentrations of Br^- and I^- in relation to their larger Cl^- concentration.

A wide variety of ionic compounds are commonly found at high concentrations in waters; some of these are SO_4^{2-}, NO_3^-, PO_4^{3-}, or CO_3^{2-}. According to the literature [11, 12] (using a conventional 1 cm pathlength flow cell) the presence of these anions at their usual concentrations in waters do not produce interferences bigger than 5% in the measurements accomplished using the $Cl^-/Hg(SCN)_2/Fe^{3+}$ reaction system.

However, if equivalent measurements are carried out using a 100-cm long pathlength LCW, the presence of the major saline components of waters can potentially interfere in the Cl^- determination by means of refractive index changes. By the last reason, several 10 mg Cl^- L^{-1} standards containing equal amounts (10 mg L^{-1} of the potential interfering compound) were analyzed (applying a 50-fold dilution factor). No interference bigger than 5% was observed for any of the tested anions, even in the case that the concentration of the anion was increased up to 100-fold the Cl^- concentration (10 mg Cl^- L^{-1} + 1,000 mg L^{-1} of the potential interfering compound). So we can conclude that the proposed method will be useful for the Cl^- determination in water samples.

Analytical performance of the proposed system

The figures of merit corresponding to the proposed system are summarized in Table 1. As can be seen in Fig. 3, the obtained linear working range is composed of two linear segments with different sensitivities in function of the Cl^- concentration. The limit of detection (LOD) and the limit of quantification were theoretically calculated as three and ten times the standard deviation of ten injections of the blank (distilled water), following the analytical procedure for Cl^- determination previously described. The injection throughput is the average time for the accomplishment of a Cl^- determination. The linear dynamic range between 2 and 8 mg L^{-1} Cl^- was chosen for later analytical applications, as a useful range for the Cl^- determination in environmental

Table 1 Analytical performance of the proposed system

Analytical parameter	Value
Linear dynamic range (mg L^{-1})	(I) 0.2–2 (II) 2–8
Limit of detection (mg L^{-1})	0.060
Limit of quantification (mg L^{-1})	0.200
Sample throughput[a] (h^{-1})	21
For linear range (II)	
Repeatability (%) (n=10; 2 mg L^{-1} Cl^-)	1.3
Sensitivity (L mg^{-1}) (n=6)	0.0192±0.0005
Regression coefficient (n=6)	0.9963±0.0015

[a] Including on-line dilution of the sample

samples. For this linear dynamic range, the repeatability of the height of the obtained absorbance peaks was calculated as the relative standard deviation from ten consecutive injections of a 2-mg L^{-1} Cl^- standard solution. The sensitivity and the regression coefficient were both calculated from six day-to-day regression curves.

Analytical application

Once the operational parameters were selected, the interfering species were tested, and the figures of merit were calculated; the proposed system was applied to the Cl^- determination in different types of water samples (mineral 1, mineral 2, tap, and well). The two mineral water samples came from two different brands of commercial bottled mineral water. Tap water sample was collected from the University of the Balearic Islands (Balearic Islands, Spain). Well water sample was collected from a well in the vicinity of an urban solid waste treatment plant (Balearic Islands, Spain). All samples were analyzed immediately after their collection.

In order to assess the reliability of the obtained results, the samples were also analyzed by the modified Volhard Titration method [18]. The results provided by both methods are shown in Table 2, and they were compared via a t test which revealed the absence of differences at a 0.05 significance level. After the comparison between both methods, the samples were spiked with a Cl^- standard. The recoveries obtained with the proposed system were between 99% and 107% in all instances.

Comparison of the proposed MSFIA-LCW system with other flow systems for Cl^- determination based on the $Cl^-/Hg(SCN)_2/Fe^{3+}$ reaction

The $Cl^-/Hg(SCN)_2/Fe^{3+}$ reaction system for the determination of Cl^- in waters was implemented for the first time in a FI system about 30 years ago [19]. On the one hand, it is still an acknowledged automated methodology [7, 8]. On the other hand, this method presents a drawback based on the generation of waste products containing a high load in Hg(II) (680 μg of Hg(II) per Cl^- determination [19]). Improved alternatives to this pioneer FI system were developed in later years [20–22], reporting similar levels of Hg(II) consumption.

Nowadays, several FI systems have appeared for this purpose, with the aim of the reduction of Hg(II) consumption, turning this methodology into a more environmentally friendly one. The required amount of Hg(II) per Cl^- determination was reduced from 680 to 120 μg [11] and 45 μg [12].

The aim of the present work is the implementation of a MSFIA-LCW system in order to get a drastic reduction in the reagent consumption. Successful results were obtained being only required 0.2 μg (1 nmol) of Hg(II) per a Cl^- determination (more than 3,000 Cl^- determinations can be performed with only 1 mg of $Hg(SCN)_2$). The total concentration of mercuric compounds in the produced waste is not higher than 50 μg Hg(II) L^{-1}. Besides, the reduction of Fe^{3+} consumption is also a remarkable fact, being only required 28 μg Fe^{3+} per Cl^- determination. This reduction of reagent consumption does not come joined with any disadvantage besides the moderate improvement of other analytical features as for example the LOD.

The low solubility of $Hg(SCN)_2$ in water is another drawback of the Cl^- determinations based on the $Cl^-/Hg(SCN)_2/Fe^{3+}$ reaction system. The classic solution to this problem is the dissolution of the $Hg(SCN)_2$ in organic solvents (e.g., methanol). The use of the MSFIA-LCW system allows us to work with less concentrated reagent solutions, avoiding $Hg(SCN)_2$ precipitation or the use of organic solvents.

The proposed MSFIA-LCW system is compared in Table 3 with the other FI systems for the same purpose commented in this point. We emphasize in this comparison

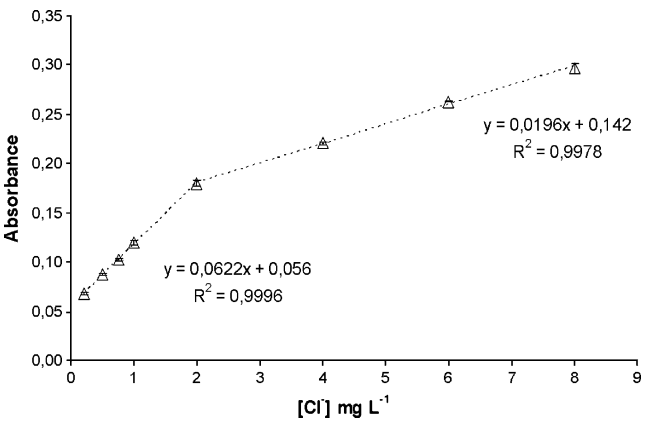

Fig. 3 Influence of the Cl^- concentration on the absorbance, reporting the two different linear dynamic ranges obtained

Table 2 Results obtained in the Cl^- determination in different types of water samples. The four samples were analyzed with the proposed MSFIA method and by means of Volhard titration

Sample	Added Cl^- (g L^{-1})	Proposed method Cl^- (g L^{-1})	Recovery %	Volhard method Cl^- (g L^{-1})
Mineral 1	0	0.0062±0.0003		0.0060±0.0005
	0.02	0.0261±0.0005	100	
Mineral 2	0	0.0432±0.0009		0.0428±0.0013
	0.02	0.0629±0.0014	99	
Tap	0	0.0664±0.0013		0.0643±0.0013
	0.02	0.0879±0.0020	107	
Well	0	0.1409±0.0013		0.1411±0.0026
	0.02	0.1834±0.0039	106	

that the most remarkable improved feature is the drastic reduction in reagents consumption, with determinations based on this reaction system approaching the principles stated by the Green Chemistry Movement.

Conclusions

In this work, the combination of multisyringe flow injection technique with a 100-cm liquid core waveguide is proposed for the spectrophotometric determination of Cl^--based on the $Cl^-/Hg(SCN)_2/Fe^{3+}$ reaction system. The proposed system allows the expeditious completely automated Cl^- determination in waters in a wide linear dynamic range due to the implementation of an on-line dilution procedure.

The major improvement of the proposed system is the drastic reduction of reagent consumption, focused overcoat, to the reduction of the used amount of Hg(II), being only required 1 nmol of Hg(II) per Cl^- determination. Furthermore, other features are improved in comparison with similar FI systems, such as the limit of detection or the autonomy of the system, avoiding $Hg(SCN)_2$ precipitation or the use of organic solvents. The proposed methodology was successfully applied to the Cl^- determination in water samples, and it was validated through an independent method which uses a different chemistry (Volhard titration).

Finally, we would like to encourage the use of liquid core waveguides as an analytical tool not only for the enhancement of sensitivity but also for its effectiveness greening classic analytical methodologies, especially if it

Table 3 Evolution of the analytical performance of several flow systems for the spectrophotometric Cl^- determination based in the $Cl^-/Hg(SCN)_2/Fe^{3+}$ reaction system

	Ref. 19	Ref. 20	Ref. 21	Ref. 22	Ref. 11	Ref. 12	Proposed
Year	1976	1991	1997	1998	2005	2008	2009
Linear range (mg L^{-1})	0–40	1–30	10–1,000	0.3–25	2–8	1–40	(1) 0.2–2 (2) 2–8
LOD (mg L^{-1})	–	0.2	–	0.3	0.5	0.2	0.06
R.S.D. (%)	–	1	<2.38	–	2.2	0.8	1.3
Injection throughput (h^{-1})	300	100	15	18	100	130	21
Type of water sample	River	Tap	Natural drink	River pond	Natural	Mineral tap-well	Mineral tap-well
Hg(II) (μg per determination)	680	1,140	1,720	600	120	45	0.2
Hg(II) consumption[a]	–	1.7-fold higher	2.5-fold higher	1.1fold lower	5.7-fold lower	15.1-fold lower	3,400-fold lower
Fe^{3+} (μg per determination)	7,210	5,620	18,400	11,100	50,000	335	28
Fe^{3+} consumption[a]	–	1.3-fold lower	2.6-fold higher	1.5-fold higher	6.9-fold higher	21.5-fold lower	257-fold lower

[a] Reported data obtained from comparison with [19]

is combined with devices for precise microfluidic handling, such as the multisyringe flow injection analysis technique.

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CAPÍTULO 4

EL ANÁLISIS POR INYECCIÓN EN FLUJO MULTIJERINGA, UNA HERRAMIENTA VERSÁTIL PARA EL DESARROLLO DE NUEVOS MÉTODOS PARA LA MEDIDA DE ÍNDICES O PARÁMETROS SUMA AMBIENTALES

Este capítulo trata sobre el desarrollo de nuevas metodologías de análisis orientadas a la obtención rápida de información analítica de interés ambiental. Por un lado, las muestras medioambientales pueden contener una gran cantidad de compuestos de diferentes familias, siendo la cuantificación de la totalidad de compuestos de una determinada familia una tarea no viable, sobre todo teniendo en cuenta que la mayoría de compuestos de interés puede que ni siquiera estén presentes en dicha muestra. Por otro lado, el establecimiento de índices ambientales nos permite de forma rápida obtener información preliminar, mediante metodologías generalmente lentas que implican tratamientos previos de muestra. Por este motivo se ha llevado a cabo un estudio de las ventajas que aportan las técnicas de análisis en flujo para el desarrollo de este tipo de metodologías analíticas además de establecer mediante la técnica MSFIA el primer método automático para la detección rápida de compuestos orgánicos halogenados en muestras de interés ambiental.

4.1. Tipos de información analítica

El objetivo en el desarrollo de una metodología de análisis reside en la obtención de información analítica relativa a alguna característica de la muestra. Existen diversos niveles de información analítica que se pueden obtener, siendo el nivel de mayor información relacionado con una mayor duración del análisis, dificultad, uso de instrumentación más sofisticada y encarecimiento del análisis.

Los diferentes niveles de información analítica pueden ir desde la obtención de información cualitativa sobre la presencia o ausencia de un determinado grupo o familia de compuestos con alguna característica en común, hasta la obtención de resultados cuantitativos para cada uno de los componentes de una muestra[1].

Para la realización de una determinación analítica es de gran utilidad el establecimiento de un orden en la aplicación de una metodología analítica concreta. Por ejemplo, en la determinación de los compuestos fenólicos contenidos en una muestra, sería útil comprobar primero si esa muestra contiene algún fenol antes de intentar cuantificar cada posible fenol presente en esta.

[1] J. R. Baena, M. Valcárcel, Trends Anal. Chem. 22 (2003) 641-646.

Este tipo de actuaciones fueron definidas como "Estrategias de Vanguardia-Retaguardia" por M. Valcárcel y S. Cárdenas[2], proponiendo el uso de técnicas rápidas y económicas con el objetivo de obtener una evidencia inicial sobre la presencia de uno o varios analitos, y su posterior confirmación mediante el uso de técnicas más precisas.

Una técnica de retaguardia debe proporcionar información cuantitativa selectiva sobre un determinado compuesto o especie. Por ejemplo el uso de técnicas de separación combinadas con ciertos detectores (usualmente cromatografía líquida o de gases acopladas con espectrómetros de masas). Una técnica de vanguardia puede ser un método cualitativo, el cual nos indica la presencia o ausencia de ese compuesto pero no si está sobrepasando algún límite establecido y por tanto entrañando peligrosidad.

De acuerdo con lo expuesto el uso de métodos basados en la expresión de resultados como índices totales puede suponer una ventaja como métodos de vanguardia en vez del uso de metodologías puramente cualitativas.

[2] M. Valcárcel, S. Cárdenas, Trends Anal. Chem. 24 (2005) 67-74.

4.1.1. Índices totales o parámetros suma de interés medioambiental

Un índice total o parámetro suma es una medida que describe un grupo o familia de compuestos químicos de similar naturaleza y/o estructura (hidrocarburos policíclicos aromáticos, bifenilos policlorados,…) y/o la producción de un efecto similar (contaminantes, antibióticos, pesticidas,…).

Un parámetro suma o índice total puede representar desde unos pocos compuestos o especies (p. ej. mercurio total en agua marina) a miles de ellos (p. ej. fenoles totales en aguas residuales). Estos compuestos pueden tener una estructura similar, o contribuir a una determinada característica de la muestra (p.ej. compuestos que contribuyen a la demanda química de oxígeno).

El uso de índices totales nos permite la obtención rápida de información analítica como en una estrategia de vanguardia. La información es además cuantitativa respecto a una determinada propiedad de la muestra, pero no a cada analito. De este modo, además de tener la primera evidencia sobre la presencia de los compuestos de interés, podemos obtener un dato que informa sobre la concentración de estos en la muestra, lo que permite saber hasta qué punto es necesario realizar posteriores análisis para la cuantificación o confirmación de cada una de las especies que contribuyen a ese parámetro o índice.

Según J. R. Baena y M. Valcárcel[3], el uso de una metodología analítica basada en un índice total proporciona entre un 50-70% de la información realmente útil para tomar una decisión rápida y oportuna.

Un índice total se obtiene a partir de una única señal, o también a partir de la obtención de una ecuación, relación o adición de una serie de señales.

El resultado puede expresarse como un número arbitrario, como la cantidad de una única de las especies o compuestos que comprende ese grupo, como un elemento común del grupo o como una función del comportamiento del grupo.

4.1.2. Aplicación de las técnicas de análisis en flujo para el desarrollo de nuevos métodos para la determinación de índices totales de interés medioambiental

Las diferentes técnicas de análisis en flujo introducidas en el Capítulo 1, han sido utilizadas para el desarrollo de nuevos adelantos en la determinación de parámetros suma o índices totales de interés medioambiental. Una recopilación de dichos adelantos puede verse detallada en el punto 4.6 de esta tesis.

Esta revisión bibliográfica aborda los recientes adelantos obtenidos mediante el uso de técnicas de análisis en flujo para la determinación de índices totales o parámetros suma de interés medioambiental ampliamente

[3] J. R. Baena, M. Valcárcel, Trends Anal. Chem. 22 (2003) 641-646.

utilizados actualmente, tales como la demanda química de oxígeno, el total de compuestos orgánicos halogenados o los fenoles totales.

4.2. Consideraciones sobre los compuestos orgánicos halogenados

Los compuestos orgánicos halogenados son compuestos con una estructura basada en el carbono, que incluyen al menos un átomo de un halógeno (flúor, cloro, bromo o yodo).

Los compuestos orgánicos halogenados pueden encontrarse de forma natural en el medio ambiente. La procedencia natural se atribuye a las plantas, tanto a las que crecen en ambientes acuáticos como terrestres. Por lo tanto, los suelos y sedimentos en los que crecen dichas plantas acumulan estos compuestos.

Los niveles de compuestos orgánicos halogenados suelen ser elevados en pantanos, y en el tratamiento del carbón. Este grupo de compuestos orgánicos halogenados producidos de forma natural se conforma por más de 3000 compuestos distintos.

Una de las características importantes de los compuestos halogenados reside en que los enlaces halógeno-carbono son muy fuertes y difíciles de romper, lo que les confiere una gran estabilidad y larga vida. Esta

característica los hizo muy atractivos, hasta que se llegó a la certidumbre que no era precisamente una propiedad deseable para los compuestos de carácter tóxico, ya que les confería una gran permanencia en el medio ambiente. Entre este tipo de compuestos indeseables se pueden citar el DDT o las PCBs, compuestos actualmente prohibidos en la UE.

La problemática sobre los compuestos orgánicos halogenados reside pues en la incorporación de nuevos compuestos de este tipo al medio ambiente, procedentes de fuentes antropogénicas. Debido a su utilidad como pesticidas, herbicidas, desinfectantes, y también en los procesos de manufacturación de productos tales como papel, plástico o tintes, existe un amplio abanico de compuestos orgánicos halogenados, siendo muchos de ellos producidos a gran escala.

Estos compuestos de origen antropogénico pueden ser contaminantes persistentes para el medio ambiente, y además pueden ser acumulados a través de la cadena alimenticia por los organismos acuáticos y los humanos, dependiendo básicamente de sus propiedades hidrofóbicas.

Por este motivo, la determinación de estos compuestos es de gran interés, tanto en el ámbito científico, como en los planes de vigilancia ambiental. Sin embargo, desarrollar metodologías analíticas para cuantificar cada uno de los compuestos orgánicos halogenados potencialmente presentes

en una muestra no es una tarea viable. Por este motivo, los métodos establecidos se basan en el uso de técnicas de separación para la determinación de un sub-grupo de compuestos orgánicos halogenados concreto. Algunos de estos sub-grupos de mayor interés son los bifenilos policlorados (PCB´s) antes mencionado, algunos pesticidas o las dioxinas y furanos.

Una estrategia para el desarrollo de un método que sirva para obtener información analítica sobre la totalidad de los compuestos orgánicos halogenados presentes en una muestra, puede ser el seguimiento de una propiedad común a todos los compuestos que conformen ese grupo. En este caso es la posesión de uno o más átomos de halógenos en su estructura molecular.

Desarrollando una metodología analítica de este tipo podríamos determinar si una muestra concreta contiene una cantidad detectable de algún/os compuestos orgánicos halogenados, y la cantidad total de estos en función de la cantidad de átomos de halógeno detectados procedentes de materia orgánica.

Las ventajas en el uso de un método basado en este principio residen en la obtención de información básica pero rápida, antes de usar técnicas más complicadas, laboriosas y más caras. Sin embargo, este tipo de metodologías

no nos permitirían el reconocimiento de cada uno de los compuestos que contribuyen al resultado obtenido, desconociendo si estos son compuestos orgánicos halogenados inocuos, o compuestos extremadamente tóxicos.

Algunos de los métodos de este tipo aplicados a la obtención de información analítica sobre compuestos orgánicos halogenados son por ejemplo, el método de los halógenos orgánicos adsorbibles (AOX, Adsorbable Organic Halogens), o el de los halógenos orgánicos extraíbles (EOX, Extractable Organic Halogens). Estos métodos, se basan en la extracción de la materia orgánica presente en una muestra, que incluye los compuestos orgánicos halogenados. Dicha extracción suele realizarse utilizando una fase sólida, tal como carbón activo (AOX), o una fase líquida, tal como n-hexano (EOX). Seguidamente, dicha fase sólida o líquida suele ser sometida a algún proceso de lavado, para eliminar los posibles restos de halógenos inorgánicos que puedan permanecer residualmente en ella. En este punto, la fase en la que son extraídos los compuestos orgánicos halogenados se somete a un tratamiento de mineralización, el cual suele ser un proceso de pirolisis a temperaturas cercanas a los 1000ºC. En este tratamiento, la materia orgánica se oxida a dióxido de carbono, y los átomos correspondientes a los halógenos inicialmente enlazados covalentemente a esta, son liberados. El paso final sería la cuantificación de los halógenos liberados con un método adecuado, como puede ser una valoración coulombimétrica.

4.3. Miniaturización y automatización de procedimientos de extracción en fase sólida basados en el uso de discos funcionalizados

La extracción en fase sólida es una técnica ampliamente utilizada para el aislamiento y la preconcentración de compuestos o especies en muestras líquidas. Los materiales utilizados como fases sólidas pueden ser de diversos tipos, dependiendo de la naturaleza de los analitos, y generalmente se emplean en forma de pequeñas partículas.

Una alternativa a la clásica extracción en fase sólida mediante materiales particulados es el uso de discos de extracción. Los discos de extracción utilizan los mismos materiales que las fases sólidas particuladas, pero en este caso las partículas se encuentran enlazadas en una matriz formada por fibras de un material inerte (generalmente teflón), formando un disco de pequeño grosor, el cual está constituido por aproximadamente un 90% de material activo y un 10% de teflón.

Algunas ventajas ofrecidas por estos discos en comparación con las fases sólidas particuladas son:

- Extracciones a mayores caudales, con un ahorro considerable de tiempo.
- Reducción en el gasto de disolventes para la elución de los compuestos previamente retenidos.

- El propio disco actúa como un filtro, previniendo el paso de pequeñas partículas, las cuales podrían atravesar una fase sólida particulada.
- Son fácilmente reemplazables.

En los sistemas de análisis en flujo donde la presión que soporta el sistema es un factor importante, el uso de discos es ventajoso debido a que estos nos permiten realizar extracciones a mayores caudales que usando materiales particulados. Por este motivo, los discos de extracción han sido implementados en sistemas de análisis en flujo para el desarrollo de diversas aplicaciones, tanto como fase sólida para la extracción[4], o como sensores que incorporan la extracción y la detección de los analitos sobre el propio disco[5].

La implementación de la extracción basada en discos en sistemas en flujo, se fundamenta en el uso de dispositivos como el que se detalla en la Figura 4.1. Estos sistemas emplean pequeños discos en un dispositivo incorporado al manifold. Se evita que el disco se deforme o se desplace mediante una frita porosa que actúa de soporte.

[4](a) C. Pons, R. Forteza, V. Cerda, Anal. Chim. Acta 550 (2005) 33-39. (b) M. Manera, M. Miro, J. M. Estela, V. Cerda, Anal. Chim. Acta 582 (2007) 41-49.
[5] (a) M. Miro, W. Frenzel, J. M. Estela, V. Cerda, Analyst 126 (2001) 1740-1746. (b) C. Pons, R. Forteza, V. Cerda, Anal. Chim. Acta 528 (2005) 197-203. (c) C. Pons, R. Forteza, V. Cerda, Talanta 66 (2005) 210-217. (d) M. Manera, M. Miro, J. M. Estela, V. Cerda, M. A. Segundo, J. L. F. C. Lima, Anal. Chim. Acta 600 (2007) 155-163.

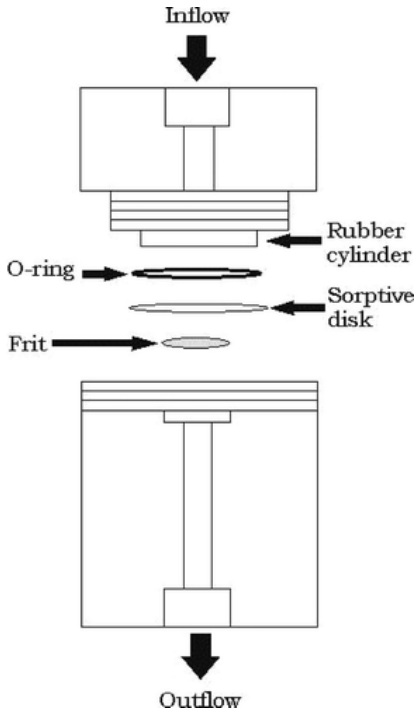

Figura 4.1. Esquema de un dispositivo de extracción en fase sólida basada en discos para su uso en sistemas de análisis en flujo.

4.4. Digestión en línea de muestras acuosas mediante de fotorreactores

Existe una amplia variedad de estrategias para la digestión de muestras. Con el fin de oxidar la materia orgánica que estas contienen estos métodos utilizan temperaturas elevadas, oxidantes químicos, ultrasonidos, microondas, radiación ultravioleta,…

Un método eficaz es la combinación de un oxidante químico y la radiación ultravioleta. Este procedimiento puede implementarse fácilmente en sistemas de análisis en flujo mediante el uso de una zona de confluencia que

permita la mezcla de la muestra con el oxidante, para seguidamente ser la mezcla resultante transportada a un bucle de reacción sometido a radiación ultravioleta. Con el objeto de que la radiación sea eficaz se enrolla la tubería, por la que circula la mezcla, a la lámpara ultravioleta.

Este tipo de mineralización ha sido utilizada en sistemas de análisis en flujo para la determinación de parámetros tales como el nitrógeno total[6], fósforo total[7], o la demanda química de oxígeno[8].

4.5. Desarrollo de un método de análisis por inyección en flujo multijeringa para la determinación del total de compuestos orgánicos halogenados en muestras acuosas

Actualmente, un parámetro o índice ambiental ampliamente utilizado es el de la medida del total de halógeno ligado orgánicamente. Existen varios índices establecidos para este propósito, siendo el de los Halógenos Orgánicos Adsorbibles (AOX, Adsorbable Organic Halogens) uno de los más utilizados. La metodología para determinar este índice es un método oficial: *European Standard EN 1485, Water-quality determination of adsorbable organically bound halogens*, el cual es similar al *EPA Method 1650 for Adsorbable Organic Halides by Adsorption and Coulometric Titration*.

[6] M. T. Oms, A. Cerdà, V. Cerdà, Talanta 59 (2003) 319-326.
[7] C. Pons, I. V. Toth, A. O. S. S. Rangel, R. Forteza, V. Cerda, Anal. Chim. Acta 572 (2006) 148-154.
[8] D. Z. Dan, R. C. Sandford, P. J. Worsfold, Analyst 130 (2005) 227-232.

Estos métodos se basan en un procedimiento manual de varias etapas, requiriendo un intervalo de tiempo de varias horas para la obtención de resultados. Este hecho es un inconveniente, ya que una de las principales ventajas que nos tiene que proporcionar un parámetro suma o índice ambiental es la obtención rápida de información analítica sobre una propiedad de interés de la muestra.

Por este motivo, el objetivo de este trabajo ha sido el sacar partido de las ventajas que nos brindan las técnicas de análisis en flujo para desarrollar una nueva metodología más rápida para la determinación de este parámetro.

Para llevar a cabo este objetivo, es necesario desarrollar un método analítico que tiene que contemplar tres pasos clave:

1- Aislamiento y preconcentración de los compuestos orgánicos halogenados de la matriz de la muestra. Para ello se decidió recurrir al uso de la extracción en fase sólida basada en discos funcionalizados.
2- Oxidación de dichos compuestos para la liberación de los halógenos inicialmente presentes en su estructura. Para ello se decidió usar un procedimiento de foto-oxidación mediante radiación UV ayudada por un oxidante químico.

3- Cuantificación de los halógenos liberados en el paso anterior. Para ello se decidió utilizar el método espectrofotométrico para la determinación de cloruros detallado en el Capítulo 3.

Debido a la complejidad de combinar estos tres procedimientos de una forma completamente automatizada, se tuvo que recurrir a la técnica MSFIA. Aprovechando las posibilidades que nos ofrece esta técnica pudimos combinar satisfactoriamente estos tres procedimientos de forma rápida y completamente automatizada. Este trabajo se presenta detalladamente en el punto 4.5 de esta tesis.

4.6. Artículo original IV

Flow Analysis Techniques as Effective tools for the Improved Environmental Analysis of Organic Compounds Expressed as Total Indices

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Review

Flow analysis techniques as effective tools for the improved environmental analysis of organic compounds expressed as total indices

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ABSTRACT

The scope of this work is the accomplishment of an overview about the current state-of-the-art flow analysis techniques applied to the environmental determination of organic compounds expressed as total indices. Flow analysis techniques are proposed as effective tools for the quick obtention of preliminary chemical information about the occurrence of organic compounds on the environment prior to the use of more complex, time-consuming and expensive instrumental techniques. Recently improved flow-based methodologies for the determination of chemical oxygen demand, halogenated organic compounds and phenols are presented and discussed in detail. The aim of the present work is to demonstrate the highlight of flow-based techniques as vanguard tools on the determination of organic compounds in environmental water samples.

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Contents

1. Introduction

In the present-day society a wide spectrum of analytical methodologies are extensively used providing us chemical information about the presence/quantity of target elements/compounds (from natural or anthropogenic sources). This chemical information becomes in a higher life-quality level.

There is different types or degrees of chemical information, from the obtention of qualitative (yes/no presence), to fully quantitative information for each individual specie contained in the sample. The obtention of more complete chemical information is related with an increase in effort, costs, time-consumed and difficulty [1].

In some situations, the analyst knows with security if the analyte is present in a sample (e.g. quality control analysis in pharmaceutical or food industry). In this case a quantitative analytical methodology with adequate features is required, in order to get the desired information.

In other situations, such as in the environmental analysis field (e.g. analysis of emerging pollutants), the most advanced analytical methodologies are usually based on the use of chromatographic techniques coupled to mass spectrometric detectors supplying precise quantitative chemical information about each analyte. In

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counterpoint of this, in some fields like the environmental analysis of pollutants, the target compounds are not often present in the analyzed samples, being the direct use of powerful separation techniques for these purposes an excessive loss of time and money, with the concomitant decrease of the chemical information obtained per time unit.

An intermediate point between qualitative (yes/no binary response) methods and fully quantitative methods are the total indices or sum parameters. Total indices are useful in order to get quantitative chemical information correlated with a concrete feature of the sample, but not for each concrete compound contained in it. Total indices provide useful chemical information about vast (up to millions of different compounds) and heterogeneous families of compounds, being the analysis of each one of them a hardly feasible task. The use of a total index determination allows the quick knowledge about the presence and the total amount of a concrete type of compounds contained in a sample, but the identification of each concrete analyte is not feasible.

Once an analytical methodology for the determination of a total index has been established, its automation simplifies the execution and increases the efficiency, approaching it to a real time measurement. Highly efficient tools for these purpose are the flow analysis techniques [2,3]. Furthermore, the required amounts of reagents/solvents are diminished and more environmentally friendly analytical methodologies can be developed. These automated methods are closer to the principles stated by the Green Chemistry movement [4], thus their relation with the Analytical Chemistry field [5]. And last but not least, the minimization of the exposure of the analyst in front of aggressive/hazardous chemicals or dangerous physical treatments.

2. Total indices or sum parameters

The role and principles of total indices in analytical chemistry were established by Baena and Valcárcel [6]. A total index or sum parameter is described as a group of compounds of similar nature, similar structure (e.g. PCBs, PAHs, etc.) or a concrete common characteristic (e.g. toxicity, hydrophobicity, acidity and oxidability). When a total index is defined, all the compounds involved in the measured property, contribute in a certain percentage to the single value obtained as a result.

As it was stated before, separation techniques coupled with mass spectrometric detectors are the most powerful techniques for environmental analysis, but when the time or costs per analysis are a predominant factor, the use of total indices becomes advantageous. It happens when the target compounds are not often found in the analyzed samples, allowing the total index measurement a fast way for the initial evidence of their potential presence. If the initial evidence is confirmed, more sophisticated techniques should be additionally used, obtaining more exhaustive chemical information. This was defined as Vanguard-Rearguard Strategies by Valcárcel and Cárdenas [7], where the total indices are defined as Vanguard Strategies, leaving the use of complex sample pretreatments followed to hyphenated techniques (e.g. gas chromatography–mass spectrometry) as Rearguard Strategies.

In Table 1, are listed various of the most commonly used total index parameters in the environmental analytical field.

3. Flow analysis techniques

Flow analysis techniques are well-established tools for the automation and miniaturization of analytical methodologies, providing advantages such as: (i) increased injections throughputs, (ii) high versatility, (iii) high robustness, (iv) new analytical improvements based on operating modes under non-stationary conditions, (v) decrease of the human exposure under hazardous chemical/physical sample pretreatments, (vi) more environmentally friendly procedures obtained due to process downscaling and (vii) use of alternative detection systems with the concomitant simplification of the operating conditions (viz. chemiluminescent detection).

The different types of flow analysis techniques are usually classified according to the device used for fluidic handling (Table 2). The use of flow analysis techniques allows the implementation of almost the totality of the sample pretreatments included on classic methodologies for the determination of environmental total indices, such as: (i) UV digestion, (ii) microwave digestion, (iii) sample distillation, (iv) liquid–liquid extraction, or (v) solid phase extraction, among others. By this reason, flow analysis techniques are useful tools for the fully automation of this type of analytical methodologies, achieving a highly efficient way for the unsupervised monitoring of total index parameters. It is also a noteworthy fact, the new applications in environmental analysis carried out by means of micro-total analysis systems (μTAS) [13], which in the next years could provide great achievements.

4. Recent developments on flow analysis techniques for the determination of environmental total indices of organic nature

The recent contributions of flow analysis techniques for the improvement of total indices for the assessment of organic com-

Table 1
Examples of total indices in environmental analysis.

Name	Abbreviation	Chemical information obtained	Result units
Chemical oxygen demand	COD	Amount of chemically oxidizable matter present in a liquid sample	Consumed mg O_2 L^{-1}
Total organic carbon	TOC	Amount of organic matter present in a sample	mg C L^{-1}
Dissolved organic carbon	DOC	Amount of organic matter remaining in a sample after filtration through a 0.45 μm pore size filter	mg C L^{-1}
Adsorbable organic halogens	AOX	Amount of Cl^-, Br^- and I^- organically bounded to organic compounds, which are adsorbable into activated charcoal	μg Cl^- L^{-1}
Extractable organic halogens	EOX	Amount of Cl^-, Br^- and I^- organically bounded to organic compounds, which are extractable in a determined organic solvent	μg Cl^- L^{-1}
Total organic chlorine	TOCl	Total Cl^- organically bounded to organic compounds, which are adsorbable on a XAD resin. Also exist TOF, TOBr and TOI	μg Cl^- L^{-1}
Biochemical oxygen demand	BOD	Amount of biologically oxidizable matter present in a sample	Consumed mg O_2 L^{-1}
Total suspended solids	TSS	Total solid matter per volume unit	mg solid matter L^{-1}
Total X (X = Hg, As, N, P, S, etc.)	TX	Total amount (from inorganic and organic nature) of a concrete element in a sample	mg X L^{-1}
Toxicity		It is the percentage of dead or property loss of an established microorganism culture, caused by contact with the sample. It can be acute or chronic	IC_{50} or LC_{50}
Phenolic index		Sum of the detectable phenols under defined conditions	μg phenol L^{-1}

Table 2
Summary of commonly used flow analysis techniques.

Technique	Abbreviation	Fluidic handling	Year	Reference
Flow injection analysis	FIA	Peristaltic pump	1975	[8]
Sequential injection analysis	SIA	Syringe pump with solenoid valve. Selection valve	1990	[9]
Multicommutated flow injection analysis	MCFIA	Peristaltic pump. Solenoid valves	1994	[10]
Multisyringe flow injection analysis	MSFIA	Up to four syringes with solenoid valves moved simultaneously by a single pump	1999	[11]
Multipumping flow systems	MPFS	Solenoid micropumps	2002	[12]

pounds in samples of environmental interest are exhibited and critically discussed. Concretely, the most notable improvements in the determination of some widely used environmental total indices like the chemical oxygen demand, the halogenated organic compounds or the phenolic index are described below.

