



Universidad de Burgos

*Departamento de Química
Área de Química Analítica
Departamento de Biotecnología y ciencia de los alimentos
Área de Ingeniería Química*

Determinación de contaminantes del mar en aguas de bateas de cultivo de mejillón: Desarrollo de sensores electroquímicos y estudios de filtración con membranas.

Lydia Pérez Ruiz

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Tesis Doctoral



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Memoria de investigación titulada “*Determinación de contaminantes del mar en aguas de bateas de cultivo de mejillón: Desarrollo de sensores electroquímicos y estudios de filtración con membranas*” presentada para optar al Grado de Doctora en la Universidad de Burgos.

Fdo.: Lydia Pérez Ruiz



Facultad de Ciencias
Pza. Misael Bañuelos,
s/n 09001 Burgos



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Dña Isabel Escudero Barbero, Profesora Titular del Departamento de Biotecnología y Ciencia de los Alimentos de la Universidad de Burgos y Dña. M. Julia Arcos Martínez, Catedrática de Universidad del Departamento de Química de la Universidad de Burgos.

INFORMAN FAVORABLEMENTE sobre la presente Tesis Doctoral, titulada “*Determinación de contaminantes del mar en aguas de bateas de cultivo de mejillón: Desarrollo de sensores electroquímicos y estudios de filtración con membranas*”, realizada en el Área de Ingeniería Química del Departamento de Biotecnología y Ciencia de los Alimentos y en el Área de Química Analítica del Departamento de Química de la Universidad de Burgos por Dña. Lydia Pérez Ruiz.

Y para que así conste, firman el presente informe en Burgos, 10 de Mayo de 2019.

Fdo.: Isabel Escudero Barbero

Fdo.: M. Julia Arcos Martínez



Facultad de Ciencias
Pza. Misael Bañuelos,
s/n 09001 Burgos

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Abreviaturas

Abreviaturas	Definición
AC	Carbono activado
AuNP	Nanopartículas de oro
ASV	Voltamperometría de redisolución anódica
BM1	Material de soporte 1 (500 μm thickness polyester film)
BM2	Material de soporte 2 (Gore-Tex® Pro-Shell Nylon Liner)
BM3	Material de soporte 3 (2-Layer Gore-Tex® Paclite® Shell Ripstop)
COD	Demanda química de oxígeno
CV	Voltamperometría cíclica
DPAdASV	Voltamperometría diferencial de impulsos de adsorción con redisolución anódica
DPASV	Voltamperometría diferencial de impulsos de redisolución anódica
DPV	Voltamperometría diferencial de impulsos
DSP	Intoxicación diarreica por marisco
DSPM	Modelo de exclusión de Donnan
ERGO	Grafeno oxidado reducido electroquímicamente
GO	Óxido de grafeno
HMDE	Electrodo de gota colgante de mercurio
LOD	Límite de detección
MF	Microfiltración
MFI	Índice modificado de ensuciamiento
MFI-UF	Índice modificado de ensuciamiento aplicado para membranas de ultrafiltración
MWNT	Nanotubos de varias capas
NF	Nanofiltración
OA	Ácido Okadaico
OR	Ósmosis inversa
PFM	Modelo de flujo de poros
p-NP	p-nitrofenol
p-NPP	p-nitrofenol fosfatado
PP	Fenil fosfato
PP1	Proteína fosfatasa tipo 1
PP2A	Proteína fosfatasa tipo 2A
RSD	Desviación estándar relativa
SD	Desviación estándar
SDI	Índice de densidad del sedimento
SDM	Modelo de disolución-difusión
SDFM	Modelo de película-solución-disolución

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SPCE	Electrodos serigrafiados de carbono
SPE	Electrodos serigrafiados
SWASV	Voltamperometría de onda cuadrada de redisolución anódica
SWCNT	Nanotubos de carbono de una sola capa
SWNT	Nanotubos de una sola capa
SWV	Voltamperometría de onda cuadrada
TMP	Presión transmembranal
UF	Ultrafiltración
UV-SW	Digestión de agua de mar con ultravioleta
4-MU	4-metilumbeliferona
4-MUP	4-meilumbeliferilo fosfatado

