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**Review**

**Sensores químicos piezoeeléctricos**

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**Resumen.** La utilización del fenómeno piezoeeléctrico para la construcción de sensores químicos es un campo emergente de indudable interés. Se revisan aquí los aspectos teóricos fundamentales sobre el funcionamiento de dos tipos diferentes de dispositivos acústicos: los basados en la utilización de ondas másicas (BAW) y los que emplean ondas superficiales (SAW). Se describen también las configuraciones de los circuitos electrónicos necesarios para el correcto funcionamiento de estos sensores. Por último se comentan las posibles aplicaciones de los mismos para el análisis de líquidos y gases, así como su idoneidad para ser utilizados como biosensores.

**Introducción**

Los sensores químicos pueden ser definidos como dispositivos formados por una interfase química, que reacciona selectivamente con determinadas sustancias en fase líquida o gasosa, y un transductor físico que convierte la señal de origen químico en señales físicas cuantificables. Magnitudes como la temperatura, el potencial eléctrico, las constantes ópticas o dieelécticas, la presión, la masa, etc., pueden servir, mediante la implementación del transductor adecuado, para cuantificar las evoluciones de una interfase química. Los transductores comúnmente utilizados con este fin son varios: termistores, transistores de efecto de campo, detectores ópticos conectados a la interfase química mediante guías de ondas ópticas, etc. [1]. Los cambios másicos asociados a procesos que tienen lugar en las interfases químicas pueden ser convertidos en señales eléctricas mediante la utilización de transductores piezoeeléctricos. Los fenómenos piezoeletricos fueron descubiertos por el matrimonio Curie en 1880, que observaron como se formaba un dipolo eléctrico cuando un cristal anisotrópico era sometido a una presión. El desplazamiento de las partículas en el sólido piezoeeléctrico conlleva una separación macroscópica de carga y la creación de un campo eléctrico. El efecto es reversible, esto es, aplicando una diferencia de potencial a un material piezoeeléctrico se generan tensiones en el mismo que dan lugar al desplazamiento de partículas y a la aparición de ondas acústicas. La frecuencia de oscilación de las mismas depende de parámetros tales como la densidad o la viscosidad del medio que está en contacto con el cristal piezoeeléctrico y, especialmente, de cambios másicos que sufre dicho cristal. Así pues, cualquier proceso químico que ocurra en el seno o la superficie del piezoeeléctrico, y que conlleve cambios en la masa del mismo, puede ser analizado a partir de los cambios de frecuencia observados cuando se aplique simultáneamente un corriente eléctrica. Existen dos tipos básicos de dispositivos sensores de masa: aquellos basados en el uso de ondas acústicas másicas (Bulk acoustic wave o BAW), también conocidos como microbalanzas de cristal de cuarzo (Quartz crystal microbalance o QCM), y los que utilizan ondas acústicas superficiales (Surface acoustic wave o SAW). Para los dos tipos de dispositivos se han encontrado aplicaciones en el campo de los sensores químicos [2-4]. El presente trabajo es una recopilación de los principios teóricos del funcionamiento de los sensores piezoeletricos, los métodos instrumentales implicaes en su utilización como sensores químicos y, por último, algunos resultados aparecidos en la bibliografía sobre investigación y desarrollo en este campo.
**Fundamentos teóricos**

*Dispositivos basados en las ondas acústicas musicales (BAW)*

Los dispositivos basados en la utilización de ondas acústicas musicales están formados por una delgada oblea de cristal piezoeléctrico, en la que se han depositado, por ambas caras, sendos recubrimientos metálicos (oro) que actúan a manera de electrodo. En la Figura 1 se presenta, de forma esquemática, el diseño típico de un BAW. Al aplicar una corriente alterna entre los dos electrodos se genera una onda acústica que viaja entre las dos caras de la oblea. Dependiendo de la simetría del cristal y del tipo de corte cristalográfico en él practicado, se pueden generar dos tipos de onda: transversales (en las que el movimiento de las partículas es perpendicular a la dirección de propagación de la onda y paralelo a la superficie del cristal) y longitudinales (con un movimiento de las partículas paralelo a la dirección de propagación de la onda y normal a la superficie del cristal). La configuración más frecuente en dispositivos BAW es aquella que utiliza una oblea de cuarzo con corte cristalográfico AT que permite la propagación de ondas transversales [3]. Debido a las dimensiones finitas del piezoeléctrico, y a las condiciones de contorno que impone la superficie, no todas las ondas pueden propagarse a través del cristal [2]. Sólo aquellas oscilaciones con número de onda $\kappa$ tal que:

$$\kappa = \frac{w}{v} = \frac{B\pi}{d}$$

serán oscilaciones resonantes y podrán, por tanto, propagarse. En esta ecuación $w$ es la frecuencia angular, $v$ es la velocidad de propagación de la onda, $d$ es el espesor del cristal y $n$ es un número entero. La velocidad de propagación depende exclusivamente de la naturaleza del material utilizado, la orientación del corte cristalográfico y el tipo de oscilación, y viene dada por:

$$v = \sqrt{\frac{\mu}{\rho}}$$

siendo $\rho$ y $\mu$ la densidad y el módulo transversal del cristal, respectivamente. Para un corte AT de cuarzo $\rho = 2.648$ $g/cm^3$ y $\mu = 2.947 \times 10^{11}$ $g/cm^3s^2$.

La frecuencia resonante del BAW, $f_0$, puede ser amplificada y cuantificada mediante el uso de un amplificador y un frecuencímetro, y sus valores son siempre inversamente proporcionales al espesor de la oblea de cuarzo:

$$f_0 = \frac{v}{2d}$$

A partir de esta ecuación Sauerbrey [5] derivó una relación entre la frecuencia resonante y cualquier incremento de masa $\Delta m$ que tenga lugar en la superficie del cristal, como es el caso de la deposición o adsorción de un material ajeno:

$$\Delta f = -C_1 f_0^2 \Delta m$$

siendo:

$$C_1 = \frac{2}{A/\sqrt{\rho \mu}}$$

donde $A$ es el área del electrodo.

Para llegar a la ecuación 4 Sauerbrey supone que las medidas son llevadas a cabo en el vacío y que el material añadido se deposita uniformemente sobre la superficie del cristal, pero no tiene en consideración la existencia de diferencias entre las propiedades elásticas y la densidad del cuarzo y el material depositado. Análisis más detallados sobre el efecto que la adición de un material tiene sobre las propiedades del piezoeléctrico de cuarzo se pueden encontrar en la bibliografía [2, 3, 6].

La sensibilidad acústica de un sensor de onda acústica puede expresarse como [7]:

$$S_m = \lim_{\Delta m \to 0} \frac{1}{f_0} \frac{\Delta f}{\Delta m}$$

luego:

$$\frac{\Delta f}{f_0} = S_m \Delta m$$

Para un sensor tipo BAW es fácil demostrar la siguiente expresión [7]:
donde \( \rho \) es la densidad del cristal piezoelectrónico y \( \lambda \) es la longitud de onda de la oscilación. Así, para un BAW que opere a 10 MHz y esté construido con cuarzo de corte cristalino AT (\( C_0 = 2.26 \times 10^{-9} \text{cm}^2 \cdot \text{s}^{-1} \)) y electrodo de 1 cm\(^2\) de superficie, la sensibilidad teórica es de 0.226 Hz-ng\(^{-1}\). El límite de detección, considerando una relación señal/ruido \( > 3 \), y tomando un nivel de ruido de fondo de 5 Hz para \( f_s = 10 \) MHz, es de 6.6 ng-cm\(^{-2}\). Se pueden mejorar la sensibilidad y el límite de detección utilizando cristales con mayores frecuencias resonantes, aunque la fabricación y el manejo de tales dispositivos se complica notablemente, pues para frecuencias mayores a 15 MHz las ondas resultan excesivamente delgadas.

Como ya hemos apuntado, estos sensores también son conocidos como microbalanzas de cristal de cuarzo (QCM), y deben este nombre al hecho de que han sido utilizadas profusamente para cuantificar masas depositadas mediante diferentes técnicas [8, 9]. Por el contrario, durante mucho tiempo se pensó que la aplicación de los BAW como instrumental analítico en fases líquidas no era de interés, debido al efecto supresor de la oscilación que el medio viscoso tenía sobre la superficie del cristal. Afortunadamente, en una serie de publicaciones aparecidas en los últimos años, se ha demostrado que los BAW pueden ser aplicados con éxito en el análisis químico de fases líquidas, principalmente como biosensores en los que el cristal se recubre con una capa de enzimas, lípidos, anticuerpos o antígenos que pueden dar lugar a interacciones específicas y selectivas con analitos en disolución [19].

Los cambios de frecuencia resonante en los dispositivos BAW no sólo son debidos a la adición de materia, sino que también están generados por cambios viscoelásticos que afectan a todo el sistema sensor [11]. Al poner en contacto la superficie del cristal piezoelectrónico con una disolución acuosa, los cambios de frecuencia resonante dependen de cambios en la densidad, viscosidad y conductividad específica de la disolución [3]. Existen algunos modelos sencillos [6, 12] para los que se ha elaborado una expresión más acertada de los cambios de frecuencia que se dan en los sensores BAW cuando éstos son sumergidos en una fase líquida:

\[
\Delta f = -4.30 \times 10^{-6} f_0 \sqrt{\eta \rho L}
\]

Donde \( \eta \) y \( \rho \) son la viscosidad y la densidad del líquido. Los cambios de frecuencia se producen por el acoplamiento entre la oscilación del cristal, debida a la existencia de la onda transversal, y la oscilación atenuada en el medio fluido, debida a la propagación de la misma onda. Según los modelos teóricos, la onda decrece exponencialmente en el medio con una distancia de atenuación característica. Sólo una delgada capa del líquido en contacto con el cristal (aprox. 1 \( \mu \)m) es desplazada, y la respuesta del dispositivo es función de dicha capa. Los posibles efectos interfaciales deben a variables como la rugosidad de la superficie, conductividad y constante dielectrica del líquido, o cambios en la energía libre superficial y viscosidad del líquido, no han sido tenidos en cuenta, a pesar de que pueden llegar a afectar drásticamente a la frecuencia resonante [3, 13, 14].

Recientemente, ha sido publicado [15] un nuevo modelo, ostensiblemente más sofisticado, en el que se tienen en cuenta estos aspectos superficiales, deteniéndose en particular en el efecto que, sobre la respuesta del sensor BAW, tienen la densidad, viscosidad, tensión superficial y ángulo de contacto del líquido.

**Dispositivos basados en las ondas acústicas superficiales (SAW)**

Los dispositivos SAW están basados en la existencia de las ondas acústicas denominadas Rayleigh, y se diferencian de los BAW en que la propagación de la onda acústica y el desplazamiento de las partículas del piezoelectrico están confinados a la superficie del mismo.

La configuración más frecuente en la que se presentan los SAW es la que se conoce como "línea de retardo" (Delay Line). La Figura 2 es una representación esquemática de esta configuración. Las ondas superficiales son generadas al aplicar una corriente alterna a un grupo de electrodos metálicos interdigitales depositados en la superficie del cristal. El campo eléctrico creado mediante estos transductores interdigitales (IDT) induce un desplazamiento de las partículas del sólido que es el causante de la aparición de la onda Rayleigh, la cual, gracias a la reversi-
bility del fenómeno de transducción, es detectada al otro extremo de la superficie con un segundo IDT.

La frecuencia de operación de los dispositivos SAW viene determinada por la velocidad de la onda en la superficie y el espaciado entre los dedos del IDT:

$$f_s = \frac{v}{2d} \quad (10)$$

El número de dedos del IDT determina la anchura del dispositivo y la distancia interdigital de los IDT, lo cual, a su vez, determina directamente la impedancia del dispositivo.

Como en el caso de las QCM, los dispositivos SAW pueden ser utilizados como elementos controladores de la frecuencia de un circuito oscilante cuya frecuencia de oscilación, $f_s$, es proporcional a la velocidad de la onda superficial. Esta velocidad de propagación puede verse afectada por varios parámetros físicos [16], entre los que se incluyen la temperatura, presión, masa superficial, rigidez, constante dieléctrica y conductividad. Cambios en la velocidad de la onda se pueden registrar como desviaciones en la frecuencia, amplitud o fase de la señal eléctrica de salida [2]. En esta correspondencia se basa la aplicación de los SAW como sensores químicos, ya que el sensor tiene una delgada capa de un material químicamente activo, los cambios de masa asociado a cualquier reacción, principalmente adsorciones de otras especies que se encuentren en el medio líquido o gaseoso que esté en contacto con el piezoelectrólito, afectarán la velocidad de propagación de la onda acústica.

Si se mantienen constantes condiciones experimentales tales como la temperatura o la presión (u otro SAW es utilizado como referencia para compensar los efectos de dichas variables) el espesor de las capas de material detector aplicadas sobre la superficie es suficientemente pequeño como para poder despreciar las contribuciones debidas a cambios de rigidez, conductividad o permitividad del medio, los cambios de frecuencia en el sensor pueden ser expresados mediante la ecuación simplificada:

$$\Delta f = (K_1 + K_2)f_s \Delta m \quad (11)$$

siendo $f_s$ la frecuencia original de oscilación del dispositivo, $K_1$ y $K_2$ constantes que dependen de la naturaleza de los materiales utilizados, y $\Delta m$ la cantidad de masa añadida por unidad de área. Cuando películas de polímeros orgánicos de espesor considerable son depositados en una línea de retardo SAW, las propiedades elásticas de dicho material deben ser tenidas en cuenta [17]:

$$\Delta f = (K_1 + K_2)f_s^2 d \rho' - K_3 f_s^2 d \left(4\mu'(\lambda' + \mu') \right. \left. \nu_s^2(\lambda' + 2\mu') \right) \quad (12)$$

En esta ecuación $\Delta y \rho'$ son el espesor y la densidad del material depositado, $\nu_s$ es la velocidad de la onda Rayleigh en dicho material, $\mu'$ es el denominado módulo transversal del material de recubrimiento y $\lambda'$ es la constante de Lamé del mismo. Para cuarzo con un corte cristalográfico ST y propagación de la onda en la dirección x, la configuración normalmente empleada en los sensores de gases SAW, $\nu_s = 4758 \text{m/s}$ y $K_3$ son $8.7 \times 10^8$ y $3.9 \times 10^8 \text{m}^2 / \text{s}$, respectivamente [17]. Debe tenerse en cuenta que las ecuaciones 11 y 12 son sólo válidas cuando el material depositado es no conductor e isotrópico, y tiene un espesor menor al 0.2% de la longitud de onda utilizada.

Una onda acústica superficial que se propaga a través de un medio piezoeléctrico es acompañada por una onda electromagnética la cual se puede ver afectada por las propiedades conductoras o semiconductoras de la capa superficial [18]. La influencia que sobre la velocidad de la onda tiene la conductividad del material depositado se puede expresar como [2, 16, 18]:

$$\frac{\Delta V}{V_0} = -\frac{K_2}{2} \Delta \left(\frac{\sigma^2}{\sigma^2 + \nu_s C_s^2}\right) \quad (13)$$

siendo $C_s$ la capacitancia por unidad de longitud de la superficie, $\sigma$ la conductividad laminar del material, y $K_2$ la constante de acoplamiento electromagnético del piezoeléctrico.

La sensibilidad acústica de un dispositivo SAW se puede definir como [7]:

$$S_m = \lim_{\Delta m \to 0} \frac{1}{\Delta m} \frac{\Delta V}{V_0} \quad (14)$$

Si el circuito que contiene el dispositivo SAW sufre cambios en la fase de la onda electromagnética que puedan ser despreciados frente a los cambios experimentados en la línea de retardo del mismo, o dicho cambio de fase es independiente de la frecuencia, entonces los cambios de frecuencia son proporcionales a los cambios de velocidad de propagación de la onda [7], y se puede escribir:

$$\frac{\Delta V}{V} = \frac{\Delta f}{f_0} = S_m \Delta m \quad (15)$$

Para los SAW que operan con ondas tipo Rayleigh la sensibilidad acústica puede expresarse como [7]:

$$S_m = -K(\sigma) \frac{1}{\rho \lambda} \quad (16)$$

donde $K(\sigma)$ es una función del coeficiente de Poisson $\sigma$ que depende de la relación entre las velocidades de las ondas transversal-máscia y longitudinal, componentes de la onda que se propaga a lo largo de la superficie. En esta misma ecuación $\rho$ es la densidad del cristal y $\lambda$ es la longitud de onda. Para la mayoría de los sólidos isotrópicos el parámetro $K(\sigma)$ varía entre 1 y 2 [7]. Como en el caso de
Los dispositivos BAW, la sensibilidad de los sensores SAW puede mejorar al operar a frecuencias más elevadas. Así, por ejemplo, los datos experimentales sobre la respuesta de un sensor SAW construido a partir de la deposición de una capa activa mediante el método de Langmuir-Blodgett, arrojan valores de $S_\mu$ de -17 y -91 cm$^{-1}$·g$^{-1}$ al operar a 31 y 112 MHz, respectivamente. A modo de comparación, nótese que la sensibilidad de un dispositivo BAW operando a 6 MHz es de -14 cm$^{-1}$·g$^{-1}$.

Los dispositivos SAW recubiertos de diferentes capas químicas activas han sido ampliamente utilizados como sensores de gases y vapores [4, 17, 19], si bien su aplicación a la detección de líquidos ha encontrado barreras tecnológicas considerables. Como ya hemos apuntado, las ondas Rayleigh que se propagan a lo largo de un piezoelectrónico presentan dos componentes, una paralela a la dirección de propagación y otra perpendicular a la misma. En contacto con una fase líquida, la componente normal genera una onda de compresión en la misma que repercute en una fuerte atenuación de la onda Rayleigh (4.2 dB MHz$^{-1}$·cm$^{-1}$) [20].

No obstante, las ondas Rayleigh no son las únicas ondas elásticas que pueden generarse en el piezoelectrónico. Dependiendo de la geometría de los IDT, de la simetría y orientación del cristal, y del diseño del circuito oscilador, se pueden generar una variedad de ondas acústicas [4, 21]. Dos tipos de ondas han sido comúnmente usados en sensores químicos operando en líquidos: ondas Lamb y ondas transversales polarizadas horizontalmente (ondas SH). La Figura 3 ayuda a visualizar la dirección de desplazamiento de las partículas para cada una de las ondas mencionadas [4].

Como en el caso de las ondas Rayleigh, las de tipo Lamb poseen componentes vertical y longitudinal, y son generadas en obleas muy finas. Si el espesor de la oblea es considerablemente menor a la longitud de onda generada [23], los dispositivos basados en ondas Lamb pueden ser utilizados como sensores en medio líquido [22, 23], puesto que en tal caso la velocidad de fase de la onda es menor que la velocidad de la onda de compresión en la mayoría de los líquidos. Los sensores químicos basados en el uso de ondas Lamb son también conocidos como sensores de onda en placa flexible (flexural-plate-wave o FPW) [22, 24, 25]. En la Figura 4 se representa esquemáticamente el diseño típico de un sensor FPW [23]. Se compone éste de una membrana de silicio en la que se han depositado sendas láminas de nitruro de silicio (2.0 µm), aluminio metálico (0.3 µm) y óxido de cinc (0.7 µm), sobre la cual, a su vez, se depositan los dos conjuntos de IDT. La membrana de silicio es grabada, formando un pozo que permite llegar hasta la capa de nitruro de silicio, dejando así una porción de las otras láminas suspendidas libremente. En el cristal de ZnO (material piezoelectrónico) se genera la onda Lamb que tendrá componentes normales en ambas caras de la oblea, pudiéndose establecer contacto con una fase líquida por la cara que no posee los IDT, y evitándose así la posibilidad de un cortocircuito eléctrico.

Cuando una onda acústica se propaga a lo largo de un cristal piezoelectrónico, una pequeña porción de su energía es transmitida a la película de líquido más próximo, produciendo una perturbación evanescente que decrece exponencialmente con la distancia a la interfase [25]. Una distancia de atenuación típica en un sensor FPW es de aproximadamente...
mente 16 μm. Por ello, variaciones de densidad en el líquido más próximo a la oblea influyen en la velocidad de la onda acústica y pueden ser detectadas por el sensor.

La velocidad de propagación de una onda tipo Lamb se puede expresar como [7]:

\[ v_r = \left( \frac{2\pi}{\lambda} \right) \sqrt{\frac{B}{M}} \]  
(17)

siendo B la rigidez efectiva de la oblea, la cual depende del espesor, el efecto de las diversas capas que la forman y la tensión; M = m + Δm es la masa total por unidad de área, en contraste con m, la masa de la membrana desnuda; y \( \lambda \) es la longitud de onda de la componente flexible de la onda. Si la velocidad de propagación del sonido \( (v_s) \) en un fluido que esté en contacto con una de las superfcies de la membrana, es mayor que la velocidad del sonido en el sólido \( (v_p) \), existe una influencia sobre la longitud de onda acústica que puede ser cuantificada por el efecto equivalente que produciría un cierta carga magnética [7]:

\[ \Delta m = \rho \delta \]  
(18)

En esta ecuación \( \rho \) es la densidad del fluido y \( \delta \) es el espesor de la película de perturbación en el fluido, el cual a su vez viene dado por [7]:

\[ \delta = \frac{\lambda}{2\pi} \sqrt{1 - \left( \frac{v_s}{v_r} \right)^2} \]  
(19)

siendo \( v_s \) la velocidad del sonido en dicho fluido. La ecuación 17, deducida para un líquido en contacto con una de las caras del piezoelectrónico, se puede reescribir como:

\[ v_r = \frac{2\pi}{\lambda} \sqrt{\frac{B}{m + \rho \delta}} \]  
(20)

La validez de esta ecuación ha sido demostrada experimentalmente para fluidos de baja viscosidad, encontrándose desviaciones menores al 0.3% para cambios de velocidad de hasta el 36% [26].

La sensibilidad de los dispositivos FPW viene dada por [7]:

\[ S_w = -\frac{1}{2} \rho d \]  
(21)

y depende sólo del espesor de la membrana y no de la frecuencia de operación, como ocurriría en el caso de los dispositivos BAW y SAW.

El diseño del sensor FPW mostrado en la Figura 4 no es el único posible. Una configuración algo más sencilla ha sido la descrita por Yamaguchi [22], la cual utiliza una membrana compuesta únicamente de una película de ZnO depositada sobre una lámina de aluminio.

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**RECUERBILMENTO QUÍMICAMENTE ACTIVO**

**Figura 5.** Desplazamiento de las ondas SH entre las caras de un cristal piezoelectrónico.

**Figura 6.** Representación esquemática de un modo de vibración transversal-horizontal y de su desplazamiento a lo largo del cristal.

Es interesante hacer notar que los sensores FPW pueden ser fabricados sin la mediación de materiales piezoelectrónicos. En efecto, Giesler y Meyer [26], han desarrollado un nuevo transductor, basado en el uso de fuerzas electrostáticas y cambios capacitativos que generan ondas de lámina flexible en una delgada membrana de nitrato de silicio cubierta con una fina película de metal.

Tal como ya hemos apuntado, existe otro tipo de ondas, conocidas como ondas transversales horizontales (shear-horizontal waves o SH), que pueden ser también generadas en cristales piezoelectrónicos. En dichas ondas el desplazamiento de las partículas es paralelo a la superficie, sin presentar ninguna componente normal que permita la onda propagarse eficientemente en la fase líquida. La componente planar está, no obstante, influenciada por cualquier perturbación de los parámetros interfaciales [20]. Las ondas SH (también conocidas como ondas acústicas más superficiales) son ondas más que pueden ser dirigidas, sin cambio de polarización, mediante reflexiones sucesivas entre las dos superfcies paralelas del cristal [27] (Figura 5). Si el espesor de la lámina es suficientemente pequeño, la interferencia entre ondas incidentes y reflejadas produce modos de vibración transversales horizontales estacionarios en las dos caras del cristal y a lo ancho del mismo.
Sensores químicos piezoeléctricos

(Figura 6). Se pueden excitar varios modos de vibración de las ondas SH que presentan una distribución de desplazamientos de partícula y velocidades ligeramente diferentes, aunque escogiendo la anchura de banda y la geometría apropiada en los transductores IDT es posible conseguir la separación de los mismos [28]. La frecuencia del n-ésimo modo de vibración con un mayor acoplamiento efectivo entre el transductor y el substrato se puede escribir como:

\[ f_n = \frac{V_o}{d} \sqrt{1 + \left( \frac{nd}{2b} \right)^2} \]  \hspace{1cm} (22)

siendo d/2 el espaciamiento entre electrodos en el IDT (Figura 6), b la anchura de la lámina, y n el índice del modo de vibración que representa el número de nodos de desplazamiento entre las caras del cristal. La sensibilidad máxima de un sensor SH se puede expresar como [28]:

\[ \frac{\Delta v}{v_o} = \frac{\Delta m}{\rho b} \Delta \omega \]  \hspace{1cm} (23)

siendo \( \rho \), la densidad del substrato piezoeléctrico y \( a_n \), un parámetro igual a 1/2 para n=0 y 1 para el resto de los modos. Como en el caso de las ondas Lamb, la sensibilidad depende del espesor de la lámina. Los cambios en las propiedades elásticas de la capa superficial influyen también la velocidad de la onda, aunque este efecto es amenazado poco importante [28].

En líquidos viscosos puede existir un acoplamiento de la onda SH con una pequeña película del líquido en contacto con la interfase, de manera que este experimenta un movimiento transversal que decela rápidamente con la distancia a la misma. La distancia de atenuación de una oscilación de frecuencia angular \( \omega \) es, aproximadamente:

\[ \delta = \frac{2\eta \omega}{\rho_0 \omega^2} \]  \hspace{1cm} (24)

donde \( \rho \) y \( \eta \) son, respectivamente, la densidad y la viscosidad del líquido. Así, por ejemplo, para un dispositivo SH operando en agua a 158 MHz, la distancia de atenuación es de unos 50 nm.

El acoplamiento viscoso de la onda con el líquido tiene como consecuencia la perturbación en la velocidad de onda y la atenuación que resulta de la disipación de energía en el líquido [20]. La expresión matemática para los cambios en la velocidad de la onda ha sido deducida por Riccio [28] mediante un análisis de perturbación. Las pérdidas en la propagación de una onda que se mueve en un substrato en contacto con un líquido vienen dadas por [20]:

\[ L = 1.2 \frac{\mathrm{cm}}{\rho_i V_s^2} \sqrt{\omega \rho_0 \eta} \]  \hspace{1cm} (25)

donde \( \rho_i \) es la densidad del substrato y \( l \) es la distancia de interacción sólido-líquido que viene determinada por las dimensiones de la celda media.

Como en el caso de las ondas Rayleigh, las ondas SH sufren acoplamiento acustoelectrico con las cargas de la disolución (iones, dipolos y dipolos inducidos). Este acoplamiento decae exponencialmente con la distancia, siendo la distancia de amortiguación de aproximadamente \( \lambda/2\pi \). Los cambios en la velocidad de la onda vienen dados por [28, 29]:

\[ \frac{\Delta v}{v_o} = -\frac{K^2}{2} \left( \frac{\varepsilon_{\omega} + \varepsilon_{\omega}}{\varepsilon_{\omega} + \varepsilon_{\omega}} \right) \frac{\sigma^2}{\omega^2} \]  \hspace{1cm} (26)

donde \( \varepsilon_{\omega} \), \( \varepsilon_{\omega} \) y \( \varepsilon_{\omega} \) son respectivamente los coeficientes dielectricos del substrato, del líquido y del vacío, y \( K \) es el coeficiente de acoplamiento elctromecánico.