4.1. Chemical oxygen demand

The chemical oxygen demand (COD) is a measure established for the quick assessment of the amount of both organic and inorganic matter contained in a water sample. The result is expressed as the required amount of oxygen (from a chemical oxidant) for the total oxidation of the compounds contained in the sample.

The classic procedure for the COD determination is based on sample mixing with strong sulphuric acid and a known excess of potassium dichromate. The mix is refluxed for 2 h. The remaining oxidant is titrated with ferrous ammonium sulphate, being the result of the titration expressed as the oxygen consumed by the sample.

This classic methodology presents important drawbacks, such as: (i) the use of hazardous chemicals including potassium dichromate, mercury sulphate (for the precipitation of the interfering chloride), silver sulphate (catalyst) and strong acids; (ii) these hazardous chemicals are used at very high temperatures; and (iii) very low analysis throughputs are obtained. In short, this is a popular analytical methodology, but implies a slow, tedious and dangerous procedure, involving the generation of big amounts of extremely hazardous wastes.

An alternative to the titrimetric quantification is the spectrophotometric (SPM) detection. SPM detection has been used since the earliest FIA systems for COD determination, involving on-line digestion by means of heating a mixture composed by the sample, a strong acid and permanganate [14] or dichromate [15] as oxidants. Increased analysis throughputs, waste reduction and less tedious operating conditions were obtained in comparison to manual methods.

Nowadays, these pioneer flow analysis methods have been improved following strategies based on advanced sample digestion procedures thus alternative detection systems, such as chemiluminescent or electrochemical detection. By this way, smart, quicker and more environmentally friendly strategies for the determination of the COD have been achieved. A summary of recently developed flow analysis systems for COD determination is presented in Table 3.

Classic FIA systems for COD determination with SPM detection have been improved recently [16]. Heating is replaced by UV-photocatalytic oxidation in the presence of acidic permanganate. COD is related with a decrease on the permanganate absorbance at 524 nm. This method provides fast, sensitive, accurate and cost-effective in situ COD determinations, thus avoiding the use of Ag_2SO_4 and $HgSO_4$. A schematic representation of a FIA manifold for this determination is shown in Fig. 1a.

Chemiluminescent (CL) detection is an efficient alternative to the classic SPM detection for COD measurements. Recent strategies based on FIA systems have been proposed for these purposes [17–20]. These systems are based on the reactivity between some products released or removed in the sample digestion procedures with appropriated chemiluminometric reagents. These are ozone [17], manganese(II) [18], chromium(III) [19] or free radicals [20].

Target compounds can be oxidized by the combined O_3–UV action, consuming O_3 with the concomitant CL signal decrease on the O_3–luminol–Co^{2+} reaction system. Studies using α-naphthol corroborate that UV-ozonation is a more efficient oxidation process than the classic $KMnO_4$ oxidation [17].

An alternative to the use of O_3, is the use of a strong cation exchange resin for the elimination of the traces of metals contained in the sample. By this way, after the digestion of the sample with $KMnO_4$, the only metal cation present is the gradually released

Table 3
Comparison of recent flow analysis systems for COD determination.

Flow technique	Digestion	Detection	Samples	LOD ($mg\,L^{-1}$)	Linear range ($mg\,L^{-1}$)	Injection throughput (h^{-1})	Reference
FIA	UV–$KMnO_4$	SPM at 524 nm	Freshwater	0.5	0.5–50	30	[16]
FIA	UV–O_3	O_3–luminol–Co^{2+} CL	Fresh and Sea water	–	0.5–20	–	[17]
FIA	$KMnO_4$	Mn^{2+}–luminol–H_2O_2 CL	Lake water	2	4–4000	40	[18]
μTAS	$K_2Cr_2O_7$	Cr^{3+}–luminol–H_2O_2 CL	Wastewater	100	270–10,000	–	[19]
FIA	UV	Luminol CL of the released free radicals	River and lake water	0.08	0.2–20	6–12	[20]
FIA	UV/TiO_2 packed bead reactor	O_2 electrode EC	Lake water	0.12	0.12–8	6	[21]
FIA	UV/TiO_2 packed bead reactor	EC using two O_2 electrodes (one placed before and one after digestion)	Water from dam reservoirs	0.5	0.5–9.5	3	[22]
FIA	Electrocatalytic oxidation at F-PbO_2 modified electrode	EC detection based in the current changes produced on the modified electrode	Wastewater	15	100–1200	–	[23]
FIA	Synergistic electrocatalytic–photocatalytic oxidation at a Ti/TiO_2/PbO_2 electrode	EC detection based in the current changes produced on the modified electrode	Wastewater	15	30–2500	–	[24]

CL, chemiluminescence detection; FIA, flow injection analysis; μTAS, micro-total analysis system; EC, electrochemical detection.

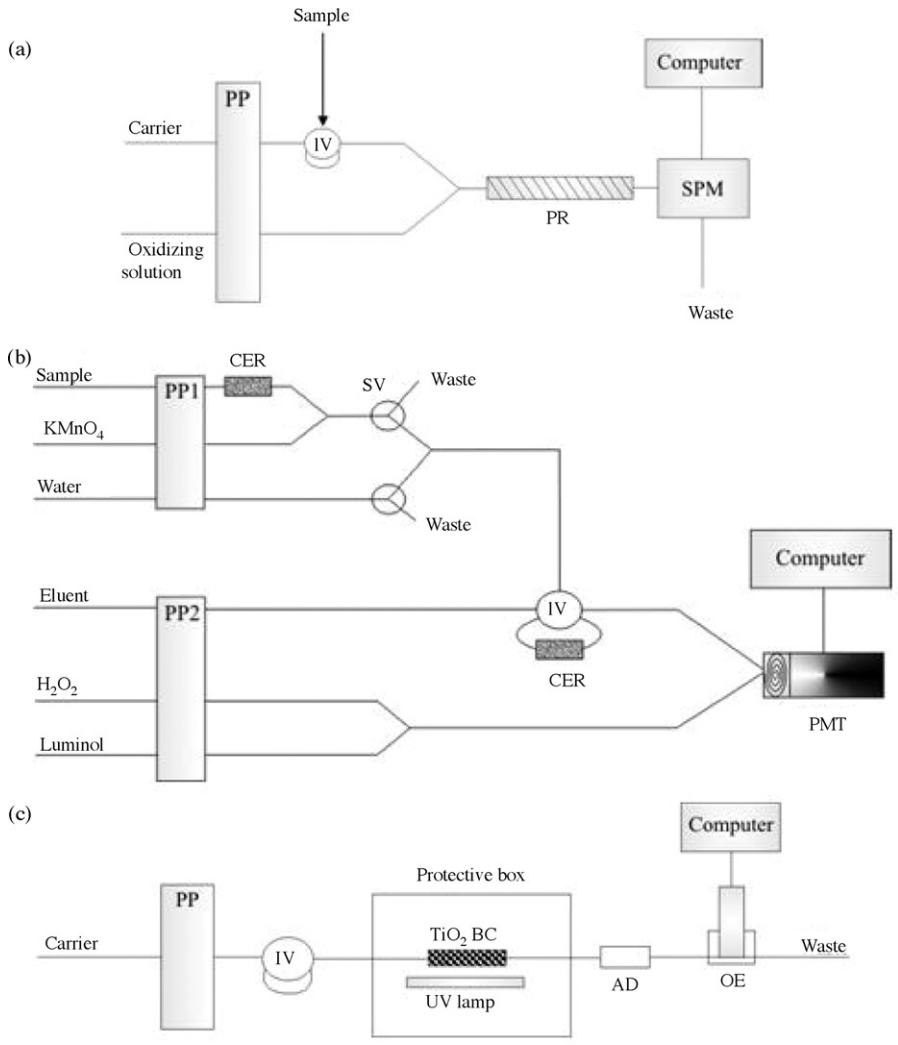

Fig. 1. Examples of FIA systems for the determination of COD using (a) spectrophotometric, (b) chemiluminescent and (c) electrochemical detection. PP, peristaltic pumps; IV, six-port rotating injection valve; PR, photo-reactor; SPM, spectrophotometer; CER, column filled with a strong cation exchange resin; SV, solenoid valves; PMT, photomultiplier equipped with a spiral shaped flow cell; TiO_2 BC, column filled with TiO_2 beads; AD, air damper; OE, oxygen electrode.

Mn^{2+} from the removed $KMnO_4$, which is preconcentrated into a second strong cation exchange resin. After elution with KCl, Mn^{2+} is quantified due to its catalytic action on the luminol–H_2O_2 reaction system, emitting a CL signal proportional to the COD value (Fig. 1b). This method provides a wide linear working range of three orders of magnitude and a high analysis throughput [18]. Other alternative to O_3 is based on the use of acidified $K_2Cr_2O_7$. The released Cr^{3+} is correlated with the COD amount. Cr^{3+} is also quantified by CL reaction with luminol–H_2O_2. The interferences caused by other metallic species are avoided by the addition of EDTA, due to the unfavorable competitive complexation of the Cr^{3+} in front of other metallic cations. This approach is not suitable for natural waters due to its low sensitivity, but it is suitable for COD measurements in wastewaters. Furthermore, it is an environmentally friendly alternative for COD measurements due to its implementation on a μTAS system using various independent syringe pumps as microfluidic handlers [19]. In natural waters, the COD determination is accomplished from the free radicals released by the single action of UV radiation and their subsequent reactivity with luminol [20]. This method allows the measurement of very low amounts of COD (LOD < 100 $\mu g\,L^{-1}$).

Electrochemical detection is probably the most environmentally friendly alternative for COD measurements [21–24]. Recently improved methods are based on the implementation of UV/TiO_2 packed bead reactors for sample digestion followed by the measurement of the O_2 concentration changes using an amperometric sensor for O_2 detection [21,22] (see Fig. 1c). Other improvements in the COD determination using EC detection are based on the development of the entire analytical procedure (sample digestion + detection) at the same electrode surface. It is achieved by using an F-PbO_2 modified electrode [23], and improved later using a Ti/TiO_2/PbO_2 electrode [24].

4.2. Halogenated organic compounds

Halogenated organic compounds (HOCs) are a huge and heterogeneous group of carbon-based compounds presenting covalently bounded halogens in their structure, being an enormous effort, the quantification of each individual HOC potentially contained in a sample. On the one hand, HOCs are naturally produced in big amounts. On the other hand HOCs are massively produced from anthropogenic sources becoming in an environmental problem, even of global extension. By this reason, several total indices have been defined for that purpose, such as: adsorbable organic halogens (AOX), extractable organic halogens (EOX) or total organic chlorine (TOCl), among others.

The adsorbable organic halogens (AOX) parameter is the measurement of the sum of the adsorbable HOCs into activated charcoal

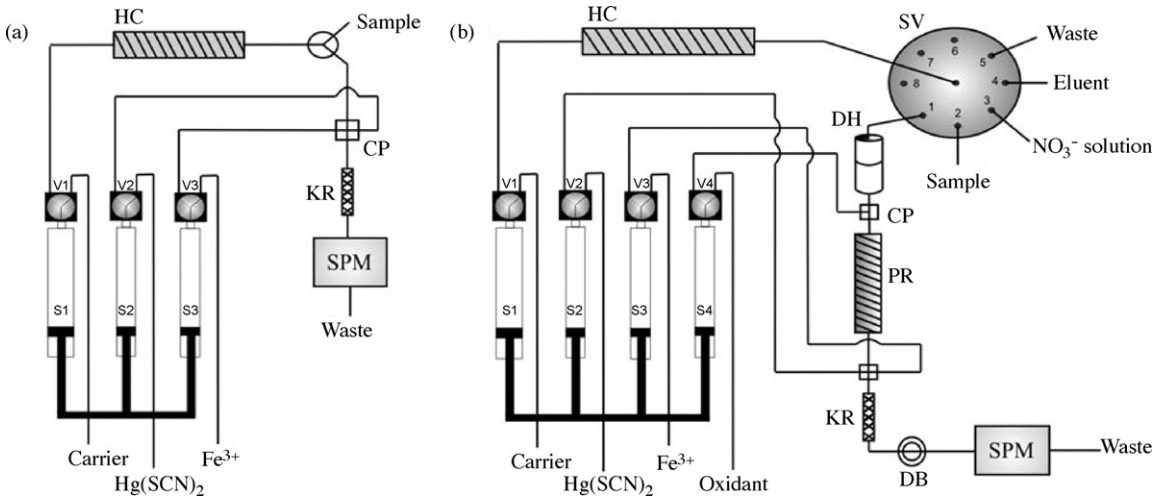

Fig. 2. Schematic representation of (a) MSFIA system for chloride determination and (b) MSFIA system for the determination of halogenated organic compounds as a total index. S1–S4, syringes; V1–V4, solenoid valves; HC, holding coil; SV, selection valve; DH, holder for the accommodation of the sorbent disk for SPE; CP, confluence point; PR, photo-reactor unit; KR, knotted reaction coil; DB, debubbler; SPM, spectrophotometer.

under specific conditions. Once the activated charcoal is free of inorganic halides, it is combusted at 950 °C in a pyrolytic oven, with the concomitant release of free hydrogen halides. These are quantified by means of coulometric titration [25]. Results are expressed as $\mu g\, Cl^-\, L^{-1}$ and represent the total Cl^-, Br^- and I^- (not F^-, due to a limitation of the technique) from organic origin. This official procedure presents a lack of automation with a concomitant low analysis throughput. By this reason, an automated quasi-continuous system for AOX monitoring was developed [26]. Exploiting a FIA system, AOX are preconcentrated and isolated in an activated charcoal cartridge. The cartridge is automatically discharged into a pyrolytic oven, being the released hydrogen halides in the HOCs digestion determined using plasma emission spectrometry. This method is also useful for TOF, TOCl and TOX determinations.

Exploiting the versatility of the MSFIA technique, a multicommutated method for the determination of Cl^- in waters using the $Hg(SCN)_2/Fe^{3+}$ SPM method (Fig. 2a) [27] was adapted for HOCs measurements (Fig. 2b) [28]. This was accomplished furnishing the MSFIA system with a miniaturized flow through device for the inline solid phase extraction (SPE) of HOCs, thus a UV-photocatalytic reactor for the digestion of the previously preconcentrated HOCs. The SPE procedure is carried out using 9 mm diameter poly(styrenedivinylbenzene) sorbent disks. Rinsing the sorbent with a $NaNO_3$ solution, the interfering inorganic halides are removed. HOCs are eluted using a 2-propanol/water solution. The eluate is mixed with a $Na_2S_2O_8$ solution and irradiated with UV light. The released hydrogen halides from the previous photolysis are quantified using the $Hg(SCN)_2/Fe^{3+}$ method. This system enables the inline completely automated determination of HOCs in a similar way to the AOX classic determination, in a matter of minutes instead of hours.

Simon et al. [29] developed a FIA system for the determination of the extractable TOCl, TOBr and TOI, being their sum the so-called EOX parameter. This system can be defined as a FIA system for the ion-selective determination of the EOX total index. This method is based on the use of a chromatomembrane cell [30] for the liquid–liquid extraction of EOX into hexane. Hexane is combusted in a pyrolytic oven, and the released hydrogen halides are absorbed into a hydrazine sulphate solution, which is injected into an ion chromatograph.

Fluorinated organic compounds are considered as emerging organic pollutants. Their fast screening as TOF is carried out satisfactorily using a FIA system [31]. Organic compounds are extracted off-line in a carbon-based solid phase (e.g. multi-walled carbon nanotubes, carbon nanofibers or activated carbon). Once the SPE has been carried out, sodium biphenyl is added directly onto the sorbent surface producing the release of free fluoride. The recovered fluoride is determined using a FIA system furnished with a fluoride ion-selective electrode. Alternatively, a FIA system with fluorimetric detection using quercetin and zirconium(IV) sulphate, was also used satisfactorily for the same purpose.

Mihalatos and Calokerinos [32] developed a FIA system for gas phase CL detection of halogenated hydrocarbons. This system is based on the injection of a current of O_2 into a photochemical reactor, where it is irradiated with UV light with the concomitant production of O_3. The released O_3 reacts with ethylene in a flow cell and the emitted CL is detected by a photomultiplier. When the sample is injected into the O_2 current, a part of the released ozone is consumed due to the oxidation of the halogenated hydrocarbons. The decrease on the CL signal is proportional to the amount of the target compounds.

4.3. Phenolic index

The presence of phenolic compounds in waters is used as an indicator of pollution from anthropogenic sources. Besides the natural presence of a plethora of natural phenolic and polyphenolic compounds, synthetic phenols are massively used by humans for diverse applications. Phenols are used directly as they are, as feed-stocks or generated as byproducts in petroleum, paper, dyes, tannery or pesticide industries, among others.

If the identification of the target compounds is not required, the use of total indices is the quickest way for the obtention of quantitative chemical information about phenol pollution. By this reason, several standard methods for the determination of phenolic compounds as total indices have been established. Some of these are based on the use of the FIA technique with SPM detection [33,34]. This classic methodology, known as the 4-aminoantipyrine (4-AAP) method is based on the oxidative coupling of some phenols with 4-AAP in alkaline medium aided with an oxidant ($K_3[Fe(CN)_6]$). The developed reaction product is SPM detected at 500 nm. But the 4-AAP method also presents some drawbacks. It is a very time-consuming methodology. The analytical procedure involves a tedious sample pretreatment. Poor recoveries for some para-substituted phenols are obtained. The method requires the use of hazardous chemicals at high concentrations with the concomi-

Table 4
Comparison of recent flow systems developed for phenolic index determination.

Flow technique	Pretreatment	Detection	Samples	LOD ($\mu g\,L^{-1}$)	Linear range ($\mu g\,L^{-1}$)	Injection throughput (h^{-1})	Reference
FIA	(1) Inline SPE (2) Reaction with 4-AAP/K_3[($Fe(CN_6)$]	SPM 490 nm	Environmental and industrial waste waters	4	10–1000	12	[35]
FIA	(1) Reaction with 4-AAP/K_3[($Fe(CN_6)$] (2) SPE (optosensing)	SPM	–	1	5–500	–	[36]
SIA	Direct reaction with 4-AAP/K_3[($Fe(CN_6)$]	SPM 510 nm	Waste waters	10	50–2500	24	[37]
MSFIA	(1) Inline SPE (2) Reaction with 4-AAP/K_3[($Fe(CN_6)$]	SPM 510 nm	Certified reference material	2	10–280	4	[38]
MCFIA	Direct reaction with 4-AAP/K_3[($Fe(CN_6)$]	SPM 500 nm	Natural and waste waters	1	10–100	90	[39]
MPFS	Direct reaction with nitroprusside/hydroxylamine	SPM 700 nm	Environmental and waste waters	13	50–3500	65	[40]
FIA	Direct reaction with rhodamine-6G/Ce^{4+}	CL	–	–	–	–	[41]
FIA	(1) Inline SPE (2) Reaction with acidic $KMnO_4$	CL	Ground and waste water	1	1–20	4	[42]
FIA	Direct reaction with acidic $KMnO_4$ sensitized with formaldehyde	CL	Waste water	3	5–1000	–	[43]
FIA	(1) Inline SPE (2) Reaction with luminol/K_3[($Fe(CN_6)$]	CL	Natural waters	0.00066	0.0047–0.47	–	[44]
FIA	Sample acidification	EC	Waste water	37.6	94–9400	180	[45]
FIA	No pretreatment	EC	Waste water	8.3	18.8–18,800	–	[46]

tant generation of toxic wastes, thus the previous distillation of samples (for the elimination of potential interferences), among other drawbacks. By this reason, several improved applications for the determination of this total index have been recently proposed [35–46] (Table 4). These improvements are based on the exploitation of alternative detection systems to classic SPM detection, such as: long pathlength spectrophotometry [39], chemiluminescence [41–44] or electrochemical detection [45,46]. Thus, the use of more advanced flow analysis techniques, such as: SIA [37], MSFIA [38], MPFS [39] and MCFIA [40].

An improved sensitivity for the 4-AAP method automated in a FIA system is obtained with the use of inline SPE prior to SPM detection. This is achieved using a microcolumn packed with Amberlite XAD-4 resin for the preconcentration/isolation of phenols prior to their SPM detection [35]. Or by direct optosensing of the developed reaction product onto the same sorbent surface by using C18-modified silica resin, packed in a microcolumn [36].

Sequential flow analysis techniques have been also applied for the phenolic index determination through the 4-AAP method. As the volumes of reagents are perfectly controlled, the volume of waste products is drastically reduced, becoming in more environmentally friendly strategies. Some examples are, the direct determination of phenols using a SIA system [37], or the MSFIA system proposed by Oliveira et al. [38] combining in a single system (Fig. 3a) the advantageous characteristics of their predecessors; inline preconcentration [35] and low reagent consumption [37].

The previous methods are improved exploiting a MCFIA system furnished with a 1m long path length liquid waveguide capillary cell (LWCC) as flow cell for SPM measurements [39]. This MCFIA–LWCC system (Fig. 3b) allows a high analysis throughput of 90 h^{-1} and a 200-fold reduction of reagent consumption. As the sensitivity is enhanced with the use of the LWCC, some additional sample treatments such as SPE are not required. Another more environmentally friendly alternative for the SPM determination of the phenolic index is based on the use of solenoid micropumps as microfluidic handling devices, using nitroprusside/hydroxylamine as reagents, instead 4-AAP/ferricyanide [40].

Chemiluminescence is also an efficient detection system for the determination of phenolic compounds, achieving higher sensitivity than with classic SPM detection. Other advantageous features for the CL detection of phenolic compounds are: (i) the obtention of good recoveries for para-substituted phenols, thus (ii) the predistillation of the samples is not required.

Cui et al. [41] accomplished using a FIA system a study about the reactivity of the chemiluminescent reaction of phenolic compounds with rhodamine-6G/Ce^{4+} and its subsequent CL emission. The system was not applied to real samples. FIA was also combined with SPE using a microcolumn packed with Amberlite XAD-4 resin, thus detection based on the acidic permanganate CL [42]. Afterwards, the CL reaction between phenolic compounds and acidic permanganate was sensitized by the addition of formaldehyde, improving the sensitivity and achieving without preconcentration a LOD of only 3 $\mu g\,L^{-1}$ phenol [43]. The LOD of the previous CL methods was improved using a FIA system with inline SPE (microcolumn filled with C18-modified silica) prior to luminol/K_3[$Fe(CN)_6$] CL detection. By this way an ultra-low limit of detection of only 0.66 $ng\,L^{-1}$ phenol was achieved [44] (Fig. 3c).

Recent improvements on the determination of phenolic compounds as a total index have been achieved using electrochemical detection. As with CL detection, pre-distillation is not required and good recoveries for para-substituted phenols are achieved. Some examples of these are: (i) a biamperometric system for an irreversible redox couple, based on coupling the oxidation of phenolic compounds at a platinum-wire electrode and the concomitant reduction of permanganate in another analogous electrode obtaining a method with a high analysis throughput [45] or, (ii) the application of self-assembled monolayer-based tyrosinase biosensors for the amperometric determination of phenols [46], achieving a wide linear dynamic range of three orders of magnitude.

When the distillation of the sample is required, Quaresma et al. [47] developed a fast alternative for this pretreatment by using focused microwave-assisted sample distillation. A sample volume of 25 mL is distilled following the proposed procedure for 15 min. Obtained recoveries were of a 95% phenol in the distilled solution,

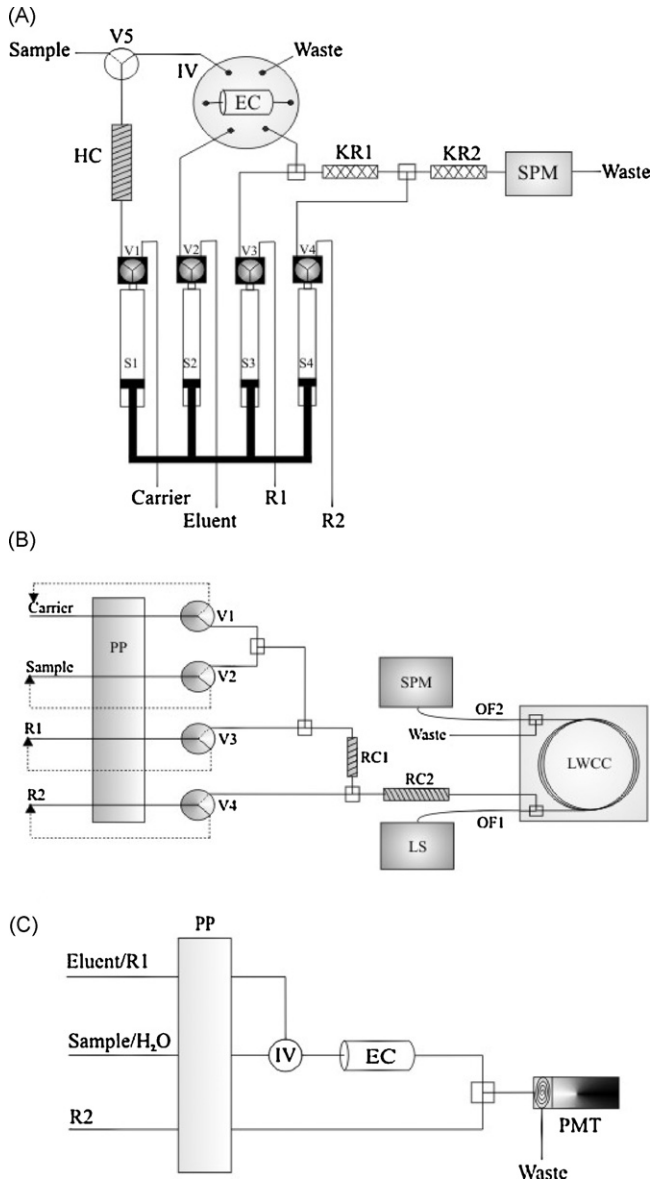

Fig. 3. Schematic representation of various alternatives based on flow analysis techniques for the determination of phenols as total indices. PP, peristaltic pump; V1–V5, solenoid valves; RC1–RC2, reaction coils; OF1–OF2, optical fibers; LS, UV–vis light source; SPM, spectrophotometer; LWCC, liquid waveguide capillary cell; HC, holding coil; IV, injection valve; S1–S4, syringes; KR1–KR2, knotted reaction coils; PMT, photomultiplier tube. (a) MSFIA system with inline SPE and spectrophotometric detection. R1, 4-aminoantipyrine; R2, $K_3[Fe(CN)_6]$. (b) MCFIA system with spectrophotometric detection based on the use of a 1 m LWCC as flow cell. EC, extraction column filled with Amberlite XAD-4 resin; R1, 4-aminoantipyrine; R2, $K_3[Fe(CN)_6]$. (c) FIA system with inline SPE and chemiluminescent detection. EC, extraction column filled with C_{18}-modified silica resin; eluent/R1, methanol–luminol (60%, v/v); R2, $K_3[Fe(CN)_6]$.

which is analyzed with the 4-AAP/$K_3[Fe(CN)_6]$ method implemented in a FIA system. Following this procedure, the obtained results are similar that those of the standard methods, but increasing the analysis throughput.

5. Concluding remarks and future outlook

From the above overview about the recent developments based on flow analysis techniques for COD, HOCs and phenolic compounds determination as total indices, we can set out the next conclusions:

- Total indices are indispensable tools for the quick obtention of preliminary chemical information about complex analytical systems.
- The classic methodologies for the determination of total indices are time-consuming, laborious, involves dangerous procedures, thus the generation of considerable amounts of toxic wastes. These disadvantageous features are diminished due to their implementation in flow analysis systems.
- In the recent years, classic FIA methods have been improved due to the development of more advanced flow analysis techniques and new types of detection systems. These advances in instrumentation induce a concomitant improvement of the analytical features of the developed methodologies, such as: higher analysis throughputs, lower detection limits, larger linear dynamic ranges, lower relative standard deviations, among others. Furthermore, they provide useful solutions to some limitations of classic techniques, thus new possibilities of chemical analysis.
- The process downscaling obtained using flow analysis-based systems enables the saving of reagents and solvents, being the new developed strategies for the determination of total indices more environmentally friendly [48,49] than the classic ones.

A possible future outlook about this topic could deal with the new possibilities offered by nanotechnology, providing new materials for improved SPE, sample digestion or developing new detectors. Other improvements could be achieved by the substitution of hazardous chemical reagents/solvents for other more benign ones (i.e. ionic liquids), besides the development of meso- and microfluidic devices, such as lab-on-valve (LOV) [50] or lab-on-chip (LOC)/μTAS systems [51].

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4.7. Artículo original V

Completely Automated System for Determining Halogenated Organic Compounds by Multisyringe Flow Injection Analysis

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Completely Automated System for Determining Halogenated Organic Compounds by Multisyringe Flow Injection Analysis

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A new, multisyringe flow injection setup was designed to develop the first completely automated flow methodology for the expeditious, accurate in-line determination of halogenated organic compounds (HOCs) in water. The target compounds are preconcentrated and isolated by solid-phase extraction. Following elution, previously organically bound halogens are released as free hydrogen halides by the combined action of UV light and a chemical oxidant for their subsequent spectrophotometric determination by reaction with $Hg(SCN)_2$ and Fe^{3+}. Optimizing the major hydrodynamic and chemical variables resulted in improved performance. Recovery of various HOCs was assessed, and potential interferents were examined. Under the selected operating conditions, the proposed method exhibits variable analytical performance depending on the particular sample volume used (e.g., a sample volume of 5 mL provides a linear working range of 140−2000 $\mu g\ L^{-1}$, a LOD of 100 $\mu g\ L^{-1}$, and a throughput of 9 samples h^{-1}). The method was successfully used to determine total adsorbable organic halogens (AOX) in well water and leachates, and the results validated against an AOX reference method. The role of the proposed system in the environmental analytical field is critically discussed.

Analytical methodologies for determining group parameters facilitate expeditious assessment of water quality.[1] The family of HOCs (see abbreviation list in Table 1) is vast and heterogeneous. A complete study of all potentially present HOCs in a concrete sample is an enormous effort. Instead, HOCs are usually determined jointly as a group parameter, which is one of the keys to assessing water quality.[2]

HOCs can come from both natural[3–5] and anthropogenic sources. The massive industrial production of HOCs has been the origin of many environmental problems. In fact, HOCs are formed in the synthesis of a variety of products (e.g., pesticides, disinfectants)[6] and in the manufacture of the most extensively used products worldwide (e.g., plastics, paper, dyes).[7] In addition, HOCs can be generated as byproduct in waters containing large amounts of OCs and X^- ions (e.g., hospital wastewater, industrial effluents).[8,9]

Table 1. List of Abbreviations

abbreviation	description
HOCs	halogenated organic compounds
OCs	nonhalogenated organic compounds
X^-	inorganic halides
HX	hydrogen halides
AOX	adsorbable organic halogens
EOX	extractable organic halogens
TOCl	total organic chloride
TOBr	total organic bromide
TOI	total organic iodide
$OrgCl^-$	Cl^-, Br^-, and I^- ions released from HOCs
DOC	dissolved organic carbon

Most available procedures for determining HOCs as a whole involve three main stages, namely: enrichment and concomitant isolation of HOCs from the matrix of the sample; mineralization procedure of HOCs, with release of CO_2 and free HX; and quantitation of the previously released HX. The most widely accepted standard methodology for the group determination of HOCs is based on their adsorption in activated charcoal, followed by pyrolysis and coulometric titration; the result is called the "AOX group parameter".[10] There are, however, alternative analytical methodologies for this purpose including the (a) organic solvent extractable organic halogens (EOX);[11] (b) the TOCl determination by resin sorption in combination with particle induced X-ray emission spectroscopy;[12] (c) the differential determination of AOX as TOCl, TOBr, and TOI with ion chromatographic rather than

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coulometric detection;[13] (d) the manual determination of AOX by resin sorption followed by microwave digestion of the resin and spectrophotometric detection;[14] (e) the determination of AOX by atomic emission spectrometry (AES) rather than coulometry;[15] (f) the AOX determination using thermal desorption rather than pyrolysis prior to AES detection;[16] and (g) an automated batch determination method involving flow injection preconcentration of AOX in activated charcoal, followed by automatic discharge of the charcoal into a pyrolytic wave and AES detection.[17] To the best of our knowledge, no existing methodology for this purpose relies on an in-line continuous procedure for the simple, rapid determination of HOCs in a completely automated manner, even though it might substantially surpass existing choices in performance. Various flow injection techniques[18–20] have proved highly effective toward automating analytical procedures. One such technique is multisyringe flow injection (MSFI),[21–24] where fluids are driven across a flow manifold by a multisyringe buret (MS). An MS with 4 syringes affords up to 32 different injection modes, thereby vastly expanding the flexibility of automated microfluid handling.

In this work, the MSFI technique was used for the automated in-line determination of HOCs as a group parameter. To this end, organic compounds were preconcentrated and isolated from their sample matrix by solid-phase extraction (SPE). The solid phase was rinsed with a nitrate solution in order to effect competitive desorption of interfering X^- ions, the target compounds being eluted with an organic solvent/water mixture. The eluate was mixed with a chemical oxidant (persulfate ion, $S_2O_8^{2-}$) and irradiated with UV light. HOCs were thus oxidized and released CO_2, which was removed with a debubbler, and HX as a result. Released HX were then reacted with $Hg(SCN)_2$ to obtain free SCN^- ions, which were concomitantly reacted with Fe^{3+} to obtain a deeply colored coordination complex. Monitoring the absorbance of the complex at 480 nm allowed the analytical signal for released Cl^-, Br^-, and I^- ions, measured as micrograms of $OrgCl^-$ L^{-1}, to be obtained.

The $X^-/Hg(SCN)_2/Fe^{3+}$ reaction system[25] has proved efficient for determining X^- ions in both aqueous[26–28] and polar organic media.[9] In this work, the ions were determined in an aqueous/organic mixed medium. We chose to use this reaction system on the grounds of its simplicity and also for comparison with the detection method typically used by AOX standard analyzers, which afford the simultaneous determination of Cl^-, Br^-, and I^- ions released from HOCs. *Mercury(II) thiocyanate is very toxic by inhalation, in contact with skin, and if swallowed. It is environmentally hazardous and highly toxic to aquatic organisms. Protective gear should be used at all times and the waste products handled as hazardous chemical waste.*

The proposed MSFI setup was initially operated as a sequential injection (SI)[19] preconcentration system; however, once the eluent was released, it was switched to a forward flow mode as in classical flow injection[18] systems.

In summary, the aim of this work was to explore the use of the MSFI technique for improving existing methodologies for the determination of HOCs as a group parameter and to develop the first approach to date to the accurate, expeditious assessment of HOCs. The applicability of the proposed system is reflected in its intrinsic features (adsorption−UV irradiation−oxidation−spectrophotometric detection). This is the first reported method for the completely automated in-line determination of the parameter AOX.