Hoy en día hay que destacar el elevado nivel de contaminación al que están expuestos muchos productos alimenticios como resultado de una mayor actividad industrial y de una insuficiente gestión de los residuos y de la contaminación medioambiental. La suma de estos factores obliga en ocasiones a tomar decisiones sobre la producción de la industria alimentaria por su repercusión en el cuidado de la salud y de la calidad de vida.

Para la industria alimentaria es igualmente importante mantener un alto nivel de calidad de la producción, como la seguridad alimentaria de los consumidores. La necesidad de aplicar sistemas de control sobre la calidad de los productos antes de entrar en el mercado es reconocida en la mayoría de las empresas. En particular, en la industria del cultivo de mejillones, antes de los controles rutinarios, se está estudiando de forma estratégica la posibilidad de desarrollar sistemas eficaces, precisos, rápidos, económicos y de alta sensibilidad para la detección de contaminantes en el agua de mar que posibiliten una recogida precoz del mejillón en las bateas. Estos estudios persiguen obtener un producto de alta calidad sin riesgo de contaminación y conseguir evitar las enormes pérdidas económicas que supone el cierre de las bateas afectadas por episodios tóxicos.

La investigación que se presenta en esta Tesis Doctoral forma parte de un proyecto más amplio (RTC-2015-4077-2) concedido por el Ministerio de Economía y Competitividad dentro de la convocatoria Retos-Colaboración del Plan Estatal 2013-2016. El proyecto titulado “Sistema de alerta temprana para la detección automática de contaminantes y predicción de episodios tóxicos en el cultivo del mejillón. El proyecto MUSSEL” se ha desarrollado durante el periodo 2015-2017. El objetivo de este proyecto es el desarrollo de un sistema complejo de alerta temprana que recoge información del agua marina mediante un sistema basado en el filtrado selectivo del agua de mar y la detección de contaminantes por medio de sensores electroquímicos, aglutinando dicha información y enviándola por medios inalámbricos a una plataforma central. El proyecto se orienta a solventar los graves problemas sanitarios y económicos que implica la presencia en el agua de mar de metales pesados como cadmio, plomo o arsénico, así como de ácido

okadaico, debido a la floración de “alga roja”, que puede provocar graves enfermedades en el ser humano.

En el marco de este proyecto, esta Tesis Doctoral recoge los estudios realizados en el ámbito de la filtración con membranas y el desarrollo de sensores electroquímicos para la determinación de contaminantes en el agua de mar que rodea las bateas del cultivo del mejillón.

En un principio se describe, en el capítulo 2, los objetivos generales y específicos que se han seguido para el desarrollo de la Tesis Doctoral. Después de definir los objetivos, se describen en el capítulo 3 las generalidades de los analitos de estudio y los antecedentes bibliográficos, seguido de los capítulos 4 y 5 donde se definen los fundamentos teóricos de las tecnologías de membrana y de la electroquímica para la comprensión de los diferentes trabajos que se describen en los capítulos siguientes.

En el capítulo 6 se presenta un trabajo realizado sobre el estudio de validación del modelo de película-solución-difusión (SDFM) para la caracterización del transporte de solutos neutros y cargados en una membrana de nanofiltración (NF). El trabajo realizado supuso un estudio de iniciación en la comprensión de los fenómenos que acontecen durante la filtración con membranas. Además, fue de gran utilidad para la interpretación de resultados y la toma de decisiones en el trabajo que se presenta en el capítulo 7 y que aborda el tratamiento del agua de mar mediante tecnologías de separación con membranas a baja presión.

