UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Facultade de Química

Departamento de Química Analítica, Nutrición e Bromatoloxía

Uso de polímeros de impronta molecular para la simplificación del análisis de mercurio total y metilmercurio

Memoria para optar al grado de Doctor que presenta

Roi Rodríguez Fernández

Santiago de Compostela, Febrero 2017


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INFORMAN

Que la presente Tesis Doctoral, titulada "Uso de polímeros de impronta molecular para la simplificación del análisis de mercurio total y metilmercurio" ha sido realizada bajo nuestra dirección por Roi Rodríguez Fernández en el Departamento de Química Analítica, Nutrición y Bromatología de la Universidad de Santiago de Compostela.

Y para que así conste firman la presente en Santiago de Compostela, a 24 de Febrero de 2017

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Que la Tesis presentada por Roi Rodríguez Fernández es idónea para ser presentada, de acuerdo con el Artículo 41 del Reglamento de Estudios de Doctorado de la Universidad de Santiago de Compostela, por la modalidad de COMPENDIO DE ARTÍCULOS, en los que el investigador en formación tuvo participación en el peso de la investigación y su contribución fue decisiva para llevar a cabo este trabajo.

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Agradecimientos

A mis directoras, Elena y Pilar, por su dedicación y sobre todo por su paciencia. Por tantas frases necesarias llenas de ánimo y optimismo. Por creer en mí y en mis posibilidades. Por su preocupación, y el empeño que ponéis en vuestro trabajo, que sigue siendo formar personas. Nunca es uno demasiado viejo para seguir aprendiendo cada día, sobre todo si tiene los mejores maestros.

A Antonio, Raquel, Mari Carmen, Manolo, Paloma y Cruz, por estar siempre, siempre disponibles para echar una mano, resolver una duda o echar una bronca cuando hacía falta, lo más necesario si cabe.

Al departamento de analítica, en especial al LIDSA por su apoyo moral y ~~bombones~~ logístico, y a María Celeiro porque siempre podía ir a saludarla y contarle mis penas, además de apuntarse siempre a las convocatorias fallidas con nuestros compañeros del máster.

A todo el grupo de maGETEEs, por tantas mañanas y tardes de risas y buenos momentos. Todos esos "coffe break" interminables resolviendo el mundo. Todos esos congresos donde había tiempo tanto para conocer gente maravillosa como para conocer la gastronomía local… o sus locales. Todas esas horas en el laboratorio, esos días en los que te marchabas a última hora sin querer volver y que al día siguiente regresabas el primero. Nuestras cañas y nuestras cenas, las performance en directo entre experimento y experimento. A Ángel, Brais, Julia, Nati, Vane, Martha y Cris, que ya no estáis porque unos encontrasteis vuestro camino y las chicas ya sois doctoras. A Pili, Ala', Iria y Vanesa, porque pronto os encontraréis ahí. A Juan, porque he sido testigo de todo el proceso que te ha llevado a convertirte en Doctor, quién lo diría hace años, cuando nos peleábamos con apuntes de materias incomprensibles y tú rasgabas la guitarra en Ycia igual que haces ahora en los Mouchos. Al fin pudimos alcanzar esta cima los dos, a hombros de gigantes.

A la *Comunidade Lexendaria*, en especial a todos los que compartimos el piso de Pasaje, porque cada vez que echo la vista atrás y recuerdo todo lo vivido, volvería a matricularme en la USC mil veces sólo por volver a compartir una pequeña fracción de esos momentos. Tantas fiestas, tantas horas en la Conchi que derivaban en incontables cafés a lo largo del día de "estudio", a ese culto al deporte, traducido en la formación de escuadras irrepetibles como el *Tuercebotas a.k.a. Raíces Galegas F.S.,* nuestro equipo de básquet cuyo nombre no reproduciré aquí o aquel intento de Rugby 7. Quizá algún día regresemos a Ons, o en una churrascada en Aldán me lleve un trofeo por ganar un *comunio*, que no creo. Pero me conformo con tener la suerte de mantener este fantástico grupo humano. Gracias.

A mis compañer@s de SOGARISA, primero por confiar en mí por dos veces para trabajar con vosotros, y segundo por formar la mejor plantilla de compañeros que uno puede tener en su trabajo, por no perder nunca la sonrisa y conseguir que a pesar de haber tenido que madrugar a las 6:30 uno se levantase siempre con ganas de llegar.

A mis amigos de toda la vida, Fernando, Adrián, Eduardo, Santi y David. Podemos llevar meses sin vernos y recibirnos siempre con un abrazo. Sé que siempre estaréis ahí.

Ós meus país e á miña irmá, porque se hai algo de proveito na miña persoa e só grazas a todo o que me ensinastes. Esta tese vai dedicada a vós, pero só é unha mínima parte da enorme débeda que teño convosco e que nunca terei tempo suficiente para agradecelo.

A Marta, mi guía y mi compañera. Desde que te conocí siempre has estado ahí para empujarme en la dirección correcta. Eres mi apoyo incondicional, mi voz de la conciencia, la imagen mental de mis canciones, desde *Extreme* hasta *Sidecars*. El sitio de mi recreo. Una tesis es un suspiro estando a tu lado. Por ésta y por tantas otras "tesis" que nos depare la vida, pero que sean junto a ti.

> *"Un científico en su laboratorio no es sólo un técnico: es también un niño colocado ante fenómenos naturales que le impresionan como un cuento de hadas."*
>
> **Marie Sklodowska-Curie**


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Introducción

Parte Experimental

Lista de acrónimos

AAS	*Espectroscopía de Absorción Atómica*
AFS	*Espectroscopía de Fluorescencia Atómica*
AIBN	*2,2'-Azobis-isobutironitrilo*
CCD	*Detector de Acoplamiento de Carga*
CILE	*Electrodo de Pasta de Carbono modificado con Líquido Iónico*
CME	*Electrodos Modificados Químicamente*
CNT	*Nanotubos de Carbono*
CPE	*Electrodo de Pasta de Carbono*
CRM	*Material de Referencia Certificado*
CV	*Vapor Frío*
DAD	*Detector de Array de Diodos*
DVB	*Divinilbenceno*
EDMA	*Etilenglicol dimetacrilato*
EDXRF	*Fluorescencia de Rayos X de energía dispersiva*
ETAAS	*Espectroscopía de Absorción Atómica ElectroTérmica*
EtHg	*Etilmercurio*
ETV	*Vaporización ElectroTérmica*
FIA	*Flow Injection Analysis*
FIMS	*Sistema de análisis de Mercurio por Inyección en Flujo*
FT-IR	*Espectroscopía Infrarroja por Transformada de Fourier*
GC	*Cromatografía de Gases*
GC	*Cromatografía de Gases*
GCE	*Electrodo de Carbono Vítreo*
HPLC	*Cromatografía Líquida de Alta Resolución*
HRCSAAS	*Espectroscopía de Absorción Atómica de Fuente Continua de Alta Resolución*
ICP-MS	*Espectrometría de Masas – Plasma de Acoplamiento Inductivo*
ICP-OES	*Espectrometría de Emisión Óptica – Plasma de Acoplamiento Inductivo*
IIP	*Polímero de Impronta Iónica*
LLE	*Extracción Líquido-Líquido*
LOD	*Límite de Detección*
LOQ	*Límite de Cuantificación*
MAA	*Ácido Metacrílico*

MAC	*Metacriloil-(L)-cisteína metilester*
MeHg	*Metilmercurio*
MIP	*Polímero de Impronta Molecular*
MSPD	*Dispersión de Matriz en Fase Sólida*
NIP	*Polímero Blanco (no improntado)*
NPs	*Nanopartículas*
PE	*Polietileno*
PFTE	*Politetrafluoroetileno*
QD	*Quantum Dot*
RGO	*Óxido de Grafeno Reducido*
RP	*Fase Reversa*
SEM	*Microscopía Electrónica de Barrido*
SPE	*Extracción en Fase Sólida*
TAR	*4-(2-thiazolyazo) resorcinol*
TMPTM	*trimetilolpropanotrimetacrilato*
TRIM	*Trimetil propano trimetacrilato*
UATR	*Reflectancia Total Atenuada Universal*
VP	*Vinilpiridina*
VTCA	*Ácido 3-metil-5-(metiltio)-4-viniltiofeno-2-carboxílico*
XRF	*Fluorescencia de Rayos X*

CAPÍTULO I

Análisis y especiación de mercurio. Polímeros de impronta molecular y quantum dots


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Resumen

Existe un gran número de especies de mercurio dentro de los medios naturales, y la forma química controla su biodisponibilidad, transporte, persistencia y su impacto en el cuerpo humano, siendo el metilmercurio la especie más tóxica. Debido a su elevada bioacumulación, las concentraciones de mercurio escalan a lo largo de la cadena alimentaria y, por ejemplo, pueden encontrase concentraciones varios órdenes de magnitud más elevadas en pescados depredadores de gran tamaño que los existentes en las aguas naturales, estando la mayoría de este mercurio presente en los organismos en forma de metilmercurio.

Debido a esto último, se hace especialmente necesario desarrollar técnicas no sólo para el análisis de mercurio sino para la determinación de sus especies. Mediante la síntesis y el empleo de polímeros de impronta molecular (MIPs), se consiguen materiales altamente selectivos a la molécula a determinar, que pueden ser utilizados en cromatografía y en procesos de extracción en fase sólida.

La gran afinidad y selectividad de estos materiales permite su empleo en la modificación de otros materiales adsorbentes o sensores electroquímicos u ópticos. El uso de nanopartículas con propiedades ópticas, como el caso de los quantum dots, modificados con polímeros de impronta molecular, es una gran herramienta que combina la gran sensibilidad de estas nanopartículas fluorescentes con la afinidad de los MIPs por el analito en cuestión.

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1. Análisis y especiación de mercurio. Polímeros de impronta molecular y quantum dots

1.1 Importancia de la determinación de mercurio y sus especies

La presencia de mercurio en el medio ambiente se ha incrementado significativamente durante los últimos años debido a la actividad humana, incluyendo la utilización de combustibles fósiles, la incineración de residuos, la minería de oro, y otras aplicaciones. El mercurio es liberado principalmente como Hg(0) a la atmósfera por fuentes naturales como la actividad volcánica o geotérmica, y sobre todo en sus formas inorgánicas por fuentes antropogénicas. Estudios recientes indican que la actividad humana constituye un 50-70% de la emisión total al medio ambiente, provocando una enorme contribución al ciclo global del mercurio [1].

La toxicidad del mercurio depende considerablemente de su forma química, y es bien conocido que los compuestos organometálicos son generalmente más perjudiciales que las especies inorgánicas. Al producirse las conversiones entre formas orgánicas e inorgánicas, se alteran las propiedades físicas y químicas del elemento, afectando a su biodisponibilidad, movilidad y regulando su reparto entre diferentes reservorios en el medio. Estas transformaciones pueden modular la toxicidad y por tanto el impacto para la salud pública y del ecosistema. El Hg tiene una gran facilidad para formar derivados organometálicos, mono y diorganometálicos (RHgX y R_2Hg) estables en medios naturales, biodegradables en algunos casos y peligrosamente tóxicos. La forma orgánica de origen natural, el metilmercurio (MeHg) es un potente neurotóxico [2, 3] debido a que es lipofílica, y su capacidad para unirse a proteínas incrementa su bioacumulación a niveles muy altos en la cadena alimentaria marina, convirtiéndolo en un peligro potencial para la salud y la reproducción de la vida salvaje [4, 5]. El catión metilmercurio (II) ha sido protagonista de alguna de las intoxicaciones masivas por compuestos metálicos más relevantes, como la de la bahía de Minamata en Japón, derivada de la descarga de los residuos industriales de una fábrica que utilizaba $HgCl_2$ y $HgSO_4$ como catalizadores para la producción de cloruro de vinilo y acetaldehído generando metilmercurio como subproducto. Otra grave intoxicación ocurrió en Irak (1972), provocada por la ingestión de harina de trigo contaminada con fungicidas organometálicos, incluyendo metilmercurio y etilmercurio, usados para tratar el grano destinado, en principio, a la siembra, y que originó la hospitalización de 6350 personas, de las que 459 murieron.

La presencia en los medios naturales de estas especies organometálicas complica considerablemente el ciclo natural del elemento (Figura 1) y también su toxicología [6]. La oxidación de Hg(0) es uno de los aspectos menos estudiados del ciclo biogeoquímico del mercurio. La oxidación es un proceso importante que disminuye el nivel mercurio gaseoso disuelto (DGM) en medios acuáticos e incrementa la concentración de Hg(II), el substrato para la metilación. En las aguas superficiales, la oxidación se lleva a cabo gracias a la luz solar, mientras que en aguas más profundas el proceso está dirigido por oxidación microbiana mediante la enzima catalasa. En el medio natural, bajo condiciones aeróbicas, el mercurio inorgánico puede ser reducido a mercurio metálico por la acción bacteriana, acumulado en la cadena alimentaria, complejado con la materia orgánica disuelta en el agua (DOC) o adsorbido por la materia orgánica particulada (POC) (Figura 1). El Hg(II) puede ser transformado a metilmercurio por procesos de metilación provocados por microorganismos, acumulándose también en la materia orgánica y/o pasando a la cadena alimentaria. Mitchell Berman [7] demostró en 1986 que en ambientes anaerobios, las bacterias reductoras de sulfato (SRB), son los principales metiladores de mercurio. Las bacterias reductoras de hierro (FeRB) juegan también un papel importante en la metilación de mercurio en el medio ambiente [8, 9]. Generalmente se acepta que la metilación es un proceso intracelular en las bacterias [10], lo cual implica que el paso del mercurio inorgánico a través de la pared celular es un paso fundamental en el proceso. El ciclo geoquímico del mercurio está íntimamente ligado al ciclo del azufre, y es posible que especies no cargadas como HgS y $Hg(SH)_2$ difundan pasivamente a través de la membrana celular.

En condiciones anaerobias, el mercurio se acumula en forma de HgS por reacciones con el H_2S presente en el medio debido a reacciones microbianas de reducción de sulfatos (Figura 1).

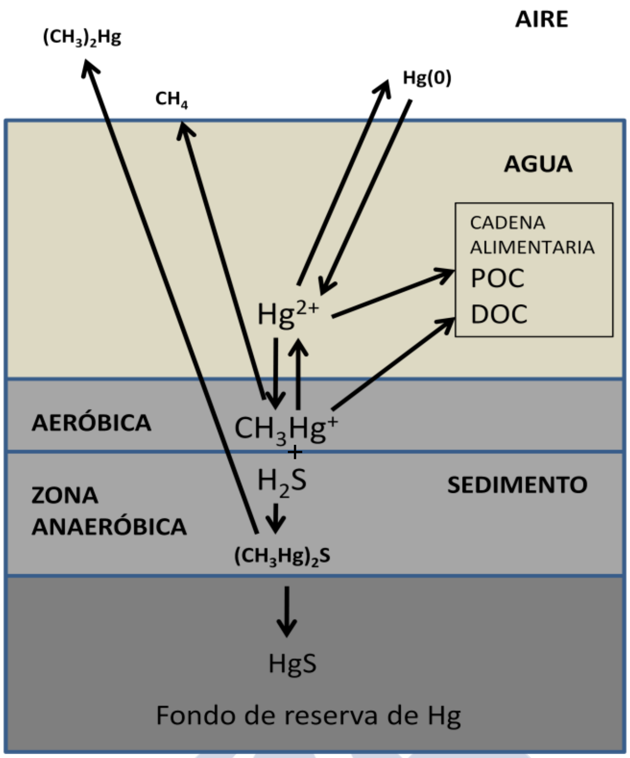

Figura 1. Ciclo bioquímico del mercurio [11].

1.2 Toxicología del mercurio

1.2.1 Toxicocinética de las formas químicas del mercurio

La absorción del Hg(0) líquido por parte del cuerpo humano es mínima, no se produce intoxicación aguda ni cuando el mercurio líquido de los termómetros es ingerido accidentalmente. El problema surge cuando el mercurio líquido se calienta y pasa a fase gaseosa, lo cual causa neumonía intersticial cuando se inhala en alta concentración. Aproximadamente el 80% del Hg(0) inhalado es absorbido por el torrente sanguíneo y traspasa fácilmente la barrera hematoencefálica en su forma no oxidada, alcanzando el cerebro y dañando el sistema nervioso central. Al mismo tiempo, el Hg(0) gaseoso en el cuerpo es oxidado a Hg(II), que se acumula en los riñones y los daña [12]. El tiempo de vida medio biológico del Hg absorbido como vapor en la sangre es de aproximadamente de 2 a 4 días cuando el 90% es excretado a través de la orina y heces. La absorción del Hg(II) a través del tracto digestivo es comparativamente menor. A pesar de esto, una gran ingesta de Hg(II), por ejemplo por ingesta accidental o suicidio, puede causar daños en el sistema digestivo y los riñones, provocando la muerte [12] El metilmercurio se absorbe fácilmente por el tracto digestivo y traspasa fácilmente la barrera hematoencefálica, causando degeneración y disfunción de las células nerviosas [12, 13]. Aunque el etilmercurio y el metilmercurio tienen unas propiedades toxicológicas similares, existen algunas diferencias importantes. Por ejemplo, el etilmercurio se degrada más fácilmente a Hg(II), y se acumula en menor medida en el cerebro si se considera la exposición a dosis equivalentes [14]. La elevada asimilación del metilmercurio por los tejidos parece que es debida a la formación del complejo metilmercurio-cisteína, que es llevado al interior de las células por una proteína transportadora de aminoácidos neutros. El cerebro de los fetos en desarrollo es muy sensible al metilmercurio. Además la concentración en sangre fetal es más alta que en la madre, debido al transporte activo de metilmercurio a través de la placenta [15, 19]. Por esto, los fetos están incluidos en el grupo de mayor riesgo para el metilmercurio, especialmente en poblaciones que consumen grandes cantidades de pescado y productos marinos [15-20].

1.2.2 Efectos tóxicos de la exposición al mercurio

La exposición al mercurio, especialmente al MeHg [21, 22], puede producir daños permanentes en el sistema nervioso, provocando una gran variedad de síntomas como parestesia, ataxia, fallos sensoriales, temblores, visión borrosa, dificultad en el habla y en el oído, ceguera o sordera. Además de ser neurotóxico, puede afectar otros sistemas y secuencialmente provocar efectos adversos, incluidos toxicidad renal, infarto de miocardio, mal funcionamiento del sistema inmunológico y presión sanguínea irregular [20-23].

La gravedad depende mucho de la magnitud de la dosis. Actualmente, episodios de envenenamiento como los de Minamata o Iraq [21] por elevada exposición al mercurio son extraños y los riesgos para la salud para la población se deben más a exposiciones crónicas al MeHg debido al consumo de pescado contaminado y otros organismos acuáticos, particularmente especies de gran tamaño [23]. La epidemia conocida como "Enfermedad de Minamata", con más de 2200 diagnosticados, fue la primera evidencia registrada de severo envenenamiento por metilmercurio causado por contaminación de origen antropogénico en la ciudad de Minamata, en Japón. Fue causada por el consumo de grandes cantidades de pescado y moluscos extremadamente contaminados con metilmercurio arrojado por una planta química [24]. El primer caso fue reportado en 1956, y la descarga de metilmercurio continuó hasta 1968. El nivel de contaminación fue extraordinariamente alto y la concentración de mercurio en pelo en los pacientes se halló en el rango de 96,8 – 705 ppm. Los síntomas principales fueron desordenes neurológicos (ataxia, deterioro sensorial, parálisis)[25].

Se considera que la exposición crónica prenatal al MeHg es la problemática más extendida, ya que interfiere con el crecimiento y la migración de las neuronas y tiene el potencial para causar daños irreversibles en el desarrollo del sistema nervioso central [21]. Debido al consumo materno de pescado con elevadas concentraciones de mercurio, los niños pueden presentar deficiencias en las conexiones sinápticas (bajos cocientes intelectuales, tono muscular anormal y decrecimiento de la función motora) [21]. Horvat y col. [26] encontraron niveles elevados de MeHg en grano de arroz (del orden de 144 ng g^{-1}) en algunas regiones cerca de minas de mercurio en China, pero dicha exposición sería un caso excepcional [27, 28].

1.2.3 Riesgos y beneficios del consumo de pescado para el desarrollo cerebral

El ácido docosahexaenoico (DHA) es importante para el cerebro fetal y para el crecimiento, y su principal fuente es el consumo de pescado por parte de la madre. Sakamoto y col. [29] demostraron que metilmercurio y DHA en la circulación están directamente relacionados. Si el pescado consumido tiene niveles bajos de metilmercurio y altos de DHA, la salud del bebé se verá mejorada, pero si el nivel de MeHg es suficientemente alto, causará deterioros en el desarrollo superiores a los posibles beneficios del DHA. Por tanto, el consumo de pescado es un riesgo para el niño en esta etapa de crecimiento, y debe tomarse como un riesgo potencial, a pesar de los beneficios del pescado en el desarrollo del menor [30].

El límite legal que fija la Unión Europea para el mercurio en pescado es de 1 mg kg^{-1} en peso fresco [31]. De igual modo fija un límite para aguas continentales de 0,07 µg L^{-1} [32]. La EFSA (European Food Safety Authority) actualizó en el año 2012 sus recomendaciones sobre el mercurio estableciendo una ingesta semanal tolerable (Tolerably Weekly Intake , TWI) de mercurio inorgánico de 4 µg kg^{-1} de peso corporal de mercurio inorgánico y de 1.3 µg kg^{-1} para el metilmercurio [33]. El Panel de expertos en Productos Dietéticos, Nutrición y Alergias (NDA) [34] concluyó en 2014 que el consumo de 1-2 raciones de productos marinos y de hasta 3-4 raciones durante el embarazo está asociado a un menor riesgo de mortalidad por problemas coronarios en adultos y a un mejor desarrollo cerebral en niños. Estas recomendaciones están de acuerdo con las existentes en algunos países europeos donde se dan recomendaciones específicas para evitar el consumo de ciertas especies (pez espada, tiburón, atún rojo, lucio) por parte de niños pequeños, embarazadas y mujeres lactantes.

En España, la Agencia Española de Consumo, Seguridad Alimentaria y Nutrición (AECOSAN) ha alertado en los últimos años sobre esta cuestión [35]. En el año 2013, un estudio llevado a cabo en Europa en muestras de pelo reveló que la concentración de Hg en pelo en los españoles era más de 6 veces superior a la media europea, probablemente debido al elevado consumo de pescado [36].

1.3 Métodos de análisis y especiación de mercurio

Las nuevas metodologías de análisis y especiación de mercurio se centran en el diseño de métodos rápidos y fiables de preparación de muestra, métodos de preconcentración y de generación de vapor más respetuosos con el medio ambiente, diseño de instrumentación miniaturizada y con menor consumo energético, y en la mejora de las características analíticas como la exactitud y la sensibilidad. Las principales aplicaciones se desarrollan en el campo de la seguridad alimentaria y la contaminación ambiental [37].

1.3.1 Preparación de la muestra

La conservación y preparación de la muestra es una etapa básica para la determinación exacta de mercurio en distintos tipos de matrices. Los mejores materiales para la conservación de muestras para análisis de especies de mercurio son el vidrio (Pyrex o cuarzo) y el Teflón. Los recipientes de plástico tipo polipropileno contribuyen a la contaminación o pérdida de mercurio en las muestras [49].

El Teflón es la mejor elección en el caso de muestras acuosas. Las especies de mercurio más volátiles presentes en el agua son el Hg(0) y el dimetilmercurio. Es recomendable extraer estas especies del agua mediante un medio de adsorción adecuado (Carbotrap o SPE) para el subsecuente análisis en el laboratorio. Para la medida de los compuestos disueltos de mercurio se requiere una filtración de la muestra, normalmente mediante el empleo de filtros de membrana de 0,45 µm. Las muestras se acidificarán previamente al análisis, con dos limitaciones: la materia en suspensión tiene que ser eliminada antes de la acidificación y, si se realiza la especiación, el Hg(0) y dimetilmercurio también tienen que ser extraídos antes de la misma, puesto que de otra forma estas especies podrían transformarse en compuestos iónicos de mercurio [38]. En general, las muestras para especiación de especies volátiles de mercurio en aguas se tratan inmediatamente tras la toma. A la hora de conservar las muestras, debemos evitar los agentes oxidantes. La estabilización con HNO_3 produce una reducción de la concentración de metilmercurio, aunque el Hg(II) permanece estable en presencia de este ácido. El HCl es el ácido más apropiado para la conservación de muestras con metilmercurio, aunque algunos autores afirman que para las determinaciones de metilmercurio, guardar las muestras a temperaturas por debajo de 5 °C (o congeladas) es mejor que añadir ácidos [39].

En el caso de las muestras biológicas, la contaminación externa de las mismas con metilmercurio es difícil que ocurra, pero hay que tomar precauciones para evitar la contaminación con mercurio inorgánico. Las muestras de sangre y pelo se emplean habitualmente para estimar la exposición de las personas al mercurio y sus compuestos. La sangre es una suspensión de componentes celulares con un elevado contenido en proteínas. Es imprescindible tomar la muestra de sangre sin la adición de ningún conservante o anticoagulante, debido a que pueden contener algún compuesto con mercurio o contaminarse con impurezas de mercurio y, por otro lado, puede romperse la relación original de especies de mercurio. Las muestras de sangre sólo se pueden congelar una vez para evitar la pérdida de metilmercurio, y además hay algunas evidencias de que el metilmercurio puede destruirse durante la liofilización [40].

El análisis de muestras de pelo ofrece algunas ventajas sobre el resto de muestras biológicas: facilidad para la recolección y almacenamiento, la concentración de metilmercurio es aproximadamente 250 veces más alta que en la sangre, y el análisis de diferentes secciones longitudinales del pelo nos da información histórica de la exposición al mercurio ingerido como alimento. El metilmercurio es estable por un período de varios años guardado en un lugar seco y a oscuras a temperatura ambiente.

Otras muestras biológicas se analizan preferentemente frescas o tras liofilización. La congelación de muestras frescas, especialmente para un almacenamiento prolongado, debe evitarse ya que se ha demostrado que en algunas matrices el metilmercurio se descompone con repetidas congelaciones y descongelaciones, especialmente en bivalvos [40]. El metilmercurio y el mercurio total en muestras biológicas liofilizadas, como en el caso de materiales de referencia certificados (CRMs), es estable durante años. A pesar de ello los CRM son esterilizados en autoclave. Es importante prevenir la actividad bacteriológica, para evitar procesos de metilación y desmetilación.