EXPERIMENTAL SECTION

Chemicals and Sorbents. All chemicals were reagent-grade and used without further purification. Millipore-quality water was used to prepare solutions. The reagent stock solutions used included the following: (a) 2 mmol L^{-1} mercury(II) thiocyanate (0.063 g of $Hg(SCN)_2$ (Fluka, Deisenhofen, Germany) in 0.1 L of a 99:1 (v/v) water−methanol mixture), which was prepared on a daily basis under gentle heating in a steam bath; (b) 0.3 mol L^{-1} iron(III) (12.12 g of $Fe(NO_3)_3 \cdot 9H_2O$ (Fluka) in 0.1 L of 1.3 mol L^{-1} HNO_3); (c) sodium persulfate (5 g of $Na_2S_2O_8$, Fluka, in 0.1 L of 0.13 mol L^{-1} HNO_3); and (d) sodium nitrate (0.2 g of $NaNO_3$, Fluka, in 0.1 L of 0.13 mol L^{-1} HNO_3). The eluents used were methanol, ethanol, 2-propanol, and acetonitrile (all from Panreac, Castellar del Valles, Spain). HOC stock standard solutions containing 1 g of $OrgCl^-$ L^{-1} were prepared by dissolving 0.362 g of 4-chlorophenol (Aldrich, Deisenhofen, Germany) in 0.1 L of 0.01 mol L^{-1} NaOH. The other HOCs studied, which are referred to in subsequent sections and were all obtained from Aldrich, were also dissolved in 0.01 mol L^{-1} NaOH or, if necessary, in the minimum amount of methanol. Working-strength solutions were prepared by appropriate dilution of the stocks and acidified to pH <2 with HNO_3. Various commercial disinfectants including Preventol BP and Preventol CMK (Lanxess), Zotal D (Zotal), and Cresovex S (SP Veterinaria) were also studied. These products contain one or more HOCs in their composition, the amount of each component being stated by the manufacturer on its commercial container. Preventol BP and Preventol CMK are solid chlorinated phenols, so they were dissolved and diluted to appropriate concentrations. Zotal D and Cresovex S are commercial solutions containing 2.65 and 18.3 g of $OrgCl^-$ L^{-1}, so they were directly diluted to appropriate concentrations. The solid reversed-phase materials used to extract HOCs were Chromabond HR-P.AOX (a modified poly(styrenedivinylbenzene) resin from Macherey-Nagel); C18-octadecyl-, SDB-XC-poly(styrenedivinylbenzene)- and SDB-RPS- poly(styrenedivinylbenzene)-modified sorbent disks (Empore 3M, St Paul, MN).

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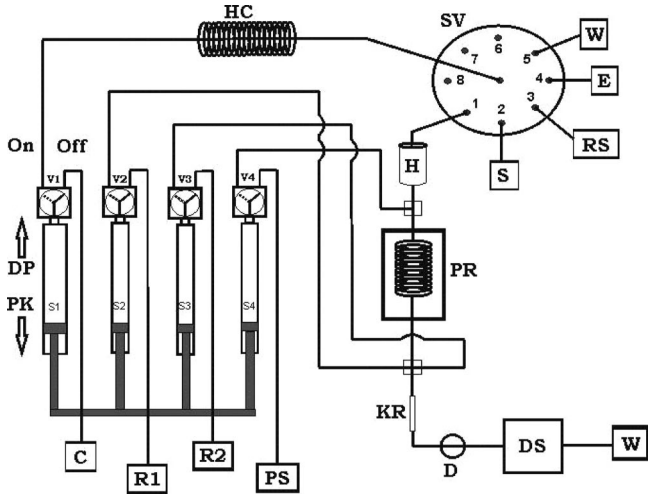

Figure 1. Schematic depiction of the proposed MSFI setup for the determination of HOCs. S1−S4 syringes, V1−V4 solenoid valves, C carrier reservoir, R1 $Hg(SCN)_2$ solution reservoir, R2 Fe^{3+} solution reservoir, PS $Na_2S_2O_8$ solution reservoir, DP dispense solutions, PK pickup solutions, Off normally open position of the solenoid valves (syringes connected with the reservoirs), On normally closed position of the solenoid valves (syringes connected to the flow network), HC holding coil, SV selection valve, S sample solution reservoir, RS rinsing solution reservoir, E eluent solution reservoir, W waste reservoir, H flow through sorbent holder, PR photoreactor, KR knotted reactor, D debubbler, and DS detection system.

Standard Reference Method. The proposed methodology was validated by using a suitable reference method for determining HOCs as a group parameter. Concretely; a Euroglas (Delft, The Netherlands) ECS 1200 AOX analyzer was used to implement the EN 1485[10] European Standard Method for AOX determination, which is similar to EPA Method 1650 for Adsorbable Organic Halides by Adsorption and Coulometric Titration. Briefly, this standard method involves the following steps: samples are acidified to pH <2 with HNO_3 and stored at 4 °C. Then, an Erlenmeyer flask is supplied with 100 mL of the water sample and 50 mg of activated charcoal and shaken for 1 h, after which the charcoal is filtered through a quartz holder and rinsed with a $NaNO_3$ solution to remove X^- ions. The quartz holder containing the filtered activated charcoal is then burnt at 950 °C to release HX compounds, which are transported to a microcoulometric cell by an O_2 stream. Finally, released HX compounds are titrated by precipitation as silver halides. This method provides a linear working range of 10−300 μg of $OrgCl^-$ L^{-1}. In theory, it can tolerate a maximum amount of X^- and DOC of 1000 and 100 mg L^{-1}, respectively; however, real measurements provided a maximum tolerated levels of only 600 mg L^{-1} X^-.

Instrumentation and Software. Figure 1 depicts the proposed flow system. A multisyringe automatic buret (MS) from Crison (Alella, Barcelona, Spain) was used to drive the liquid to the flow network. The buret was equipped with four syringes (S1−S4), and each syringe is mounted between a common metallic bar and its own three-way solenoid valve (V1−V4; N-Research, West Caldwell, NJ); as a result, the four syringes were operated simultaneously. Depending on the position of the solenoid valves, the fluids contained in syringes were loaded (PK, pickup) or dispensed (DP, dispense) to the flow network (on) or the reservoirs (off). Syringe S1 (10 mL) acted as a flow carrier and was connected to an eight-port selection valve (SV) from

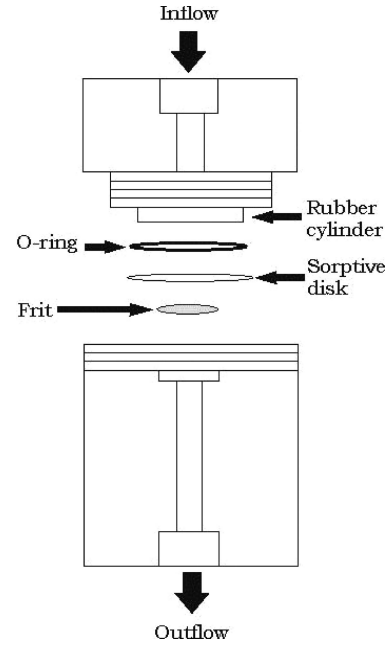

Figure 2. Schematic depiction of the device used for in-line solid-phase extraction with sorbent disks 9 mm in diameter.

Crison, which allowed the sequential automated microfluid handling of the sample (S), rinsing solution (RS), and eluent (E). The other three syringes, however, were used for injection of their respective solutions in the forward flow mode, thereby avoiding the typical mixing problems of sequential injection systems. S2 (5 mL) and S3 (5 mL) contained $Hg(SCN)_2$ and Fe^{3+} solutions, respectively, and S4 (2.5 mL) was filled with $Na_2S_2O_8$ solution.

Miniaturized solid-phase extraction was performed in a laboratory-made holder (H) shown in Figure 2. The holder was a Perspex cylindrical block (2 cm × long 1.3 cm i.d.) with a central cavity in which an extraction disk 9 mm in diameter was accommodated in order to pass the different fluids involved in the SPE procedure. The PR consisted of a laboratory-made wood box accommodating an 8-W mercury lamp (λ_{max} = 254 nm) with a poly(tetrafluoroethylene) (PTFE) tube 200 cm long × 0.8 mm i.d. directly wound around it. Just in the outlet of the PR, a PTFE tube knotted reactor is placed (50 cm × 0.8 mm i.d.) to facilitate the reaction product development and also with a debubbler (D, Trace Analytics, Braunschweig, Germany). All other tubing was also PTFE (0.8 mm i.d.), and merging connectors were made from poly(methyl methacrylate) (PMMA). The DS consisted of a deuterium−halogen light source (Mikropack, Ostfildern, Germany), two optical fibers 400 μm in diameter (Ocean Optics, Dunedin, FL), a 18-μL glass flow cell of 1-cm path length (Hellma, Mülheim, Germany), and a USB 2000 miniature fiber-optic spectrometer (SPM) (Ocean Optics) connected to a computer via a USB interface. We chose to use dual-wavelength spectrophotometry (480 and 700 nm) in order to minimize the Schlieren effect.[30]

Execution of the analytical protocol was computerized by using the AutoAnalysis 5.0. software package (Sciware, Palma de Mallorca, Spain). The basic program contains appropriate dynamic link libraries[31] to implement MS, SV, and SPM.

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Table 2. Main Steps of the AutoAnalysis 5.0 Standard Analytical Procedure for AOX Determination

step	inst	system protocol	analytical description
1		start loop A	start AOX determination protocol
2		start loop B	start preconcentration cycle
3	SV	move to position 2	connecting S1 with S reservoir
4	MS	PK 5 mL at 5 mL min^{-1} (on/off/off/off)	S loading
5	SV	move to position 1	connecting S1 with the flow network
6	MS	DP 6 mL at 3 mL min^{-1} (on/off/off/off)	preconcentration of the target species
7		end loop B	end cycle; repeat *X* times
8	SV	move to position 3	connecting S1 with RS reservoir
9	MS	PK 0.5 mL at 5 mL min^{-1} (on/off/off/off)	RS loading
10	SV	move to position 1	connecting S1 with the flow network
11	MS	DP 2 mL at 3 mL min^{-1} (on/off/off/off)	removing possible X^- residues
12	SV	move to position 4	connecting S1 with E reservoir
13	MS	PK 0.5 mL at 2 mL min^{-1} (on/off/off/off)	eluent loading
14	SV	move to position 1	connecting S1 with the flow network
15	MS	DP 1 mL at 1 mL min^{-1} (on/off/off/on)	elution and mixing with oxidant
16		wait (stop flow for 60 s)	UV oxidation
17	SPM	start reading every 0.4 s (A, 480; R, 700)	start absorbance data acquisition
18	MS	DP 2 mL at 1.5 mL min^{-1} (on/on/on/off)	analytical signal acquisition
19	SPM	stop measure	finish absorbance data acquisition
20		end loop A	determination finished; repeat *X* times

Analytical Procedure. Table 2 summarizes the general analytical procedure for the automated determination of HOCs as a group parameter using the MSFI technique. The procedure is based on a main cycle (loop A) that includes the steps needed for complete sample pretreatment and subsequent determination of HOCs as $OrgCl^-$. Loop A includes a subcycle (loop B) that is used to preconcentrate 5 mL of sample—the maximum volume that can be loaded into HC. Loop B can be repeated as many times as required to increase the preconcentration factor of the target compounds. Once the preconcentration cycle is completed, the procedure continues with solid-phase rinsing prior to elution of the target species. Finally, an in-line approach is also used to oxidize the target species with $S_2O_8^{2-}$/UV light and detect them spectrophotometrically as $OrgCl^-$.

RESULTS AND DISCUSSION

In-Line Solid-Phase Extraction. According to DIN 38409-H22, the efficiency of activated charcoal, which is the classical sorbent for HOCs, can be increased by using copolymeric poly(styrenedivinylbenzene)-modified resins.[32] We tested this material as a solid phase and assessed its HOC extraction capabilities in two different forms, namely: (a) resin (HR-P.AOX) of pore diameter 60 Å, surface area 1270 $m^2\ g^{-1}$ and average particle size 85.80 μm packed in PMMA cylinders 18 mm long × 2 mm i.d.; and (b) disks (SDB-RPS) 47 mm in diameter consisting of 90% adsorbent particles 0.5 ± 0.05 mm in width linked by 10% PTFE that were used at a maximum flow rate of 100 mL min^{-1} (the particles had a pore diameter of 60 Å, a surface area of 450 $m^2\ g^{-1}$, and an average size of 12 μm, and a portion 9 mm in diameter of disk was placed in the holder of Figure 2, the resulting maximum flow rate in the SPE device being 4 mL min^{-1}). The sorbents were compared in terms of performance by using a solution containing 1.5 mg L^{-1} $OrgCl^-$ (from 4-chlorophenol) that was eluted with a 20:80 (v/v) water−ethanol mixture. The sorption disks were found to exhibit better analytical performance in terms of blank and signal repeatability, the absence of overpressure or particle compaction, a longer useful life of the sorbent material, no carryover, and a 56% increase in peak height. We thus chose to use the sorbent in disk form and tested C18 and SDB-XC as sorbents for the solid-phase extraction of HOCs. C18 disks were found to result in poor repeatability, significant carryover, and a 43% lower peak height than SDB-RPS disks. On the other hand, SDB-XC disks retained the favorable properties of SDB-RPS disks in addition to providing improved signal repeatability and 23% higher peak height. This led us to adopt SDB-XC disks as the sorbent material for SPE.

We studied methanol, ethanol, 2-propanol, and acetonitrile as potentially effective solvents for HOCs. To this end, we used 80:20 (v/v) solvent−water mixtures to elute a volume of 5 mL of the previous 1.5 mg L^{-1} 4-chlorophenol solution. The highest recoveries under these conditions were obtained with 2-propanol, those provided by methanol and ethanol being 30 and 60% lower, respectively. Also, acetonitrile was discarded owing to its incompatibility with the postcolorimetric reaction. Finally, the optimum proportion of 2-propanol in the water−alcohol mixed eluent was found to be 60% (v/v).

Once the most suitable sorbent material and eluent were chosen, we examined the influence of the operational variables related to their use. The effect of the eluent volume was studied over the range of 0.1−1 mL and a value of 0.5 mL selected. The effects of the preconcentration and elution flow rates were both studied over the range 1−4 mL min^{-1} (see Figure 3a). The flow rate adopted was 3 mL min^{-1} for preconcentration and 1 mL min^{-1} for elution. Mass calibration was done by using the selected SPE configuration to inject sampling volumes of 1−40 mL containing an identical amount of $OrgCl^-$ (4 μg). Calibration was linear over the range 1−30 mL, and 13% analyte breakthrough was obtained by using a 40-mL sample volume.

Oxidation of Halogenated Organic Compounds. Determining halogens bound to organic compounds requires their prior decomposition in order to release free hydrogen halides in solution. One easy, highly effective way of accomplishing such decomposition is by UV photooxidation,[33] which had previously

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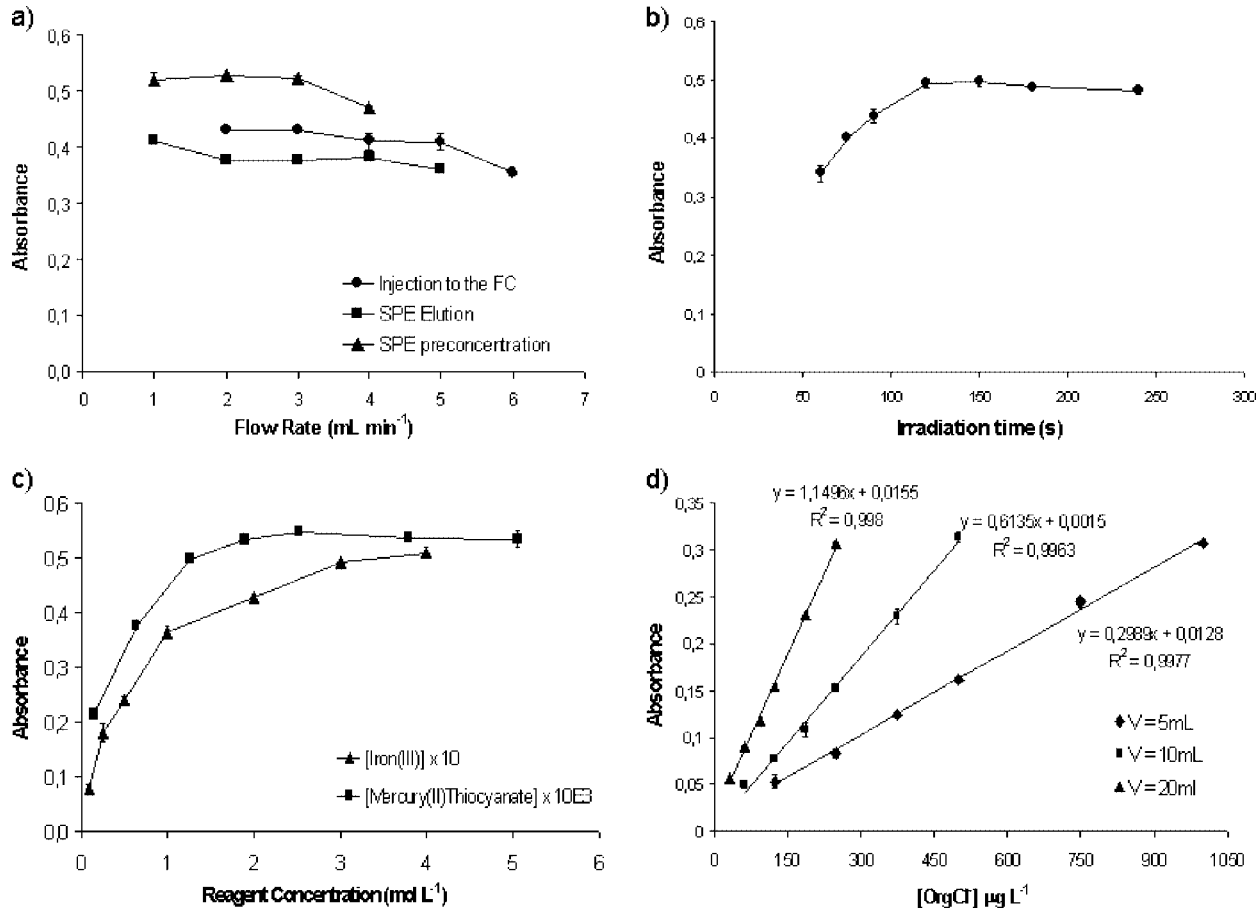

Figure 3. Influence on the net absorbance of (a) preconcentration, elution, and injection flow rates; (b) irradiation time; (c) $Hg(SCN)_2$ and Fe^{3+} concentrations; and (d) $OrgCl^-$ concentration in three different linear dynamic ranges. Each point in the graphs represents an average value (n = 3) ± its standard deviation.

proved quite efficient in the flow injection determination of other parameters such as total nitrogen,[34] total phosphorus,[35] and chemical oxygen demand.[36] In this work, the oxidation potential of UV light was combined with the action of a strong chemical oxidant (viz., 50 g L^{-1} $Na_2S_2O_8$, which was quite compatible with the other chemicals used). As shown in Figure 3b, the efficiency of the photooxidation step was assessed by examining the influence of the irradiation time on the net absorbance obtained by analyzing a solution containing 1.5 mg L^{-1} $OrgCl^-$ from 4-chlorophenol (the AOX standard compound used in the EN method). An irradiation time of 120 s was selected for further testing. The choice was supported by the results obtained in the analysis of a solution containing 1.5 mg L^{-1} $OrgCl^-$from 2,4,6-trichlorophenol (Aldrich), which is the AOX standard compound used in the EPA method.

Spectrophotometric Detection. The last step in the proposed methodology was detection of the previously released free Cl^-, Br^-, and I^- ions with the $X^-/Hg(SCN)_2/Fe^{3+}$ reaction system. In order to minimize the amount of reagents used, we examined the influence of the concentrations of $Hg(SCN)_2$ and Fe^{3+} on the net absorbance. As can be seen from Figure 3c, best results were provided by 2×10^{-3} mol of $Hg(SCN)_2$ L^{-1} and 0.3 mol of Fe^{3+} L^{-1}, which were thus adopted for subsequent experiments. After the flowing segment containing the target species was mixed with the $Hg(SCN)_2$ and Fe^{3+} solutions in the knotted reactor, the resulting reaction plug was injected into the FC. The effect of the injection flow rate was studied over the range 2−5 mL min^{-1}, and as can be seen from Figure 3a, the best results were obtained with 3 mL min^{-1} (1.5 mL min^{-1} relative to S1).

Analytical Performance. Once the final setup was established and the influence of the main operational variables examined, the proposed methodology was assessed in terms of performance.

A sample volume of 5 mL was found to provide a linear dynamic working range of 140−2000 μg of $OrgCl^-$ L^{-1}. The limits of detection (LOD) and quantitation (LOQ) were estimated as 3 and 10 times the standard deviation of the absorbance for 10 injections of the blank (a 5×10^{-2} mol L^{-1} HNO_3 solution) and found to be 100 and 140 μg L^{-1}, respectively. The sensitivity (slope) and regression coefficient were calculated from eight day-to-day regression curves and found to be 0.2997 ± 0.0044 L μg^{-1} and 0.9973 ± 0.0010, respectively. The repeatability of the proposed method was estimated as the relative standard deviation (RSD) for 10 consecutive injections of a 1 mg of $OrgCl^-$ L^{-1} solution; under these conditions, the RSD was 2.6%. The reproducibility was calculated from the RSD of the slopes of eight day-to-day regression curves and found to be 1.7%, which is roughly 5 times

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Table 3. Recoveries Obtained in the Analysis of Various HOCs with the Proposed System.[a]

target chemical	recovery (%)
4-chlorophenol	99
2-chlorobenzoic acid	99
3-methyl-4-chlorophenol	99
2-benzyl-4-chlorophenol	99
2-chlorophenol	98
2,4-dichlorophenol	98
2,4,6-trichlorophenol	94
pentachlorophenol	60
2-bromophenol	97
5-chloro-8-hydroxyquinoline	82
3,5-dibromosalicylic acid	98
3,5-diiodosalicylic acid	95
linuron	99
diuron	99
dieldrin	21
trichloroacetic acid	63
dichloroacetic acid	17
Preventol BP	99
Preventol CMK	98
Zotal D	98
Cresovex S	88

[a] All solutions contained 1 mg of $OrgCl^-$ L^{-1}.

better than that of AOX/EOX commercial analyzers.[2,10] The injection throughput (IT) as determined in accordance with the standard procedure was 9 samples h^{-1}. Although the operating endurance of the system was limited by the useful life of the SPE disk portion, the flow system was able to operate unattended for at least 12 h (110 injections). Consistent with the mass calibration, two additional linear working ranges obtained with 10 (LOD = 51 μg L^{-1}, LOQ = 70 μg L^{-1}, IT = 6 samples h^{-1}) and 20 mL (LOD = 27 μg L^{-1}, LOQ = 36 μg L^{-1}, IT = 3 samples h^{-1}) were established for better suitability to samples containing lower HOC levels. Figure 3d illustrates the response of the proposed system to variable sample volumes.

Response of the Proposed System to Various Halogenated Organic Compounds. We examined the response of the proposed system to various HOCs. To this end, we studied 4-chlorophenol recovery by comparing the results obtained in the direct spectrophotometric determination of 10 mg L^{-1} Cl^- from NaCl (Panreac) and the in-line UV photooxidation/spectrophotometric detection of the same amount of $OrgCl^-$. The recoveries of the other HOCs studied were established by subjecting a 1 mg L^{-1} $OrgCl^-$ solution from each to the above-described in-line procedure and comparing the results with those obtained for a solution containing 1 mg L^{-1} $OrgCl^-$ from 4-chlorophenol. The recoveries thus obtained from the commercial and chemical products studied are listed in Table 3. As can be seen, most were quite good. However, some strongly persistent polyhalogenated organic compounds with very strong carbon−halogen bonds (e.g., dieldrin) were only partially oxidized and provided low recoveries; such compounds require stronger oxidation conditions (e.g., a more powerful UV lamp or a pyrolytic oven). Highly polar HOCs were also poorly recovered; as in the AOX standard method, this was a result of their being only partially retained by the SPE sorbent (viz., dichloroacetic acid).

Interference Test. The main requirement for the selective determination of $OrgCl^-$ was complete removal of X^- ions from the flow network. This was accomplished by rinsing the sorbent material with an acidified NO_3^- solution. The effect was checked by spiking several solutions containing 100 μg of $OrgCl^-$ L^{-1} with a given amount of Cl^- and subjecting them to the above-described analytical procedure. No interference was found upon addition of Cl^- ion at concentrations up to 2 g L^{-1}; however, a concentration of 3 g L^{-1} Cl^- increased peak height by 30%. In any case, repeating the rinsing procedure should allow water samples with higher salt loads to be analyzed, at the expense of a decreased injection throughput.

One must also consider the potential interference of nonhalogenated dissolved OCs, the presence of which can reduce SPE efficiency through partial or complete saturation of the solid support and the consequent reduction in UV oxidation efficiency. In order to study the effect of OCs on the SPE-UV oxidation in-line pretreatment, several solutions containing 100 μg of $OrgCl^-$ L^{-1} were spiked with a given amount of DOC from phenol (Aldrich), a nonhalogenated OC for which the selected SPE sorbent material has a high retention affinity. The addition of 1 g of DOC L^{-1} was found to result in no interference; however, higher concentrations decreased the analytical signal through progressive saturation of the sorbent disk portion. In conclusion, the MSFI system provides increased selectivity in the determination of AOX relative to the AOX standard method; thus, it raises the maximum tolerated levels of X^- from 0.6 to 2 g L^{-1}, and those of DOC from 0.1 to 1 g L^{-1}. Samples with higher X^- and DOC contents (up to 100 and 1 g L^{-1}, respectively) can be subjected to the manual cleanup pretreatment described in Appendix 1 of the EN1485 standard method (see also DIN 38409-H22).

Application and Validation of the Proposed MSFI Procedure. The accuracy of the proposed methodology was assessed by applying it to the analysis of HOCs in real samples. Samples were collected in glass bottles, acidified to pH <2 with HNO_3, and stored at 4 °C. None exhibited high levels of potential interferents (X^- <2 g L^{-1} and DOC <0.5 g L^{-1}). Therefore, in order to validate the proposed methodology, various samples with a matrix of appropriate composition were analyzed with the AOX standard procedure (X^- <0.6 g L^{-1} and DOC <0.1 g L^{-1}) and the proposed MSFI method for comparison. Using a sample volume of 20 mL with the MSFI method provided a linear dynamic range similar to that for the standard method (36−500 μg of $OrgCl^-$ L^{-1} versus 10−300 μg of $OrgCl^-$ L^{-1}).

Water samples from three different wells in the vicinity of an urban solid waste treatment plant (Mallorca, Spain) and three samples from various demolition waste treatment plant leaching dumps (Mallorca, Spain) were analyzed. The well 1, leachate 1 and leachate 2 samples (all with X^- <0.6 g L^{-1} and DOC <0.1 g L^{-1}) had a suitable matrix composition for analysis with the MSFI and standard method. The results were compared via a *t*-test[37] that revealed the absence of differences at the 0.05 significance level. The samples leachate 3 (1 g of X^- L^{-1} and 0.2 g of DOC L^{-1}), well 2 and well 3 (both with 1.5 g of X^- L^{-1}) contained higher X^- or DOC levels than those afforded by the standard method; however, they were satisfactorily analyzed with the MSFI method and their recoveries (102−107%) verified by addition of 4-chlorophenol. The results are shown in Table 4. In summary, the proposed MSFI system allows the accurate determination of AOX.

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Table 4. Results Obtained in the Determination of Parameter AOX in Environmental Samples Using the Proposed MSFI Method and the EN-1485 Reference Method

Samples with a Matrix Suitable to Analysis with Both Methods

	added (μg L^{-1})	MSFI method ($n = 3$) found (μg L^{-1})	reference method ($n = 3$) found (μg L^{-1})
well 1	0	LOQ	
	150	148 ± 8	152 ± 2
leachate 1	0	194 ± 4	201 ± 5
leachate 2	0	228 ± 4	225 ± 3

Samples Suitable to Analysis with the MSFI Method Only

	added (μg L^{-1})	MSFI method ($n = 3$) found (μg L^{-1})	recovery (%)
well 2	0	LOQ	
	60	62 ± 6	103
well 3	0	59 ± 6	
	60	123 ± 11	107
leachate 3	0	240 ± 11	
	200	444 ± 12	102

Comparison with Other Methods of Similar Features. We compared the proposed method with others for the same purpose. No flow-based analytical methodology was seemingly reported previously, and only manual or automated batch methods for determining HOCs existed. The most similar choice for this purpose was AOX pyrolytic−coulometric commercial analyzers based on HOC sorption in activated charcoal HOCs followed by pyrolysis and coulometric detection; this approach, however, is rather different from the proposed MSFI methodology. By virtue of their intrinsic characteristics, these analysers provide a powerful mineralization procedure and better sensitivity levels than does the proposed MSFI system. On the other hand, the proposed system uses a faster, alternative in-line flow injection procedure and provides better standard deviations, in addition to markedly increased automation, robustness, cost-effectiveness, and simplicity.

Incorporation of the Proposed System into the Current Environmental Analytical Field. According to Valcárcel et al.,[38] analytical systems can be classified as vanguard analytical systems (VAS) and rearguard analytical systems (RAS). Separation techniques, which are by now widely established not only for detection, but also for identification, of HOCs,[39–41] are of the RAS type. However, vanguard systems for HOCs include various (AOX, EOX, TOX) analyzers that are useful for determining the total amount of HOCs without identifying them and can in principle be deemed VAS. In any case, the three main features a VAS are a rapid, reliable response, simplified analytical protocols, and instrumental robustness and cost-effectiveness; therefore, the previous analyzers are redefined as RAS for HOC group parameter determination and, in consequence of this, being defenseless for the VAS zone for HOC group determinations.

The proposed MSFI constitutes a true VAS for HOC assessment inasmuch as it avoids the use of complex, time-consuming equipment to determine total parameters. As such, it can help expand the range of existing avant-garde methodologies for the nonseparative determination of HOCs in water samples, which was done here with simple analytical instrumentation including an in-line flow manifold; this can reduce costs, servicing needs, work, and time, thereby increasing operating endurance and miniaturizability.

CONCLUSIONS

Above all, the proposed MSFI methodology clearly surpasses existing analytical alternatives for the same purpose. Thus, the proposed MSFI system provides a simple and smart tool for the completely automated in-line determination of HOCs, expressed as AOX, in water. Also, it features better standard deviations, throughput, simplicity, and flexibility than pyrolytic−coulometric AOX analyzers. In addition, the ensuing methodology uses highly cost-effective, compact equipment and can be easily applied to samples containing a wide variety of matrixes.

The primary aim of this work was to develop a simple, automated vanguard methodology for the rapid group determination of HOCs without the need to assess each individual compound, which requires using more complex, expensive, and time-consuming techniques such as gas chromatography−mass spectrometry.

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CAPÍTULO 5

NUEVAS APLICACIONES DE LA DETECCIÓN QUIMIOLUMINISCENTE MEDIANTE LA TÉCNICA DE ANÁLISIS POR INYECCIÓN EN FLUJO MULTIJERINGA COMBINADA CON MÉTODOS DE SEPARACIÓN EN LÍNEA

Este capítulo trata sobre el desarrollo de tratamientos en línea previos a la detección quimioluminiscente, mejorando de este modo la selectividad de este tipo de detección, y permitiendo el desarrollo de nuevas metodologías potencialmente útiles en las que la detección quimioluminiscente puede ser aplicada a muestras de matrices complejas. Para ello se utiliza por una parte el luminol como reactivo quimioluminiscente y una cámara de difusión gaseosa con membrana como dispositivo sencillo y efectivo para la separación de compuestos volátiles, conjuntamente con la técnica MSFIA. Esto ha permitido desarrollar una nueva metodología para la determinación de sulfuro en muestras medioambientales. Por otra parte, se propone la utilización conjunta del sistema quimioluminiscente Tris(2,2´-bipiridil)rutenio(II) con materiales monolíticos microporosos, para el desarrollo de métodos cromatográficos a baja presión mediante la técnica MSFIA, y procedimientos de extracción en fase sólida para el desarrollo de una metodología analítica para la determinación selectiva de trazas de varias tiazidas con acción diurética, tanto en muestras medioambientales como en muestras de orina. Además, se ha estudiado la modificación de columnas monolíticas mediante el uso de surfactantes para poder convertirlas en columnas aptas para la realización de separaciones de especies cargadas. Ello ha permitido establecer un nuevo método MSFIA basado en el sistema quimioluminiscente del Tris(2,2´-bipiridil)rutenio(II), para la determinación rápida de oxalato en muestras de cerveza y orina.

5.1. Aplicaciones analíticas de reactivos quimioluminiscentes

Actualmente hay una variedad de compuestos que emiten quimioluminiscencia (QL) en determinadas condiciones. Basándose en este hecho se han propuesto multitud de metodologías analíticas, correlacionando la respuesta obtenida con la presencia o cantidad de algún compuesto o especie de interés en la muestra. De entre todos los reactivos quimioluminiscentes, el luminol y el Tris(2,2´-bipiridil)rutenio(II) han sido dos de los más utilizados.

5.1.1. Luminol

La 5-aminoftalhidrazida más conocida como Luminol, es posiblemente el reactivo más ampliamente utilizado en reacciones quimioluminiscentes. La QL emitida por el luminol tiene lugar cuando este reacciona con agentes oxidantes. Algunos de estos agentes oxidantes satisfactoriamente usados para tal propósito son, entre otros, el peróxido de hidrógeno, hexacianoferrato(III), permanganato, N-Bromo- ó N-Clorosuccinimida, periodato, dicromato, persulfato, diclorocianurato, clorato ó hipobromito electrogenerado en medio básico.

La especie ó intermedio de reacción encargado de la emisión de luz es el anión 3-aminoftalato en estado excitado. La desactivación de este anión da un máximo de emisión en la zona del azul, a 425nm.

La importancia y amplio uso del luminol para la detección QL de compuestos o especies de interés recae no sólo en su eficiencia de emisión,

sino también en el amplio abanico de posibilidades que nos brinda la QL de este compuesto, debido a las diferentes formas en las que esta puede ser influida por la presencia de un analito. El analito puede participar en el mecanismo de reacción de la oxidación del luminol como potenciador, inhibidor ó catalizador, siendo la QL emitida proporcional a la concentración de este compuesto o especie, permitiéndonos no sólo su detección, sino también su cuantificación.

En la Figura 5.1 se expone el mecanismo de reacción abreviado del luminol. En ella pueden observarse las estructuras correspondientes al reactivo, al estado excitado emisor de luz y al producto final de la reacción.

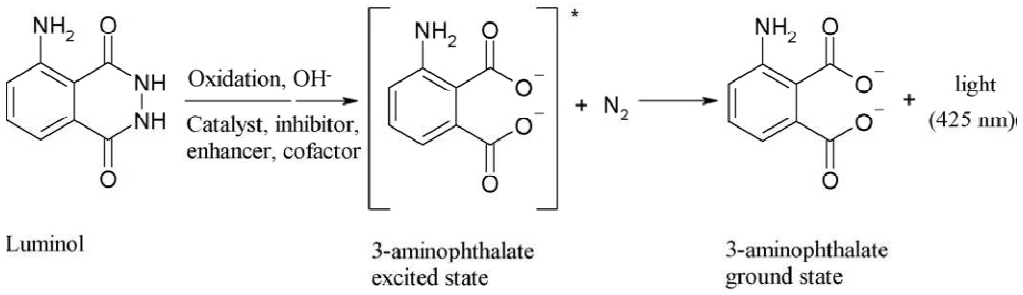

Figura 5.1. Mecanismo de reacción del luminol.

Como se ha comentado previamente, la emisión QL del luminol puede producirse con una gran variedad de compuestos con actividad catalítica sobre la reacción. La eficiencia de un catalizador está relacionada con su especificidad para reaccionar con un oxidante en particular. La enzima

peroxidasa, y más en concreto la extraída de la rúcula (armoracia rusticana) es considerada como el catalizador más eficiente para la reacción de luminol, debido a su alta especificidad en su reacción con el peróxido de hidrógeno. La reacción se produce en medios débilmente básicos (pH = 8-10), y se utiliza en muchos bio- e inmunoensayos. La reacción de luminol en este caso se utiliza para revelar posibles interacciones con la peroxidasa ó para la detección del peróxido de hidrógeno producido por una reacción específica.

La segunda vía considerada de mayor eficiencia para la generación de QL con luminol es mediante su oxidación electroquímica, usando el peróxido de hidrógeno como co-oxidante.