A continuación, en los capítulos 8 y 9, se utiliza la tecnología serigráfica para el desarrollo de sensores electroquímicos capaces de determinar concentraciones muy pequeñas sin la necesidad de un pretratamiento complejo de la muestra. Además de ser una tecnología que nos permite el uso de diversas combinaciones de técnicas para lograr sensibilidades y selectividades mayores de un analito de interés.

Los resultados de esta tesis pueden ser de gran utilidad y de interés para futuras investigaciones dentro del campo de las tecnologías con membranas, así como para la monitorización de contaminantes por medio de sensores electroquímicos, que presentan importantes ventajas respecto de los métodos de análisis y control de toxinas que se aplican en la actualidad.

Con la investigación que se plantea en esta Tesis se pretende avanzar en el diseño de un sistema de alerta temprana para la detección de contaminantes en el agua de mar que rodea las bateas de cultivo del mejillón. En concreto se abordan dos objetivos generales:

- A. Diseñar un sistema de filtración selectiva del agua de mar, utilizando membranas de microfiltración / ultrafiltración.

- B. Desarrollar sensores electroquímicos desechables para la detección de contaminantes en el agua de mar.

Para alcanzar estos objetivos generales, se plantearon los siguientes objetivos específicos:

1. Estudiar el rendimiento de una membrana de nanofiltración (NF) para diferentes solutos cargados y no cargados.

2. Validar el modelo de película- solución -difusión (SDFM) en membranas de nanofiltración y estudiar la información aportada por este modelo como base de estudio en la comprensión de los fenómenos que pueden acontecer en las operaciones posteriores de filtración de agua de mar con membranas.

3. Estudiar un sistema de filtración selectiva del agua de mar, utilizando diferentes membranas de microfiltración / ultrafiltración en condiciones de baja presión.
 - 3.1.- Estudiar de ensuciamiento de las membranas utilizando el índice de ensuciamiento modificado.
 - 3.2.- Seleccionar las condiciones de operación óptimas para la obtención de flujos estables durante tiempos prolongados.
 - 3.3.- Analizar los rechazos de los contaminantes del agua de mar para obtener permeados exentos de materia coloidal e interferentes de los analitos de interés que puedan afectar al sistema de medición.

4. Diseñar sensores electroquímicos desechables, para la detección de contaminantes en agua de mar, capaces de detectar concentraciones submolar de manera rápida y precisa.
 - 4.1.- Poner a punto un sensor para la detección de plomo en concentraciones trazas, capaz de poder alertar de manera temprana.
 - 4.2.- Poner a punto un sensor para la detección del ácido okadaico en concentraciones bajas, capaz de alertar de manera temprana.

5. Validar los sensores específicos para la determinación de contaminantes de forma directa en el agua de mar, sin necesidad de realizar un tratamiento previo a la muestra.
 - 5.1. - Validar el sensor para medir el contenido de plomo en agua de mar.
 - 5.2. - Validar el sensor para medir el contenido de ácido okadaico en agua de mar.

3.1.- El agua

El agua es el recurso más importante de nuestro planeta, indispensable para la vida y para la sociedad. Es el compuesto químico más abundante en la biosfera, formando el 71% de la superficie de la Tierra y siendo principalmente agua salina. Se puede encontrar en diferentes lugares como en el suelo, el aire e incluso dentro de los organismos vivos [1,2]. Es un elemento que está presente de manera natural y es fundamental para la vida de todos los seres vivos. También es utilizado en la sociedad para generar y mantener el crecimiento económico y la prosperidad a través de diversas actividades terrestres.

El cambio climático, el crecimiento de la población y el aumento de la actividad industrial, a lo largo de los años, están planteando mayores desafíos para el suministro de agua, mayores demandas y creando también conflictos en la calidad del agua [1,3]. La contaminación causa la degradación de los hábitats y genera un alto riesgo para los organismos que habitan en el ecosistema, afectando a la cadena alimentaria y creando un riesgo para la población humana. La gran producción industrial, la agricultura y el crecimiento de la población son las principales causas de la contaminación de los ecosistemas acuáticos y sobretodo de las zonas costeras, debido al efecto que están ejerciendo los ríos con el transporte y la acumulación de la contaminación interior [4–6].