En muestras biológicas como el pescado u otros productos marinos se han extraído los compuestos organomercuriales para su posterior determinación (Figura 2) como cloruros tras una acidificación de la muestra homogeneizada y una posterior extracción con un disolvente orgánico (benceno o tolueno) [41, 42]. Los extractos orgánicos son sometidos a una reextracción con una solución acuosa de cisteína. Este proceso se repite con una nueva extracción y reextracción para eliminar interferencias. En general, los procedimientos de extracción con disolventes consumen mucho tiempo, la recuperación varía entre muestras y en muestras ricas en lípidos, la separación de fases es complicada debido a la formación de emulsiones. Otros métodos de extracción utilizados para el tratamiento de las muestras biológicas a la hora de obtener el metilmercurio para su determinación son: la extracción asistida por microondas en medio ácido [43], la extracción mediante ultrasonidos [44], la extracción alcalina [45], la extracción con L-cisteína [46], extracción con tolueno [47] y la digestión enzimática [48].

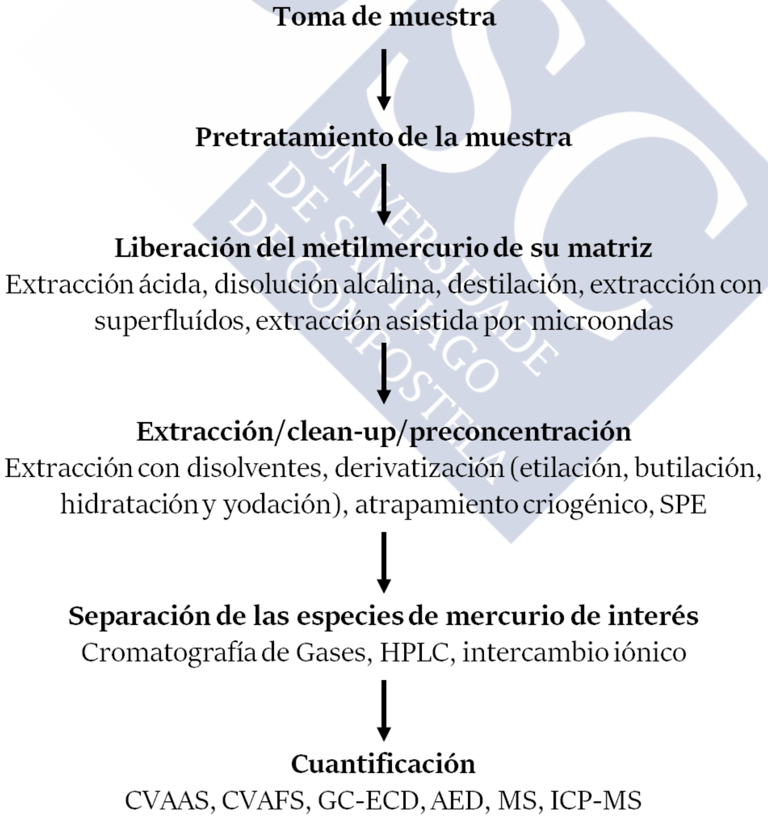

Figura 2. Etapas para la determinación de compuestos organomercuriales [49]

1.3.2 Determinación de mercurio total

La generación química de vapor (CVG) es la técnica más popular para la determinación de mercurio ya que permite la separación del analito de la matriz de la muestra y mejora la eficiencia de la introducción de la misma. La técnica de vapor frío empleando tetrahidroborato o cloruro de estaño como reductor es la técnica más extendida para la determinación de mercurio en distintos tipos de muestras como aguas naturales, muestras biológicas o geológicas. Sin embargo, las interferencias de metales de transición, la contaminación de los reactivos y la inestabilidad de las disoluciones cuando se emplea tetrahidroborato, han causado que se hayan desarrollado otras técnicas de generación de vapor (p.ej. generación de vapor electroquímica, generación fotoquímica de vapor, generación de vapor promovida por ultrasonidos) para mejorar los límites de detección y la reproducibilidad [37].

También se han producido desarrollos instrumentales de sistemas analíticos miniaturizados, como los microplasmas para espectroscopía de emisión óptica [50-52] que permiten reducir el consumo de gas y la potencia requerida, son de pequeño tamaño y de un bajo coste de manufacturación.

Dada la baja concentración de mercurio en muchos tipos de muestra y la interferencia de la matriz es necesario llevar a cabo etapas de preconcentración y separación de la matriz, empleando técnicas como la extracción líquido-líquido (LLE) y la extracción en fase sólida (SPE). Entre las técnicas de extracción líquido-líquido destaca el desarrollo de métodos empleando la extracción en punto de nube [53-56], y los métodos de preconcentración miniaturizada como la microextracción líquido-líquido dispersiva (DLLME) que es adecuada para utilizar en combinación con ETAAS y ETV-ICP-MS debido al mínimo volumen de la fase de enriquecimiento [57, 58].

La extracción o microextracción en fase sólida (SPE/SPME) son las técnicas más populares de preconcentración de mercurio debido a su simplicidad, alto factor de enriquecimiento y bajo consumo de disolventes orgánicos. Los sorbentes utilizados se derivan de la inmovilización a distintos tipos de soportes de agentes orgánicos, la mayoría moléculas conteniendo azufre (por ejemplo, 5.5'-ditiobis ácido 2-nitrobenzoico, 1-aciltiosemicarbazida, 2-mercaptobenzimidazol, 1,5-difenilcarbazida [37]) dada su elevada afinidad por el analito. La aplicación de la SPE/SPME en espacio de cabeza también se ha utilizado para el análisis de mercurio empleando metales nobles como sorbentes, tales como hilos de plata [59] o de paladio para microextracción [60].

La utilización de novedosos materiales de tamaño nanométrico lleva a una alta eficiencia y rápida dinámica de extracción. La elevada afinidad del mercurio por el oro ha llevado a Leopold y col. [61, 62] al desarrollo de un método para la determinación del elemento en aguas naturales mediante AFS tras la adsorción en nanopartículas de oro sobre partículas de sílica y una etapa de desorción térmica. También se han empleado nanopartículas de sílica recubiertas de ácido piridina-2,6- dicarboxílico [63], o nanopartículas magnéticas recubiertas para separar el elemento de la matriz y reducir el tiempo de análisis [64-66].

Actualmente, los polímeros de impronta molecular se investigan como sorbentes altamente selectivos para Extracción en Fase Sólida (SPE) con el fin de concentrar y eliminar interferentes de muestras complejas previamente al análisis. La retención selectiva del analito en la matriz polimérica unida a una etapa de elución nos permite la determinación de la especie de interés, evitando la necesidad de una separación cromatográfica previa [67].

1.3.3 Métodos de especiación

Los métodos para la especiación del mercurio se pueden dividir en dos clases: métodos cromatográficos (que incluirían cromatografía de gases, cromatografía líquida y electroforesis capilar) y métodos no cromatográficos, basados en las propiedades físicas y químicas de las diferentes especies de mercurio.

1.3.3.1 Métodos cromatográficos

La separación cromatográfica en combinación con un detector específico es el procedimiento más habitual para la especiación del mercurio. Los detectores más empleados son el ICP-MS y CV-AFS debido a su mayor sensibilidad [37].

Para la determinación del mercurio y sus especies mediante cromatografía de gases es necesaria una derivatización previa del analito, normalmente tetraetilborato sódico ($NaBEt_4$). Una vez realizada la separación cromatográfica, las especies de mercurio se pueden preconcentrar mediante SPME o directamente detectar mediante fluorescencia atómica [68-71], emisión [72, 73] o ICP-MS [74-76]. Sin embargo, en el caso de los analitos Hg(II) y EtHg ambos pueden ser transferidos al mismo producto, dietilmercurio. Por ello, la etilación en medio acuoso no es útil para la detección simultánea de ambas especies. Una posible alternativa sería el uso de sodio tetrapropilborato o sodio tetrafenilborato [37]. Las técnicas de dilución isotópica en combinación con GC-ICP-MS/AFS han supuesto una mejora significativa en las características analíticas. Así, Jackson y col. [74], utilizaron la técnica de dilución isotópica, el método de purga y trampa y GC-ICP-MS para la determinación de ultratrazas de metilmercurio en aguas con un límite de detección de 0,003 ng L^{-1}.

La cromatografía líquida ofrece diversas ventajas sobre la cromatografía de gases al poder ser aplicada a compuestos no volátiles y con una gran versatilidad al emplear distintos modos de separación (fase reversa, cambio iónico, exclusión por tamaño). En el caso de la cromatografía líquida, la especiación se lleva a cabo mediante HPLC acoplado a ICP-MS [77, 78], o mediante detección por fluorescencia atómica [79, 80] o absorción [81].

Cuando el acoplamiento del HPLC a una técnica de detección no es suficientemente sensible para analizar muestras reales, como muestras de aguas, es necesario emplear una técnica de de preconcentración previa a la separación y la detección. Aplicaciones recientes para la especiación de mercurio con preconcentración mediante SPE previa a HPLC-ICP-MS o HPLC-AFS incluyen la utilización de resinas de cambio aniónico [82], y catiónico [83], o la combinación de C18 con el empleo de agentes complejantes para aumentar la interacción del analito con el sorbente [80-86]. La generación de vapor post-columna se ha empleado también para mejorar la sensibilidad y reducir el efecto matriz [80, 87]. Con el desarrollo de la proteómica y metalómica la combinación HPLC-ICP-MS jugará un papel importante para entender las rutas metabólicas, la biodisponibilidad y la toxicidad de las especies de mercurio en el organismo [37].

La aplicación de la electroforesis capilar a la especiación de mercurio en muestras reales todavía es escasa en comparación a los métodos de CG y HPLC. Sus principales ventajas son la separación rápida con alta eficiencia y la utilización de pequeños volúmenes de muestra [37], lo que hace necesario el empleo de detectores adecuados. Se ha utilizado el acoplamiento a un ICP-MS con un nebulizador MicroMist para aumentar la eficiencia de nebulización [88], y también una interfase acoplando la electroforesis capilar con generación de vapor frío y la detección mediante absorción atómica en un tubo de cuarzo [89].

1.3.3.2 Métodos no cromatográficos

Los métodos de especiación no cromatográficos se basan en el comportamiento químico y físico de las distintas especies de mercurio. Un ejemplo es el empleo de distintas condiciones de atomización para el análisis de mercurio inorgánico y de metilmercurio, o la generación selectiva de vapor. El empleo de reductores suaves como Sn(II) se utiliza para la determinación selectiva de Hg(II), mientras que para la determinación de las especies orgánicas de mercurio o para el análisis de mercurio total se realiza la conversión de las formas orgánicas a Hg(II) mediante irradiación UV, oxidación química, o el tratamiento con reductores más fuertes como el tetraborato sódio o potásico [37].

Otra aproximación a la especiación del mercurio mediante un método no cromatográfico es la realización de una extracción y/o elución selectiva de las diferentes especies en base a las diferencias en la interacción con diferentes complejantes [90, 91], resinas y polímeros [92-94], amalgamas de oro [95-97] o radiación UV [98]. La tendencia es desarrollar métodos verdes de análisis que no empleen reactivos o que minimicen la cantidad de disolventes utilizados. Así, se ha utilizado la microextracción con fibras huecas (*hollow fiber microextraction*, HFME) para determinar metilmercurio en muestras biológicas y de fangos [99].

1.4 Polímeros de impronta molecular: conceptos generales

La técnica de la impronta molecular se basa en la preparación de un polímero altamente entrecruzado alrededor de un analito "plantilla" (*template*), en presencia de un monómero apropiado. En primer lugar es necesario poner el analito en contacto con el monómero en un disolvente adecuado con el objetivo de que se forme un complejo de pre-polimerización estable. A continuación, se realiza la polimerización en presencia del entrecruzante. Por último, el polímero debe ser sometido a un proceso de limpieza para eliminar el analito plantilla de las cavidades formadas, que serán capaces, posteriormente, de retener selectivamente el analito en una muestra compleja.

Mediante la tecnología de impronta molecular es posible sintetizar polímeros muy estables que presentan sitios de reconocimiento en la matriz polimérica complementarios en tamaño y forma con el analito para el que fueron generados, presentado constantes de afinidad y selectividad comparables a las del reconocimiento antigénico. Una de las mayores ventajas que presentan estos polímeros, en comparación con los anticuerpos, es la sencillez y bajo coste de su síntesis [100]. Debido a estas ventajas, el interés por los polímeros de impronta molecular (Molecularly Imprinted Polymers, MIPs) ha ido en aumento en los últimos años, fundamentalmente como materiales selectivos para cromatografía y procesos de extracción en fase sólida.

1.4.1 Síntesis de polímeros de impronta molecular

Las características químicas (selectividad, afinidad, capacidad…) y propiedades físicas del polímero obtenido (porosidad, rigidez, área superficial…) van a estar directamente relacionadas con la estructura y grupos funcionales que presente el analito plantilla, así como con el monómero, el entrecruzante y el disolvente en el que se lleve a cabo la polimerización (*porogen*). A continuación se describe brevemente la función de cada uno de ellos, y las etapas de la síntesis.

1.4.1.1 Analito y monómeros.

Por lo general, los MIPs son capaces de distinguir entre la molécula para la que fueron generados y moléculas muy parecidas, si estas presentan alguna pequeña diferencia estructural o conformacional. Por este motivo, la forma y el tamaño del analito plantilla van a tener una gran influencia en la selectividad que presente el polímero posteriormente.

El monómero ha de contener en su estructura grupos funcionales capaces de interaccionar con el analito con energía suficiente para generar un complejo que se mantenga estable durante la polimerización. La formación de este complejo puede realizarse mediante una unión covalente o mediante uniones no covalentes, que impliquen interacciones electrostáticas, puentes de hidrógeno o enlaces de coordinación con iones metálicos.

El primer trabajo sobre impronta molecular mediante enlace covalente, fue publicado por Wulff y Sarhan en 1972 [101]. En él se describe la preparación de un polímero cuyas cavidades presentan propiedades quirales que permiten la separación de especies en una mezcla racémica. Por lo general, la técnica de impronta molecular por formación de enlaces covalentes permite una gran definición estructural en la forma y el tamaño de la cavidad generada por el analito. Sin embargo, resulta poco versátil, ya que existen pocos monómeros y analitos que presenten grupos funcionales apropiados para formar dichos enlaces.

Arshady y col. [102] fueron los primeros en emplear interacciones no covalentes como base para la impronta molecular en 1981. En este caso, existe un número mucho mayor de monómeros capaces de interaccionar con los analitos, lo que ha permitido aplicar la impronta molecular a diferentes tipos de compuestos. Por este motivo, el interés por la técnica ha ido en aumento en los últimos años, y muchos han sido los trabajos publicados que versan sobre diferentes aspectos a cerca de la tecnología de impronta molecular. En cualquier caso, independientemente del monómero elegido resulta recomendable emplear una gran cantidad de monómero con el fin de desplazar el equilibrio hacia la formación del complejo de prepolimerización. Por lo general, una relación molar 1:2 (plantilla:monómero) es suficiente para lograr el objetivo deseado. Sin embargo, el exceso de monómero libre tras la polimerización puede provocar la presencia de sitios de unión no específicos en el polímero, lo que hace necesario optimizar las etapas de carga, lavado y elución del analito.

El ácido metacrílico (MAA) ha sido el monómero más empleado hasta la fecha, resultando muy apropiado para aquellos analitos que presentan grupos funcionales básicos en su estructura. Del mismo modo, la vinilpiridina (VP) resulta muy adecuada para analitos que contengan grupos ácidos en su estructura [103].

1.4.1.2 Entrecruzante y porogen.

La morfología del polímero va a venir determinada tanto por las propiedades del disolvente (*porogen*) como del entrecruzante (*cross-linker*). Ambos tienen una influencia conjunta en las propiedades físicas y químicas del polímero obtenido, particularmente en su porosidad, la cual ha de ser suficiente para permitir el acceso del analito a los sitios de unión generados; aunque en algunos casos, la porosidad del polímero puede variar en los tratamientos posteriores a la polimerización.

Las interacciones que se producen entre el monómero y la plantilla van a estar muy influenciadas por el disolvente empleado durante la polimerización. Por lo general, la estabilización de los enlaces por puentes de hidrógeno, o por interacciones electroestáticas entre los monómeros y la plantilla, se va a ver muy favorecida por el empleo de disolventes con una constante dieléctrica baja, como el tolueno o el cloroformo. Los polímeros obtenidos al utilizar otros disolventes, con mayores constantes dieléctricas (por ejemplo, acetonitrilo) presentan, generalmente, una menor afinidad en el reconocimiento posterior del analito.

El uso de disolventes más polares, como el agua o el metanol, en principio no es recomendable puesto que van a competir con el analito por la formación de puentes de hidrógeno con el monómero, interfiriendo significativamente en la formación de los enlaces con la plantilla. Sin embargo, cada vez es mayor el número de aplicaciones en literatura científica en las que están presentes en la composición del porogen. El empleo de la mezcla metanol:agua [104], sería apropiada en el caso de que otro tipo de disolventes pudiesen interferir en la correcta interacción entre la molécula plantilla y el monómero [105].

Una vez formado el complejo, es necesario preservar su integridad estructural durante el proceso de polimerización con el fin de generar cavidades complementarias en tamaño y forma al analito plantilla. Para ello resulta necesario conferir al polímero un alto grado de entrecruzamiento, de modo que la fijación espacial de los grupos funcionales en la matriz polimérica se lleve a cabo de una forma estable.

El etilen glicol-dimetacrilato (EDMA) es el entrecruzante más comúnmente empleado en combinación con el MAA, resultando muy adecuado ya que preserva los sitios de unión durante la polimerización, al mismo tiempo que confiere al polímero una gran estabilidad mecánica y térmica, buena humectación y transferencia de masa.

Únicamente el trimetil propano trimetacrilato (TRIM) posee características similares, y en algún caso mejores, que el EDMA [103].

1.4.1.3 Polimerización.

Como se indicó anteriormente, la primera etapa en el proceso de síntesis consiste en la formación del complejo plantilla:monómero en un pequeño volumen del porogen apropiado, al que posteriormente se le añade el entrecruzante (Figura 3). En los sistemas basados en el MAA como monómero, y el EDMA como entrecruzante, la relación molar más adecuada es 1:4:20 (plantilla:monómero:entrecruzante) [103].

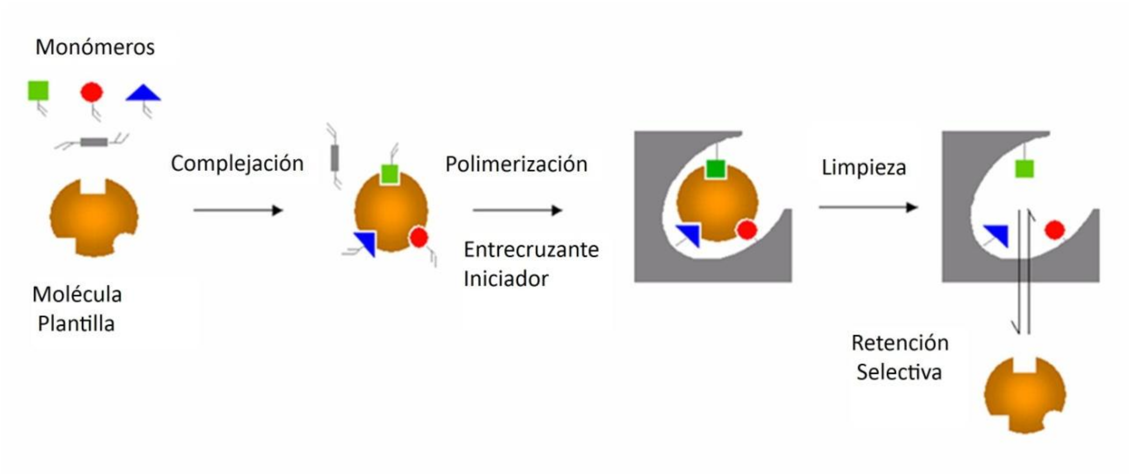

Figura 3. Esquema general de la síntesis de un polímero de impronta molecular (MIP)

La polimerización se inicia añadiendo un iniciador de tipo azo, normalmente 2,2'-azobis-isobutironitrilo (AIBN), y sometiendo la mezcla a una temperatura igual o superior a 60°C o a radiación ultravioleta. Por lo general, la polimerización se lleva a cabo durante 24 horas.

El proceso de polimerización más ampliamente utilizado es la *polimerización en bloque* (*bulk polymerization*) [106]. Mediante este proceso se obtienen polímeros de forma idéntica al recipiente empleado para realizar la polimerización (generalmente un tubo de ensayo), el cual ha de romperse para recuperar el polímero. Posteriormente es necesario triturarlo y tamizarlo hasta el tamaño de partícula deseado (25-38 µm para cromatografía, o 50-200 µm para la extracción en fase sólida). Este tipo de polimerización es el método más sencillo y barato. Sin embargo, presenta varios inconvenientes; por un lado, las partículas obtenidas tras la trituración y tamizado no son homogéneas en cuanto a tamaño y forma; por otro lado, tras el proceso completo la cantidad de polímero que puede ser usado con fines analíticos se reduce a menos de un 50% del inicial. Además, una parte de los sitios específicos generados se destruyen en el proceso de trituración. Todo ello conlleva una pérdida importante de la capacidad de carga del polímero. Aunque las partículas obtenidas mediante el proceso de polimerización en bloque resultan apropiadas para SPE, son necesarios varios tratamientos antes de poder empaquetarlas en una columna cromatográfica.

Otro tipo de polimerizaciones permiten obtener el polímero en forma de partículas más o menos esféricas, como es el caso de la *polimerización por dispersión* [107] o *por precipitación* [108]. En ambos métodos se emplea un gran volumen de disolvente, diferenciándose en la manera de estabilizar las partículas formadas. En los métodos de polimerización por dispersión se utilizan estabilizantes o surfactantes que encapsulen las partículas formadas para su posterior separación, mientras que en la polimerización por precipitación la rigidez y entrecruzamiento de las partículas formadas es suficiente para permitir la separación. Las partículas obtenidas mediante estas técnicas son muy homogéneas en tamaño y forma, sin embargo, resulta difícil establecer unas reglas generales que permitan la obtención de partículas con un tamaño concreto.

Otra opción consiste en la *polimerización sobre la superficie de partículas esféricas de sílica*, o de politrimetil propano trimetacrilato [109]. En este caso, el polímero se obtiene en forma de partículas mixtas, cuyo interior es el material empleado como soporte, por lo que solo la superficie de la partícula obtenida tiene las propiedades de reconocimiento adecuadas. También es posible realizar la polimerización en los poros de estos materiales, de modo que el tamaño y la forma de las partículas obtenidas se corresponderán con el de los poros del material empleado.

En los últimos años se han empleado otro tipo de soportes para MIPs, mejorando o ampliando sus características, como es el caso de la funcionalización de nanopartículas o nanomateriales, principalmente con el objeto de aumentar considerablemente el área superficial del mismo y por tanto su capacidad de adsorción. Si por otro lado se utilizan NPs magnéticas, se puede aprovechar esta cualidad para facilitar la agitación durante el proceso de extracción y la posterior separación de las partículas en la disolución. Por último, si interesa que el material adquiera propiedades ópticas para una posterior determinación por espectrofotometría, se puede realizar una polimerización sobre la superficie de nanopartículas fluorescentes o quantum dots, que serán mencionados más adelante.

1.4.1.4 Limpieza.

Una vez obtenido el polímero con el tamaño de partícula deseado es necesario someterlo a un proceso exhaustivo de limpieza, con el objetivo de extraer el analito plantilla de la matriz polimérica. Generalmente estos procesos se llevan a cabo mediante lavados del polímero con disolventes capaces de destruir las interacciones plantilla:monómero, o mediante procesos de extracción Soxhlet, aunque en la mayoría de los casos resulta prácticamente imposible la eliminación total del analito plantilla de la matriz polimérica, lo que puede provocar la aparición de residuos del mismo durante las etapas de elución en procesos de extracción que se lleven a cabo con el polímero [110].

1.4.2 Extracción en fase sólida sobre MIPs (MIP-SPE)

El primer estudio de extracción en fase sólida sobre un polímero de impronta molecular fue realizado por Sellergreen en el año 1994 [111]. Los procesos de extracción en fase sólida sobre MIPs pueden llevarse a cabo en modo "*off-line*" y en modo "*on-line*", sin embargo, la mayor parte de los trabajos publicados hasta el momento han sido desarrollados en modo "*off-line*", debido a que resulta más sencillo y económico.

El modo "*on-line*" presenta la ventaja de que no es necesario manipular la muestra entre la etapa de preconcentración y la etapa de análisis, de modo que se reduce la posibilidad de que se pierda una parte del analito, al tiempo que se reduce el riesgo de contaminación de la muestra. Otras ventajas que presenta esta modalidad es que el volumen de muestra necesario es más pequeño, el consumo de disolventes menor y resulta más fácil automatizar el procedimiento. Los límites de detección y la reproducibilidad también mejoran al emplear este modo.

El proceso de extracción en fase sólida sobre MIPs es idéntico a cualquier otro procedimiento de extracción en fase sólida. En primer lugar es necesario trasvasar una pequeña cantidad del polímero (generalmente 50-200 mg) a una columna (extracciones en continuo) o a un cartucho (extracciones en discontinuo) y, a continuación, es necesario acondicionar el polímero con el disolvente en el que, posteriormente, se realizará la carga de la muestra (Figura 4). Tras la fijación selectiva de los analitos se llevará a cabo un proceso de lavado, para eliminar aquellos compuestos interferentes que hayan podido quedar retenidos de forma inespecífica en la matriz polimérica y, por último, los analitos serán eluídos convenientemente.

Una parte de los analitos de interés puede quedar retenida en la matriz polimérica mediante interacciones no-específicas que se producen habitualmente debido al exceso de monómero empleado durante el proceso de polimerización. La manera más sencilla de evaluar estas interacciones consiste en realizar un estudio de recuperación sobre un polímero blanco (Non Imprinted Polymer, NIP), que se obtiene mediante un proceso de síntesis similar al del MIP, pero sin adicionar la plantilla a la mezcla inicial, de forma que las matrices de ambos polímeros sean similares. En algún caso, también se ha utilizado como blanco un polímero preparado para algún compuesto similar al analito de interés [112].