Una tercera alternativa eficaz para la producción de QL mediante el sistema luminol-peróxido de hidrógeno se basa en su catálisis mediante la adición de trazas de metales de transición tales como Co^{2+}, Cu^{2+}, Cr^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, Mn^{4+}, Ni^{2+},... o mediante el uso de algunos de sus complejos de coordinación tales como el ferroceno o el ferricianuro. Sin embargo, en este caso existen varias limitaciones, como una peor relación señal/ruido, así como el uso de medios de reacción más básicos.

La implementación de la detección quimioluminiscente se ve facilitada por su automatización mediante técnicas de análisis en flujo. A lo largo de los últimos años han aparecido un elevado número de publicaciones científicas consistentes en el desarrollo de nuevas metodologías automáticas basadas en

la reacción del luminol, para la detección de compuestos tan diversos como aminoácidos, cationes metálicos, pesticidas u otros tipos de contaminantes.

La principal ventaja de este tipo de detección reside en sus bajos limites de detección y amplios rangos dinámicos de trabajo, llegándose a detectarse en algunas aplicaciones cantidades de las especies de interés por debajo del orden de los femtomoles por litro (10^{-15}M). Sin embargo, el número de aplicaciones se ve limitado por la baja selectividad de la reacción del luminol. En muestras reales es bastante probable que se den casos de reactividad cruzada con otros posibles catalizadores o inhibidores presentes además del propio analito, obteniéndose falsos aumentos o disminuciones de la señal analítica.

Las reacciones QL tienen una selectividad limitada a muestras que en principio solo contengan una especie activa QL. Este hecho puede darse en el análisis de procesos de un determinado producto, por ejemplo, en la producción de alimentos o fármacos. En estos casos la detección QL no sólo es ventajosa por su sensibilidad, sino que permite la detección de un componente de la muestra el análisis del cual puede no ser selectivo mediante técnicas espectrofotométricas. En estos casos la detección quimioluminiscente será una alternativa eficaz al uso de otras técnicas.

Sin embargo, en el caso de muestras de una mayor complejidad como muestras biológicas o ambientales, el uso de la detección quimioluminiscente sólo es eficaz en técnicas de separación.

En estos casos se puede operar de dos modos distinto: (1) Separación de los compuestos y posterior adición del reactivo quimioluminiscente, ó (2) realización de un determinado proceso de derivatización enlazando los analitos con la especie QL y añadiendo el oxidante una vez la separación ha sido llevada a cabo. Para este último propósito se han propuesto una amplia variedad de compuestos derivados del luminol para un apropiado marcaje de péptidos, aminoácidos y varios compuestos con interés farmacológico.

Una alternativa desarrollada recientemente y que ha conferido una mayor selectividad a la detección QL, es su combinación con el uso de polímeros de impresión molecular[1]. Estos polímeros son sintetizados siguiendo un proceso en el cual se incorpora el compuesto de interés actuando éste como patrón, quedando, una vez sintetizado el polímero y eliminado el patrón, una serie de sitios específicos que permitirán el reconocimiento de nuevo de dicha molécula patrón. La mayor selectividad de estos materiales en comparación con los típicos materiales de extracción en fase sólida, será por lo tanto, una alternativa eficaz para ampliar la aplicabilidad de la reacción de QL del luminol.

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5.1.2. Tris(2,2´-bipiridil)rutenio(II)

El compuesto Tris(2,2´-bipiridil)rutenio(II) ($Ru(bipy)_3^{2+}$) fue sintetizado por F. H. Burstall en 1936[2] (Figura 5.2).

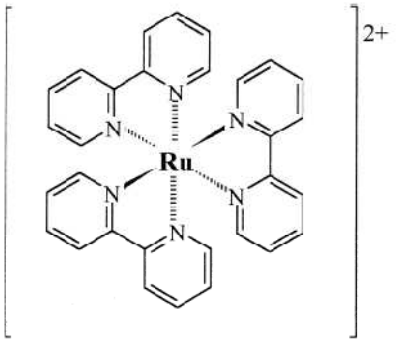

Figura 5.2. Estructura química del Tris(2,2´-bipiridil)rutenio(II)

Tres décadas después se publicó la evidencia de la emisión QL de este compuesto[3]. El $Ru(bipy)_3^{2+}$ disuelto en H_2SO_4 9N se oxidó a $Ru(bipy)_3^{3+}$ usando dióxido de plomo, el cual fue eliminado por centrifugación. Al reaccionar este compuesto con NaOH 9N se observó a simple vista una emisión de luz naranja durante un breve periodo de tiempo (menos de 1 segundo). Doce años después apareció la primera aplicación analítica de este compuesto[4], el cual fue utilizado para la determinación de una serie de compuestos farmacéuticos. En esta primera aplicación de la reacción QL del $Ru(bipy)_3^{2+}$, le especie activa QL $Ru(bipy)_3^{3+}$ fue electrogenerada, lo cual evita el uso de reactivos químicos

[2] F. H. Burstall, J. Chem. Soc. (1936) 173-175.
[3] D. M. Hercules, F. E. Lytle, J. Am. Chem. Soc. 88 (1966) 4745-4746.
[4] W. K. Nonidez, D. E. Leyden, Anal. Chim. Acta 96 (1978) 401.

adicionales así como posteriores procedimientos de centrifugación o filtración. En el año 1978 se publicó la primera aplicación analítica utilizando $Ru(bipy)_3^{3+}$ químicamente generado[5], para ello se sustituyó el uso del dióxido de plomo por sulfato de cerio (IV).

Independientemente del modo en que la especie activa QL se produce, el mecanismo general de la reacción es el siguiente:

$$Ru(bipy)_3^{2+} \xrightarrow{\text{oxidation}} Ru(bipy)_3^{3+} \xrightarrow{\text{analyte}} [Ru(bipy)_3^{2+}]^* \longrightarrow Ru(bipy)_3^{2+} + h\nu\ (610\ nm)$$

Desde entonces ha aumentado el uso de este reactivo en química analítica debido a la alta estabilidad del compuesto precursor ($Ru(bipy)_3^{2+}$) en medio acuoso y las bajas señales de fondo obtenidas. Pero sobre todo debido a su reactividad con una amplia variedad de compuestos, especialmente compuestos orgánicos. Los métodos desarrollados presentan en general una muy buena sensibilidad y amplios rangos de trabajo.

El principal problema de este sistema es la limitada estabilidad de la especie activa QL, es decir el $Ru(bipy)_3^{3+}$, siendo usualmente generado a partir de su precursor estable en los instantes previos a su reacción. Para ello, la generación electroquímica ha sido desde siempre la alternativa más utilizada. Sin embargo, en los últimos 10 años ha aumentado el uso de la generación química por oxidación mediante reacción con sulfato de cerio (IV), permanganato potásico o dióxido de plomo. Las diferencias obtenidas en las

[5] A. D. Karavaev, D. V. Kazakov, G. A. Tolstikov, V. V. Yakshin, N. L. Khokhlova, Zh. Anal. Khim. 41 (1986) 42.

características analíticas de un método, usando un sistema de generación u otro son muy pequeñas[6]. Sin embargo, la generación electroquímica permite evitar el uso de compuestos químicos adicionales, mientras que la generación química permite una mayor simplicidad instrumental.

Una información más exhaustiva sobre las propiedades QL de este compuesto, así como de sus aplicaciones puede verse recopilada en una serie revisiones bibliográficas[7] y libros[8].

5.2. Separación por difusión gaseosa

En muchas ocasiones, la medida directa de muestras en técnicas de análisis en flujo es difícil debido a la presencia de compuestos que pueden interferir en la determinación. Una alternativa para aumentar la selectividad en este tipo de metodologías analíticas es el uso de membranas para el pretratamiento de las muestras. Ejemplos de este tipo de pretratamientos son la diálisis, la pervaporación, o la difusión gaseosa.

La técnica de difusión gaseosa ha sido utilizada en técnicas de análisis en flujo para la separación en línea de compuestos volátiles previa a su detección, como por ejemplo en el caso del amoniaco[9]. Dicha técnica también

[6] J. M. Gonzalez, G. M. Greenway, T. McCreedy, S. Qijun, Analyst 125 (2000) 765-769.
[7] (a) W. Y. Lee, Mikrochim. Acta 127 (1997) 19-39. (b) R. D. Gerardi, N. W. Barnett, S. W. Lewis, Anal. Chim. Acta 378 (1999) 1-41. (c) A. W. Knight, Trends Anal. Chem. 18 (1999) 47-62. (d) K. A. Fahnrich, M. Pravda, G. G. Guilbault, Talanta 54 (2001) 531-559. (e) M. M. Richter, Chem. Rev. 104 (2004) 3003-3036. (f) B. A. Gorman, P. S. Francis, N. W. Barnett, Analyst 131 (2006) 616-639.
[8] Electrogenerated Chemiluminescence, ed. A. J. Bard, Marcel Dekker, New York, 2004.
[9] J. R. Clinch, P. J. Worsfold, F. W. Sweeting, Anal. Chim. Acta 214 (1988) 401-407.

es útil para la determinación de especies no volátiles que pueden ser convertidas en línea en especies volátiles, tales como el mercurio (II)[10] o el cianuro[11].

En la Figura 5.3 puede apreciarse una fotografía de la cámara de difusión gaseosa utilizada en esta tesis. Dicha cámara está compuesta por dos placas de metacrilato, teniendo cada una grabado un canal semi-circular en forma de U alargada de modo que superponiendo las dos placas nos quedaría un único canal.

Las características de dichos canales son: 10cm de longitud, 0.08cm de anchura, 0.04cm de profundidad, 0.025ml de volumen interno.

Estos canales se separan colocando entre ellos una membrana hidrofóbica. Se utilizan membranas de politetrafluoroetileno (PTFE) o de fluoruro de polivinilideno (PVDF) que son permeables a los gases a través de poros de pequeño diámetro (200nm de diámetro).

Uno de los dos canales se usa como canal dador, es decir, por él circula la disolución que inicialmente contiene el analito que posteriormente atravesará la membrana. El otro canal, conocido como canal aceptor, contiene una solución capaz de realizar la captación de la especie proveniente del otro canal de la cámara. Por ejemplo, en el caso del amoniaco, la disolución aceptora podría ser una disolución ácida, para de este modo captar el

[10] N. Amini, S. D. Kolev, Anal. Chim.Acta 582 (2007) 103-108.
[11] H. Sulistyarti, T. J. Cardwell, S. D. Kolev, Anal. Chim. Acta 357 (1997) 103-109.

amoniaco convirtiéndolo en ión amonio, o simplemente agua dada la gran solubilidad del amoniaco en ella).

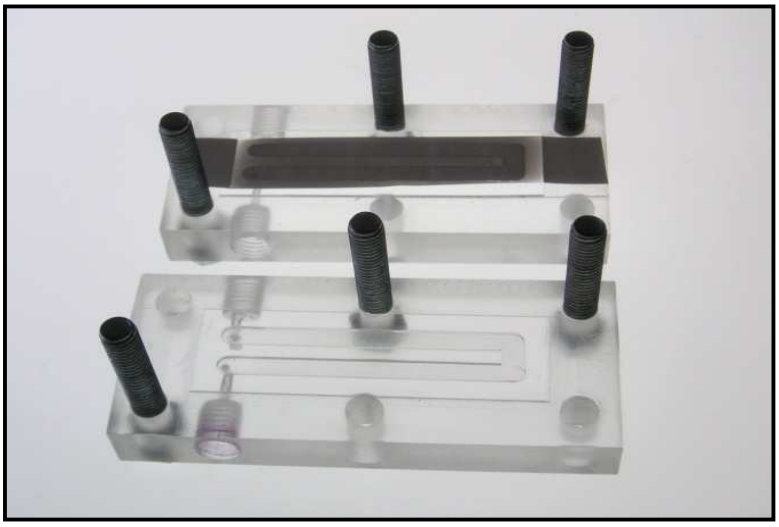

Figura 5.3. Cámara de difusión gaseosa.

Las membranas utilizadas para este propósito tienen una permeabilidad a los gases de aproximadamente un 15%. Es decir, un 15% del analito llegará al detector en unas condiciones mucho más selectivas que la inyección directa de la muestra. Sin embargo, el 85% restante del analito presente en la muestra no será aprovechado, obteniendo una sensibilidad mucho menor que en el caso de la inyección directa. Para solucionar este inconveniente, pueden utilizarse alternativas para la manipulación de los fluidos basadas en el uso de un sistema de propulsión para cada canal, o recurriendo al uso de válvulas adicionales para el manejo independiente de los flujos que circulan por los

canales dador y aceptor[12]. Utilizando diversas estrategias en la manipulación de fluidos podemos compensar la inicial pérdida de sensibilidad, e incluso llegar a obtener un cierto grado de pre-concentración en comparación con un procedimiento de inyección directa.

Algunas de estas estrategias son:

- Disolución aceptora y dadora, ambas en circulación.
- Disolución aceptora detenida y disolución dadora en circulación. Pre-concentrando los compuestos volátiles que van atravesando la membrana progresivamente en el pequeño volumen interno de la cámara donde se encuentra detenida la disolución aceptora.
- Disolución aceptora detenida y disolución dadora en circulación realizando varias pasadas en contacto con la membrana mediante inversión del flujo. De este modo se puede aumentar el factor de pre-concentración en la disolución aceptora detenida mediante la aspiración de la muestra una vez esta ya ha fluido por el interior de la cámara dadora. De este modo, el 85% de gases que teóricamente no han atravesado la membrana vuelven a estar en contacto con esta, como mínimo una segunda vez, permitiendo incrementar la cantidad de gas que ha atravesado dicha membrana.

[12] (a) M. T. Oms, A. Cerda, A. Cladera, V. Cerda, R. Forteza, Anal. Chim. Acta 318 (1996) 251-260. (b) S. D. Kolev, P. R. L. V. Fernandes, D. Satinsky, P. Solich, Talanta 79 (2009) 1021-1025.

5.3. Consideraciones sobre el sulfuro

La presencia de sulfuro en el medio ambiente puede proceder de fuentes naturales. El sulfuro de hidrógeno es emitido a la atmósfera debido a procesos geotérmicos, así como por erupciones volcánicas. También es liberado al medio acuoso debido a la descomposición de la materia orgánica, o como resultado de diversos procesos químicos anaeróbicos. Este sulfuro en medio acuoso, dependiendo de la acidez del medio, estará presente como ión hidrogenosulfuro (HS^-) o como ácido sulfhídrico (H_2S), desprendiéndose este último a la atmosfera.

Sin embargo, también existe un aporte de sulfuro al medio ambiente debido a fuentes antropogénicas como resultado del proceso de curtidos, plantas de tratamiento de aguas residuales, alcantarillados, fábricas textiles, fundiciones de hierro, procesado de alimentos, fabricación de asfalto, plantas de gas natural y plantas petroquímicas[13]. Además de las fuentes anteriores, el sulfuro de hidrógeno también se forma en vertederos debido a la descomposición de la materia orgánica. El sulfuro de hidrógeno llega a constituir hasta el 1% de los gases que se emiten en un vertedero[14].

El H_2S es uno de los componentes principales en el ciclo biogeoquímico del azufre. El H_2S directamente liberado en el agua o que proviene de la atmosfera, es fácilmente oxidado por el oxígeno presente en las aguas superficiales. Además el H_2S puede encontrarse en el medio acuoso formando

[13] D. C. Fuller, A. J. Suruda, J. Occup. Environ. Med. 42 (2000) 939-942.
[14] Agency for Toxic Substances and Disease Registry, US (2001), Annual Report. www.atsdr.cdc.gov.

parte del material particulado en suspensión, o en disolución en aguas que presenten escasez de oxígeno.

Existen en el medio acuoso, bacterias y hongos que producen la liberación de H_2S a partir del proceso de degradación de la materia orgánica, y más en concreto de las proteínas. Las bacterias anaeróbicas sulfato-reductoras, suelen encontrarse presentes en los sedimentos, y liberan H_2S mediante la reducción directa de los sulfatos presentes en el medio.

El sulfuro juega un papel importante dentro de los procesos biogeoquímicos, ya que forma sales insolubles con varios metales (Cu, Zn, Fe, Ni,…) que pueden estar presentes en los sedimentos o en el agua. También puede producirse el caso contrario, es decir, la liberación de metales al medio acuoso por la movilización de dichos sedimentos debido a la actividad bacteriana.

El H_2S se desprende fácilmente del agua. Dicha volatilidad depende sobre todo del pH del agua, pero también de otros factores como la temperatura o la concentración de ciertos iones metálicos.

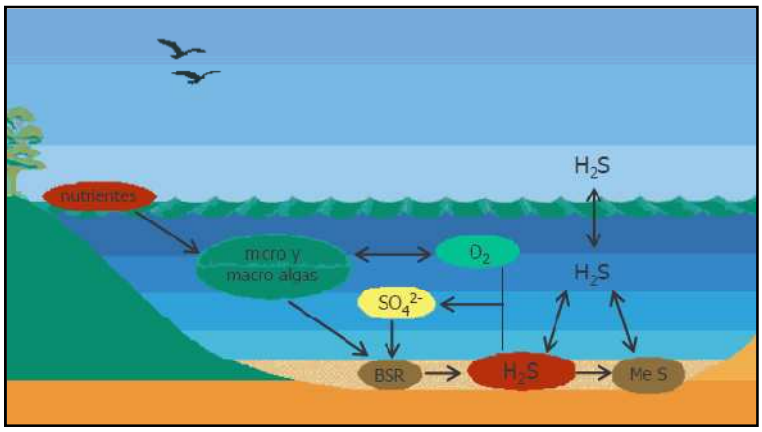

Figura 5.4. Modelo conceptual del H_2S en un lago. BSR: bacterias sulfato reductoras. MeS: sulfuros de metales

El H_2S en medio acuoso se comporta como un ácido débil, sus constantes de acidez son: pKa_1= 6.88, pKa_2= 14.15. Por lo tanto, sus formas mayoritarias en medio acuoso serán como H_2S ó HS^-. Estas especies son rápidamente oxidadas por el oxígeno presente en las aguas superficiales. Los tiempos de vida media para dichas especies en agua a 25ºC y pH= 8 son de 50 y 26 horas para el agua dulce y el agua marina, respectivamente. En las aguas residuales la concentración de sulfuro puede ser controlada mediante la adición de oxidantes químicos.

En aguas naturales se pueden producir sulfuros en ambientes anóxicos. Sin embargo, niveles más elevados pueden encontrarse debido a factores antropogénicos. Mientras que en aguas naturales los sulfuros pueden encontrarse en el rango de los µg L^{-1}, en desechos urbanos e industriales estos pueden encontrarse a niveles de hasta varias decenas de mg L^{-1}. En los

efluentes de las plantas de procesamiento de pieles pueden encontrarse niveles de hasta 200mg L^{-1}, ya que el sulfuro sódico es usado en altas concentraciones (2g L^{-1}) en las propias disoluciones de procesado de la piel[15].

La presencia de sulfuros puede ser detectada a partir del mal olor que produce el desprendimiento de H_2S, incluso a niveles de traza. La mínima concentración de H_2S detectable por la nariz humana es de 0.02mg L^{-1}, pero la máxima sensibilidad se halla a una concentración de aproximadamente 5mg L^{-1}. Por encima de este nivel, o a través de una exposición prolongada, la percepción de este gas se ve afectada debido a los efectos neurotóxicos que produce este sobre los nervios olfatorios[16].

El sulfuro actúa como un veneno celular causando la desactivación de la respiración aeróbica, pudiendo causar la muerte por asfixia. Dosis letales, dependiendo del tiempo de exposición, pueden ser de entre 0.3-1g L^{-1}. A dosis similares, el H_2S presenta una mayor toxicidad que el cianuro de hidrógeno (HCN). Sin embargo, un mayor número de intoxicaciones son producidas por el HCN, ya que al contrario que el H_2S, este es difícilmente detectable olfativamente. Casos clínicos de intoxicación por H_2S presentan usualmente niveles de entre 0.03-3mg L^{-1} de esta sustancia. Debido a las bajas cantidades de H_2S necesarias para causar problemas fisiológicos, los métodos

[15] N. S. Lawrence, J. Davis, R. G. Compton, Talanta 52 (2000) 771-784
[16] (a) P. Patnaik, A Comprehensive Guide to the Hazardous Properties of Chemical Substances, second ed., Wiley, New York, NY, 1999. (b) R. P. Pohanish, S. A. Greene, Hazardous Materials Handbook, Van Nostrand Reinhold, New York, 1996. (c) W. Puacz, W. Szahun, Analyst 120 (1995) 939-941.

desarrollados para su detección deben ser rápidos y a su vez altamente sensibles.

5.4. Desarrollo de un nuevo método para la determinación rápida y selectiva de trazas de sulfuro en muestras complejas

Tal como se ha descrito en el anterior apartado, dos de las características más importantes que debe poseer un método para la determinación de sulfuro son sensibilidad y velocidad de análisis.

En la Figura 5.5 podemos ver distintas estrategias para la determinación de sulfuro. Como podemos apreciar, la mayor parte de las posibilidades que tenemos para su detección, se basan en la conversión del sulfuro en H_2S.

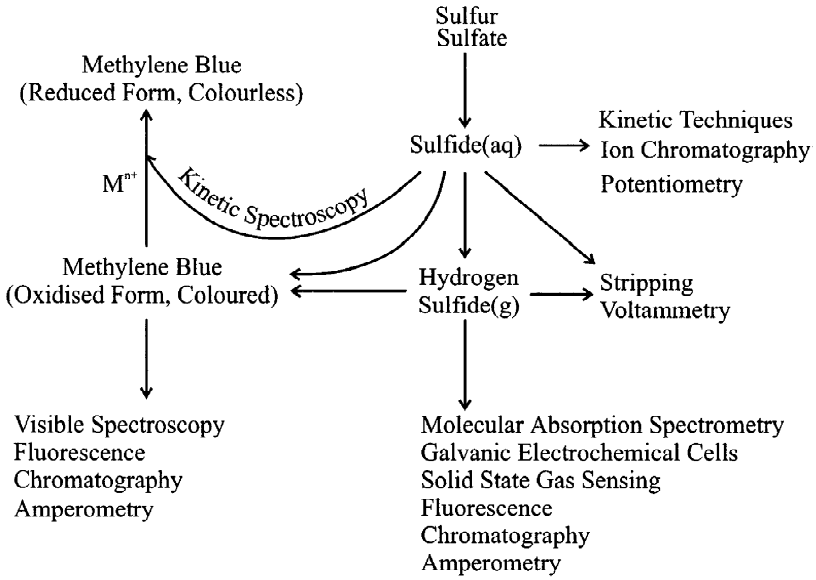

Figura 5.5. Estrategias utilizadas para la detección de sulfuro.

La detección de sulfuro puede realizarse mediante métodos cinéticos, electroquímicos, espectrofotométricos o por fluorescencia. Sin embargo, un gran número de métodos se basan en su transformación en el colorante azul de metileno, o en su separación cromatográfica previa a la detección. Incluso se puede combinar el proceso de transformación previo a la separación cromatográfica, por lo que se separa el colorante en vez del sulfuro.

Independientemente del sistema de detección, la velocidad de análisis de estos métodos se verá considerablemente incrementada al ser automatizados mediante técnicas de análisis en flujo, tal como puede apreciarse en la revisión bibliográfica realizada por L. Ferrer et al[17]. Sin embargo, en la mayoría de los casos los métodos más rápidos presentan una baja sensibilidad, mientras que los métodos con mayor sensibilidad presentan velocidades de análisis relativamente bajas.

Entre los años 2001 y 2002 fueron propuestos varios métodos para la detección quimioluminiscente de sulfuros, con una alta sensibilidad y simultáneamente altas velocidades de análisis debido a que las reacciones eran muy rápidas y a que dichos métodos fueron automatizados mediante la técnica FIA. Estos métodos se basaban en reacciones entre el sulfuro y el luminol[18], la N-bromosuccinimida[19] (ó N-clorosuccinimida), ó con isocianuratos clorados[20].

[17] L. Ferrer, M. Miró, J. M. Estela, V. Cerdà, Trends Anal. Chem. 26 (2007) 413-422.
[18](a) J. X. Du, Y. H. Li, J. R. Lu, Chinese J. Anal. Chem. 29 (2001) 189-191. (b) H. X. Du, Y. H. Li, J. R. Lu, Anal. Chim. Acta 448 (2001) 79-83.
[19] A. Safavi, M. A. Karimi, Talanta 57 (2002) 491-500.
[20] A. Safavi, M. A. Karimi, M. R. Hormozinezhad, Anal. Lett. 35 (2002) 2023-2037.

Sin embargo carecían de selectividad, lo que imposibilita su aplicación a muestras con matriz compleja, tal como son las muestras ambientales.

De acuerdo con lo anterior y teniendo en cuenta trabajos previamente realizados mediante la técnica MSFIA, se concluye que esta puede ser una herramienta eficaz para la automatización de métodos directos de análisis basados en la detección QL[21]. Además la técnica MSFIA, debido a sus diferentes posibilidades en el manejo automático de fluidos nos permite la utilización flexible de dispositivos de separación por difusión gaseosa a través de membrana, tal como se ha comentado anteriormente.

Por lo tanto, el desarrollo de una nueva metodología de análisis completamente automática que combine la separación por difusión gaseosa con la detección QL podría ser un método rápido y altamente sensible para la determinación de sulfuro. Además al realizar una separación por membrana obtenemos una mayor selectividad permitiéndonos la aplicación de dicho método a muestras complejas.

La metodología analítica que se pretende desarrollar se basa en la acidificación de la muestra para la conversión de los sulfuros en ácido sulfhídrico, con lo que una parte de este se volatiliza atravesando la membrana de difusión gaseosa. También se utiliza una disolución de pH básico, en el canal aceptor de la celda de difusión gaseosa, que podemos combinar

[21] (a) N. Pizà, M. Miró, G. de Armas, E. Becerra, J. M. Estela, V. Cerdà, Anal. Chim. Acta 467 (2002) 155-166. (b) M. Miró, J. M. Estela, V. Cerdà, Anal. Chim. Acta 541 (2005) 55-66.

perfectamente con la detección QL basada en la reacción del luminol, ya que esta también se efectúa también en un medio básico.

Finalmente se decidió montar un sistema con dos buretas multijeringa. La primera bureta se encarga de la toma de muestra, su acidificación, y su inyección a través del canal dador de la cámara de difusión gaseosa. La segunda bureta se encarga del manejo de la disolución básica aceptora del sulfuro transferido, y su traslado al detector de quimioluminiscencia mezclándose previamente con los reactivos adecuados (luminol y peróxido de hidrógeno). Es decir, la primera bureta se utiliza como sistema de introducción de los compuestos volátiles en medio ácido presentes en la muestra en el sistema de detección QL, el manejo de fluidos se regula a través de la segunda bureta multijeringa.

El desarrollo de un método rápido y sensible para la determinación de sulfuro en muestras ambientales se encuentra detallado en el punto 5.12.

5.5. Contaminantes emergentes en el medio ambiente

En los últimos años la presencia y el destino de compuestos farmacéuticos en el medio acuático, ha sido reconocido como una de las temáticas más importantes dentro del campo de la química ambiental[22]. No sólo debido al desecho de medicamentos no usados a través de las tuberías del agua, sino

[22] (a) B. Halling-Sorensen, N. Nielsen, P. F. Lansky, F. Ingerslev, L. Hansen, H. C. Lutzhoft, S. E. Jorgensen, Chemosphere 36 (1998) 357-394. (b) C. G. Daughton, T. A. Ternes, Environ. Health Perspect. 107 (1999) 907-938.

también a la incompleta eliminación de estos compuestos una vez ha sido realizada su ingestión. A menudo estos compuestos son excretados por el cuerpo humano sin ser metabolizados, o habiendo sido ligeramente transformados. Una vez en el sistema de aguas residuales pueden pasar a través de las plantas de tratamiento sin ser eliminados, siendo finalmente liberados al medio ambiente. Estos compuestos farmacéuticos pueden acabar en los acuíferos, pudiendo ser consumidos de nuevo por el ser humano, esta vez de forma accidental.

Otras fuentes para la introducción de compuestos farmacéuticos en el medio ambiente es a través de lixiviaciones producidas en vertederos incontrolados, o el vertido ilegal de residuos procedentes de la industria farmacéutica, lo cual ocurre principalmente en países subdesarrollados donde las legislaciones vigentes son más tolerantes o incluso inexistentes en este sentido. También es de gran relevancia el uso de grandes cantidades de fármacos en la industria ganadera. La Figura 5.6 presenta un esquema sobre las posibles vías que pueden seguir los compuestos farmacéuticos en el medio ambiente.

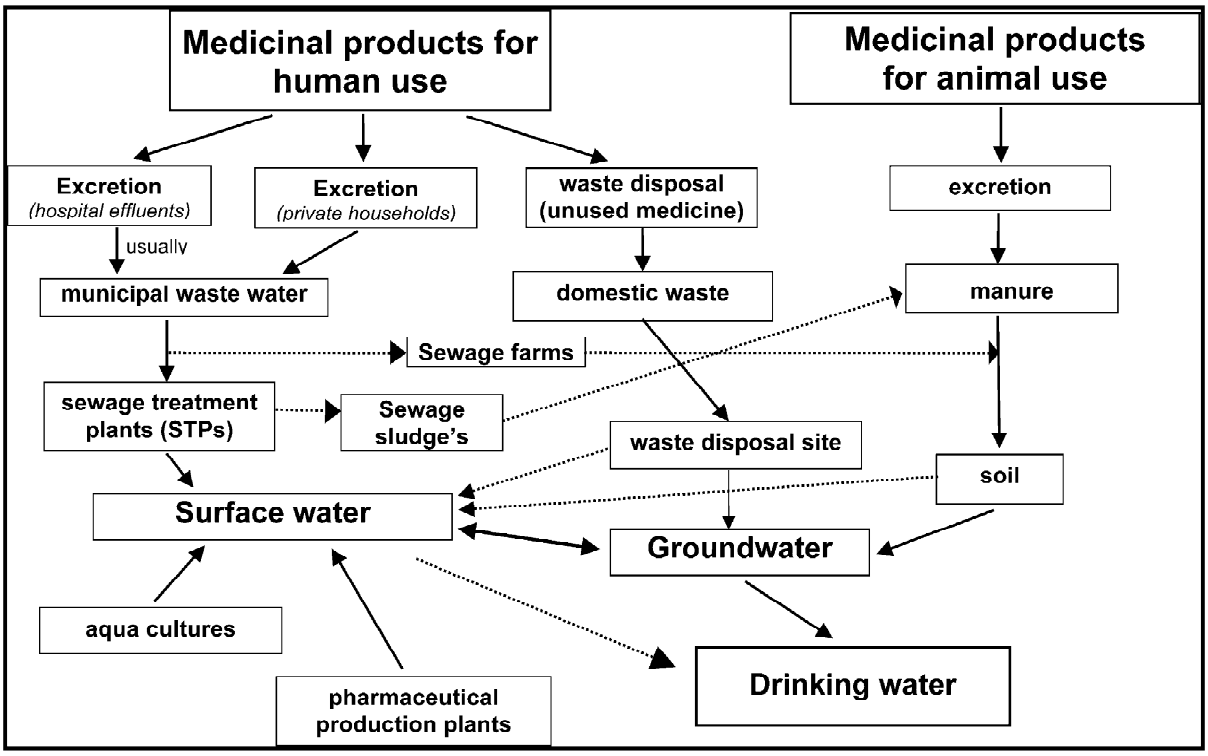

Figura 5.6. Posibles fuentes y vías para el transporte de compuestos farmacéuticos en el medio acuoso.

Los fármacos más usados en humanos, y que pueden ser encontrados en el medio ambiente, son generalmente analgésicos, antibióticos, diuréticos, antiasmáticos, hipotensivos ó psicolépticos. También pueden hallarse fármacos administrados a animales en el sector de la ganadería.

5.6. Columnas monolíticas y cromatografía líquida en sistemas de análisis en flujo

Uno de los temas importantes de la Química Analítica está relacionado con las técnicas de separación, puesto que son necesarias para la realización de determinaciones analíticas selectivas en áreas tales como la medicina, el medio ambiente o la industria farmacéutica.

Uno de los métodos más ampliamente utilizados es la cromatografía líquida, sin embargo, el uso de columnas cromatográficas convencionales de partículas de pequeño diámetro ha demostrado ciertas limitaciones. Por este motivo, el desarrollo de nuevos materiales para su uso como fases estacionarias en cromatografía líquida es un campo en constante desarrollo.

A lo largo de estos últimos años ha suscitado una gran expectación un nuevo tipo de fases estacionarias que utilizan materiales monolíticos. Debido a su facilidad de preparación, sus excelentes propiedades, así como a sus altas prestaciones en comparación con las columnas cromatográficas convencionales, principalmente en la separación o purificación de biomoléculas de gran tamaño.

Una columna monolítica es una estructura continua que conforma un esqueleto poroso. Dicha estructura se prepara in-situ en el mismo tubo o dispositivo que la va a contener. La preparación de dichos materiales tuvo sus inicios a principios de la década de los 70, siendo unos 20 años más tarde cuando se empezaron a introducir las columnas monolíticas en el campo de las

separaciones tal y como las conocemos actualmente[23]. Estas columnas estaban basadas en polímeros orgánicos rígidos producidos siguiendo mecanismos de reacción sencillos, lo que en años posteriores supuso una amplia expansión en el uso de este tipo de materiales. Posteriormente, varios grupos de investigación desarrollaron columnas monolíticas de sílice[24].

De entre estos dos tipos de columnas monolíticas, las que han tenido una amplia aceptación son las de sílice frecuentemente funcionalizadas con grupos octadecilo. Estas columnas, además de para la separación de moléculas de gran tamaño, presentan una buena eficiencia para la separación de pequeñas moléculas. El método más reconocido para su síntesis obteniendo una porosidad homogénea, es conocido como método sol-gel. Este método se basa en la condensación hidrolítica de un alcoxisilano en presencia de polímeros orgánicos solubles en agua. El material poroso obtenido puede ser posteriormente modificado, por ejemplo, con grupos octadecilo (C18).

Las columnas monolíticas han sido utilizadas para el desarrollo de un gran número de aplicaciones de cromatografía líquida de alta presión (HPLC)[25].

[23] F. Svec, J. M. J. Frechet, Anal. Chem. 64 (1992) 820-822.
[24] (a) S. M. Fields, Anal. Chem. 68 (1996) 2709-2712. (b) H. Minakuchi, K. Nakanishi, N. Soga, N. Ishizuka, N. Tanaka, Anal. Chem. 68 (1996) 3498-3501.
[25] (a) H. F. Zou, X. D. Huang, M. L. Ye, Q. Z. Luo, J. Chromatogr. A 954 (2002) 5-32. (b) N- Ishizuka, H. Kobayashi, H. Minakuchi, K. Nakanishi, K. Hirao, K. Hosoya, T. Ikegami, N. Tanaka, J. Chromatogr. A 960 (2002) 85-96. (c) N. Tanaka, H. Kobayashi, N. Ishizuka, H. Minakuchi, K. Nakanishi, K. Hosoya, T. Ikegami, J. Chromatogr. A 965 (2002) 35-49. (d) K. Cabrera, J. Sep. Sci. 27 (2004) 843-852. (e) F. Svec, J. Sep. Sci. 27 (2004) 747-766. (f) G. Guiochon, J. Chromatogr. A 1168 (2007) 101-168.

Además, han abierto un nuevo abanico de posibilidades permitiendo la realización de separaciones cromatográficas en sistemas de análisis en flujo[26].

En la Figura 5.7 se representa una ilustración del perfil de un fluido atravesando una columna de cromatografía líquida particulada donde en la separación predomina la transferencia de masa por difusión, y una columna monolítica donde predomina la transferencia de masa por convección.