Hoy en día se sabe que aunque la gran mayoría de la contaminación es causada por el hombre, como se ha indicado anteriormente, no es la única contaminación acuática. También existe una contaminación de manera natural, con efectos importante a tener en cuenta. Los factores naturales son capaces de contaminar, alterar el ecosistema y perjudicar el normal desarrollo de los seres vivos. Entre estos factores se encuentran las cenizas procedentes de erupciones volcánicas, mercurio de la corteza terrestre u toxinas. Estas toxinas son menos conocidas y son generadas por los afloramientos de algas o microalgas, las cuales son dañinas para los peces, el ganado e incluso para los humanos [7].

El problema principal y más importante siempre se plantea desde el peligro y riesgo del ser humano a sufrir enfermedades o muertes. Por lo tanto, las muestras tomadas en el control de contaminantes en el agua de mar están relacionadas directamente con organismos acuáticos de consumo humano [8]. Sin embargo, actualmente también se plantean nuevos estudios que abordan los efectos negativos provocados por los contaminantes marinos en los animales que realizan un consumo del medio acuático y son expuestos a su contaminación [9–11]. La contaminación se produce dentro de la cadena alimentaria por la inadecuada o nula eliminación de los organismos a algunos contaminantes, generándose una bioacumulación en su interior y una alta toxicidad a sus consumidores. Si la toxicidad de algunos contaminantes marinos es muy alta, no se requieren concentraciones elevadas para producir problemas importantes con la ingesta [11].

Los metales pesados se consideran contaminantes muy tóxicos y altamente peligrosos. Como se ha indicado anteriormente, estos contaminantes presentan una gran persistencia en los medios acuáticos y son fácilmente acumulables en los organismos, produciendo de este modo una biomagnificación en las cadenas alimentarias [12,13]. Los metales pueden dividirse en elementos esenciales como (Se, Zn, Cu y Mn) o en no esenciales como (Hg, Pb, Cd, Ni...), siendo algunos de ellos muy peligrosos para la salud.

Hay organismos como los bivalvos que por su alta capacidad de bioacumulación de metales pesados en su organismo se utilizan como bioindicadores para la monitorización de la contaminación marina. [8,9,11,14]. El proceso de bioacumulación que se produce es debido a la imposibilidad de los organismos contaminados de mantener los niveles necesarios de excreción [15]. Como consecuencia de este proceso, las especies y entre ellas las de origen marino, pueden sobrepasar los límites máximos fijados por la Unión Europea (UE) (Reglamento 1881/2006) [16] y verse afectada su comercialización con una repercusión económica y social.

En el agua de mar existen organismos de una sola célula, filamentosos o en cadenas de células que conforman lo que se conoce con el nombre de fitoplancton. En este grupo se pueden encontrar una gran variedad de microalgas que se alimentan de sustancias químicas como el nitrógeno, fósforo y sílice. Muchas de ellas presentan grandes floraciones (“algae bloom”) que pueden ser perjudiciales para los mariscos, larvas y peces que se alimentan de ellas. Estos acontecimientos se producen por excesos de nutrientes debido a la contaminación provocada por el hombre o de manera natural y están influenciados por las circunstancias climáticas e hidrográficas, haciendo imposible su control [17].

Las floraciones pueden darse por diferentes tipos de algas, pero cuando las algas son nocivas representan un peligro que puede causar intoxicaciones en los humanos por consumo de mariscos, muerte masivas de peces y mariscos, e incluso problemas industriales [18]. Para evitar estos problemas se han desarrollado diferentes métodos de control, los cuales han resultado poco exitosos hasta el momento [19]. Como consecuencia de la poca eficacia de estos intentos las toxinas siguen acumulándose en el interior de los animales y prevaleciendo desde meses hasta incluso años. [7,20].