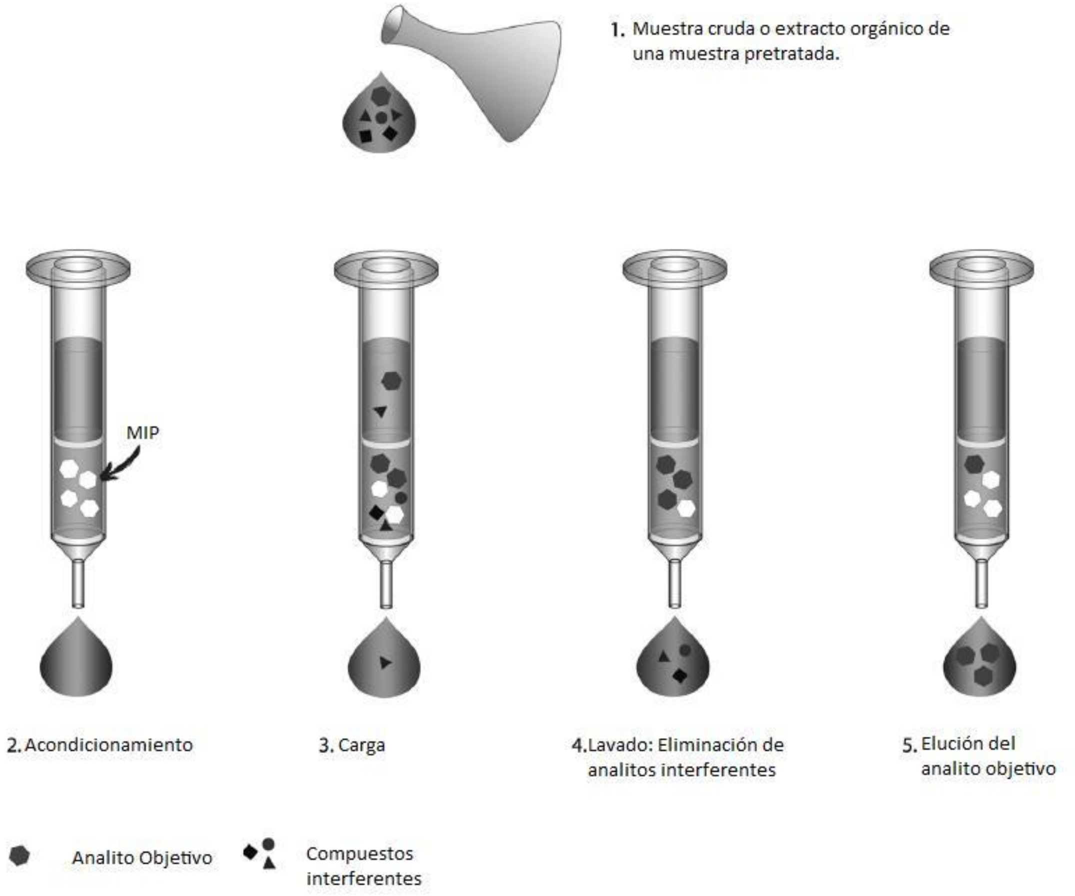

Figura 4. Esquema de la utilización de un MIP como cartucho para SPE [113]

1.4.2.1 Carga de la muestra.

La mayoría de los polímeros empleados actualmente se basan en interacciones del tipo “puente de hidrógeno”. Por tanto, en este tipo de polímero, el disolvente más adecuado para la carga de la muestra será aquel que favorezca la formación de este tipo de uniones, mientras que el disolvente de elución más adecuado será aquel que permita destruir los enlaces formados.

1.4.2.2 Lavado.

Con el objetivo de conseguir una buena limpieza de la muestra para el análisis resulta necesario realizar una etapa de lavado previa a la elución, empleando algún disolvente que permita destruir las interacciones no-específicas de los compuestos presentes en la matriz de la muestra, sin perder los analitos de interés.

1.4.2.3 Elución.

Una vez que los compuestos interferentes han sido eliminados de la matriz polimérica, se pueden eluir los analitos retenidos mediante la adición de un disolvente adecuado (polar, prótico, un ácido o una base). De esta forma, se destruyen los enlaces formados entre los analitos y los monómeros presentes en las cavidades del polímero.

La etapa de elución es determinante en el tiempo de vida del polímero, pues los cambios en la polaridad de los disolventes empleados pueden provocar variaciones en su morfología (contracciones o hinchamientos de la matriz polimérica), que pueden afectar a la accesibilidad de los sitios de unión. Después de numerosas extracciones, la contracción de los huecos puede provocar una falta total del reconocimiento del analito plantilla.

Otro problema asociado a la etapa de elución se debe al analito plantilla que no ha sido eliminado de la matriz polimérica tras el proceso de síntesis. La extracción de la plantilla tras la polimerización raramente es totalmente efectiva, lo que puede originar contaminaciones aleatorias durante las etapas de elución (*leaking*) y, por tanto, errores considerables en las determinaciones a niveles traza. Para evitar estas contaminaciones se ha sometido a los polímeros a procesos de limpieza más agresivos o a elevadas temperaturas, que no han dado buenos resultados, debido a los problemas relacionados con cambios de morfología.

Un procedimiento empleado para corregir las contaminaciones aleatorias en los procesos de elución ha sido la extracción paralela de una disolución "blanco" sobre otro cartucho que contenga la misma cantidad de polímero. La determinación final se realiza por diferencia de las concentraciones encontradas en la muestra y en el blanco, para lo cual resulta necesario asumir que las contaminaciones van a ser las mismas en ambos cartuchos. Otra opción empleada, ha sido la preparación de polímeros utilizando como plantilla un compuesto estructuralmente análogo al analito que se quiere determinar, de tal forma que la posible contaminación no afecte a la cuantificación del analito de interés. Estos materiales, sin embargo, han resultado ser menos selectivos que los polímeros de impronta convencionales [114].

Naturalmente, los polímeros de impronta molecular han sido objeto de investigación para la determinación de MeHg en muestras acuosas y biológicas, desarrollando diferentes compuestos y rutas para su síntesis. Yongwen Liu y col. [115] desarrollaron un polímero de impronta molecular para determinar selectivamente metilmercurio en muestras de pelo humano y muestras de suelo. Sintetizaron un monómero ((4-etenilfenil)-4-formato-6-fenil-2,2'-bipiridina), utilizaron DVB como entrecruzante y AIBN como iniciador, y la polimerización por precipitación. El metilmercurio fue analizado mediante CVAAS tras la extracción de la muestra y la retención selectiva del analito con el MIP sintetizado. Büyüktiryaki y col. [116] sintetizaron un MIP para SPE para la separación y preconcentración de metilmercurio en muestras acuosas en presencia de otros iones metálicos y su posterior análisis por ICP-OES y HPLC-DAD. Partiendo de la formación de un complejo de pre-polimerización entre el metilmercurio y el metacriloil-(L)-cisteína metilester (MAC), llevaron a cabo la síntesis utilizando EDMA como entrecruzante y AIBN como iniciador.

La ditizona (Figura 5), uno de los complejantes que se utiliza en esta tesis, es uno de los reactivos comunes para la complejación con metales pesados, tales como cobalto, cobre, plomo o mercurio [117-120], y se ha empleado en polímeros de impronta iónica (IIP) para diversos metales [121 123]. El fenobarbital se utiliza como anticonvulsivo para el tratamiento de la epilepsia [124], aunque también posee propiedades sedantes e hipnóticas. La afinidad de la molécula de fenobarbital con el mercurio y metilmercurio ya ha sido estudiada con anterioridad, y se ha empleado como método de detoxificación en el caso de envenenamiento [125]. Asimismo, el complejo ditizona-mercurio se ha empleado en los primeros métodos colorimétricos semicuantitativos y de screening de barbitúricos en orina, plasma o contenidos gástricos [126-128]. En el caso del fenobarbital, existen trabajos que emplean polímeros de impronta molecular para la determinación del propio fenobarbital y otros barbitúricos [129-131], realizando la polimerización también con el MAA y el EDMA, pero no tenemos conocimiento de la utilización del fenobarbital como ligando para la síntesis de MIPs.

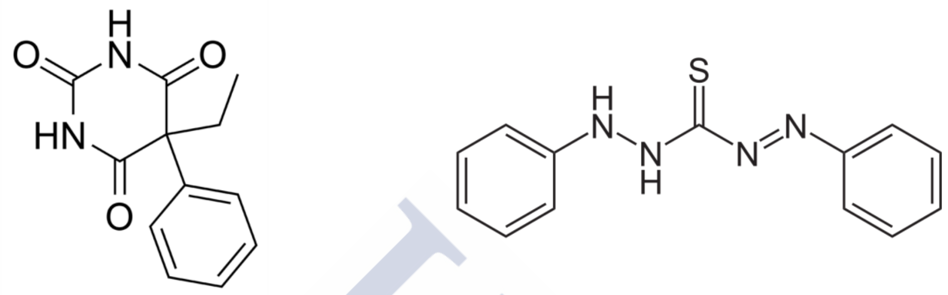

Figura 5. Estructura química de los ligandos: Fenobarbital (izquierda) y Ditizona (derecha)

1.4.3 Síntesis de polímeros de impronta molecular sobre quantum dots para el desarrollo de sensores

1.4.3.1 Quantum dots

Un quantum dot (QD) es una estructura cristalina, típicamente esférica, con dimensiones en la escala de los nanómetros (diámetro comprendido entre 2 y 10 nm). Este tipo de nanopartículas inorgánicas han recibido una atención cada vez mayor en los últimos años.

Cualquiera que sea la longitud de onda de excitación (visible, ultravioleta o infrarrojo), el QD emite un haz de luz monocromático, intenso y con un espectro de emisión siempre muy estrecho, simétrico y sin solapamientos. Estos factores mejoran notablemente su fotoestabilidad comparados con los colorantes fluorescentes comunes. Gracias a dicha propiedad, una vez fotoexcitado, un QD puede brillar durante horas o días, mientras que los fluoróforos convencionales producen una emisión estable sólo durante unos pocos segundos o minutos. Estas cualidades les otorgan una enorme una gran sensibilidad y versatilidad analítica [132]. En la Tabla 1 se comparan las propiedades más importantes de los quantum dots y de los fluoróforos orgánicos [133].

Tabla 1. Comparación de las propiedades de los compuestos fluoróforos orgánicos y los quantum dots

Propiedad	Fluoróforos	Quantum dots
Espectro de absorción	Estrecho	Ancho, incremento hacia el UV
Coeficiente de extinción molar	Pequeño, < 200.000 $M^{-1}cm^{-1}$	Alto, > 2.000.000 $M^{-1}cm^{-1}$
Espectro de emisión	Ancho, asimétrico, con cola	Estrecho
Desplazamiento de Stokes	Menor de 100 nm	Puede ser mayor de 200 nm
Rendimiento cuántico	Rango amplio	La mayoría alto
Tiempo de vida de fluorescencia	Menos de 5 ns	Más de 10 ns
Fotoestabilidad	Variable	Estable, con poco photobleaching dependiendo de las condiciones
Capacidad multiplex	Poco frecuente	Excelente
FRET	Variable, baja eficiencia	La sintonización de la emisión incrementa la eficiencia
Resistencia química	Variable	Muy estable
Reactividad	Variable, múltiple	Variable, menos reactivo en comparación
Tamaño	< 1 nm	La mayoría entre 2-10 nm
Electrocromicidad	Poco frecuente	Frecuente

Una de las propiedades más interesantes de los quantum dots es su rango de emisión dependiente del tamaño de partícula, de forma que es posible ajustar este rango según la aplicación que se quiera dar a los mismos si podemos controlar el tamaño de partícula durante nuestra síntesis. Por ejemplo, los QDs de CdSe tienen una longitud de onda de emisión entre 500-700 nm cuando varía su tamaño entre 3 y 7 nm. QDs basados en el Zn, como los de ZnS o ZnSe emiten alrededor de los 400 nm, mientras que los basados en Pb tienen una banda de emisión en la región del infrarrojo cercano [134].

Los QDs de ZnS dopados con Mn empleados en esta tesis presentan algunas ventajas respecto de los QDs basados en el Cd. La síntesis de los mismos se realiza a temperatura ambiente, mientras que en el caso del CdSe se necesita calentar la mezcla por encima de los 300°C [135]. La baja toxicidad de los ZnS:Mn respecto a otros QDs y la posibilidad de aprovechar la fosforescencia que emiten les proporciona una mayor versatilidad [136].

1.4.3.2 Principales aplicaciones de los quantum dots recubiertos por polímeros de impronta molecular

El empleo de los QDs en sensores se debe al hecho de que la emisión de radiación fluorescente es muy sensible a los cambios en su superficie.

Cheng y Rosenzweig [137] fueron los primeros en demostrar su utilidad. La funcionalización de QDs con ligandos de L-cisteína (sistema soluble en agua) permitió observar un aumento en la fluorescencia en presencia de iones Zn(II), mientras que cuando se utilizaban ligandos tioglicerol disminuía la fluorescencia en presencia de iones Cu(II). Lo mismo se observó con QDs recubiertos con trietanoolamina que en presencia de iones Hg(II) perdían la fluorescencia.

Existen muchos ejemplos de la utilización de estos sistemas como sensores de distintos analitos como por ejemplo la determinación de cianuro [138], de enoxacina (antibiótico) [139], pentaclorofenol [140], 4-nitrofenol [141], metimazol (medicamento antitiroideo) [142], y atropina [143].

Es posible combinar quantum dots y polímeros de impronta molecular para la detección de analitos no fluorescentes. Estas nanopartículas resultantes se llaman MIOM (*Molecularly Imprinted Optosensing Materials*). Por ejemplo, QDs de CdSe/ZnS recubiertos de un MIP selectivo al antioxidante tocoferol se han empleado para su detección en muestras de arroz [144, 145]. Otra configuración emplea nanopartículas de sílice como soporte para inmovilizar QDs de CdTe recubiertas de MIPs para detectar hemoglobina bovina y albúmina de suero [146, 147]. Más ejemplos de la combinación QD/MIP son los sensores de nitrofenol en agua corriente y continental [148], clembuterol y melanina [149], la micotoxina zearalenona en muestras de cereales

[150], ractopamina [151] y cisteína [152]. También se ha aprovechado la fosforescencia emitida por QDs de Mn:ZnS recubiertos de MIP para la detección selectiva del ácido domoico, una toxina [153].

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CAPÍTULO II

Polymers and composite materials for removal, analysis and speciation of mercury


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Resumen

Dentro de los metales pesados, el mercurio es el más estudiado debido a su elevada toxicidad, persistencia en el medio ambiente y acumulación en la cadena alimentaria. Por ello, en los últimos años se ha incrementado de forma drástica la bibliografía relativa a este elemento, también en el campo de los polímeros y los polímeros de impronta molecular.

Se ha realizado una revisión de la bibliografía reciente sobre la utilización de los materiales poliméricos aplicados a la Extracción en Fase Sólida (SPE) como método de pretratamiento de muestra o para eliminar el elemento de las aguas, y al desarrollo de sensores. La determinación de iones metálicos mediante el uso de electrodos selectivos es una buena alternativa debido al bajo coste de la instrumentación utilizada y la gran versatilidad que presentan sobre todo los electrodos de carbono a la hora de ser modificados (con nanopartículas metálicas, polímeros o nanotubos de carbono, entre otros).

Se hace especial énfasis en las aplicaciones de los polímeros de impronta que pueden ser sintetizados fácilmente, ofrecen una alta selectividad, capacidad para preconcentración y estabilidad.

Por último, se consideran las distintas aplicaciones de los polímeros y materiales compuestos (combinación con nanopartículas, materiales inorgánicos, polímeros naturales, etc.) al análisis de diferentes tipos de muestras (matrices de interés ambiental, productos alimentarios, medios biológicos), y de sus aplicaciones al análisis de especiación.


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2. Polymers and composite materials for removal and analysis of mercury (II) and methylmercury

Roi Rodríguez Fernández, Elena Peña Vázquez, Pilar Bermejo Barrera

ABSTRACT

Mercury, especially its organometallic forms, has severe adverse effects on the environment and living beings at very low concentrations. Methodologies using novel materials are needed for quick and economical detection. This review deals with the recent literature on the use of polymers and their composites, focusing on their sorptive characteristics and analytical performance. Special emphasis will be placed on molecularly and ionic imprinted polymers due to their selectivity, stability, and low cost of synthesis. Polymers can be combined with other materials (nanoparticles, solgels, natural polymers...), and used for the pretreatment of samples, purification of water, or the development of electrochemical and optical sensors, due to their great versatility. The main applications of the polymers for analysis of different samples and speciation will be also considered.

Keywords

Mercury determination, polymer, imprinted polymer, composite materials, speciation

2.1 Introduction

Mercury is one of the most harmful pollutants present in natural waters and affecting wildlife. In recent years, mercury levels in the environment have increased significantly be cause of human action, mainly due to mining, incineration of waste or use of fossil fuels. Inorganic mercury (Hg(II) is the main species present in environmental matrices (waters, sediments, soils); whereas, methylmercury (MeHg) is the predominant form accumulated in biological organisms. Mercury causes several types of irreversible damages, such as neurological problems, multiorgan damage or malformations [1]. That is the reason why the removal of mercury from natural and wastewaters is crucial.

The number of scientific papers devoted to the analysis of mercury has increased sharply in recent years in different fields: solid phase extraction [2], speciation in environmental and biological samples [3, 4], and detection [5-12]. However, to the best of our knowledge, there is still not any review in the bibliography that integrates the information available concerning the use of polymers, imprinted polymers, and their composites materials for mercury removal, analysis and speciation. We think that a comprehensive review is necessary due to the growing body of literature and the different characteristics of the polymers (operating procedures, preconcentration factors, adsorption capacity, etc...).

This review deals with the study of the polymers and related composite materials that interact with mercury (combination with magnetic nanoparticles, solgels, natural polymers...). We´ll make a special emphasis in latest developments and the synthesis and the studies concerning molecularly and ionic imprinted polymers that offer a high selectivity, capacity for preconcentration and stability. They can be easily synthesized and used as solid phase extraction sorbents, for the development of sensors and chemical speciation. Finally, we will consider the applications of polymers and composite materials to the analysis of different samples: environmental samples (natural waters, wastewaters and sediments), food products (fish, mollusks, wine) and biological materials (hair, blood and serum).

2.2 Solid phase extraction sorbents for mercury analysis and removal from waters

2.2.1 Polymers and composite materials

The Solid Phase Extraction (SPE) technique was introduced in 1940, but the developments leading to its widespread use and adaptation into current analytical methods were started in the 1970s [13], and has been developed under many different methods in both static and dynamic processes. Advances in improving the extraction and selectivity of these methods have been summarized by Mehdinia and Aziz-Zanjani [14] in 2013. The use of polymers as SPE sorbents has two main advantages: a large adsorption surface and the capacity of modifying that surface with different functional groups. The possibility of anchoring these polymers to a large number of supports gives them an enormous versatility. The recent developments of nanomaterials is also of great interest in the field of SPE due to their high extraction capacity because of their high surface/volume ratio, low reagent consumption, high selectivity and simple separation methods [15]. Several reviews on polymers for metal ions SPE and wastewater removal, including mercury, have been published in recent years [13, 16-21].

In Table 1, there is a summary of the most recent articles applying polymers for mercury removal or mercury determination using solid phase extraction. The table shows the type of sorbent used, the type of sample studied (mainly aqueous samples), the technique used for Hg determination, the enrichment factor if available, the adsorption capacity of the polymer expressed in mg of Hg g-1 of sorbent, and the operating mode (batch or column). It can be observed that some of the studies were only performed with the objective of removing Hg from waters, and most of the experiments were carried in batch mode, using atomic spectroscopy (mainly CV-AAS) as detection technique.

Table 1. Polymers and composite materials for SPE of mercury and methylmercury

Material	Sample	Hg determination	Enrichment factor	Adsorption mg g^{-1}	Mode	Reference
Acrylamide surface grafts on polystyrene beads	Water (Hg removal)	UV-VIS (diphenyl carbazide)	-	722	Batch	[22]
ß-cyclodextrin polymer loaded with 1,3-di-(4-nitrodiazoamino)-benzene	Water, soil and dust	UV-VIS (1,3-di-(4-nitrodiazoamino)-benzene)	25-500	221	Batch	[23]
Magnetic resin particles with magnetite (Fe_3O_4) core and glycidyl methacrylate/ divinylbenzene resin shell	Water (Hg removal)	Titration with EDTA	-	963	Batch, column	[24]
Magnetic chitosan resin modified with Schiff's base derived from thiourea and glutaraldehyde	Water (Hg removal)	Titration with EDTA	-	572	Batch, column	[25]
Passive sampler consisting of a 47 mm Empore^{TM} chelating disk with iminodiacetic groups and a polyethersulphone membrane	Contaminated river water	ICP-MS	-	903 mg $disk^{-1}$	Batch	[26]

Table 1. Polymers and composite materials for SPE of mercury and methylmercury

Material	**Sample**	**Hg determination**	**Enrichment factor**	**Adsorption mg g^{-1}**	**Mode**	**Reference**
Poly(vinyl alcohol)/poly(4-vinylpyridine) complexing membrane	Water (Hg removal)	FAAS	-	450	Batch	[27]
Thymine-modified polymeric sorbent	Tap water (Hg removal)	UV-VIS (dithizone), ICP-OES	-	200	Batch	[28]
Porous cross-linked polystyrene containing sulfoxide and 2-aminopyridine	Water (Hg removal)	CV-AAS	-	363	Batch	[29]
3-mercaptopropyl and 3-mercaptopropyl functionalized ethenylene mesoporous organosilica included in a Diffusive Gradients in Thin film (DGT)	Pond water	CV-AAS	-	69	Batch	[30]
CYPHOS IL 101-impregnated Amberlite XAD-1180 resin	Mineral, tap and river water	CV-AAS	51	0.5 mg $column^{-1}$	Column	[31]

Table 1. Polymers and composite materials for SPE of mercury and methylmercury

Material	Sample	Hg determination	Enrichment factor	Adsorption mg g^{-1}	Mode	Reference
Poly-o-anisidine Sn(IV) tungstate nano-composite cation exchanger synthesized via sol–gel	Aqueous synthetic mixture of ions (Hg removal)	Titration (EDTA)	-	-	Column	[32]
Nanofiber based on a uracil-functionalized polymer	Water	ICP-MS	-	543.9	Batch	[33]
Polybenzimidazole hybrid membranes	Drinking, tap, lake and seawater	ICP-OES	-	2.48	Batch	[34]
Ag nanoparticle-embedded poly(acrylamide)-grafted poly(propylene) sheet and 1,8-octanedithiol-functionalized gold-coated poly(propylene) sheet	Groundwater and seawater	EDXRF, CV-AAS	12	-	Batch	[35]
Polymer based on the use of a vinyl derivative of 8-hydroxyquinoline	Fish, seawater	AFS	40	4.6 (Hg(II)), 1.6 (MeHg)	Column	[36]

Table 1. Polymers and composite materials for SPE of mercury and methylmercury

Material	Sample	Hg determination	Enrichment factor	Adsorption mg g^{-1}	Mode	Reference
Divinylbenzene polymer/Fe_3O_4 hybrid monolithic column	Fish, tap, lake and river water	AFS	-	20 ng MeHg $monolith^{-1}$	Column	[37]
Fe_3O_4@SiO_2@polythiophene magnetic nanocomposite	Seafood	CV-AAS	267	59	Batch	[38]
Fe_3O_4-2,5-dimercapto-1,3,4-thiadiazole)/metal–organic framework nanocomposite	Seafood	CV-AAS	363	185	Batch	[39]
C,N-bipyrazole receptor grafted onto a porous silica surface	Water	AAS	-	10.4	Batch	[40]
Poly(allyl acetoacetate)-grafted graphene oxide	Industrial wastewater, Tehran well water (Hg removal)	CV-AAS	-	282.7	Batch	[41]

Table 1. Polymers and composite materials for SPE of mercury and methylmercury

Material	**Sample**	**Hg determination**	**Enrichment factor**	**Adsorption mg g^{-1}**	**Mode**	**Reference**
Polymer inclusion membrane made of cellulose triacetate and containing the task specific ionic liquid trioctylmethylammonium thiosalicylate (Diffusive Gradient in Thin film)	River water	Gold amalgam-AAS	-	0.44	Batch	[42]

Most of the sorbents contain functional groups containing N or S that interacts with Hg: acrylamide surface grafts on polystyrene beads [22], a 1,3-di-(4-nitrodiazoamino)-benzene loaded on a β-cyclodextrin polymer to increase the sensitivity (25-500 fold) [23], poly(vinyl alcohol)/poly(4-vinylpyridine) [27], a copolymer of allyl thymine [28], chelating resins modified with 2-amino-pyridine [29], polybenzimidazole hybrid membranes [32], or a polymer based on the use of a vinyl derivative of 8-hydroxyquinoline [36].

Some studies combine the adsorptive capacity of the polymers with metallic nanoparticles, such as magnetic nanoparticles, to produce composite materials, e.g. a magnetic chitosan resin modified with a Shiff's base [25], a Fe_3O_4@SiO_2@polythiophene nanocomposite [38], or a [(Fe_3O_4-2,5-dimercapto-1,3,4-thiadiazole)/metal–organic framework] [39]. The magnetic materials have the advantage that they can be easily retrieved from their suspensions using an external magnetic field, reducing analysis time and providing relatively high preconcentration factors. Atia et al. [24] prepared a resin with magnetite (Fe_3O_4) core and glycidyl methacrylate/divinylbenzene shell, with a big adsorption capacity (963 mg g^{-1}). The resins with the longest chain length and high amine content had a higher rate of adsorption and higher efficiency. Most of these studies were developed in batch mode, but some authors used packed columns and even coupled them online mode with a detector, as in the case of Ma et al. [37], where a divinylbenzene polymer (DVB)/Fe_3O_4 monolithic column was coupled to an atomic fluorescence spectrophotometer. Methylmercury is adsorbed on the column and subsequently a thermal desorption occurs, and the analyte is dragged into AFS.

Thakur et al. [35] synthesized two flatsheet polymers with acrylamide and thiol groups binding Hg(II) ions, while Ag ions reduces Hg(II) to Hg(0), which is retained on the sheet by amalgam formation. After sorption a change of color is produced due to the presence of silver nanoparticles, which can be utilized for qualitative screening of Hg(II) with the naked eye in the field before determination using EDXRF/CV-AAS in the laboratory. Inorganic-nanocomposites provide good ion-exchange capacity and stability, e.g. the poly-o-anisidine Sn(IV) tungstate nano-composite cation exchanger [34], or the use of a C,N-bipyrazole receptor grafted onto a porous silica surface [40]. The high surface of graphene can be an advantage as a sorbent; thus, a material consisting of poly(allyl acetoacetate)-grafted graphene oxide was employed as an adsorbent for Hg(II) removal from aqueous solutions [41]. It exhibited higher adsorption capacity (282.7 mg g^{-1}) with regard to graphene oxide (56 mg g^{-1}) because of its effective functionalities, especially the dicarbonyl groups which are significant chelating agents. The results showed that adsorption reaches up to 95 % of its maximum in less than 2 min.