Métodos de medida

Dispositivos BAW

Existen dos tipos de métodos diferentes para la caracterización de los sensores químicos basados en el uso de cristales piezoeléctricos. El más utilizado comúnmente es el método activo, también conocido como método oscilador. En el mismo, el dispositivo piezoeléctrico forma parte de un circuito eléctrico oscilante conectado a la entrada y a la salida de un amplificador, el cual suministra el feedback positivo causante de la oscilación. Un circuito integrado oscilante estándar (TTL) es utilizado usualmente con esta finalidad [30], aunque también es posible servirse de un amplificador RF de banda ancha. Una configuración típica para la medida de la frecuencia resonante de un dispositivo BAW es la representada en la Figura 7. La ventaja más no-

![Figura 7. Dispositivo experimental típico para medidas de frecuencia resonante en sensores BAW.](Image)
Cada una de las componentes es función de los parámetros equivalentes del circuito y de la frecuencia. Las cantidades medibles de esta variable son su magnitud:

\[ |Z| = \sqrt{R^2 + X^2} \]  \hspace{1cm} (28)

y su ángulo de fase:

\[ \theta = \frac{1}{\tan \frac{X}{R}} \]  \hspace{1cm} (29)

Por razones de conveniencia operacional se suele tratar con la inversa de las impedancias, conocida como admitancia:

\[ Y = \frac{1}{Z} = G + jB \]  \hspace{1cm} (30)

siendo \( G \) la conductancia y \( B \) la susceptancia. Para el circuito equivalente de la Figura 8:

\[ G = \frac{R_m}{R_m^2 + \left( \omega L_m - \frac{1}{\omega C_m} \right)^2} \]  \hspace{1cm} (31)

\[ B = \frac{\left( \omega L_m - \frac{1}{\omega C_m} \right)}{R_m^2 + \left( \omega L_m - \frac{1}{\omega C_m} \right)^2} + \omega C_0 \]  \hspace{1cm} (32)

\[ R = \frac{G}{G^2 + B^2} \]  \hspace{1cm} y  \hspace{1cm} \[ X = \frac{B}{G^2 + B^2} \]  \hspace{1cm} (33)

La condición que se debe cumplir para que un circuito sea resonante es que tanto la reactancia \( X \) como el ángulo de fase \( \theta \) sean nulos. Se puede demostrar [13, 14] que existen dos frecuencias resonantes (frecuencia resonante en serie \( f_s \), y frecuencia resonante en paralelo \( f_p \)) cuando \( Z \) es una resistencia pura:

\[ f_s = \frac{1}{2\pi} \sqrt{\frac{1}{L_m C_m}} \]  \hspace{1cm} (34)

\[ f_p = \frac{1}{2\pi} \sqrt{\frac{1}{L_m C_m} + \frac{1}{L_n C_n}} \]  \hspace{1cm} (35)

La frecuencia resonante en serie es la cantidad medida a través del método oscilador. Las dos últimas ecuaciones han sido obtenidas asumiendo \( R_n = 0 \) (resistencia dinámica nula), lo cual es razonable para dispositivos BAW que operen en fase gaseosa, donde la dissipación de energía por par-
Sensores químicos piezoelectricos

de del circuito resonante puede ser despreciada [13]. En un
medio líquido, \( R_m \) es del orden de \( 10^4 \, \Omega \) [3] y las ecuacio-
nes 34 y 35 deben ser corregidas [13]. Un incremento del
producto densidad-viscosidad del líquido conlleva un au-
mento de la dissipación de la energía \( \gamma \), por tanto, un in-
cremento de \( R_m \) que se puede expresar como [13]:

\[
R_m \approx A \sqrt{\omega \rho \eta} \quad (36)
\]

donde \( A \) es el área del electrodo, \( \omega \) es la frecuencia an-
gular, \( \rho \) y \( \eta \) son la densidad y la viscosidad del líquido.

Algunos resultados experimentales [13] sugieren que,
para la determinación de la propiedades másicas del líqui-
da, la resistencia dinámica es un parámetro más adecua-
dado que la frecuencia resonante, el cual, como hemos visto, es
función compleja de varios parámetros.

Los aumentos de \( R_m \) llevan asociados ensanchamientos
del pico resonante y disminución en los cambios del ángu-
ilo de fase. Para determinados valores de \( R_m \), no llegan a se-
pararse las frecuencias resonantes paralela y en serie, y el
ángulo de fase es negativo en todo el intervalo de frecuen-
cias. En dichas condiciones el método activo no es adecua-
da.

La capacitancia dinámica, \( C_m \), es equivalente a la elasticidad
del óbolo de cuarzo, y se considera constante e in-
dependiente de la carga másica. En fase gaseosa, la
inductancia dinámica, \( L_m \), es equivalente a la masa de la
óbolo, y aumenta con la carga másica de la misma. Cuando
el piezoelectrónico está en contacto con un líquido, \( L_m \) es
equivalente a la energía cinética intercambiada entre el
cuarzo y el fluido, y es proporcional a \( (\rho \eta)^{1/2} \). \( L_m \) es tam-
bién sensible a cambios en las propiedades de la interfase
sólido-líquido, de manera que se verá afectada por ciertos
<incomprehensible> cambios estructurales del líquido que conllevan variacio-
nes en su densidad o viscosidad [13].

Como resumen de los métodos de medida utilizados con
las disposiciones BAW cabe resaltar que la frecuencia de las
señales eléctricas en los mismos es función compleja de las
propiedades del cuarzo y del medio que lo rodea. Cuando
el sensor BAW se utiliza para mediciones en fase gaseosa,
ambos métodos, activo y pasivo, generan información si-
nilar, siendo preferible el método activo por su mayor
sencillez. En fase líquida, las propiedades del sistema
<incomprehensible> un cristal de cuarzo con recubrimiento quími-
<incomprehensible> <incomprehensible> de varios paráme-
<incomprehensible> de manera que se necesitará el uso de un analizador de
<incomprehensible> para conseguir una caracterización satisfactoria del
<incomprehensible> y el análisis. Por último, remarcar que el método os-
<incomprehensible> no es aplicable para fases líquidas bajo ciertas con-
<incomprehensible> experimentales.

Dispositivos SAW

† Como ya hemos mencionado, la información obtenida
de la interacción de un elemento SAW con el medio provie-
de directamente de cambios en la velocidad superficial de

la onda acústica (\( \Delta v/v \)). Como en el caso de los sensores
SAW, se pueden emplear dos métodos de medida con estos
dispositivos. En el método oscilador, el SAW, que reali-
menta al amplificador, se utiliza como elemento de control
de un circuito oscilante. Los cambios en la frecuencia de
oscilación (\( \Delta f \)) son proporcionales (ver ecuación 15) a
los cambios en la velocidad superficial de la onda acústica.
Los elementos de una malla oscilante sencilla son represen-
tados esquemáticamente en la Figura 9 [28, 33]. Para con-
seguir un circuito oscilante estable, la señal que recorre la
malla debe retornar a su punto de partida con la misma am-
plitud y un desfase de 2\( \pi \) radianes [28]. Para compensar pos-
ibles desfasos en la malla de realimentación se suele
utilizar un ajustador de fase. Se necesitan, así mismo, un fil-
tro que elimine cualquier otra frecuencia que no sea la de
interés, un atenuador variable que sirva para ajustar la ga-

Figura 9. Circuito electrónico para el funcionamiento de un oscilador
SAW (M=red de ajuste de impedancia).

Figura 10. Dispositivo experimental para la medida de amplitudes y fases
de las señales eléctricas en una línea de retardo SAW. (M=red de ajuste de
impedancia; T=resistencia terminal de 50\( \Omega \)).
nancia del circuito, y un acoplador que deriva una cierta porción de la corriente hacia un contador de frecuencia.

Uno de los inconvenientes de este método estriba en la imposibilidad de separar totalmente los diferentes modos de vibración, por lo que el dispositivo trabaja con varias frecuencias, facilitando la existencia de intercambios entre ellas si se producen perturbaciones rápidas en el medio [28, 34].

Para los dispositivos SAW, la frecuencia y los cambios de fase están relacionados mediante la siguiente ecuación [35]:

\[ \phi = -360 \frac{\Delta f}{v_p} \]  

(37)

siendo \( \phi \) la fase en grados, \( f_p \) la frecuencia, \( \Delta f \) el espaciado interdigital y \( v_p \) la velocidad de fase. De la ecuación 37 se sigue que los cambios de fase en un dispositivo SAW que opera con una frecuencia constante son inversamente proporcionales a la velocidad de la onda superficial.

La Figura 10 muestra un montaje experimental para la medición de amplitud y fase de una línea de retardo SAW [28, 29]. En el mismo, el acoplador divide en dos partes iguales la señal de salida de frecuencia mixta y amplitud constante de la malla (=500 mV). Una parte es desviada hacia la línea de retardo, y la otra es utilizada como referencia, y alimenta a un voltímetro vectorial que mide la diferencia entre las dos señales. De esta manera se pueden cuantificar los cambios de fase y atenuación. No obstante, los cambios de frecuencia pueden ser también medidos a partir de la cantidad de ajuste necesaria para que la frecuencia de la señal del circuito oscilante vuelva a su valor inicial [29]. Para este tipo de medidas es más conveniente la utilización de un analizador de red [36]. Por comparación entre ambos se observa que el método oscilador proporciona mayor precisión que el método de cambio de fase [28] o atenuación.

**Aplicaciones de los sensores piezoeeléctricos**

Los dispositivos piezoeeléctricos han sido especialmente utilizados como sensores de cambios de masa e interacciones químicas. A continuación se recogen los avances más recientes que sobre este campo se pueden encontrar en la bibliografía. Para obtener una información más detallada recomendamos la lectura de otros artículos recopilatorios sobre BAWs [3, 10, 38, 39] y SAWs [17, 19, 28, 40].

**Sensores de gases**

Los dispositivos piezoeeléctricos pueden convertirse en sensores químicos mediante la aplicación de una delgada película de material activo, el cual interacciona con el analito de interés y confiere sensibilidad y selectividad al dispositivo.

Diferentes recubrimientos orgánicos han sido investigados al respecto [41], encontrándose una particularidad, inherente a los mismos y, por tanto, difícil de salvar, que dificulta su aplicación. Tal fenómeno es la divergencia existente entre la capacidad selectiva del recubrimiento y la reversibilidad de las reacciones en las que participa. Si el analito sufre una fisiorsión debido a interacciones débiles ácido-base o a la actuación de fuerzas de van der Waals, la respuesta del sensor es reversible, pero la selectividad es pobre, pues la membrana experimenta el mismo tipo de interacciones con una gran variedad de especies diferentes. Si la detección del analito involucra una quimisorción y, por tanto, la formación de una unión covalente, la selectividad será adecuada, pero el proceso de respuesta será irreversible a temperatura ambiente. El problema de la carencia de selectividad puede ser obviado mediante el uso de series de sensores con distintos recubrimientos [17], los cuales reaccionan con cada uno de los analitos de manera ligeramente diferente. La respuesta compleja de la serie de sensores es tratada estadísticamente empleando las denominadas técnicas de Pattern Recognition. Siguiendo este planteamiento se han desarrollado series de sensores SAW para gases, capaces de determinar cada uno de los componentes de mezclas gaseosas formadas por vapor de agua, metanol, octano y gasolina [42].

Los dispositivos BAW han sido aplicados como detectores en cromatografía de gases [3, 38]. Debido a que la finalidad principal de este método analítico es la detección de la mayor cantidad de posibles compuestos carbonados, se utilizaron recubrimientos no selectivos. La elección de determinados tipos de polímeros permitió obtener selectividad respecto a moléculas polares tales como compuestos aromáticos, oxigenados o inyectados. Para mejorar la selectividad de los dispositivos se han utilizado películas de compuestos organometálicos depositadas sobre los polímeros orgánicos de recubrimiento. De esta manera se consigue catalystizar una determinada reacción. Así por ejemplo, se ha descrito en la bibliografía [43] la construcción de un sensor de vapor de etileno basado en este principio. Aunque la interacción resultó irreversible, se pudo regenerar el recubrimiento mediante exposición a un atmósfera de gas etileno.

Para la detección de vapores de disolventes orgánicos se han utilizado varios recubrimientos poliméricos [41, 44]. Aunque mucho menos sencillo, y de resultados igualmente positivos, ha sido la aplicación de fases estacionarias comerciales para cromatografía de gases [45].

Para la detección de gases inorgánicos, como NO₂, se han depositado sobre los piezoeeléctricos ftaicolaminas de varios metales [46-48]. Cabe recordar que la respuesta de los dispositivos SAW es también función de las propiedades fisicoquímicas del recubrimiento. Este aspecto es importante en el caso de los sensores de ftaicolaminas, pues la respuesta no solo se origina a partir de cambios másicos debido a reacciones con la fase gaseosa, sino que depende también de cambios en la conductividad del recubrimiento [16, 49].
Sensores químicos piezoeléctricos

Varios métodos han sido utilizados para la deposición de los recubrimientos en la superficie del sensor. Entre ellos destacan los conocidos como extracción del disolvente [41], vaporización térmica [48], y la técnica de Langmuir-Blodgett [50]. Una alternativa a esta última, y con la que se consiguen unas propiedades laminares similares, es la técnica de monocapas autoensambladas [51-53] basada en la formación de alcanotioles en la superficie de una película de oro. Los sensores obtenidos de esta manera se han mostrado especialmente adecuados para la detección de organofósforicos. Otra metodología, que está en fase de implantación, es el anclaje químico directo a la superficie del cristal de moléculas reactivas como los paracidofoles [54]. Estas especies interaccionan con el analito siguiendo los principios selectivos de la química supramolecular.

La humedad, al igual que la temperatura, puede afectar drásticamente las características del sensor cuando el recubrimiento contiene polares. Los efectos de la humedad han sido estudiados con un sensor SAW cuya superficie fue modificada mediante el anclaje de amino-propilretiioxilano [55]. Los resultados obtenidos por Hubiere [47] al utilizar sensores SAW recubiertos con flácianinas diversas revelaron que los cambios de humedad afectan no sólo a la deriva de la línea de base, sino que además implican cambios en la sensibilidad y tiempo de respuesta del sensor.

Sensores de líquidos

Los sensores BAW han sido utilizados, de manera satisfactoria, como microbalanzas electroquímicas de cuarzo para la determinación en disolución de cambios de masa durante la electrodeposición o electrodissolución de materiales de recubrimiento de los electrodos. La deposición/disolución de cobre [56] y plomo [57] ha sido estudiada utilizando cristales de cuarzo de 5 MHz de frecuencia resonante y sensibilidad máxima de 19.5±0.1 ng/Hz⁻¹-cm⁻². También se han utilizado dispositivos QCM para investigar los procesos de intercambio iónico con la disolución de recubrimientos de polipirrol y poli-N-metilpirrol, depositados sobre los electrodos de oro del mismo dispositivo [58].

La bondad de la ecuación 9, que describe los cambios de frecuencia debidos a cambios de la densidad y viscosidad del medio, ha sido probada en varios trabajos [32, 51]. A partir de medidas de impedancia [51] ha sido posible separar los efectos debidos a cambios en la densidad, las viscoelásticas y dielectrísticas de un líquido en contacto con un dispositivo QCM. Kurosawa [32], tras comparar métodos activos y pasivos, encontró que los cambios de frecuencia resonante, obtenidos a partir de medidas de impedancia, son unos de los mejores parámetros de caracterización de un sensor. El mismo autor obtiene dependencias lineales de δf frente a (ρnp)¹/² en un rango de valores de (ρnp)³/² entre 1 y 8. Por contra, los valores de frecuencia resonante obtenidos mediante el método oscilador se apartan claramente de la linealidad.

Otros sensores piezoeléctricos, como los dispositivos APM (Acoustic Plate Mode) o los basados en el uso de ondas Lamb [22, 23, 59], han servido para estudiar cambios en la viscosidad de una disolución. En general, se ha observado que, en soluciones de baja viscosidad (agua), el efecto que los cambios de este parámetro tienen en la frecuencia de oscilación es pequeño, dependiendo de la respuesta del sensor de los cambios de densidad. La sensibilidad relativa de los sensores a los cambios de densidad es de ~2.3·10⁻² [23]. Este fenómeno llevó a Wang [25] a utilizar un sensor, FPW para medir la disolución de etanol, NaCl y sacarosa en una fase gelatinosa de agarosa, agar-agar o alginito de calcio. Los coeficientes de disolución obtenidos de esta manera fueron comparables a los encontrados mediante métodos convencionales. En ese trabajo se afirma que la utilización de un sensor piezoeléctrico para la medida de coeficientes de disolución es válida, no sólo para fases gelatinosas, sino para toda aquella fase que actúe a modo de filtro [25].

En soluciones acuosas, la viscosidad y la densidad no se ven alteradas de forma notable por cambios de concentración iónica, y por lo tanto es posible la utilización de una sonda electrolítica de carácter piezoeléctrico [29, 60]. Por ejemplo, se ha demostrado que un dispositivo APM fabricado con un corte ZX de LiNbO₃, el cual posee un elevado coeficiente de acoplamiento electromecánico, permite realizar medidas de conductividad y mobiliidad iónica de electrolitos fuertes (cloruros de metales alcalinos) a dilución infinita a partir de cambios y pérdidas de frecuencia [29]. Los valores así obtenidos están en buen acuerdo con los datos teóricos y experimentales preexistentes. Para disoluciones de electrolitos débiles (ácidos o bases débiles) los cambios o pérdidas de frecuencia sólo pueden ser atribuidos a cambios en la conductividad de la disolución para concentraciones muy bajas (no mayores a 1 % en peso) [29]. Cuando la concentración aumenta, los cambios de viscosidad y densidad juegan un papel preponderante. No obstante, es posible separar los distintos efectos [60]. Kondoh [60, 61] ha estudiado las interacciones acuostoelectrás entre el potencial piezoeléctrico, generado en un dispositivo de ondas transversales-horizontales (SH) cuyo piezoeléctrico era un corte cristalino de LiTaO₃, y las propiedades eléctricas (conductividad y permitividad relativa) de la solución electrolítica en contacto. Cálculos realizados con este dispositivo muestran que las interacciones acuostoelectrás ocurren en una capa interfacial unas 150 veces más ancha que la zona de desplazamiento transversal de la onda. Cuando se utiliza una línea de retardo dual con una de las líneas cubierta por una película de metal para prevenir su interacción electroacústica con la solución, se pueden eliminar las perturbaciones mecánicas (carga de masa correspondiente al líquido y cambios de la viscosidad) mediante medidas diferenciales, registrando sólo cambios en los parámetros eléctricos de la interfase sólido-líquido [61]. Aprovechando esta fenomenología se han podido construir sensores de conductividad y pH [60], que a su vez se han utilizado como
elementos de base para el desarrollo de biosensores enzimáticos [61, 62].

Para conseguir sensibilidad y selectividad hacia un cierto ion en disolución se ha experimentado también con la utilización de membranas selectivas de iones [63] o superficies modificadas químicamente [28]. Caltiendo [63] se ha servido de membranas de PVC impregnadas de un agente plastificante y un ionóforo (valinomicina) para operar una celda de flujo continuo con un dispositivo APM. La respuesta del sensor respecto a los cambios en concentración de iones K⁺ en solución se muestra reversible y lineal en el rango de pH 1.5 a 4. La sensibilidad del dispositivo en la región lineal, definida como $S = (\Delta f / f) / pK^+$, es, aproximadamente, de 8.5·10⁻⁴. Ricco [28] también ha utilizado una celda de flujo continuo con un sensor APM fabricado con un corte ST de cuarzo. Después de modificar la superficie del cuarzo con el anclaje de N-2-aminoetilaminopropiltriétiloxiilano, que puede considerarse una molécula análoga a la etilendiamina por su capacidad de formar complejos biidentados con los iones de los metales de transición, el sensor experimentaba cambios de frecuencia cuando a través de la celda de flujo se hacía pasar una disolución de Cu²⁺ 0.25 M. Estos resultados indican que se genera una carga másica de iones Cu²⁺ en la superficie del sensor, con una densidad de 5·10⁻⁸ iones·cm⁻². La superficie puede ser fácilmente regenerada mediante lavado de la misma con disolución de HCl, lo que causa la protonación de la diamina inmovilizada y la subsecuente desorción del ion Cu²⁺. Desafortunadamente, los autores no aportan datos sobre la dependencia de la respuesta respecto a la concentración.

Biosensores

El campo en el que las posibilidades de aplicación de los sensores piezoeléctricos son más prometedoras es, sin duda, el de la fabricación de biosensores. Diferentes compuestos bioquímicos activos como enzimas, anticuerpos/antígenos, fragmentos de DNA, etc., pueden ser inmovilizados en la superficie de un piezoeléctrico y participar en reacciones altamente específicas, lo que le confiere al dispositivo un carácter muy selectivo. Los sensores acústicos másicos han sido ya utilizados con esta finalidad [10], y los sensores SAW, tomando las precauciones necesarias, son potencialmente aplicables.

Así, por ejemplo, la inmovilización de lectinas sobre cristales piezoeléctricos de cuarzo AT [11, 64] ha servido para desarrollar un biosensor capaz de la determinación selectiva de azúcares y eritrocitos, permitiendo, incluso, la distinción entre eritrocitos de tipo A y B. Un modelo viscoelástico [64] ha sido utilizado para explicar los resultados, pues los incrementos de masa, por si solos, no pueden justificar los cambios de frecuencias resonantes. Otro sensor para la determinación de eritrocitos [65] se ha desarrollado a partir de la inmovilización de anticuerpos A de la antiligilcoforina en un capa de polietilenimina depositada sobre la superficie de una QCM, la cual operaba a 10 MHz. El sensor puede ser utilizado para la detección de eritrocitos humanos en sangre, proporcionando una respuesta de frecuencia que es lineal en el intervalo de concentración celular entre 10⁸ y 5·10¹⁰ células. La respuesta es atribuible a cambios de masa en la región interfacial. Otro ejemplo de biosensor basado en el uso de dispositivos SAW ha sido el desarrollado para la determinación del virus HIV [66], causante del SIDA.

Varios sensores SAW con diferentes tipos de modos acústicos de placa (APM) han sido aplicados como biosensores [34, 36, 67]. Hemos mencionado ya el trabajo de Kondoh y col. [60, 61] sobre la utilización de un sensor SAW del tipo SH para el estudio de las interacciones acustoelectróléticas con la disolución. Este grupo consiguió, mediante la utilización de colinesterasa y ureasa, un sensor enzimático que respondía a cambios de pH causados por las reacciones catalizadas enzimáticamente que tenían lugar en la interfase. El mismo tipo de interacciones acustoelectróléticas ha sido empleado para la preparación de un inmunosensor altamente selectivo [68]. Con el mismo se midió la respuesta inmunoespecifica de un anticuerpo (antiglucosa oxidasa monoclonal) depositado sobre la superficie de un SAW. Las medidas se llevaron a cabo haciendo pasar una disolución de glucosa oxidasa a través de una celda de flujo de 80 µl. La respuesta obtenida fue reproducible, si bien la deriva de la línea de base resultó excesivamente alta.

Otro inmunosensor [4] del tipo APM, y con una configuración de línea de retardo dual, ha sido construido recurriendo una de sus líneas con el antígeno IgG de cabra, mientras que para la otra línea, que actúa de referencia y reduce interferencias tales como las adosaciones no específicas, se utilizó un recubrimiento de IgG de cabra inmunizada con un anticuerpo que se ha mostrado adecuado para la detección de anticuerpos en niveles inferiores a los 20 ng. El mismo grupo que publicó estos resultados ha construido un sensor SAW con recubrimientos de ADN, para la detección de hybridaciones entre cadenas simples y complementaria de ADN. De esta forma han conseguido detectar cantidades específicas de secuencias determinadas de ADN a nivel de nanogramos o subnanogramos. Esta sensibilidad es equiparable a la obtenida con otros métodos más convencionales, como el análisis de radioisótopos, el marcado fluorescente, o las técnicas de amplificación enzimática.

Otra posibilidad de aplicación de un biosensor piezoeléctrico ha sido mostrada por Costello [24], quien ha utilizado un sensor FPW para la monitorización del metabolismo microbiano a partir de los cambios de densidad que tienen lugar en las disoluciones de glucosa durante los procesos de fermentación.

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Original Research Papers

Direct analysis of foods as solids or slurries by atomic spectrometry

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Abstract. Recent developments in direct analyses of foods (particularly as slurries) by atomic spectroscopic techniques are reviewed. Electrothermal atomization is by far the most frequently used atomization method for food slurry analysis. Most reviewed applications involve animal tissues and vegetables, but relatively few fish or fruits. Some elements whose contents in foods are of a high nutritional and toxicological significance have only occasionally been determined in this way.

Key words: Direct solid and slurry sampling, Atomic spectrometry, Foods, Review.

Introduction

Improving the preliminary operations of the analytical process by reducing human participation to minimal manipulation of real samples is a major goal of today’s analytical chemistry as acknowledged by instrument manufacturers, who are beginning to commercialize off-line and on-line instrumentation for establishing a fluent link between the unsampled, untreated, unmeasured sample and detection. In this context, direct analyses of solid and slurry samples by atomizing the components directly from the solid state offers some advantages in the analytical process arising from simplified the preliminary operations - chiefly from avoidance of time-consuming decomposition steps, as well as avoidance of contamination, no losses of volatile components, simplicity and costs. Atomic spectrometry (particularly graphite furnace atomic absorption spectrometry, GFAAS) has proved to be a useful technique for the direct analysis of solids thanks to its high sensitivity and selectivity. General information [1-7], atomization systems [1, 2, 4, 5], sample introduction techniques, dilution, calibration, accuracy and precision [1, 5], and applications [1-3, 5, 7] were reviewed in a series of seven papers on solid or slurry sampling in atomic absorption and emission spectrometry; however, no specific, comprehensive review in relation to food analysis has so far been published.

Potential applications of analytical chemistry to food technology will undoubtedly arise in response to some current industrial trends that can be summarized as follows: (a) the food industry is highly regulated and likely to remain so; (b) quality control during processing and distribution is becoming increasingly frequent practice and will likely be implemented throughout the industry in the near future and (c) the attention focused by food scientists on toxicology will continue to increase as environmental concerns remain high. All of these trends will increase the number of chemical assays needed in the food analysis laboratory; the development of inexpensive, rapid and precise automated analytical techniques should prove to be extremely useful to the food chemist [8, 9].

Minerals in foods are the constituents remaining as ash after incineration of plant and animal tissues; they may be divided into two categories: main elements and trace elements. The use of atomic absorption spectrometry for the direct analyses for trace elements in food is as old as the technique itself. Sample digestion and preconcentration methods for the determination of trace metals in foods by atomic absorption spectrometry (AAS) have been reported [10]; GFAAS enables solid sampling, which thus offers
some assets (e.g., minimum sample handling and avoidance of contamination).

This review surveys direct analyses of solid and slurry food samples by atomic spectrometry in terms of the features, advantages, shortcomings, and applications of each approach.

**Solid versus slurry sampling**

Analytical alternatives avoiding digestion (e.g., solid analyses) have aroused considerable interest in recent years. In addressing direct solid analyses, several principal considerations: solid and slurry sampling. A brief definition of both is thus in order so as to establish any differences. In solid sampling, the sample is directly introduced into an atomizer and atomized from the solid state. In slurry sampling, the sample is introduced as an aliquot of a stabilized suspension into the atomizer. Both approaches have some advantages and shortcomings that are discussed below.

**Direct solid sampling**

Interest in solid sampling has grown steadily ever since the first application was reported [11]; an average of 15 papers have been published yearly in the intervening period (1970-1994). Solid sampling is a powerful technique in as much as it requires no dissolution, so it avoids sample contamination and provides decreased detection limits. As a result, the analytical process is simplified, less intensive manipulation is required, and the hazards associated with the use of acids are avoided. However, direct solid sampling has some limitations such as the difficulty involved in automating the introduction of a small mass of powdered material into an atomizer and weighing micro amounts of sample. Other occasional limitations include the lack of suitable calibration standards, the presence of a matrix interferences (which requires a good background correction), sample and particle size non-homogeneity, poor precision in the results, and the need to design special atomizers [5].

Laser ablation (LA) extends the capabilities of atomic spectroscopy to the direct analysis of solid samples. This is particularly advantageous for materials such as alloys, ceramics, glasses, powders, and geological samples [12, 13]. Bulk samples or thin sections can be analyzed directly, while powders only requiring pressing into pellets. Absorption at the laser wavelength by a biological sample can differ from that of inorganic materials, because the bonding energies of molecules may be similar to photon energies and some molecules can undergo photochemical changes. Therefore, in contrast to ablation of inorganic samples, organic material can show little or no evidence of melting or thermal deterioration. Owing to the complex nature of the ablation process which detracts from accuracy and calibration, few authors have used LA-ICP-MS for elemental determinations in biological samples [14]. This hyphenated approach may be a reliable means for analyzing foods; however, no applications in this area have so far been reported, despite the high potential of the technique for processing lyophilized foods.

**Slurry sampling**

This technique, originally developed by Brady et al. [15, 16], combines the advantages of liquid and solid sampling [17]. Conventional autosamplers, pipettes, and flow systems can be used to inject slurries into an atomizer for analysis. Also, slurries can be introduced fairly easily into ICP-MS [18], ICP-AES [19], FAAS [20], ETA-AFS [21, 22] and GFAAS instrument [24]. This expands the scope of application of these spectroscopic sampling techniques; in fact, atomizers used for liquid sampling can also be used for atomization of slurries. However, the most critical factor in this technique is the need to preserve slurry homogeneity during the time required for sample introduction. Two other limiting factors are related to the particle size differences and dilution of the solid sample, both which affect accuracy and precision.

**Advances in problem solving**

L’vov [25] suggests addressing many of the above mentioned shortcomings of the two sampling techniques by using new components and state-of-the-art technology and equipment in powder atomization. Some advances in solid sampling based on new, special graphite atomizers for handling solid samples, and the automation of solid sample introduction have been achieved [5, 6]. Some progress in this direction is due to the STPF (Stabilized Temperature Platform Furnace) introduced in 1981 [26, 27], which ensures more isothermal conditions for atomization. Also, direct analyses of slurry samples can frequently be done by using aqueous standards for calibration, thereby simplifying routine determinations [6]. Using the Smith-Hieftje background corrector, the Zeeeman effect, and, in most instances, the deuterium corrector, enables compensation for background signals [28]. The precision can be affected by the sample non-homogeneity. Such devices agitation as vortex mixers, magnetic stirring bars, ultrasonic baths or gas bubblers are commonly used to minimize non-homogeneity of the slurry; also, by using a less sensitive wavelength, larger amounts of material may be used and problems arising from a lack of representativeness of sample size can thus be avoided. A number of stabilizing agents have also been tested in order to inject a representative aliquot of slurry into the atomizer. Finally, introducing oxygen during the charring step in the furnace programme can avoid deposition of residual material in the graphite furnace.