3.2.- Principales contaminantes en el agua de mar

3.2.1.- Plomo

El plomo es uno de los metales pesados más tóxicos que existe en medios acuáticos [21]. Es un elemento que se puede encontrar de manera natural en este ecosistema y que al incrementarse la concentración puede atraer graves consecuencias sobre el medio acuático e indirectamente también en el medio terrestre.

La concentración del plomo en aguas sin contaminar está alrededor de 0,002µg/L hasta 0,2µg/L. El plomo llega al medio acuático de manera natural o por fuentes antropológicas [4]. En las aguas costeras es donde se producen las mayores concentraciones de plomo, superando el 1,0 µg/L. Este aumento considerable de

contaminación es debido a la gran exposición humana, como consecuencia de las emisiones que se proceden del tráfico de vehículos, de las fuentes industriales, de la fundición del plomo, de la combustión de carbón, del uso de pinturas a base de plomo, tuberías en los suministros de agua o baterías [22]. No obstante, es destacable la reducción de la contaminación por plomo que se ha producido en los últimos años por la prohibición de su uso en las gasolinas, pinturas, tuberías y otros productos en la mayoría de los países desarrollados [23].

En comparación con otros metales pesados, el plomo en el agua no resulta especialmente tóxico, sino que su toxicidad depende de la forma química. Esto significa que cuando el plomo se compleja presenta un proceso en el que la biodisponibilidad se reduce. El proceso se produce porque el plomo en ambientes acuáticos naturales, a pH neutros o superiores, se compleja fácilmente produciendo compuestos orgánicos estables y sales insolubles o muy poco solubles en el agua, a excepción de las sales de nitrato, clorato y cloruro que presentan una gran solubilidad. El ion plomo se puede encontrar de manera diferente en agua dulce y en agua salada. En el agua dulce las especies más abundantes son los carbonatos e hidróxidos. Aunque también se une a la materia orgánica natural en suspensión con una alta afinidad, lo que puede utilizarse como protección contra la toxicidad del ion libre [24]. Se ha estimado que la materia orgánica compleja la mayoría del plomo en concentración alta, como por ejemplo en las aguas costeras de ríos o mares [25]. La gran diferencia que hay que destacar del agua salada con respecto a la dulce es la influencia del ion cloruro, pudiendo formar complejos de cloruro. Se ha estimado que entre el 50 y el 70% del plomo en los mares se encuentra ligado orgánicamente [26].

El plomo no se bioamplifica en la cadena trófica, pero sigue existiendo el riesgo de una bioacumulación en los organismos vivos y una intoxicación directa o indirecta mediante la cadena alimentaria [27]. Hay algunos organismos que acumulan elevadas concentraciones de plomo inorgánico sin verse afectados, aparentemente. Un ejemplo de estos organismos son los mejillones [8]. En zonas contaminadas, dichos organismos, pueden llegar a acumular elevadas concentraciones de plomo, por su almacenamiento en forma de gránulos en la glándula digestiva [28]. Sin embargo, otros organismos marinos,

como los crustáceos y las algas, se ven afectados a concentraciones mayores de 10ppm de una manera directa en su crecimiento [29,30].

Otros peligros asociados a este metal para organismos no marinos son los efectos dañinos al sistema nervioso central, al riñón, al hígado, al sistema reproductivo, a las funciones cerebrales e incluso pueden alterar los procesos celulares básicos. Los síntomas tóxicos pueden aparecer como anemia, insomnio, dolor de cabeza, mareos, irritabilidad, debilidad de los músculos, alucinaciones y daños renales [13,31]. En particular, en el caso del ser humano, los niños presentan un mayor riesgo debido a la alta capacidad de absorción para el plomo. Un niño puede acumular en su cuerpo hasta un 35 – 40% del metal ingerido, que es 4 o 5 veces mayor que un adulto [22]. Se cree que los efectos neurológicos y conductuales asociados a las acumulaciones del plomo en los organismos son irreversibles [21].