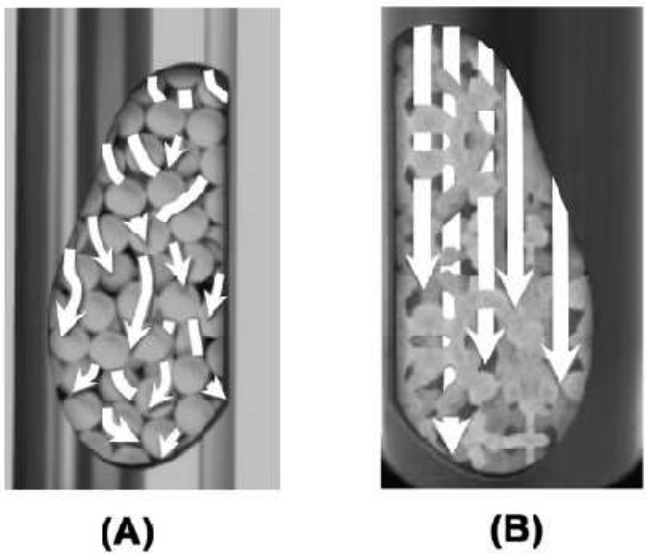

Figura 5.7. Perfiles del flujo a través de columnas de cromatografía líquida: (A) Columna basada en partículas y (B) columna monolítica.

La posibilidad de efectuar separaciones cromatográficas a bajas presiones mediante el uso de sistemas de análisis en flujo, se debe principalmente al esqueleto en forma de un único bloque con elevada porosidad que presentan

[26] (a) P. Chocholous, P. Solich, D. Satinsky, Anal. Chim. Acta 600 (2007) 129-135. (b) H. M. Gonzalez-San Miguel, M. Fernandez, J. M. Estela, V. Cerda, Trends Anal. Chem. 28 (2009) 336-346. (c) F. S. Kika, J. Chromatogr. Sci. 47 (2009) 648-655.

estas columnas. Una típica columna monolítica de sílice contiene dos tipos diferentes de poros. Los macroporos (2µm de diámetro) permiten el transporte de los analitos a través de la columna a presiones menores que en las columnas particuladas convencionales, mientras que los mesoporos (13nm de diámetro) es donde se efectúa el proceso de separación debido a su gran área efectiva (aproximadamente 300m^2 g^{-1}) (Figura 5.8).

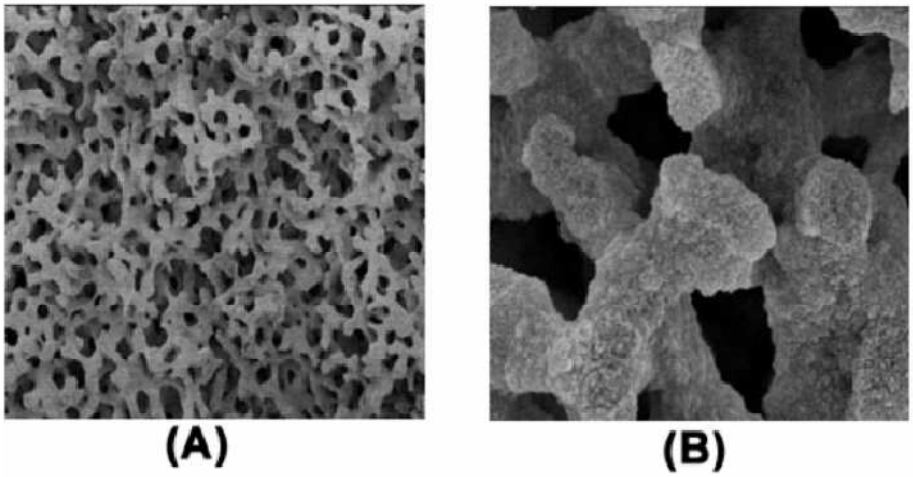

Figura 5.8. Estructura de una columna monolítica de sílica: (A) Macroporos y (B) mesoporos.

Por una parte, la porosidad total de este tipo de columnas es muy elevada (aproximadamente un 80%), siendo la resistencia que opone la columna monolítica a que un fluido la atraviese, sensiblemente menor que en el caso de una columna particulada convencional de las mismas características, lo que permite trabajar con caudales más elevados y de este modo obtener una mayor

velocidad de análisis. Por otra parte, es posible realizar separaciones cromatográficas en dispositivos más simples y que soporten menos presión que en el caso de la utilización de las bombas convencionales para sistemas HPLC.

Existen varias técnicas de flujo, que han sido combinadas con las columnas monolíticas, tales como FIA, SIA ó MSFIA. En este caso la combinación de las técnicas SIA ó MSFIA con columnas monolíticas es conocida como técnica SIC[27] (Sequential Injection Chromatography) o técnica MSC[28] (Multisyringe Chromatography), respectivamente. También cabe destacar el sistema híbrido FIA-HPLC desarrollado J. L. Adcock *et al.*[29]. Sin embargo, la mayoría de las aplicaciones de técnicas en flujo combinadas con columnas monolíticas que pueden encontrarse en la literatura científica se basan en aplicaciones simples en el campo de la química farmacéutica, siendo actualmente la aplicación de este tipo de técnicas a muestras más complejas, una línea de investigación aun prácticamente sin explorar.

5.7. Consideraciones sobre tiazidas con acción diurética

La función de los diuréticos sobre el organismo humano es la de incrementar la producción de orina por parte del riñón. Esta función se consigue alterando la forma en la que el riñón trata el sodio. Aumentando la cantidad de sodio excretada por el riñón, la excreción de agua también se ve incrementada. Esta alteración se basa frecuentemente en la inhibición de la reabsorción del sodio

[27] D. Santinsky, P. Solich, P. Chocholous, R. Karlicek, Anal. Chim. Acta 499 (2003) 205-214.
[28] H. M. Gonzalez-San Miguel, J. M. Alpizar-Lorenzo, V. Cerdà-Martin, Talanta 72 (2007) 296-300.
[29] J. L. Adcock, P. S. Francis, K. M. Agg, G. D. Marshall, N. W. Barnett, Anal. Chim. Acta 600 (2007) 136-141.

en diferentes segmentos del sistema renal tubular. A menudo es necesaria la combinación de dos diuréticos para obtener una mayor eficacia que utilizando uno solo, ya que varias combinaciones de estos tienen un efecto sinérgico. Esto se debe a que un determinado diurético tiene una acción de mayor eficacia en una determinada zona del riñón, por tanto, con la combinación de dos diuréticos distintos se puede conseguir una acción más uniforme en todo el riñón.

Entre los diferentes tipos de diuréticos las tiazidas son los más comúnmente utilizados. Estos diuréticos inhiben el transporte de sodio-cloruro en el túbulo distal. A pesar de no ser los diuréticos más eficaces, son ampliamente utilizados ya que son lo suficientemente potentes para satisfacer la mayoría de necesidades terapéuticas en las que el uso de diuréticos sea requerido.

Mediante sus efectos sobre el balance de sodio y agua, los diuréticos disminuyen el volumen y la presión sanguínea. Esto produce la disminución del volumen ventricular y el ritmo cardíaco, lo que conlleva a una caída de la presión arterial.

Los diuréticos son altamente efectivos para el tratamiento de la hipertensión, siendo eficaces en el 90-95% de los casos. Dicha efectividad se ve aumentada al combinar el uso de diuréticos con una dieta baja en sodio. La gran mayoría de los pacientes con hipertensión son tratados con diuréticos de la familia de las tiazidas. Aparte de ser un tratamiento eficaz para la

hipertensión, los diuréticos también son utilizados para combatir la insuficiencia cardíaca, así como edemas pulmonares y sistémicos.

5.8. Desarrollo de un nuevo método para la determinación rápida de trazas de diuréticos en muestras complejas

El interés en el desarrollo de métodos analíticos para la determinación de diuréticos se inicia en el momento en que dichos compuestos se empiezan a comercializar debido a su acción farmacológica. El objetivo de estas metodologías es la determinación de los diuréticos en las propias formulaciones farmacéuticas que los contienen, para establecer una metodología de control de calidad[30]. Siendo el desarrollo de nuevas técnicas para la mejora de estas metodologías un campo actual de investigación[31].

Además de dichas aplicaciones, se han desarrollado una amplia variedad de metodologías analíticas para la determinación de diuréticos en orina humana. La importancia de esta determinación estriba en que los diuréticos son compuestos que se encuentran incluidos en la lista de sustancias dopantes prohibidas elaboradas por la Comisión Médica del Comité Olímpico Internacional (COI), y por todas las federaciones deportivas profesionales. Ello se debe a su uso con una finalidad clara de causar fraude en las competiciones deportivas. Concretamente, dichos fraudes han sido relacionados con

[30] E. B. Domingo, M. J. M. Hernandez, G. R. Ramos, M. Celia, G. Alvarez-Coque, Analyst 117 (1992) 843-847.
[31] K. Tolba, D. Belder, Electrophoresis 28 (2007) 2934-2941.

competiciones de deportes en los cuales se establecen categorías por pesos, como por ejemplo el boxeo. Por un lado, con la ingesta de diuréticos se obtiene una pérdida de peso artificial, debido a la rápida perdida de agua por parte del organismo, lo cual posibilita competir en categorías de pesos inferiores. Por otro lado, los diuréticos pueden ser utilizados en general con la intención de impedir la detección de otras sustancias dopantes en los controles de orina. Al conseguir la producción de una elevada cantidad de orina, la concentración de dichos agentes dopantes en el organismo puede verse reducida rápidamente.

Existe una amplia variedad de técnicas instrumentales que han sido aplicadas a la determinación de diuréticos en muestras de orina. Dichas técnicas son principalmente técnicas de separación, existiendo varias metodologías establecidas para la determinación de diversos diuréticos mediante cromatografía de gases[32], cromatografía líquida[33] y electroforesis capilar[34].

Recientemente, como ya se ha detallado en el punto 5.5, una amplia variedad de compuestos farmacéuticos han sido detectados a niveles de trazas en el medio ambiente, entre ellos varios diuréticos. Las tiazidas son excretadas por el cuerpo humano prácticamente sin metabolizar, manteniendo su estructura inicial.

[32] L. Amendola, C. Colamonici, M. Mazzarino, F. Botre, Anal. Chim. Acta 475 (2003) 125-136.
[33] M. B. Barroso, R. M. Jimenez, R. M. Alonso, E. Ortiz, J. Chromatogr. B 675 (1996) 303-312.
[34] E. Gonzalez, A. Becerra, J. J. Laserna, J. Chromatogr. B 687 (1996) 145-150.

Tanto en los análisis en el área de los controles anti-dopaje como en el análisis medioambiental, el uso de técnicas de separación convencionales tiene una serie de desventajas tales como:

- Bajas velocidades de análisis, siendo este el factor limitante cuando se debe realizar un elevado número de análisis en un corto intervalo de tiempo. Por ejemplo, antes de un gran evento deportivo tal como los Juegos Olímpicos.
- En el campo del análisis medioambiental, cuando el objetivo es detectar un determinado contaminante en una serie de muestras. Generalmente dicho compuesto estará ausente en prácticamente la totalidad de estas. Las técnicas de separación convencionales suelen acarrear un elevado gasto económico, tanto en su adquisición, como en su funcionamiento.

Por lo tanto, sería interesante el desarrollo de técnicas analíticas económicas que nos ofreciesen la posibilidad de una determinación rápida de trazas de varios diuréticos. De este modo tendríamos una rápida alternativa en los controles de dopaje, así como una alternativa económica para análisis ambiental de contaminantes emergentes como primer paso hacía una confirmación más precisa sólo en el caso de que esta fuese necesaria.

Para llevar a cabo una aplicación inicial en este campo hemos decidido utilizar la técnica MSFIA como plataforma analítica para combinar por primera vez la extracción miniaturizada en fase sólida, la cromatografía líquida de baja presión utilizando columnas monolíticas y la detección quimioluminiscente

para la determinación simultánea de varios diuréticos de la familia de las tiazidas. Se han seleccionado tres tiazidas con propiedades diuréticas conocidas como Hidroflumetiazida, Furosemida y Bendroflumetiazida.

Estos diuréticos son preconcentrados y aislados de la matriz de la muestra utilizando un dispositivo miniaturizado de extracción en fase sólida en el interior de los cuales se acomodan pequeños discos de extracción, tal como se ha detallado en el punto 4.3. Una vez seleccionado un disco de material adecuado para esta extracción, se estudió la composición de una fase móvil que tuviese la capacidad de eluir dichos compuestos del disco de extracción y a su vez separarlos en su paso a través de una columna monolítica (Octadecil-Silica, 25x4.6mm) tal como se ha explicado en el punto 5.6. Finalmente los diuréticos, ya separados, a su salida de la columna son mezclados con una disolución que contiene $Ru(bipy)_3^{3+}$, generado previamente en un bucle de reacción a partir de $Ru(bipy)_3^{2+}$ y Ce^{4+}, utilizando para tal efecto dos jeringas adicionales. Dicha mezcla se efectúa justo antes de su entrada en el detector de quimioluminiscencia.

Este trabajo está expuesto en su totalidad en el punto 5.13.

5.9. Modificación de columnas monolíticas utilizando surfactantes

Las columnas monolíticas convencionales (Sílice-C18) pueden ser recubiertas semi-permanentemente mediante surfactantes, adquiriendo de este modo la capacidad de ser utilizadas como columnas para cromatografía iónica[35]. Estos surfactantes pueden ser fácilmente aplicados, re-aplicados, o eliminados de la columna.

Fluyendo a través de la columna una solución acuosa que contenga un surfactante determinado, el extremo hidrofóbico de este se adhiere a las terminaciones C18 de la columna monolítica. Añadiendo un porcentaje concreto de modificador orgánico, generalmente acetonitrilo, a la disolución del surfactante, la capacidad de intercambio iónico de la columna puede ser controlada. Existe un descenso lineal en la capacidad de la columna al incrementar el porcentaje de acetonitrilo en la disolución de surfactante entre un 0-30%[36].

En la Figura 5.9 se muestra un esquema de la estructura de una columna C18 recubierta por el surfactante CTAB (Bromuro de cetiltrimetilamonio).

[35] (a) B. Paull, P. N. Nesterenko, Trends Anal. Chem. 24 (2005) 295-303. (b) S. D. Chambers, K. M. Glenn, C. A. Lucy, J. Sep. Sci. 30 (2007) 1628-1645.
[36] P. Hatsis, C. A. Lucy, Anal. Chem. 75 (2003) 995-1001.

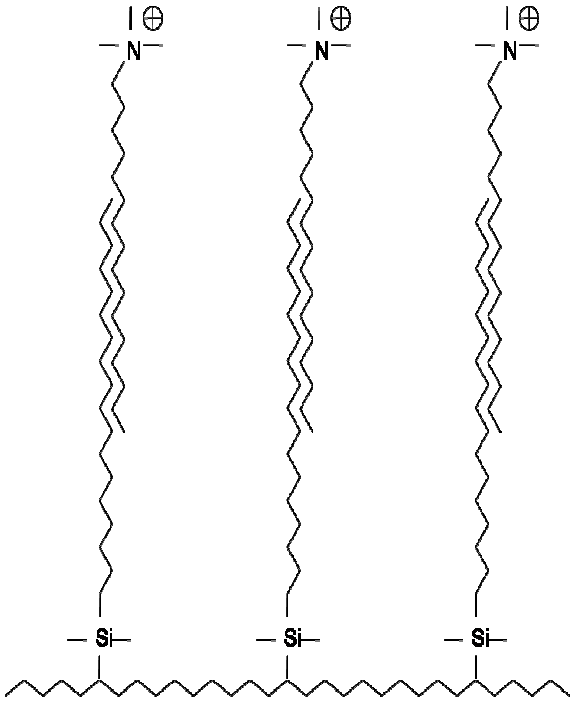

Figura 5.9. Representación esquemática de una columna C18 recubierta con el surfactante CTAB.

5.10. Consideraciones sobre el oxalato

El oxalato es la base conjugada del ácido oxálico, el cual es el ácido dicarboxílico más pequeño. El ácido oxálico es un ácido relativamente fuerte, a pesar de ser un ácido carboxílico:

$$C_2O_4H_2 \rightarrow C_2O_4H^- + H^+;\ pK_a = 1.27$$

$$C_2O_4H^- \rightarrow C_2O_4^{2-} + H^+;\ pK_a = 4.28$$

El oxalato produce un efecto negativo en el proceso de fabricación de la cerveza, y por tanto, a su calidad final. La concentración de oxalato en cerveza

suele ser de entre 4 y 32 mg L^{-1} y se relaciona con la germinación de la cebada. Una alta concentración de oxalato produce la formación de precipitados, generalmente de oxalato cálcico. Estos precipitados pueden causar problemas en las superficies internas de los tanques de fermentación y almacenamiento, en las tuberías y en los intercambiadores de calor que conforman el sistema de producción de la cerveza. Además, una elevada concentración de oxalato provoca inestabilidad en el producto una vez este ya ha sido envasado.

La determinación de oxalato es importante en la orina humana para el diagnostico de la hiperoxaluria entérica ó hereditaria, y en la evaluación y tratamiento de pacientes que padecen cálculos renales. Los cálculos renales suelen originarse por cristalización de oxalato cálcico, siendo la formación de estos favorecida evidentemente a concentraciones altas de oxalato.

5.11. Desarrollo de un nuevo método para la determinación rápida y selectiva de oxalato en muestras de matriz compleja

Tal como se ha comentado en el punto anterior, la determinación de oxalato es importante en muestras de orina ó cerveza. Por este motivo se han ido proponiendo diversas metodologías analíticas para este fin. Dichas metodologías suelen estar basadas en:

- Procedimientos manuales con reactivos selectivos (enzimas), siendo métodos lentos, laboriosos, y que requieren el uso de reactivos caros e inestables.
- El uso de técnicas de separación tales como: cromatografía iónica con detección conductimétrica, cromatografía líquida de pares iónicos con detección quimioluminiscente, electroforesis capilar en chip con detección conductimétrica, electroforesis capilar de zona con detección UV. Estas técnicas no requieren el uso de reactivos selectivos, pero sin embargo, siguen siendo técnicas con velocidades de análisis relativamente bajas, y en algunos casos su coste de adquisición y mantenimiento es elevado.

El objetivo de esta investigación es el desarrollo de un nuevo método para la determinación rápida y selectiva del oxalato en muestras con matrices complejas, tales como la cerveza o la orina humana. Para ello se ha combinado la detección quimioluminiscente utilizando el compuesto $Ru(bipy)_3^{2+}$ mencionado en el punto 5.1.2, con la selectividad que confieren el uso de

columnas monolíticas recubiertas con surfactantes, para el desarrollo de separaciones de iones, las cuales han sido introducidas en el punto 5.9. La posibilidad de realizar por primera vez dicha combinación ha sido facilitada mediante su implementación en la técnica MSFIA. El trabajo experimental desarrollado se muestra en su totalidad en el punto 5.1.

5.12. Artículo original VI

Improving the Chemiluminescence-based Determination of Sulphide in Complex Environmental Samples by Using a New, Automated Multi-syringe Flow Injection Analysis System Coupled to a Gas Diffusion unit

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Improving the chemiluminescence-based determination of sulphide in complex environmental samples by using a new, automated multi-syringe flow injection analysis system coupled to a gas diffusion unit

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ABSTRACT

A new, completely automated multi-syringe flow injection analysis (MSFIA) system coupled to a gas diffusion unit (GDU) was used for the chemiluminescence (CL)-based determination of sulphide ion in various types of environmental matrices with a high sensitivity and selectivity, and the need for no manual sample pretreatment. Sulphide ions are transferred as H_2S from the donor channel of the GDU to its acceptor channel (AC) through a hydrophobic membrane inserted between the two streams. The solution held in AC replaces the initial sample matrix, which may contain a wide variety of interferents, with one suitable for the CL determination of the analyte. Once sulphide ions have been isolated from the sample matrix, they are determined by their catalytic action on the luminol/H_2O_2 chemiluminescent reaction system. The influence of various chemical and hydrodynamic variables is discussed and the performance of the proposed system compared with that of existing flow systems for the same purpose. Under the operating conditions used, the proposed method features a linear working range of 0.02–2 mg L^{-1}, a limit of detection ($3\sigma_{blank}$) of 0.003 mg L^{-1}, a throughput of 20 samples h^{-1} and a coefficient of variation of 2.4% ($n = 10$) for a 1 mg L^{-1} sulphide concentration. The method was used to determine sulphide in leachates and various types of water samples.

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1. Introduction

The presence of sulphide in water is one major factor to be monitored in order to avoid the highly toxic effects of hydrogen sulphide on aquatic species. A wide range of approaches and methodologies for this purpose have to date been reported [1]. Classical methods for sulphide involve time-consuming manual sample pretreatments (*e.g.* flocculation of suspended solids) [2] that delay delivery of the results. Many have been superseded by automated methods [3,4] for highly efficient for sulphide monitoring. Some such methods use a flow system [5–8] and are coupled to a separation technique in order to suppress matrix effects and improve selectivity as a result [9,10].

Chemiluminescence provides sensitive, economic, fast detection [11]. Chemiluminescent reactions require some degree of automation for proper monitoring, a requirement that is effectively met by flow analysis techniques. A number of simple, sensitive flow systems for the chemiluminescence-based determination of sulphide ion have been reported

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[12–15]. However, CL reactions are typically poorly selective by effect of the interference of a variety of species including metals, anions, oxidants and organic compounds. Avoiding the deleterious effects of the wide range of interferents present in some specially complex matrices usually involves implementing off-line chemical separation procedures.

No analytical flow method for S^{2-} using a gas diffusion unit to isolate the analyte from the matrix in combination with CL detection, which might be a useful tool for the sensitive, selective determination of sulphide in highly complex matrices, was included in a recent review on the determination of S^{2-} using flow techniques [16].

Multi-syringe flow injection analysis (MSFIA) is a recent, highly flexible flow technique the intrinsic features of which make it specially suitable for the isolation of S^{2-} by gas diffusion with a view to its subsequent CL detection. In this work, we used an autoburette with four syringes soldered into a common metal bar, each syringe bearing a solenoid valve at the top, in order to develop a low regent consumption system as in sequential injection systems (SIA), but due to the use of four syringes instead of one, MSFIA systems also let us to work in a forward flow scheme as in flow injection analysis (FIA), obtaining good sample–reagents mixing, an higher injection throughputs than in SIA. In previous work, MSFIA proved especially suitable for implementing flow methods based on CL detection [17,18].

Sulphide ions catalyse the chemiluminescent reaction between luminol and hydrogen peroxide. Free S^{2-} in an alkaline medium reduces dissolved oxygen to superoxide anion radical, which reacts with H_2O_2 to give hydroxyl radical (a co-oxidant for the luminol/H_2O_2 system that enhances the CL emission as a result).

In this work, we used MSFIA to demonstrate the potential of the luminol/H_2O_2 system in combination with a GDU [19] for the determination of S^{2-}. The proposed method removes major interferents [13,18], thereby affording application to environmental samples with complex matrices. The method combines the high selectivity of flow systems using a GDU and the high sensitivity of CL flow systems with no GDU, thereby allowing S^{2-} to be highly efficiently determined in such matrices.

2. Experimental

2.1. Reagents and solutions

All reagents were analytical grade and solutions prepared in Milli-Q water (Millipore, Molsheim, France). A 5×10^{-2} mol L^{-1} NaOH solution (Merck, Darmstadt, Germany) was used as DC carrier. The acidifying solution used to form H_2S from S^{2-} was obtained by appropriately diluting concentrated HCl from Scharlau Chemie (Barcelona, Spain). The acceptor solution for H_2S consisted of 0.1 mol L^{-1} bicarbonate/carbonate buffer containing 5×10^{-3} mol L^{-1} ethylenediaminetetraacetic acid (EDTA); the solution was prepared by dissolving 10.6 g of Na_2CO_3 (Probus, Barcelona, Spain) and 1.86 g of EDTA disodium salt (Probus) in 0.8 L of water, adjusting to pH 11.5 with 1 mol L^{-1} NaOH and making to 1 L with water. A 10^{-2} mol L^{-1} stock solution of the luminescent reagent was prepared by dissolving 1.7710 g of 3-aminophthalhydrazide (luminol, Acros Organics, Morris Plains, NJ) in the previous buffer/EDTA solution and diluting to 1 L with the same solution. The oxidant solutions were made by diluting 30% (v/v) H_2O_2 (Panreac, Barcelona, Spain) in buffer/EDTA solution. A sulphide stock solution approximately 1000 mg L^{-1} in S^{2-} was prepared on a daily basis by dissolving the required amount of $Na_2S.9H_2O$ (Panreac) in 0.05 mol L^{-1} NaOH; the stock solution was standardized iodometrically [20] and used to prepare standard working-strength solutions by appropriate dilution with 0.05 mol L^{-1} NaOH.

2.2. Manifold and software

The proposed MSFIA manifold, Fig. 1, consisted of two multi-syringe modules (MS1 and MS2) from Crison (Barcelona, Spain) equipped with four syringes (Hamilton, Bonaduz, Switzerland) each; only five of the eight syringes were used, however. A three-way solenoid valve (S1-S5, N-Research, West Caldwell, NJ) was mounted on the head of each syringe and an additional valve (V6, STV-3-1/4, UKG, Takasago, Japan) was employed to facilitate loading from the autosampler (AS) into the holding coil (HC) in order to avoid carry-over in S1. In their OFF position, the solenoid valves had a common port connected to the normally open (NO) port; in their ON position, the common port was connected to the normally closed (NC) port. The flow system was connected to a personal computer (PC) via an RS232C interface.

The holding coil was 8 m in length and made from polytetrafluoroethylene (PTFE) of 0.8 mm i.d. All other connectors were also made from PTFE tubing and the two confluence points used (CP1, CP2) from polymethylmethacrylate (PMMA).

The gas diffusion membrane used was a polyvinylidene difluoride (PVDF) Durapore model (Millipore, GVHP29325, Bedford, USA) 125 μm thick and 0.2 μm in pore size. The gas diffusion unit (GDU), of the sandwich type, was laboratory-made by using two overlapped PMMA blocks into which an elongated U-shaped semi-tubular channel (10 cm long, 0.08 cm wide, 0.04 cm deep, 25 μL inner volume, 0.8 cm^2 surface area) was carved. The channels were the mirror images of each other. The hydrophobic membrane was placed between the two channels in order to hinder the transfer of non-gaseous and non-volatile compounds.

The flow-cell (FC) was also laboratory-made by using a PMMA plate to keep a PTFE body airtight. To this end, a spiral-shaped channel (4 cm long, 0.14 cm wide, 0.09 cm deep, 50 μL inner volume, 0.8 cm^2 effective light emission surface area) was carved into it; the spiral diameter was 0.9 cm. The flow-cell was placed in front of a model H5784 photomultiplier tube (PMT) from Hamamatsu (Hamamatsu City, Japan) with a photosensitive wavelength range 04 (195–850 nm), a maximum selectivity wavelength of 400 nm and a photosensitive area of 0.5 cm^2. The maximum emission wavelength of luminol was 425 nm and no wavelength discrimination was done in the detector.

The flow-cell and photomultiplier tube were both accommodated in a plastic box in order to protect the detection system from external light.

A signal amplifier module (AM) previously reported by Miró et al. [18] was used to supply the PMT with a feedback voltage

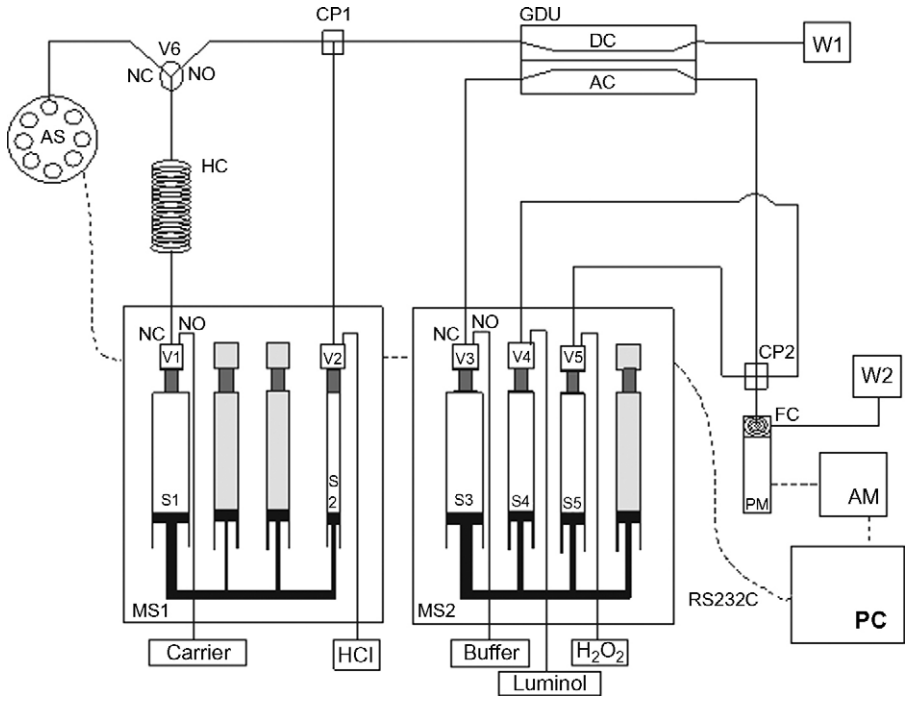

Fig. 1 – Schematic depiction of the proposed MSFIA–CL set-up. MS1 and MS2 multi-syringe modules, S1–S5 syringes (S1 = S3 = 10 mL, S2 = 1 mL, S4 = S5 = 5 mL; the other three syringes were not used), V1–V6 three-way solenoid valves, NO solenoid valve normally open port, NC solenoid valve normally closed port, HC holding coil, AS autosampler, CP1 and CP2 confluence points, GDU gas-diffusion unit, DC donor channel, AC acceptor channel, FC flow-cell, PMT photomultiplier tube, AM signal amplification module, PC personal computer, W1 and W2 waste.

of 0.3–0.8 V and obtain a signal gain (*G*) linear over the range 1–1000. The best results were obtained with a voltage of 0.65 V (*G* = 700).

Instrumental control and data acquisition were accomplished by using the Autoanalysis 5.0 software package (Sciware, Palmanyola, Spain), which is based on dynamic link libraries (DLLs) [21]. Appropriate DLLs were used to operate the multi-syringes, autosampler and CL detection system. The multi-syringe DLL provided for the presence of the additional solenoid valve (V6).

2.3. *Procedure for determining sulphide by MSFIA coupled with gas diffusion and chemiluminescence-based detection (MSFIA–GD–CL)*

The analytical protocol used to determine sulphide by MSFIA–GD–CL involved the following steps (flow-rates are stated in a subsequent section):

(1) The syringes in MS1 and MS2 were loaded with the following reagents (with all valves OFF): DC carrier (5×10^{-2} mol L^{-1} NaOH) (S1), acidifying solution (HCl) (S2), AC carrier (0.1 mol L^{-1} bicarbonate/carbonate buffer/EDTA solution) (S3), reagent 1 (luminol) (S4) and reagent 2 (H_2O_2) (S5).
(2) A sample volume of 2.5 mL was loaded into the HC, with valves V1 and V6 ON, and all others OFF.
(3) A volume of 4 mL (*viz.* the previously loaded 2.5 mL of sample plus 1.5 mL of carrier in order to prevent carry-over) was injected with V1 and V2 ON. This allowed the sample to be acidified (with 0.4 mL of HCl solution) and S^{2-} initially present in the sample transferred across the PVDF membrane as H_2S from the DC to the AC of the GDU while the sample matrix was sent to waste (W1). Transferred H_2S was then back-converted into S^{2-} by effect of the alkalinity of the AC solution.
(4) Sulphide ions then in the AC were injected into the FC by multi-commutated injection in two steps. In the first, the CL detector took readings at 0.1 s intervals, with no points of average; then, syringes S3, S4 and S5 were used to inject 0.4, 0.2 and 0.2 mL of their respective solutions with V3, V4 and V5 ON; S^{2-} ions were mixed with luminol and H_2O_2, and injected into FC in order to obtain a CL peak. In the second step, valves V4 and V5 were switched OFF in order to flush FC with 0.4 mL of acceptor solution and the CL detector was stopped. These two steps produced waste that was evacuated via W2.
(5) All syringes were filled with their respective reagents with all valves OFF. At that point, the procedure was either finished or restarted with a new injection.

3. Results and discussion

From univariant studies the different designs of the manifold were assessed and the chemical and hydrodynamic parameters of the MSFIA system were selected.

3.1. Design and selection of the manifold configuration

The proposed system consists of two multi-syringe modules mutually connected via a GDU. The unit used in this work initially decreased the sensitivity relative to a similar flow system containing no GDU. We thus constructed an MSFIA–CL system with no GDU (*i.e.* one consisting of one multi-syringe module with S1 = bicarbonate/carbonate buffer/EDTA solution, S2 = luminol and S3 = H_2O_2). A sample volume of 0.2 mL was loaded into the buffer solution and injected into the system for mixing with luminol and H_2O_2 immediately before entering the flow-cell. This system without provided a linear range of 0.005–4 mg L^{-1} S^{2-}. A plot of relative CL intensity (*viz.* analytical peak/blank peak ratio) *versus* sulphide concentration (in mg L^{-1}) provided the following regression equation: $Y = 27.34[S^{2-}] + 0.79$ ($R^2 = 0.9997$). The relative CL intensity can be interpreted as the number of times CL emission is increased by effect of the catalytic action of S^{2-}.

Once chemical and hydrodynamic conditions were selected, the GDU was incorporated into the system. The most effective way of transferring S^{2-} across the membrane was determined from the following results:

- In *forward flow* mode A, the AC and DC solutions were continuously circulated at the same flow-rate, so no preconcentration was obtained. A comparison of the slope of the resulting calibration graph with the results provided by the MSFIA–CL system including no GDU revealed that the H_2S transfer efficiency across the membrane was only 7%. In *forward flow* mode B, AC and DC were also circulated in a continuous manner, but the AC flow-rate was 10 times lower than the DC flow-rate, which afforded some preconcentration.
- Preconcentration was greater in the *stop-flow* mode, where the AC solution remained stagnant. Such was also the case with the *reversal flow* mode, where the DC solution performed a forward/backward/forward movement across the membrane.
- The highest sensitivity was obtained with the *reversal flow* mode. This, however, was rejected on the grounds of its decreased regression coefficient ($R^2 = 0.9897$) and poor signal repeatability (R.S.D. > 5%), also resulting in a lower injection throughput.

As can be seen from Fig. 2, the *stop-flow* configuration was the most suitable for our purpose, so we chose to adopt it for subsequent tests. The analytical results obtained with it were on a par with those provided by the system including no GDU; also, its selectivity was vastly superior.

3.2. Study and selection of chemical variables

Once the most suitable manifold type was chosen, the chemical variables involved in the target determination were studied and selected.

The extent of formation of H_2S was found to depend on the HCl concentration used in S2, the influence of which was examined over the range 1–3 mol L^{-1}. The best results were obtained with a 2 mol L^{-1} HCl solution.

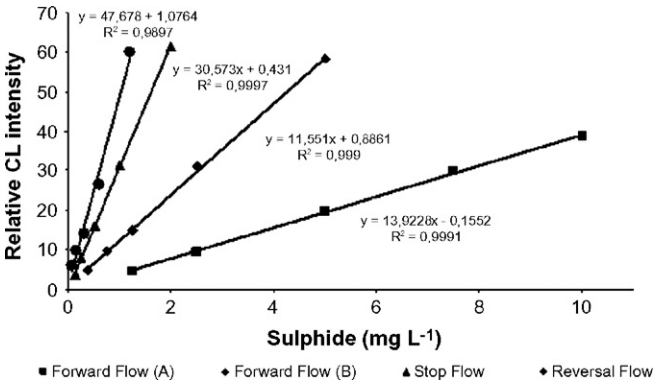

Fig. 2 – Influence of the way S^{2-} is transferred across the PVDF membrane on the sensitivity of the proposed system.