**The alternative of choice**

Choosing between solid and slurry sampling for analysis of solid samples should rely on a critical review of some
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Factors involved in the analytical procedure concerned such as sample pretreatment, homogeneity, introduction mode, calibration, etc. Both techniques occasionally require some sample pretreatment such as grinding or dilution. If dilution is needed, it can be achieved more readily by using aqueous solutions for slurry sampling—solid samples require graphite powder or a solid chemical modifier. Use of a chemical reagent is less essential in solid sampling, owing to the typically poor interactions between chemical modifiers and solid samples (various solid chemical modifiers have been tested in order to increase interactions). The slurry technique allows aqueous standards to be used for calibration in many instances, in contrast to direct solid sampling, which requires certified reference materials, synthetic solid standards (of similar composition to the samples) or the standard-addition method for calibration [1, 6]. Solid sampling requires higher atomization temperatures than does slurry sampling in order to avoid interferences from refractory metals that result in incomplete release and occlusion of the solid matrix.

Both techniques have been extensively reviewed [1-7]. Authors tend to prefer the slurry technique [5-7] since it excels direct solid sampling in analytical performance. To our minds, slurry sampling has a few other advantages over solid sampling, namely: the ability to automate sample pretreatment and addition of reagents and/or matrix modifiers by using a flow system and the ease with which the chief shortcoming of this technique (non-homogeneity) can be minimized [29-31].

Preparation of the food slurries

Samples of high analyte contents can be analysed more readily by the slurry technique than by direct solid sampling since slurries can be readily diluted in accordance with the analyte concentration. It is interesting to note that slurries can only be diluted within a narrow range since precision is diminished when highly diluted slurries are used [5, 32].

Characteristics of the slurry: particle size and concentration

Several authors have reported the optimal particle size for such samples as rocks, sediments and soils; however, there is little information on the optimal particle size for foods. The particle size of the solid material used to prepare the slurry sample influences its stability, deposition and atomization efficiency, which in turn affect the accuracy and precision of analyses as shown by Bendicho et al. [5]. The optimal particle size range depends on the sample composition. In any case, flame and plasma atomization systems are more markedly dependent on particle size than are electrothermal atomization systems, which may account for the wider use of the latter for slurry atomization. In a

elaborate study on slurry sample preparation with GFAAS, Miller-Ihli [29] suggested that very small particle sizes (< 30 μm) were not essential for precise slurry analyses of biological or botanical materials, which could be extrapolated to food samples. On this assumption, Hernández-Córdoba and López-García [33] performed a number of experiments on commercial paprika samples containing lead; the lead signals from slurries prepared from the 100-200, 50-100 and 30-50 μm particle sizes were 10.5, 2.4 and 1.1% higher, respectively, of that obtained from the slurry prepared from the less than 30 μm fraction. Also, signal reproducibility was lower for the coarser fractions. The RSD was 15.6 % and 5.6 % for the 50-100 and less than 30 μm fraction, respectively. This suggests a marked effect of particle size, but only above 30 μm. In the work reviewed here, ICP was the technique involving the smallest particles sizes (lower than 5 μm) [34, 35]. The fundamental parameters of slurry nebulization in ICP-AES were recently reviewed by Goodall et al. [36]. Theoretical considerations and empirical data suggest that, for accurate analysis of reference materials (e.g. hay powder and spruce needles) by slurry nebulization, the particle size distribution of the slurry should not exceed a value that is determined by density (viz. 2.9 and 1.5 μm for materials with a density of 1 and 7 g cm3, respectively). Particles smaller than 10 μm can seemingly be readily transported through the spray chamber to the flame [37]. In this context, Andrade et al. [21] developed a flow injection system for introduction of food slurries of a mean particle size of 6.7 μm into a modified Babington nebulizer in order to determine iron and zinc by FAAS.

Errors associated with particle size can be lessened by grinding the original sample. Foods can be ground by using various devices such as mixers/mills [21, 34], agate ball mills [32, 38], household grinders [39, 40], blown zirconia spheres [41], etc. Some authors use powdered reference materials, so no grinding or sieving is required [29, 35]. The grinding time (10 min to 1.5 h) of food depends on the sample composition, initial sample weight and particle size required, which are in turn dictated by the particular technique employed. Thus, reducing the particle size of spinach samples, to less than 17 μm for lead determination required grinding for 1.5 h according to Stephen et al. [17]. Others authors [33] have reported grinding times as short as 10 min. Even though grinding is fairly uncomplicated, due care should be exercised in order to avoid sample contamination.

The required concentration range can be more readily achieved by using slurry sampling than direct solid sampling because diluting a slurry is much easier. However, slurries cannot be diluted at will because the precision suffer when highly diluted slurries are used [42]. Lynch and Littlejohn [32] established an optimal slurry concentration range for food analysis. Slurry concentrations above 5% (w/v) result in inefficient deposition of the slurry aliquot; however, the authors [43]
achieved quantitative recoveries for some slurries at concentrations up to 10% (w/v), but recommended a maximum slurry concentration of ca. 5% (w/v) for general use.

**Slurry stabilization-homogenization and introduction**

The need for stabilizing agents arises from the sedimentation rate of the suspended material. In aqueous solutions, these materials undergo rapid sedimentation, probably as a result of their hydrophobic nature; the sedimentation rate depends on the radius of the sample particles and the viscosity of the diluent medium. Slurries can also be stabilized by using some device to foster mixing in order to facilitate pipetting and subsequent introduction into the atomizer.

Stabilizing or dispersant agents to keep the slurry homogeneous or to disperse the particles and prevent agglomeration, respectively, have been used. The more frequently used in food analysis include Triton X-100 [18, 29, 33, 34, 38, 44-46], HMP-hexametaphosphate [24], Viscalex HV30 [17, 39], Antifoam B emulsion [32, 43] and sodium pyrophosphate or aerosol OT [36], which prevent foaming. López-García et al. [24] used 0.05% HMP as stabilizing agent to determine titanium in vegetables by GFAAS and the same HMP concentration in aqueous standards. Littlejohn et al. [39] avoid sample sedimentation by using a highly viscous medium: Viscalex at concentration above 2-3%. However, the precision is decreased as a result of the increased difficulty of pipetting the slurry diluted in the viscous stabilizer [17]; also, an additional pyrolysis step is required to excess stabilizer and the blank level is raised when a high Viscalex concentration is used. Mochizuki et al. [18] determined 18 elements in bovine liver and rice flour by ICP-MS; samples were wet-milled in Triton X-100 (1%) and after 30 min of grinding, the contents were diluted to appropriate volumes with the same solution. The nebulizer system was also washed with Triton X-100 prior to sample introduction. Miller-Ilili [29] noted that there are no real differences between standards prepared with or without Triton X-100 if peak-area rather than peak-height measurements are made in the simultaneous determination of eight elements in plant materials by GFAAS. Similar data based on peak height measurements showed dramatic differences between standards prepared with and without Triton X-100. Tetrasodium pyrophosphate and Aerosol OT, as dispersants [36], have also been used to minimize aggregation and improve the transport efficiency of the powder suspension in the nebulizer system.

Slurry stability can be preserved during the time required for sample introduction by using several homogenization approaches involving magnetic stirring [24, 31-34, 44, 47], vortex mixing [48, 49], ultrasonic agitation [45, 49, 50] or manual agitation after grinding [21, 43, 51]. By magnetic or vortex mixing, the slurry can be prepared in a beaker and stirred to the degree of homogeneity needed. Ultrasonic agitation is more effective than other approaches because the analyte of interest is partly extracted into the liquid phase under the effect of ultrasounds when slurries are prepared in an acid medium. Manual shaking was used by Andrade et al. [21] to facilitate slurry mixing and for the determination of zinc and iron in foods with good results; after vigorous manual shaking, the slurry was introduced into the flow-injection system coupled to the FAAS. By using a vortex mixing and ultrasonic agitation [49], a comparative study was made of slurry homogenization in GFAAS; the precision was not significantly different for the two systems.

As a rule, a micropipette is used to introduce slurries into GFAAS instruments following magnetic or vortex mixing because of the difficulties involved in these fitting systems to autosamplers. In order to circumvent this shortcoming, Lynch and Littlejohn [32] developed a mini-magnetic stirrer that can be used in combination with an autosampler for the determination of lead in food slurries; by using small magnetic bars coated with PTFE, slurries were homogenized in the autosampler cups. A similar device was developed by Haraldsen and Pougnet [30] the same year. Ultrasonic agitation can be used in combination with both manual and automated introduction of slurries as these can be prepared directly in the autosampler cups. Miller-Ilili [49, 52] automated the process by mounting a titanium ultrasonic probe above the autosampler tray and synchronizing its operation with that of the autosampler in order to facilitate slurry mixing. A similar design is commercially available from Perkin-Elmer (US-100) [53].

In summary, applications of GFAAS involving the use of autosamplers for introduction of food slurry samples [29-32, 39, 43, 54-57] amply exceed those based on manual introduction [33, 37, 51]. Flow-injection systems have been used in combination with FAAS [21, 38, 40, 44, 45, 58] to introduce slurry aliquots because continuous introduction into the flame was impossible; this coupled approach minimizes manipulation, volatile analyte losses and atmospheric contamination, and results in favourable instrumental responses. When employed ICP-MS [18] a conventional system to slurry introduction by using PTFE Babington-type nebulizer have been chosen. By ICP-AES slurry introduction has been carried out by using Légère-Babington [34], V-groove [48] and PTFE Babington [50] nebulizer systems.

**Pre-digestion of slurries**

Pre-treatment of a slurry can be an efficient means for extracting the analyte of interest into the liquid phase because only partial decomposition of the slurry is required, and so much time is saved relative to conventional digestion procedures. Short digestion times, digestion of troublesome matrices and dissolution in what is essentially an environmentally tight system are some of the advantages of microwave digestion procedure [59, 60].
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The combination of a microwave oven for digestion and flow-injection interface and FAAS detector provides increased sample throughput and minimal sample contamination [40, 44, 45, 58].

Chemical modifiers

Originally proposed by Ediger in 1975 [61], chemical modifier serves two essential functions: diminishing production of thermal compounds from interfering ions or analyte volatility. Chemical modifiers used in slurry analyses lead to similar appearance times for the atomic absorption signals of slurries and aqueous standards [62]. Oxygen ashing to avoid buildup of carbonaceous material has also been used successfully in some studies [17, 39, 51, 59]. Applications of chemical modifiers in the slurry technique were discussed by Tsalev [63].

Lynch and Littlejohn [32] employed 800 mg L\(^{-1}\) of Pd to stabilize lead compounds in food slurries up to a charring temperature of 900 °C while avoiding a high background absorption; NH\(_4\)H\(_2\)PO\(_4\) was also tested, but it stabilized lead up to 750 °C only. In order to reduce the loss of volatile Cr compounds, Wagley et al. [57] used Mg(NO\(_3\))\(_2\) as the chemical modifier in analysis of milk and milk powder, with which a charring and atomization temperature of 1600 and 2400 °C, respectively, was achieved. In order to avoid Cd losses, Lynch and Littlejohn [35] utilized Pd as a chemical modifier for milk, bovine liver and olive leaf slurries; cadmium compounds were stable up to 800 °C in all the samples studied. The possibility of dispensing with both the chemical modifier and the pyrolysis step in slurry sampling has also been investigated. Isothermal conditions for atomization and an efficient background correction system such as the Zeeman effect are required for this purpose. Bradshaw and Shaw [64] reported a method that avoids both pyrolysis and the use of a chemical modifier while providing rapid furnace analyses. The precision for replicates of slurries varied from 2 to 10% depending on the amount of solid sample delivered to the furnace.

Figures of merit

Calibration curves

Calibration curves for solids or slurries can be constructed by using different standardization methods including sample measurements against commercially available reference materials, the standard-addition technique or aqueous standard solutions in some cases. In most of the applications reviewed here the calibration curve was obtained from aqueous standard solutions, which is one of the salient advantages of the slurry technique. In some instances, standardization was done by using the standard-addition method [17, 24, 37, 39, 41, 44, 46, 50, 56, 57].

Standardization of solids that are analysed directly entails using a standard that is similar in composition to the samples. However, it is often difficult to obtain suitable references samples, in which case one can make up substitute synthetic standards from carefully weighed pure constituents subsequently combined into the desired form [4, 5]. Some samples can be calibrated with liquid standards [65, 66].

Accuracy and precision

The accuracy of food slurry introduction methods has been checked by different approaches such as the use of various methods, certified and standard reference materials (CRM and SRM), recovery tests and the standard-addition method. In food analyses, the accuracy is usually measured with CRMs or SRMs supplied by the Bureau of Community Reference (BCR) or the National Institute of Standards and Technology (NIST), among others. Analytical quality assurance in solid sampling of bovine liver and bovine kidney [67-69] has been achieved by using a wet digestion decomposition procedure with acceptable accuracy.

The precision of the measurements in food slurry analysis can be affected by such factors as particle size distribution, the amount of solid used and the analyte solubility in the solvent. The precision can be improved by either decreasing the particle size or increasing the mass used for the determination, which is essential with non-homogeneous samples. As noted earlier, precision in this context has been extensively studied by Holcombe and Majidi [42]. On average, the reported relative standard deviation (RSD) for the papers reviewed here ranged between 5 and 10%, though it approached 20% in a few cases [18, 31, 32, 35, 44, 50, 57].

Applications

The applications of slurry and solid sample introduction in atomic absorption and emission spectrometry dealt with this review are summarized in Table 1. Food samples are tabulated alphabetically. The table shows information on the elements determined, the type of atomic spectroscopic technique used, the typical relative standard deviation (RSD) of the method in question as well as some relevant comments. A comparison with late reviews of general applications [3, 5-7] reflects an increased awareness of an interest in food composition. However, much of the work in this area has been carried out on inorganic materials (air, minerals, coal, water, glass, sediments) and biological materials (plants, pine needles, citrus leaves) and only ca. 10% on foods and foodstuffs [5, 6].

The applications shown in Table 1 allow one to draw the following conclusions:

a) Most involved animal tissues (bovine liver, mussels) and vegetables (peppers, spinach, potatoes, tomatoes), but few fish or fruits; b) none has so far been concerned with
cheese or yogurt, all of which are included in the human daily intake, probably because of the lack of certified materials for assessing the accuracy; c) the most frequently determined elements in foods were Zn, Pb, Fe, Cu, Mn, Cd, Ca, Mg and K, followed by Se, Cr, Mo, Na, Hg and other elements whose contents in foods are pivotal in nutritional or toxicological terms (e.g. P, Co, Ti, V, Ce, Pd, Al and As) have only occasionally been determined; d) GFAAS was the most widely used atomic spectroscopic technique for slurry analyses; and e) the majority of applications for introducing slurries in the flame mode have been carried out by means of a continuous flow system involving injection of the slurry into an aqueous stream; only two applications of this continuous technique in non-flame atomization mode has been reported [70, 71].

Acknowledgements

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<table>
<thead>
<tr>
<th>Sample</th>
<th>Element(s)</th>
<th>Method</th>
<th>Concentration</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brussels sprouts</td>
<td>Cd, Cu, Pb</td>
<td>ETAAS</td>
<td></td>
<td>NH$_4$H$_2$PO$_4$ as matrix modifier Viscalex HV30 as thixotropic thickening agent</td>
</tr>
<tr>
<td>Se</td>
<td></td>
<td>GFAAS</td>
<td>12.9</td>
<td>Calibration by using standard-addition method</td>
</tr>
<tr>
<td>Zn, Mn, Cu, Ca, Se, Hg, K, Na, P</td>
<td></td>
<td>GFAAS</td>
<td>2.0-4.0</td>
<td>Subsample weight: 0.5 mg</td>
</tr>
<tr>
<td>Fe, Zn</td>
<td></td>
<td>FAAS</td>
<td>2.0-3.0</td>
<td>Fl system for slurry introduction</td>
</tr>
<tr>
<td>Mn, Cu</td>
<td></td>
<td>FAAS</td>
<td>4.3-5.3</td>
<td>Fl system with microwave oven for online slurry decomposition</td>
</tr>
<tr>
<td>Fe, Cu</td>
<td></td>
<td>FAAS</td>
<td>6.9-43.2</td>
<td>Slurry introduction by Fl system / 30-fold online slurry dilution</td>
</tr>
<tr>
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<td></td>
<td>FAAS</td>
<td>1.2-17.2</td>
<td>Fl system with online digestions</td>
</tr>
<tr>
<td>Ca</td>
<td></td>
<td>FAAS</td>
<td>3.0</td>
<td>Results are not dependent on the particle size of the slurry samples</td>
</tr>
<tr>
<td>Cr</td>
<td></td>
<td>GFAAS</td>
<td>33.0</td>
<td>Three-point estimation-standard addition method</td>
</tr>
<tr>
<td>Zn,Mn,Cu,Ca,Se,Hg,K,Na,P</td>
<td></td>
<td>GFAAS</td>
<td>1.0-10.0</td>
<td>Subsamples weight: 0.5 mg</td>
</tr>
<tr>
<td>Pb, Cd</td>
<td></td>
<td>GFAAS</td>
<td>7.1-18.6</td>
<td>Calibration performed with liquid standards solutions</td>
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<tr>
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<td></td>
<td>GFAAS</td>
<td>13.6</td>
<td>Pd as chemical modifier</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Slurry concentrations up to 4% (w/v)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Miniature magnetic agitation device</td>
</tr>
<tr>
<td>Cd, Pb</td>
<td></td>
<td>GFAAS</td>
<td>13.0</td>
<td>Amount of solid sample: 15-300 µg in 5 µl of slurry</td>
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<td>FAAS</td>
<td>1.2-2.0</td>
<td>Fl system for slurry introduction</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>Sampling frequency: 60 h$^{-1}$</td>
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<tr>
<td>Al</td>
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<td>GFAAS</td>
<td>3.7-4.7</td>
<td>Fl system for automatic dilution, addition of the chemical modifier and filtration of the slurry</td>
</tr>
<tr>
<td>Se</td>
<td></td>
<td>GFAAS</td>
<td>4.4-12.0</td>
<td>Fl system for automatic dilution, addition of the chemical modifier and filtration of the slurry</td>
</tr>
<tr>
<td>Ca, Mg, P</td>
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<td>ICP-AES</td>
<td>1.8-4.2</td>
<td>Aerosol OT as dispersant of carbonized sample</td>
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<tr>
<td>Pb</td>
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<td>HGAAS</td>
<td>4.3</td>
<td>Hydride generation in a lactic acid-potassium dichromate medium</td>
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<tr>
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<td>GFAAS</td>
<td>0.9-9.9</td>
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<td>FAAS</td>
<td>0.7-6.6</td>
<td>Sampling of carbonaceous slurry</td>
</tr>
<tr>
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<td>FAAS</td>
<td>2.1-14.0</td>
<td>Fl system with on-line digestions</td>
</tr>
<tr>
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<td></td>
<td>HGAAS</td>
<td>10.0</td>
<td>Triton X-100 as slurry stabilizer</td>
</tr>
<tr>
<td>Pb</td>
<td></td>
<td>GFAAS</td>
<td>10.0</td>
<td>Pd as chemical modifier</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Slurry concentrations up to 4% (w/v)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Miniature magnetic agitation device</td>
</tr>
<tr>
<td>Cr</td>
<td></td>
<td>GFAAS</td>
<td>33.0</td>
<td>Three-point estimation standard addition method</td>
</tr>
<tr>
<td>Ti</td>
<td></td>
<td>GFAAS</td>
<td>4.2</td>
<td>0.03% HMP as thixotropic agent</td>
</tr>
<tr>
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<td>13.8</td>
<td>Hydride generation in a lactic acid-potassium dichromate medium</td>
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<td>HGAAS</td>
<td>5.6</td>
<td>Calibration by standard-addition method</td>
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<tr>
<td>Pb</td>
<td></td>
<td>HGAAS</td>
<td>5.5</td>
<td>Triton X-100 as slurry stabilizer</td>
</tr>
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<td>Elements</td>
<td>Detection Method</td>
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<td>--------------------</td>
<td>-------------------</td>
<td>------------------</td>
<td>---------------------</td>
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<td>0.7-6.6</td>
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<td>GFAAS</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Marine foodstuffs</td>
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<td>GFAAS</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Milk crackers</td>
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<td>2.0-3.0</td>
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<td>2.0-4.0</td>
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<td>7.9</td>
<td></td>
</tr>
<tr>
<td>Mussels</td>
<td>Ca, Mg, Fe, Zn</td>
<td>FAAS</td>
<td>1.4-7.6</td>
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<tr>
<td>Mussels</td>
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<td>HGAAS</td>
<td>7.9</td>
<td></td>
</tr>
<tr>
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<td>2.8-37.5</td>
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<tr>
<td>Mussels</td>
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<td>HGAAS</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>Non-fat milk powder</td>
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<td>1.1-4.8</td>
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<td>-</td>
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<td>2.0-3.0</td>
<td></td>
</tr>
<tr>
<td>Peas</td>
<td>Ti</td>
<td>GFAAS</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>Peas</td>
<td>Fe, Zn</td>
<td>FAAS</td>
<td>2.0-3.0</td>
<td></td>
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<tr>
<td>Pepper</td>
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<td></td>
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<tr>
<td>Pepper</td>
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<tr>
<td>Pig liver</td>
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<td>GFAAS</td>
<td>-</td>
<td></td>
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<td>Pollen</td>
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<tr>
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<td>2.0-3.0</td>
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<tr>
<td>Poultry meal</td>
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<td>GFAAS</td>
<td>-</td>
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<tr>
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<td>Pb</td>
<td>HGAAS</td>
<td>7.1</td>
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<tr>
<td>Sardines</td>
<td>Pb</td>
<td>HGAAS</td>
<td>7.1</td>
<td></td>
</tr>
</tbody>
</table>

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- Sampling of carbonaceous slurry
- Boat atomizer
- Subsample weight: 3 - 15 mg
- Boat atomizer
- FI system for slurry introduction
- Particle size < 5 μm
- Pd as chemical modifier
- PTFE as fluorinating agent
- Lanthanum was added to avoid chemical and ionization interferences
- Rh as chemical modifier for Se
- Pd as chemical modifier for Cd and Pb
- Mg(NO₃)₂ plus NH₄H₂PO₄ for Cu and Zn
- Subsample weight: 0.5 mg
- Hydride generation in a lactic acid-potassium dichromate medium
- Fl system with microwave oven
- Calibration by standard-addition method
- Air-ashing
- Triton X-100 as slurry stabilizer
- V-groove nebulizer with peristaltic pump for sample aspiration
- Triton X-100 as slurry stabilizer
- Rotating arc design and furnace interface
- Three-point estimation standard addition method
- Platform-tube atomizer
- Particle size < 30 μm
- Fl system for slurry introduction
- 0.03% HMP as thiotropic agent
- Fl system for slurry introduction
- Hot-chamber system/Légeré-Babington nebulizer
- Fl system with microwave oven for slurry digestion
- Boat atomizer
- Amount of sample: 0.5 - 5.5 mg
- PTFE as fluorinating agent
- Fl system for slurry introduction
- Platform-tube atomizer
- Particle size < 3 μm
- Suspensions prepared in 20% ethanol and 4% hydrogen peroxide medium
- Hydride generation in a lactic acid-potassium dichromate medium
- Calibration by standard-addition method
<table>
<thead>
<tr>
<th>Substance</th>
<th>Analyte</th>
<th>Method</th>
<th>Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sausage</td>
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<td>FAAS</td>
<td>2.0-3.0</td>
</tr>
<tr>
<td>Sugo flour</td>
<td>Pb</td>
<td>GFAAS</td>
<td>10.6</td>
</tr>
<tr>
<td>Spinach</td>
<td>Pb</td>
<td>GFAAS</td>
<td>3.0</td>
</tr>
<tr>
<td>Spinach</td>
<td>Ti</td>
<td>GFAAS</td>
<td>6.5</td>
</tr>
<tr>
<td>Spinach</td>
<td>Fe, Mn, Zn, Cu, Cr</td>
<td>SIMAAC</td>
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<td>Pb</td>
<td>GFAAS</td>
<td></td>
</tr>
<tr>
<td>Spinach</td>
<td>Mo</td>
<td>ETV-ICP-AES</td>
<td>22.2</td>
</tr>
<tr>
<td>Spinach</td>
<td>Pb</td>
<td>GFAAS</td>
<td>6.8</td>
</tr>
<tr>
<td>Tomato paste</td>
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<td>FAAS</td>
<td>2.0-3.0</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>Pb</td>
<td>GFAAS</td>
<td></td>
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<tr>
<td>Wheat flour</td>
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<td>FAAS</td>
<td>2.0-3.0</td>
</tr>
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<td>Wheat flour</td>
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<td>&lt;20.0</td>
</tr>
<tr>
<td>Wheat flour</td>
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<td>ICP AES</td>
<td>1.3-13.2</td>
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<tr>
<td>Wheat flour</td>
<td>Mo</td>
<td>ETV-ICP-AES</td>
<td>14.3</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>Cd</td>
<td>GFAAS</td>
<td></td>
</tr>
<tr>
<td>Wheat flour</td>
<td>Zn, Mn, Mg, Cu, Ca, Fe, K</td>
<td>FAAS</td>
<td>0.7-6.6</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>Zn, Mn, Cr, Ca, Se, Hg, K, Na, P</td>
<td>GFAAS</td>
<td>2.0-4.0</td>
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<td>Wheat flour</td>
<td>Pb</td>
<td>ETAAS</td>
<td>28.6</td>
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FAAS: Flame atomic absorption spectrometry; GFAAS: Graphite furnace atomic absorption spectrometry; SIMAAC: Simultaneous multi-element atomic absorption spectrometer with a continuous source; ETV-ICP-AES: Electrothermal atomic emission spectrometry; ETAAS: Electrothermal atomic absorption spectrometry; PTFE: Polytetrafluoroethylene; Viscalex: Viscalex as stabilizer.

References
Evaluation of two different acid digestion methods in closed systems for trace element determinations in plants

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Abstract. Two acid digestion methods under pressure have been evaluated for determining potentially toxic elements in plants. The accuracy and precision of each method were calculated with different plant tissue samples obtained from the Measurement and Testing Programs (formerly Community Bureau of Reference, BCR).

The results suggest that both digestion methods were equivalent and suitable for the investigated elements: copper, lead, mercury and cadmium. Likewise, the coefficient of variation was adequate for each method, considering a maximum of 10% in the bias calculation.

Keywords: Autoclave, Pressure digestor, Lead, Cadmium, Copper, Mercury.

Introduction

At present, few analytical techniques allow direct determination of trace elements in solid samples (IR, FRX, NAA). The transformation of the sample into a solution is a critical step in the analytical procedure. Digestion of organic and inorganic matrices is one of the most essential and critical steps in analysis of trace elements and determines the precision and accuracy of the results obtained.

Acid digestion under pressure is a relatively new total digestion technique [1, 2]. This system is very effective for dissolving samples, minimizing losses by volatilization and contamination [1, 3]. Furthermore, this method presents several advantages compared to an open acid digestion: 1) it is a faster procedure, 2) it has lower blank values, 3) it allows control over some element volatilizations (Hg, As, and Se) and 4) it allows good control over toxic vapour fumes, reducing health hazards and limiting possible damages to other instruments [1].

The purpose of this study is to evaluate two different acid digestion methods under pressure for determining the concentrations of potentially toxic metals in plant tissues.

Material and methods

Biological materials

Different biological samples were used: Evernia prunastri lichen* (TP24), Parmelia sulcata lichen* (TP25) [4], lyophilized maize leaves* (FD8), lyophilized algae* (VL/93-605), kenaf stems (Hibiscus cannabinus, tropical herbaceous), Laminaria species alga and Undaria pinnatifida alga. The effectiveness of both digestions was observed over these plant matrices.

Some of these samples (those marked with an asterisk) came from the Measurement and Testing Programme, MAT (formerly named Community Bureau of Reference, BCR). Since the metal concentration was already known, it was possible to evaluate the accuracy and precision of the proposed methods, doing away with necessity to use internal standards for the yield determination.

Experimental design

a) Pressure Digestor. Samples were dried and homogenized by milling. From each sample, portions of 0.1000 ± 0.0001 g were weighed and placed in 100 mL Teflon vessels (PTFE). Then, 3 mL HNO3 (65%) and 2 mL H2O2 (35%) (Merck-Suprapur quality) were added [5].

The PTFE vessels were put in the stainless steel shields, were hermetically locked and remained in an oven at 130°C for 4 hours [1, 2].

Five replicates from each sample were digested. In a similar way, blank study was made with seven replicates.
Table 1. Effect on Cd, Pb, Cu and Hg concentrations in Parmelia sulcata lichen digested in autoclave using: * 5mL H$_2$O$_2$ + 4mL HNO$_3$ + 2mL H$_2$O = 10mL H$_2$O + 3mL HNO$_3$ + 2mL H$_2$O$_2$ (3 replicates).