Different polymers have also been employed as a part of passive sampler devices: membranes of polyethersulphone [26], 3-mercaptopropyl functionalized materials [30] or polymer inclusion membranes made of cellulose triacetate and a task specific ionic liquid (IL) [42] for Diffusive Gradients in Thin film (DGT) probes. These materials allow to measure only the labile fraction of mercury in water that diffuses across the polymeric membrane. However, they can help to determine Hg(II) in some places where its concentration is below the detection limit of spot sampling [26]. The use of polymer-supported ionic liquid solid phase extraction can be also useful for speciation, trapping Hg(II) as an anionic chlorocomplex ($HgCl_4^{2-}$) [31].

2.2.2 Hg(II) and methylmercury imprinted polymers

SPE is the most common technique when using molecular imprinting polymers for the determination of mercury and its species [43]. Molecular and ionic imprinting technologies are a powerful tool in the development of highly selective analytical methods. These materials are great candidates as adsorbents for simple preparation, due to their high selectivity and their ability to preconcentrate the analytes from complex sample matrices [18, 19]

Table 2 displays a summary of different Hg(II) or methylmercury imprinted polymers used for the analysis of mercury and water purification, the type of template, the method of synthesis, the different reagents used for the synthesis (ligand, functional monomer, crosslinker, initiator and porogen) and template removal.

Table 2. Imprinted Polymers based materials for Solid Phase Extraction

Template	**Synthesis**	**Ligand, functional monomer/Crosslinker**	**Initiator**	**Porogen**	**Template removal**	**Reference**
Hg(II)	Bulk polymerization	Diaminobenzene, VP/EDMA	AIBN	Methanol	0.5M thiourea in 0.1M HCl	[44]
MeHg	Bulk polymerization	(4-ethenylphenyl)-4-formate-6-phenyl-2,2'-bipyridine/DVB	AIBN	DMF	1M thiourea in 4-6 M HCl	[45]
Hg(II)	Silica gel surface imprinting	3-mercaptopropyltrimethoxysilane/ tetraethoxysilane (TEOS)	-	CTAB, water, NH_3H_2O 150:1:8	0.6M HNO_3	[46]
Hg(II)	Suspension polymerization	N-Methacryloyl-(L)-cysteine, 2-hydroxyethyl methacrylate (HEMA)/EDMA	Benzoyl peroxide	Toluene	0.5% thiourea in 0.05M HCl	[47]
Hg, CTAB	Silica gel imprinting, emulsion	N-[3-(trimethoxy-silyl)propyl]ethylenediamine (TPED)/ tetraethoxysilane (TEOS)	1M NaOH	Water	4M HCl	[48]

Table 2. Imprinted Polymers based materials for Solid Phase Extraction

Template	**Synthesis**	**Ligand, functional monomer/Crosslinker**	**Initiator**	**Porogen**	**Template removal**	**Reference**
MeHg	Suspension polymerization	Methacryloyl-(L)-cysteine /EDMA	AIBN	Toluene	1M thiourea and 8 M HCl	[49]
Hg(II)	Silica gel surface imprinting	3-aminopropyltrimethoxysilane/silicagel	-	Methanol	6 M HCl	[50]
Hg(II)	Dispersion polymerization	1-(2-thiazolylazo)-2-naphthol (TAN), MAA/ trimethylolpropane trimethacrylate TMPTM	AIBN	ACN	4M HNO_3	[51]
Hg(II)	Silica gel surface imprinting	3-mercaptopropyltrimethoxysilane (MPS) /Methanesulfonic acid functionalized silicagel	-	Methanol	1M HNO_3	[52]
Hg(II)	Bulk polymerization	4-(2-thiazolylazo) resorcinol, MAA/EDMA	AIBN	Cyclohexanol	1M thiourea in 0.5M HCl	[53]

Table 2. Imprinted Polymers based materials for Solid Phase Extraction

Template	Synthesis	Ligand, functional monomer/Crosslinker	Initiator	Porogen	Template removal	Reference
Hg(II)	Emulsion polymerization	Dithizone, phenyl methacrylate and methyl metacrylate/1,6-hexanediol diacrylate	Potassium persulfate	Water	-	[54]
Hg(II)	Bulk polymerization	5, 10, 15, 20-tetrakis(3-hydroxyphenyl)-porphyrin, MAA/EDMA	AIBN	Acetonitrile, DMSO 4:1	1M HNO_3	[55]
Hg(II)	Bulk polymerization	N-methacryloyl-2-mercaptoethylamine, MAA/EDMA	AIBN	DMSO/ toluene	Thiourea 5% in 2M HCl	[56]
Hg(II)	Sol-gel process	Thymine, 3-isocyanatopropyltriethoxysilane (IPTS)/tetraethoxysilane (TEOS)	-	Ethanol, aqueous ammonia	0.5M HCl	[57]
Hg(II)	Silica gel surface imprinting Suspension	1-(2-thiazolyazo)-2-naphthol and 1-pyrrolidinedithiocarboxylic acid , MAA/silica gel modified with trimethylolpropane trimethacrylate	AIBN	ACN	4M HNO_3/0.5 M thiourea in 0.5 M HCl	[58]

Table 2. Imprinted Polymers based materials for Solid Phase Extraction

Template	Synthesis	Ligand, functional monomer/Crosslinker	Initiator	Porogen	Template removal	Reference
Hg(II),CTAB	Silica gel imprinting, emulsion	N'-[3-(trimethoxysilyl)-propyl] diethylenetriamine (TPET)/tetraethoxysilane (TEOS)	-	Water	3M HNO_3	[59]
Hg(II)	Surface modification of Fe_3O_4 nanoparticles	N-(pyridin-2-ylmethyl)ethenamine, vinyl Fe_3O_4 /EDMA	AIBN	Methanol	0.1M EDTA in 0.1M HCl	[60]
Hg(II)	Polyamination and imprinting of chitosan	Tetraethylenepentamine (TEPA), chitosan/ Epichlorohydrin	-	10 % NaOH, metanol 1:1	0.1M H_2SO_4	[61]
Hg(II)	Graft copolymerization of PET backbone	Thiosemicarbazide, polyacrylonitrile (PAN) copolymerized with poly(ethylene terephthalate) /Formaldehyde	KMnO4 and oxalic acid	Diluted HCl	0.5M HNO_3	[62]
Hg(II) or MeHg	Thermal polymerization	3-methyl-5-(methylthio)-4-vinylthiophene-2-carboxylic acid (VTCA) /EDMA	Benzoyl peroxide (BPO)	Acetonitrile	Methanol/ acetic acid 9:1	[63]

Table 2. Imprinted Polymers based materials for Solid Phase Extraction

Template	Synthesis	Ligand, functional monomer/Crosslinker	Initiator	Porogen	Template removal	Reference
Hg(II)	Dispersion polymerization	1-pyrrolidinedithiocarboxylic acid , MAA/ trimethylolpropane trimethacrylate	AIBN	ACN	4M HNO_3/ 0.5 M thiourea in 0.5 M HCl	[64]
Hg(II)	Sol-gel process	Dithizone/3-aminopropyltriethoxylane/tetraethoxysilane (TEOS)	-	Ethanol, aqueous ammonia	0.5M HCl	[65]
Hg(II)	Modification of cellulosic cotton fiber	Thiourea modified cotton fibers/Formaldehyde	-	Diluted HCl	0.1 M HNO_3	[66]
Hg(II)	Precipitation polymerization	1-(2-acetatophenyl)-3-(4-methylphenyl)triazine, MAA/EDMA	AIBN	DMSO, acetonitrile 1:4	1M HCl	[67]
Hg(II)	Precipitation polymerization	2, 2'-di pyrydyl amine, MAA/EDMA	AIBN	Methanol	50% HCl	[68]

Table 2. Imprinted Polymers based materials for Solid Phase Extraction

Template	Synthesis	Ligand, functional monomer/Crosslinker	Initiator	Porogen	Template removal	Reference
Hg(II)	Precipitation polymerization	4-aminothiophenol	Ammonium persulfate	Ethanol, water 2:3	5% thiourea in 2M HCl	[69]
Hg(II)	Bulk polymerization	8-hydroxyquinoline, 4-vinylpiridine/EDMA	AIBN	DMSO	0.1M EDTA, 10% HNO_3	[70]
Hg(II)	Bulk polymerization	Cysteine, MAA, HEMA/EDMA	Benzoyl peroxide (BPO)	Methanol	0.5% thiourea in 0.05M HCl	[71]
MeHg	Precipitation polymerization	Phenobarbital, MAA/EDMA	AIBN	ACN, water 4:1	1M thiourea in 1M HCl	[72] [73]
Hg(II)	Surface modification of silica coated maghemite nanoparticles	Mercaptoethylamino/EDMA	Ammonium persulfate	Water	0.5M thiourea, 0.05M HNO_3 1:1	[74]

Table 2. Imprinted Polymers based materials for Solid Phase Extraction

Template	**Synthesis**	**Ligand, functional monomer/Crosslinker**	**Initiator**	**Porogen**	**Template removal**	**Reference**
Hg(II)	Dispersion polymerization	2-vinylpiridine/EDMA	AIBN	Methanol	1M EDTA	[75]
Hg(II)	Bulk polymerization	5, 10, 15, 20-tetrakis(3-hydroxyphenyl)-porphyrin, MAA/EDMA	AIBN	Acetonitrile, DMSO 4:1	1M HNO_3	[76]
Hg(II), Cd(II), Ni(II), Cu(II)	Sol-gel process	Dithizone/3-aminopropyltriethoxylane/ tetraethoxysilane (TEOS)	-	Ethanol, aqueous ammonia	0.5M HCl	[77]
Hg(II)	Surface modification of silica coated Fe_3O_4 nanoparticles	Allylthiourea/EDMA	AIBN	Methanol, DMF 1:1	5% thiourea in 0.5M HNO_3	[78]

The most common method of synthesis is bulk or precipitation polymerization. The process of precipitation polymerization differs from bulk polymerization in that the former is performed in a large volume of solvent. Therefore, precipitation polymerization is carried out over an extended period of time to facilitate the contact between the template and the functional monomer [21]. Only a few studies used methylmercury as a template [45, 49, 63, 72]. Liu et al. [45] synthesized a methylmercury-imprinted polymer with (4-ethenylphenyl)-4-formate-6-phenyl-2,2'-bipyridine, divinylbenzene (DVB) as crosslinking agent and 2,2′-azobisisobutyronitrile (AIBN) as initiator. They packed the polymer in columns and used it for the determination of methylmercury in aqueous and biological samples (human hair). Büyüktiryaki et al. [49] used the dispersion polymerization technique to synthesize imprinted beads that were used as SPE support for the determination of methylmercury and mercury ions in marine food and seawater samples. The methylmercury-methacryloyl-(L)-cysteine (MM-MAC) complex was used as monomer, and ethylene dimethacrylate (EDMA) was used as crosslinking agent. Rodriguez et al. [72] synthesized a molecularly imprinted polymer (MIP) using phenobarbital as ligand, methacrylic acid (MMA) as monomer, and EDMA as cross-linking agent, for analysis of wastewater [79], seafood [72] and speciation in seawater after a chromatographic separation [73]. MAA (functional monomer) and EDMA (crosslinker) are two of the most used reagents in the synthesis of the imprinted polymers. Acidic thiourea, is the most common reagent to remove the template, sometimes in combination with ultrasonic energy to accelerate the process [72].

Wu et al. [48], and Batlokwa et al. [59] used a double-imprinting technique with inorganic mercury and cetyltrimethylammonium bromide (CTAB) as a second template to improve the efficiency of a hybrid organic-inorganic polymer. Silica gel surface imprinting provides good accessibility to the analyte and low-mass transfer resistance, and it is now one of the favorite method of synthesis of composites using different ligands for mercury: 3-mercaptopropyltrimethoxysilane [46], N-[3-(trimethoxy-silyl)propyl]ethylenediamine)] (TPED) [48], 3-aminopropyltrimethoxysilane [50], methanesulfonic acid [52], or 1-(2-thiazolyazo)-2-naphthol and 1-pyrrolidinedithiocarboxylic acid [51]. The sol-gel process has also been used to produce hybrid materials with ligands such as thymine [57], or dithizone [65], [77]. Magnetic nanoparticles have been also modified with imprinted polymers [60], in some cases after a surface modification with silica [74, 65]. Mercury (II) imprinted materials such as polyaminated chitosan derivatives [61], thiourea modified cotton fibers [66], or chelating fibers based on poly(ethylene terephthalate) [62], have also been developed for water purification.

Table 3 collects the information from studies applying imprinting polymers for mercury removal or mercury determination using solid phase extraction. The table shows the type of sample studied, the technique used for Hg determination, the enrichment factor, the adsorption capacity of the polymer, and the operating mode (batch or column).

Table 3. Methods of analysis of Hg (II) or methylmercury using molecularly imprinted polymers

Template	Sample	Hg determination	Enrichment factor	Adsorption (mg g^{-1})	Mode	Reference
Hg(II)	River sediments, water samples	CV-AAS	200	41.12	Batch and column	[44]
MeHg	Human hair, soil samples	CV-AAS	20	34.10	Batch and column	[45]
Hg(II)	Seawater, river water, human hair, soil	ICP-OES	150	78.5	Batch and column	[46]
Hg(II)	Human serum	CV-AAS	-	0.45	Batch	[47]
Hg, CTAB	Water samples	CV-AAS	-	39.92	Batch	[48]
MeHg	Synthetic seawater, seafood	ICP-OES, HPLC-DAD	450-1700	92.40	Batch and column	[49]
Hg(II)	Water, certified river sediment, Chinese traditional medicine samples	ICP-OES	75	29.89	Batch and column	[50]

Table 3. Methods of analysis of Hg (II) or methylmercury using molecularly imprinted polymers

Template	**Sample**	**Hg determination**	**Enrichment factor**	**Adsorption (mg g^{-1})**	**Mode**	**Reference**
Hg(II)	Mineral, river and seawater	CV-AAS	50	6.42	Batch	[51]
Hg(II)	Water	CV-AAS	75	92.27	Batch	[52]
Hg(II)	Spiked tap water and river water samples	CV-AAS	2.5	25.07	Batch	[53]
Hg(II)	Water (Hg removal)	CV-AAS	-	4	Column	[54]
Hg(II)	Seawater and tap water, fish	CV-AAS	160	249	Batch	[55]
Hg(II)	Tap water and seawater	AFS, ICP-MS	-	28	Batch	[56]
Hg(II)	River and tap water	AFS	200	4.2	Batch and column	[57]
Hg(II)	Wines	CV-AAS	10	22.06	Column	[58]
Hg(II),CTAB	Water	ICP-OES	-	0.71	Batch	[59]
Hg(II)	Fish	ICP-OES	20	147	Batch	[60]

Table 3. Methods of analysis of Hg (II) or methylmercury using molecularly imprinted polymers

Template	Sample	Hg determination	Enrichment factor	Adsorption (mg g^{-1})	Mode	Reference
Hg(II)	Water (Hg removal)	ICP-OES	-	9.07	Batch	[61]
Hg(II)	Water (Hg removal)	CV-AAS	-	137.13	Batch	[62]
Hg(II) and MeHg	Seawater (Hg removal)	ICP-OES	-	92.8	Batch	[63]
Hg(II)	Surface waters	CV-AAS	25	12.8	Batch	[64]
Hg(II)	Water, fish, human hair	AFS, ICP-MS	20	0.74	Batch, column	[65]
Hg(II)	Water (Hg removal)	CV-AAS	-	110.3	Batch	[66]
Hg(II)	Water	GF-AAS	83.33	2.75	Batch	[67]
Hg(II)	Water, fish	CV-AAS	120	27.96	Batch	[68]
Hg(II)	Water (Hg removal)	Colorimetry (complex with diphenyl carbazide), AAS	-	400	Batch	[69]
Hg(II)	Tap and river water	VG-AFS	20	143.8	Batch	[70]

Table 3. Methods of analysis of Hg (II) or methylmercury using molecularly imprinted polymers

Template	**Sample**	**Hg determination**	**Enrichment factor**	**Adsorption (mg g^{-1})**	**Mode**	**Reference**
Hg(II)	Sludge, petroleum oil (Hg removal)	ICP-MS	-	1.10	Batch	[71]
MeHg	Fish, wastewater	HR-CSAAS, ICP-OES	40	0.02	Column	[72] [79]
MeHg	Water	HPLC-ICPMS	50	-	Column	[73]
Hg(II)	Tap water and wastewater	FAAS	100	72.8	Batch	[74]
Hg(II)	Seawater and tap water, fish	CV-AAS, FAAS	50	24.6	Column	[75]
Hg(II)	Water, human hair	FIA-Photometry (complex with dithizone)	160	-	Column	[76]
Hg(II),Cd(II), Ni(II), Cu(II)	Seawater	AFS	20	16.55	Batch, column	[77]
Hg(II)	Water	AFS	-	78.3	Batch	[78]

Most of the materials including imprinted polymers were mainly developed for analytical purposes. The highest adsorption capacity (249 mg g^{-1}) corresponds to the polymer synthesized by Shamsipur et al. [55] that includes 5, 10, 15, 20-tetrakis(3-hydroxyphenyl)-porphyrin as ligand, and provides an enrichment factor of 160 operating in batch mode. The sorbent based on Fe_3O_4 nanoparticles developed by Najafi et al. [60] provided enrichment factors of 20, with a capacity of 147 mg g^{-1}. Imprinted thiourea modified cotton fibers [66], and the chelating fibers based on poly(ethylene terephthalate) [62] designed for Hg removal from waters have some of the best adsorption capacities (110.3 mg g^{-1} and 137.12 mg g^{-1}, respectively). The adsorption capacity of the polymer synthesized by Büyüktiryaki et al. [49] is 92.40 mg g^{-1}, with preconcentration factors of 450 (Hg(II)) and 1700 (methylmercury) in batch mode. Finally, the porphyrin-based polymer developed by Rajabi et al. [76] was packed in a minicolumn and was loaded with sample volumes ranging from 10 to 800 mL; therefore, the preconcentration factor of 160 could be obtained for 5.0 mL of elution. After elution, the mercury content was determined photometrically after reaction with dithizone using a flow injection system. The study by Khairi et al. [71] must also be mentioned because of the interesting application of their sorbent: Hg removal from sludges and petroleum oil.

2.3 Sensors for mercury determination

2.3.1 Electrochemical sensors based on polymer modified electrodes

Chemically modified electrodes (CME) have advantages over unmodified electrodes (usually carbon and gold electrodes), such as improved sensitivity and selectivity when used for the electrochemical determination of many analytes. CME have been used for the analysis of heavy metals at trace levels [8], and methods are based on the interaction of metals with a functional group of the compound used to modify the electrode. This interaction is usually selective or only occurs with a very specific group of metals, and the discrimination depends on the potential used for electrochemical measurements. Polymers normally exhibit good conductivity or electrocatalytic ability. The most typical strategies for the synthesis of modified electrodes are an electropolymerization onto the electrode surface or a simple adsorption of the polymer. Conductive polymers are one of the materials most commonly applied to modify electrode surfaces [8]. These polymers typically contain groups that bind selectively to mercury or that can function as ion exchangers. Other compounds to modify electrodes are complexing agents, DNA or ion imprinting polymers (IIP) [8, 15, 19, 80]. The electrochemical analysis of mercury is sensitive, inexpensive, simple, and fast and can be performed with miniaturized, portable instrumentation [81]. It should be noted that, despite some very promising results, many of the electrodes have only been applied to aqueous standards.

Table 4 summarizes the latest work on the development of modified polymer electrodes for the detection of mercury. The type of modified electrode, the type of sample, the limit of detection (LOD) and the linear range, are shown.

Table 4. Electrochemical sensors for mercury using electrodes modified with polymers and polymeric composites

Electrode composition	**Sample**	**LOD (mol L^{-1})**	**Linear Range (mol L^{-1})**	**Reference**
GCE, Nafion modified	Water	4.5×10^{-8}	Up to 5×10^{-7}	[82]
ISE, nano-composite poly-o-toluidine Th(IV) phosphate	Water, titrations	5×10^{-5}	10^{-1} - 5×10^{-5}	[83]
Sol–gel and sol–gel- poly(vinylsulfonic acid) carbon composite electrode	Synthetic water and wastewater	1.5×10^{-8}	5.0×10^{-8} - 5.0×10^{-5}	[84]
Graphite electrode and PVC polymeric membrane electrode incorporating furan-2-yl-4-(4-nitrophenyl)-2-phenyl-5H-imidazole-3-oxide	Synthetic water, insecticide, dental amalgam	8.91×10^{-7} and 6.3×10^{-6}	10^{-1} - 10^{-6} and 10^{-1} - 10^{-5}	[85]
Pt modified with poly(vinylferrocenium)	Tap and natural spring water	5×10^{-10}	10^{-3} - 5×10^{-5}	[86]
ISE, polymeric-inorganic composite cation-exchanger Nylon-6,6 Sn(IV) phosphate	Drain water	10^{-7}	10^{-1} - 10^{-7}	[87]
Screen-printed carbon electrode (SPCE) modified with polyaniline- poly(2,2-dithiodianiline) co-polymer	Water	2.1×10^{-7}	10^{-8} - 10^{-5}	[88]

Table 4. Electrochemical sensors for mercury using electrodes modified with polymers and polymeric composites

Electrode composition	**Sample**	**LOD ($mol\ L^{-1}$)**	**Linear Range ($mol\ L^{-1}$)**	**Reference**
Screen-printed carbon electrode (SPCE) modified with a polyaniline-methylene blue polymer layer	Water	2.7×10^{-7}	10^{-8} - 10^{-5}	[89]
Screen-printed carbon electrode (SPCE) modified with polyaniline or polyaniline- poly(2,2-dithiodianiline) co-polymer	River water	8.4×10^{-11} and 2.2×10^{-13}	10^{-6} - 10^{-3} and 10^{-9} - 10^{-6}	[90]
GCE, poly(3,4-ethylenedioxythiophene), gold nanoparticles	Water	4.14×10^{-9}	2.5×10^{-9} – 1.5×10^{-8} and 2.5×10^{-8} – 5.5×10^{-8}	[91]
Ir microelectrode/ AuNPs covered with poly(2-hydroxyethyl methacrylate) brushes modified with a metallothionein derived peptide	Water	5×10^{-10}	0–1.6×10^{-5}	[92]
GCE, AuNPs-polydopamine carbon nanospheres and a dual hairpin structure based on interaction thymine–Hg^{2+}–thymine	Water	5.2×10^{-11}	10^{-10} – 10^{-6}	[93]
Organic thin-film transistors (OTFTs)/block copolymer-templated AuNPs functionalized with a mercury-binding oligonucleotide sequence	Water	10^{-6}	Up to 5×10^{-7}	[94]
Au modified with short-chain, thymine-rich, ss-DNA oligonucleotides	Water	3×10^{-9}	3×10^{-9} – 5×10^{-8}	[95]

Table 4. Electrochemical sensors for mercury using electrodes modified with polymers and polymeric composites

Electrode composition	**Sample**	**LOD (mol L^{-1})**	**Linear Range (mol L^{-1})**	**Reference**
Ni electrode, carbon paste, polypyrrole/cellulose composite papers in solution	Groundwater	10^{-6}	Up to 10^{-5}	[96]
FET, reduced graphene oxide –polyfuran nanohybrids	Water	10^{-11}	10^{-11}-10^{-7}	[97]
Pt, copolymer of aniline and dithiodianiline with immobilized horseradish peroxidase	Tap water and river water	3.9×10^{-12}	0– 5×10^{-9}	[98]
Polypyrrole -functionalized three dimensional porous electrode of electrochemically reduced graphene oxide	Wastewater	3×10^{-11}	10^{-10}-1.1×10^{-7}	[99]
Heterogeneous precipitate- based membrane electrode ISE, polyaniline into the gel of Sn(II)SiO_3 followed by functionalized carbon nanotubes	Water	10^{-6}	10^{-7}-10^{-1}	[100]
Polymer electrode prepared from polystyrene grafted with acrylonitrile as monomer	Water	10^{-4}	2.5×10^{-3}-10^{-2}	[101]
GCE, NiNPs-decorated carbon porous materials	Fish extracts	2.1×10^{-9}	0-2.1×10^{-5}	[102]

The lowest limits of detection were obtained by Somerset et al. [90] using screen-printed carbon electrodes (SPCE) modified with polyaniline or polyaniline- poly(2,2-dithiodianiline) co-polymer (LOD = 8.4×10^{-11} M and 2.2×10^{-13} M, respectively) for the voltammetric determination of the ion Hg(II), and with application to river waters. The Hg(II) ions were determined in two steps: (i) pre-concentration and reduction on the modified electrode surface and (ii) subsequent stripping during the positive potential sweep. Other electrodes modified with aniline derivatives were developed by the same authors [88, 89], that also prepared an enzyme polymeric electrode based on a co-polymer of aniline and dithiodianiline as a mediator for the immobilization of the enzyme horseradish peroxidase in the biosensor construction [98]. In this case, the sensor was applied to the analysis of tap and river water, and the limit of detection was 3.9×10^{-12} M, lower than the concentrations levels recommended by the World Health Organization and Environmental Protection Agencies.

Carramolino et al. [84] synthesized a sol–gel- poly(vinylsulfonic acid) carbon composite electrode. The functional group is encapsulated in the sol–gel material but maintaining their ability to interact with Hg(II) species that diffuse into the matrix. The composite electrode benefits from the mechanical properties of the silicate backbone to increase sensitivity and selectivity.

A poly(vinylferrocenium)-modified platinum electrode was developed for determination of Hg(II) ions in aqueous solutions [86]. The ion was preconcentrated at the polymeric matrix by adsorption and also complexation reaction with Cl^{-}. Detection of Hg(II) was carried out by differential pulse anodic stripping voltammetry (DPASV) after reduction. Mercury ion concentration as low as 5×10^{-10} M could be detected with the prepared electrode, and the electrode was applied to the determination of Hg(II) in tap and spring water samples.

Gold nanoparticles (AuNPs) were also used in several modified electrodes to increase sensitivity [91-94]. Schwer et al. [92] modified Ir microelectrodes with AuNPs covered with poly(2-hydroxyethyl methacrylate) brushes modified with a metallothionein derived peptide. Polymer brushes are dense assemblies of polymer chains that are attached by one chain end to an underlying substrate, and they are attractive because they allow access to sensory coatings that present a high surface density of functional groups. Wang et al. [93] developed an AuNPs based electrode that combines the specificity of the thymine–Hg(II)–thymine interaction with the high sensitivity of enzymatic signal amplification, providing low limits of detection (5.11×10^{-11} M). The thymine interaction with mercury was also used by Gorski et al. [95] to create a modified gold electrode with thymine-rich DNA chains.