In Fig. 3, is illustrated the influence of the luminol and hydrogen peroxide concentrations. The effect of the former was examined over the range 10^{-2}–10^{-5} mol L^{-1}. The experimental conditions used in the tests were as follows: $[S^{2-}] = 2$ mg L^{-1}, $[H_2O_2] = 2$ mol L^{-1}, S^{2-} transfer flow-rate = 2 mL min^{-1} and injection flow-rate = 10 mL min^{-1}. The best response for luminol concentration was found to be 8×10^{-4} mol L^{-1}—higher levels resulted in decreased repeatability in the analytical signal. The effect of the H_2O_2 concentration was examined over the range 0.01–6 mol L^{-1}, using the previous experimental conditions and the previously selected luminol concentration. The maximum relative CL intensity was obtained with 3 mol L^{-1} H_2O_2, which was thus chosen for subsequent tests. Higher peroxide concentrations caused the blank signal to rise to a greater extent than the analytical signal and decreased the relative CL intensity as a result.

The composition of the reaction medium was selected as described elsewhere [13]. A 0.1 mol L^{-1} bicarbonate/carbonate buffering solution at pH 11.5 was chosen as it resulted in the highest possible stability in the CL peaks. The buffer was supplied with EDTA in order to minimize interferences from metal ions present as reagent impurities. The effect of the EDTA concentration was examined over the range 10^{-2}–10^{-3} mol L^{-1} and a value of 5×10^{-3} mol L^{-1} found to suffice in order to efficiently minimize interferences.

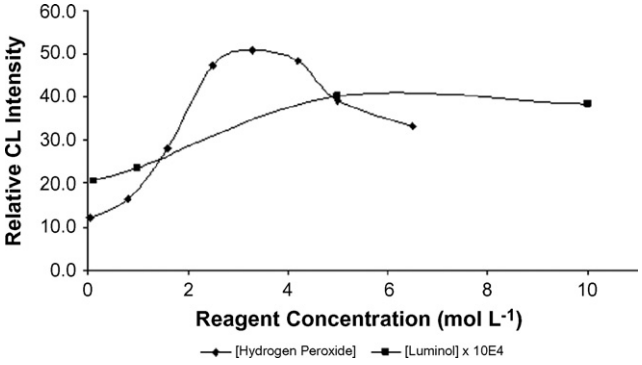

Fig. 3 – Influence of the reagent concentrations on the relative CL emission intensity.

Table 1 – Studied ranges and selected values of the hydrodynamic variables for the proposed MSFIA–CL–GDU system

Variable	Range	Selected
Sample volume (mL)	0.5–4.5	2.5
DC flow-rate (mL min^{-1})	2–20	2
Reaction plug volume (mL)	0.4–2	0.8
Injection flow-rate (mL min^{-1})	5–20	15

3.3. *Study and selection of hydrodynamic variables*

We studied the effect of various hydrodynamic variables the most critical of which are shown in Table 1.

The selected sample volume was taken to be that sufficing to obtain a high enough preconcentration level in the AC stagnant solution in order to offset the low efficiency of S^{2-} transfer across the gas-diffusion membrane while preserving the sensitivity relative to a system involving no separation of the analyte.

The reaction plug consisted of a 2:1 mixture of AC solution containing transferred S^{2-} and each reagent solution (luminol and H_2O_2). The total injected volume adopted was 0.8 mL.

The influence of the flow-rate of the DC solution used in the S^{2-} transfer step is illustrated in Fig. 4. The experimental conditions used were $[S^{2-}] = 2\,mg\,L^{-1}$, $[luminol] = 8 \times 10^{-4}\,mol\,L^{-1}$, $[H_2O_2] = 3\,mol\,L^{-1}$ and injection flow-rate = 10 mL min^{-1}. The figure also shows the influence of the flow-rate of injection of the reaction plug into the flow-cell, which was examined under the previous experimental conditions and an S^{2-} transfer flow-rate of 2 mL min^{-1}. Best results were obtained with a flow-rate of 15 mL min^{-1} (in relation to S3), so the selected total flow-rate of injection into FC was 30 mL min^{-1}.

Chemiluminescence-based flow systems use no reaction coils as they can result in the loss of radiation intensity from the flow-cell. The optimum length of tubing (0.8 mm i.d. PTFE) between CP2 and FC was found to be 6 cm.

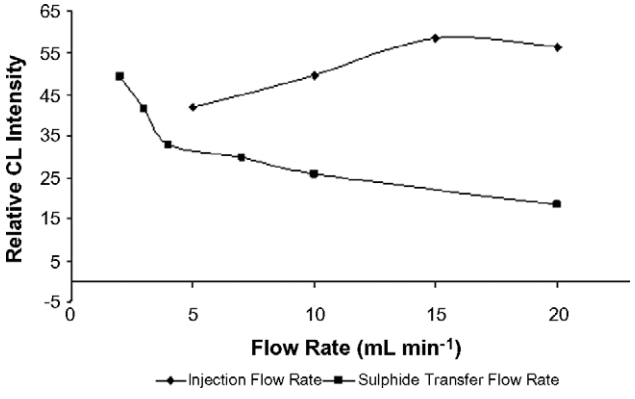

Fig. 4 – Influence of the reaction plug injection flow-rate and donor channel flow-rate in the S^{2-} transfer step on the relative CL emission intensity.

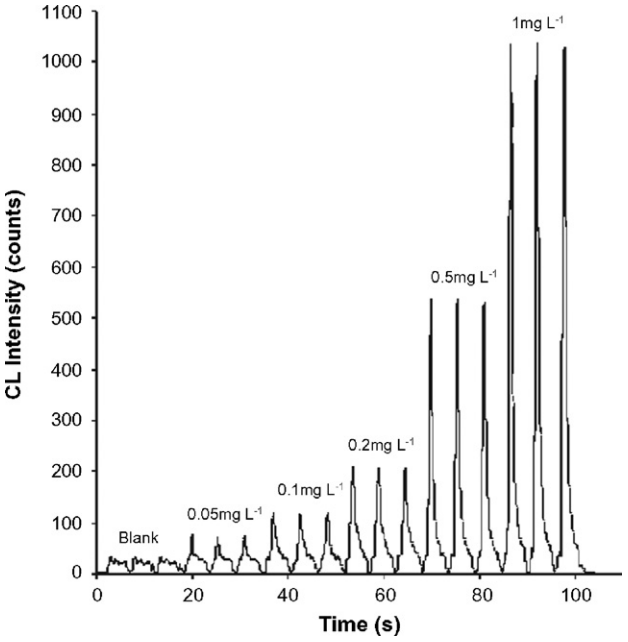

Fig. 5 – Response of the proposed MSFIA–CL–GDU system to variable concentrations of S^{2-} shown above the corresponding signals.

3.4. *Analytical performance*

Once the final operational parameter values were selected, the resultant system configuration was used to determine its figures of merit. Fig. 5 shows its response to variable S^{2-} concentrations.

The analytical figures of merit of the proposed configuration are summarized in Table 2. A linear working range spanning two orders of magnitude was obtained. The repeatability of the proposed method was estimated as the relative standard deviation (R.S.D.) for 10 consecutive injections of a $1\,mg\,L^{-1}\,S^{2-}$ standard solution. Its reproducibility, also expressed as R.S.D., was estimated from seven standard solutions of $1\,mg\,L^{-1}\,S^{2-}$ measured on different days. The obtained limit of detection was equal to $0.003\,mg\,L^{-1}$, and it was calcu-

Table 2 – Analytical performance of the proposed MSFIA–CL–GDU system for determining S^{2-}

Analytical parameter	Value
Limit of detection (mg L^{-1})	0.003
Limit of quantitation (mg L^{-1})	0.01
Sensitivity (L mg^{-1}) ($n = 6$)	31.34 ± 0.97
Regression coefficient ($n = 6$)	0.9993 ± 0.0006
Reproducibility (%) ($1\,mg\,L^{-1}\,S^{2-}$) ($n = 7$)	3.8
Linear dynamic range (mg L^{-1})	0.02–2
Repeatability (%) ($1\,mg\,L^{-1}\,S^{2-}$) ($n = 10$)	2.4
Injection throughput (h^{-1})	20
Sample throughput (h^{-1})	6

Table 3 – Study of the interferences

System	Interferent (amount added to a 1 mg L^{-1} S^{2-} solution)					
	None added	Thiol groups	Metals		Anions	
		L-Cysteine 10,000 mg L^{-1}	Co^{2+} 1 mg L^{-1}	Co^{2+} 1000 mg L^{-1}	SO_3^{2-} 1 mg L^{-1}	SO_3^{2-} 1000 mg L^{-1}
MSFIA–CL without GDU	29	–[a]	–[a]	–[a]	29.1	3.6[b]
MSFIA–CL with GDU	29.6	28.7	24.6[b]	5.7[b]	29.5	4.6[b]

Results are expressed as relative CL intensity.

[a] CL signal peak height falling outside the working range. Analysis is not feasible with this method.

[b] Decreased signal due to the decreased concentration of free S^{2-} in the medium by effect of the presence of the interferent.

lated in accordance with IUPAC's criterion [22] (*i.e.* as three times the standard deviation of the CL emission, 3σ blank, in 10 consecutive injections of 2.5 mL of distilled water). The sensitivity and regression coefficient were calculated from 6-day-to-day regression curves. The injection frequency was calculated as the number of injections the system could realize in 1 h, and the sampling frequency as the number of samples analysed in 1 h at a rate of three injections per sample and with provision for the time needed to flush the tubing between injections of different samples.

The robustness of the system is dictated by the durability of the PVDF gas-diffusion membrane, which was estimated to be at least 70 injections. This allows the system to operate uninterruptedly for at least 3.5 h. The first symptom of membrane deformation is a decreased repeatability and reproducibility in the CL signal.

3.5. *Study of interferences*

The reaction system used is not specific for S^{2-}. In fact, metal traces, compounds containing thiol groups and some volatile compounds in an acid medium are also effective catalysts – even more so than S^{2-} itself in some cases – for the luminol/H_2O_2 reaction [13].

The effect of each interference was examined by measuring the relative CL intensity obtained from 1 mg L^{-1} solutions of S^{2-} containing one of the different potential interferents. Each solution was analysed with the proposed MSFIA–CL–GDU system and with that excluding the GDU (see Section 3.1) in order to confirm the increased selectivity obtained by using a gas-diffusion unit.

Potential interferents (Fe^{2+}, Fe^{3+}, Cu^{2+}, Al^{3+}, Zn^{2+}, Cr^{3+}, Co^{2+}, L-cysteine, SO_3^{2-}, NO_2^-, $S_2O_3^{2-}$ and SCN^-) were studied by adding a 1000-fold amount of each to a 1 mg L^{-1} solution of the analyte.

Metal ions contained in the sample, which were catalysts for the reaction under the operating conditions used, were removed from the DC solution by effect of the membrane acting as a barrier for charged hydrophilic compounds. On the other hand, anions in the acid medium were possibly transferred across the membrane; however, they had no catalytic effect on the reaction under our operating conditions. Also, although samples can contain suspended solids, they can be retained by the PVDF membrane owing to their typically large particle size.

Compounds containing thiol groups can also interfere. However, because they occur in large particle sizes and are not volatile in acid media, they should be removed by the PVDF membrane, as confirmed by adding a 1000-fold amount of L-cysteine to a 1 mg L^{-1} S^{2-} solution. Methyl mercaptan (CH_3SH) is one exception owing to its small size and its being a volatile compound, which can result in its interfering with the proposed method. According to Toda et al. [23], the transfer efficiency of CH_3SH across a PVDF membrane is roughly 10 times lower than that of H_2S. Therefore, this compound can significantly interfere with the determination if present at a

Table 4 – Results obtained in the analysis of environmental samples using the proposed MSFIA–CL–GDU method and the APHA-AWWA-WPCF reference method

Sample	S^{2-} added (mg L^{-1})	MSFIA–CL–GDU method		Official method	
		Found (mg L^{-1})	Recovery (%)	Found (mg L^{-1})	Recovery (%)
Tap water[a]	0	<LOQ[b]	–	<LOQ	–
	0.2	0.230 ± 0.020	115	0.221 ± 0.008	110
Wastewater[c]	0	0.158 ± 0.005	–	0.152 ± 0.012	–
	0.3	0.464 ± 0.016	102	0.472 ± 0.019	107
Leachates[d]	0	1.213 ± 0.036	–	1.223 ± 0.018	–
	0.5	1.691 ± 0.010	96	1.695 ± 0.030	94

[a] BOD_5 < 10 mg L^{-1}; pH 7.3; COD 2 mg L^{-1}.

[b] LOQ, limit of quantitation for each method.

[c] BOD_5 < 10 mg L^{-1}; pH 9.0; COD 44 mg L^{-1}; SS 25 mg L^{-1}.

[d] BOD_5 12 mg L^{-1}; pH 7.9; COD 240 mg L^{-1}; SS 155 mg L^{-1}.

Table 5 – Comparison of the proposed MSFIA–CL–GDU method with other flow methods for determining S^{2-}

Analytical parameter	Flow system			
	MSFIA–CL–GDU[a]	rFIA (CL)[b]	MSFIA–GDU[c]	MSFIA–RE–GDU[d]
Linear range ($mg\,L^{-1}$)	0.02–2	0.002–0.8	0.5–20	0.05–1
Limit of detection ($mg\,L^{-1}$)	0.003	0.0006	0.03	0.0046
Injection throughput (h^{-1})	20	10	13	5

[a] Proposed system.
[b] Reversed FIA system with CL detection [13].
[c] MSFIA system with GDU and spectrophotometric detection [9].
[d] Reflectometric MSFIA system with GDU and spectrophotometric detection [10].

concentration ratio above 1:2 with respect to S^{2-} in the sample. Ethyl mercaptan and higher thiols are not significantly transferred across a PVDF membrane.

If is suspected the presence of CH_3SH in the samples to be analysed for S^{2-}, its interference can be avoided by increasing membrane polarity (*e.g.* by replacing the PVDF membrane with a Nafion membrane [23]).

Sulphur-containing compounds bearing no thiol groups are not catalysts for the analytical reaction and should thus pose no interference.

The presence of large amounts of some interferents resulted in a markedly decreased analytical signal by effect of S^{2-} being partly removed via precipitation reactions with metals or redox reactions with some anions. Therefore, their effect cannot be considered a negative interference. Table 3 shows representative results for each type of interferent.

3.6. *Analysis of real samples*

The accuracy of the proposed method was assessed by applying it to the analysis for S^{2-} in tap water (UIB, Balearic Islands), water from a lagoon biological wastewater treatment station (UIB, Balearic Islands) and leachates from a demolition waste treatment plant (CTP Manacor, Balearic Islands). Samples were collected by filling up bottles in order to minimize the presence of air and analysed immediately upon collection.

Table 4 shows the analytical results obtained with the proposed method and the APHA-AWWA-WPCF manual standard method [2], which uses a different chemistry involving a reaction between S^{2-}, Fe^{3+} and *N*,*N*-dimethyl-*p*-phenylenediamine (DMPD) to obtain methylene blue, the dye being spectrophotometrically measured at 666 nm. Methylene blue dye is generated mixing 7.5 mL of sample or standard, with 0.15 mL of $FeCl_3$ 0.9 $mol\,L^{-1}$ and 0.5 mL of DMPD 1.6 $mol\,L^{-1}$ (prepared in H_2SO_4 50%). In all instances, recovery was verified by using S^{2-} standard additions.

None of the real samples studied contained any potential interferents in large amounts. In any case, the combination of components of the sample matrix precluded analysis of most of the samples without a GDU. A separation step was therefore indispensable for the fully automated determination of S^{2-} with CL detection in these environmental samples. Table 4 shows the most salient properties of the samples, namely: biochemical oxygen demand (BOD_5), chemical oxygen demand (COD) and suspended solids (SS). The results provided by the proposed and standard methods were compared via a *t*-test [24] that revealed the absence of significant differences at a 0.05 significance level.

3.7. *Comparison of the proposed MSFIA-CL-GDU system with similar flow systems for determining sulphide*

A number of flow systems for the CL determination of S^{2-} have been reported [12–14] none of which uses a GDU to isolate the analyte. Thus, Du et al. [13] proposed a reversed FIA CL system using the same reaction conditions as the proposed method but no analyte separation. The proposed method performs similarly to this method, but affords a higher degree of automation in sample pretreatment, which results in higher selectivity than with the reversed FIA system.

Two MSFIA methods for S^{2-} do use a GDU [9,10], but rely on the production of methylene blue and its spectrophotometric detection. One [9] uses an analytical procedure similar to that of the proposed CL method; the other [10], preconcentrates S^{2-} by using an optrode. The proposed MSFIA–CL–GDU method has a lower linear working range than the former method [9] and a higher throughput than the latter [10].

The analytical performance of the four methods is compared in Table 5.

4. Conclusions

A sensitive, selective, completely automated flow system for the determination of S^{2-} in complex environmental samples is proposed. The method combines multi-syringe flow injection with gas diffusion in order to isolate the analyte prior to its chemiluminescence-based detection. The proposed method opens up a wide range of possibilities for the CL determination of S^{2-} in real samples which cannot be realized without isolating the analyte.

Using the *stop-flow* mode results in improved performance relative to other flow methods and reduces production of contaminating waste. Also, the system is highly flexible for adaptation to a variety of determinations.

The method was validated by applying it to the determination of S^{2-} in environmental samples of variable complexity.

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5.13. Artículo original VII

Interfacing On-line Solid Phase Extraction With Monolithic Column Multisyringe Chromatography and Chemiluminescence Detection: An Effective Tool for Fast, Sensitive and Selective Determination of Thiazide Diuretics

Autores: Fernando Maya, José Manuel Estela, Víctor Cerdà

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Interfacing on-line solid phase extraction with monolithic column multisyringe chromatography and chemiluminescence detection: An effective tool for fast, sensitive and selective determination of thiazide diuretics

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ABSTRACT

A new, multisyringe flow injection set-up has been developed for the completely automated determination of trace thiazide compounds with diuretic action in different types of samples. The proposed instrumental set-up exploits for the first time, a low pressure on-line solid phase extraction–liquid chromatography–chemiluminescence detection method. This novel combination of sample treatments in flow systems expands the current applicability of low pressure liquid chromatography due to the isolation/preconcentration of the target compounds, besides high selectivity and sensitivity.

For the determination of three thiazide compounds named hydroflumethiazide, furosemide and bendroflumethiazide, the proposed set-up provided with the preconcentration of only 1 mL of sample, limits of detection of 3, 60 and 40 $\mu g\,L^{-1}$, respectively. Furthermore wide linear dynamic ranges of 6–4000, 140–20,000 and 90–40,000 $\mu g\,L^{-1}$, respectively, were obtained. Besides of this, a high injection throughput of $12\,h^{-1}$ was also achieved. As in sports, thiazide diuretics are prohibited substances, the proposed method has been applied to their determination in urine samples. Furthermore the potential of the proposed method as a fast-screening approach for emerging contaminants in waters has been also tested by applying it to well water and leachates from a solid waste landfill.

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1. Introduction

Flow injection/sequential injection techniques (FI/SI) [1] are well established tools for the automation of analytical methodologies, but their initial capability for multi-analyte determinations, is basically limited to (i) simple two analyte determinations based on speciation [2], (ii) the chemometric deconvolution of overlapped spectras [3] and (iii) determinations based on the sum parameters or total indices [4,5].

Initially, the separation and quantification of several organic compounds with similar molecular structures in a FI/SI manifold was not a feasible task. Nonetheless, the development of hybrid porous silica monolithic columns (MC) [6] for liquid chromatography (LC), provided high performance LC separations at relatively lower back-pressures, making feasible its accomplishment in Sequential Injection Analysis (SIA) systems [7,8]. This technique was defined as Sequential Injection Chromatography (SIC) [9,10], allowing faster, cheaper and environmentally friendlier [11] LC separations.

Among the different FI techniques, Multisyringe Flow Injection Analysis (MSFIA) [12–15] also revealed its potential for the accomplishment of LC schemes [16], this variant was named Multisyringe Chromatography technique (MSC) [17]. Due to its inherent characteristics the MSC technique owns high capabilities in terms of versatility, simplifying the implementation of in-line pre- and post-column sample treatments commonly used in LC separations. But at the moment the major part of the current applications of this technique have been focused to the direct analysis with UV spectrophotometric detection of pharmaceuticals (in pharmaceutical formulations) [18–20]. So the high versatility of SIC and overcoat MSC techniques for the implementation of automated sample treatments has been scarcely exploited.

On the one hand, the few studies accomplished by the moment about this topic, are based on: (i) the determination of amino acids after automated derivatization with o-phthalaldehyde by using SIC technique [21], (ii) the automated sample filtration and dilution for the accomplishment of pharmacological studies about acyclovir also exploiting SIC [22], (iii) the development of an hybrid FIA-HPLC system with chemiluminescence (CL) detection for the generation of solvent gradients [23], or the (iv) implementation of in-line solid phase extraction (SPE) on the MSC technique for the determination of hydrochlorothiazide and losartan potassium in different types of water samples containing high loads of them [24].

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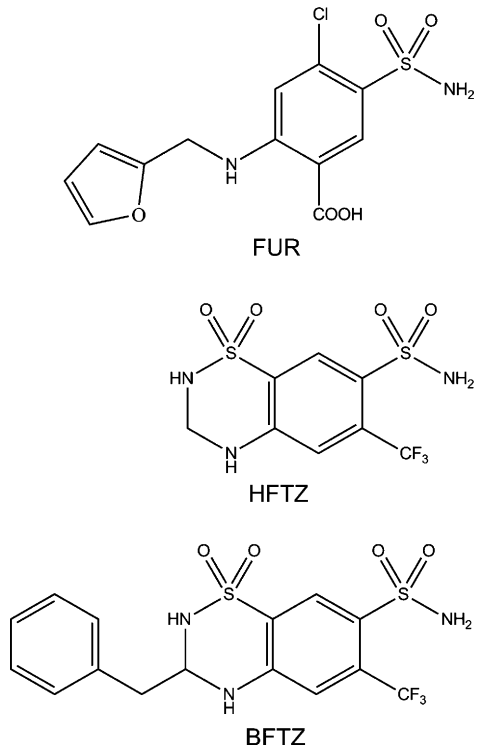

Fig. 1. Molecular structures of: furosemide (FUR, $pK_{a_1} = 3.9$), hydroflumethiazide (HFTZ, $pK_{a_1} = 8.9$), and bendroflumethiazide (BFTZ, $pK_{a_1} = 8.5$).

On the other hand, the relatively low selectivity and sensitivity achieved by this type of techniques remains a drawback. It is widely known that some very advantageous sample treatments for improved selectivity and sensitivity are SPE and CL detection. The development of a SPE–LC–CL system would be a complicated task, but this could be simplified, exploiting the high versatility of the MSC technique.

The development of a fast SPE–LC–CL system could be a powerful tool for the determination, as for example, of thiazide compounds such as hydroflumethiazide (HFTZ), furosemide (FUR) and bendroflumethiazide (BFTZ) (Fig. 1), which present a diuretic action in humans. These compounds present CL emission by reaction with Tris(2,2′-bipyridylruthenium(III)) ($[Ru(bipy)_3]^{3+}$) [25,26]. Diuretics are a group of substances with pharmacological action over hypertension and heart or renal failure [27], but also are misused by athletes for fast weight loss (before competition in sports divided by weight categories) and also for dilution of other doping agents in the urine (prior to anti-doping controls). Diuretics are on the list of forbidden substances by the World Anti-Doping Agency (WADA) [28]. By this reason, fast and quick methods for the determination of diuretics in human urine samples are essential. The most recently developed analytical methodologies for the determination of diuretics in urine are based on complex methodologies and instrumentation, such as: (i) capillary electrophoresis–electrospray ionization–mass spectrometry [29], (ii) gas chromatography–mass spectrometry (after derivatization) [30], (iii) capillary electrophoresis–amperometric detection [31] or (iv) hollow fiber-based liquid–liquid–liquid microextraction coupled to high performance liquid chromatography with UV detection [32]. Beside this, several pharmaceuticals used commonly as diuretics are recognized as emerging contaminants in waste wasters [33]. In fact, in several cities from Italy, some diuretics have been detected in wastewaters by means of SPE combined with HPLC–MS/MS [34]. They have been found even at low $\mu g\ L^{-1}$ concentrations.

This work is aimed to solve an important drawback of low pressure chromatographic techniques, this is their limited selectivity and sensitivity. By this reason, the first on-line SPE–MSC–CL system has been developed and characterized for the determination of diuretics in potentially polluted well water, wastewater and human urine samples.

2. Experimental

2.1. Reagents and sorbents

Hydroflumethiazide (HFTZ), furosemide (FUR) and bendroflumethiazide (BFTZ) were purchased from Sigma–Aldrich (Germany). Stock solutions of 1000 mg L^{-1} HFTZ, BFTZ and FUR were prepared in methanol (Scharlau, Spain) and were stored at 4 °C in dark glass flasks. Standards were obtained from proper dilution of stock solutions and acidified to pH = 3 with H_2SO_4.

Other chemicals were of analytical-grade quality and they were used without purification. Millipore-quality water was used to prepare solutions. Acidified water to pH = 3 by using H_2SO_4 (Scharlau) was used as carrier for SPE. The $[Ru(bipy)_3]^{2+}$ reagent 10 mmol L^{-1} stock solution was prepared by dissolving 0.374 g Tris(2,2′-bipyridyl)dichlororuthenium(II) hexahydrate 99.95% metals basis (Sigma–Aldrich) in 0.05 L water. The oxidant 20 mmol L^{-1} stock solution was prepared by dissolving 0.664 g of ammonium cerium(IV) sulfate dihydrate 95% (Sigma–Aldrich) in 0.05 L of diluted H_2SO_4.

Acetonitrile (Scharlau) was used as mobile phase organic modifier. Potassium dihydrogen phosphate (Scharlau) 10 mmol L^{-1} is used as mobile phase buffer. Mobile phase was degassed for 15 min in an ultrasonic bath and filtered through a 0.45-μm Nylon membrane.

The solid reversed-phase materials tested for SPE were C18 (octadecyl), SDB-XC (poly(styrenedivinylbenzene)) and SDB-RPS (poly(styrenedivinylbenzene) modified with sulphonic groups) sorbent disks (Empore 3M, St. Paul, MN).

2.2. Instrumentation and software

Fig. 2 depicts the proposed flow system. A multisyringe automatic burette (MS) from Crison (Alella, Barcelona, Spain) was used to drive the liquids to the flow network. The burette was equipped with four syringes (S1–S4), which were all mounted on a common metallic bar and every one of them were provided with a solenoid valve (V1–V4); as a result, the four syringes were operated simultaneously. Depending on the position of the solenoid valves, the fluids contained in syringes were loaded (PK, pickup) or dispensed (DP, dispense) to the flow network (on) or the reservoirs (off). Syringe S1 (5 mL) acted as a flow carrier and was connected to an additional three-way solenoid valve (V5, MTV-3-N1/4UKG, 2 bar maximum pressure, Takasago, Japan) allowing the sample introduction into the flow network. A second syringe of 5 mL (S2) is used for the handling of the mobile phase for the elution, separation and detection of the previously isolated target compounds. S3 and S4 are both of 1 mL, and are used for the post-column injection of the two reagents required for the CL emission. V1, V3 and V4 do not suffer any significantly high pressure, so N-Research (West Caldwell, NJ) solenoid valves (maximum pressure 2 bar) were used. V2 suffers higher pressures than 2 bar, by this reason a two way connector made of polyoxymethylene was used to connect S2 with V2, which is situated outside the MS module. V2 is a Takasago MTV-3-1/4UKGH (Takasago), which allows a maximum pressure of 6 bar.

All the tubing of the system was made of polytetrafluoroethylene (PTFE) 0.8 mm i.d. except the holding coil (HC), which is a

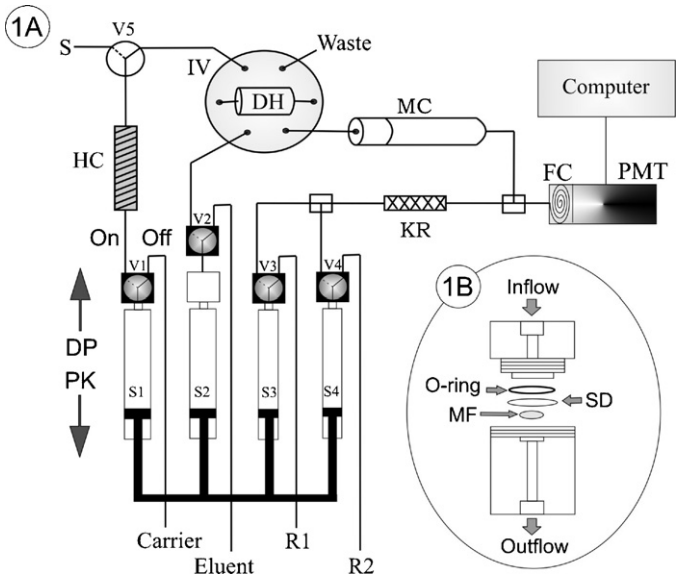

Fig. 2. Schematic depiction of the proposed multisyringe flow set-up for the determination of thiazide compounds. (A) General set-up: S, sample; HC, holding coil; V1–V5, three-way solenoid valves; DP, dispense; PK, pickup; off, normally open position of the solenoid valves (syringes connected with the reservoirs); On, normally closed position of the solenoid valves (syringes connected to the flow network); S1–S4, syringes; R1, Tris(2,2′-bipyridyl)dichlororuthenium(II) solution; R2, cerium(IV) solution; IV, injection valve; DH, disk holder for SPE; MC, monolithic column; KR, knotted reactor; FC, spiral-shaped flow cell; PMT, photomultiplier. (B) Amplified view of the DH: SD, sorbent disk; MF; microporous silica frit.

4 m long × 1.5 mm i.d. PTFE tube (7 mL volume). The two three-way confluence points were made also from PTFE.

A six-port injection valve (IV) (Crison) is used as interface between the sample loading – SPE – waste and the elution–LC–CL flow networks. In the injection loop of the IV is placed a miniaturized SPE device (Sciware, Palma de Mallorca, Spain) (Fig. 2), which is named disk holder (DH). The DH was a polyoxymethylene cylindrical block (2 cm long × 0.8 mm i.d.) with a central cavity of 1 cm diameter, in which an extraction disk of 0.9 cm diameter was accommodated in order to pass through it the different fluids involved in the SPE.

Extracted compounds are separated at room temperature by means of LC on a reversed-phase monolithic porous silica MC functionalized with octadecyl (C18) groups (25 mm length × 4.6 mm i.d.; Phenomenex) coupled with a guard column of the same material (5 mm length × 4.6 mm i.d.). The column was conditioned and cleaned according to the specifications of the manufacturer. A knotted reactor (KR; 60 cm length × 0.8 mm i.d.) is used for the generation of the $[Ru(bipy)_3]^{3+}$ from $[Ru(bipy)_3]^{2+}$ and Ce^{4+}.

The flow cell (FC; Sciware) was made from a PEEK plate to keep a PTFE body airtight. To this end, a spiral-shaped channel (4 cm long, 0.14 cm wide, 0.09 cm deep, 50 μl inner volume, 0.8 cm^2 effective light emission surface area) was carved into it; the spiral diameter was 0.9 cm. The FC was placed in front of a model H5784 photomultiplier tube (PMT, Hamamatsu, Japan) with a photosensitive wavelength range 04 (195–850 nm) and a photosensitive area of 0.5 cm^2. No wavelength discrimination was done in the detector. The FC and the PMT were both accommodated in a black plastic box protecting the detection system from external light.

A signal amplifier module [35] (Sciware) was used to supply the PMT with a feedback voltage of 0.3–0.8 V and obtain a signal gain (G) linear over the range 1–1000. The best results were obtained using a voltage of 0.80 V (G = 1000). The resultant scale for CL measurements is from 0 to 10000 counts. Instrumental control and data acquisition were accomplished by using the AutoAnalysis 5.0 (Sciware), which is based on dynamic link libraries (DLLs) [36] appropriate DLLs were used to operate the MS, the IV and the CL detector. MS DLL contemplates the use of the additional V5.

2.3. Analytical procedure

Table 1 summarizes the analytical procedure developed for the determination of HFTZ, FUR and BFTZ with the proposed SPE–MSC–CL system. The entire procedure complains 12 steps. On the steps 1–5 the SPE procedure is carried out, adjusting the volumes or repeating the steps, the volume of preconcentration can be easily modified. Once the sample matrix is isolated from the flow network, the elution of the previously retained species in the DH is accomplished, followed by LC with CL detection (steps 6–12).

3. Results and discussion

3.1. Chemiluminescence detection of diuretics

For the sensitive detection of the target compounds, chemiluminescence detection was chosen. The MSFIA technique is a useful tool for the micro-fluidic handling of complex matrix samples prior to CL detection [37].

The on-line chemical generation of $[Ru(bipy)_3]^{3+}$ from $[Ru(bipy)_3]^{2+}$ and Ce^{4+} was selected as a potentially appropriate detection system for the target compounds and preliminary studies were carried out by direct CL measurements (100 μL of sample were loaded into the HC and subsequently injected to the detector). The initial conditions were: 10 mmol L^{-1} $[Ru(bipy)_3]^{2+}$; 20 mmol L^{-1} Ce^{4+} in 0.25 mol L^{-1} H_2SO_4; 1 mg L^{-1} HFTZ, 10 mg L^{-1} FUR and 10 mg L^{-1} BFTZ, injection flow rate = 7 mL min^{-1}. With these experimental conditions the three target compounds exhibited repeatable CL signal (RSD's: HFTZ = 2.3%, FUR = 3.7%, BFTZ = 2.1%).

The concentration of $[Ru(bipy)_3]^{2+}$ was studied between 1 and 10 mmol L^{-1} (Fig. 3A) and a concentration of 7.5 mmol L^{-1} was selected. Once the previous concentration was adopted, the con-

Table 1
Main steps of the Autoanalysis 5.0 standard analytical SPE–MSC–CL procedure for the determination of HFTZ, FUR and BFTZ (for a sample volume of 1 mL).