<table>
<thead>
<tr>
<th>Elem.</th>
<th>MAT certified conc. (µg/g)</th>
<th>Conc. found mixt. acid* (µg/g)</th>
<th>Coeff. Variat. (%)</th>
<th>Conc. found mixt. acid** (µg/g)</th>
<th>Coeff. Variat. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>26.5 ± 3.1</td>
<td>25.1 ± 2.6</td>
<td>10.4</td>
<td>25.9 ± 0.7</td>
<td>2.6</td>
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<tr>
<td>Cd</td>
<td>0.93 ± 0.22</td>
<td>0.89 ± 0.06</td>
<td>6.7</td>
<td>0.85 ± 0.04</td>
<td>4.7</td>
</tr>
<tr>
<td>Hg</td>
<td>0.25 ± 0.04</td>
<td>0.23 ± 0.05</td>
<td>21.7</td>
<td>0.23 ± 0.01</td>
<td>4.3</td>
</tr>
<tr>
<td>Pb</td>
<td>145 ± 11</td>
<td>150 ± 3</td>
<td>1.8</td>
<td>147 ± 3</td>
<td>2.3</td>
</tr>
</tbody>
</table>

The final volume of sample-acids must not exceed 10% of the total volume of the PTFE vessel, in order to avoid problems due to nitrous gas compression which is generated during the mineralization.

b) Autoclave Digestion. Less than 0.5 g of each plant powder were weighed into a 100 mL glass autoclave bottle. 10 mL H$_2$O (Milli-Q) were added and, also, 3 mL HNO$_3$ (65%) and 2 mL H$_2$O$_2$ (35%) (Merk-Suprapur) were added.

Borosilicate (low in alkaline content) bottles with turn-caps, resistant to heat and pressure, and pouring rings were used. They all were provided by POBEL and manufactured under ISO rules.

For sample digestion, the glass bottles were placed in an autoclave (SELECTA Autoester-G) at 125°C, 24.5 104 N/m$^2$ of pressure for 30 minutes [1, 5].

Five replicates from each sample and seven blank replicates were made.

A short preliminary investigation on the autoclave method was undertaken by comparing two different concentrated acid mixtures. Parmelia sulcata lichen was treated with 5mL H$_2$O$_2$ + 4mL HNO$_3$ + 2mL H$_2$O$_2$ or with 10 mL H$_2$O + 3 mL HNO$_3$ + 2 mL H$_2$O$_2$. Three replicates were made of both mixture digestions.

The final volume of sample-acids must not exceed 2/3 of the total volume contained in the autoclave-bottles.

Analytical instrumentation
An Atomic Absorption Spectrometer (Perkin Elmer 2100), equipped with graphite furnace and cold vapour technique with flow injection system (FIA) was used to measure the metals. Deuterium arc background corrector and monoelemental lamps EMI (Pb and Hg) and HCL (Cd, Cu, Ni, Cr and Zn) were employed.

The elements analysed in the samples were: Cu, Cd, Pb (in graphite furnace) and Hg (by cold vapour-FIA). In addition to this, Ni, Cr, Zn, (in graphite furnace) were determined in the blanks of both methods.

Statistical analysis
The results obtained for the MAT-samples describe the accuracy, precision and recovery coefficient of both digestions. The statistical method "student analysis for means from two normal populations" was used, with a 99% confidence interval. Likewise, a maximum of 10% in the coefficient of variation for the bias calculation from the proposed digestion procedures was considered [6].

The same statistical analysis was used to compare data from samples, which did not come from the MAT programme, but from the preliminary autoclave study.

Results and discussion
Effect of acid mixture concentration on autoclave-digestion
The matrix is not completely broken down in the acid autoclave treatment of some samples and the silicates remained without being mineralized in all of them.

Table 2. Concentration averages of Cd, Cu, Pb, Cr, Ni and Zn in blank and detection limits from both proposed digestion methods (7 replicates).

<table>
<thead>
<tr>
<th>Elem.</th>
<th>Pressure digestors</th>
<th>Autoclave</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD (µg/L)</td>
<td>Lim. Detect. (µg/L)</td>
</tr>
<tr>
<td>Cd</td>
<td>0.01 ± 0.02</td>
<td>0.06</td>
</tr>
<tr>
<td>Hg</td>
<td>0.06 ± 0.06</td>
<td>0.18</td>
</tr>
<tr>
<td>Pb</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Cr</td>
<td>0.3 ± 0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Zn</td>
<td>1.4 ± 0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>Ni</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

After digestion, samples were filtered through Whatman n° 54 filter paper. Although it did not seem to be necessary, but in order to prevent variations, the digested samples in pressure digestors and the blanks from both attacks were also filtered.

The results showed (Table 1) that there were no significant differences between the two acid mixtures in the analytes. So, metals were not occluded or absorbed in the residue.

Furthermore, slightly higher blanks were obtained using the most concentrated acid mixture (*). Therefore, the diluted acid solution (**-Table 1) was used in the rest of the study.

Blank study
The main contamination sources are from two types: 1) at random and 2) systematic [6]. The second one is the most important and it is the one that we tried to reduce and to quantify. Table 2 shows small Zn and Cr concentrations in the pressure digestion blanks. The blank caused by the other elements was negligible.

In the autoclave digestion, blank absorbances were slightly higher than those in the pressure digestor. Nevertheless, autoclave blanks remained within an acceptable interval.

The detection limits were also determined (Table 2). These correspond to the concentration in the measured solution, which provides an absorbance reading three times (99% confidence interval) the standard deviation of the blank (n=7). The detection limits were also calculated in micrograms of elements per gram of dry sample [6].
Evaluation of two different acid digestion methods in closed systems for trace element determinations in plants

Table 3. Concentration averages, coefficients of variation and recovery of Cd, Cu, Pb and Hg in the samples (5 replicates).

<table>
<thead>
<tr>
<th>Element</th>
<th>MAT certified conc. (µg/g)</th>
<th>Pressure digestors</th>
<th>Autoclave</th>
<th>SD equiv. at 99% conf. limit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD (µg/g)</td>
<td>C.V. (%)</td>
<td>Recov. (%)</td>
<td>Mean ± SD (µg/g)</td>
</tr>
<tr>
<td>Water (FD8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>16.27 ± 3.49</td>
<td>17.61 ± 0.24</td>
<td>1.36</td>
<td>108.2</td>
</tr>
<tr>
<td>Cd</td>
<td>39.93 ± 11.54</td>
<td>41.14 ± 0.95</td>
<td>2.31</td>
<td>103.0</td>
</tr>
<tr>
<td>Fe</td>
<td>12.52 ± 5.33</td>
<td>13.39 ± 0.50</td>
<td>3.73</td>
<td>106.9</td>
</tr>
<tr>
<td>Hg</td>
<td>0.28 ± 0.40</td>
<td>0.35 ± 0.03</td>
<td>10.00</td>
<td>106.1</td>
</tr>
<tr>
<td>Lichen PS (TP25)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>26.5 ± 3.1</td>
<td>26.05 ± 0.70</td>
<td>2.59</td>
<td>101.7</td>
</tr>
<tr>
<td>Cd</td>
<td>0.93 ± 0.22</td>
<td>0.85 ± 0.08</td>
<td>9.41</td>
<td>91.4</td>
</tr>
<tr>
<td>Fe</td>
<td>145 ± 11</td>
<td>143.19 ± 2.71</td>
<td>0.89</td>
<td>98.8</td>
</tr>
<tr>
<td>Hg</td>
<td>0.25 ± 0.004</td>
<td>0.28 ± 0.02</td>
<td>7.14</td>
<td>112.0</td>
</tr>
<tr>
<td>Lichen EP (TP24)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>4.80 ± 0.64</td>
<td>5.06 ± 0.39</td>
<td>7.70</td>
<td>105.4</td>
</tr>
<tr>
<td>Cd</td>
<td>0.16 ± 0.04</td>
<td>0.18 ± 0.01</td>
<td>5.55</td>
<td>112.5</td>
</tr>
<tr>
<td>Fe</td>
<td>5.61 ± 1.11</td>
<td>5.77 ± 0.23</td>
<td>3.99</td>
<td>102.8</td>
</tr>
<tr>
<td>Hg</td>
<td>0.18 ± 0.03</td>
<td>0.21 ± 0.02</td>
<td>9.52</td>
<td>116.7</td>
</tr>
<tr>
<td>Slime (VL93-695)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>13.29 ± 2.68</td>
<td>14.20 ± 0.43</td>
<td>3.03</td>
<td>106.8</td>
</tr>
<tr>
<td>Cd</td>
<td>0.30 ± 0.12</td>
<td>0.29 ± 0.02</td>
<td>6.90</td>
<td>96.7</td>
</tr>
<tr>
<td>Fe</td>
<td>12.96 ± 4.71</td>
<td>12.34 ± 0.88</td>
<td>7.13</td>
<td>95.2</td>
</tr>
<tr>
<td>Hg</td>
<td>0.23 ± 0.21</td>
<td>0.35 ± 0.03</td>
<td>8.57</td>
<td>152.2</td>
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<tr>
<td>Slime LS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>9.59 ± 0.28</td>
<td>5.50</td>
<td>4.04</td>
<td>116.8</td>
</tr>
<tr>
<td>Cd</td>
<td>1.00 ± 0.06</td>
<td>6.00</td>
<td>1.06</td>
<td>112.5</td>
</tr>
<tr>
<td>Fe</td>
<td>1.46 ± 0.10</td>
<td>6.85</td>
<td>7.56</td>
<td>95.2</td>
</tr>
<tr>
<td>Hg</td>
<td>98.67 ± 9.81</td>
<td>9.94</td>
<td>7.56</td>
<td>95.2</td>
</tr>
<tr>
<td>Slime UP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>9.25 ± 0.92</td>
<td>9.94</td>
<td>1.06</td>
<td>112.5</td>
</tr>
<tr>
<td>Cd</td>
<td>1.63 ± 0.12</td>
<td>7.36</td>
<td>7.56</td>
<td>95.2</td>
</tr>
<tr>
<td>Fe</td>
<td>1.15 ± 0.15</td>
<td>9.68</td>
<td>1.06</td>
<td>112.5</td>
</tr>
<tr>
<td>Hg</td>
<td>103.50 ± 10.21</td>
<td>9.86</td>
<td>7.56</td>
<td>95.2</td>
</tr>
<tr>
<td>Seaweed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>6.62 ± 0.37</td>
<td>5.59</td>
<td>4.04</td>
<td>116.8</td>
</tr>
<tr>
<td>Cd</td>
<td>0.16 ± 0.01</td>
<td>6.25</td>
<td>7.56</td>
<td>95.2</td>
</tr>
<tr>
<td>Fe</td>
<td>5.59 ± 0.40</td>
<td>7.16</td>
<td>7.56</td>
<td>95.2</td>
</tr>
<tr>
<td>Hg</td>
<td>0.27 ± 0.02</td>
<td>7.41</td>
<td>7.56</td>
<td>95.2</td>
</tr>
</tbody>
</table>

Biological sample analysis

The statistical analysis revealed that there were no significant differences between the autoclave method and the pressure digestor method (Table 3). Moreover, no effect was observed due to the different sample matrix compositions.

Likewise, no differences in the "certified" samples were found between data from each digestion, and between values from both mineralizations and the provided ones by the MAT. As Table 3 shows, the values obtained are in accordance with those offered by Measurement and Testing.

The coefficients of variation for five independent determinations of each element in each sample were satisfactory (Table 3), as well as their recovery coefficients.

Both digestion methods are simple, can be carried out with basic equipment and require small acid volumes. No significant losses of volatile elements and lower possibility to contaminate make them suitable for treatment of small samples. Moreover, they were more adequate to determine trace elements, compared with traditional wet digestion systems in an open or semiclosed way. These results are in accordance with Matusiewicz, 1991 [1], in his review of
accordance with Matusiewicz, 1991 [1], in his review of acid vapour-phase sample digestion of inorganic and organic matrices.

The autoclave method has the additional advantage of needing shorter digestion time per sample. This characteristic places it close to microwave digestion, with a treatment time ca. 10 minutes [3, 7]. On the other hand, to employ higher acid volume in microwave attack increases element concentrations in the blanks.

Autoclave digestion is a useful and low cost technique customarily used to determine Cd, Pb, Cu and Hg in plant tissues. When analyte is found in the composition of autoclave glass bottles (like B, Si or Al), the use of PTFE vessels to autoclave might be appropriate.

Conclusions

The results obtained in the “certified” samples indicate the suitable accuracy and precision of the methods used. The data verify that the proposed digestions are adequate for routine determination of Cd, Cu, Pb and Hg in plant tissues.

Autoclave digestion is suggested for biological samples, with regard to pressure digestors, in routine laboratory analysis. The autoclave procedure presents some advantages: low cost, low digestion time and possibility to use high sample amount (to concentrate sample solution).

On the other hand, the autoclave treatment could be considered a possible alternative to microwave attack, to mineralize samples. Although, the former is more time consuming in sample preparation [3, 5, 7], it produces lower blank responses. Blank absorbances and the extension to other elements in autoclave digestion could be optimized by employing PTFE vessels to autoclave.

References

Additional advantages of the use of immobilized enzymes: immobilization of $\beta$-D-galactosidase for magnesium determination

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Abstract. In addition to the lower cost of the analyses, the configuration of immobilized enzymes endows these biocatalysts with higher stability than that they show in solution. The stabilization effect that some metal ions have on enzymes in solution can be supplied by immobilization. This phenomenon has been observed in $\beta$-D-galactosidase, which requires the presence of magnesium as stabilizer. The loss of these ions in the immobilization process is suitable for the determination of magnesium based on the activation effect of the analyte.

Key words: Enzymatic activation, Immobilized $\beta$-D-galactosidase, Magnesium, Serum.

Introduction

The use of enzymes as analytical reagents has contributed to a larger development of novel analytical methodologies. The catalytic effects of enzymes clearly improve the selectivity of the methods proposed by using these biocatalysts. However, the use of enzymes in solution involves some drawbacks (i.e. high cost, loss of activity in storage and manipulation, etc.) that has hindered their massive use [1, 2].

The immobilization of enzymes on a suitable support has contributed to the decrease of the drawbacks involved in their use. The immobilization process enables the reusability of the biocatalyst, thus decreasing analysis costs. The immobilization produces changes in the enzyme structure that increase its stability, probably because the configuration of the active sites under immobilization becomes similar to that in their natural medium. This improvement in stability allows the development of the enzymatic reaction under less restrictive working conditions (i.e. temperature, pH, etc.). Usually, the use of immobilized enzymes also increases the selectivity of the methods as it is a very easy task to remove the interferents from the immobilized enzyme-support conjugate [3-5].

The presence of some species is essential in a number of storage enzymatic media as they contribute to stabilize the biocatalyst, whereas there are some compounds which modify its stability by activation or inhibition of its activity [6,7].

$\beta$-D-galactosidase ($\beta$-D-galactoside galactohydrolase, E.C. 3.2.1.23) catalyzes the hydrolysis of $\alpha$-nitrophenyl-$\beta$-D-galactopyranoside (ONPG) according to the following equation:

$$\text{ONPG} \rightarrow \text{\alpha-nitrophenol + \beta-D-galactose} \quad \text{Na(I) Mg(II)}$$

Sodium and magnesium are specific activators of this system. Under favorable conditions (substrate concentration, pH and temperature) the analytical signal is proportional to the sodium and/or magnesium concentration. Berry et al. [8], and more recently our research group [9] have proposed methods for the determination of sodium based on the activation of $\beta$-D-galactosidase. It is also possible to develop an enzymatic method for the determination of magnesium based on the activation of the enzyme by this ion.
When the enzyme is used in solution the presence of magnesium in commercial enzyme preparations produces a fast evolution of the blank signal; so the method exhibits a poor sensitivity in the determination of this analyte. The elimination of Mg(II) in the medium gives rise to the sharp loss of sensitivity, probably due to an irreversible enzyme denaturation.

In order to stabilize β-D-galactosidase in the absence of magnesium, different studies were performed. Only the immobilization of the enzyme on a suitable support as controlled-pore glass (CPG) allows the enzyme activity to remain constant.

The stabilization of the enzyme via immobilization is reported here. After proving this phenomenon, the immobilized biocatalyst has been used to develop a continuous unsegmented method for the determination of magnesium ion.

**Experimental**

**Apparatus**

An RA-1000™ discrete batch autoanalyzer (Technicon Instruments Co., Tarry-Town N.Y.) was used. A Phillips PU 8700 spectrophotometer furnished with a Hellma 178.12QS flow-cell (18 μL inner volume) and a Julabo-5 recirculating thermostat, a Gilson Minipuls-2 peristaltic pump provided with a rate selector, a “laboratory-made” double injection system consisting of two Rheodyne 3041 injection valves and Teflon tubing of 0.5 mm inner diameter were used to build the flow injection (FI) manifold. A Leo PC system equipped with a DAS-SPEGA interface (metabyte Co) was used as active interface and also for absorbance-time data acquisition, processing and delivery. Microsep™ microconcentrator 10K (Filitron Technology Co.) was used in the ultrafiltration process. Whenever necessary, the pH was measured by means of a Beckman 072 pH meter. A Perkin Elmer 380 atomic absorption spectrophotometer was also employed.

**Reagents**

β-D-galactopyranoside (grade VIII, from E. coli), o-nitrophenyl-β-D-galactopyranoside (ONPG), and Dl-dithiothreitol (d-DTT) were purchased from Sigma Chemical Co. St. Louis MO-63178. Sodium chloride, ethylene bis(oxyethylenemethylene)tetraacetic acid (EGTA, Trirplex VI), magnesium chloride, tris(hydroxymethyl)aminomethane (TRIS) and all other reagents were supplied by Merck Co. Darmstadt, Germany.

Reagent A was an aqueous solution containing 100 mmol·L⁻¹ TRIS, 4 mmol·L⁻¹ dl-DTT and 0.4 mmol·L⁻¹ EGTA; the pH was adjusted with 6 mol·L⁻¹ HCl. Reagent B was prepared by diluting 4.7 g of NaCl and 60.3 mg of ONPG in 11 of reagent A. Reagent C was a 1 N NaOH aqueous solution.

A standard solution of magnesium was prepared by dissolving 1 g·L⁻¹ of MgCl₂·6H₂O, Calgon calibration reference material (cat. no B5160-1) from Baxter Healthcare Co. (Miami, USA) was used. Serum samples from a routine clinical laboratory diluted to 1:100 with reagent A were also used.

All solutions were prepared in bidistilled water of high purity obtained from a Millipore Milli-Ro system.

**Ultrafiltration procedure.** The enzyme stock solution was dissolved by addition of 2 ml buffer phosphate 100 mmol·L⁻¹ pH 7.0 and was placed in a microsep™ and was centrifuged and refrigerated (4°C) at 4500 rpm for 2 h. Then, the final volume (0.2 ml) was diluted to 1.8 ml in the previous buffer, after which the procedure was repeated once more. The purified enzyme was diluted in buffer and was stored in the refrigerator at 4°C.

**Immobilization of β-D-galactosidase.** The enzyme was immobilized on controlled-pore glass (CPG 120-200 mesh, from Electromonolecotics, Fairfield, USA) by using the Masson and Townshend's procedure [10]. Glass tubing of different lengths and 0.5 mm i.d. were then packed with the support-enzyme conjugate and stored in buffer solution at 4°C. Under these conditions the enzyme activity remained constant at least for two months.

**Procedure**

a) **Kinetic manual method.** For the development of the kinetic method [11] 1 ml of the reagent solution (1500 U/L β-D-galactosidase, 200 mmol·L⁻¹ sodium chloride and 1 mmol·L⁻¹ ONPG diluted in Tris buffer solution) is added to a test tube containing 100 μL of magnesium standard solution or sample. The mixture is placed in the spectrophotometer at 37°C and the absorbance is then measured at 405 nm for 300 seconds.

b) **Automatic batch method.** The above-reported kinetic method was also adapted to a discrete batch autoanalyzer (RA-1000TM) which operates in the zero order kinetic mode. Four μL of magnesium standard solution and 350 μL of reagent-1 (1.14 mmol·L⁻¹ ONPG, 2.25 mmol·L⁻¹ sodium chloride diluted in Tris buffer) are dispensed into a vial. After incubation for 30 seconds, 45 μL of reagent-2 (14 KU/L β-D-galactosidase diluted in Tris buffer) are added and the enzymatic reaction starts. Reaction-rate measurements are made at 405 nm for 120 seconds.

c) **Unsegmented continuous method.** A method based on the flow injection (FI) technique [12] was also implemented, but using the enzyme immobilized on CPG. The configuration used is depicted in Fig. 1. In order to minimize the reagent consumption, a symmetrical merging-zones approach was used. The manifold consists of a peristaltic pump (P) which propels the reagent stream A through two channels. A dual injection valve (DIV) inserts simultaneously the sample and a solution containing reagent B into two reagent streams. A single bead string reactor (SBR) [13] favors the mixing of both injected plugs after the merging point and the hydrolysis reaction occurs on passage of the mixed plug through the immobilized enzyme reactor (IMER). At the second merging point (b) the plug is mixed with a basic stream (reagent C) to enhance the colour of the reaction product.

![Fig. 1. Flow injection merging-zones manifold with an immobilized enzyme reactor for determination of magnesium based on an activation effect on β-D-galactosidase.](image-url)
Additional advantages of the use of immobilized enzymes: immobilization of β-D-galactosidase for magnesium determination

Table 1. Optimization of variables.

<table>
<thead>
<tr>
<th>Type</th>
<th>Variable</th>
<th>Range studied</th>
<th>Conventional Method</th>
<th>Flow Injection Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical</td>
<td>Temperature, °C</td>
<td>20-45</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>Chemical</td>
<td>[TRIS], mmol-L⁻¹</td>
<td>50-500</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>6-9</td>
<td>7.5</td>
<td>7.5</td>
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<tr>
<td></td>
<td>[β-D-galactosidase], U·L⁻¹</td>
<td>500-3000</td>
<td>1500</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>[sodium chloride], mmol·L⁻¹</td>
<td>1-1000</td>
<td>200</td>
<td>800</td>
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<tr>
<td></td>
<td>[ONPG], mmol·L⁻¹</td>
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<tr>
<td></td>
<td>[EGTA], mmol·L⁻¹</td>
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<td>4</td>
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<tr>
<td></td>
<td>[NaOH], N</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Flow-rate, ml·min⁻¹</td>
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<td>-</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>Injected sample volume, µL</td>
<td>40-200</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Injected reagent volume, µL</td>
<td>40-200</td>
<td>-</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Length of SBSR, cm</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Length of IMER, cm</td>
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<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Length of reactor L₀, cm</td>
<td>50-200</td>
<td>-</td>
<td>100</td>
</tr>
</tbody>
</table>

Fig. 2. Influence of 8-hydroxyquinoline in the activity of the enzyme in solution via to complexation of the magnesium ion. For (1) = 0, (2) = 2.26, (3) = 2.82, (4) = 3.4 and (5) = 3.94 mmol-L⁻¹ 8-hydroxyquinoline, respectively.

Table 2. Features of the Kinetic methods.

<table>
<thead>
<tr>
<th>Features</th>
<th>Conventional method</th>
<th>Flow injection method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration graph</td>
<td>A=5·10⁻⁴ C+4.6·10⁻⁵</td>
<td>A=14.9·10⁻² C+0.47·10⁻³</td>
</tr>
<tr>
<td>Regression coefficient</td>
<td>0.991</td>
<td>0.997</td>
</tr>
<tr>
<td>Linear range (v)</td>
<td>3-12 (n=6)</td>
<td>5-20 (n=8)</td>
</tr>
<tr>
<td>Sampling frequency (h⁻¹)</td>
<td>12</td>
<td>40</td>
</tr>
</tbody>
</table>

In order to establish a method for the determination of magnesium based on enzyme activation, different kinetic studies with both the enzyme in solution and immobilized were performed.

Use of β-D-galactosidase in solution

First of all, the presence of magnesium in commercial preparations of β-D-galactosidase was checked by atomic absorption spectroscopy.

In a second experiment it was proved that the use of a selective complexing agent of magnesium in solution produced the deactivation of the enzyme. The kinetics of the reaction in the presence of different concentrations of 8-hydroxyquinoline were studied and the results in Fig. 2 show that increasing the 8-hydroxyquinoline concentration increased the deactivation of the enzyme by magnesium complexation.

The magnesium present in solution was removed by ultrafiltration at low temperature by following the procedure described under experimental. The purified β-D-galactosidase solution was used to develop a kinetic manual method.

The influence of the different variables involved in the system were systematically studied by the univariate...
method. Table 1 shows the variables, the ranges studied and the optimal values found.

The features of this manual method implemented using purified enzyme in solution are shown in Table 2.

The behavior of the purified dissolved enzyme was also checked in an automatic batch analyzer as the RA-1000™ is by using the procedure under experimental. The kinetic automatic method showed similar features to its manual counterpart, with a three times higher sensitivity expressed as slope of the calibration graph. Nevertheless, the drawback involved on the use of the dissolved enzyme remained: the sharp loss of activity within the two h after the purification process.

It was concluded that β-D-galactosidase in solution is not suitable for the implementation of a new enzymatic method for determination of magnesium in serum for two main reasons: first, because the original enzyme solution showed a low sensitivity for magnesium, and then due to the fact that the enzyme purified by ultrafiltration did not remain stable in solution for long periods.

Use of immobilized β-D-galactosidase

The immobilization of β-D-galactosidase gave the enzyme a special configuration with higher stability than in solution in absence of magnesium. This was an unexpected advantage of the immobilized enzyme in comparison with its use in solution. This feature enabled the development of an FI system based on immobilized β-D-galactosidase for the determination of magnesium in serum, which was implemented using the configuration in Fig. 1 and the procedure under experimental.

A study of the different variables influencing the system was performed after grouping them into physical, chemical and hydrodynamic. Table 1 shows the range over which each variable was studied and the optimum value found.

The calibration graph was run for different solutions of Cation cal serum reference, and the features of the linear range (between 5 and 20 μmol·L⁻¹) are summarized in Table 2.

The precision of the method was studied on 11 solutions prepared from Cation cal serum at two magnesium concentration levels (5.84 and 17.6 μmol·L⁻¹). The results obtained (2.9 and 0.7 % for within-run and 3.2 and 1.7 for between-run, respectively) were acceptable in all instances. The sampling frequency of the method depended on the flow-rate, the valve load and injection times and the reaction time. It was estimated in 40 h⁻¹. The study of potential interferences was focused on monovalent and divalent cations commonly present in serum. These interferences were added to the samples at higher concentration levels than usually found in real samples. The results showed that sodium and potassium are tolerated in a 800:1 (interferent/Mg) ratio, while ammonium and lithium cause no interference in a 160:1 ratio. There is no interference from calcium in a 20:1 ratio. Other species like Cu(II) and Zn(II) are not significant at a 1:100 dilution of serum samples, which was mandatory for the application of the proposed method.

The method was also applied to six samples of human serum from both healthy and sick individuals to validate it. The analyte concentration in each sample was previously determined by the conventional atomic absorption method. Samples were diluted 100-fold and subjected to two additions of standard (4.01 and 8.04 μmol·L⁻¹) to determine the recovery of magnesium. Table 3 lists the results obtained. The analyte recovery ranged between 96 and 113 %.

Final remarks

This paper shows the significant advantage gained by coupling the use of immobilized enzyme with the implementation of a continuous method as a result of two synergistic effects:

1. The enzyme immobilization permits the elimination of interferent species present in original, commercial or laboratory enzyme preparations. In the case here presented the elimination of magnesium occurring in the immobilization step enabled the use of β-D-galactosidase to propose a new enzymatic method for the determination of magnesium.
2. The immobilization process modifies the enzyme configuration contributing to increase the stability of the enzymatic system.

Acknowledgements

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References


Premio "Sociedad Española de Química Analítica" para investigadores novedos

Convocatorias 1993 y 1994

Por acuerdo de la Junta Directiva de la SEQA se convoca el Premio para Investigadores Novedos correspondiente a las convocatorias de 1993 y 1994.

Bases

1) El premio, indivisible y que podrá ser declarado desierto, consistirá en un diploma acreditativo y 75.000 Pesos. En caso excepcional podrá concederse un accesit hono-

2) Podrán participar los socios de la SEQA con un máximo de tres años de investigación, tras la lectura de su tesis doctoral. (A partir del 1 de Enero de 1990 para convocatoria 1993 y 1 de Enero de 1991 para convocatoria 1994).

3) Los socios participantes enviarán junto con una carta de solicitud un currículum vitae en el que, al menos, se incluirá una certificación acreditativa de la fecha de lectura de la Tesis Doctoral, así como las separatas de sus publicaciones, a:

Dr. E. Lorenzo, Secretaría de la SEQA
Dpto. Química Analítica. Facultad de Ciencias. Cantoblanco. 28049 MADRID

4) El plazo de presentación de solicitudes se abrirá el 15 de Octubre de 1994 y concluirá el 15 de Enero de 1995.

5) El jurado para la concesión de este premio estará constituido por cinco miembros y será nombrado por la Junta Directiva de la SEQA.

6) Entre los criterios a considerar por el jurado se tendrá en cuenta de manera especial, la calidad científica y originalidad de las publicaciones de los solicitantes.

7) Las decisiones del jurado se tomarán por votación nominal y secreta y serán inapelables.

8) Este premio se entregará en la reunión bianual de la SEQA que se celebrará en Madrid en el año 1995.

El Presidente de la SEQA La Secretaría de la SEQA
Spectrophotometric study on metallothionein and related molecules

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Abstract. A spectrophotometric study of Cd,ZnThioneins (MT) and of one molecule intrinsic to the metallothionein structure: Lys-Cys-Thr-Cys-Cys-Ala thionein fragment [56-61] MT I was performed.