Graphene based materials were also used in the development of electrochemical sensors. A new type of field-effect transistor (FET) sensor, based on reduced graphene oxide–polyfuran nanohybrids [97], exhibited a rapid response (<1 s) and high sensitivity (10^{-11} M). Wang et al. [99] designed a polypyrrole -functionalized electrode of electrochemically reduced graphene oxide that exhibited high sensitivity and selectivity, with a limit of detection of 3×10^{-11} M. The good performance of this electrode was attributed to its oriented porous microstructure that facilitates the strong and selective adsorption of the target ions.

Electrodes modified with imprinted polymers have very promising characteristics. However, they have the disadvantage that to obtain low LODs very high accumulation times, usually greater than 10 minutes, are required. An improvement of the materials and the development of new polymers will facilitate the obtaining of electrodes with better yields and shorter analysis times [8].

Table 5 summarizes the latest work on the development of electrodes modified with IIP for the detection of methylmercury and especially Hg(II).

Table 5. Electrochemical sensors for mercury using electrodes modified with molecularly imprinted polymers.

Electrode	**Sample**	**LOD (mol L^{-1})**	**Linear range (mol L^{-1})**	**Reference**
GCE, tetra pyrrole appended EDTA-like derivative IIP	Water	5×10^{-10}	10^{-8} -8×10^{-6}	[103]
GCE, N,N'-ethylenebis[N-[(3-(pyrrole-1-yl)propyl) carbamoyl)methyl]-glycine] IIP	Water	10^{-8}	10^{-8} -10^{-6}	[104]
CPE, nanocomposite including 5%MWCNT, 1% nanosilica, 10% ionic liquid, 15% 4-vinylpiridine IIP	Wastewater	10^{-7}	10^{-7} - 10^{-2}	[105]
CPE, 4-vinylpyridine IIP	Tap, river and lake water	5.2×10^{-10}	2.5×10^{-9} – 5×10^{-7}	[106]
GCE, poly(2-mercaptobenzothiazole) (MPMBT) IIP	River water	10^{-10}	10^{-9}-1.60×10^{-7}	[107]
GCE, SWCNTS, AuNPS, poly(2-mercaptobenzothiazole) (MPMBT) IIP	River and tap water	8×10^{-11}	4×10^{-10} – 9.6×10^{-8}	[108]
CPE, MWCNT & N-(pyridin-2- ylmethyl)ethenamine) IIP	Tap water, seawater, sewage water, fish samples	6.3×10^{-8}	8×10^{-8} – 3.5×10^{-3}	[109]
GCE, MWCNT, 5, 10, 15, 20-tetrakis(3-hydroxyphenyl)-porphyrin IIP	Ground and wastewater	5×10^{-9}	10^{-8}- 7×10^{-4}	[110]

Table 5. Electrochemical sensors for mercury using electrodes modified with molecularly imprinted polymers.

Electrode	**Sample**	**LOD (mol L^{-1})**	**Linear range (mol L^{-1})**	**Reference**
ASS, conductive composite covered by a PVC membrane including 4-vinylpiridine IIP	Wastewater	3.2×10^{-9}	10^{-8} - 10^{-3}	[111]
CPE, graphene nanosheets, Al_2O_3 nanoparticles, ionic liquid and thiazolylazo) resorcinol IIP	Tap water, river water, wastewaters, the waste of dental amalgam, tuna fish, shrimp and human hair samples	1.95×10^{-9}	4×10^{-9} – 1.3×10^{-3}	[112]
CILE, dithizone IIP	Wastewater	10^{-10}	5×10^{-10} – 10^{-8} and 8×10^{-8} – 2×10^{-6}	[113]
CPE, SiO_2 coated magnetite nanoparticles modified with a mercaptoethylamino IIP	River water, waste water, potato, carrot and lettuce samples.	2×10^{-10}	10^{-9} – 8×10^{-6}	[114]
GCE, reduced graphene oxide modified with 2,2′–((9E,10E)–1,4–dihydroxyanthracene–9,10– diylidene) bis(hydrazine–1–carbothioamide) (DDBHCT) IIP	Water samples	10^{-10}	3.5×10^{-10} – 4×10^{-7}	[115]

Ionic imprinted polymers have been used together with carbon materials of high surface area to decrease accumulation time: nano-composite pastes containing multi-walled carbon nanotubes (MWCNTs) in combination with IIP with different reagents for Hg(II) (4-vinylpiridine [105], N-(pyridin-2- ylmethyl)ethenamine) [109], 5, 10, 15, 20-tetrakis(3-hydroxyphenyl)-porphyrin [110], composites with graphene oxide [111, 115], and graphene nanosheets and alumina nanoparticles [112]. Fu et al. [108] performed the electrochemical detection of Hg(II) in river and tap water with a composite sensor consisting of electropolymerized ion imprinting poly(2-mercaptobenzothiazole) films at the surface of Au nanoparticles/single-walled C nanotube nanohybrids modified glassy C electrode. The sensor showed a good selectivity and provided low detection limits (8×10^{-11} M).

Other electrodes were modified with IIP with pyrrole derivatives [103, 104], or SiO_2 coated magnetite nanoparticles with a mercaptoethylamino IIP [114] providing relatively low detection limits (approximately 10^{-10} M). It should be noted that some of the electrodes were applied to complex matrices: fish [109], waste of dental amalgam, fish, shrimp and human hair samples [112], and vegetables [114].

2.3.2 Optical sensors based on polymers and composites materials

The application of polymers and nanomaterials to design optical sensors for Hg(II) is nowadays one of the most active research fields due to their cost-effectiveness, simplicity and rapidity [9, 15].

Table 6 shows a summary of the latest publications on optical sensors that integrate polymers for the analysis of mercury. The table includes the type of response, and the composition of the sensor or probe for mercury, the type of sample, the detection limit achieved and the dynamic range.

Table 6. Optical sensors for mercury using polymers and composites

Type of response. Composition	**Sample**	**LOD (mol L^{-1})**	**Dynamic range (mol L^{-1})**	**Reference**
Fluorescence (turn off). Hg(II) imprinted polymer membrane with 4-vinylpyridine as monomer and 9-vinylcarbazole	Water	-	5×10^{-7} - 10^{-4}	[116]
Fluorescence resonance energy transfer (FRET). Fluorescein isothiocyanate and a spirolactam rhodamine derivative attached onto polyethylenimine and polyacrylic acid respectively.	Water	2×10^{-7}	Up to 8×10^{-5}	[117]
Colorimetric, naked eye. Membranes of a copolymer of methacrylamide and vinylpyrrolidone with a rhodamine B derivative	Tap water	7.7×10^{-8}	Up to 10^{-3}	[118]
Fluuorescence (turn off). Nanocomposite substituted of a carbazole-based conjugated polymer and silica-coated magnetic iron oxide nanoparticles	Tetrahydrofuran	4.17×10^{-7}	Up to 3.33×10^{-8}	[119]
Fluorescence (turn on) Composite nanospheres containing dithizone, luminescent $LaVO_4:Eu^{3+}$ nanoparticles and the methylmethacrylate-co-methacrylicacid copolymer	Tap water, cell culture media	3.2×10^{-8}	4×10^{-8} – 4×10^{-6}	[120]
Electroluminescence. Polymer of 4H-cyclopenta[2,1-b:3,4-b′]dithiophene (CPDT)-linked ruthenium phenanthroline complexes carrying azacrown ethers	Acetonitrile/PBS buffer	<5×10^{-5}	Up to 10^{-4}	[121]

Table 6. Optical sensors for mercury using polymers and composites

Type of response. Composition	**Sample**	**LOD (mol L^{-1})**	**Dynamic range (mol L^{-1})**	**Reference**
Colorimetric and fluorescence (turn on). Thiocarbonyl quinacridone-based conjugated polymer nanoparticles	Water, Hela cell lines	5×10^{-9}	$2\times10^{-6} - 1.48\times10^{-5}$	[122]
Fluorescence (turn on). Tetracyanoethylene-decorated triphenylamine-based conjugated polymer	Tetrahydrofuran	6.9×10^{-9}	$3.27\times10^{-4} - 3.3\times10^{-7}$	[123]
Colorimetric. Polyaniline based immobilized sensor	Water	5×10^{-9}	$5\times10^{-9} - 1.5\times10^{-4}$	[124]
UV-VIS and fluorescence (turn off). D- π -A conjugated polymer chemosensor based on benzo[c][1,2,5] selenadiazole moiety and fluorene groups	$CHCl_3$	1.9×10^{-7}	Up to 1.8×10^{-5}	[125]
UV-VIS and fluorescence (turn off). Solid polymer and metallogel networks based on a fluorene derivative	Acetonitrile: Water	8.6×10^{-5}	$4.90\times10^{-7} - 2.92\times10^{-3}$	[126]
Colorimetric and fluorescence (turn on). Water-soluble poly(vinyl alcohol) with pendant rhodamine B	Water	5.72×10^{-7}	Up to 8.0×10^{-5}	[127]
Fluorescence (turn off). Polymer incorporating triazole moieties	Dimethylformamide	2.6×10^{-7}	$3.0\times10^{-5} - 10^{-4}$	[128]
Colorimetric and fluorescence resonance energy transfer (FRET). Au NPs functionalized with terthiophene appended rhodamine derivatives.	Dimethyl sulfoxide	1.34×10^{-6}	-	[129]

Table 6. Optical sensors for mercury using polymers and composites

Type of response. Composition	Sample	LOD (mol L^{-1})	Dynamic range (mol L^{-1})	Reference
Colorimetric and luminescence (turn on). Hydrophobic cyclometallated ruthenium complex as a chemodosimeter to assemble on amphiphilic polymer-coating upconversion nanophosphors	Water	4.1×10^{-8}	Up to 8×10^{-6}	[130]
Fluorescence (turn on). Polymer with functional monomer glycidyl methacrylate-rhodamine hydrazine	Water	5.23×10^{-8}	Up to 2.5×10^{-4}	[131]
Fluorescence (turn off). Benzoselenadiazole-based conjugated polymer fluorescent sensor		2.1×10^{-7}	Up to 2.5×10^{-5}	[132]
Colorimetric and fluorescence. Polymer supporting dibutylaniline substituents in a microfluidic chip	Water	5×10^{-4}	Up to 0.5×10^{-2}	[133]
Colorimetric. TiO2/poly(acrylamide-co-methylenbisacrylamide) nanocomposite	Tap, river and seawater	5×10^{-9}	5×10^{-9} – 5×10^{-5}	[134]
Fluorescence resonance energy transfer (FRET). Host-guest-recognition-based polymer brush-functionalized mesoporous silica nanoparticles loaded with conjugated polymers (spirolactam rhodamine derivative for recognition)	Ethanol/water	4.2×10^{-6}	4×10^{-6} – 10^{-5}	[135]
Colorimetric, naked eye. Porphyrin-functionalized polyacrylonitrile fiber	River water	10^{-7}	-	[136]
Colorimetric. Based on aggregation of AuNPS-DNA-phthalic diglycol diacrylate	Lake water	1.5×10^{-10}	2.5×10^{-10} – 5×10^{-7}	[137]

Table 6. Optical sensors for mercury using polymers and composites

Type of response. Composition	**Sample**	**LOD (mol L^{-1})**	**Dynamic range (mol L^{-1})**	**Reference**
Colorimetric, naked eye. Aldoxime-functionalized water-soluble polymeric probe	Water	6×10^{-3}	Up to 2.4×10^{-2}	[138]
Colorimetric, naked eye. Polymeric probes with dithioacetal units in the side chains	Water	$<4\times10^{-6}$	Up to 9.6×10^{-5}	[139]
Colorimetric. Silver embedded chitosan matrix nanocomposite	Water	7.2×10^{-8}	Up to 8×10^{-4}	[140]
Fluorescence resonance energy transfer (FRET). Spirolactam rhodamine-B derivative co-functionalized mesoporous silica nanoparticles	Ethanol/water	2×10^{-7}	$2\times10^{-5} – 1.2\times10^{-4}$	[141]
Fluorescence (turn off). Polymer incorporating thiazole moiety	Tetrahydrofuran	5.1×10^{-7}	$2\times10^{-6} –10^{-5}$	[142]
UV-VIS absorption. Amphiphilic acrylamide-based polymer with porphyrin pendants	Water	5×10^{-8}	$5\times10^{-8}–10^{-4}$	[143]
Fluorescence (turn off). Hypercrosslinked conjugated 2,4,6-trichloro-1,3,5-triazine and dibenzofuran microporous polymer	Water	5×10^{-8}	Up to 4×10^{-4}	[144]
Fluorescence (turn on). Polymer containing N-(4-azidophenyl)-4-(5-(1-oxo-indan-5-yl)pyrimidin-2-yl)piperazin-1-carbothioamide	Water, fish	6.6×10^{-6}	Up to 2×10^{-3}	[145]
Fluorescence (turn off). Lanthanide coordination polymer probe of Tb- ciprofloxacin /adenosine monophosphate	Drinking water, urine	1.6×10^{-10}	$1.5\times10^{-9} – 7\times10^{-8}$	[146]

Table 6. Optical sensors for mercury using polymers and composites

Type of response. Composition	**Sample**	**LOD (mol L^{-1})**	**Dynamic range (mol L^{-1})**	**Reference**
Fluorescence (turn on). Gd-based coordination polymer with 5-triazole isopthalic acid	Water	5×10^{-7}	Up to 10^{-5}	[147]
Fluorescence (turn off). Graphene oxide quantum dots and polymer-like quantum dots (obtained after the pyrolysis of citric acid and diethylenetriamine)	Water	5×10^{-8}	Up to 10^{-5}	[148]

Optical sensors employing molecular imprinting polymers are typically based on the inclusion of a chromophore or fluorophore in its structure which provides its optical characteristics. The selectivity of the polymer to the analyte of interest is bound to the interaction of the latter with the fluorescent molecule and will allow its determination by spectrophotometric techniques. Güney et al. [116] synthesized an IIP using a fluorescent monomer as complexing agent 9-vinylcarbazole, in addition to 4-vinylpyridine and EDMA as a crosslinker. The polymer membranes with semicylindrical shapes were synthesized in a Teflon mold and were directly applied to aqueous samples. The values of the Hg(II) binding ratio, sensitivity and selectivity were superior for the imprinted polymeric membranes than for non imprinted polymeric membranes, probably due to the rougher surface of the IIP, which provided an increase in the surface area and in complexation performance. The authors deduced that the synthesized IIP had the drawback of long equilibration and elution times because of the thickness of the polymeric membrane. The literature about optical sensors and IIP is still scarce; the use of ion-imprinted sol-gel fluorescent films for recognition of mercury could be a better strategy for analysis [149].

Different sensing schemes based on the use of polymers with rhodamine were developed for mercury sensing [117, 118, 127, 129, 131, 135, 141]. Ma et al. [117] described the use of a fluorescence resonance energy transfer (FRET)-based ratiometric sensor with a polymer nanoparticle as scaffold for detecting Hg(II) in aqueous media. A fluorescent dye fluorescein isothiocyanate (donor) and a spirolactam rhodamine derivative (mercury ion probe) were covalently attached onto polyethylenimine and polyacrylic acid respectively. Under optimized conditions, the system exhibited a stable response for Hg(II) in aqueous media in a pH range of 4.6-7.3 in water, and the donor-acceptor distance between the layers of polymer and energy transfer efficiency could be precisely controlled. Membranes of a copolymer of methacrylamide and vinylpyrrolidone with a rhodamine B derivative were used by El Kaoutit et al. [118] for colorimetric detection of Hg(II) in aqueous media. The copolymer structures were designed to have both good mechanical properties, and suitable water swelling. The color response can be tuned for detection with the naked eye, or with conventional cameras (e.g. smartphone). Nanomolar concentration of Hg(II) could be detected by the naked eye, and the concentration of the ion could be quantified, within the millimolar to nanomolar range, by means of analyzing the digital information of pictures taken of the membranes after dipping them in water containing the cation for 25 minutes. Kaewtong et al. [129] designed a multifunction sensor based on Hg(II)-induced spirocycle opening leading to fluorescence and colorimetric enhancement. Moreover, they developed a new composite (Au NPs functionalized with terthiophene appended rhodamine derivatives). These nanoparticles aggregated in solution (dimethyl sulfoxide) in the presence of Hg(II) with a measurable change in the emission spectrum.

One of the main problems of many optical sensors is that they only work in organic solvents or mixed solutions, and they are not applicable to the analysis of waters (Table 6). Lanthanide coordination polymers have emerged as attractive biosensor materials due to their flexible components, high tailorable properties and unique luminescence features [120, 146, 147]. Composite nanospheres containing dithizone, luminescent $LaVO_4:Eu^{3+}$ nanoparticles, and amphiphilic polymer have been produced via ultrasonication for the simple, selective, and visual luminescence turn-on detection of Hg(II) in water [120]. Due to the absorption of dithizone, the strong red luminescence of $LaVO_4:Eu^{3+}$ is quenched; however, in the presence of Hg(II) can be tuned. Liu et al. [146] designed a lanthanide coordination polymer probe of Tb-ciprofloxacin /adenosine monophosphate (AMP) for Hg(II) detection. The polymer emits strong green luminescence due to the inclusion of AMP, which withdraws the coordinated water molecules and shields Tb(III) from the quenching effect of O-H vibration in water molecules. The addition of Hg(II) quenches the fluorescence because of the specific coordination interaction with AMP. The probe has long fluorescence lifetime up to millisecond and was applied to analyze drinking water and human urine samples with satisfactory results, and a detection limit of $1.6x10^{-10}$M.

Other sensors are based on porphyrin-functionalized polyacrylonitrile fibers [136], amphiphilic acrylamide-based polymer with porphyrin pendants [143], or polyaniline based materials [124]. Petzoldt et al. [133] introduced a polymer supporting dibutylaniline substituents in a microfluidic chip. The device is capable of monitoring Hg(II) concentrations continuously and without time delay, at a 500 µM concentration. Balamurugan et al. [139] used polymeric probes with dithioacetal units in the side chains for the colorimetric sensing of Hg(II) ions in aqueous solutions, with reaction times of less than 1 min at low pH. Then, these polymeric probes are potential platforms for integrating stimuli-responsive water-soluble polymers with tunable sensing behaviors. These authors [138] also designed aldoxime-functionalized water-soluble polymeric probes for the consecutive colorimetric sensing of multiples analytes, including Hg(II), Ag(I), ClO^-, and cysteine. The consecutive sensing of the analytes can be achieved and this design principle could be extended to higher level applications, such as molecular logic gates, chemical passwords for keypad locks, etc.

The detection of ultralow concentrations of mercury is a challenge. Zhu et al. [137] proposed the colorimetric detection of Hg(II) based on the aggregation of gold nanoparticles (AuNPs) driven by a cationic polymer. In a three-component system, DNA combines electrostatically with phthalic diglycol diacrylate (PDDA) in a solution of AuNPs. In the presence of Hg(II), thymine (T)-Hg(II)-T induced hairpin turns are formed in the DNA strands, facilitating the aggregation of the AuNPs. Then, a change of color from wine-red to blue-purple is produced, and it can be observed with the naked eye. A linear range of 0.25-500nM was obtained by absorption spectroscopy with a detection limit of 0.15 nM. The proposed method, with high selectivity in the presence of other heavy metal ions, was evaluated with the analysis of lake water. Nanocomposites of TiO_2/poly(acrylamide-co-methylenbisacrylamide) [134] were used for the preconcentration of Hg(II) and Pb(II) at trace levels in environmental samples. Afterwards, they selective monitored the target ions by complexation with dithizone. The method was applied to the analysis of tap, river, and sea water samples. The hydrogel sensor could be used as simple preconcentrator leading to simultaneous visual inspection and complete removal of these metal ions over a wide range of concentrations.

2.4 Methods of analysis using polymers, and Hg(II) or methylmercury imprinted polymers

2.4.1 Analysis of environmental samples

The main technique applied to mercury analysis in combination with SPE is atomic absorption coupled to a cold vapor generation system (CV-AAS) (Tables 1 and 3). Despite the need for a larger volume of sample, the reasonable limits of detection achieved, and especially the higher cost of other techniques such as ICP-OES and ICP-MS, make CV-AAS the most frequent technique used in the literature, especially for water analysis [31, 30] after solid phase extraction. UV-VIS detection is an inexpensive alternative to the aforementioned techniques for water analysis, since these samples usually have simple matrices that do not interfere in the formation of the colored complexes on which these colorimetric techniques are based. Thus, Liu et al. [23] applied SPE with UV-VIS detection for mercury determination in water, soil and dust samples using a β-cyclodextrin based polymer as sorbent. Thakur et al. [35] developed a method for the determination of Hg(II) in groundwater and seawater samples in the presence of a high concentration of interfering ions. A polymer based on the use of a vinyl derivative of 8-quinolinone was applied to the analysis of fish and seawater using AFS as detector [36]. This type of detector was also used to analyze fish and various kinds of water after preconcentration with a monolithic column including a divinylbenzene polymer and magnetite [37].

The robustness of the imprinted polymers makes possible the use of these materials as supports for SPE. Liu et al. [44] used imprinted polymer preconcentration in column mode for the subsequent determination of mercury in waters and river sediments. As described above, IIP-SPE combined with CV-AAS detection is the most common technique described for the preconcentration and determination of mercury in water samples [48, 51-54, 62, 64, 66]. Xu et al. [85] synthesized a IIP based on the interaction of Hg (II) and thymine anchored on the surface of a support polymer. The IIP was used for the preconcentration of mercury in samples of river water and tap water, and its subsequent determination by atomic fluorescence. Firouzzare et al. [84] employed AFS following batch extraction using a 2-mercaptoethylamine IIP. Luo et al. [99] also coupled a sequential injection system and vapor generation to AFS for the determination of mercury in water samples, after preconcentration by a IIP based on 4-vinylpyridine and 8-hydroxyquinoline. Magnetic nanocomposites were also applied to the analysis of waters [78], tap water and wastewaters [74].

There are many applications in the literature for the analysis of aqueous solutions and waters using electrodes modified with polymers and imprinted polymers (Tables 4 and 5). However, very low limits of detection (10^{-10}-10^{-13} M) are need to measure the concentrations in natural waters (spring waters [86], river waters [90, 98, 106, 107, 108]), seawaters [109], or even some samples of wastewaters [99, 113]).

Traditionally, the problem of many optical sensors has been the interaction of the probes with the element in water, but modern composites allow the quantification of Hg(II) in some natural samples with limit of detection still relatively high in comparison to those obtained with the electrochemical sensors. Applications include the analysis of tap water [118, 120, 134, 146], river [134, 136], lake [137] and seawater[134].

2.4.2 Analysis of foods and biological materials

Solid phase extraction is a good alternative as pre-concentration and cleaning technique for biological and food samples. In the case of fish samples, the SPE is usually carried out on aqueous extracts. Zarco et al. [36] performed on-column SPE extraction using an 8-hydroxyquinoline based polymer and fluorescence detection for fish and seawater samples. Ma et al. [37] also determined Hg by fluorescence after polymeric SPE in a polydivynilbenzene column. Recoveries of methylmercury were approximately 96%, and a standard reference material GBW 10029 tuna muscle tissue was analyzed for validation of the methodology. The magnetic composites developed by Abolhasani et al.[38] and Ghorbani-Kalhor et al. [39] also provided an accurate and rapid extraction in the analysis of seafood. The parameters affecting the preconcentration procedure were selected after applying a Box-Behnken design through response surface methodology.

Numerous applications of IIPs and MIPs, such as SPE or biomimetic sensors have been successfully developed to solve problems in food analysis [150]. The separation and cleanup step provided by the SPE applying IIPs is useful in dealing with the complex matrices that food presents. Dakova et al. [58] carried out the determination and speciation of mercury in wine samples. The extraction was carried out in batch mode and column mode, using silica gel functionalized with a 1-(2-thiazolylazo)-2-naphthol imprinted polymer. Büyüktiryaki et al. [49], and Rodriguez et al. [72] performed speciation in seafood. The determination was performed by CV-AAS. Najafi et al. [60] analyzed fish samples by ICP-OES with a previous separation assisted by Fe_3O_4 magnetic nanoparticles coated with an imprinted polymer. Shamsipur et al. [55], Roushani et al. [68] and Soleimani et al. [75] also performed mercury determination in fish samples by CV-AAS. Zheng et al. [50] applied a IIP coated silica gel material for Hg determination in Chinese tradicional medicines by ICP-OES.

Hg(II) and methylmercury imprinted polymers have been applied to the analysis of other biological samples. Hg (II) has been determined in human serum by CV-AAS using an imprinted polymer with cysteine groups [47]. Liu et al. [45] synthesized a methylmercury imprinted polymer with (4-ethenylphenyl)-4-formate-6-phenyl-2,2'-bipyridine to extract the compound from human hair and perform the analysis by CV-AAS. Fan et al. [46] determined mercury in human hair by SPE using an inorganic-organic composite for extraction and subsequent determination by ICP-OES. Zhang et al. [65] applied fluorescence detection and a dithizone based imprinted polymer, and Rajabi et al. [76] performed an on-line flow injection extraction with photometric detection for analysis of hair.

There are also some examples of electrodes modified with imprinted polymers and applications to the analysis of fish extracts [109, 112], hair samples [112] or vegetables [114]. There are still few applications using optical sensors with polymeric composites or polymeric probes for the analysis of cell culture media [120], fish extracts [145], urine [146], and bioimaging of Hela cell lines [122].

2.4.3 Speciation of mercury

Most of the methodologies for mercury speciation require a chromatographic separation of the species [2,3,4]. In most cases, the alternative option is to determine organometallic species by difference between inorganic mercury and total mercury [31]. In the cases of the electrochemical and optical sensors, most of them are devoted to the analysis of Hg(II), with a few exceptions that study the interactions of the electrode polymeric modifier with methylmercury [82].