Step	Inst	System protocol	Analytical description
1	MS	DP 1 mL at 15 mL min^{-1} (off/off/off/off/off)	Adjustment of syringe volumes
2	MS	PK 1 mL at 5 mL min^{-1} (on/off/off/off/on)	Sample loading
3	IV	Move to "Load" position	Connecting S1 with SPE unit and waste
4	MS	DP 1.5 mL at 2.5 mL min^{-1} (on/off/off/off/off)	SPE of the target compounds
5	MS	PK 1.5 mL at 15 mL min^{-1} (off/off/off/off/off)	Adjustment of syringe volumes
6	IV	Move to "Inject" position	Connecting S2 with SPE unit and LC–CL system
7	CL	Starts data acquisition every 0.1 s	
8	MS	DP 1.0 mL at 1.30 mL min^{-1} (off/on/off/off/off)	Elution from disk and LC separation
9	MS	DP 3.0 mL at 1.30 mL min^{-1} (off/on/on/on/off)	Continue the LC separation, and start the injection of reagents
10	MS	DP 0.7 mL at 1.30 mL min^{-1} (off/on/off/off/off)	System cleaning
11	CL	Stop data acquisition	
12	MS	PK 4.7 mL at 15 mL min^{-1} (off/off/off/off/off)	Adjustment of syringe volumes

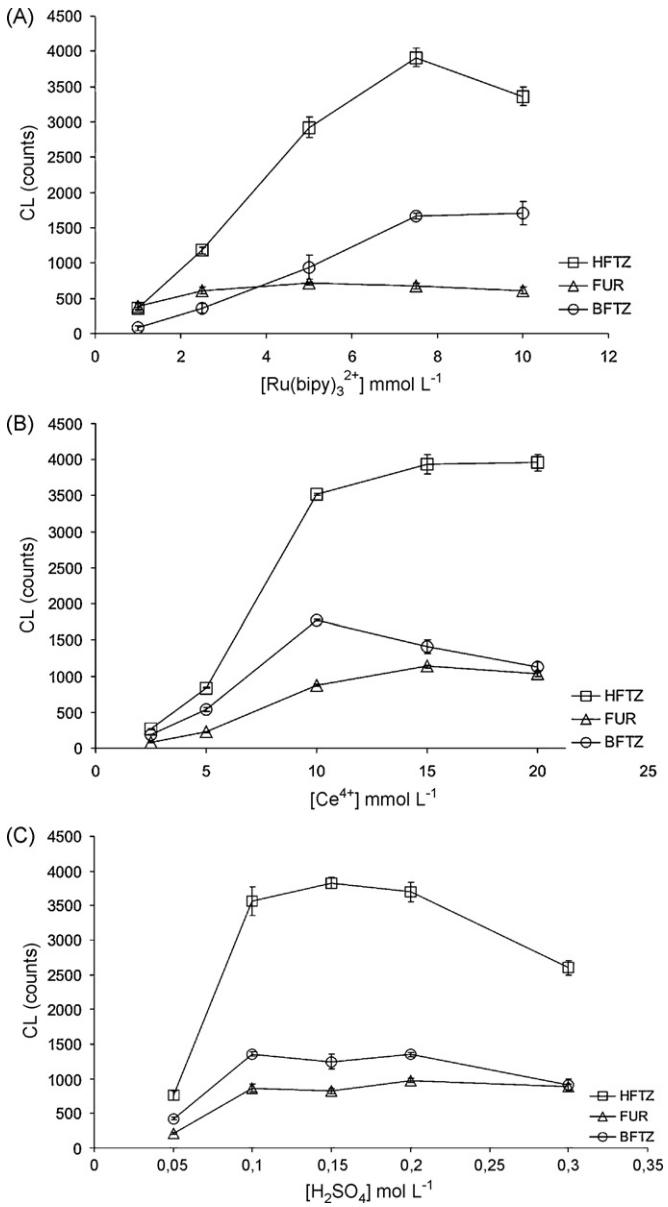

Fig. 3. Influence on the emitted CL (peak height) of (A) the concentration of Tris(2,2′-bipyridyl)dichlororuthenium(II) in S3, (B) the concentration of Ce^{4+} in S4 and (C) the concentration of H_2SO_4 in S4.

centration of Ce^{4+} was studied in the range of 2.5–20 mmol L^{-1}, as is shown in Fig. 3B a concentration of 10 mmol L^{-1} Ce^{4+} was selected for further experiments. The next experiment consists in the adjustment of the H_2SO_4 concentration of the Ce^{4+} solution, this was studied between 0.05 and 0.3 mol L^{-1} (Fig. 3C), lower H_2SO_4 concentrations induced short-term stability of Ce^{4+} solution, in a compromise between signal peak height and solution stability, a concentration of 0.2 mol L^{-1} H_2SO_4 was adopted for further experiments.

3.2. Solid phase extraction

In order to achieve the SPE of the target compounds for sample matrix clean-up and/or preconcentration, thus accomplishing it without increase significantly the back-pressure, sorbent disks were chosen instead of particulate resins. By this way, from the 6 bar maximum pressure allowed by V2, only 0.25 bar are employed for SPE procedure. Remaining a total of 5.75 free bars, that could be employed for the post-SPE liquid chromatographic separation. The three target compounds are highly polar by this reason an appropriate sorbent material could be SDB-RPS disks, but also were tested SDB-XC and C18 disks. The three types of disks were of an initial diameter of 47 mm, of which a smaller portion of 0.9 mm diameter is placed on the miniaturized SPE device, being the final effective surface extraction area of 0.6 mm diameter.

Changing the initial carrier composition by a mixture of 0.01 mol L^{-1} H_3PO_4–$H_2PO_4^-$/ACN (60:40) adjusted to pH = 3, no significant differences are observed in the obtained CL signals. So this was selected as the initial composition of eluent, and also 2 mL min^{-1} flow rates for preconcentration and elution were initially adopted. ACN was selected as organic modifier according to the specifications of the MC manufacturer. A preconcentration volume of 1 mL of sample was adopted.

The instrumental set-up for these experiments is the same as in Fig. 2, but without the MC. So, with this SPE–CL instrumental set-up, the three types of disks were tested. As can be seen in Table 2, SDB-RPS disks showed the best performance in terms of repeatability, obtaining adequate standard deviation values for the three target diuretics (RSD ≤ 3%). RSD's higher than a 5% were obtained for BFTZ using SDB-XC and for BFTZ and HFTZ using C18. In terms of sensitivity, higher signals were obtained for HFTZ and BFTZ by using SDB-RPS, but for FUR higher signals were obtained by using C18. Finally, SDB-RPS disks (90% adsorbent particles linked by 10% PTFE, 0.5 ± 0.05 mm in width, 60 Å pore diameter, 450 m^2 g^{-1} surface area and 12 μm average particle size) were chosen for further experiments.

The flow rate for the preconcentration of the target compounds was studied (Fig. 4A) in the range of 1–3 mL min^{-1}. Higher flow rates caused higher RSD's and a decrease of the life time of the sorbent disk. A flow rate of 2.5 mL min^{-1} was selected for further experiments. The percentage of organic modifier for the elution of the target compounds from the sorbent disk was studied between 20 and 50% ACN, as is seen in Fig. 4B, at least a 30% ACN is required in the mobile phase for the satisfactory elution of the three compounds.

In order to study the durability of the sorbent disk, a standard solution (mixture of 1 mg L^{-1} HFTZ, 10 mg L^{-1} FUR, 10 mg L^{-1} BFTZ) was analyzed 40 times with the same disk portion. For the first ten injections, the RSD was a 3%. For the next ten injection series, the RSD's were of 3.5% (11–20 injections), 4.5% (21–30 injections) and slightly higher than a 5% (31–40 injections). In conclusion, the same disk portion could be used satisfactorily for at least 30 injections.

3.3. Chromatographic separation

By using the complete SPE–MSC–CL set-up, the separation of the target compounds in the short 25 × 4.6 mm MC was studied follow-

Table 2
Results obtained in the selection of an appropriate sorbent material for SPE of HFTZ, FUR and BFTZ (n = 3). CL emission is expressed in hundreds of counts.

Sorbent material	HFTZ (1 mg L^{-1})		FUR (10 mg L^{-1})		BFTZ (10 mg L^{-1})	
	RSD (%)	CL emission	RSD (%)	CL emission	RSD (%)	CL emission
SDB-RPS	1.6	17	3.0	6	1.4	15
SDB-XC	0.6	10	2.6	6	8.2	5
C18	9.2	3	1.5	12	7.0	10

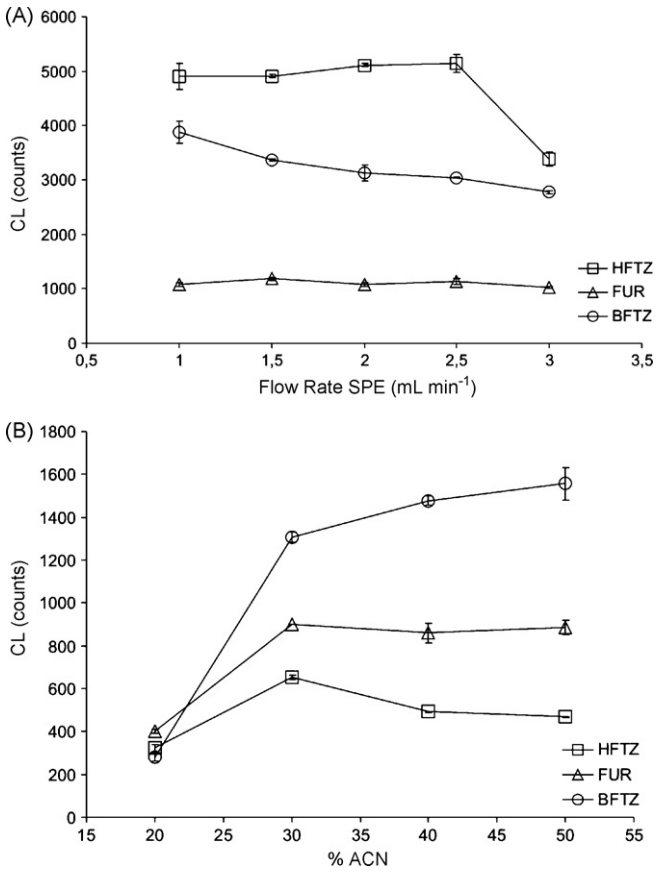

Fig. 4. Influence on the emitted CL (peak height) of (A) the flow rate for the SPE of the target compounds and (B) the percentage of organic modifier.

ing the procedure detailed in Table 1. The maximum mobile phase flow rate allowed by V2 (maximum pressure 6 bar) was estimated measuring the column back-pressure and increasing the flow rate. The maximum allowable flow rate (for mobile phases containing between 20 and 50% ACN) was 1.5 mL min^{-1} (pressure = 5.75 bar). Finally, in order to avoid short-term damage of V2, a final flow rate for the LC separation of 1.3 mL min^{-1} was selected for further experiments.

The percentage of organic modifier (ACN) was studied in the range of 20–50% ACN. By using a 20% ACN the three peaks were separated. Increasing the content of ACN to a 30%, no peak overlapping was observed, thus improved separation conditions were achieved. With a 40% ACN, HFTZ and FUR peaks partially co-eluted obtaining these two signals overlapped, and with a 50% ACN, the three compounds co-eluted in a single peak. Finally a 35% ACN was tested, achieving a good peak separation and a reduced mobile phase and reagents consumption.

In order to improve the LC procedure, the addition of methanol to the mobile phase was studied between 1 and 10% methanol. Best results were obtained with a 10% methanol, but this induced a severe precipitation of Ce^{4+} inside the flow cell, causing overpressure problems and decreasing signal repeatability. Finally, a 3% methanol was selected. The final eluent composition is H_3PO_4–$H_2PO_4^-$/ACN/CH_3OH (62:35:3) adjusted to pH = 3. By the addition of methanol peak tailing was reduced, thus signal peak height and elution of BFTZ were slightly improved. The selected composition of the mobile phase showed good compatibility with the CL reagents, a factor to have in consideration as one of the main sources of problems in LC–CL systems. Fig. 5A shows a typical chromatogram obtained by working under the selected conditions.

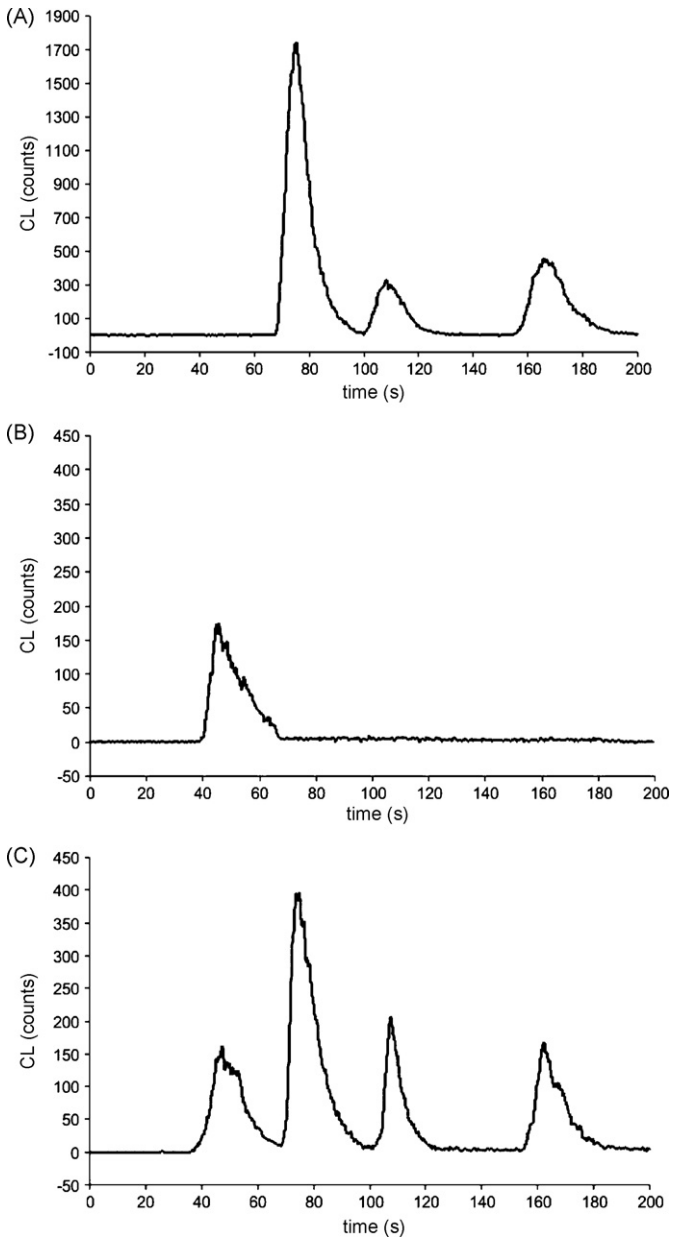

Fig. 5. Some examples of the obtained chromatograms working with the proposed SPE–MSC–CL system under the final experimental conditions. (A) Standard solution (HFTZ = 1.5 mg L^{-1}, FUR = 5 mg L^{-1}, BFTZ = 5 mg L^{-1}), (B) blank of an urine sample, (C) spiked urine sample (HFTZ = 1 mg L^{-1}, FUR = 10 mg L^{-1}, BFTZ = 5 mg L^{-1}). Standard/sample volume in (A) is 1 mL, in (B) and (C) is 3 mL. (B) and (C) have been diluted 10-fold.

Mass calibration was done injecting sample volumes between 1 – 20 mL containing an identical amount of total mass (2 μg HFTZ, 2 μg FUR, 2 μg BFTZ). No significant analyte loss (<5%) was observed when the preconcentration volume was increased from 1 to 7.5 mL. If the volume is increased up to 10 mL, a 10% analyte loss for HFTZ is observed, and FUR and BFTZ are still completely retained. Finally for a 20 mL sample volume, a 50% and a 60% of analyte breakthrough are observed for HFTZ and FUR, respectively, while no significant breakthrough is observed for BFTZ.

3.4. Analytical performance

Once the final instrumental set-up was established and the influence of the main variables examined, the performance of the

Table 3
Figures of merit of the proposed SPE–MSC–CL system. Parameters were calculated by using 1 mL of sample volume.

Analytical parameter	HFTZ	FUR	BFTZ
Linear dynamic range ($\mu g\,L^{-1}$)	6–4000	140–20,000	90–40,000
Limit of detection ($\mu g\,L^{-1}$)	3	60	40
Limit of quantification ($\mu g\,L^{-1}$)	6	140	90
Repeatability (%) ($n = 10$)	4.6	4.9	3.5
Sensitivity ($L\,\mu g^{-1}$) ($n = 6$)	1.244 ± 0.059	0.066 ± 0.002	0.093 ± 0.001
Regression coefficient ($n = 6$)	0.997 ± 0.002	0.994 ± 0.002	0.996 ± 0.003
Retention time (s)	75	108	165

proposed methodology was assessed. The analytical figures of merit of the proposed set-up are summarized in Table 3. Wide linear working ranges were achieved for the three analytes, which is typical in methods based on CL detection. The limits of detection (LOD) and quantification (LOQ) were estimated as 3 and 10 times the standard deviation of the absorbance for 10 injections of the blank. LOD and LOQ were calculated by using a sample volume of 1 mL, and they could be improved by increasing sample volume, in accordance with the mass calibration done. The repeatability of the proposed method was estimated as the relative standard deviation (RSD) for 10 consecutive injections of a standard containing 1 mg L^{-1} HFTZ, 10 mg L^{-1} FUR and 10 mg L^{-1} BFTZ. The sensitivity (slope) and regression coefficient were calculated from six day-to-day regression curves. The injection throughput (IT) obtained by using the entire SPE–MSC–CL method was 12 h^{-1}, if a sample volume of 1 mL is used (it is decreased to 7 and 5 h^{-1} for sample volumes of 5 and 10 mL, respectively). Another highlight of the proposed system is the low mobile phase consumption, with the concomitant saving of organic solvents. Only are required 1.65 mL ACN and 0.14 mL MeOH per determination. Furthermore is also presented a low consumption of CL reagents being achieved 400 determinations with only 1 g of $[Ru(bipy)_3]^{2+}$.

3.5. Interference tests

The CL evoked by $[Ru(bipy)_3]^{2+}$ can be used to determine a wide variety of organic compounds, but also, this CL reaction system cannot be applied directly to samples with a matrix of high complexity. In this work, we attempt to remove the potential interferences by means of on-line SPE–MSC prior CL detection, expanding by this way the analytical applicability of this CL reaction system. By this reason, in order to ensure this selectivity, a group of chemical compounds were tested as potential interfering compounds.

In order to do this, 100 mg L^{-1} standards of each one of the selected compounds were prepared and analyzed with the proposed configuration of the SPE–MSC–CL set-up. Different types of responses were obtained:

(1) Compounds which are removed by SPE and/or LC, or that are no CL emitters in the proposed experimental conditions. This was observed when ibuprofen, ketoprofen, trimethylamine, uric acid, glycine, phenol, ethylenediaminetetraacetic acid and p-aminocinnamic acid standards were analyzed.
(2) Compounds that exhibited CL emission with the proposed experimental conditions, but does not interfere due to their different retention times in the MC. This was observed analyzing a standard of Naproxen, this compound was extracted by SPE and quantified with good sensitivity by MSC–CL, obtaining similar results in sensitivity than for BFTZ, but with a retention time (tret = 192 s) higher than those of BFTZ.
(3) Compounds as oxalic acid, ascorbic acid or hydrazine that evoke intense CL emission with $[Ru(bipy)_3]^{2+}$ but are poorly retained by SPE and MC (approximately tret = 50 s). Experimental results confirmed that oxalic and ascorbic acids are almost totally removed by SPE, and due to the high concentration of the tested standard, only a small residual part of them arrives to the detector. Anyway, the retention times of these compounds are lower than those of HFTZ, not being interferences. Considerably higher concentration than 100 mg L^{-1} of these compounds could be a real interference. Nonetheless, hydrazine seems that is slightly more retained by SPE than oxalic and ascorbic acids. A concentration of hydrazine 20-fold higher than HFTZ causes a positive interference of a 5%, if the concentration of hydrazine is higher than 1 mg L^{-1}.
(4) Compounds which were CL emitters in the proposed experimental conditions, and have similar retention times than some of the analytes. This is the case of the thiazide diuretic hydrochlorothiazide (HCTZ), which has a retention time similar than HFTZ. The interference of 1-fold HCTZ over HFTZ is a 8%, if HCTZ concentration is at least 0.1 mg L^{-1}.

3.6. Application of the proposed SPE–MSC–CL system

The proposed methodology was assessed by analyzing HFTZ, FUR and BFTZ in spiked environmental and biological samples. The two environmental samples were a water sample from a well located in the vicinity of an urban solid waste landfill and a leachate from a solid urban waste landfill. The two biological samples were two human urine samples collected from healthy volunteers.

All samples were filtered through a 0.45 μm pore filter, spiked with a concrete amount of target compounds and adjusted to pH = 3. Furthermore, non-spiked samples were analyzed in order to test the baseline behavior and if they contain significant interferences. None of the two environmental samples exhibited significantly higher CL emission than the blank, nonetheless the two urine samples exhibited CL emission at low retention times, this drawback was avoided by means of diluting 10-fold the urine samples, avoiding possible interferences with the HFTZ signal peak. An example of a blank and a spiked urine samples are shown in Fig. 5B and C, respectively.

Table 4 shows the results obtained from the analysis of the detailed spiked samples with the proposed system. Environmental samples were spiked at the $\mu g\,L^{-1}$ level and a volume of 5 mL of sample was used. Urine samples were spiked at the mg L^{-1} level, and after dilution, a volume of 2.5 mL of urine 1 and 1 mL of urine 2 were used. In all instances, relative standard deviations between 1.8 and 4.4% for environmental and 2.7–5.5% for urine samples, were obtained. Furthermore, the absolute recoveries were between 94 and 108% in all instances.

The proposed method due to its wide linear dynamic range, can be applied satisfactorily to samples containing $\mu g\,L^{-1}$ levels of the target compounds, and also at mg L^{-1} level in high concentrated matrices, allowing sample dilution.

3.7. Comparison with other current analytical methodologies for the determination of diuretics

Due to the current relevance about the determination of diuretics in urine samples for anti-doping controls, several improved methodologies have been developed recently [29–32]. They are

Table 4
Results obtained in the determination of HFTZ, FUR and BFTZ in spiked environmental and biological samples by means of the proposed SPE–MSC–CL system. For all samples $n = 3$.

	Samples			
	Added ($\mu g\,L^{-1}$)	Found ($\mu g\,L^{-1}$)	RSD (%)	Absolute recovery (%)
Environmental				
HFTZ				
Well	2	2.13	4.3	107
Leach	5	5.06	3.4	101
FUR				
Well	20	20.6	4.4	103
Leach	50	53.4	2.9	107
BFTZ				
Well	20	21.4	3.1	107
Leach	50	49.6	1.8	99
	Samples			
	Added ($mg\,L^{-1}$)	Found ($mg\,L^{-1}$)	RSD (%)	Absolute recovery (%)
Human urine				
HFTZ				
Urine 1	1	1.03	5.5	103
Urine 2	10	10.8	4.5	108
FUR				
Urine 1	1	0.94	4.1	94
Urine 2	10	10.2	2.7	102
BFTZ				
Urine 1	1	1.06	3.9	106
Urine 2	10	9.57	4.1	96

based on highly expensive and time-consuming instrumentation, thus lower levels in terms of automation, being required in some cases time-consuming manual sample derivatization (at least 2 h) [30] or extraction (50 min) [32] treatments prior to GC–MS and HPLC-UV, respectively.

As an alternative to chromatography, recently developed capillary electrophoresis (CE) methods for diuretics have provided improved injection throughputs, as for example a highly selective and sensitive CE-ESI-MS method for the determination of 12 diuretics [29], providing almost no sample pretreatment and an IT = 3 h^{-1}. Another alternative is based on CE with amperometric detection [31], which provides an improved IT = 6 h^{-1} and a wider linear dynamic range of three order of magnitude, but with a concomitant lower selectivity than CE-ESI-MS.

On the one hand, the proposed SPE–MSC–CL system provides a higher automation degree in front of the previously commented systems, is a low cost system, both referring to the low cost of the used instrumentation and furthermore the low cost per analysis. It provides a wide linear range as with CE-amperometry, and similar sensitivity than the previous systems with only 1 mL of sample. The proposed method provides higher injection throughputs (IT = 12 h^{-1}), and is highly versatile being easily adapted to different preconcentration volumes or types of samples.

On the other hand, the multi-analyte determination by the proposed system becomes limited in comparison with high performance chromatographic or electrophoretic techniques coupled with mass spectrometric detectors. But having in account the necessity to have fast-screening systems for organic compounds (pharmaceuticals, pesticides, personal care products, etc.), the proposed system provides an standard analytical tool – which is applied in this work to the determination of some thiazide diuretics – that meets the main corollaries for fast-screening, such as: rapid and reliable response, simplification of operations, thus avoiding the extensive use of high cost and maintenance instrumentation.

If we are talking about the development of simplified methodologies for the determination of emerging organic pollutants in environmental samples, the proposed SPE–MSC–CL provides an smart, versatile and downsized efficient instrument to carry out analytical tasks in this field, but it is still work to be done in this field, in terms of selectivity, and overcoat in the obtention of improved sensitivity to the low ng L^{-1} range.

4. Conclusions

For the first time, the potential of the versatility of monolithic column-based chromatographic separations in low/medium-pressure flow systems have been fully exploited, allowing the implementation of on-line pre- and post-column sample treatments in a smart, miniaturized and expeditious fashion, being this achieved through the versatile fluidic handling allowed by the MSFIA technique. To prove this, a novel methodology for the determination of three known diuretics (HFTZ, FUR and BFTZ) has been developed. The proposed method is the first on-line completely automated method which combines SPE with low pressure LC and CL detection. This allowed the fast, selective and sensitive determination of the target compounds, thus a concomitant saving of reagents/solvents. This improved performance, allowed for the first time the application of syringe-based (SIC and MSC techniques) multicommuted LC separations to the determination of target compounds in complex samples (environmental and human urine samples) at the $\mu g\,L^{-1}$ level, instead their usual applications in simple commercial formulations of pharmaceuticals, herbicides and other types of products at the $mg\,L^{-1}$ level. This is transduced in an improved applicability of this type of techniques, being fast and low cost screening valuable tools, for vanguard strategies to carry out difficult tasks as the anti-doping controls, or the detection of emerging contaminants in waters.

Acknowledgements

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5.14. Artículo original VIII

Multisyringe Ion Chromatography With Chemiluminescence Detection for the Determination of Oxalate in Beer and Urine Samples

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ORIGINAL PAPER

Multisyringe ion chromatography with chemiluminescence detection for the determination of oxalate in beer and urine samples

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Abstract The first multisyringe-based low-pressure ion chromatographic method is presented. It is based on the use of short surfactant coated octadecyl-silica monolithic columns. As a first application, we have determined oxalate in beer and human urine via post-column chemiluminescence detection. Oxalate is separated from the sample matrix in the monolithic column by precise programmable fluid handling, and then detected by reaction with on-line generated tris(2,2′-bipyridyl)ruthenium(III). Column coating, un-coating, ion chromatography and chemiluminescence detection are quickly performed by using a simple low-pressure multi-burette. The factors influencing the separation of oxalate and its subsequent detection, including the column coating with surfactants and its stability have been studied. The chromatographic behavior of the oxalate in presence of potentially interfering species also was assessed. The method has limits of detection and quantification of 0.025 and 0.035 mg L^{-1}, respectively, a relative standard deviation of 3.1% (for 10 consecutive measurements without column re-coating) and a throughput of 48 h^{-1}. The results obtained with real samples were validated by using an enzymatic spectrophotometric test. The method is critically compared to recent methods for the determination of oxalate.

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Keywords Multisyringe flow injection analysis · Monolithic columns · Surfactant coatings · Ion chromatography · Chemiluminescence · Oxalate · Urine · Beer

Introduction

One of the current demands on the analytical chemistry field is the development of new tools for the efficient production of high quality analytical information. This is intended as the quick generation of information related with a property of interest from a sample including high sensitivity, selectivity and reproducibility levels. This information is often contradictory, inasmuch as the achievement of good analytical features involves a concomitant decrease in the analysis throughput, and vice versa. For example, flow analysis techniques are well established instrumental tools for the development of fast analytical methodologies [1, 2], thus downscaling analytical processes reducing both costs and produced waste products [3, 4]. The main drawback of flow analytical techniques is their limited selectivity for the accomplishment of multi-analyte determinations, or the determination of a single compound in samples with a highly complex matrix. This kind of determinations are practically limited to: (i) speciation procedures [5, 6], (ii) use of chemometric approaches [7, 8], thus (iii) the development of analytical methods based on the expression of the obtained results as a total index or sum parameter [9, 10].

The development of alternatives for the increase of the selectivity of flow-based techniques would be of interest, being the implementation of monolithic materials [11, 12] as stationary phases in flow techniques [13, 14] a practical approach to overcome this limitation. Monolithic columns (MC) enable the possibility to achieve liquid chromato-

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graphic separations at lower back-pressures than using classic particle-based columns. By this way, the combination of MC´s with flow analysis techniques is a current research field on the development of improved analytical methodologies [15, 16]. The development of low-pressure high-performance fast chromatographic methods started with the combination of the sequential injection analysis (SIA) technique with 25×4.6 mm silica-based C18 monolithic columns, this technique was named sequential injection chromatography (SIC) [13, 14]. It was followed by the combination of the multisyringe flow injection analysis (MSFIA) [17, 18] with the same type of MC´s defining the resultant technique as multisyringe chromatography (MSC) [19, 20].

On the one hand, the initial low sensitivity obtained by these techniques was improved taking advantage of their inherent versatility, implementing miniaturized disk-based solid phase extraction [21], post-column chemiluminescence detection [22] or a combination of both [23]. On the other hand, the combination of flow techniques with monolithic columns has also been used for the separation of charged species like common anions [24, 25] or alkaline earth metals in water samples [26] by coating C18 monolithic columns with appropriate anionic or cationic surfactants [27]. However, the potential of flow-based techniques has not been still exploited in the determination of charged species by low-pressure ion chromatography in complex samples.

As starting point for the development of this initial concept we can state that the determination of oxalate in urine samples is interesting due to the function of oxalate as a precursor in the formation of renal stones. Furthermore, the determination of oxalate in the quality control of beer production is also a relevant application. Oxalate presents a good reactivity with the chemiluminometric reagent Tris (2,2′-bipyridylruthenium(III)) ($[Ru(bipy)_3]^{3+}$) [28, 29], and as it is an anion, it will be potentially separable from the sample matrix by using a cationic surfactant-coated monolithic column. The available methods for oxalate determination in complex sample matrices are based on the use of manual tests [30, 31], or the use of separation techniques such as high-performance liquid chromatography (HPLC) [32, 33], ion chromatography (IC) [34] or capillary electrophoresis (CE) [35, 36]. But all of them present relatively low analysis throughputs.

The aim of the present work is the development of the first multisyringe low-pressure ion chromatographic (MSIC) system with chemiluminescence detection. The development of this system is facilitated due to the high versatility inherent to the MSFIA technique and the use of short silica-based MC´s. In this case, the potential of the proposed MSIC approach has been demonstrated exploiting chemiluminescence detection (MSIC-CL) serving as an expeditious tool for the determination of oxalate in different types of complex samples, covering the lack of fast methodologies for the determination of this compound.

Experimental section

Reagents and solutions

All chemicals used were of analytical-grade quality and they were used without further purification. Millipore-quality water was used to prepare solutions. Different solutions of Potassium hydrogen phthalate (KHF), sodium carbonate and sodium sulphate purchased from Panreac (http://www.panreac.com) were used as eluents. These solutions were degassed for 15 min in an ultrasonic bath and filtered through a 0.45 μm Nylon membrane (Scharlab, http://www.scharlab.com).

The $[Ru(bipy)_3]^{2+}$ reagent 10 mmol L^{-1} stock solution was prepared by dissolving 0.374 g Tris(2.2´-bipyridyl) dichlororuthenium(II) hexahydrate 99.95% metals basis (Sigma-Aldrich, http://www.sigmaaldrich.com) in 0.05 L water. The oxidant 15 mmol L^{-1} stock solution was prepared by dissolving 0.664 g of Ammonium Cerium (IV) Sulfate Dihydrate 95% (Sigma-Aldrich) in 0.05 L of 0.2 mol L^{-1} H_2SO_4. The use of H_2SO_4 is required for the preparation of stable aqueous solutions of the Cerium(IV) reagent.

Coating solution was a 5 mmol L^{-1} N-Cetyl-N,N,N-trimethylammonium bromide (CTAB, Merck, http://www.merck-chemicals.com) solution, prepared by dissolving 0.456 g CTAB in 0.25 L of an acetonitrile/water (5:95) mixture. As oxalate standard, sodium oxalate (Sigma-Aldrich) was used.

Instrumentation and software

Figure 1 depicts schematically the proposed set-up. A multisyringe burette (Crison, http://www.crison.es) was equipped with three syringes (S1−S3), which were all mounted on a common metallic bar and every one of them were provided with a solenoid valve (V1-V3). As a result, all syringes were operated simultaneously. Depending on the position of the solenoid valves, the fluids contained in syringes were loaded (PK, pickup) or dispensed (DP, dispense) towards the flow network (on) or the reservoirs (off). Syringe S1 (5 mL) performs sample loading and the oxalate separation. S1 was connected to a two-way connector made from polyoxymethylene, allowing the connection of S1 with an external three-way solenoid valve V1 (Takasago, MTV-3-1/4UKGH, http://www.takasago-elec.com). V1 bears higher pressure (6 bar) than conventional solenoid valves (2 bar) enabling higher flow rates for the oxalate separation.

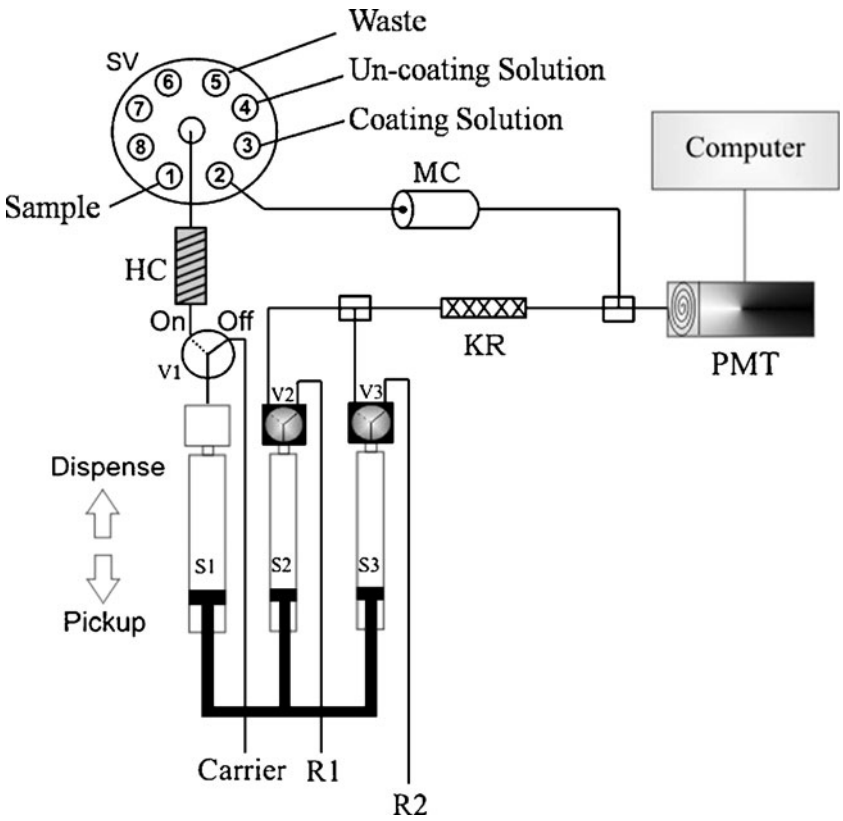

Fig. 1 Schematic depiction of the proposed flow system: S1–S3, syringes; V1–V3, three-way solenoid valves; R1, Tris(2.2′-bipyridyl)dichlororuthenium(II) solution; R2, Cerium(IV) solution; HC, holding coil; SV, selection valve; MC, monolithic column; KR, knotted reactor; PMT, photomultiplier with an spiral-shaped flow cell placed in front of it

S2 and S3 (both of 1 mL) are used for the post-column injection of the two CL reagents. V2 and V3 do not suffer any significantly high pressure, so N-Research (http://www.nresearch.com) solenoid valves (max. pressure 2 bar) were used. All the tubing of the system was made of polytetrafluoroethylene (PTFE) 0.8 mm i.d.

The introduction of samples, coating solution, un-coating solution into the flow network was performed by using an eight-port selection valve (SV, Crison). These solutions are loaded into a holding coil (HC, 5 m length). The two three way confluence points were made also from PTFE. A knotted reactor (KR; 60 cm length x 0.8 mm i.d.) is used for the generation of the $[Ru(bipy)_3]^{3+}$ from $[Ru(bipy)_3]^{2+}$ and Ce(IV).

As stationary phase for the separation of oxalate from the sample matrix components, a silica short monolithic column functionalized with octadecyl (C18) groups (10 mm length× 4.6 mm i.d.; Phenomenex, http://www.phenomenex.com) was used.

The chemiluminescence detector (Sciware, http://www.sciware-sl.com) was constituted by a flow cell (FC) made from a PEEK plate to keep a PTFE body airtight. To this end, a spiral-shaped channel (4 cm long, 0.14 cm wide, 0.09 cm deep, 50 μl inner volume, 0.8 cm^2 effective light emission surface area) was carved into it; the spiral diameter was 0.9 cm. The FC was placed in front of a model H5784 photomultiplier tube (PMT, Hamamatsu, http://www.hamamatsu.com) with a photosensitive wavelength range 04 (195–850 nm) and a photosensitive area of 0.5 cm^2. No wavelength discrimination was done in the detector. The FC and the PMT were both accommodated in a black plastic box protecting the detection system from external light.