The influence of different parameters, such as the medium, TRIS and phosphate buffers, the pH of solution and the addition of metal elements, cadmium and zinc was investigated. The apparent acid-base dissociation constant of the peptidic fragment [56-61] MT I was evaluated. The obtained value close to 9 corresponds to the dissociation of protons from the thiol groups. The peptidic fragment forms stable complexes with cadmium and zinc at pH higher than 6. It was assumed that the main complex of Cd-peptidic fragment possessed a stoichiometry 1:1, but probably two or more different complexes co-existed. The apparent acid-base dissociation constants of two MT, MT rabbit liver I + II and MT rabbit liver II were also estimated. The reversibility of the equilibrium MT ⇌ M + T was investigated in dependence of the pH from basic to acid solutions and vice versa.

The analytical characteristics of the quantitative determination of commercial MT of different origin, presenting different isoforms and with a big range of total contents in cadmium and zinc were established for spectrophotometric method. Absorbance as a function of the molar MT concentration was a single calibration curve for all metallothioneins. UV spectrophotometry is proposed as a determination method.

Key words: Spectrophotometry, Metallothioneins, Thionein Fragment, MTI.

Introduction

Metallothioneins (MT) are ubiquitous low molecular weight proteins. The structure and characteristics have been studied by a variety of biophysical and biochemical methods, including UV, ESR and NRM spectroscopy, X-ray diffraction, amino acid sequence and partial proteolysis [1-3].

The UV spectrophotometric characteristics of metallothioneins (MT) are composed of two parts, one of which corresponds to the complex polypeptidic molecule, the other to the metal bonded protein.

The following characteristics can be mentioned: due to the lack of aromatic amino acids there is no protein absorption around 280 nm. A large absorption band with a maximum at 190-200 is present in the UV region of amide absorption, corresponding to the transitions of two primary and 61 secondary amides and to the 20 cysteinyl residues [4-6]. Another characteristic is the presence of a shoulder assigned to charge-transfer transitions typical for the cation-thiol bonds [7-9]; this shoulder appears at a wavelength depending on the nature of the cation bound to the thionein, from λ = 230 nm for Zn-thionein to λ = 400 nm for Pb-thionein [4, 6, 10-21].

When one cation is replaced by another one, consequently a shift of the shoulder is observed which is due to the difference of cations bound to the thiol group and not to the phenomena of conformational modifications in the molecule [22].

At low pH upon removal of metals bound to the protein, the broad absorption shoulder disappears [4, 10, 11, 13, 14, 21, 23-27]. Furthermore, the molar absorptivity coefficient depends on the content of metal ion in the metallothionein [12].

The application of the spectrophotometry to study MT is very widespread and a great number of publications exist. These can be classified into two big groups according to
Spectrophotometric study on metallothionein and related molecules

The objective of using this technique: first, to determine and characterize MT and the metals associated [4-6, 10, 11, 14, 15, 20, 21, 24-34], and, second, to be used as a control or detection method during some different procedures. In the latter case spectrophotometry is coupled in general with one or more other techniques [12, 13, 17-19, 21, 23, 35-52]. Likewise the separation of the MT isoforms can be confirmed because the band structures of their spectra are rather different due to their different aminoacidic composition and metallic contents [14, 11, 27, 34]. Spectrophotometry is used as well to observe changes caused by the removed cations previously bound to the protein, either by addition of other metal ions, such as copper, cadmium, zinc, mercury and silver [10, 13, 20, 29, 34, 41] or by addition of complexing agents such as EDTA [47].

With the aim of comparing the spectrophotometric behaviour of metallothioneins with that of similar compounds, one less complex molecule being part of the proteinic structure of metallothioneins has been chosen, the peptidic fragment Lys-Cys-Thr-Cys-Cys-Ala [56-61] MT I (FT) containing three cysteinyl residues.

There are numerous publications on spectrophotometric characterization of cysteine, particularly concerning its complexing properties with several metal cations [53, 54, 55]. Likewise, UV spectrophotometry has been used in basic studies on several compounds containing thiol groups in their molecule, such as mercaptans, alkyl sulphides and polypeptides [54, 56, 57]. However, to our knowledge, the only investigation on peptidic fragment [56-61] MT I describes its metal-binding activity and detoxification effect, using spectrophotometric technique [58]. No systematic studies on FT are mentioned in the literature.

The purpose of this work is, on the one hand, the systematic study of the spectrophotometric behaviour of the FT [56-61] and Cd,ZnThioneins as a function of different parameters, such as the pH of solution, which is an important parameter for spectrophotometric response as well as for the MT stability.

On the other hand, the study of the absorbance as a function of the concentration of the different compounds chosen allows their quantitative determination in a given medium. This absorbance can be measured at the wavelength corresponding to the contribution from the organic part of the molecule and at the one corresponding to the thiol-metal bonds. To our knowledge, no publication dealing with the systematic determination of commercial Cd,ZnThionein exists studying, from an analytical point of view, the figures of merit of the spectrophotometry applied as a determination method.

Experimental

Instrumentation

UV/VIS Spectrophotometry. All spectrophotometric measurements were made with a Perkin Elmer Lambda 7 Model Spectrophotometer. The pH's were measured with a Tacussel Minisys 5000 model. A glovebox with a nitrogen atmosphere was used in several cases in order to avoid interferences of oxygen in the UV region under investigation (O₂ < 0.1%). Control of atmosphere was achieved with an oxygen analyser Abiss Hotgas "D" model.


Differential Pulse Polarography (DPP). EG&G Princeton Applied Research Polarographic/Voltametric Analyser model 384B, EG&G model 303 A Electrode housing with a medium size mercury drop as the working electrode, a platinum wire as the counter electrode and a silver/silver chloride reference electrode. Houston Plotter DMP-40.

Barnstead Nanopure II water deionisation system in conjunction with a Jencons double distillation still.

Chemicals

Cd,ZnThioneins Sigma (MT); MT I + II horse kidney M 4766 lot 79 F 9670, MT I + II horse kidney M 4766 lot 28 F 9545, MT I + II rabbit liver M 7641 lot 20 H 9560, MT I rabbit liver M 5267 lot 129 H 9575, MT II rabbit liver M 5392 lot 79 H 9510, Lys-Cys-Thr-Cys-Cys-Ala Peptidic Fragment [56-61] MT I, acetate salt, synthesized from mouse liver, containing mercaptoethanol as stabilizer (87% of purity) L 4512 lot 39 F 5810 (Sigma). (For calculation of concentrations only the peptide content was taken into account.) TRIS (hydroxymethyl) amino methane - Merck, Na₂HPO₄ - Merck, Triolol standard solutions Merck 1000 ± 2 μg ml⁻¹ CuCl₂ and ZnCl₂, H₃PO₄/Mg(NO₃)₂, as matrix modifier for cadmium analysis in ETAAS.

Analytical procedure

ETAAS. The instrumental parameters were optimised. Linearity range, detection limit and characteristic concentration were established. Standard solutions of Cd²⁺ and Zn²⁺ between 0.2 and 10 μg ml⁻¹ were prepared for the calibration curve. Determinations of Cd and Zn concentration were carried out by direct standard calibration and by the method of standard additions.

DPP. The instrumental parameters were first optimised and then the analytical characteristics of the method were established. Standard solutions of Cd²⁺ and Zn²⁺, from 1 to 5 μg ml⁻¹ were prepared in TRIS-HCl 2.5·10⁻³ M pH = 2. For sample analysis the method of standard additions was used.

UV Spectrometry. Difference absorption spectra were recorded versus a reference solution prepared in two buffer media: TRIS-HCl (pH 1.38 to 11.8) and phosphate (pH 1.5 to 12).

Results and discussion

Determination of total metal concentrations in Cd, Zn Thioneins

ETAAS and DPP were applied to determine the metal concentrations in the only commercial metallothioneins available. The values obtained (Table 1) using two independent methods are in good agreement. At pH = 2 the concentrations of cations determined by DPP are very similar to the total amount found by AAS indicating that in acid solutions cadmium and zinc are completely dissociated from the MT.
Table 1. Determination of Cadmium and Zinc concentrations in Sigma Metallothioneins.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration mg/g</th>
<th>Cd ETAAS</th>
<th>Cd DPP</th>
<th>SIGMA indicative value</th>
<th>Cd ETAAS</th>
<th>Cd DPP</th>
<th>SIGMA indicative value</th>
<th>Zn ETAAS</th>
<th>Zn DPP</th>
<th>SIGMA indicative value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT Horse kidney</td>
<td>45.10</td>
<td>44.64</td>
<td>±1.46</td>
<td>16.78</td>
<td>±0.43</td>
<td>±0.43</td>
<td>16.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 4766 Lot 79 F 9670</td>
<td>±1.46</td>
<td>±1.71</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MT Horse kidney</td>
<td>35.96</td>
<td>36.79</td>
<td>±0.70</td>
<td>8.80</td>
<td>±0.23</td>
<td>±0.41</td>
<td>9.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 4766 Lot 28 F 9545</td>
<td>±0.70</td>
<td>±0.22</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>MT Rabbit liver</td>
<td>83.22</td>
<td>87.39</td>
<td>±1.50</td>
<td>13.25</td>
<td>±0.38</td>
<td>±0.15</td>
<td>11.2</td>
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<tr>
<td>M 7641 Lot 20 H 9650</td>
<td>±1.50</td>
<td>±0.97</td>
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<td></td>
</tr>
<tr>
<td>MT I Rabbit liver</td>
<td>64.32</td>
<td>68.6</td>
<td>±1.40</td>
<td>11.07</td>
<td>±0.43</td>
<td>±0.43</td>
<td>11.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 5267 Lot 129 F 9575</td>
<td>±1.40</td>
<td>±1.71</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MT II Rabbit liver</td>
<td>72.05</td>
<td>68.67</td>
<td>±1.42</td>
<td>10.02</td>
<td>±0.28</td>
<td>±0.45</td>
<td>9.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M 5392 Lot 79 F 9510</td>
<td>±1.42</td>
<td>±0.35</td>
<td></td>
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</tr>
</tbody>
</table>

Fig. 1. Absorption spectra of the peptidic fragment [56-61] MT I, c = 2.65 × 10⁻² M in several media
1) Phosphate 4×10⁻³ M, pH 7.4
2) TRIS 2.5×10⁻² M, pH 7.5
3) TRIS 5×10⁻² M, pH 7.5

The big range of metal content in the MT studied should be noticed, e.g. MT I + II horse kidney, lot 28 F 9545 and MT I + II rabbit liver lot 20 H 9650, 44.76 and 96.97 mg (Cd and Zn)/g (MT), respectively. This fact is difficult to explain assuming that metallothioneins synthesized in vivo have all their thiol groups saturated, as mentioned in most of the publications.

Spectrophotometric study

Influence of the medium. TRIS is an organic molecule which can exhibit absorption in the UV range studied. To verify the influence of this compound, absorption measurements at four different concentrations of TRIS lying between 5×10⁻³ and 5×10⁻² M were carried out from 190 to 250 nm. TRIS exhibits an absorption band between 190 and 210 nm. The intensity of absorption at the maximum does not depend linearly on the concentration, but the band structure of the spectrum changes: the absorption band is broader and the maximum shifts to larger wavelengths with increasing TRIS concentration.

An experiment was performed to verify the interactions between metal cations as Cd and/or Zn and TRIS. The λ_max is similar for both cations but is dependent on the TRIS concentration. The absorption for a given cation is similar in the two studied media. The influence of TRIS is negligible.

Peptidic Fragment [56-61] of MT I.

Influence of the medium. The absorption spectra of FT are shown in Figure 1, in phosphate and TRIS solutions. The spectra are similar in all media investigated with a peak lying between 200 and 208 nm representative for the absorption of amide and thiol groups. In this case the shoulder is not observed at a wavelength higher than λ_max.

However, the intensity at the maximum depends on the
Table 2. Acid-base constant of the peptic fragment [56-61] MT I.

<table>
<thead>
<tr>
<th>Media</th>
<th>pK_a</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRIS (λ = 240 nm)</td>
<td>8.92</td>
</tr>
<tr>
<td>TRIS (λ = 220 nm)</td>
<td>8.87</td>
</tr>
<tr>
<td>Phosphate (λ = 250 nm)</td>
<td>9.07</td>
</tr>
<tr>
<td>Phosphate (λ = 240 nm)</td>
<td>9.40</td>
</tr>
<tr>
<td>Phosphate (λ = 220 nm)</td>
<td>9.49</td>
</tr>
</tbody>
</table>

medium; for the same concentration of FT the signal is lower in phosphate than in TRIS and for the latter medium the signal is higher for the lower concentration of TRIS in the solution.

**Influence of the pH.** The peptic fragment contains several acid-basic groups, particularly the amino and thiol groups (pK = 9) and the carboxylic group (pK = 4). In consequence its chemical form depends on the pH.

The study on change of spectral band structure with the pH were carried out in phosphate 5·10^{-2} M and in TRIS HCl 2.5·10^{-2} M and 5·10^{-2} M. Most of them were performed in a nitrogen atmosphere. The change of spectra is similar in all studied cases. As an example Figure 2 shows spectra in phosphate solutions at several significant pH values. The most important modifications are observed in the region between 190 and 240 nm. The maximum, initially at 195-200 nm (pH 1.5) shifts to higher wavelengths (210-220 nm) with the pH increasing from 1.5 to 12; simultaneously the absorption intensity decreases. This bathochromic shift and decreasing of intensity are attributed to the deprotonation of thiol groups. Additionally, the appearance of a shoulder at approximately λ = 230 nm is observed. The absorbance at this wavelength attributed to the thiolate groups increases from pH = 7 to pH = 12.

Plots of absorbance, measured at several wavelengths in different media versus the solution pH allow to establish the acid-base dissociation constants. The obtained value close to 9 in all media corresponds to the dissociation of the protons from the thiol groups. The interpretation of the ensemble of results, (A = f (pH)), using the MiniSpect program, leads to the values of apparent acid-base constants given in Table 2.

The pK value of the peptic fragment [56-61] MT I is slightly greater in phosphate solution than in TRIS. All values are similar and close to those determined using different methods for other organic compounds possessing analogous characteristics and containing thiol groups in their structure [53, 54].

**Addition of the metal elements.** The peptic structure contains three thiol groups and two amino groups; consequently, this molecule can have complexing properties with respect to metal ions.

**Addition of Cadmium.** The addition of cadmium was carried out in the three media mentioned above. The change of the spectral band structure with increasing cadmium concentration is similar in all cases. After the first addition of Cd, at pH higher than 4, a broad shoulder extended from 235 nm to 280 nm appears. It is attributed to the S-Cd bonds. Different absorption spectra were recorded versus a reference solution, now containing the same concentration of peptic fragment but no cadmium. In this case the absorption band is representative only of the Cd-fragment complexes. The intensity at the absorption maximum remains unchanged from ratios Cd/FT greater than a certain value.

The complex stability was studied as a function of the pH of the solution with a constant ratio of concentration of cadmium and fragment, with pH values ranging from 1.5 to 12. Plots of absorbance versus the pH at different wavelengths are given in Figure 3. The absorbance at the wavelength corresponding to the shoulder due to the S-Cd bonds appears at pH > 4, increases between 4 < pH < 8 and remains constant at pH > 9. Therefore, the complex cadmium-fragment is stable in basic solution.

The continuous variations and the molar-ratio methods were used to determine the stoichiometry of the Cd-FT complex at pH = 7.5. From these results it can be assumed that the main complex is Cd:FT 1:1, but probably other species exist as well. Furthermore, it is suspected that this 1:1 complex does not necessarily consist of Cd:FT only, but instead of two or more different complexes, the constituents of which sum up to an overall 1:1 Cd:FT ratio. This hypothesis was confirmed using electrochemical methods [59].

**Addition of Zinc.** Only a few experiments were performed on the interaction of zinc with the peptic fragment in
TRIS $2.5 \times 10^{-3}$ M and $5 \times 10^{-3}$ M. The form of the spectra is similar to that previously described in the case of Cd complexes. There is a shoulder at approximately $\lambda = 250$ nm attributed to the Zn-S bonds.

The variation of the absorbance with the amount of zinc was measured. A constant value is obtained from a ratio $C_{Zn}/C_{PT}$ greater than that corresponding to the Cd-Fragment. This is probably due to the participation of amino groups in the coordination of zinc. This interaction stabilizes the complexation of Zn with SH-containing peptides [60].

Cd/ZnThioineins

**Influence of the medium.** Two different media were chosen, phosphate and TRIS, the latter at two different concentrations, both at pH = 7.0. The band structure of spectra is similar, with a well defined peak approximately at 200 nm and a broad shoulder between 230-250 nm, as already described in several publications dealing with other metallothioneins of different origin. The maximum shifts to higher wavelength with increasing TRIS concentration and with changing from TRIS to phosphate buffer. Additionally, the maximum intensity is raised in TRIS media with respect to the phosphate, in fact $A_{TRIS} = 2 A_{Phos}$ for comparable MT concentration. This exaltation of the response is similar to that found in the case of the peptic fragment [56-61] MT I under the same experimental conditions.

**Stability of MT.** The metallothionein is a protein, therefore the question arose if it was stable with time. A consequence of instability would be the loss of the quaternary structure and consequently the removal of cations bound to the protein. In this case free thiol groups would exist and possibly form disulfur bridges. To check the stability of MT the spectrum as a function of time was recorded. Solutions were kept at 4°C between measurements. The absorbance was measured at three characteristic wavelengths as a function of time. It remains unchanged at the wavelength corresponding to the peak attributed to the organic molecule as well as at those corresponding to the shoulder attributed to the cation-thiol bonds.

**Influence of the pH.** This study was performed using two different kinds of metallothioneins, MT rabbit liver I and II containing two different isoforms and MT r.I, containing only isoform II, in three different media: phosphate $5 \times 10^{-2}$ M, TRIS $2.5 \times 10^{-2}$ M and TRIS $5 \times 10^{-2}$ M. The pH values ranged between 1.3 and 12. The general features of spectra are similar for both MT in the different media studied. The absorption maximum shifts to higher wavelengths with increasing pH. The maximal shift is ~ 20 nm from pH = 1.5 to pH = 12. The intensity at the peak is lower in basic pH solutions than in acid solutions; for instance, $A_{酸} (pH = 1.70) = 5A_{酸} (pH = 12)$ in phosphate. The shoulder attributed to the metal-thiol bands is abolished in acid solutions, as expected. In basic solutions the shoulder remains constant, hence, at pH > 6 the cations are bound to the organic molecule in a stable manner.

The major difference between the band structure of MT and those of cysteine and the peptide fragment [56-61] MT I is that the MT spectra remained unchanged for a large ratio of pH whereas the spectra of the cysteine and the peptic fragment are very different in acid and in basic solutions. This seems to indicate that in the case of MT there is no ionisation of the HS group in S-bcause the thiol groups are always saturated by protons at acid pH or by metal ions ($\text{Cd}^{2+}$ and $\text{Zn}^{2+}$) at basic pH.

Plots of absorbance measured at different wavelengths in different media versus the solution pH (Figure 4) allow to estimate the acid-base dissociation constants of two MT studied. The value of pKa for both compounds lying between 3.5 and 4 is in good agreement with a value published earlier (pKa = 4) for an equine thioneine [25] and with our own results obtained using electrochemical methods [59]. This value of the acid-base dissociation constant can be attributed to the deprotonation of carboxylic groups of the organic molecule.

**Reversibility of the equilibrium $M + T \Leftrightarrow MT$ in dependence of the pH.**

It is known that MT exhibits a spatial structure suitable to bind metallic ions. At acid pH the quaternary structure is modified and cations are removed. The purpose of the experiment described below was to know if the system $M \Leftrightarrow M+T$ is reversible, i.e. if the modification from acid to basic solutions reveals a spectrophotometric response similar to that obtained for a solution freshly prepared in a basic medium.

Several spectra sequentially obtained with the same sample to which small volumes of HCl were added to modify the pH but not the MT concentration are shown in Figure 5(a). The same evolution of the band structure as that previously described is observed; the peak shifts to lower wavelengths and its intensity increases with the decreasing pH; at pH < 6 the wavelength corresponding to the maximum remains unchanged and the intensity decreases in more acid solutions. The shoulder is abolished at pH < 4.
Table 3. Molar absorptivity coefficients for MT.

<table>
<thead>
<tr>
<th>Sample</th>
<th>(\epsilon_{\text{325}}(\text{MT}))</th>
<th>(\epsilon_{\text{325}}(\text{Cd+Zn}))</th>
<th>(\epsilon_{\text{325}}(\text{Cd+Zn}))</th>
<th>(\epsilon_{\text{325}}(\text{Zn}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT h.k.</td>
<td>3.38 \times 10^4</td>
<td>7.91 \times 10^4</td>
<td>1.33 \times 10^4</td>
<td>1.05 \times 10^4</td>
</tr>
<tr>
<td>79 F 9670</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MT h.k.</td>
<td>3.22 \times 10^4</td>
<td>1.09 \times 10^4</td>
<td>1.63 \times 10^4</td>
<td>1.18 \times 10^4</td>
</tr>
<tr>
<td>28 F 9545</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MT r.l.</td>
<td>2.90 \times 10^4</td>
<td>4.69 \times 10^4</td>
<td>1.13 \times 10^4</td>
<td>0.83 \times 10^4</td>
</tr>
<tr>
<td>201 F 9650</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MT r.l.</td>
<td>3.18 \times 10^4</td>
<td>6.60 \times 10^4</td>
<td>1.29 \times 10^4</td>
<td>1.02 \times 10^4</td>
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<tr>
<td>129 F 9575</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MT r.l.</td>
<td>3.37 \times 10^4</td>
<td>6.45 \times 10^4</td>
<td>1.15 \times 10^4</td>
<td>1.19 \times 10^4</td>
</tr>
<tr>
<td>79 F 9150</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(MT) and (Cd+Zn) indicate the molar concentration of MT and of total metal, respectively.

From these results it is assumed that the equilibrium MT ⇨ M+T is reversible and consequently the protein in acid solution is still able to reconstruct its structure and in basic solution to complex metal ions through its thiol groups. However, it is also likely that the structure is not the same as that synthesized in vivo. The equilibrium between cadmium and zinc cations and apothionine which apparently is quickly reached gives rise to a Cd,Zn Thionine with a spatial structure different to that synthesized in vivo. Cadmium forms at least two different complexes with the thionein possessing different nature and stability inferred from electrochemical methods [59]. It is probable that zinc exhibits a similar behaviour but no data are available up to now. Consequently, the reconstructed MT could possess different structure and properties and therefore form different kinds of Cd and Zn complexes when the pH changes from acid to basic solutions.

Absorbance as a function of concentration

Choice of experimental conditions. In these preliminary experiments two kinds of metalllothioneins of the same origin but containing different isoforms, MT rabbit liver I and II and MT r.l. II were used. The influence of buffer (phosphate or TRIS, the latter at two different concentrations) was checked. Furthermore, the pH values of solutions were 7.5 or 9. From these results the TRIS 2.5 \times 10^{-2} at pH 7.5 was selected as the suitable medium for MT absorbance measurements.

Metalllothioneins. The range of concentrations of the five metalllothioneins was 1.76 \times 10^{-4} M to 7.0 \times 10^{-3} M calculated assuming a molecular weight of 6500 Da. Firstly, it should be pointed out that the absorption bands of the five MT of different origin and containing different isoforms are similar and analogous to those previously described. Secondly, in all cases linear relationships are observed between absorbance at different wavelengths and concentration. Plots of absorbance as a function of concentration, expressed in different manners (molar concentration of MT, total metal concentration and
Table 4. Molar absorptivity coefficients for the peptidic fragment under different conditions.

<table>
<thead>
<tr>
<th>medium TRIS</th>
<th>concentration range (x10^7)</th>
<th>ε_{max}</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0x10^{-7}M absence of O_2</td>
<td>1.85 - 7.33</td>
<td>1.38x10^4</td>
</tr>
<tr>
<td>2.5x10^{-7}M absence of O_2</td>
<td>1.80 - 6.80</td>
<td>2.24x10^4</td>
</tr>
<tr>
<td>2.5x10^{-7}M presence of O_2</td>
<td>1.79 - 8.82</td>
<td>2.28x10^4</td>
</tr>
</tbody>
</table>

The value of absorptivity coefficient, 3.22x10^4, is in good agreement with the previous published data for other metallothioneins as well as with the value estimated for adding the different contributions of organic functional groups combined in the molecule [4].

Plots of the absorbance measured at the same wavelength, 204 nm, as a function of the total metal concentration (C_{Cd-Zn}) lead to a sheaf of curves, each corresponding to one MT with values of slopes lying between 4.69x10^4 and 10.9x10^4. The sequence of these values Ε(MT hk 28F > MT hk 79F > MT rI. I > MT rI. II > MT rI. I + II) is in the reverse order as the total content of cadmium and zinc in the metallothioneins C_{Cd-Zn}(MT rI. I + II > MT rI. II > MT rI. I > MT hk 79F > MT hk 28F). This fact can be explained assuming that with lower metal content in MT the number of free thiol groups in the molecule increases contributing to an increase of absorption intensity at this wavelength. Moreover this fact implies that, in different metallothioneins, not all thiol groups are saturated by the cations.

Absorbances measured at the shoulder of the spectra, 250 nm are graphically presented in Figure 8 as a function of the metal concentration. Contrary to the case previously described, a single straight line is not obtained as could be expected. Slopes of different curves are similar, ranging from 1.13x10^4 to 1.63x10^4, but not identical. It is probably that this difference is due to the total absorption corresponding to the sum of the contributions of Cd-thiol and Zn-thiol absorptions. Furthermore, the ratios of cadmium and zinc concentrations in the five metallothioneins are very different (C_{Cd-Zn} are ranged from 1.6 to 4.3). Spectrophotometry does not allow to discriminate between both cations. However, a direct dependence between slopes of curves and the total concentration is not found.

Peptidic fragment [56-61] MT I (FT). In this case, several measurements were carried out in a glove box with a nitrogen atmosphere (< 0.1 %) to avoid the possible interference of oxygen in the wavelength region explored (190 - 260 nm). The range of concentrations, the composition of different media and the experimental conditions concerning either the presence or the absence of oxygen are presented in Table 4, as well as the molar absorptivity coefficients found. The interference of oxygen is negligible.
Table 5. Analytical characteristics of spectrophotometric method.

<table>
<thead>
<tr>
<th>compound</th>
<th>range of concentration</th>
<th>equation line</th>
<th>variation coefficient (%)</th>
<th>detection limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>peptidic fragment</td>
<td>2.32·10⁻⁴-8.53·10⁻⁴ M</td>
<td>( y=1.38·10^{10}x +1.99·10^{-2} )</td>
<td>18.0</td>
<td>1.9·10⁻⁵</td>
</tr>
<tr>
<td>metallothioneins</td>
<td>3.5·10⁻¹-1.72·10⁻⁴ M</td>
<td>( y=3.22·10^{-9}x +6.6·10^{-4} )</td>
<td>7.6</td>
<td>3.0·10⁻⁷</td>
</tr>
</tbody>
</table>

The most important analytical characteristics, such as linearity range, detection limit and variation coefficient were established for spectrophotometric method applied to the different compounds: peptidic fragments and Cd,ZnThioneins from horse kidney and rabbit liver (Table 5). Medium was TRIS 2.5·10⁻² pH 7.5. Concerning MT the equation of the straight line corresponds to the single calibration curve presented in Figure 7. In all cases the detection limit, expressed as a concentration, was estimated taking three times the standard deviation obtained for the intercept of the calibration curve.

Conclusions

Similarities as well as differences have been found between the spectrophotometric characteristics of MT and of the peptidic fragment [56-61] (FT) MT I.

In the presence of metal ions, Cd and/or Zn, in basic solutions FT exhibits spectra similar to the MT; complexing properties of FT seem to be similar to those of MT. The spectrophotometric behaviour of FT in the absence of cations and of MT as a function of solution pH is different. In the former case the acid-base dissociation constant corresponds to the deprotonation of thiol groups contained in the FT structure. In the latter case, MT with thiol groups saturated by metal ions the acid-base dissociation is attributed to the protons of carboxylic group.

The 'reconstruction' of MT from acid to basic solutions leads to a compound not exhibiting identical spectrophotometric properties in the UV region where the absorption of the organic part of the molecule occurs, neither in the wavelength range corresponding to the absorption of the metal-thiol bonds. It is possible that when the pH varies from acid to basic the CdZnT obtained possesses properties and characteristics which are different to their native form.

The absorbance as a function of concentration for all compounds is linear in the range of concentration investigated. Values of molar absorptivity coefficients depend on the nature of the molecule. At the wavelength corresponding to the peak maximum, \( \varepsilon_{\text{max}}(\text{MT}) > \varepsilon_{\text{max}}(\text{FT}) \), as expected.

The absorbance as a function of concentration for all compounds is linear in the range of concentration investigated. Values of molar absorptivity coefficients depend on the nature of the molecule. At the wavelength corresponding to the peak maximum, \( \varepsilon_{\text{max}}(\text{MT}) > \varepsilon_{\text{max}}(\text{FT}) \), as expected.

A more general conclusions, but results obtained are oriented to confirm this hypothesis. However, plots of absorbance measured at the wavelength corresponding to the shoulder as a function of metal concentration do not lead to a single calibration curve.

UV spectrophotometry is a simple and easily accessible method used in order to get rapid information on the characteristics of analysed compounds and to know whether or not the metal ions are complexed by the thiol groups.