Mercury speciation using molecularly imprinted polymers is a complex process, since the great structural similarity between the different species makes it almost impossible for the materials to have an exclusive affinity for one of the species. Thus, Yordanova et al. [151] digested surface water samples, and they assumed that the difference between the amount in the digested sample and the Hg (II) in solution is methylmercury, discarding other species that are usually in negligible concentrations in water samples, such as ethylmercury. Zhang et al. [152] synthesized a polymeric hybrid material using a sol-gel process and dithizone as a ligand with good selectivity to Hg(II) for the speciation of mercury in waters and biological samples (human hair and fish meat), also determining methylmercury by difference. The same procedure was applied by Dakova to analyze mineral water and sea water [51]. These authors found that the polymer using the interaction between Hg(II) and 1-(2-thiazolylazo)-2-naphthol was highly selective to that ion, with extraction efficiencies of 97.5% and 3% respectively for Hg(II) and methylmercury. The selectivity coefficients were the highest for methylmercury (1633) in comparison to other ions such us Cd(II), Co(II), Cu(II), Ni(II), Pb(II) or Zn(II). The same authors developed a core-shell imprinted polymer with 1-(2-thiazolylazo)-2-naphthol and 1-pyrrolidinedithiocarbamate acid as ligands that was used for the selective speciation of inorganic mercury in wines [58]. The speciation was performed with the polymer in column mode, and the limit of detection was 0.02 µg L^{-1}, much lower than the limits defined by the International Organization of Vine and Wine (5 µg L^{-1}).

Liu et al. [45] developed a methylmercury imprinted polymer and validated the method for the determination of methylmercury content in a certified reference material of human hair (IAEA-086) and soil (BCR CRM 580). The selectivity factor of the methylmercury imprinted polymer was 24.0 for MeHg/Hg(II). Büyüktiyaki et al. [49] used their methylmercury imprinted sorbent previously to HPLC-DAD for speciation. They tested the accuracy of the method after determining the content of methylmercury and Hg(II) in a non-defatted lobster hepathopancreas certified reference material (LUTS-1), and in spiked synthetic seawater samples. Rodriguez et al. [72] also synthesized a methylmercury imprinted polymer to analyze seafood after a separation of this organic form in toluene. The polymer was also applied for the determination of inorganic mercury, methylmercury and ethylmercury in seawater by high performance liquid chromatography coupled to ICP-MS detection [73].

Recently, a polymer was proposed for direct inorganic mercury and methylmercury speciation without the need of chromatography or quantification by difference [36]. The polymer was employed to determine inorganic mercury and methylmercury after a sequential elution applied in the same cartridge. First, inorganic mercury was completely recovered after elution with 3 mL of 2 M HCl in methanol and second, methylmercury was eluted with 5 mL of NaClO 20% in 1 M HCl. In the case of the analysis of seawater a preconcentration step was needed previously to the analysis. The procedure was validated by analyzing the BCR 464 certified reference material and applied to commercial fish samples (swordfish and tuna fish).

2.5 Outlook

In recent years, the development of Hg(II) and methylmercury imprinted polymers has increased the range of applications of polymers to the analysis of the element using solid phase extraction and chemically modified electrodes. This trend will continue in the following years with the development of new composite materials. The use of nanomaterials contributes to the rapid separation and preconcentration of the element (e.g. with magnetic nanoparticles), and to the development of optical and electrochemical sensors. The combination of polymers and different carbonaceous materials (graphene, carbon nanotubes) contributes to increase the superficial area of the sorbents, and inorganic materials like sol-gels can be easily manipulated to increase selectivity and facilitate the diffusion and the interaction with the element. Natural materials combined with imprinted techniques are also a promising technique for mercury removal from natural waters, while cellulose composite papers are starting to be applied to develop cheap and flexible electrochemical sensors, appropriate for wearable devices.

A great advance will probably be produced in the field of optical sensors, with the development of more amphiphilic polymers, and the use of lanthanide coordination based polymers, with their tailorable properties and unique luminescence features that can also be applied for bioimaging, or for visual detection with the naked eye or the camera of a mobile phone. The control of the distance between the different layers of modified polymers can help to modify FRET interactions and develop ratiometric sensors. The consecutive sensing of different analytes can also be achieved with some probes, and this design principle could be extended to higher level applications, such as molecular logic gates.

On the other hand, the use of more environmentally friendly reagents should be investigated in the synthesis, especially to try to eliminate solvents such as toluene or acetonitrile, or to develop biocompatible mercury imprinted polymers to be used as treatment methods for acute heavy metal poisoning.

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OBJETIVOS

Objetivos

Esta tesis doctoral tiene por objetivo el desarrollo de nuevos materiales basados en polímeros de impronta molecular para el análisis de mercurio en muestras medioambientales.

En primer lugar se sintetizará y caracterizará un polímero de impronta molecular basado en la interacción con ditizona como método de extracción en fase sólida y posterior determinación mediante CV-HRCSAAS para el análisis de metilmercurio.

En segundo lugar, se desarrollará un polímero de impronta molecular basado en la interacción con fenobarbital como método de SPE y posterior determinación mediante CV-HRCSAAS para el análisis de metilmercurio en extractos orgánicos de productos marinos. Posteriormente se utilizará el polímero de fenobarbital sintetizado para la determinación de mercurio en aguas residuales mediante CV-ICP-OES. Se realizará un estudio para la preconcentración y especiación de mercurio en agua de mar mediante extracción en fase sólida y posterior determinación por HPLC-ICP-MS.

Por último, se sintetizarán quantum dots de ZnS dopados con Mn y recubiertos de un polímero de impronta molecular para la detección fluorescente de mercurio.


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CAPÍTULO III

Synthesis, characterization and analytical performance of a methylmercury imprinted polymer for solid phase extraction

Roi Rodríguez-Fernández, Elena Peña-Vázquez and Pilar Bermejo-Barrera

Resumen

En el siguiente capítulo se describe la síntesis y aplicación de un polímero que emplea cloruro de metilmercurio como plantilla, ditizona como ligando, 4-vinilpiridina como monómero y etilenglicoldimetacrilato como entrecruzante. El polímero se ha sintetizado mediante polimerización por precipitación, siendo el porogen una mezcla acetonitrilo:tolueno 3:1, y con AIBN como iniciador.

Se ha sintetizado de igual forma un polímero no improntado, NIP, que emplea los mismos reactivos que el MIP salvo la plantilla de metilmercurio. El MIP ha sido evaluado tanto en modo *batch* como en modo columna, dando unos resultados más satisfactorios para el primer modo. Empleando el MIP en modo *batch* se consigue una recuperación cuantitativa. El MIP sintetizado se ha caracterizado empleando diferentes técnicas, como análisis elemental, espectroscopía infrarroja, fluorescencia de rayos-X y microscopía electrónica de barrido. Los espectros de fluorescencia permiten observar la desaparición de la señal de Hg, de forma que se confirma el correcto sangrado del polímero. Por medio de las micrografías del SEM se aprecian diferencias en la superficie entre el MIP y el NIP.

Tanto la eliminación de la plantilla de metilmercurio como la elución del analito retenido tras la extracción, se realiza mediante una disolución de tiourea 1M en HCl 1M. Los extractos de tiourea se analizan utilizando un Espectrómetro de Absorción Atómica de Fuente Continua de Alta Resolución (HRCSAAS) acoplado a un sistema de generación de vapor empleando una disolución 0,2% de $NaBH_4$ estabilizada con NaOH al 0,05% como agente reductor. La exactitud se ha evaluado utilizando un material de referencia certificado BCR-463, con una buena concordancia entre el valor experimental ($3,27 \pm 0,29$ µg g^{-1}) y el certificado ($3,04 \pm 0,16$µg g^{-1}).

3. Synthesis, characterization and analytical performance of a methylmercury imprinted polymer for solid phase extraction

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Abstract

A molecularly imprinted polymer (MIP) was synthesized using methylmercury chloride as the template, dithizone as ligand, 4-vynil pyridine (4-VP) as monomer, and ethylene glycoldimethacrylate (EDMA) as cross-linking agent. The MIP was characterized using elemental analysis, infrared spectroscopy, energy dispersive X-ray fluorescence and scanning electron microscopy, and the operating conditions for solid phase extraction (SPE) were optimized in batch mode. SPE was carried out at pH 9.3 during 2 h, and the methylmercury was extracted from the MIP particles by sonication with 0.5 M thiourea/ 1M HCl for 20 min. The MIP was used as SPE sorbent with a good interday reproducibility (RSD: 11.4%) and accuracy to determine methylmercury in the tuna fish CRM BCR-463.

Keywords. Methylmercury, molecularly imprinted polymer (MIP), SPE, HRCSAAS, fish

3.1 Introduction

Molecularly imprinted polymers (MIPs) are materials that are synthesized using a template molecule whose structure and functionalities define the properties of the binding sites of the polymer [1]. Template molecules usually interact with functional monomers through hydrogen bonds or Van der Waals interactions. However, in the case of ion-imprinted molecules (IIP), a ligand is generally required to form a complex with the ion to produce selective binding sites after the leaching of the template [2]. Imprinted polymers are used as selective phases in various analytical techniques such as liquid chromatography, capillary electrochromatography, binding assays and biosensors [1]. Moreover, one of the most important applications has been the use as sorbents for solid phase extraction (SPE) in sample preparation [3, 4].

More than 140 nations signed a treaty on reduction in human use and the release of mercury into the environment, but terrestrial and oceanic mercury reservoirs continue to increase [5]. This is the reason why different materials including MIPs have been developed for SPE of mercury with applications to environmental remediation or analysis. The number of publications in the field has increased in recent years, mainly for the extraction of Hg(II); however, only a few studies deal with the development of imprinted polymers using methylmercury as a template [6, 7]. The first study found in the bibliography for the determination of mercury using polymers was from 1976. Nishide et al.[8] synthesized poly(4-vinylpyridine) resins (PVP) using partially-quaternized PVP, poly(styrene sulfonate) and Cu(II), Fe(III), Co(II), Zn(II) or Hg(II) as templates. The resins adsorbed percentages of Hg(II) ranging from 76-90%. However, the crosslinking structure was partially destroyed with the addition of acid.

Different types of ligands have been used for complexing metal ions [9]. Many of the imprinted polymers developed include a ligand with a sulfur donating atom to form the complex. Büyüktiryaki et al. [6] synthesized methylmercury-imprinted beads by a dispersion polymerization technique with methylmercury-methacryloyl-(L)-cysteine (MM-MAC) complex monomer and EDMA. These beads were used as a SPE support for the determination of methylmercury and mercury ions in LUTs (a non-defatted lobster hepatopancreas certified reference material) and in several spiked synthetic seawaters. These authors also synthesized a polymer for the selective removal of Hg(II) from human serum MAC as the complexing monomer [10]. Firouzzare and Wang [11] synthesized an aminothiol monomer (N-methacryloyl-2-mercaptoethylamine) to complex Hg(II) before polymerization with methacrylic acid (MAA) and EDMA. The polymer was used for the determination of Hg(II) in tap and seawater samples. Dakova et al.[12] prepared a metal IIP by copolymerization of MAA as monomer, trimethylolpropane trimethacrylate as cross-linking agent and 2-2'-azobisisobutyronitrile (AIBN) as initiator, in the presence of Hg(II)-1-(2-thiazolylazo)-2-naphthol complex. The polymer was used for the speciation of Hg in river and mineral waters working in batch mode. Singh and Mishra [13] synthesized a Hg(II) imprinted polymer using 4-(2-thiazolyazo) resorcinol (TAR) as the complexing ligand, MAA (monomer) and EDMA as crosslinker.

The amino sites present in some natural compounds such as chitosan are also reactive groups for capturing heavy metals. A chitosan derivative adsorbent was prepared by polyamination and imprinted technologies for improving Hg(II) removal from aqueous solutions [14]. In another study, the functional monomer 3-isocyanatopropyltriethoxysilane (IPTS) [15] bearing thymine (T) bases was synthesized by sol-gel process for imprinting Hg(II) and preconcentrating the element in water samples. Liu et al. [7] used precipitation polymerization to synthesize a methylmercury-imprinted polymer with (4-ethenylphenyl)-4-formate-6-phenyl-2,2'-bipyridine, DVB as crosslinking agent and AIBN as initiator. They packed the polymer in columns and used it for the determination of methylmercury in aqueous and biological samples (human hair). These authors also developed a method for the determination of Hg(II) after preconcentration with Hg(II)-imprinted diazoaminobenzene-vinylpyridine copolymers, and they applied it to the analysis of tap water, river water and seawater samples [16].

Surface imprinting is a technique that provides good accessibility to the target species and low-mass transfer resistance [2]. Thus, Fan [17] prepared a Hg(II) imprinted thiol-functionalized mesoporous sorbent by a sol-gel process using 3-mercaptopropyltrimethoxysilane (MPTMS) as a coupling agent. Wang et al. [18] synthesized an ion-imprinted thiol-functionalized sorbent using silica gel and methanesulfonic acid. They obtained a selective separation of Hg(II) from other ions that also bind thiols, such as Cu(II) and Cd(II). These authors [19] developed an organic-inorganic hybrid material by a hierarchically double-imprinting method with Hg(II) and cetyltrimethylammonium bromide (CTAB) as templates, N-[3-(trimethoxysilyl)propyl]ethylenediamine (TPED) as functional monomer and tetraethoxysilane (TEOS) as cross-linking agent. Dakova et al. [20] synthesized a core-shell type IIP using silica gel chemically modified with 3-(trimethoxysilyl)propyl methacrylate (TSPM) as supporting material. The outer Hg(II)-IIP layer was prepared with MAA as monomer, trimethylolpropane trimethacrylate (TMPTM) as a cross-linking agent, and complexes of Hg(II) with pyrrolidine dithiocarbamate (PDC) or with 1-(2-thiazolyazo)-2-naphthol (TAN) as templates. The sorbent was used for the speciation of mercury in samples of wine, with or without digestion. Najafi et al. [21] developed a magnetic ion-imprinting polymer based on N-(pyridine-2-ylmethyl)ethenamine coated on Fe_3O_4 nanoparticles, and they used it for the determination of Hg(II) in fish samples.

The ion imprinting is now also being used for the development of modified carbon electrodes [22-25] for the detection of Hg(II). Moreover, Güney and Cebeci [26] developed a chemosensor for the optical detection of Hg(II). They synthesized polymer membranes with 4-VP as the functional monomer and 9-vinylcarbazole as complex-forming agent and fluorescent probe.

Finally, dithizone (diphenylthiocarbazone) has been used for solid phase extraction of metal and organometallic species in several studies. Chow and Buksak [27] used a dithizone-treated polyurethane foam for the extraction of Hg(II) and methylmercury(II) chloride over a wide pH range from aqueous solutions including domestic water, river water, raw sewage and secondary-treated sewage. Shah and Devi [28] anchored dithizone on a poly(VP)-divinyl benzene copolymer and used this matrix for the preconcentration and separation of Hg(II) from Zn, Cd and Pd. Salih et al. [29] developed dithizone-anchored poly(ethylene glycol dimethacrylate-hydroxyethylmethacrylate) microbeads for the removal of heavy metal ions (Cd(II), Hg(II), Cr(III) and Pb(II)) from aqueous media. The authors used the poly(EDMA-HEMA) microbeads for the determination of inorganic and organic mercury compounds [30], and inorganic and organolead [31] compounds by gas chromatography coupled with atomic absorption spectrometry. Ray and Gupta [32] prepared the metal dithizone complexes of Cu(II), Ni(II), Co(II) and Zn(II), that were used for making template sites for the elements in four types of polymeric films: polystyrene, poly(methyl methacrylate), poly(vinyl chloride) and polycarbonate. Each ion imprinted polymer (IIP) showed the maximum absorption capacity for the ion from which the template was constructed, regardless of the stability constants. Saraji and Yousefi [33] synthesized an IIP for the determination of Ni using the Ni-dithizone complex as a template. This IIP polymer was packed in a column and used for the determination of the element in river and waste water samples. Ashraf et al. [34] used emulsion polymerization for the preparation of a hydrophobic imprinted resin using methylmethacrylate and phenylmethacrylate as monomers, a mercury(II) dithyzonate complex and a non-ionic alkyl-oligo(ethylene glycol) surfactant. Recently, Zhang et al. [35] synthesized an IIP by a sol-gel process using dithizone-Hg(II) chelate as template and 3-aminopropyltriethoxysilane as functional monomer for mercury speciation analysis in environmental and biological samples by atomic fluorescence spectroscopy. The same research group [36] developed dithizone-based multi-ion imprinted polymers by sol-gel polymerization for simultaneous removal of Hg(II), Cd(II), Ni(II) and Cu(II) from aqueous solutions.

The objective of the present study is the development of a simple procedure to synthesize a methylmercury imprinted polymer using a readily available ligand (dithizone). The polymer has been characterized using different techniques. The conditions for operation were studied, the polymer was used for the analysis of methylmercury in a tuna CRM.

3.2 Experimental

3.2.1 Instrumentation

The concentrations of mercury and methylmercury were measured using a High Resolution Continuum Source Atomic Absorption Spectrometer (HRCSAAS) (Analytik Jena ContrAA 300 model, Jena, Germany), equipped with a flow injection system to perform the cold vapor generation.

The concentrations of Cd, Ni, Pb and Zn were measured by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) using an Optima 3300DV equipped with a Gem-cone cross-flow nebulizer (Perkin Elmer, Norwalk, USA).

The temperature controlled Boxcult incubation camera from Selecta (Barcelona, Spain) equipped with a low profile roller (Stovall, Greensboro, NC, USA) was used to synthesize the polymer. The Boxcult camera has a orbital-horizontal shaker that was used to perform the SPE in batch mode. A pH-meter model 720 (ThermoOrion, Waltham, USA) was used to adjust the pHs needed for the experiments.

A peristaltic pump (Gilson, Villiers, France) was used in the SPE experiments in column mode. In this case, the polymer was packed into 5 mL SPE cartridges.

The elemental analyzer FLASH 1112 from Thermo Finnigan (Waltham, MA, USA) was used to analyze the content of nitrogen, carbon, oxygen, hydrogen and sulfur in the polymer. The EVO LS 15 microscope (Zeiss, Oberkochen, Germany) was used to obtain the micrographs of the MIP and NIP (non imprinted polymer). The spectrometer 670 IR from Varian (CA, USA) was used to obtain the IR spectra. Two labmade spectrometers (Servicios Generales USC) with anodes of Mo and pirographite-iron were used for energy dispersive x-ray dispersion fluorescence measurements.

3.2.2 Reagents

Ultra-pure water of 18Ωcm resistance obtained from a Milli-Q purification device (Millipore Co., Massachusetts, USA) was used to prepare all the solutions. Standard solutions (1000 mg L^{-1}) of Cd, Hg, Ni, Pb and Zn were from Merck (Darmstadt, Germany). Methylmercury chloride, 4-VP, EDMA and thiourea were supplied by Sigma (Steinhelm, Germany). Diphenylthiocarbazone (dithizone) was obtained from Merck. Azobisisobutyronitrile (AIBN) was purchased from Fluka (Steinhelm, Germany). Tuna fish (BCR-463) certified reference material was obtained from the Community Bureau of Reference (Brussels, Belgium). All the other chemicals were purchased from Panreac and Scharlau (Barcelona, Spain). All glass and plastic material were rigorously cleaned and kept in 10% (w/w) nitric acid for at least 48 h. The material was then rinsed three times with ultra-pure water before being used.

3.2.3 Synthesis of the Molecularly Imprinted Polymer

The procedure developed in the present study is based on that used by Otero-Romaní et al. [37] in a previous work for nickel determination. For the preparation of approximately 0.5 g of polymer: 9.2 mg of MeHgCl and 19.1 mg of dithizone were weighed and mixed with 15.7 µL of 4-VP in a clean glass tube. A volume of 6 mL of the porogen (acetonitrile:toluene 3:1) were added and stirred for 5 min with a vortex and let to stand for another 5 min. In case of precipitate formation, the solution was either filtered or decanted. After placing the tube in an ice bath, 467 µL of the cross-linker EDMA and 21.6 mg of AIBN (initiator) were added. The mixture was stirred again for 1 min and purged with argon before closing the tube. Afterwards, the tube was set in the temperature-controlled incubator camera on a low-profile roller at 60^0C, and the polymerization was completed after 36 h. The non-imprinted polymer (NIP) was synthesized following the same procedure but without adding the methylmercury template. The polymers obtained (course orange powder) were dried at room temperature and pulverized in a mortar.

3.2.4 Template removal procedure

Approximately 0.5 g of polymer were transferred to a 100 mL Erlenmeyer flask with 75 mL of an acidic thiourea solution (1 M thiourea in 1 M HCl). The vessel was placed in an ultrasonic bath for 25 min. Afterwards, the polymer particles were vacuum filtered through a 0.22 µm membrane (Durapore®, Millipore Co., Massachusetts, USA). The particles were collected and transferred back to the Erlenmeyer flask with a fresh portion of thiourea solution. The template was completely removed after 4 repetitions of the process.

3.2.5 Solid phase extraction procedure

3.2.5.1 Batch mode

Mercury (II) and methylmercury aqueous standard solutions were prepared in a NH_4Cl/NH_3 buffer at the optimized pH (9.3). Acidified fish sample extracts were neutralized with 1 M NaOH and the pH was adjusted to 9.3 with 0.5 M ammonia. A mass of 50 mg of polymer was introduced into the alkaline standard/sample solution and stirred with the orbital-horizontal shaker at 180 rpm for 2 h. The polymer particles were vacuum filtered and transferred to a polyethylene tube. For methylmercury elution, 10 mL of acidic thiourea (0.5M thiourea in 1M HCl) were added and the mixture was sonicated for 20 min. Once the MIP particles were filtered, the thiourea extract was collected and stored at 4 ^{0}C for further analysis.

3.2.5.2 On-column mode

MIP fractions of 150 mg were packed in 5 mL syringes between Teflon frits. Before the SPE procedure, MIP must be conditioned with the NH_4Cl/NH_3 buffer at the proper pH (9.3). Standards or samples (buffered at pH 9.3) were loaded at a low flow rate of 0.5 mL min^{-1} using a peristaltic pump and conditioned with 5 mL of buffer before the elution process. The retained methylmercury was eluted with 10 mL of acidic thiourea (0.5M thiourea in 1M HCl) at the same flow rate (0.5 mL min^{-1}). Finally, thiourea extracts were stored in PE tubes at 4 ^{0}C.

3.2.6 Determination of Hg(II) and methylmercury by CV-HRCSAAS

A flow injection system was used to perform the cold vapor generation previous to the determination by HRCSAAS (Figure 1). Samples were transported by a 3% (v/v) HCl solution (carrier), and mixed with the reductant, a 0.2% (w/v) $NaBH_4$ solution stabilized with 0.05% (w/v) NaOH. An 8-way Gilson peristaltic pump (Gilson, Villiers, France) equipped with a 3.18 mm i.d. Tygon tube was used for extracting the waste from the gas-liquid separator. The reagents were transferred to the gas-liquid separator through a 500 µL reaction loop. The Hg vapor was separated from the liquid mixture and was swept to the quartz cell using a 25 L h^{-1} Ar flow.

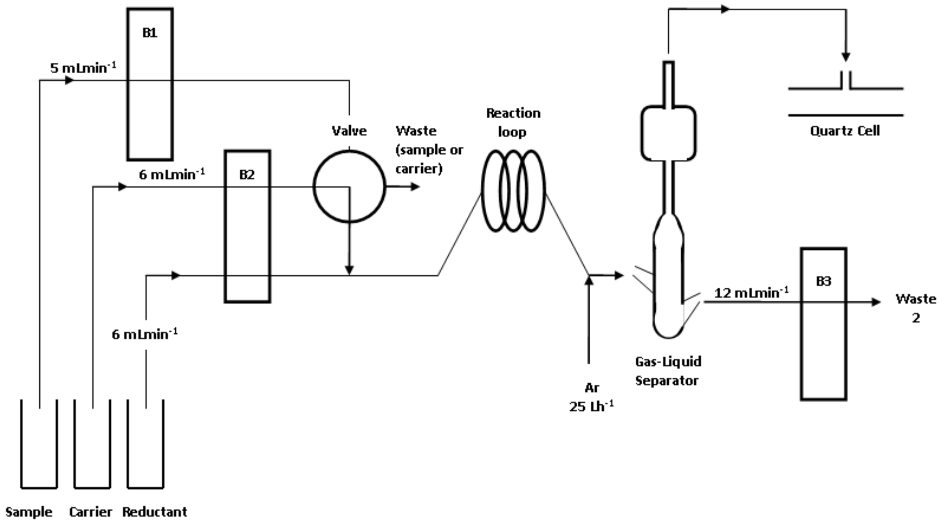

Figure 1. Vapor generation system for methylmercury determination by HRCSAAS

The signal registration was performed by using peak volume selected absorbance ($A_{\lambda\Sigma}$). The line used for Hg determination was 253.6492 nm; two hundred pixels were registered, and three analytical pixels (central pixel ± 1) were used to calculate the $A_{\lambda\Sigma}$. Detector integration time was 45 s (300 spectra recordings), and area mode was used. A reference spectrum (blank: 0.5M thiourea in 1M HCl) was used for background correction, and the instrument selected automatically the pixels used for correction in each measurement (dynamic background correction). The operating parameters for CV-HRCSAAS are shown in Table 1.

Table 1. Operating parameters for CV-HRCSAAS

Vapor generation system					
Step	Pump 1 (mL min^{-1})	Pump 2 (mL min^{-1})	Waste	Time	Reading
Load	5	6	Sample	10	-
Auto zero	0	6	Sample	10	Yes
Reaction	5	6	Carrier	20	Yes
Washing	0	6	Sample	35	Yes
Spectrometer					
Current /A			13		
Spectral range /pixels			200		
Analytical line for Hg/nm			253.6492		
Evaluated pixels			1 or 3 (CP ± 1)		
Background correction mode			With reference		
Background correction fit			Dynamic		
Read time / s			45		
Integration mode			Mean		
Number of spectra			300		
Temperature of the quartz cell			150		

3.2.7 ICP-OES measurements

Cd, Ni, Pb and Zn were measured by ICP-OES (axial configuration) using a radiofrequency power of 1300 W and plasma, auxiliary and nebulizer argon flows of 15, 0.5 and 0.8 L min^{-1}, respectively. A sample uptake rate of 1.5 mL min^{-1} was used in combination with a stabilization delay of 45s. Afterwards, 5 replicates were measured. The detection wavelengths were 214.440, 231.604, 220.353 and 206.200 nm for Cd, Ni, Pb and Zn, respectively. Calibrations were performed by using aqueous standards in 3% (v/v) HCl. The calibration covered Ni and Zn concentrations within the 50-1000 µg L^{-1} range, and within the 500-10000 µg L^{-1} range for Cd and Pb.

3.3 Results and discussions

3.3.1 Characterization studies

Elemental analysis, infrared spectroscopy and energy dispersive X-ray fluorescence were used for the characterization of the MIP (with and without the methylmercury template) and the non-imprinted polymer (NIP). Scanning electron microscopy (SEM) was employed to examine morphological differences between MIP and NIP.