A signal amplifier module (Sciware) was used to supply the PMT with a feedback voltage of 0.3–0.8 V and obtain a signal gain (G) linear over the range 1–1000. The best results were obtained using a voltage of 0.80 V (G=1000). The resultant scale for CL measurements is from 0–10000 counts. Instrumental control and data acquisition were accomplished by using the AutoAnalysis 5.0 software package (Sciware) [37].

Analytical procedure

In Table 1 is summarized the procedure developed for the determination of oxalate with the developed multisyringe set-up. The entire procedure complains 16 steps. Steps 1 to 6 are used for the automatic re-coating of the monolithic column. The analytical procedure for the oxalate determination is carried out between the steps 7 to 16, including a multicommutated step (step 13), allowing the saving of CL reagents by enabling their injection just when these are required.

Table 1 Main steps of the Autoanalysis 5.0 analytical procedure developed for the determination of oxalate

Step	Inst	System protocol	Analytical description
1	MS	DP 1.5 mL at 15 mL min^{-1} (off/off/off)	Adjustment of syringe volumes
2	SV	Move to position 3	Connecting S1 with coating solution
3	MS	PK 1.5 mL at 5 mL min^{-1} (on/off/off)	Loading coating solution into HC
4	SV	Move to position 2	Connecting S1 with IC-CL system
5	MS	DP 3.0 mL at 2 mL min^{-1} (on/off/off)	Column coating
6	MS	PK 3.0 mL at 15 mL min^{-1} (off/off/off)	Adjustment of syringe volumes
7		Start LOOP	Start procedure for oxalate determination
8	MS	DP 0.1 mL at 5 mL min^{-1} (off/off/off)	Adjustment of syringe volumes
9	SV	Move to position 1	Connecting S1 with sample reservoir
10	MS	PK 0.1 mL at 1.5 mL min^{-1} (on/off/off)	Sample loading into HC
11	SV	Move to position 2	Connecting S1 with IC-CL system
12	CL	Starts data acquisition every 0.1 s	
13	MS	Multicommuted step: 1) DP 1 mL at 3.5 mL min^{-1} (on/off/off) 2) DP 1.2 mL at 3.5 mL min^{-1} (on/on/on) 3) DP 1 mL at 3.5 mL min^{-1} (on/off/off)	Multicommuted step for reagent saving
14	CL	Stop data acquisition	
15	MS	PK 3.2 mL at 15 mL min^{-1} (off/off/off)	Adjustment of syringe volumes
16		End LOOP	Repeat oxalate determination X times

Results and discussion

Column coating

CTAB has been selected as an adequate surfactant for this application according with the results of Glenn and Lucy [38]. CTAB enables efficient column coatings, thus avoiding some of the drawbacks inherent to the two-tailed surfactants like didodecyldimethyl-ammonium bromide (DDAB), which can provide reproducibility and back-pressure problems.

In order to determine the maximum capacity of CTAB coating the MC column, this was equilibrated with the un-coating solution, which was Acetonitrile/water (50/50). After this, the column was flushed with water followed by a 5 mmol L^{-1} CTAB in 5% ACN solution at 2 mL min^{-1}. Column breakthrough was determined by taking 0.5 mL aliquots from the column outlet. These were diluted with water up to a volume of 5 mL, and the conductivity of the resultant solution was measured. As is seen in Fig. 2a, the first 2 mL of CTAB solution are almost completely retained in the column. The measured conductivity is similar to that of distilled water. From 3 mL onwards the CTAB solution is completely percolated through the column without surfactant retention, being the measured conductivity the same to that of the surfactant solution. Therefore, an initial coating of at least 3 mL of 5 mmol L^{-1} CTAB (in 5% ACN) is required for the complete column coating.

The CTAB column coating is removed by flushing the MC again with un-coating solution [38].

Validation method

The obtained results by the developed method are compared with those obtained analyzing the same samples with an enzymatic UV-spectrophotometric test [30]. Shortly, oxalic acid is cleaved to formic acid and CO_2 at pH=5 in the presence of oxalate decarboxylase. Formic acid is quantitatively oxidized to bicarbonate by nicotinamide-adenine dinucleotide (NAD) at pH=7.5 in the presence of the enzyme formate dehydrogenase (FDH). The amount of NADH (followed at 340 nm) released in the previous reaction is stoichiometric to the amount of oxalic acid. The limit of detection of this method is 1.6 mg L^{-1} of oxalate.

Chemiluminescence detection of oxalate using the MSFIA technique

The MSFIA technique enables the smart handling of sample and reagents plus the required sample treatments prior the CL detection [39, 40]. In this case, the on-line chemical generation of $[Ru(bipy)_3]^{3+}$ from the oxidation of $[Ru(bipy)_3]^{2+}$ by means of Ce(IV) was selected as an appropriate CL reaction system for the detection of oxalate. Preliminary studies were carried out by direct CL measurements (100 μL of sample were loaded into the HC and subsequently injected towards the detector). The initial conditions were: 10 mmol L^{-1} $[Ru(bipy)_3]^{2+}$; 10 mmol L^{-1} Ce(IV) in 0.2 mol L^{-1} H_2SO_4; 1 mg L^{-1} Oxalate, total injection flow rate=7 mL min^{-1}. The concentration of $[Ru(bipy)_3]^{2+}$ was studied among 1–10 mmol L^{-1} (Fig. 2b)

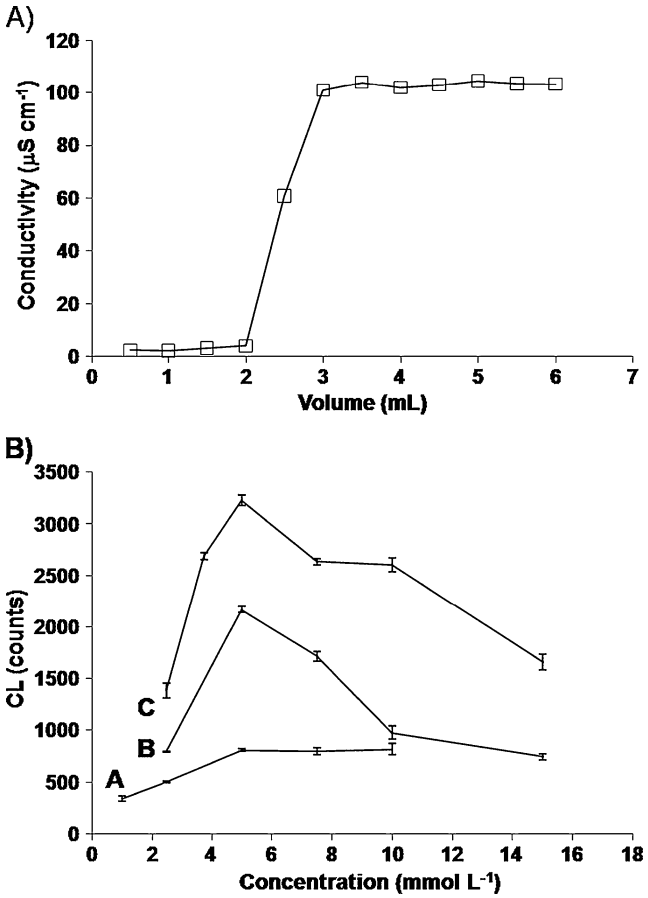

Fig. 2 **a** Column breakthrough represented as the increase in conductivity as a function of the coating solution volume (5 mmol L^{-1} CTAB in 5% ACN). **b** Effect of the concentrations of post-column reagents on the obtained CL signal (A=[sulphuric acid]/20; B=[Ce(IV)]; C=[Ru(bipy)])

and a concentration of 5 mmol L^{-1} was selected. Once the previous concentration was adopted, the concentration of Ce(IV) was studied in the range among 2.5–15 mmol L^{-1}, as is shown in Fig. 2b a concentration of 5 mmol L^{-1} Ce (IV) was selected for further experiments. The next experiment consisted in the adjustment of the concentration of H_2SO_4 contained in the Ce(IV) solution, this was studied between 0.05–0.3 mol L^{-1} (Fig. 2b), lower H_2SO_4 concentrations induced short term stability of Ce(IV) solution, in a compromise between signal peak height and solution stability, a concentration of 0.1 mol L^{-1} H_2SO_4 was adopted for further experiments.

Studies about the oxalate determination by means of the developed MSIC-CL technique

A 10×4.6 mm monolithic column was then implemented into the proposed MSFIA set-up. Initially, the maximum mobile phase flow rate allowable by the multisyringe burette was studied. This was carried out injecting water solutions through the monolithic column (which was coated under the previously detailed conditions), measuring the pressure obtained in the inlet of the column. As can be seen in Fig. 3a, the maximum flow rate allowable by V1 (max. pressure 6 bar) is 4 mL min^{-1}.

A 10 mmol L^{-1} SO_4^{2-} pH=7 solution was tested initially as eluent obtaining a retention time for oxalate of 37 s but with a concomitant decrease of the post-column CL emission. Substituting the previous eluent by a 10 mmol L^{-1} KHF solution at pH=7, CL emission was increased 3-fold, thus obtaining a retention time of 16 s. This was confirmed by direct injection of oxalate solutions prepared in water and in a 10 mmol L^{-1} KHF solution, not obtaining significant differences in the measured peak heights.

Decreasing the initial concentration of KHF from 10 to 2.5 mmol L^{-1} the blank was minimized and the retention time of oxalate was increased from 16 to 29 s. In Fig. 3b is shown the effect of the surfactant coating in the monolithic column on the retention of oxalate. The death volume of the manifold causes a delay of 5 s in the direct injection of the sample from the HC to the detector. By the presence of the

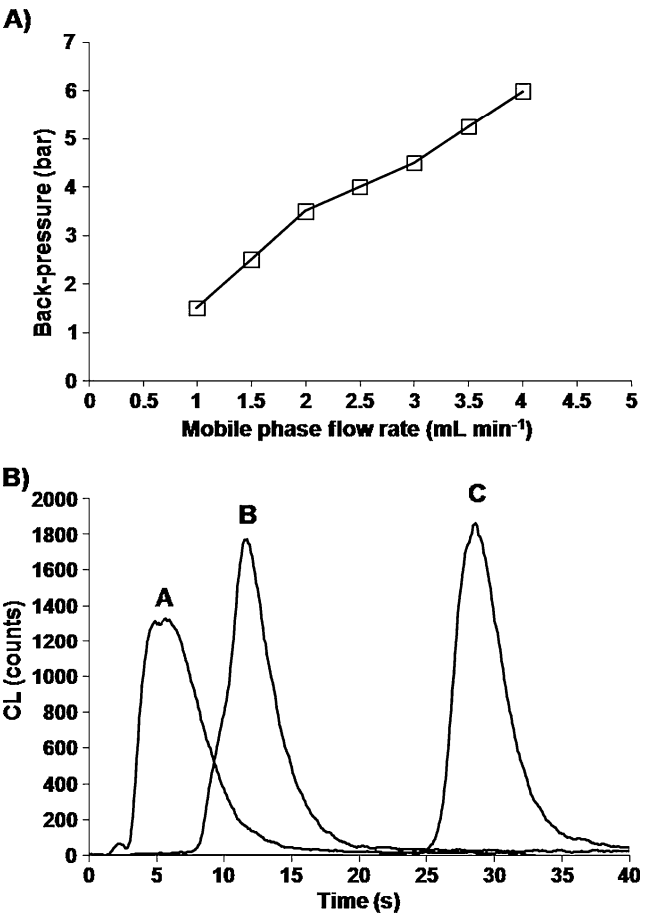

Fig. 3 **a** Influence of the flow rate on the obtained back-pressure of the monolithic column. **b** CL signals obtained analyzing the same 2 mg L^{-1} oxalate standard without column (Signal A), with the uncoated column (Signal B) and with a CTAB coated column (Signal C)

uncoated column this time is increased to 12 s. If the column is coated with CTAB under the reported conditions, the retention time is increased up to 29 s.

Two different columns were compared in order to evaluate possible inter-column differences in the obtained results. The relative standard deviation obtained was a 3.8% in peak height and a 0.5% in retention time, being negligible the errors obtained from changing one column by another. The sample volume was studied between 50–200 μL, being selected a final volume of 100 μL. Lower sample volumes provided considerably lower peak heights. Higher sample volumes provided a modest increase in peak height but a concomitant high increase in peak width.

The anion exchange capacity of the column is modulated by the addition of ACN to the coating solution. Coating solutions with a 1, 5 and 10% of ACN were tested maintaining a fixed volume of coating solution. The obtained RSD´s in peak height were 5.7, 2.7 and 3.7%, respectively. The obtained retention times were 29, 29 and 24 s, respectively.

Therefore, the inclusion of a 5% ACN in the coating solution maintained almost the same retention time than using just an aqueous CTAB solution, but improving the coating stability and reproducibility of the results for the first injections accomplished, in comparison with a 100% aqueous CTAB coating solution.

The flow rate of the mobile phase was studied between 1–4 mL min^{-1}. A flow rate of 1 mL min^{-1} is the minimum permitted flow rate for the multisyringe module used in this work (5000 step motor) if we are working with a 5 mL syringe. A flow rate of 4 mL min^{-1} is the maximum allowed by the external valve (V1).

Concerning the mobile phase flow rate, higher peak heights are obtained by using at least a flow rate of 2.5 mL min^{-1}. Finally, in a compromise between obtain a highest injection throughput possible, thus avoiding premature valve damage caused by overpressure, a final flow rate of 3.5 mL min^{-1} was adopted for further studies. The detailed flow rate is referred to the S1, being the total flow rate in the flow cell of 4.9 mL min^{-1}.

Working under the previous experimental conditions, the peak capacity was 1.7 for oxalate, which is a similar capacity than the obtained in order studies using coated monolithic columns of similar length [25].

In order to study the stability of the CTAB coating on the MC, 30 consecutive oxalate determinations without re-coat the column were accomplished. Relative standard deviations of 3.1 (injections 1–10), 6.3 (injections 11–20) and 8.4% (injections 21–30) were obtained for the measured CL peak heights. For the same number of injections relative standard deviations of 1.4, 1.9 and 2.3% for the retention time of the oxalate peak were obtained. Anyway column re-coating procedures can be accomplished automatically in a short time.

Analytical performance

Once the final instrumental MSFIA set-up was established and the influence of the main variables was examined, the analytical figures of merit were calculated.

A wide linear dynamic range for oxalate between 0.035–10 mg L^{-1} was found, which is common in CL detection systems involving the use of $[Ru(bipy)_3]^{3+}$. The limits of detection (LOD) and quantification (LOQ) were estimated as 3 and 10 times the standard deviation of the CL peak height for 10 injections of the blank. The obtained LOD and LOQ were 0.025 and 0.035 mg L^{-1}, respectively.

The repeatability was calculated as the relative standard deviation (RSD) for ten consecutive injections of a standard containing 1 mg L^{-1} oxalate. As was stated in the previous section the RSD obtained with a single coating is a 3.1%, being this improved re-coating the column (with 1.5 mL of CTAB solution) prior each injection obtaining a RSD of a 1.9%.

The sensitivity (slope) and regression coefficient were calculated from six day-to-day regression curves: Sensitivity= 0.923±0.041, regression coefficient=0.9978±0.0009. The RSD between the different slopes was a 4.5%. The injection throughput (IT) is 48 h^{-1}. Including a column re-coating each three injections (as detailed in Table 1) the average IT is 33 h^{-1}.

A complete removal of the column coating followed with the accomplishment of a new complete coating can be performed in 320 s. Flushing the column with 2 mL of the un-coating solution, followed with 3 mL of the coating solution.

Interference test

In this work the CL determination of oxalate is provided with selectivity recurring to the use of short surfactant-coated monolithic columns as ion chromatographic support for sample clean-up. By this reason, in order to ensure this selectivity, a group of chemical compounds were tested as potential interfering compounds. In order to accomplish this task, standards with a concrete amount of each one of the selected compounds were prepared and analyzed with the proposed set-up. As a reference for this study, a signal provided by the tested compounds, which is equal to 50 counts will be equal to a 5% error in the measurement of a 1 mg L^{-1} oxalate.

(i) Besides oxalate, the behavior of several carboxylic acids that elicited CL by reaction with $[Ru(bipy)_3]^{3+}$ were tested. Succinic, tartaric and citric acids do not interfere at a 1000 mg L^{-1} level. A maximum tolerable level of gluconic acid was found at 250 mg L^{-1}.

(ii) The behavior of various major urine components such as uric acid and urea were analyzed at their usual concentration in un-diluted urine. No interference higher than a 5% was found by analyzing a 1000 mg L^{-1} uric acid solution and a 20000 mg L^{-1} urea solution, being this study useful in order to predict the potential applicability of the proposed method in urine samples.

(iii) Other compounds that elicit CL by reaction with $[Ru(bipy)_3]^{3+}$ under similar conditions than the detailed in this work, were tested. A 1000 mg L^{-1} L-Cysteine solution elicited similar CL than a 0.2 mg L^{-1} oxalate solution, but at a retention time of 13 s, not being a real interference. Ascorbic acid is also a reactive compound with $[Ru(bipy)_3]^{3+}$, it can be presented in urine at high concentrations (hundreds of mg L^{-1}), overcoat in people consuming vitamin supplements. Furthermore it is potential interfering specie in enzymatic tests for oxalate determination. As is shown in Fig. 4a, a 1000 mg L^{-1} solution of ascorbic acid provides a strong CL signal with a retention time of 13.5 s, being this peak completely separated from the peak of oxalate.

(iv) Several organic compounds used commonly as pharmaceuticals can react with $[Ru(bipy)_3]^{3+}$ producing a positive interference. Hydrochlorothiazide, bendroflumethiazide and naproxen were tested at a 100 mg L^{-1} level, not causing any significant interference. Some reproducibility problems can be attained to the retention of these compounds in possible non-coated sites of the column. So, in urine samples with high loads of pharmaceuticals a coating-recoating procedure after each injection can avoid possible carry-over interferences.

Application of the proposed system

The developed methodology was assessed by determining oxalate in beer and human urine samples. The two beer samples were from two different brands of commercially available lager beer. The two urine samples were collected from healthy volunteers. All samples were filtered through a 0.45 μm pore Nylon filter, diluted (if it was necessary) and adjusted to pH=3 (Oxalic acid, pKa1=1.27, pKa2=4.28). The pH was adjusted in order to minimize the possible interferences of other compounds presenting negative charge at lower pH values.

In order to work in the linear region of the method, thus minimize potential interferences, the samples were diluted 100-fold prior to analysis. Table 2 shows the results obtained analyzing the previously commented samples by the developed method and validated with the previously described batch enzymatic test.

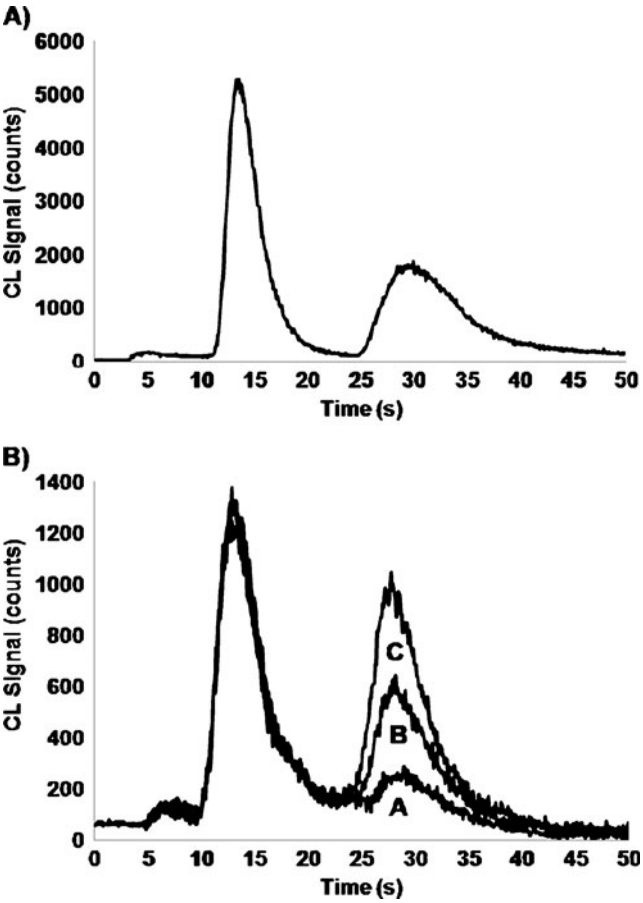

Fig. 4 **a** Oxalate (t_{ret}=29 s) in presence of 500-fold ascorbic acid (t_{ret}=13.5 s). **b** Chromatographic behavior of a urine sample (A), an urine sample spiked at a 400 μg L^{-1} level (B) and an urine sample spiked at a 800 μg L^{-1} level (C)

A good agreement between the obtained results by both methods was obtained. The relative errors found between the proposed method and the commercially available tests were between 1.2–4.6% for all the samples. The relative standard deviations obtained with the developed method (n=3) were between 1.8–6.0%, and with the enzymatic method (n=2) between 5.6–7.1%. In Fig. 4b, we can see the behavior of the chromatogram of a diluted urine sample and two consecutive oxalate spikings (0.4 mg L^{-1} each).

Table 2 Results obtained in the determination of oxalate in beer and urine samples by means of the developed MSIC method (n=3) and the enzymatic test (n=2). Results expressed in mg L^{-1}

Sample	MSIC method	Enzymatic method	Relative error[a]
Beer 1	16.2±1.0	16.0±1.1	1.2
Beer 2	29.8±0.9	29.2±1.7	2.1
Urine 1	31.5±0.5	32.6±2.0	3.4
Urine 2	47.5±2.0	45.4±2.5	4.6

[a] Relative error between the MSIC method and the enzymatic test.

We can appreciate a first signal at a retention time of 14 s, attributed to the total of non retained compounds in the column that elicited CL.

Finally, we would emphasize on the simplicity of the chromatograms obtained in comparison with UV or conductivity detection, due to the inexistent reactivity between the CL regents used and the major components of the sample matrix.

Comparison with other recently described methods

On the one hand oxalate can be determined by using commercially available enzymatic tests [30], but this type of methodologies are time consuming (analysis time > 1 h). But certainly, the analysis throughput can be increased accomplishing several runs in parallel.

They also involve the use of expensive and unstable compounds. Anyway the development of improved manual test for oxalate determination still being a current research field, for example, substituting the use of enzymes for synthetic compounds [31].

On the other hand, oxalate can be determined by separation techniques like IC with conductivity detection [34] or ion-pair HPLC with $[Ru(bipy)_3]^{2+}$ based CL detection [32]. Being the developed MSIC system the first one which hyphenates the ion chromatography with the CL detection, providing a versatile, completely automated and miniaturized system for the determination of oxalate. This new combination provides improved analytical features in comparison with the previously described methods. The most relevant improvement is the high injection throughput achieved with the developed method (48 h^{-1}), in comparison with those of its precedent separation techniques (5–6 h^{-1}).

In the last years new methodologies for oxalate determination have been reported in the scientific literature like: (i) CE integrated on a chip with conductivity detection [35]. (ii) The combined determination of citric, malic, tartaric, lactic and oxalic acids with HPLC including post-column photochemical reaction with visible light in the presence of Fe^{3+} and UO_2^{2+}, being the released Fe^{2+} quantified by luminol CL [33]. (iii) The determination of oxalate, citrate, uric acid and creatinine by zone CE with UV detection [36]. These systems presents some advantages in comparison with the developed system like a higher level of miniaturization (chip-CE) or a higher multi-analyte capacity (conventional HPLC/CE systems). But also, there is a common advantage of the proposed system in comparison with all of them that is the possibility to achieve a completely automated high analysis throughput oxalate determination maintaining high selectivity and sensitivity levels by using simple and low-cost instrumentation, allowing an analysis throughput of 48 h^{-1} (being the analysis throughputs for the rest of reported methods ranging from 6 to 18 h^{-1}, approximately).

Conclusions

In this work, the first low-pressure ion chromatographic method with chemiluminescence detection based on flow analysis techniques has been accomplished. This has been achieved by implementing the use of CTAB-coated short C18 monolithic columns to the multisyringe flow injection technique obtaining a simple and smart tool for the completely automated determination of oxalate in complex matrices, such as beer and urine.

The proposed system operates like a sequential injection analysis system for sample loading and column coating, and like a multicommuted flow injection analysis system for the oxalate clean-up and its concomitant chemiluminescence detection, being a useful tool for fast oxalate analysis.

Prospective work could be done studying the possibilities that offer this technique by means of the use of longer monolithic columns, testing new monolithic materials, surfactants, alternative CL reagents. Thus new analytical applications for the combination of CTAB-coated C18 monoliths with $[Ru(bipy)_3]^{2+}$ CL-based detection.

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Conclusions

In the presented doctoral thesis entitled “Development of new analytical methods of environmental and clinical interest exploiting the multisyringe flow injection analysis technique” new analytical applications of the Multisyringe Flow Injection Analysis Technique have been developed. These new applications have been focused on the accomplishment of new alternatives to contribute on the field of environmentally friendly or green analytical chemistry, the development of simplified methods for the measurement of total indices of environmental relevance, and the improvement of the selectivity of chemiluminescence based detection systems applied to complex matrix samples.

The main highlights of the performed works exploiting the MSFIA technique are:

- Development of the first method combining gas diffusion separation as a front end to chemiluminescence detection for the determination of sulphide. The obtained method provided improved analysis throughputs and sensitivity than previously reported methodologies for same purposes.

- Development of a multicommutated method for the spectrophotometric determination of chloride. This method provided a new strategy for the routine determination of chloride in waters with improved analysis throughputs and reagent consumption, which is of high relevance in methodologies requiring the use of toxic chemicals like this one.
- The development of a novel combination based on in-line disk-based solid phase extraction, UV oxidation and spectrophotometric detection applied to the determination of the total organic halogens in waters samples, obtaining the first automated method for the accomplishment of this task, furthermore obtaining a huge improvement in analysis throughput in comparison with other total organic halogens analyzers.
- The combination of long pathlength spectrophotometry based on liquid core fiber optics with the MSFIA technique, applied to the spectrophotometric determination of chloride in waters. This novel instrumental set-up provided improved sensitivity for this determination, and an important reduction in reagent consumption in comparison with any other flow system for the automation of this methodology.
- The development of the first flow systems combining on-line disk-based solid phase extraction, liquid chromatography and chemiluminescence detection, enabling in a completely automated and miniaturized fashion the isolation preconcentration of three thiazidic diuretics, and their subsequent separation and sensitive detection.

- The development of a novel instrumental combination based on the use of short-surfactant coated monoliths as a front end to chemiluminescence detection, resulting on a fast and low cost methodology for the determination of oxalate in samples with a complex matrix.

ANEXO

PUBLICACIONES DERIVADAS DE ESTA TESIS

I. Improving the Chemiluminescence-based Determination of Sulphide in Complex Environmental Samples by Using a New, Automated Multi-syringe Flow Injection Analysis System Coupled to a Gas Diffusion unit

Autores: Fernando Maya, José Manuel Estela, Víctor Cerdà

Revista: Analytica Chimica Acta **Año:** 2007 **Número:** 601 **Páginas:** 87-94

II. Spectrophotometric Determination of Chloride in Waters Using a Multisyringe Flow Injection System

Autores: Fernando Maya, José Manuel Estela, Víctor Cerdà

Revista: Talanta **Número:** 74 **Año:** 2008 **Páginas:** 1534-1538

III. Completely Automated System for Determining Halogenated Organic Compounds by Multisyringe Flow Injection Analysis

Autores: Fernando Maya, José Manuel Estela, Víctor Cerdà

Revista: Analytical Chemistry **Número:** 80 **Año:** 2008 **Páginas:** 5799-5805

IV. Multisyringe Flow Injection Analysis Hyphenated With Liquid Core Waveguides for the Development of Cleaner Spectroscopic Analytical Methods: Improved Determination of Chloride in Waters

Autores: Fernando Maya, José Manuel Estela, Víctor Cerdà

Revista: Analytical and Bioanalytical Chemistry **Número:** 394 **Año:** 2009

Páginas: 1577-1583

V. Multisyringe Flow Injection Technique for Development of Green Spectroscopic Analytical Methodologies

Autores: Fernando Maya, José Manuel Estela, Víctor Cerdà

Revista: Spectroscopy Letters **Número:** 42 **AÑO:** 2009 **Páginas:** 312-319

VI. Interfacing On-line Solid Phase Extraction With Monolithic Column Multisyringe Chromatography and Chemiluminescence Detection: An Effective Tool for Fast, Sensitive and Selective Determination of Thiazide Diuretics

Autores: Fernando Maya, José Manuel Estela, Víctor Cerdà

Revista: Talanta **Número:** 80 **Año:** 2010 **Páginas:** 1333-1340

VII. Flow analysis techniques as affective tools for the improved environmental analysis of organic compounds expressed as total indices

Autores: Fernando Maya, José Manuel Estela, Víctor Cerdà

Revista: Talanta **Número:** 81 **Año:** 2010 **Páginas:** 1-8

VIII. Multisyringe low-pressure ion chromatography exploiting short monoliths as a front end to chemiluminescence detection

Autores: Fernando Maya, José Manuel Estela, Víctor Cerdà

Revista: Microchimica Acta (Aceptado)

CONTRIBUCIONES A CONGRESOS DERIVADAS DE ESTA TESIS

I. Improvement of flow chemiluminescence analysis of sulphide in complex environmental samples using a new multisyringe flow injection system coupled to a gas diffusion unit

Autores: Fernando Maya, José Manuel Estela, Víctor Cerdà

Congreso: IX International Symposium on Analytical Methodology in the Environmental Field

Año: 2007 **Lugar:** Pollença, España **Tipo de Comunicación:** Póster

II. Simple and fast spectrophotometric determination of chloride in waters using a multisyringe flow injection system

Autores: Fernando Maya, José Manuel Estela, Víctor Cerdà

Congreso: IX International Symposium on Analytical Methodology in the Environmental Field

Año: 2007 **Lugar:** Pollença, España **Tipo de Comunicación:** Póster

III. Desenvolupament d´un sistema d´anàlisi en flux multixeringa per a la determinació completament automatitzada del total de compostos organohalogenats (AOX) presents en mostres d´interés mediambiental

Autores: Fernando Maya, José Manuel Estela, Víctor Cerdà

Congreso: V Jornades de Medi Ambient de les Illes Balears

Año: 2008 **Lugar:** Palma de Mallorca, España **Tipo de Comunicación:** Póster

IV. Quick assessment of halogenated organic compounds in wáter samples based on multisyringe flow technique

Autores: Fernando Maya, José Manuel Estela, Víctor Cerdà

Congreso: SIA 2008

Año: 2008 **Lugar:** Hradec Kralove, República Checa

Tipo de Comunicación: Póster

V. Drastic reduction in reagent consumption in spectrophotometric chloride determination

Autores: Fernando Maya, José Manuel Estela, Víctor Cerdà

Congreso: SIA 2008

Año: 2008 **Lugar:** Hradec Kralove, República Checa

Tipo de Comunicación: Póster

VI. Development of a multisyringe flow injection system for the completely automated determination of halogenated organic compounds as a group parameter

Autores: Fernando Maya, José Manuel Estela, Víctor Cerdà

Congreso: 12º Jornadas de Análisis Instrumental

Año: 2008 **Lugar:** Barcelona, España **Tipo de Comunicación:** Póster

VII. Greener spectrophotometric determination of chloride in waters based on the use of liquid core waveguides in combination with multisyringe flow injection technique

Autores: Fernando Maya, José Manuel Estela, Víctor Cerdà

Congreso: 12º Jornadas de Análisis Instrumental

Año: 2008 **Lugar:** Barcelona, España **Tipo de Comunicación:** Póster

VIII. Halogenated compounds assessment based on the development of a completely automated multisyringe flow injection system

Autores: Fernando Maya, José Manuel Estela, Víctor Cerdà

Congreso: 15th International Conference on Flow Injection Analysis

Año: 2008 **Lugar:** Nagoya, Japón **Tipo de Comunicación:** Póster

IX. Multisyringe flow injection technique allows fast and sensitive ion chromatographic separations based on the use of surfactant coated short monolithic columns and post-column chemiluminescence detection

Autores: Fernando Maya, José Manuel Estela, Víctor Cerdà

Congreso: XV EuroAnalysis

Año: 2009 **Lugar:** Innsbruck, Austria **Tipo de Comunicación:** Poster

X. Implementation of in-line pre- and post-column sample treatments in Multi-Syringe Chromatography and their applicability to the determination of trace pollutants in environmental samples

Autores: Fernando Maya, José Manuel Estela, Víctor Cerdà

Congreso: 10th International Conference on the Biogeochemistry of Trace Elements

Año: 2009 **Lugar:** Chihuahua, México **Tipo de Comunicación:** Oral

XI. Flow analysis techniques as effective tools for simplified and improved environmental analysis of organic compounds as total indices

Autores: Fernando Maya, José Manuel Estela, Víctor Cerdà

Congreso: 11th Flow Analysis Conference

Año: 2009 **Lugar:** Pollença, España **Tipo de Comunicación:** Póster

XII. The potential of multisyringe chromatography for fast, sensitive and selective determination of trace pharmaceuticals in complex samples

Autores: Fernando Maya, José Manuel Estela, Víctor Cerdà

Congreso: 11th Flow Analysis Conference

Año: 2009 **Lugar:** Pollença, España **Tipo de Comunicación:** Oral

XIII. A novel multisyringe flow injection set-up for fast oxalate determination based on surfactant-coated short monolithic columns and chemiluminescence detection

Autores: Fernando Maya, José M. Estela, Víctor Cerdà

Congreso: 16th International Conference on Flow Injection Analysis

Año: 2010 **Lugar:** Pattaya, Tailandia **Tipo de Comunicación:** Oral

XIV. The use of short monoliths for low-pressure ion separations: Fast determination of oxalate in complex sample matrices

Autores: Fernando Maya, José M. Estela, Víctor Cerdà

Congreso: II International Workshop on Analytical Miniaturization

Año: 2010 **Lugar:** Oviedo, España

Tipo de Comunicación: Póster + Oral (Flash Communication)

OTROS ARTÍCULOS PUBLICADOS

I. Automatic screening of the effect of phenolic compounds on luminol-hydrogen peroxide-peroxidase system by a flow system

Autores: Andre R. T. S. Araujo, Fernando Maya, M. Lúcia M. F. S. Saraiva, José L. F. C. Lima, José M. Estela, Víctor Cerdà

Revista: Luminescence (aceptado)

II. Sequential injection chromatography combined with long pathlength spectrophotometry for the evaluation of anticoccidial agents in aqueous samples

Autores: Erland Björklund, Fernando Maya, Soren A. Bak, Martin Hansen, José M. Estela, Víctor Cerdà

Revista: Microchemical Journal (enviado)

OTRAS CONTRIBUCIONES A CONGRESOS

I. Automatic assessment of the effect of the different phenol derivates on the chemiluminescence luminol-H_2O_2-horseradish peroxidase system exploiting multisyringe flow injection analysis

Autores: Andre R. T. S. Araujo, Fernando Maya, M. Lúcia M. F. S. Saraiva, José L. F. C. Lima, José M. Estela, Víctor Cerdà

Congreso: 11th Flow Analysis Conference

Año: 2009 **Lugar:** Pollença, España

Tipo de Comunicación: Oral

II. Evaluation of sequential injection chromatography for the analysis of anticoccidial agents in aqueous matrices

Autores: Fernando Maya, E. Björklund, José M. Estela, Víctor Cerdà

Congreso: 28th International Symposium on Chromatography

Año: 2010 **Lugar:** Valencia, España

Tipo de Comunicación: Poster