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We would like to thank Dr. B. Ribas (Instituto de Salud Carlos III, Madrid, Spain) for his initiation of and interest in the metallothionein subject. The authors are also grateful to the Analytical Chemistry Department (Universidad Autónoma de Barcelona) for determining acid-base constants using Minispef program. This investigation was supported by a grant from the European Communities.

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36. J.H.R. Kägi, S.R. Himmelhoch, P.D. Whanger, J.L. Bethune, and
Separation of selenite and selenate anions by free solution capillary electrophoresis

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Abstract. The behaviour of Se(IV) and Se(VI) on a capillary zone electrophoresis system with on column UV detection (190 nm) was studied. The influence of concentration and pH of buffer carrier on mobility of selenite and selenate was determined and both ions were separated in less than 20 minutes using a fused silica capillary (50 μm i.d., 72 cm long) and 0.015 M NaH₂PO₄ (pH=2.55) buffer as carrier. A linear relationship between peak area and concentration was observed for both ions, the low mass detection limits were 150 pg for Se(VI) and 15 pg for Se(IV).

Introduction

It is now recognized that selenium is an essential trace nutrient, and it is present, for example, in glutathione-peroxidase, an enzyme important in the metabolism of hydroperoxides [1], and its toxic and indispensable doses are very close [2].

Methods for determination of selenium include UV spectrophotometry, fluorimetry, atomic absorption spectroscopy (AAS), inductively coupled plasma emission (ICP) and gas chromatography (GC) [3]. In these methods a previous reduction of Se(VI) to Se(IV) is necessary in order to apply the procedure, i.e. AAS and ICP involve the hydride generation, colorimetric and fluorimetric methods the formation of piazelenol or selenotrisulfide, reactions that only take place with Se(IV).

On the other hand several analytical methods have been reported for differentiation and quantification of both species [4, 5]. Se(IV) and Se(VI) can be separated by coprecipitation, chemical treatment or liquid chromatography followed by detection using AAS, ICP, spectrofluorimetry or X-ray fluorescence.

An alternative approach can be free solution capillary electrophoresis (FSCE). This technique was firstly introduced by Mikkers et al. [6] and by Jorgenson and Lukacs [7, 8] and extensive reviews have been published in different journals [9-13]. Capillary electrophoresis is a powerful separation technique in which the analyte is introduced into a capillary tube and subjected to electrokinetic separation. This separation is based on differences in the electrophoretic mobilities of species and the formation of a electrosomotic flow. These parameters depend on solute characteristics (size, shape and charge) and are also affected by the properties of the carrier electrolyte, such as pH, ionic strength, viscosity and dielectric constant. Many ion such as iodate and periodate [14], metal ions [15, 16], hexacyanoferate(II) and (III) ions [17], bromide, bromate, iodide iodate, nitrite, nitrate and selenite ions [18] can be separated by free solution capillary electrophoresis (FSCE). Moreover, the separation of organic and inorganic selenium compounds has been recently reported [19].

In this paper we study the separation of selenite and selenate by FSCE. The influence of buffer (pH and concentration) on mobility was determined and both selenium ions separated in less than 20 minutes using a cathodic injection-anodic detection scheme.

Experimental

Instrumentation. An Applied Biosystems (Foster City, CA, USA) Model 270A capillary electrophoresis system was used for this work. A 72 cm
long x 50 μm I.d. silica capillary filled with phosphate buffer served as the separation tube operated at high voltage (10-30 kV). A small section of the polyimide coating of the capillary was removed, prior to filling, to get an optical window for UV detection. The on-column detection was performed at 190 nm at an absorbance range of 0.01 APU and a rise time of 1 s. The capillary was thermostated at 40 °C to avoid changes in mobility due to temperature variations. Samples were injected by the vacuum technique. Between analysis the capillary was washed with 0.1 M NaOH solution for 5 minutes and flushed with the buffer solution for 15 minutes. All electrophoregrams were recorded using a Spectra-physics (San José, CA, USA) Chromatix Model SP4400 integrator.

Reagents. Stock solutions of selenium compounds were prepared of 1000 mg.L⁻¹ Se as follows:

Selenite, Na₂SeO₃·5H₂O (Merck, purity 99%) dissolved in water; selenate, Na₂SeO₄·10H₂O (Aldrich, purity 99%) dissolved in water. These reagents were used without further purification.

Bidistilled and filtered (<0.2 μm) water was used for dilutions or as buffer solutions.

Carrier buffer solutions were prepared from a stock solution of NaH₂PO₄ (25 mM) and adjusted to the desired pH with H₃PO₄.

Results and discussion

Electrophoretic separations are strongly dependent on analytes electric charge. For a diprotic compound, H₂A, as selenite and selenate are, the apparent charge, Q_APP, can be calculated by the following equation:

$$Q_{APP} = \frac{2[A^2] + [HA^-]}{[A^2] + [HA^-] + [H_2A]}$$  \hspace{1cm} (1)

If K₁ and K₂ are the dissociation constants of H₂A, equation 1 can be rearranged to give:

$$Q_{APP} = \frac{2K_1K_2 + K_1[H^+]}{K_1K_2 + [H^+] + [H^+]^2}$$ \hspace{1cm} (2)

So the degree of ionization of selenite and selenate depends on their pKᵣ values and on the pH of the carrier electrolyte. In Figure 1 the apparent charge of both ions as a function of pH is plotted. We can observe that below pH=6 the charge difference between both ions became important, so we decided to study the influence of pH on separation below pH=6.

First experiments were carried out at pH=5.59 with a 25 mM phosphate buffer as carrier, and a applied voltage of +30 kV (cathode: detection / anode: injection). Only one signal peak was obtained, later identified as selenite. A change of polarity (-30 kV; cathode: injection / anode: detection) yielded again one signal peak, but this time was identified as selenate. So in this conditions Se(IV) migrates to the cathode and Se(VI) to the anode. This different behaviour is due to electroosmotic flow.

The ion mobility in a free solution capillary electrophoretic system depends on two components [10, 18], one electrostatic and other one electroosmotic. So for an anion the total, or apparent, mobility, μ_APP, can be expressed as a difference between electroosmotic, μ_ESM, and electrophoretic, μ_APP, mobilities. The electroosmotic mobility is due to a double layer formed at capillary wall by ionization of silanols groups, [10, 18] and depends on the charge density by surface unit [10], so the μ_ESM depends on the degree of ionization of silanol groups. For this reason the pH change modifies the ion mobility in a free solution capillary electrophoretic system.

Apparent mobility can be experimentally calculated from the following equation [8, 12]:

$$\mu_{APP} = \frac{L_{L}}{t_{mV}}$$ \hspace{1cm} (3)

where Lₘ is the length of the capillary from the inlet to the detection window (in cm), Lₗ is the capillary total length (in cm), tₘ is the measured migration time (in seconds) and V is the applied voltage (in Volts). In Table 1 the apparent mobility, for two different applied voltages (-30 and -20 kV), as a function of carrier buffer pH is given. We can observe that Se(VI) apparent mobility increases at low pH values to reach a maximum at pH values below 3. For Se(IV) the maximum mobility is

\begin{table}[h]
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
pH & \multicolumn{3}{c|}{μ_APP (x10⁻⁵ cm²/Vs)} & \multicolumn{3}{c|}{μ_APP (x10⁻⁵ cm²/Vs)} \\
\hline
 & Se(IV) & Se(VI) & Se(IV) & Se(VI) & Se(IV) & Se(VI) \\
\hline
-20 kV & -30 kV & μ_APP & -20 kV & -30 kV & μ_APP & -20 kV & -30 kV & μ_APP \\
\hline
4.60 & 0.72 & -0.62 & 3.54 & 2.94 & 3.20 & 3.54 & 2.94 & 3.20 \\
4.03 & 2.04 & 1.99 & 2.03 & 5.00 & 4.88 & 4.94 & 5.00 & 4.88 & 4.94 \\
3.05 & 2.78 & 2.79 & 2.79 & 6.67 & 6.97 & 6.82 & 6.67 & 6.97 & 6.82 \\
2.55 & 2.44 & 2.44 & 2.44 & 6.52 & 6.97 & 6.75 & 6.52 & 6.97 & 6.75 \\
2.02 & - & - & - & 6.79 & 6.79 & -6.79 & 6.79 & -6.79 & -6.79 \\
\hline
\end{tabular}
\end{table}

\textsuperscript{a}: Mean of apparent mobility at both voltages.

\textsuperscript{b}: No peak observed after 45 minutes of sample running.

\textsuperscript{c}: Not performed.
reached between pH values from 3 to 3.5. At pH=4.5 (-30 kV) and pH=2.5 (both voltages) no peak is obtained after 45 minutes of sample running. At pH=2.5 the apparent charge of selenite is near to 0.5 (see Figure 1), so the mobility is poor and the migration time increases over 45 minutes. At pH near 4.5 the electroosmotic mobility of selenite must have a similar value than its electrophoretic mobility, because a change of migration sense is observed with the change of voltage applied, that increase the value of electroosmotic flow. For further experiments a pH between 2.5 and 3.0 was selected, because it gives the greater mobility values, with short analysis time, in a good separation conditions.

In Figure 2 the influence of buffer concentration on mobility is shown. The plotted results are the mean of apparent mobilities at -20 and -30 kV. We can observe that for both selenium ions their apparent mobilities are constant in the concentration range from 20 to 10 mM. Figure 3 shows the electropherograms obtained at -20 kV when the buffer concentration changes. A loss of efficacy (wider peaks) is observed for lower concentrations. On the other hand, greater signal was obtained for Se(VI) at buffer concentrations of 20 and 15 mM. For further experiments a 15 mM buffer concentration was selected because little changes in buffer concentration have little influence on mobilities and signal to background ratio is good.

From this studies a separation voltage of -20 kV was selected as working voltage, because the signal to background ratio is lower than at -30 kV. However, at the higher voltage the time of analysis is shorter and the efficacy greater (sharper peaks).

Finally the peak area relationship with the volume of sample injected was studied. Hydrodynamic (vacuum) and electrokinetic injection systems were tested. Electrokinetic injection gives very poor peaks for selenite and no peaks for selenite. This is probably due to the low injection potential (±5 kV), fixed by the instrument and the working conditions, that yield a low electroosmotic flow. So for all the work hydrodynamic injection was used.

In Table 2 the peak area injected amount ratio is given as a function of the injected amount or volume. The sample used was a solution containing 100 mg.L⁻¹ of Se (IV) and 10 mg.L⁻¹ of Se(VI). The peak area measured are the mean of two injections, and the mean and the standard relative deviation (RSD) of peak area-injected amount ratio is also
Figure 5. Separation of selenate (a) and selenite (b) with the optimized conditions. Capillary: 72 cm x 50 μm i.d.; carrier: 15 mM phosphate buffer, pH = 2.55; voltage applied: -20 kV; temperature: 40°C; injected volume: 15 nL; sample: 100 mg L⁻¹ Se(VI) and 10 mg L⁻¹ Se(IV). Detection: UV at 190 nm.

Table 2. Relationship between sample injected volume and peak area response. Capillary: 72 cm x 50 μm i.d.; carrier: 15 mM phosphate buffer, pH = 2.55; temperature: 40°C; voltage: -20 kV; sample: 100 mg L⁻¹ Se(VI) and 10 mg L⁻¹ Se(IV).

<table>
<thead>
<tr>
<th>T_i (s)</th>
<th>V_i (nL)</th>
<th>Q_i (ng)</th>
<th>A(Q)</th>
<th>A/Q</th>
<th>A/Q mean</th>
<th>A/Q %RSD</th>
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<tbody>
<tr>
<td>Se(IV)</td>
<td></td>
<td></td>
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<td>2</td>
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<td>6</td>
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<td>8</td>
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<tr>
<td>10</td>
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<td>159151</td>
<td>530503</td>
<td>521281</td>
<td>4.52</td>
<td></td>
</tr>
<tr>
<td>Se(VI)</td>
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<td></td>
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</tr>
<tr>
<td>6</td>
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<td>32520</td>
<td>31501</td>
<td>4.19</td>
<td></td>
</tr>
</tbody>
</table>

T_i: injection time; V_i: injected volume; Q_i: injected amount; A_i: Peak area; (a): mean of two measurements.

Table 3. Linearity of peak area response versus injected sample amount. Same conditions that in Figure 4.

<table>
<thead>
<tr>
<th>Q_i (ng)</th>
<th>Se(IV)</th>
<th>Se(VI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A(Q)</td>
<td>A/Q (x10⁻³)</td>
</tr>
<tr>
<td>0.150</td>
<td>69019</td>
<td>464</td>
</tr>
<tr>
<td>0.120</td>
<td>52841</td>
<td>440</td>
</tr>
<tr>
<td>0.090</td>
<td>40538</td>
<td>450</td>
</tr>
<tr>
<td>0.060</td>
<td>26663</td>
<td>444</td>
</tr>
<tr>
<td>0.030</td>
<td>11240</td>
<td>374</td>
</tr>
<tr>
<td>0.015</td>
<td>5396</td>
<td>359</td>
</tr>
<tr>
<td>0.0075</td>
<td>3840</td>
<td>512</td>
</tr>
</tbody>
</table>

Q_i: injected amount; A_i: peak area; (a): mean of three measurements

given. A good linear response was obtained in the range studied as can be observed in the results. A 15 nL injection volume (injection time of 5 s) was selected to study linearity of peak area response versus sample concentration.

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In Figure 4 the peak area measured is plotted versus sample concentration for seven standards containing different amounts of both selenium ions, and the calibration curve is also drawn. All the results are the mean of three measurements. Good correlation coefficients were obtained, r = 0.9987 for Se(VI) and r = 0.9989 for Se(IV), but some deviation of linearity can be observed for lower concentrations in the range studied. This fact becomes more evident by comparing the measured peak area-injected amount ratio for the different standards, as it is shown at Table 3.

Under selected conditions, 15 mM phosphate buffer (pH = 2.55), injected volume of 15 nL, applied voltage -20 kV, temperature 40°C, selenate (t_r = 4.58 min) and selenite (t_r = 14.58 min) give well resolved peaks in less than 20 minutes, as it is shown in Figure 5. To obtain signal background ratios equal to three, the sample concentration must be of 10 mg L⁻¹ for Se(VI) and 1 mg L⁻¹ for Se(IV). Taking into account the little volume injected (15 nL) this yields a low mass detection limit of 150 pg for selenate and 15 pg for selenite.

Acknowledgements

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References

Electroanalytical study of the herbicides dinoseb, methoprotryne and terbutryn

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Abstract. A polarographic study of the herbicides dinoseb, methoprotryne and terbutryn is reported. Two well-defined peaks were obtained for dinoseb within all the pH range studied, and one well-defined peak was obtained for methoprotryne and terbutryn below pH 6.0 in differential pulse polarography (dpp). The peak potentials were, in all cases, shifted to more negative values with increasing pH, pk, values of 4.0, 4.0, and 4.4 being obtained for dinoseb, methoprotryne and terbutryn, respectively. The characteristics of the electrode processes were examined at pH 3.0 for the three herbicides, and also at pH 10.0 for dinoseb. The diffusion coefficients were calculated, as well as the kinetic parameters on, and k∞. The mechanisms of the polarographic reduction processes were proposed. Linear calibration plots were obtained using dpp over different concentration ranges, the analytical characteristics of the methods having been determined. The effect of the presence of other herbicides on the iC and EC values of each one of the three herbicides was studied. The developed methods were applied to the determination of dinoseb in spiked apple samples and of methoprotryne and terbutryn in spiked pear samples with good recoveries.

Key words: Dinoseb, Methoprotryne, Terbutryn, Polarography, Voltammetry, Fruits.

Introduction

In the last decades, the agricultural production has suffered from a quick growth due to the use of pesticides in the protection of crops, nitrophenolic and s-triazine derivatives being widely used for this purpose. Dinoseb (2-sec-butyl-4,6-dinitrophenol) is a nitrophenol widely employed as herbicide, fungicide and insecticide. It is toxic to mammals, both oral and dermatologically presenting a long persistence in blood which is probably due to the formation of a complex with the albumin, and in the brain due to its slow metabolism [1]. On the other hand, methoprotryne (2-isopropylamino-4-(3-methoxypropylamino)-6-methylthio-1,3,5-triazine) and terbutryn (2-tertbutylamino-4-ethylamino-6-methylthio-1,3,5-triazine) are methylthio derivatives belonging to the s-triazine family. Like other 1,3,5-triazines, they are widely employed in agriculture and industry as effective components of selective herbicides. Their low solubility in water as well as their chemical stability provoke a high persistence of their residues. Thus, they are accumulated in soils [2] and crops [3] directly treated, and they have also been found as pollutants in fruits [4], waters [5], and even milk [6]. Although their toxicity to mammals is low when compared with other plaguicides, they can provoke renal disorders, inhibit the DNA, RNA and proteins synthesis, and they can even play an important role in the evolution of ovarian cancer [7].

The maximum content allowed for each one of these herbicides is of 0.05 mg Kg⁻¹ in all kinds of vegetables [8], analytical methods of high sensitivity being required for their determination. Although GC with EC [9, 10], MS [5, 11], and NP [3, 4] detectors are the most widely employed techniques for the determination of either nitrophenol and 1,3,5-triazines, electroanalytical techniques, mainly those working in the differential pulse mode, are a valuable alternative approach offering fast procedures and low cost of instrumental devices. Nevertheless, electroanalytical techniques have scarcely been used for the determination of nitrophenol [12-14] and 1,3,5-triazine [15-19] herbicides. Thus, in this work a study of the polarographic

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behaviour of dinoseb, methoprotryne and terbutryn is presented, as a previous step for their further determination in fruit samples.

Experimental

Apparatus
A Metrohm E 626 Polarecord equipped with a Metrohm 663 VA stand and a Metrohm E 506 Polarecord equipped with an E 505 polarographic stand were used. A Metrohm E 612 VA-Scanner coupled with a L Y 1600 Linseis X-Y recorder, a Metrohm E 510 pH-meter, a P-Selecta rotary vacuum evaporator and a Visiprep (Supelco) vacuum station were also used.

Electrodes and electrochemical cell
The electrochemical cell consisted of either a Metrohm 6.1230.000 dropping mercury electrode, an Ingold 10-303-3000 saturated calomel reference electrode and a platinum wire counter electrode in a double-walled Metrohm EA 867-20 vessel, or a Metrohm 6.1246.020 multimode mercury electrode, a Metrohm 6.078.000 Ag/AgCl/(3 mol L^-1 KC1) reference electrode and a Metrohm 6.1247.000 glassy carbon electrode in a double-walled Metrohm 6.1415.0210 vessel. A Metrohm AG-9100 combined electrode was used for pH measurements.

Reagents and solutions
Dinoseb, methoprotryne and terbutryn were obtained from Riedel-de Haën, and 1.0x10^-3 mol L^-1 stock solutions of each herbicide in acetonitrile (Carlo Erba) were prepared by weighing. More dilute standards were prepared by suitable dilution with water from a Millipore Milli-Q system. A Britton-Robinson buffer solution containing each component acid at a final concentration of 0.1 mol L^-1 was used as supporting electrolyte. Methylene chloride and n-hexane (Carlo Erba) together with Sep Pak Florisil cartridges (1 g, 6.0 mL, Waters) were employed for the determination of these herbicides in spiked fruit samples.

Procedures
Polarographic and cyclic voltammetric studies. The test solutions (50 mL) were transferred into the electrochemical cell and deaerated by passing an argon stream through them for 10 min. Polarograms and voltammograms were recorded at 20±1 ºC keeping an inert atmosphere in the cell. The analytical response was measured in all instances against the background current.

Determination of dinoseb in spiked apple samples and of methoprotryne and terbutryn in spiked pear samples. About 50 g of cut apple or pear sample were weighed accurately and transferred into a blender. In the recovery studies, the appropriate volume of each herbicide stock solution was added. Once 100 mL of de-ionised water had been added, the mixture was homo-

![Figure 1](https://via.placeholder.com/150)

Figure 1. Polarograms obtained for (a) 5.0x10^-3 mol L^-1 dinoseb, (b) 1.0x10^-4 mol L^-1 methoprotryne and (c) 1.0x10^-3 mol L^-1 terbutryn by differential pulse polarography on the dropping mercury electrode; 0.1 mol L^-1 Britton-Robinson at pH 5.5; v 10 mV s^-1; t 0.5 s.

genized at 18500 r.p.m. for 5 minutes and quantitatively transferred into six 35 mL centrifuge tubes. Next, 100 mL of either methylene chloride, to extract dinoseb from apple samples, or n-hexane to extract terbutryn or methoprotryne from pear samples, were added to the centrifuge tubes. The corresponding herbicide was extracted in the organic solvent by mechanical stirring for 5 min. After centrifugation at 4000 r.p.m. for 20 min, the organic phase was transferred into the 250 mL vessel of the rotary vacuum evaporator and concentrated to a final volume of ca. 3 mL. Then, this extract was passed through a Sep Pak Florisil cartridge previously activated with 10 mL of methylene chloride when determining dinoseb, or with 10 mL n-hexane when determining either methoprotryne or terbutryn. Next, dinoseb was eluted with 40 mL of methylene chloride, methoprotryne and terbutryn being eluted with 10 mL of the same solvent. The organic phase was evaporated to dryness in the rotary vacuum evaporator, and the residues were dissolved in 50 mL of a 0.1 mol L^-1 Britton-Robinson (pH 10.0) solution when working with samples spiked with dinoseb, or in 50 mL of a 0.1 mol L^-1 Britton-Robinson (pH 3.0) solution when samples had been spiked either with methoprotryne or with terbutryn. The determination of the herbicides was carried out by differential pulse polarography (dpp) using calibration graphs obtained by adding aliquots of the respective herbicide to a blank solution of the sample subjected to the above treatment.

Results and discussion

Dinoseb exhibits two well-defined reduction peaks (Figure 1a) by dpp which are probably due to the reduction of its two nitro groups to the corresponding amino groups with a six-electron exchange each one of them [20]. Regarding methoprotryne and terbutryn, they give rise to a well-defined reduction peak (Figure 1b, c) which has been attributed to the reduction of the -C=N- bond in the heterocyclic ring with an exchange of two electrons [15].
Electroanalytical study of the herbicides dinoseb, methoprene and terbutryn (Figure 2), from whose intersection points pK₆ values of 4.0 and 4.4 were obtained for methoprene and terbutryn, respectively. Regarding dinoseb a decrease in the slope of the E_p vs pH plot was observed from pH 5.3 for its first reduction process (Figure 3), and consequently this value has been assigned to a polarographic pK₆ [21]. However, from the intersection point of the two linear plots obtained for the second reduction process a pK₆ of 4.2 was obtained. This fact suggests that the nitro group involved in the second reduction process is the one in ortho with respect to the phenolic group.

Regarding peak currents values, i_p for the first peak of dinoseb increased slightly when pH increased up to a value of 10.0, where a maximum was observed. However, the second peak tended to decrease from pH 3.0-3.5 with increasing pH. Regarding the 1,3,5-triazines, their peak currents increased with pH being highest at pH 3.0 for both herbicides and disappearing above pH 6.0 due to the hydrolysis of both 1,3,5-triazines to their hydroxide-derivatives [22].

The results obtained by current-sampled dc polarography were very similar to those described above. Consequently, pH 3.0 was chosen as working pH for further studies, both for methoprene and terbutryn. Regarding dinoseb, two pH values were chosen: 3.0 and 10.0; at the acid pH the narrowest peaks were obtained by dpp, while at pH 10.0 the maximum difference between the two reduction potentials was observed, also being the pH value at which the highest first peak current was obtained.

**Characteristics of the electrode processes**

The limiting current is diffusion controlled for both 1,3,5-triazines, as deduced from the slope values, 0.67 and 0.65 for methoprene and terbutryn, respectively, of the log i_p vs log h_concentration plot using current-sampled dc polarography for a 1.0x10⁻³ mol L⁻¹ concentration, from the value of the temperature coefficients, 1.90±0.01 and 1.83±0.01, respectively, and from the linear relationship between the limiting currents and the 1,3,5-triazines concentration in the range 1.0x10⁻⁴ - 1.0x10⁻³ mol L⁻¹ (r=0.999 in both cases). Regarding dinoseb, the limiting current of the first wave is also diffusion controlled following the same criteria, either at pH 3.0 or at pH 10.0; nevertheless, the slopes of the log i_p vs log h_concentration plots for the second wave were 1.5 and 1.1 at pH 3.0 and 10.0, respectively, suggesting that this wave is not only diffusion controlled, adsorption phenomena being also probably involved in this process.

The diffusion coefficients of the three herbicides were determined from the slopes of the above i_p vs concentration plots, assuming a six-electron exchange for each dinoseb reduction process and a two-electron exchange for methoprene and terbutryn. The results are summarized in Table 1.

The results obtained by applying several conventional criteria, such as logarithmic analysis and E_3⁰-E_1⁰ values of

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**Figure 2.** Effect of pH on E_p by differential pulse polarography. (a) 1.0x10⁻⁴ mol L⁻¹ methoprene, (b) 1.0x10⁻⁴ mol L⁻¹ terbutryn 0.1 mol L⁻¹ Britton-Robinson; v 10 mV s⁻¹; t_p 0.5 s.

**Figure 3.** Effect of pH on E_p by differential pulse polarography. (●) First peak; (▲) Second peak. 5.0x10⁻⁴ mol L⁻¹ dinoseb, 0.1 mol L⁻¹ Britton-Robinson; v 10 mV s⁻¹; t_p 0.5 s.

**Effect of pH**

The influence of pH on E₁⁄₂ and the limiting current, i_p, using current-sampled direct current (dc) polarography and on peak potential, E_p, and peak current, i_p, using dpp has been examined for the three pesticides studied. In every case, both E₁⁄₂ and E_p were shifted to more negative values when increasing pH which indicates that protonation reactions are coupled to the respective reduction processes. Two linear plots were observed on E_p vs pH graphs for both methoprene and terbutryn.
Table 1. Determination of the diffusion coefficients by current-sampled dc polarography.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>pH</th>
<th>Slope, µA L mol⁻¹</th>
<th>n°e</th>
<th>D, cm² s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dinosob</td>
<td>3.0</td>
<td>5.1</td>
<td>6</td>
<td>8.56 x 10⁻⁷</td>
</tr>
<tr>
<td>(1st wave)</td>
<td>10.0</td>
<td>6.0</td>
<td>6</td>
<td>1.18 x 10⁻⁴</td>
</tr>
<tr>
<td>Dinosob</td>
<td>3.0</td>
<td>6.3</td>
<td>6</td>
<td>1.30 x 10⁻⁴</td>
</tr>
<tr>
<td>(2nd wave)</td>
<td>10.0</td>
<td>11.0</td>
<td>6</td>
<td>3.98 x 10⁻⁴</td>
</tr>
<tr>
<td>Methoprotryne</td>
<td>3.0</td>
<td>7.1</td>
<td>2</td>
<td>6.72 x 10⁻⁴</td>
</tr>
<tr>
<td>Terbutryn</td>
<td>3.0</td>
<td>11.7</td>
<td>2</td>
<td>1.81 x 10⁻⁴</td>
</tr>
</tbody>
</table>

The current-sampled dc polarograms, showed that the polarographic reduction processes were irreversible for all the herbicides studied.

Logarithmic analysis yielded linear plots for 1.0 x 10⁻⁵ mol L⁻¹ methoprotryne and terbutryn solutions (r=0.999 in both cases) with slopes of -0.045 and -0.036 V, respectively. A similar behaviour was obtained for the first wave of dinosob at pH 3.0 (r=0.998) and 10.0 (r=0.999), as well as for the second wave of this compound at pH 3.0 (r=0.999) with slopes of -0.034, -0.033 and -0.032 V, respectively. However, the E vs log (i/1L-i) plot for the second wave of dinosob, when working at pH 10.0 is more complex, showing a sigmoidal shape with two linear sections at the beginning and at the end of the plot (Figure 4). This suggests an overlapping of two reduction processes which would occur at two very close potentials for the nitro group in ortho position in relation to the phenolic group. The slopes for both linear sections were -0.056 (line a) and -0.069 (line b).

The nonreversibility of the polarographic reduction processes for the three herbicides studied was confirmed by recording their cyclic voltammograms on a hanging mercury drop electrode (Figure 5). The cyclic voltammograms obtained for 5.0 x 10⁻⁵ mol L⁻¹ dinosob (Figure 5 a, b) showed two reduction peaks (A and B in the Figure) in the first scan, which are probably due to the reduction of each one of the nitro groups in the molecule to the respective amino groups. The amine thus produced is subsequently oxidized in the reverse scan (peak C) to the corresponding imine, as is usually observed for nitroaromatic compounds [23]. The second scan towards negative potentials showed a new cathodic peak (peak D) corresponding to the reversible reduction of the imine derivative.

Regarding methoprotryne and terbutryn, a single well-defined cathodic peak was observed (Figure 5 c, d), no oxidation peak appearing in the reverse potential scan, thus confirming the irreversibility of the electrode processes.