3.3.1.1 Microanalysis studies

Samples of MIP with methylmercury template, MIP without template and NIP were analyzed to determine the percentage of nitrogen, carbon, hydrogen, sulfur and oxygen. Results are shown in Table 2. An increase in the amount of nitrogen and sulfur can be observed in the MIP without template; this variation is due to the presence of the thiourea used for the extraction of the template.

Table 2. Elemental composition of the polymers

Element	MIP (with template)	MIP (without template)	NIP
Carbon	59.65 %	40.55 %	60.03 %
Hydrogen	6.92 %	6.58 %	7.34 %
Nitrogen	1.54 %	9.17 %	1.48 %
Sulfur	0.24 %	9.41 %	0.29 %
Oxygen	26.25 %	22.75 %	26.62 %

3.3.1.2 Scanning electron microscopy (SEM)

Digital micrographs of MIP and NIP (Figure 2) were obtained using Scanning Electron Microscopy (SEM). The pictures were taken after applying the procedure for the extraction of the template. It was observed that the surface of the MIP (Figure 2A) is more porous than that of the NIP (Figure 2B). This fact was attributed to the imprinted effect of the methylmercury template.

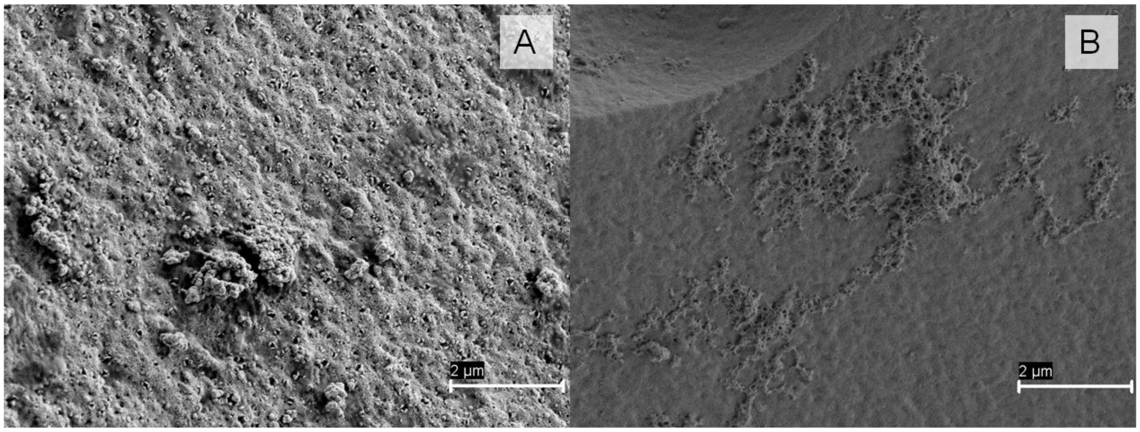

Figure 2. Micrographs of a) MIP without methylmercury template b) NIP

3.3.1.3 Infrared spectroscopy

The IR spectra in a KBr pellet of the MIP (with and without template) and the NIP were registered, and the bands observed at 3000 cm^{-1} and 1460 cm^{-1} confirmed the trapping of the dithizone ligand. The spectra of the MIP with template and NIP are similar, but there are some differences in comparison with the spectra of the MIP without template. These differences were attributed to the presence of thiourea bands (bands at 3200 and 1650 cm^{-1}), as a result of the template removal treatment.

3.3.1.4 Energy dispersive X-ray fluorescence

The results obtained using this technique (Figure 3) indicated a total elimination of the template from the MIP and the absence of methylmercury in the polymeric matrix of the NIP. In addition, the presence of the sulfur signal from the dithizone molecule could also be observed using energy dispersive x-ray fluorescence.

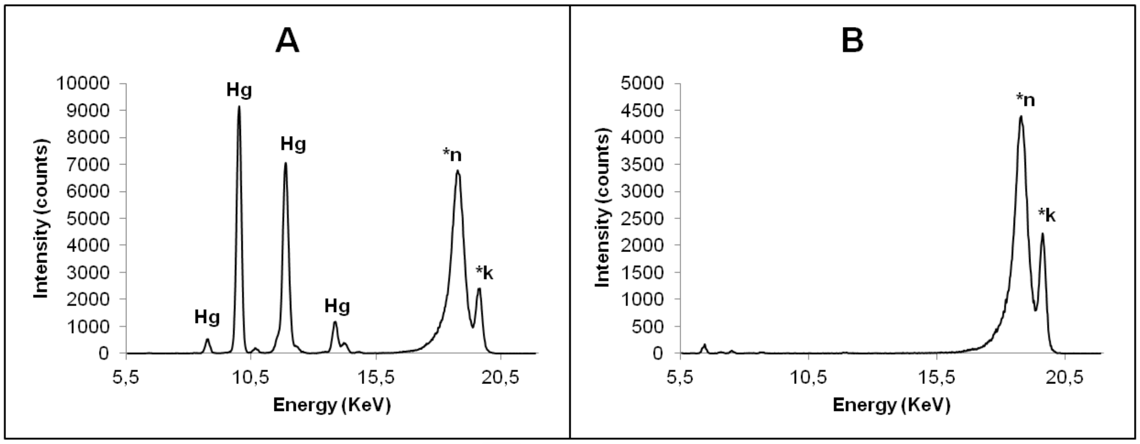

Figure 3. Energy dispersive X-ray fluorescence a) MIP with methylmercury template b) MIP without template

3.3.2 Optimization of working conditions (Batch mode)

Preliminary experiments were performed in column mode to extract the template using 0.1 and 0.5 M thiosulfate or 0.1 and 1 M thiourea in 1 M HCl, but the quantitative extraction was only achieved using the ultrasound (US) assisted procedure reported in section 2.4. The SPE on-column mode (procedure in section 2.5.2) was tested, but retention percentages of only 10-30% of methylmercury were achieved. Quantitative extraction was achieved in the column mode that showed a better reproducibility. Batch mode was also used by Liu et al. [7] for the analysis of methylmercury in soil samples, while they used the column mode for the determination of the compound in human hair samples. Zhang et al. [36] and Fu et al. [35] also used their dithizone based-polymers in batch and column mode.

3.3.2.1 Influence of pH on MIP retention

The influence of pH on MIP retention during the SPE procedure in batch mode was evaluated. The experiment was performed using 50 mg of polymer that were introduced into 25 mL of 50 µg L^{-1} methylmercury standards buffered at different pH values between 5.3-11.3. As can be observed (Figure 4A), there is a retention maximum at pH 9.3. The retention value decreases sharply at lower and higher pH ranges. This optimum pH (9.3) was selected for performing all the following experiments.

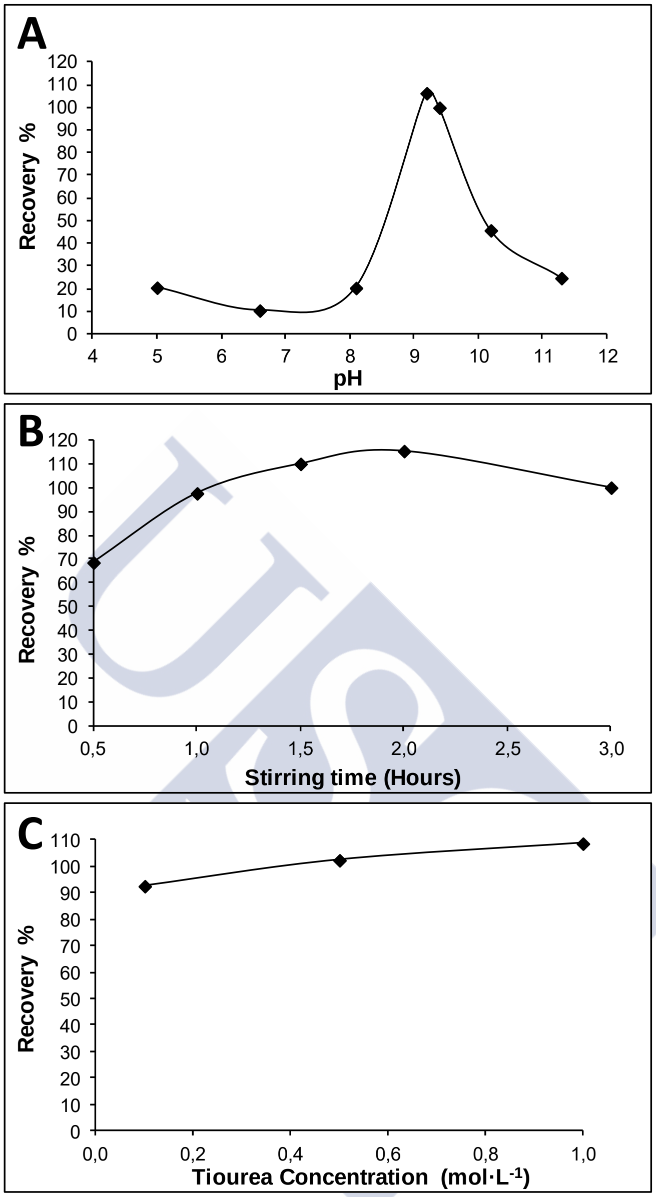

Figure 4. Optimization of SPE conditions (batch mode). Influence of: a) pH b) Time of contact c) Concentration of the thiourea solution used for elution

The selected working pH was higher than the pH used by other authors who included dithizone in their polymeric supports. The maximum exchange capacity for Hg(II) was at pH 3.0 in a 0.2 M acetate buffer using the dithizone anchored poly(VP) support developed by Shah and Devi and working in column mode [28]. Salih et al. [29] observed that the adsorption of heavy metals (Cd(II), Cr(III), Hg(II) and Pb(II)) onto dithizone anchored poly (EGDMA-HEMA) microbeads increased with the pH, reaching a plateau value at pH 5.0. These authors [30] also performed the speciation by gas chromatography of inorganic mercury, methyl mercury chloride and ethyl mercury chloride after an extraction at pH 6.0 during one hour.

The methylmercury imprinted polymer with MAC also had its optimum working pH at 7.0 [6], and Liu et al. [7] selected a working pH of 5.0 for the methylmercury imprinted polymer using (4-ethenylphenyl)-4-formate-6-phenyl-2,2'-bipyridine as ligand. However, Saraji and Yousefi [33] obtained a very similar result to the present study with their MIP for Ni using dithizone as ligand. They observed an adsorption maximum at pH 8 with a decrease at lower and higher pH values. The optimum pH for the adsorption of Hg^{2+} from aqueous solutions ranged from 7.0 to 8.0 when using the polymer developed by Zhang et al. [35]. Finally, Ashraf et al. [34] performed an experiment loading Hg(II) dithizonate in 80% acetonitrile in water into a column containing 200 mg of MIP, but they didn´t studied the effect of pH.

3.3.2.2 Influence of contact time

The influence of contact time between a standard (25 mL of 50 µg L^{-1} methylmercury, pH=9.3), and the MIP (50 mg) during the SPE procedure was optimized. Different contact times (0.5, 1, 1.5 and 2 hours) were used in the experiments that were performed in duplicate stirring at 180 rpm. Results indicated that the maximum retention capacity occurs at 2 h, and it decreases over time (Figure 4B).

3.3.2.3 Influence of thiourea concentration on the extraction efficiency

The SPE procedure was carried out using a standard (25 mL of 50 µg L^{-1} methylmercury, pH=9.3), 50 mg of MIP, and changing the concentration of thiourea used for the extraction. The concentrations studied were 0.1, 0.5 and 1 M, and the experiments were performed in duplicate. The results (Figure 4C) showed that a concentration of 0.5 M can be used with a minor change in the recovery in comparison to 1 M, with the consequent saving of reagents.

The link between methylmercury and the MIP seems to be very strong, requiring an efficient solvent for elution. A similar solvent composition was used by Singh and Mishra (1M thiourea in 0.5 M HCl) for extracting Hg(II) from their Hg(II)-TAR imprinted polymer [13]. Liu et al. [7] used 1M thiourea in 6M HCl, and Büyüktiryaki et al. [6] used 1M thiourea in 8M HCl to remove the methylmercury template. Both authors stirred the polymer for two hours in the extracting solution. US-assisted extraction accelerates the process and allows for the use of these reagents in a lower concentration.

3.3.2.4 MIP-SPE with PP membranes

Experiments consisting in packing MIP portions (50 mg) in polypropylene (PP) membranes were performed [38, 39]. The objective of this experiment was to carry out SPE in batch mode with the MIP confined into the PP membranes and avoiding the filtration step.

An *Accurel PP 2E HF* (Membrana, Wuppertal, Germany) membrane was used. Membrane fragments of 2x2 cm were cut to create a bag using a heat sealer. Because of the membrane's hydrophobic character, a zirconium pearl was introduced to keep the device into the solution. A portion of 50 mg of MIP was placed into the bag that was then sealed. Before the SPE procedure, membranes were sonicated for 10 minutes in methanol. After this process, the devices were dried (previous to the SPE procedure). As a consequence of the slow kinetic of the adsorption reaction, the membrane decreased this process even more and the recovery rates (25%) were lower than those obtained using the conventional batch mode.

3.3.3 Analytical Performance

The ASPECTS CS software used for the control of the HRCSAAS spectrometer allows the selection of the optimum number of pixels for providing the best sensitivity and reproducibility. All the measurements and calculations during the optimization of the method were done using 3 analytical pixels (central pixel ± 1), as recommended by the Analytik Jena manufacturer.

3.3.3.1 Calibration graphs

Calibration graphs were prepared with concentrations of 0, 2.5, 5.0 and 10.0 µg L^{-1} of methylmercury or Hg(II) in 3%(v/v) HCl and in 0.5 M thiourea/1M HCl. The following equations were obtained when using 3% (v/v) HCl as solvent to prepare the standards: A = 0.0965 [Hg(II)]-0.0109 , r= 1.000 and A = 0.0547 [Hg(MeHg)]-0.0118, r = 0.998. The following equation was used for methylmercury in 0.5 M thiourea/1M HCl: A = 0.0795 [Hg(MeHg)]-0.0204 , r= 0.997. For recovery studies, a internal calibration through the polymer was performed, and the following equation A = 0.011 [Hg(II)]-0.2031 , r=0.994 was obtained. The slopes of the calibration graphs were observed to be statistically different after performing a comparison using a t-test at a 95% confidence level.

3.3.3.2 Limit of detection and quantification

The limit of detection (LOD) is defined as the element concentration that corresponds to three times the standard deviation of the measurement of a blank (n =11). Similarly, the limit of quantification (LOQ) is calculated as ten times the standard deviation of the measurement of a blank. Limits of detection were calculated using the central pixel (CP), 3 pixels (CP ± 1) and 5 pixels (CP ± 2), and the values obtained are shown in Table 3. The best results were obtained using the central pixel (LOD = 5.3 μg kg^{-1}, LOQ = 17.7 μg kg^{-1}). The values obtained are very small in comparison with the limit value established by European Legislation for mercury in fish (1 mg kg^{-1}) [40]. It is assumed that from 60 to 90% of the element is present as methylmercury [41]

Table 3. Analytical performance

Number of pixels	LOD (μg L^{-1})	LOD (μg Kg^{-1})	RSD(%)
CP	0.16	5.31	6.9
CP ± 1 pixel	0.39	12.8	6.8
CP ± 2 pixels	0.67	22.2	6.6

3.3.3.3 Reproducibility of the method

The instrumental repeatability was estimated as the relative standard deviation (RSD(%)) of eleven measurements of a 10 μg L^{-1} standard. Results obtained using 1, 3 or 5 pixels are listed in Table 3. All the values were very similar, ranging from 6.6 to 6.9%.

The interday reproducibility of the method was also calculated after applying the SPE procedure to a 50 μg L^{-1} standard (n = 6). The average recovery was 106.6 % and the standard deviation was 0.12. Therefore, the RSD(%) was 11.4%, a percentage that was considered acceptable because it includes the whole procedure (SPE and analysis by CV-HRCSAAS). The polymer was used repeatedly at least in 10 cycles of SPE without major changes in its performance.

3.3.3.4 Comparison of extraction of Hg(II) and methylmercury

SPE in batch mode was performed using 25mL of 50 µg L^{-1} standards of methylmercury or Hg(II), and mixtures of both compounds. Extraction was performed under the optimized conditions (pH: 9.3, extraction time: 2 hours; solvent for extraction: 0.5 M/1 M HCl; sonication time: 20 min). The results of the experiments stated that the molecularly imprinted polymer retains mercury and methylmercury interchangeably.

3.3.3.5 Cross reactivity studies between methylmercury and other trace elements

Cross reactivity between methylmercury and other metal ions in the MIP-SPE process was investigated. The interferences caused by Cd, Ni, Pb and Zn were studied because they complex strongly with dithizone; additionally, Cd and Pb are well known to be dangerous elements found in fish and seafood samples.

Mixtures of standard solutions with increasing concentrations of methylmercury (50. 100, 150 and 150 µg L^{-1}) and Cd, Ni, Pb and Zn (at the same concentration than mercury) were prepared. After the MIP-SPE procedure, the extracts were separated into two aliquots: one aliquot to determine the methylmercury concentration by CV-HRCSAAS, and another aliquot to analyze the other elements by ICP-OES.

Results indicated that the MIP retention capacity (97.29%) was apparently not affected by the presence of other ions, and in the case of the elements studied, the MIP retained them in relatively low proportions (12-33% of Ni, 1-10% of Cd, 1-15% of Pb, <5% of Zn). Moreover, the distribution coefficients and the selectivities were calculated. The distribution coefficient (D) is the ratio between the amount of the metal remaining in the sample solution and the amount of metal in the extract. The following values were obtained: 35.90 (D_{MeHg}), 0.50 (D_{Ni}), 0.086 (D_{Cd}) and 0.146(D_{Pb}). The selectivity coefficient ($S_{MeHg/M}$) is defined as the distribution coefficient for MeHg divided by the distribution coefficient for the studied metal. The values obtained for the selectivities were: 71.73 ($S_{MeHg/Ni}$), 418.19 ($S_{MeHg/Cd}$) and 245.11 ($S_{MeHg/Pb}$). The MIP was more selective for the template (MeHg) than for the other elements, thus showing the effectiveness of the imprinting effect.

3.3.3.6 Accuracy

To evaluate the accuracy of the SPE method, the tuna fish Certified Reference Material BCR-463 was analyzed. The method proposed by Cabañero Ortiz et al. [42] was used for methylmercury extraction from the fish tissue. A portion of sample (approximately 300 mg) was taken and placed into centrifuge tubes with 5 mL of 5 M HCl, and was sonicated for 5 min. The acidic extracts were centrifuged at 3500 rpm for 10 min and the supernatant was taken for SPE after adjusting the pH at 9.2 and diluting to 25 mL. The CRM was analyzed in triplicate. Results revealed an agreement (t-test, $P=0.05$) between the experimental concentration of methylmercury (3.27 ± 0.29 µg g^{-1}) and the certified value (3.04 ± 0.16 µg g^{-1}).

3.4 Conclusions

A MIP using methylmercury as a template was synthesized. This polymer uses dithizone as ligand, 4-VP as monomer and EDMA as cross-linker. The MIP was characterized by elemental analysis, infrared spectroscopy, energy dispersive x-ray fluorescence and scanning electron microscopy. Operating parameters for SPE such as pH, time of contact with the sample and thiourea concentration were optimized. A good retention capacity was obtained in batch mode, and sonication in acidic thiourea was an effective method for the removal of the template. The accuracy of the method was evaluated using a CRM for tuna fish samples (BCR 463) with good results.

3.5 Acknowledgements

The authors are grateful for the financial support provided by the Xunta de Galicia (project number: 10PXIB209032PR).

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CAPÍTULO IV

Synthesis of an imprinted polymer for the determination of methylmercury in marine products

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Talanta, 144 **(2015)** 636–641


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Rodríguez-Fernández, R.; Peña-Vázquez, E.; Bermejo-Barrera, P., *Talanta*. 144 (2015) 636–641.

http://www.sciencedirect.com/science/article/pii/S0039914015300758


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CAPÍTULO V

Determination of mercury in wastewaters using a molecularly imprinted polymer as a solid phase extraction sorbent and CV-ICP-OES

Roi Rodríguez-Fernández, Elena Peña-Vázquez, and Pilar Bermejo-Barrera

Atomic Spectroscopy 37, nº 6 (2016) 238


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Rodríguez-fernández, R.; Peña-vázquez, E.; Bermejo-barrera, P., At. Spectrosc. 37 (2016) 238–243.

Atomic Spectroscopy

Editor: PerkinElmer Inc.

ISBN ISSN: 0195-5373

Subscription Information:

Atomic Spectroscopy
P.O. Box 3674
Barrington, IL 60011 USA
E-mail: atsonline@yahoo.com

Article indexing services:

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CAPÍTULO VI

Mercury speciation in seawater by HPLC-ICP-MS following SPE preconcentration by using an ionic imprinted polymer based on methylmercury-phenobarbital interaction

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Journal of Chromatography A. 1391 **(2015)** 9-17


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Rodríguez-Reino, M.P.; Rodríguez-Fernández, R.; Peña-Vázquez, E.; Domínguez-González, R.; Bermejo-Barrera, P.; Moreda-Piñeiro, A., *J. Chromatogr. A*. 1391 (2015) 9–17.

http://www.sciencedirect.com/science/article/pii/S0021967315003416


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CAPÍTULO VII

Synthesis and characterization of molecularly imprinted polymer – coated Mn-doped ZnS quantum dots for fluorescent detection of methylmercury

Roi Rodríguez-Fernández, Elena Peña-Vázquez and Pilar Bermejo-Barrera


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Resumen

En este trabajo se han sintetizado QDs de ZnS:Mn funcionalizados con el MIP de fenobarbital previamente desarrollado y descrito en esta tesis. La superficie de los QDs se modifica con polietilenglicol, sobre la que se produce el anclaje del polímero. El MIP emplea fenobarbital como ligando, ácido metaacrílico como monómero y EDMA como entrecruzante. El MIP polimeriza directamente sobre la superficie de los QD-PEG mediante un proceso asistido por ultrasonidos.

Se ha estudiado la respuesta fluorescente del material y se han optimizado diversos parámetros, como el tiempo de contacto entre el analito y la suspensión de QDs (20 min.), la concentración de los mismos o el pH del medio. Las partículas sintetizadas de QD-MIP, previamente tratadas con una solución de tiourea 0,5M a pH 2 para eliminar la plantilla de MeHg, se añaden como suspensión (600 mg L^{-1}) a un tampón NH_4Cl/NaOH a pH 6.5. Las medidas puntuales se llevan a cabo en un espectrofotómetro de fluorescencia (λ_{ex}: 258nm, λ_{em}: 518nm). Mediante este procedimiento se ha alcanzado un límite de detección de 7 nM.

7. Synthesis and characterization of molecularly imprinted polymer – coated Mn-doped ZnS quantum dots for fluorescent detection of methylmercury

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Abstract

Mn-doped ZnS quantum dots were synthesized and capped with a molecularly imprinted polymer consisting of methacrylic acid as monomer, ethylene dimethacrylate as crosslinker, and phenobarbital as ligand. Methylmercury was used as template, and eliminated from the polymer with acidic thiourea. The ultrasound-assisted procedure is relatively simple, inexpensive, and it can be performed at room temperature. After the preparation, the characteristics of the new material were studied by transmission electronic microscopy, X-ray diffraction spectrometry, and infrared spectroscopy. The fluorescent response to methylmercury was studied, and the operating conditions were selected (contact time with the analyte before measurements: 20 minutes; concentration of the material: 0.6 g L^{-1}; pH of the reaction media: 6.5). The limit of detection of the proposed method was 7.0 nM.

7.1 Introduction

The use of quantum dots (QDs) for inorganic-trace analysis [1] has increased in recent years because of their advantages in comparison to organic dyes: chemical and photoluminescence stability and superior optical properties [2] (tunability of emission properties, high quantum yield, narrow emission spectra, ability for multiplexing analysis). Chen and Rosenzweig [3] first used CdS QDs modified with L-cysteine and thioglycerol to detect zinc and copper ions in physiological buffer samples. As a consequence, several research groups developed this type of nanoparticles as chemical sensors for cations, mercury (II) being the most studied [4].

Most of the systems for detection of Hg(II) were based on Cd-chalcogenide QDs as it can be observed in some bibliographic reviews about the use of nanomaterials for analysis and speciation of mercury [46], and trace elements [1, 7]. Recent developments include the use of photoelectrochemical sensors [8, 9], the incorporation of fluorescence CdTe nanoparticles on the surface of self-propelled tubular micromotors [10], and electroluminescent inmunoassays [11-14]. Carbon based nanomaterials are also emerging as an alternative to semiconducting QDs due to inexpensive production, high biocompatibility, ease of conjugation to biomolecules, and high efficiency of energy transfer and luminescence [15-20].

Cadmium was also replaced in semiconducting QDs by other elements such as zinc to avoid toxicity problems. Duan et al. [21] synthesized N-acetyl-L-cysteine-capped zinc sulfide QDs in aqueous medium via a one-step method as an eco-friendly sensor for Hg (II). Chitosan-stabilized ZnS QDs [22] were used for sensing and removal of heavy metal ions (Hg(II), Ag(I), and Pb(II)) in water. The employment of cysteine-capped ZnS QDs assembled on the surface of indium tin oxide was studied with the objective of developing a photoelectrochemical sensor [23].

Zinc sulfide QDs are frequently doped by other transition elements, mainly manganese, to decrease self-quenching and avoid thermal, chemical, and photochemical disturbances [24]. Water soluble Mn-doped ZnS QDs were prepared using 3-mercaptopropionic acid (MPA) and L-cysteine as stabilizers [25]. The presence of Cu(II), Mn(II), Fe(II), Co(II), Pb(II) and Hg(II) quenched the phosphorescence of the 3-mercaptopropionic acid QDs, while Cd(II) enhanced the signal. All the ions examined quenched the fluorescence of L-cysteine-capped QDs. Tan et al. [26] evaluated the analytical characteristics provided by the utilization of the phosphorescent L-cysteine-capped Mn-doped ZnS QDs, obtaining a limit of detection of 3.8 nM. The use of other modifiers such as tiopronin [27] led to the successful analysis of real samples such as tap water, lake water and human serum. The phosphorescence of cetyltrimethylammonium bromide-capped Mn-doped ZnS QDs was also quenched by the complex formed by the mercury ion and a label-free rich thymine aptamer [28]. The decrease in the signal was explained through electron transfer and aggregation of the QDs. Ke et al. [29] also observed this effect that was investigated by infrared analysis. These authors synthesized MPA-coated Mn-doped ZnSe/ZnS QDs for ultrasensitive detection of Hg(II), and they deduced that the original ligands were separated from the surface of the colloidal nanoparticles due to the strength of the chelation between mercury ion and thiols.