On the other hand, the log i vs log v plots, within the range 10-1000 mV s⁻¹ yielded linear relationships for both dinosob peaks, with slopes of 0.78 and 0.75 for the first peak and of 0.74 and 0.69 for the second peak, at pH 3.0 and 10.0, respectively, these values being close neither to the one theoretically expected for diffusion controlled systems (0.5), nor to that theoretically expected when there is adsorption on the electrode (1.0) [24]. This behaviour suggests a mixed diffusive-adsorptive control in every case. Regarding methoprotryne and terbutryn, slopes of 0.46 and 0.59, respectively, were obtained for scan rates lower than 50 mV s⁻¹, while for higher scan rates the slopes of the log i vs log v plots were 0.91 and 0.86, respectively. Consequently, both 1,3,5-triazines reduction processes showed a diffusion control for scan rates lower than 50 mV s⁻¹, while for higher scan rates the change in the slopes values indicates the existence of adsorption phenomena on the electrode. This behaviour is the one expected for compounds weakly adsorbed on the electrode surface.
Electroanalytical study of the herbicides dinoseb, methoprotryne and terbutryn

Table 2. Heterogeneous rate constants, $k^*_{p}$, and $\alpha_n$ values for the polarographic reduction of dinoseb, methoprotryne and terbutryn

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>pH</th>
<th>$\alpha_n$</th>
<th>$k^*_{p}$, cm s$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dinoseb (1st wave)</td>
<td>3.0</td>
<td>1.66</td>
<td>6.0x10$^{-6}$</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>1.55</td>
<td>1.5x10$^{-3}$</td>
</tr>
<tr>
<td>Dinoseb (2nd wave)</td>
<td>3.0</td>
<td>1.74</td>
<td>1.9x10$^{-9}$</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>0.94</td>
<td>8.8x10$^{-3}$</td>
</tr>
<tr>
<td>Methoprotryne</td>
<td>3.0</td>
<td>1.07</td>
<td>8.0x10$^{-3}$</td>
</tr>
<tr>
<td>Terbutryn</td>
<td>3.0</td>
<td>1.38</td>
<td>4.5x10$^{-3}$</td>
</tr>
</tbody>
</table>

Table 3. Determination of $\alpha_n$ values by current-sampled dc polarography.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>pH</th>
<th>$\alpha_n$</th>
<th>$E_{2M-E_{1/2}}$</th>
<th>$E_{m-E_{1/2}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dinoseb (1st wave)</td>
<td>3.0</td>
<td>1.61</td>
<td>-0.034</td>
<td>1.52</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>1.64</td>
<td>-0.032</td>
<td>1.62</td>
</tr>
<tr>
<td>Dinoseb (2nd wave)</td>
<td>3.0</td>
<td>1.72</td>
<td>-0.030</td>
<td>1.72</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>0.97</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Methoprotryne</td>
<td>3.0</td>
<td>1.20</td>
<td>-0.046</td>
<td>1.12</td>
</tr>
<tr>
<td>Terbutryn</td>
<td>3.0</td>
<td>1.30</td>
<td>-0.035</td>
<td>1.47</td>
</tr>
</tbody>
</table>

Dinoseb polarograms show two waves which should correspond to the reduction of each of the nitro groups in the molecule to the corresponding amino groups with a six-electron exchange [20]. These processes would be represented by the following equations:

(1)

(2)

From the results obtained in the pH dependence over the reduction potentials of dinoseb, the first wave was assumed to be due to the reduction of the nitro group in para with respect to the phenolic group. This is probably owed to, apart from inductive and resonance effects, steric effects between the phenolic and the nitro groups situated in ortho because of their proximity. On the other hand, owing to the presence of weak adsorptive phenomena, two types of reactions could be involved in this mechanism: the reduction of the adsorbed dinoseb and the reduction of the dinoseb in the solution. Moreover, the observed dependence of $E_{1/2}$ on pH shows that protons are involved in the rate-determining electron-transfer step, which for aromatic nitrocompounds usually is the irreversible reduction of the nitro to nitroso group provided the preceding protonation of the involved nitro group [26, 27]. In this work, only the successive reactions of the dinoseb which diffuses from the solution to the electrode surface are proposed.

Thus, in acid medium and for the first reduction wave, the first step would probably be a quick protonation of the nitro group in para with respect to the phenolic group:

(3)

followed by its slow reduction to the nitroso group:

(4)

The subsequent reduction of the nitroso group to amine group would occur in two electrochemical steps whose potentials would be less negative than that of the nitro group reduction [26]. In the first step a hydroxylamine group would be formed:

Mechanisms of the polarographic reductions

Based on the above results, and assuming that, under the experimental conditions used, adsorption contribution to the electrochemical responses can be neglected, reduction mechanisms can be proposed for dinoseb, methoprotryne and terbutryn.
Regarding methoprotroine and terbutryn, each of their reduction mechanisms involves two electrons and one proton. Taking into account the obtained results and the data reported in literature [16, 28] the proposed mechanisms would involve a stepwise reaction including the reduction of chemical bonds within the ring proceeding in a similar way to that of pyrimidine derivatives. Since the polarographic study of these herbicides was done at pH 3.0 and their calculated $pK_a$ values were of 4.0 and 4.4 for methoprotroine and terbutryn, respectively, the reduction processes should take place over the protonated form of the 1,3,5-triazine molecules. Consequently, the following mechanism can be proposed for the methoprotroine and terbutryn electrochemical reductions:

![Chemical structures showing the reduction process of methoprotroine and terbutryn](image)

Although there is only a slight difference of electronegativity between the nitrogen and sulphur atoms, the protonation probably occurs on one of the nitrogen atoms placed in ortho with respect to the methylthio group due to its higher basicity. Nevertheless, given the great similarity between these two nitrogen atoms, it cannot be ensured which one is protonated, probably existing a mixture of both compounds. The proposed subsequent reduction of the $-C=N-$ bond involves the protonated nitrogen atom, although for the same reasons stated above this could take place involving either the carbon atom bound to the secondary amino group or the carbon atom bound to the methylthio group.

**Polarographic determination of dinoseb, methoprotroine and terbutryn and analytical characteristics of the methods**

The characteristics of the linear calibration graphs obtained by dpp ($\Delta E=50 \text{mV}$) for the three herbicides are summarized in Table 4, the analytical characteristics of the methods based on these calibration graphs being listed in Table 5. Regarding dinoseb, the analytical characteristics obtained from the most sensitive peak (the second peak at pH 3.0 and the first peak at pH 10.0) are given. Relative standard deviations (RSD) were calculated at a concentration level of 5.0x$10^{-4}$ mol L$^{-1}$ dinoseb, 6.0x$10^{-4}$ mol L$^{-1}$ methoprotroine and 5.0x$10^{-5}$ mol L$^{-1}$ terbutryn (n=10). The limits of determination were calculated according the 10s criterion [29] and the detection limits were defined as $3 \sigma_m$ [30], where m is the respective slope of the calibration plots corresponding to the lowest ranges of linearity for each herbicide, and $\sigma_b$ is the standard deviation (n=10) of the signal for 6.0x$10^{-7}$ mol L$^{-1}$
Electroanalytical study of the herbicides dinoseb, methopropryn and terbutryn

Table 4. Characteristics of the dpp calibration graphs for dinoseb, methopropryn and terbutryn.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>pH</th>
<th>Range of analysis mol L⁻¹</th>
<th>r</th>
<th>Slope x10⁻⁵ nA·mol⁻¹</th>
<th>Intercept nA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dinoseb</td>
<td>3.0*</td>
<td>6.0x10⁻⁵-1.0x10⁻⁴</td>
<td>0.999</td>
<td>5.7±0.2</td>
<td>-121</td>
</tr>
<tr>
<td></td>
<td>10.0*</td>
<td>1.0x10⁻⁵-1.0x10⁻³</td>
<td>0.998</td>
<td>9.4±0.7</td>
<td>-243</td>
</tr>
<tr>
<td>Methopropryn</td>
<td>3.0</td>
<td>2.0x10⁻⁵-1.0x10⁻³</td>
<td>0.999</td>
<td>6.4±0.2</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td></td>
<td>10.0*</td>
<td>1.0x10⁻⁵-6.0x10⁻⁴</td>
<td>0.998</td>
<td>6.4±0.1</td>
<td>7±1</td>
</tr>
<tr>
<td>Terbutryn</td>
<td>3.0</td>
<td>1.0x10⁻⁴-1.0x10⁻³</td>
<td>0.999</td>
<td>12±1</td>
<td>9±2</td>
</tr>
<tr>
<td></td>
<td>10.0*</td>
<td>1.0x10⁻⁴-4.0x10⁻³</td>
<td>0.999</td>
<td>11±1</td>
<td>13±2</td>
</tr>
</tbody>
</table>

*, 2nd peak and **, 1st peak

Table 5. Analytical characteristics of the dpp methods for the determination of dinoseb, methopropryn and terbutryn.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>pH</th>
<th>RSD %</th>
<th>Determination Limit mol L⁻¹</th>
<th>Detection Limit mol L⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dinoseb</td>
<td>3.0*</td>
<td>2.8</td>
<td>6.2x10⁻⁷</td>
<td>1.9x10⁻⁷</td>
</tr>
<tr>
<td></td>
<td>10.0*</td>
<td>2.4</td>
<td>4.7x10⁻⁷</td>
<td>1.4x10⁻⁷</td>
</tr>
<tr>
<td>Methopropryn</td>
<td>3.0</td>
<td>4.6</td>
<td>2.3x10⁻⁷</td>
<td>6.9x10⁻⁸</td>
</tr>
<tr>
<td>Terbutryn</td>
<td>3.0</td>
<td>4.7</td>
<td>9.4x10⁻⁸</td>
<td>2.8x10⁻⁸</td>
</tr>
</tbody>
</table>

*, 2nd peak and **, 1st peak

dinoseb at pH 3.0, 4.0x10⁻⁵ mol L⁻¹ dinoseb at pH 10.0, 2.0x10⁻⁷ mol L⁻¹ methopropryn and 1.0x10⁻⁷ mol L⁻¹ terbutryn.

All the RSD values obtained are lower than 5%, which demonstrates the good precision of the developed methods. On the other hand, the lowest determination and detection limits were obtained for terbutryn, those obtained for dinoseb being significantly higher.

Study of interferences

The effect of the presence of some other herbicides on the ip and Eₚ values of dinoseb, methopropryn and terbutryn was studied. All the possible pairs of three herbicides were prepared and studied by measuring ip and Eₚ for one of them at a fixed concentration level, 5.0x10⁻⁶ mol L⁻¹ for dinoseb and 6.0x10⁻⁷ mol L⁻¹ for methopropryn and terbutryn, while varying the concentration of the other within the ranges 1.0x10⁻⁷-1.0x10⁻³ mol L⁻¹ or 0.6x10⁻⁸, 1.0x10⁻⁶ mol L⁻¹, respectively. The influence of another nitrophenol such as DNOC (4,6-dinitroorthocresol) and of two other 1,3,5-triazines, such as propazine (2-chloro-4,6-diisopropylamino-1,3,5-triazine) and simazine (2-chloro-4,6-diethylamino-1,3,5-triazine) was also tested. Under the same experimental conditions used for dinoseb, methopropryn and terbutryn, DNOC showed two reduction peaks at the same potentials (-0.09 V and -0.29 V at pH 3.0, and -0.50 V and -0.82 V at pH 10.0) as dinoseb. On the other hand, while none of the tested chlorotriazines showed any reduction peaks when working at pH 10.0, at pH 3.0 a peak was observed at -1.00 V and -0.99 V for propazine and simazine, respectively.

None of the tested 1,3,5-triazines interfered in the dinoseb determination due to the separation among their peak potentials while in the presence of DNOC, dinoseb peaks increased giving errors higher than 10% from dinoseb:DNOC ratios of 1:1 at pH 3.0 (by using the second peak due to its highest sensitivity) and 1:1.02 (measuring the first dinoseb peak) at pH 10.0.

On the other hand and for the same reason stated above, none of the tested nitrophenols showed interferences over the methopropryn and terbutryn reduction signals, whereas all the tested 1,3,5-triazines provoked an increase on methopropryn and terbutryn ip which was higher on increasing the interferent concentration. Thus relative errors higher than 10% were obtained for methopropryn:interferent molar ratios of 1:0.2, 1:1.4 and 1:0.2 for simazine, propazine and terbutryn, respectively, and for terbutryn:interferent molar ratios of 1:0.3, 1:1.5 and 1:0.3 for simazine, propazine and methopropryn, respectively.

Determination of dinoseb in spiked apple samples and of methopropryn and terbutryn in spiked pear samples

Differential pulse polarography, under the experimental conditions mentioned above was used to determine dinoseb in spiked apples and methopropryn and terbutryn in spiked pear samples. While the apple samples were spiked with 0.24 µg of dinoseb per gram of apple, the pear samples were spiked either with 0.19 µg of methopropryn or with 0.17 µg of terbutryn per gram of pear. Calibration graphs in the ranges 6.0x10⁻⁷-4.0x10⁻⁴ mol L⁻¹ and 2.0x10⁻⁷-1.0x10⁻⁶ mol L⁻¹ were established by adding aliquots of dinoseb and methopropryn or terbutryn stock solutions, respectively, to blanks of each sample which were subjected to the same treatment as the spiked samples. The slopes and intercepts of these plots were (2.9±0.5)x10⁶ nA L mol⁻¹ and -(0.22±1) nA, respectively, for dinoseb at pH 10.0, (5±1)x10⁶ nA L mol⁻¹ and -(0.2±0.8) nA, respectively, for methopropryn, and (1.1±0.2)x10⁷ nA L mol⁻¹ and -(0.2±1) nA, respectively, for terbutryn. Recovery studies yielded the following results for five determinations: dinoseb found in spiked apple samples, 0.23±0.01 µg g⁻¹ (96±5% recovery); methopropryn and terbutryn found in spiked pear samples, 0.17±0.02 µg g⁻¹ (90±9% recovery) and 0.15±0.01 µg g⁻¹ (92±8% recovery), respectively, the confidence intervals being calculated for a significance level of 0.05, with relative standard deviations of 4.3, 7.9 and 6.9% for dinoseb, methopropryn and terbutryn, respectively.
Conclusions

The results obtained in the study of the polarographic behaviour of dinoseb, methoprotyne and terbutryn has led to a better understanding of the polarographic reduction processes and of the reduction mechanisms of these herbicides. The obtained results also demonstrate the suitability of the developed dpp methods for the determination of these herbicides in fruit samples, although the possible interference of some other herbicides belonging to the same families must be checked previously.

Acknowledgements

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References

Interlaboratory exercises in quality assurance. I. Heavy metals in environmental analysis

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Abstract. The harmonization of measurements performed in European countries is the main purpose of the Community Bureau of Reference (BCR), now Measurements and Testing Programme. This objective can be achieved by means of collaborative efforts with Organizations from Member States such as the Spanish Society for Analytical Chemistry (SEQA). This paper summarizes the results of four Intercomparison Exercises on trace metal determination in environmental and food matrices organized jointly by these two Institutions, and reflects the current state situation of Spanish laboratories active in this area. The present report on the discussions and the results is a subject of interest not only for all the participants but for other laboratories which are involved in this type of analysis.

Key words: Quality assurance, Measurements and Testing Programme, Trace metals, Environment, Food.

Introduction

At the end of 1988, the Spanish Society for Analytical Chemistry (Sociedad Española de Química Analítica, SEQA), presented in Spain the activities of the Community Bureau of Reference (BCR), now Measurements and Testing Programme. The aim of BCR was to support the technical collaboration between laboratories of European countries and to help the laboratories of the Member States to provide reliable and accurate results in areas considered of priority by the European Community. The meeting revealed the great interest of Spanish laboratories in topics related with Quality Assurance and especially in their participation in interlaboratory exercises, and desire to establish a collaboration between the SEQA and the BCR. It was therefore decided to start a collaborative effort by organizing some interlaboratory exercises to ascertain the performance of Spanish laboratories and to familiarize them with participation in this kind of exercise which is essential in Quality Assurance [1-3]. The aim of this collaboration was to improve the quality of the measurements in environmental and food samples by means of a joint training programme.

These collaborative efforts facilitate the exchange of information and serve to increase mutual credibility among laboratories from different European countries.

The training programme designed started in 1989 and to date four exercises, starting in February 1989, April 1990, June 1991 and June 1993 respectively, have been carried out. This training programme dealt with the determination of organochlorinated pesticides (OCP's), polychlorinated biphenyls (PCB's) and heavy metals, in environmental and food matrices. These elements and organic compounds were chosen because they are considered priority pollutants in the environment and in food [4, 5].

In this work the results as well as the technical discussion concerning heavy metals, arsenic and selenium are reported. This information, which includes specific conditions and recommendations to overcome sources of error and difficulties in the determination of the elements and matrices analyzed are relevant data not only for all the participants in the Exercises but for other laboratories which are involved in this type of analysis.

The results and discussion about the organic compounds is reported in the same issue [6].
Design of the exercises

The aim of the first intercomparison exercise was to familiarize the participants with this kind of training. All the participants received a lyophilized plant sample to analyze. From the results it was concluded that the main discrepancy was due to calibration errors. Calibration was therefore the focus of the second exercise in which the laboratories received a single sample of a nitric solution containing heavy metals.

The design of the third exercise was quite different from the previous two. Two materials with different complexity levels were analyzed in order to discuss and evaluate the calibration methods used and the sample pretreatments. In this exercise each participant received two materials: a solution of heavy metals in nitric acid and a lyophilized plant sample to be decomposed by means of an acidic attack. Each participant also received a standard nitric solution containing the metals to be determined in order to verify their calibrants. The last exercise was designed in the same way as the third one but the solution for calibration was not sent to the participants.

The samples, the appropriate laboratory identification, instructions concerning the handling of the samples, and the reporting forms for the results were sent to the participants. The date in which the exercise took place, the objective of each exercise, as well as the type of material and the elements to be analyzed in each occasion, are given in Table 1.

Participants

The exercises were addressed to experienced laboratories from the public and private sectors and universities, and each exercise was open to new participants. Figure 1 shows the distribution of the laboratories who replied (according to their origin). A significant increase of the number of participants over the time can be observed, especially for the laboratories from the public sector.

Table 2 summarizes the participation of the laboratories in the four exercises. X means participation and - means non-participation; each column corresponds to one exercise, and the number of participants is reported at the end of the row. The total number of laboratories was 102, of which only 7 participated in all the exercises. In each exercise approximately 50% of participants were new. The percentage of laboratories participating again increased from 53% in the first exercise to the second to 71% from the third to the fourth, showing an increasing interest for these exercises.

Materials

The solutions were prepared by gravimetric dilution with 5 mol/l HNO₃ from 1 mg/ml standard (Carlo Erba Analyticals Solutions) for each metal and bottled in sealed vials by the CETS Institut Quimic de Sarria (Barcelona, Spain). The stability and homogeneity of the solutions was tested by the same Institution. The nitric solution had to be diluted with water up to an appropriate volume before analysis. The elements in the solutions are given in Table 1.

Lyophilized plants: The preparation of the lyophilized plant samples was done by the Joint Research Centre in Ispra (Italy). The stability and homogeneity of the materials used in the interlaboratory exercises were tested by the same Centre. The elements determined are given in Table 1.

Table 1. Date, objective, type of material and elements analyzed in each exercise.

<table>
<thead>
<tr>
<th>Exercise</th>
<th>Date</th>
<th>Materials</th>
<th>Elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>Feb. 1989</td>
<td>Lyophilized Plant</td>
<td>Cu, Cd, Cr, Pb, Hg, Ni, Zn</td>
</tr>
<tr>
<td>2nd</td>
<td>Apr. 1990</td>
<td>Nitric Solution</td>
<td>Cu, Cd, Cr, Pb, Hg, Ni, Zn, Se, As</td>
</tr>
<tr>
<td>3rd</td>
<td>Jun. 1991</td>
<td>Lyophilized Plant</td>
<td>Cu, Cd, Cr, Pb, Hg, Ni, Zn, Se, As</td>
</tr>
<tr>
<td>4th</td>
<td>Jun. 1992</td>
<td>Nitric Solution</td>
<td>Cu, Cd, Cr, Pb, Hg, Ni, Zn, Se, As</td>
</tr>
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Table 2. Summary of the participation of the laboratories in the four exercises.

<table>
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<tr>
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<th>3rd</th>
<th>4th</th>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>9</td>
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<td>-</td>
<td>-</td>
<td>3</td>
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<td>-</td>
<td>-</td>
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<td>-</td>
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<td>10</td>
</tr>
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<td>-</td>
<td>-</td>
<td>x</td>
<td>x</td>
<td>27</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>19</td>
</tr>
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<td>-</td>
<td>-</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>9</td>
</tr>
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<td>-</td>
<td>x</td>
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</tr>
</tbody>
</table>

Total: 28, 33, 52, 67, 102
Analytical techniques used in the intercomparison

The techniques used to determine the metals were mainly FAAS (Flame Atomic Absorption Spectroscopy) or ETAAS (Electrothermal Atomic Absorption Spectroscopy) although other techniques such as ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy), ASV (Anodic Stripping Voltammetry) or DP-ASV (Differential Pulse Anodic Stripping Voltammetry) were also used by some laboratories. CVAAS (Cold Vapour Atomic Absorption Spectroscopy) was used for mercury. HGAAS (Hydride Generation Atomic Absorption Spectroscopy) or HG-ICP-OES (Hydride Generation Inductively Coupled Plasma Optical Emission Spectroscopy) were used by several laboratories to determine arsenic.

Results and discussion

The results obtained from nitric solutions are reported in Table 3. For each exercise, second, third and fourth, the elements determined, the number of laboratories determining each element, the real value, the mean value, the standard deviation, the relative standard deviation obtained, considering all the results sent by the participants and the number of outliers are reported. The last four columns give the mean value, the standard deviation, the relative standard deviation and the percentage of error calculated from the selected data. In Table 4, as in Table 3, information is reported for lyophilized plant sample. In this case the real value is unknown, and so the percentage of error is not reported.

No statistical criteria were followed to select data since the results from some laboratories may have been biased by systematic errors.

The selected data for copper and chromium in the solution samples, corresponding to the fourth exercise, are represented in Figures 2a and 2b. In these figures the percentage of error and the standard deviation of each laboratory are depicted. These two elements have been chosen as examples to show the different resulting pictures when the element is difficult to determine accurately and precisely, as for instance chromium [7] (Fig. 2b), or when the element is easier to determine, as for instance copper (Fig. 2a).

To evaluate the laboratories’ performance the Z score values [8] were calculated for each element, considering the selected data. This parameter was calculated according to the following expression:

\[ Z = \frac{(x - \mu)}{\delta} \]

where \( x \) is the mean value of laboratory \( i \), \( \mu \) the true value, \( \delta \) the reference standard deviation, was chosen “by perception” [9] by multiplying by an arbitrary factor the standard deviation of the laboratory which prepared the solution [10]. As an example, Figure 3 represents the Z score values obtained from the determination of nickel in the solution in the fourth exercise.
Table 3. Results obtained from the solution samples.

<table>
<thead>
<tr>
<th>Net</th>
<th>N Labs</th>
<th>Real</th>
<th>Mean all</th>
<th>SD all</th>
<th>%RSD all</th>
<th>Out</th>
<th>Mean sel.</th>
<th>SD sel.</th>
<th>%RSD sel.</th>
<th>% Error</th>
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<td>20.00</td>
<td>20.421</td>
<td>5.662</td>
<td>27.7</td>
<td>3</td>
<td>20.197</td>
<td>1.415</td>
<td>7.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Cd</td>
<td>28</td>
<td>0.50</td>
<td>0.873</td>
<td>0.493</td>
<td>56.5</td>
<td>5</td>
<td>0.681</td>
<td>0.185</td>
<td>27.1</td>
<td>36.2</td>
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<tr>
<td>Cr</td>
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<td>3.270</td>
<td>0.673</td>
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<td>4</td>
<td>3.041</td>
<td>1.191</td>
<td>39.2</td>
<td>52.1</td>
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<tr>
<td>Hg</td>
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<td>1.00</td>
<td>0.965</td>
<td>0.268</td>
<td>27.8</td>
<td>3</td>
<td>0.928</td>
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<td>Ni</td>
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<td>18.565</td>
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<td>18.388</td>
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<td>7.7</td>
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<tr>
<td>Zn</td>
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<td>200.00</td>
<td>217.318</td>
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<td>56.8</td>
<td>3</td>
<td>205.879</td>
<td>16.596</td>
<td>8.1</td>
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<tr>
<td>Se</td>
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<td>4.637</td>
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<td>2.911</td>
<td>0.321</td>
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Table 4. Results obtained from the lyophilized samples.

<table>
<thead>
<tr>
<th>Net</th>
<th>N Labs</th>
<th>Mean all</th>
<th>SD all</th>
<th>%RSD all</th>
<th>Out</th>
<th>Mean sel.</th>
<th>SD sel.</th>
<th>%RSD sel.</th>
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<td>5.786</td>
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<tr>
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<tr>
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<tr>
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<td>23.427</td>
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<td>1</td>
<td>108.809</td>
<td>21.264</td>
<td>19.5</td>
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Table 4. Results obtained from the lyophilized samples.

<table>
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<tr>
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<th>N Labs</th>
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<th>SD all</th>
<th>%RSD all</th>
<th>Out</th>
<th>Mean sel.</th>
<th>SD sel.</th>
<th>%RSD sel.</th>
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<table>
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<tr>
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<th>SD all</th>
<th>%RSD all</th>
<th>Out</th>
<th>Mean sel.</th>
<th>SD sel.</th>
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<td>0.229</td>
<td>0.215</td>
<td>94.1</td>
</tr>
<tr>
<td>Zn</td>
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<td>53.596</td>
<td>20.575</td>
<td>38.4</td>
<td>3</td>
<td>51.411</td>
<td>7.847</td>
<td>15.3</td>
</tr>
<tr>
<td>Se</td>
<td>17</td>
<td>0.242</td>
<td>0.331</td>
<td>136.6</td>
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<tr>
<td>As</td>
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<td>2.119</td>
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<td>5</td>
<td>2.315</td>
<td>0.840</td>
<td>36.3</td>
</tr>
</tbody>
</table>
Figure 4 shows the percentage error of the mean from the selected data reported in Table 3, for all the elements analyzed in the solutions in the second, third and fourth exercises. A significant decrease of error can be observed for cadmium and lead in comparing the first exercise with the two others.

The number of laboratories that sent results of lyophilized samples was lower than for solution samples. Figure 5 shows the percentage of error obtained in the determination of the metals in the fourth exercise in which target value was already known. The high percentage of error obtained for mercury is noticeable in comparison with the other elements. Arsenic and selenium results display negative bias. Figure 6 represents the percentage of relative standard deviation obtained for the elements in the lyophilized samples analyzed in the first, third and fourth exercises. Mercury results display high relative standard deviation in all exercises, and the highest value for selenium was obtained in the second exercise.

**General comments**

Participants discussed their results in technical meetings at the end of each exercise. In order to study the repeatability, all the laboratories were asked to carry out five independent replicate determinations. The results obtained in the first trial showed in general poor agreement, which was mainly attributed to calibration errors. Relative standard deviation values were found to be smaller than expected. In the discussion it was recommended that the five replicates should be analyzed in at least two different days and, if an outlier appeared in a series of replicates, the cause should be identified and corrected; a new series should be started, avoiding the subjective selection of the best data. One of the possible reasons for the spread of the results was assumed to be due to the different moisture content in the lyophilized samples after opening the original bottle. The need for proper moisture correction, in order to decrease discrepancies between laboratories, was made clear.

The use of commercial calibrant solutions was accepted but the need for proper checking of these solutions against primary calibrants was emphasized. The use of the calibrant solutions to analyze Certified Reference Materials to ensure traceability was strongly recommended.

The use of run charts in laboratories to verify the stability of the calibrants was also recommended.

A discussion about the cleaning of the laboratory glassware took place at the last meeting. Some laboratories recommended the use of a particular glassware for each sample type, and different cleaning systems were proposed by the participants with the common recommendation of using high quality acids with a guarantee of their metal content. Some laboratories indicated that the mercury content of some of these acids is sometimes significant for
trace analysis purposes. The use of the same glassware for the same matrices was also suggested by some participants.

As far as the decomposition of the plant materials was concerned, no differences were observed when the acidic attack was performed using microwave ovens or using electrical heaters.

**Arsenic determination**

Highest values were found when ETAAS was used for the final determination and the use of the standard addition method for calibration was recommended. Highest standard deviation was observed when using hydride generation, a finding which was attributed to an incomplete transformation to arsine. It is well known that the arsine yield is highly dependent on the degree of oxidation of the inorganic arsenic.

The need to eliminate the nitrous vapours using ammonium oxalate or an acid mixture of sulfuric and perchloric acids was also discussed. One laboratory pointed out that no differences were found if the sample was dried or wet attacked and another laboratory pointed out the difficulty of determining arsenic when HF was used for the sample attack.

**Cadmium determination**

The best results were obtained by using ETAAS and ASV. It was considered that the cigarette smoke was responsible for laboratory contamination; this was especially important in the analysis of the lyophilized sample in which the content of Cd is very low. Participants were reminded that smoking is absolutely forbidden in a trace analysis laboratory.

**Copper determination**

The results obtained for copper were in general good. No special difficulties were observed in the determination of this element.

**Chromium determination**

The participants recognized that the determination of traces of chromium by FAAS and ETAAS is difficult. The risk of sample contamination if a steel knife is used to open the vials was also emphasized. One participant stated that the best results were obtained by FAAS, using air-acetylene flame and oxine or ammonium persulfate as releaser, and pyrolytic furnace and L’vov platform when ETAAS was used.

**Lead determination**

Two different groups of results were observed in the first exercise, a finding that was attributed to sample contamination. It was recommended that the measurements by ETAAS should be made using L’vov platform, ammonium phosphate or magnesium nitrate as modifiers and stop flow option. The use of dry attack for lyophilized plant samples was rejected although one laboratory stated that no differences in the results were observed when using either dry or wet attack.

**Mercury determination**

Mercury was considered to be a difficult element to measure because of the possible contamination as was shown for cadmium due to the laboratory environment. The highest values were correlated with highest standard deviation in some cases, and volatilization during acid attack were observed by other participants. Some laboratories obtained higher sensitivity when using stannous chloride instead of sodium borohydride as reducing agent. The low levels in the lyophilized samples hampered its determination by many laboratories.

**Nickel determination**

Highest variation coefficients were observed when ETAAS was used.

Possible losses of nickel were attributed to absorption on the vessel walls and to insufficient digestion. As observed for chromium, contamination could have been due to the use of a steel knife to open the vial.

**Selenium determination**

Few laboratories participated in the determination of this element. In general the participants using ETAAS reported the highest results. No explanations were found from the discussion.

**Zinc determination**

In all the samples the concentration levels of zinc were substantially higher than for other elements. For this element the results obtained were good. Some participants pointed out the problems of contamination from the plastic material used.

**Conclusions**

From the results it can be stated that the performance of Spanish laboratories on trace metal determination is satisfactory.

In general noticeable improvements were observed in the laboratories which have participated in all the exercises.

Moreover a homogeneous group working in close collaboration, have been established.

**References**

FIRST MEDITERRANEAN BASIN
CONFERENCE ON ANALYTICAL
CHEMISTRY

November 5-10, 1995

Córdoba, Spain

Organised by “Grupo Espectroquímico” of the Spanish Royal Society of Chemistry.

The meeting will try to promote the collaboration among scientists from the Mediterranean Sea area and stimulate the progress in Analytical Chemistry in order to solve analytical problems affecting the Mediterranean Basin.

The scientific program topics will focus on education of Analytical Chemistry as well as its application in the environment, agriculture, food analysis, geoanalysis and beneficiation of minerals, biomedical analysis, archeometry and art objects preservation and quality assurance and harmonization of procedures.

Publishers and producers of analytical equipments will be welcome.

Titles of presentations (oral communication and posters) must be submitted before May 30th. Abstracts deadline: June 30th. (1995)

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Short Communication

Rapid IR-spectrophotometric determination of total nitrogen in silicon dioxide - silicon nitride mixtures

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Abstract. Analytical studies were carried out on determination of nitrogen in SiO2-Si3N4 mixtures. An analytical wavelength was selected in the infrared spectrum (575 cm⁻¹), and a rapid method was developed for evaluating the yield of silicon nitride synthesis from silicon dioxide and ammonia. The method is based on nitrogen determination in the reaction mixture. The method is selective in the presence of standard admixtures of lithium and magnesium salts (up to 5 % of initial SiO2 mass).

Key words: Silicon nitride analysis, Nitrogen determination, Infrared spectrophotometry.

Introduction

Silicon nitride is one of the most attractive materials used for manufacture of ceramics of special properties. One of the methods of its synthesis is the reaction of silicon dioxide with ammonia. Optimization of the process requires an analytical control of the product obtained, which is a mixture of SiO2 and Si3N4. The yield of the reaction may be evaluated by determining total nitrogen in the product mixture. The classical methods of analysis, based on melting the sample with hydroxide or dissolving it under pressure in acid mixtures followed by determination of ammonia [1, 2] are accurate but costly and time-consuming. The aim of this work was to develop an analytical method suitable for rapid evaluation of total nitrogen in SiO2-Si3N4 mixtures with the use of commonly applied instrumentation, e.g. infrared spectrophotometer or X-ray diffraction spectrophotometer for phase analysis.

Experimental

Apparatus
Infrared spectra were recorded by means of SPECORD 75IR (Carl Zeiss, Jena, Germany) using standard procedure with KBr tablets. Powder X-ray diffractograms were obtained with the use of TUR M-62 (Germany) apparatus. Samples for classical determination of nitrogen were dissolved in a pressure setup distributed by DHN, Poland.

Reagents
Silicon dioxide, pure (ZCh Radniki, Poland). Silicon nitride, pure (Stark, Germany). Potassium bromide, spec. pure (Fluka, Switzerland).
Sodium tetraborate, saturated solution.
Hydrochloric acid, 0.2000 M standard solution.
Sodium hydroxide, 0.2000 M standard solution.

Procedure for classical determination of nitrogen. A weighed sample (0.2-0.3 g) of SiO2-Si3N4 mixture was placed in a polytetrafluoroethylene (PTFE) vessel, to which 2.5 ml of concentrated H2SO4 and 10 ml of concentrated HF were then added. The closed vessel was placed in the brass container, which was then carefully screwed up and heated for 48 h at 150 °C. After cooling, the content of the vessel was diluted with 20 ml of sodium tetraborate solution, transferred to a 100-ml polyethylene calibrated flask and filled with the sodium tetraborate solution to the mark. An aliquot of 25 ml was transferred to a Kjeldahl's distilling apparatus, to which...
Rapid IR-spectrophotometric determination of total nitrogen in silicon dioxide - silicon nitride mixtures

Figure 1. Infrared spectra of: (a) silicon dioxide (1.3 mg + 220 mg KBr); (b) silicon nitride (1.0 mg + 205 mg KBr); (c) silicon dioxide-silicon nitride mixture (36.5 % N, 1.2 mg + 210 mg KBr).

20 ml of water was added, 10 ml of the hydrochloric acid solution was poured into the receiver and the condenser outlet was immersed in it. The nitrogen flow was opened, 20 ml of 20 % sodium hydroxide solution was added and distillation was carried out for 60 min. The excess of unneutralized acid in the receiver was titrated with the sodium hydroxide solution.

Procedure for nitrogen determination by infrared spectrophotometry. A weighed sample of SiO₂-Si₃N₄ mixture (1.0-1.5 mg) was ground with 200 mg KBr, pressed into tablet and placed in the IR spectrophotometer. The absorption spectrum was recorded in the region 2000-400 cm⁻¹ and the transmittance at 575 cm⁻¹ and 1450 cm⁻¹ was measured. The relative absorbance \( A = \log \frac{T_{1450}}{T_{575}} \) was calculated and the content of nitrogen in the sample was found with the aid of calibration curve.

Results and discussion

A former work aiming at determination of the compositions of Al₂O₃-AlN mixtures enabled to develop a rapid analytical method for nitrogen determination based on measurements of intensities of X-ray beams reflected [3]. Preliminary attempts of applying that method for determination of composition of SiO₂-Si₃N₄ mixtures failed. The amorphous mixture of one or both components [4] (following the synthesis) does not allow analytically useful X-ray patterns to be obtained.

Careful analysis of infrared absorption spectra of pure substances and their mixtures (Fig. 1) showed, that the ratio of signal intensity at 575 cm⁻¹ and that corresponding to the background (1450 cm⁻¹), where no signal of SiO₂ or Si₃N₄ is observed, is in proportion to the content of nitrogen in the mixtures. The relative absorbance was calculated from the formula:

\[
A = \log \frac{T_{1450}}{T_{575}}
\]

where: \( T_{1450} \) is the transmittance of the background at 1450 cm⁻¹ and \( T_{575} \) is the transmittance of the analytical band at 575 cm⁻¹.

Analyses of SiO₂-Si₃N₄ mixtures of different composition showed that the values of the parameter \( A \) are enclosed in the area between two parallel lines (Fig. 2). The contents of nitrogen in the samples were also
determined volumetrically after dissolving in acids and distillation of ammonia. It has been found that an analytically useful signal in the spectrum does not appear as for nitrogen contents in the sample above 20%. Lower contents cannot be determined, but this fact is meaningless for evaluation of the yield of silicon nitride synthesis, in which the product should contain near 40% of nitrogen.

The proposed method enables quick estimation (about 0.5 h) of nitrogen content in SiO₂·Si₃N₄ mixtures. Although it cannot serve as a mean for precise analysis of nitride preparations, it may be successfully employed for preliminary evaluation of the yield of their synthesis. Data concerning the precision of the method (evaluation of A values) in analysis of materials of different composition have been shown in Table 1. The method may also be used for determination of nitrogen in reaction mixtures containing additions of other salts (e.g. lithium or magnesium nitride) in amounts up to 5% with respect to initial mass of silicon dioxide.

References

Planes de Estudio

La Química Analítica en los nuevos planes de estudio de la Universidad de Valencia

Con esta breve noticia de la situación actual de las enseñanzas de Química Analítica en los nuevos planes de estudio de la Universidad de Valencia, tratamos de abrir una sección en las páginas de la revista Química Analítica para que ésta se convierta en el órgano de difusión de las experiencias y propuestas de los departamentos de Química Analítica de las distintas universidades españolas.

Por ello, desde aquí, y siguiendo las directrices de la Junta Directiva de la SEQA, os rogamos a todos los compañeros que ya tenéis en marcha los nuevos planes de estudio que envieis noticias sobre las materias obligatorias u optativas de nuestra disciplina que se estén impartiendo o que hayan sido aprobadas ya por los órganos correspondientes.

La Química Analítica está presente en los nuevos planes de estudio de la Universidad de Valencia en los títulos de: Licenciado en Química, Licenciado en Biología, Ingeniero Químico, Licenciado en Bioquímica y Licenciado en Farmacia.

Las tres primeras titulaciones constan de primer y segundo ciclo y ya están en su segundo año de aplicación de los nuevos planes de estudio. La de Bioquímica consta solo de segundo ciclo, está pendiente de su aprobación final y se iniciará el curso 1996/97, y la de Farmacia todavía se encuentra en fase de preparación. Todas ellas, están estructuradas en cuatrimestres y sobre la base de cinco años de estudios.

Dado que las materias troncales son comunes en todas las universidades y obedecen a las directrices publicadas en el BOE, a continuación se comentarán únicamente los créditos y descriptores de las materias obligatorias y optativas de cada título.

Licenciado en Química

En el primer ciclo, se incluyen además de las materias troncales, una materia obligatoria y tres optativas.

“Análisis instrumental”, es una materia de carácter obligatorio, que se imparte en el sexto cuatrimestre, consta de dos módulos, uno de 4 créditos teóricos y otro de 1 crédito de problemas. Se incluyen los métodos electroanalíticos, espectroscópicos y cromatográficos. (Hay que tener en cuenta que hay una materia troncal: “Introducción a la experimentación en Química Analítica III” (2 créditos) en la que se imparten las enseñanzas prácticas correspondientes al análisis instrumental).

“Técnicas de trabajo y seguridad en el laboratorio”, con dos módulos de 2.5 créditos (teoría) y 1.5 créditos (prácticas), es una materia optativa que se imparte en el tercer cuatrimestre en la que se trata la toxicidad de las sustancias, su manipulación, etc.

“Preparación y tratamiento específico de muestras” con dos módulos de 1.5 créditos (teoría) y 1.5 créditos (prácticas) es una materia optativa y se imparte en el cuarto cuatrimestre, incluyéndose contenidos acerca de las operaciones previas del análisis, selección de ácidos y disolventes, sistemas clásicos de digestión, tratamiento en hornos convencionales y de microondas, etc.

“Química analítica en disolventes orgánicos”, con 2 módulos de 2 créditos (teoría) y 1 crédito (prácticas) es también optativa y se imparte en el quinto cuatrimestre”, incluyéndose los equilibrios ácido-base y redox, la extracción, las reacciones catalíticas en disolventes orgánicos, etc.

Como optativas de segundo ciclo se incluyen 15 materias: “Espectrometría analítica” (5 créditos teóricos y 2 prácticos), que incluye espectrometría atómica y molecular; “Métodos de separación” (3 créditos teóricos y 1 práctico), dedicada solo a los métodos clásicos; “Análisis mediante reactivos bioquímicos (4 créditos teóricos), en la que se trata del uso de enzimas y otros reactivos; “Laboratorio de análisis aplicado” (6 créditos prácticos); “Electroanálisis” (4 créditos teóricos y 1 práctico); “Análisis cromatográfico” (5 créditos teóricos y 1 práctico); “Química analítica medioambiental” (4 créditos teóricos y 1 práctico), sobre aplicación al análisis...
atmosférico, de Aguas y Sedimentos y la evaluación del impacto medioambiental; “Química clínica analítica” (4 créditos teóricos) relativa a los métodos analíticos de interés en muestras clínicas; “Análisis orgánico” (2 créditos teóricos y 1 práctico), incluye análisis de grupos funcionales cualitativo y cuantitativo; “Análisis, homologación y control de calidad” (4,5 créditos teóricos y 1,5 prácticos), en donde se explican los métodos y se tratan algunos aspectos estadísticos de las GLP; “Análisis químico agroalimentario” (4 créditos teóricos y 1 práctico), de Aguas, bebidas, lácteos, cereales, alimentos preparados, aditivos, etc.; “Microanalisis y análisis de superficies” (4 créditos teóricos y 1 práctico), sobre microscopía electrónica, XPS, PIXE, EELS, etc.; “Análisis radioquímico” (3 créditos teóricos y 1 práctico); “Experimentación instrumental avanzada” (6 créditos prácticos) relacionada con la materia troncal correspondiente; y “Análisis de silicatos y productos cerámicos” (4 créditos teóricos).

Licenciado en Biología

En el primer ciclo solamente hay una materia optativa de Química Analítica: “Técnicas de análisis químico” con 4,5 créditos prácticos y 1,5 créditos teóricos en que se tratan contenidos de análisis clásico e instrumental.

En el segundo ciclo, se incluye la materia optativa “Química Analítica Ambiental”, similar a la de la licenciatura en Química, con la misma distribución de créditos.

Ingeniero Químico

En el tercer cuatrimestre se imparte la “Química Analítica”, que es obligatoria, con 4,5 créditos teóricos y 1,5 de problemas y la “Experimentación en Química Analítica” con 2,5 créditos.

Las materias optativas son: En primer ciclo, “Análisis Instrumental” con 5 créditos teóricos y 2 prácticos. En segundo ciclo, “Análisis, homologación y control de calidad” similar a la de Química; “Análisis industrial”, con 4,5 créditos teóricos y 1,5 créditos prácticos.

En la licenciatura en Bioquímica, se han propuesto las siguientes materias optativas: “Técnicas en microanálisis” con 3 créditos teóricos y 2 prácticos; y las materias “Química Analítica medioambiental” y “Química clínica analítica” similares a las de la licenciatura en Química.

En estos momentos todavía no está estructurado el nuevo plan de estudios de la licenciatura en Farmacia, pero probablemente además de la materia troncal denominada “Técnicas analíticas” compartida con el departamento de Química Física, se podrían ofertar dos optativas propias de la Facultad de Farmacia “Análisis y control de calidad en medicamentos” y “Métodos analíticos instrumentales” y algunas de las optativas de la licenciatura en Química.

En resumen, la Química Analítica ha sido bien acogida en el nuevo plan de estudios de la licenciatura en Química, habiéndose aceptado un gran número de materias optativas y el análisis instrumental como materia obligatoria, a pesar de que nuestro departamento está ausente de las enseñanzas de Química General, excepto en la Licenciatura en Física (en donde se imparte una materia optativa, con adscripción común a todos los departamentos de química).

Por el contrario, en las licenciaturas en las que existe una escasa o nula troncalidad de Química Analítica, ha sido muy difícil introducir materias de nuestra disciplina.

En estos momentos, todavía se puede evaluar las ventajas e inconvenientes que los nuevos planes de estudio tienen sobre la formación de los estudiantes y la organización académica, aunque sí se puede apuntar que el mayor grado de libertad de los estudiantes a la hora de escoger su propio currículum, les dota de un protagonismo mayor en su formación, ya que tienen como contrapartida los problemas que se crean a nivel de organización académica y compatibilidad de horarios.

Miguel de la Guardia y Amparo Salvador
News

XXV Reunión del W.P.A.C.

El día 12 de Junio de 1994 tuvo lugar en Toledo la "XXV Reunión del WPAC", que contó con la participación de 17 delegados de las correspondientes Sociedades de Química europeas, en representación de 14 países a la que se sumaron otros 8 miembros, en calidad de observadores o invitados en representación de diversos países europeos, africanos y australianos.

El presidente del WPAC, Prof. R. Kellner informó, entre otras cosas:

- De la publicación de la "Columna Analítica" en varias revistas nacionales e internacionales, incluyendo una traducción en chino.
- De la excelente acogida que ha tenido, por parte del Consejo de la F.E.C.S., reunido en Smolenice el 17 de marzo de 1994, el Informe Anual presentado por el WPAC, así como de modo especial su iniciativa en relación con el "EUROCURRICULUM", considerando que la actividad, enfoque y dinamismo mostrados por el WPAC constituye un modelo a imitar por los restantes Grupos de Trabajo de la F.E.C.S.
- Del nombramiento previsto, en calidad de presidente de la FECS a favor del Prof. Niinistö, ex-presidente del WPAC
- Del otorgamiento al Prof. H. Malissa de una distinción especial de la F.E.C.S.
- Del ingreso de nuevos miembros en la FECS, pendiente de confirmación en septiembre próximo, de nuevos países: Croacia, Lituania, Ucrania, así como la Sociedad Catalana de Química, Egipto y Turquía adquirirán la condición de miembros correspondientes.
- El Prof. E. Pungor, fue distinguido con el ofrecimiento de un diploma honorífico del WPAC, firmado por los Sres. Presidente y Secretario.
- La conferencia internacional, celebrada en El Cairo en enero 1994, resultó un gran éxito.
- La F.E.C.S. está contemplando iniciativas para integrar Sociedades Europeas correspondientes a diversas ramas de la Química, abiertas a miembros individuales. Se trata de una iniciativa que debe ser estudiada cuidadosamente para evitar que se puedan producir situaciones indeseables.

En ausencia del Prof. Burns, el Dr. Newman presentó un informe sobre el "EUROANALYSIS VIII", celebrado en Edimburgo (5-11 septiembre, 1993) indicando, entre otras cosas, que las Actas del mismo, que contienen unos 25 artículos, están próximas a publicarse.

El Prof. Zambonin informó sobre el progreso de los preparativos de "Euroanalysis IX", a celebrar en Bolonia en 1996.

El Prof. Kellner informó sobre el progreso del Grupo de Estudio sobre Educación, el proyecto "EUROCURRICULUM", que ha merecido muy favorables comentarios después de haber sido publicado y comentado en la revista "Analytical Chemistry". Igualmente informó sobre su proyecto de enviar un impreso-circular a todas las universidades europeas para realizar un sondeo sobre la adecuación del "Eurocurriculum" a los enfoques y programas actualmente vigentes en la enseñanza de la "Química Analítica Avanzada" en Europa.

Además, el Prof. Kellner informó de la recepción de aproximadamente el 80% del material correspondiente al Libro de Texto "WPAC", que se prevé tener editado y podrá ser presentado con ocasión de la celebración del "EUROANALYSIS IX" en 1996 en Bolonia. Finalmente, informó el Prof. Kellner sobre la "III Conferencia Internacional FECEM sobre Filosofía, Historia y Educación en Química Analítica" que tendrá lugar en Viena en los días 6-7 de octubre de 1995, presentando un programa provisional de la misma.

El Dr. B. te Nijenhuis presentó un informe sobre el progreso realizado por el Grupo del WPAC "Quality assurance and accreditation", enfatizando su relación con EURACHEM, la representación del WPAC en la estructura mun-
El Prof. Scollary informó sobre la situación de la Química Analítica en Australia al igual que lo hizo el Prof. Dachraoui sobre la Sociedad Química de Túnez y el Prof. Van Staaden informó sobre la situación en África del Sur.

La Prof. Khater resumió brevemente el satisfactorio resultado obtenido en la Conferencia de El Cairo indicando que las correspondientes Actas se hallan en proceso de edición y que la próxima Conferencia tendrá lugar en el año 1997.

El WPAC acogió receptivamente la propuesta de celebrar Reuniones Regionales y Encuentros Especiales existiendo una iniciativa para una Conferencia de Países Mediterráneos en el momento actual.

Se suministró información sobre otras actividades del WPAC, ya celebradas, o de próxima celebración en Florencia ("EUROANALYSIS"), Essen ("EUCMAS XII"), Matrafűred ("Electrochemical Sensors"), Costanza ("XII Conferencia de Química Analítica"), Hull (preparatorios para la Conferencia SAC), así como sobre otras iniciativas patrocinadas por el WPAC ("26th International Symposium Environmental Analytical Chemistry", Viena 1996; "5th Conference on Analytical Chemistry", 4-10 septiembre 1995, Danzig (Polonia).

La próxima reunión anual del WPAC ("Encuentro XXVI") tendrá lugar en Viena el 8 de octubre de 1995, en conexión con el ya citado Simposio FEChem de Educación.
Book Reviews


Se trata de una obra que resalta los aspectos fundamentales del "laboratory information management systems (LIMS)" y las etapas necesarias para poner a punto estos sistemas. Con acierto se abarcan las actividades de validación y la directa relación e implicaciones de los LIMS en los sistemas de Garantías de Calidad. El enfoque es general y cubre la práctica totalidad de consideraciones que se plantean al introducirse en el conocimiento de estos sistemas y su posible implantación en el laboratorio. A veces, sin embargo, resulta un tanto escueto ("telegráfico") su exposición. Los apéndices son muy útiles y confieren a la obra una gran profundidad. Primero, una bibliografía complementaria muy exhaustiva y actualizada, que permite una valiosa ampliación de la información proporcionada por el libro. Segundo, un glosario de términos y siglas que ayuda a entender mejor la literatura característica de este tipo de obras. Por último, una lista amplia de vendedores de LIMS y una presentación documentada (comparativa) de las características de los LIMS de las 16 firmas comerciales más representativas.


Es una obra multiactor que aborda diferentes aspectos del análisis medioambiental y desde diferentes puntos de vista. Las aportaciones de los expertos participantes en los capítulos se han dividido en cuatro grupos, que constituyen la estructura del libro, tan difícil de organizar en obras como esta. Uno de los hitos fundamentales que subyace a lo largo del material que se presenta es el Control de Calidad de las técnicas analíticas implicadas en el estudio de la contaminación medioambiental. Resulta una obra útil como referencia general para post-graduados así como de consulta práctica para los analistas que trabajan en el campo de la contaminación ambiental.


Contenido: Medida y detección basadas en el empleo del láser en microseparaciones de alta resolución. Detección luminiscente en cromatografía de líquidos. Detección quimioluminiscente en HPLC. Detección en el IR próximo en HPLC. Detección electroquímica en cromatografía líquida. Detectores fotoeléctricos para HPLC. Uso de IR-FT para la detección en HPLC. Espectrometría de masas para la detección en HPLC. Acorrelación en línea de RMN del proton en HPLC.

El libro, que es multiactor, proporciona una recopilación de técnicas de detección útiles y poco convencionales en HPLC. La principal aportación es demostrar que las posibilidades de la cromatografía líquida pueden ser claramente ampliadas con la incorporación de estas técnicas de detección más recientes. El propósito no es presentar sistemáticamente los diferentes tipos de detección en HPLC, a los que se remite a otras obras ya existentes en la actualidad. La orientación del libro es, fundamentalmente, a cromatógrafos, ya habituados con el uso de las técnicas convencionales de detección, que pueden encontrar soluciones o mejorar sus métodos de trabajo introduciendo estas nuevas técnicas. Puede ser también de utilidad como material complementario en cursos de post-grado. Aunque no excesivamente amplio, está -en general- bien documentado con ejemplos y múltiples ilustraciones que apoyan las explicaciones teóricas e instrumentales.
El libro (multiautor) recoge una serie de temas que fueron impartidos en los cursos “Recientes Desarrollos en el Laboratorio Analítico de Control”, celebrados en las Universidades de Córdoba y Málaga y organizados por el Grupo Regional Andaluz de la SEQA. Constituye una muestra significativa de los últimos avances en el laboratorio analítico, con una orientación fundamentalmente docente, sin olvidar del todo la vertiente profesional, al tratarse de temas de vanguardia. El libro se organiza en cuatro bloques de temáticas diferenciadas, combinando aspectos más circunscritos al campo de la investigación con técnicas ya establecidas. En el primer bloque se presentan dispositivos y elementos instrumentales recientemente implantados en los laboratorios o que representan una clara tendencia. En el segundo bloque se incluyen varias técnicas de interés general. En el tercero, técnicas de separación más recientes; y en el último -más heterogéneo- se incluye la especificación y los métodos quimiométricos para el análisis de multicomponentes.

Angel Ritos

Errata

Spatial and temporal variability of trace metal concentrations in North Sea sediments
Michael Kersten, Ina Bendler, Wolfgang Kienz, Volker Klatt
Química Analítica, volume 13, supplement 1, 1994, pp S57-S63

The following Figure was omitted on page S61:

Figure 3. Change in concentration in topmost (5 cm) sediment layer in percent of initial value as function of time (months) since flux have been increased by 30%, 50%, 100% and 200%, respectively. Preconditions were a fixed sedimentation rate (1 cm a-1) and an instant homogenization process to a mixing depth of 5 cm. Dotted line indicate detection limit for analytical + spatial dis = 20%.
Congresses

Abril 1995


* 6th International Symposium on Pharmaceutical and Biomedical Analysis. 23-26 Abril, St. Louis, MO. Contacto: Shirley E. Schlessinger, Suite 1015, 400 E. Randolph Dr., Chicago, IL. 60601.

* Symposium on Validation Practice for Biotechnology Products. 24-25 Abril, Gaithersburg, MD. Contacto: James K. Shillenn, Bioprocessing Resource Center, Penn State University, 519 Wartik Laboratory, University Park, PA 16802-9959. Fax: 814-863-1357.


Mayo 1995

* 7th Symposium on Handling of Environmental and Biological Samples in Chromatography. 7-10 Mayo, Lund, Suecia. Contacto: M. Frei-Hausler, JAEAC Secretariat, Postfach 46, CH-4123 Allschweil 2, Suiza. Fax: 41-61-4820805.


* 187th Meeting of the Electrochemical Society. 21-26 Mayo, Reno, NV. Contacto: Meetings Dept, The Electrochemical Society, 10 S. Main St, Pennington, NJ 08534-2896 (609-737-1902).

* 43rd ASMS Conference on Mass Spectrometry and Allied Topics. 21-26 Mayo, Atlanta, GA. Contacto: ASMS, 1201 Don Diego Ave., Santa Fe, NM 87501. Fax: 505989-1073.


Julio 1995


Agosto 1995

* Colloquium Spectroscopicum Internationale XXIX. 27 Agosto-1 Septiembre, Leipzig, Alemania. Contacto: Gesellschaft Deutscher Chemiker, Abt. Tagungen, P.O. Box. 900440 D-60444 Frankfurt/Main, Germany.

* 46th Annual Meeting of the International Society of Electrochemistry. 27 Agosto - 1 Septiembre, Xiamen, China. Contacto: Secretariat 46th ISE Annual Meeting, P.O. Box 1995, Xiamen University, Xiamen 361005 China. Fax: 86-592-208-5349.

Septiembre 1995


* BIOS Europe '95. Conference on Medical Sensors III and Fibre Optic Sensors II. 12-16 Septiembre, Barcelona, Spain. Contacto: c/o Direct Communications GmbH, BIOS Europe '95, EUROPTO, Xantener Strasse 22, D-10707 Berlin, FRG. Fax: 49-30-8815040.

* Electrochem '95. 10-14 Septiembre, Bangor, Gales. Contacto: Electrochem '95 Conference Department SCI 14/15 Belgrave Square, London SW1X 8PS. Fax: 171-823-1698.

Octubre 1995


* International The Sixth Beijing Conference and Exhibition on Instrumental Analysis. 24-28 Octubre, Pekin, China. Contacto: General Service Office, The International BCEIA, Room 585, Building of CAS (Chinese Academy of Science) San Li He, Xi Jiao, P.O. Box 2143, Beijing 1000445, China.

1996

* 4th International Symposium on Hyphenated Techniques in Chromatography HTC4. 6-9 Febbrero, Brujas, Bélgica. Contacto: Royal Flemish Chemical Society, c/o Dr. R. Smits, BASF Antwerpen N.U. Control Laboratorium, Scheldelaan 600, Haven 725, B-2040 Antwerpen, Belgium. Fax: 32-3-561-3250

* ESEAC'96. 6th European Conference on Electroanalysis 25-29 Marzo, Durham, Inglaterra. Contacto: Prof. A.K. Covington, Electrochemistry Research Laboratories, Department of Chemistry, University of Newcastle, Newcastle upon Tyne NE1 7RU, U.K. Fax: 44-91-2226929

* CAC'96. Chemometrics in Analytical Chemistry. 25-29 Junio, Tarragona, España. Contacto: Departament de Química, Universitat Rovira i Virgili, Pl. Imperial Tarraco 1, E-43005 Tarragona, Spain. Fax: 34-77-55 95 97.

* Euroanalysis IX, 1-7 Septiembre, Bolonia, Italia. Contacto: Prof. L. Sabbatini, Università di Bari, Via Orabona 4, 70162 Bari, Italy.

J.M. Pingarrón
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