Molecularly and ion imprinted polymers are synthesized using the analyte as template to define the properties of the binding sites of the polymer [30]. Imprinting technology is one of the most promising tools for the selective monitoring of metals due to the selective response, good stability, simplicity and low cost [31]. Electrochemical sensors [32-37] for Hg(II) based on imprinted polymers as recognition elements have recently been developed. The chemically modified electrodes for sensing ions [31] are very promising because of their selectivity and sensitivity, but they require accumulation times higher than ten minutes to attain low limits of detection of mercury [38]. Molecularly imprinted fluorescence polymers with 9-vinylcarbazole [39] and sol–gel-derived fluorescent films [40] have also been designed for optical sensing.

In this study Mn-doped ZnS quantum dots were synthesized and covered with a molecularly imprinted polymer based on the use of phenobarbital as ligand and methylmercury as template. This molecularly imprinted polymer has been described in previous studies for the analysis of marine products [41] and waters [42]. After the synthesis, the characteristics of the new material and its fluorescent response to methylmercury were evaluated.

7.2 Experimental

7.2.1 Chemicals

All the solutions were prepared using ultra-pure water of 18Ωcm resistance obtained from a Milli-Q purification device (Millipore Co., Massachusetts, USA). Mn-doped ZnS QDs were synthesized by using heptahydrate zinc sulfate (Panreac, Barcelona, Spain), sodium sulfide (Fluka, Buchs, Switzerland), and manganese dichloride (Merck, Darmstadt, Germany). Methylmercury chloride, methacrylic acid (MMA), ethylene dimethacrylate (EDMA), phenobarbital and thiourea were supplied by Sigma (Steinhelm, Germany). The initiator 2,2′-azobisisobutyronitrile (AIBN) was purchased from Fluka (Steinhelm, Germany), and the porogen dimethylformamide (DMF) from Merck. Potassium dihydrogen phosphate was from BDH (Poole, UK). All the other chemicals (polyethylene glycol (PEG 6000), hydrochloric acid, sodium hydroxide for the preparation of the buffer,...) were purchased from Panreac and Scharlau (Barcelona, Spain).

All glass and plastic material was cleaned and kept in 10% (w/w) nitric acid for at least 48 h. The material was then rinsed three times with ultra-pure water before use.

7.2.2 Instrumentation.

Fluorescence measurements were performed using an F-2500 Hitachi fluorescence spectrophotometer (Schaumburg, IL, USA) equipped with a xenon lamp and 10 mm quartz cells. The confirmation of the removal of the template (methylmercury) was carried out using a High Resolution Continuum Source Atomic Absorption Spectrometer (HRCSAAS) (Analytik Jena ContrAA 300 Model, Jena, Germany) equipped with a flow injection system to perform vapor generation. A Raypa model UCI-150 ultrasonic bath with a frequency of 35 kHz from R. Espinar S.L. (Barcelona, Spain), was used for preparing the MIP-coated Mn-doped ZnS quantum dots. The characterization was performed using transmission electron microscopy (JEOL JEM-1010 100 kV, JEOL USA, Inc., Peabody, MA, USA), an energy dispersive X-ray fluorescence spectrometer (Philips PW1710, PANalytical, Almelo, Netherlands) equipped with a PW1820/00 goniometer, and infrared spectroscopy (Spectrum Two FTIR, Perkin Elmer, Waltham, MA, USA). A pH-meter model 720 (Thermo Orion, Waltham, USA) was used to adjust the pHs needed for the experiments. After preparation, MIP-coated quantum dots were filtered using 0.20 µm Durapore circles and a vacuum pump (Millipore Co).

7.2.3 Synthesis of polyethylene glycol-coated Mn-doped ZnS quantum dots (QDs).

The method used for the synthesis of Mn-doped ZnS quantum dots was a modification of the procedure described by Wang et al. [43], using polyethylene glycol to cover the quantum dots and ultrasound irradiation [44]. The synthesis was carried out at room temperature: 12.5 mmol of $ZnSO_4$, 1 mmol of $MnCl_2$ and 40 mL of ultrapure water were introduced into a three-necked flask, under magnetic shaking and nitrogen stream. After 10 minutes, 10 mL of a 1.25M Na_2S solution was added dropwise using an addition funnel. The mixture was stirred for 30 minutes, and afterwards a mass of 3.33 g of polyethylene glycol dissolved in 10 mL of ultrapure water was added. After 4 hours of ultrasonic irradiation (37 kHz), the content of the three-necked flask was distributed into four centrifuge tubes. The synthesized polyethylene glycol-coated quantum dots were separated after centrifugation at 3000 rpm for 20 minutes. Centrifugation was repeated three times, rinsing the material with 5 mL of methanol between each step. The nanoparticles synthesized were kept in the dark and dried at room temperature inside a desiccator, and finally stored at 4^{o}C.

7.2.4 Synthesis of MIP-coated QDs (MIP-QDs).

Polyethylene glycol-coated QDs were functionalized with a phenobarbital-based molecularly imprinted polymer. First, a pre-polymerization mixture was prepared by dissolving 0.0375 mmol of MeHgCl, 0.15 mmol of phenobarbital and 0.375 mmol of MAA in 4 mL of dimethylformamide. This mixture was stored in the dark for 12 h to allow the assembly of template and monomer. Afterwards, 0.5 g of the previously synthesized QDs were introduced into an Erlenmeyer flask and manually stirred with 25 mL of ultrapure water, the pre-polymerization mixture, 2.87 mmol of EDMA, and 0.125 mmol of AIBN. Ultrasound-assisted polymerization was carried out at room temperature for 4 h. Several advantages of sonication have been previously reported in the synthesis of MIP-QDs: better homogenization of quantum dots size, reduction of nanoparticles agglomeration, and decrease of reaction time [44].

After polymerization, MIP-QDs were cleaned three times with methanol and separated by centrifugation (3000 rpm, 20 min). The material was kept in the dark, dried at room temperature inside a desiccator, and finally stored at 4^{o}C.

7.2.5 Template removal procedure.

Methylmercury was eliminated from MIP-QDs following this procedure: a mass of 0.5 g of MIP- QDs was added to 75 mL of an acidic thiourea solution (0.5M thiourea, pH 2.0 adjusted with HCl). The mixture was sonicated for 30 minutes and the material was vacuum filtered using a 0.20 µm Durapore membrane filter. The procedure was repeated 8 times for complete template removal. The extractant solution was analyzed after use by HRCSAAS [41] to check the complete removal of mercury.

Different extractants were previously tested for template removal. First, a solution of 0.5% of mercaptoethanol, 15% MeOH, and 10% acetic acid [42] was used, but this mixture didn´t allow the total elimination the methylmercury template. An extractant consisting of 1M thiourea in 1M HCl produced a significant loss of mass of material. Finally, the selected extractant was a low acidic 0.5M thiourea solution (pH 2.0).

7.2.6 Study of the fluorescence of MIP-QDs.

Fluorescence measurements were performed using an excitation wavelength of 258 nm (slit widths: 10 nm, photomultiplier tube voltage: 700 V), and recording the emission spectrum between 480-560 nm. The maximum of emission was observed at 518 nm.

In optimum conditions, a volume of 1.5 mL of MIP coated QDs solution (0.6 g L^{-1} prepared in a 0.1M/0.1M potassium dihydrogen phosphate-sodium hydroxide buffer, pH 6.5) was completed to 2 mL in a glass vial with buffer and/or the corresponding methylmercury buffered standard solution. Glass vials were stirred in a vortex, and afterwards they were kept in the dark at 4°C for at least 20 minutes before analysis. Finally, the samples were transferred to a 10 mm-quartz cell, and their fluorescence spectra were recorded.

7.3 Results and discussion

7.3.1 Characterization.

The synthesized MIP coated QDs were characterized by transmission electronic microscopy (TEM), X-ray diffraction spectrometry, infrared spectroscopy, and fluorescence spectroscopy.

The transmission electron microscopy (TEM) images of polyethylene glycol-coated quantum dots (QDs) and MIP-QDs are shown in Figure 1, and it is possible to observe that the size of the particles is smaller than 20 nm. Figure 2 shows the X-ray diffraction pattern of MIP-QDs that exhibits the structure of zinc blende with peaks for (111), (220), and (311) planes [43]. The average size of the particles was 2.2 nm and it was calculated using the Debye-Scherrer equation [45].

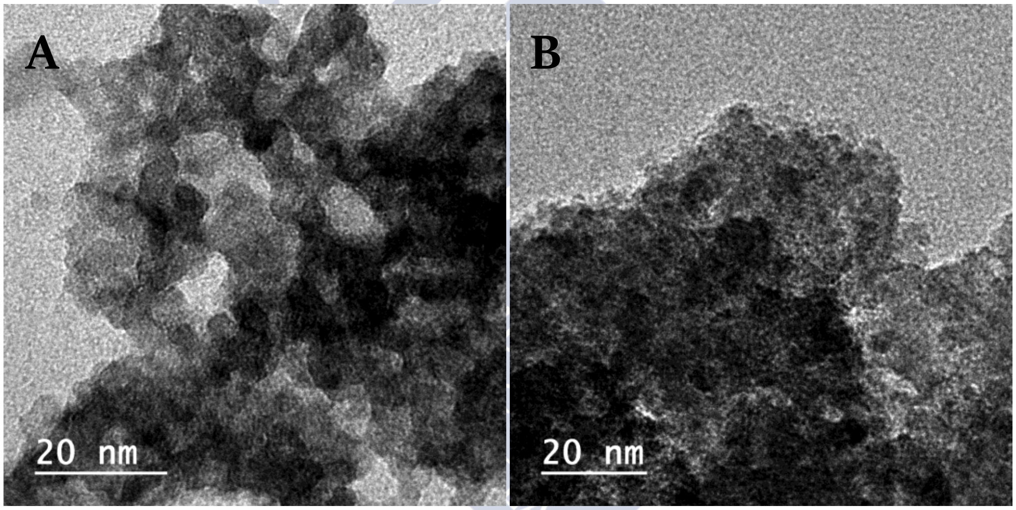

Figure 1. TEM images: (a) Polyethylene glycol-coated quantum dots (QDs), (b) MIP- QDs.

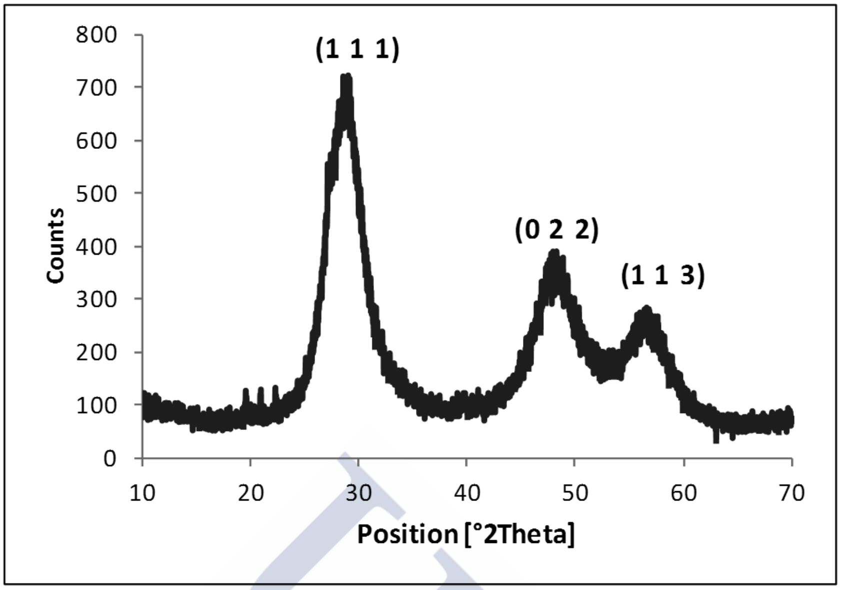

Figure 2. X-ray diffraction pattern of MIP-QDs.

Attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) enabled the direct examination without sample preparation of MIP-coated QDs and the MIP. Figure 3 shows that both spectra are very similar, but an increase of the intensity of the bands at 1640, 1445, 1050, 950, 810 and 650 cm^{-1} was observed for MIP-QDs. Those signals were associated to the presence of the inorganic ions from the QDs (650, 1050, 1640 cm^{-1}), and polyethylene glycol (1445, 950, 810 cm^{-1}).

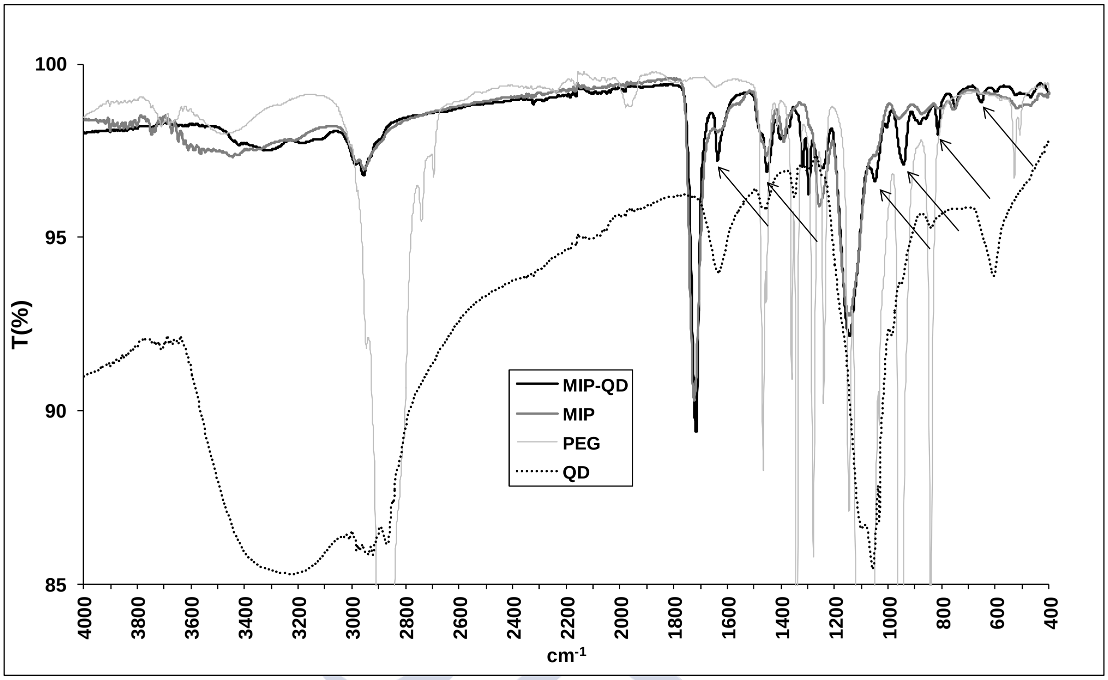

Figure 3. FTIR spectra of MIP-QDs, MIP, polyethylene glycol (PEG), and PEG-coated quantum dots (QDs).

Finally, the fluorescence excitation spectrum of MIP-QDs showed two excitation maximum at 258 and 297 nm. The emission spectra were recorded from 400 to 800 nm, and the corresponding maximums of emissions for both excitation wavelengths were at 518 (more intense) and 599 nm, respectively. A weak emission signal was also observed at 775 nm after excitation at 258 nm. Similar results were obtained in our research group by Chantada et al. [44] after the synthesis of MIP-coated Mn-doped quantum dots for the fluorescent detection of cocaine.

7.3.2 Optimization of operating conditions.

Three different parameters affecting fluorescence detection were studied: interaction time of methylmercury and MIP-coated QDs, concentration of MIP-QD, and the effect of pH. To evaluate the effect of interaction time, the fluorescence spectrum of a MIP-QDs solution was recorded every two minutes (excitation wavelength: 258 nm), and the height of the emission peak was measured. The solution consisted of a mixture of 0.4 g L^{-1} of MIP-QDs buffered at pH 6.5 with the template to obtain a final concentration of 50 µg L^{-1} of methylmercury. A time of 20 minutes after mixing the reagents was needed to achieve a stable fluorescence signal (Figure 4). One advantage of the fluorescent probes is that they can allow rapid signal read-out, but the incubation or reaction time (in some exceptional cases 1-2 hours) must be also considered [6]. In the present study, the sensitivity decreases at 20 minutes but the stabilization of the signal allows the sequential measurement of the standards that were prepared simultaneously. Thus, total analysis time is decreased and standard preparation is simpler.

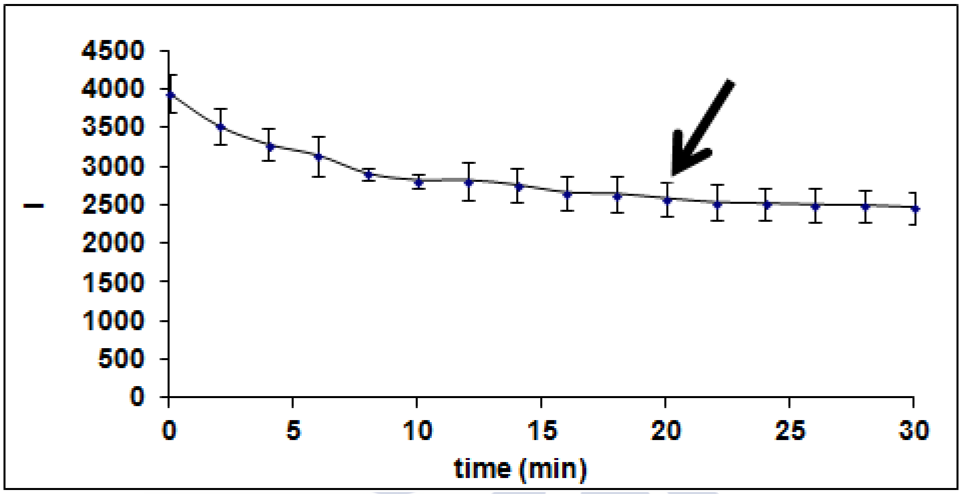

Figure 4. Variation of the intensity of emission after mixing MIP-QDs and methylmercury.

Different MIP-QDs concentrations were tested between 0.2-0.6 g L^{-1}. The quenching of fluorescence increased with the concentration; however, it was difficult to maintain a proper MIP-QD suspension at concentrations higher than 0.6 g L^{-1}. Therefore, this was the concentration selected to perform the following experiments.

Finally, four different pHs (4.4, 6.5, 8.0 and 9.7) were tested for the MIP-QDs fluorescence measurements, and the slopes of the responses to methylmercury were compared. Standards were prepared in duplicate using a mixture of 0.1M/0.1M KH_2PO_4/NaOH buffered at the different pHs, with a concentration of MIP-QDs of 0.6 mg L^{-1}, and MeHg concentrations ranging from 2.5 µg L^{-1} to 100 µg L^{-1}. Figure 5 shows the variation of Io/I versus [MeHg], where Io is the intensity of fluorescence in the absence of the analyte, I is the intensity of fluorescence in the presence of analyte, and [MeHg] is the concentration of methylmercury expressed in µg L^{-1}. The biggest variation of the signal was achieved at pH 6.5, and this value was selected for subsequent experiments. In a previous research, the molecularly imprinted polymer with phenobarbital showed a 100% recovery on the analysis of methylmercury from pH 7.0 onwards [41], but this factor seems to be more critical in the fluorescence measurements.

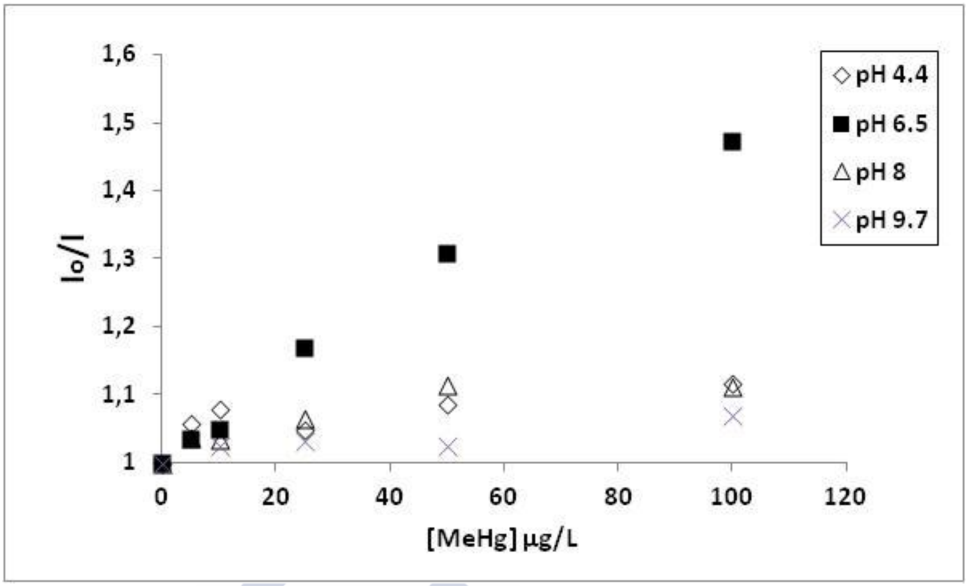

Figure 5. Variation of the fluorescence signal (quenching) at different pHs.

7.3.3 Analytical Performance.

The equation of the calibration graph obtained using the optimum MIP-QDs concentration (0.6 g L^{-1}), concentrations of methylmercury of 0, 5, 10, 25, and 50 µg L^{-1}, and the optimum pH (6.5), was I = -533.1 [MeHg]+111713, with a regression coefficient of 0.993. The fluorescence signal decreases with concentration until 100 µg L^{-1}. The excitation and emission wavelengths were 258 nm and 518 nm, respectively; measurements were carried out using standards prepared in duplicate, and peak area was used for the calculations. The experimental Stern–Volmer relationship was: Io/I = 1 + 0.0062 [MeHg], with a regression coefficient of 0.997; therefore, the Stern-Volmer constant characteristic of the quenching process was 0.0062.

The repeatability of the method was calculated after the preparation of 6 replicates of a 50 µg L^{-1} standard that were incubated with the MIP-QDs for 20 minutes, and afterwards the fluorescence was measured. The experimental value obtained was 49.4 ± 8.0 µg L^{-1} (mean ± standard deviation), with a relative standard deviation of 16% for the whole procedure. An experimental concentration of 81.9±0.3 µg L^{-1} (recovery of 109% of the analyte) was obtained for a concentration of 75 µg L^{-1} of MeHg, with a relative standard deviation in the measurements of 1% (n=15).

The limit of detection (LOD) is defined as the element concentration corresponding to three times the standard deviation of the measurement of a blank, and the limit of quantification (LOQ) is calculated as ten times the standard deviation of the measurement of a blank. The blank was prepared by diluting 1.5 mL of 0.6 g L^{-1} a QD-MIP solution to 2 mL with buffer at pH 6.5. A LOD of 1.5 µg L^{-1} of methylmercury (7.0 nM), and a LOQ of 5.0 µg L^{-1} were obtained. The limit of detection was of the same order than those obtained for the detection of Hg (II) using the measurements of the fluorescence of Mn-doped ZnS quantum dots modified with tiopronin (8.9 nM). The detection limits decreased in those studies that evaluated phosphorescence at room temperature: LOD: 3.8 nM for L-cysteine-capped Mn-doped ZnS quantum dots [26], and 1.5 nM for CTAB-capped Mn-doped ZnS in combination with a rich thymine aptamer [27]. The sensitivity could be increased with the synthesis of core-shell semiconducting nanoparticles; thus, mercaptopropionic acid-coated ZnSe/ZnS quantum dots allowed to achieve a limit of detection for Hg(II) of 0.1 nM.

7.4 Conclusion

To the best of our knowledge, this is the first study to design a material that combines the optical properties of quantum dots and the characteristics of imprinted polymers for methylmercury determination. The synthesis proposed is relatively simple, and it can be performed at room temperature and at a low cost. After studying the operating conditions for fluorescence measurements, the limit of detection achieved was of 7 nM of methylmercury. Future studies should focus on the use of phosphorescence measurements and the synthesis of core-shell semiconductors as supports for the polymer, with the objective of decreasing the detection limits and develop future applications.

7.5 Acknowledgments

This research was funded by the Government of Galicia, Spain (Consellería de Cultura, Educación e Ordenación Universitaria, GRC 2014/016).

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CONCLUSIONES


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Conclusiones

A continuación se muestra un breve resumen de las conclusiones obtenidas tras la finalización de esta tesis doctoral:

1. Se ha desarrollado un polímero de impronta molecular MIP, basado en la interacción ditizona-MeHg para la determinación de MeHg en muestras de atún mediante un método de extracción en fase sólida en modo *batch* y determinación por HRCSAAS. La exactitud del método ha sido evaluada mediante el análisis de un material de referencia (BCR-463, tejido de atún), con buena coincidencia entre el valor experimental (3,27 ± 0,29 µg/g) y el certificado (3,04 ± 0,16 µg/g).

2. Se ha desarrollado un MIP basado en la interacción fenobarbital-MeHg para la determinación de MeHg en extractos orgánicos de muestras de pescado mediante un método de SPE en modo columna. La exactitud del método fue comprobada empleando extractos en tolueno de dos materiales de referencia certificados, BCR-463 (tejido de atún) y TORT-2 (hepatopáncreas de langosta).

3. Se ha desarrollado un método para la aplicación del polímero basado en la formación del complejo con fenobarbital para el análisis de aguas mediante CV-ICP-OES. El método se ha aplicado a aguas residuales.

4. Se ha desarrollado una aplicación para la determinación y especiación de mercurio en agua de mar mediante HPLC-ICP-MS empleando el MIP de fenobarbital. El polímero presenta afinidad para Hg(II), MeHg y EtHg, y se ha realizado una separación/pre-concentración cuantitativa de las especies en muestras de agua de mar. La especiación de mercurio ha sido llevada a cabo mediante cromatografía en fase reversa. El MIP presenta un factor de preconcentración de 50, y los límites de detección alcanzados son inferiores a la concentración máxima permitida por la legislación europea (70 ng/L).

5. Se ha desarrollado un material consistente en quantum dots de ZnS dopados con Mn recubiertos de un polímero de impronta molecular y se ha estudiado su respuesta fluorescente. Mediante este método se ha alcanzado un límite de detección para el metilmercurio de 7 nM.

6. Con el trabajo desarrollado en esta tesis se ha demostrado que el uso de los polímeros de impronta molecular es una buena opción para la separación, preconcentración y análisis de especies de mercurio.