El plomo presenta una toxicidad a bajas concentraciones y como consecuencia los niveles máximos permitidos en los alimentos son muy bajos. El alimento con más concentración de plomo permitido en el mercado corresponde a los moluscos con 1,5 mg/kg peso fresco y el alimento con menos contenido de plomo es la leche, junto con otros preparados para lactantes que no debe sobrepasar el 0,020 mg/kg peso fresco [16].

3.2.2.- Cadmio

El cadmio es un metal pesado que presenta una toxicidad muy similar a la del plomo, y que incluso a concentraciones bajas en el medio marino puede ocasionar consecuencias graves [21].

La presencia del cadmio en suelos y aguas ocasiona una contaminación de la cadena trófica, provocando un riesgo en la salud. Este metal se encuentra en la corteza terrestre de manera natural y puede llegar a contaminar el medio acuático por su propia descomposición, por actividades volcánicas y por la movilización de sedimentos a través de los ríos. La contaminación por fuentes antropológicas ha aumentado en los últimos

tiempos como consecuencia de la combustión de fuel, del tabaco, de la fabricación de baterías, del uso de estabilizadores de plástico, del uso de fertilizantes en la agricultura y de los lodos procedentes del tratamiento de aguas residuales que contienen cadmio [32–35].

El cadmio no se encuentra en la naturaleza en estado libre, sino que se asocia con otros metales por afinidad química. Da lugar a sales inorgánicas como óxido de cadmio, cloruro de cadmio, sulfatos o sulfuros de cadmio. En el medio acuático a baja salinidad el cadmio está presente como ion Cd^{2+} formando hidróxidos de cadmio ($\text{Cd}(\text{OH})_2$) y complejos orgánicos que dependen del pH y de la materia orgánica disuelta. A medida que la salinidad aumenta se van formando los cloruros de cadmio con mayor afinidad (CdCl_2 y CdCl). Con la dependencia del pH se puede observar diferentes comportamientos. En los medios donde los valores de pH comienzan a ser ácidos predomina la forma del cadmio inorgánico (Cd^{2+}) y en los medios donde el pH sea mayor o igual que 6 el cadmio comienza a precipitar en carbonatos [36]. El cadmio se absorbe mejor en los organismos cuando se encuentra en su forma libre (Cd^{2+}), luego con el aumento de salinidad se consigue disminuir la bioacumulación.

La biodisponibilidad del cadmio, retención y toxicidad depende de varios factores, como el bajo contenido de hierro en el cuerpo, la existencia de enfermedades preexistentes o en el caso de las mujeres, el embarazo. El cadmio ha sido clasificado como sustancia carcinógena en humanos por la agencia internacional para la investigación del cáncer [35]. En los seres humanos puede desencadenar enfermedades renales [37], hipertensión [38], diabetes [39], anemia, osteoporosis, lesiones en los órganos reproductivos y algunos tipos de cáncer [40,41]. En el caso de peces, aves y mamíferos también ocasionan problemas como anemia y reducción en el crecimiento [34,42]. Además pueden crearse problemas indirectos a los futuros consumidores de moluscos y crustáceos cuando el cadmio se encuentra en el agua y se produce una bioacumulación dentro de estos organismos por el mecanismo de filtración que utilizan para su alimentación [4]. Algunos estudios han sugerido que el cadmio se puede acumular pero no biomagnificar, con alguna excepción en la cadena alimentaria acuática [27].

En los últimos estudios se ha observado que la presencia de cadmio en la dieta tiene un impacto bajo en las enfermedades renales (0,2%). No obstante, hay estudios que afirman que la reducción del cadmio en la dieta redundaría en un impacto positivo sobre la salud pública [43]. El cadmio se absorbe entre un 3 – 5% de la cantidad ingerida en la dieta. Este dato es relativamente bajo, pero se hace relevante cuando se tiene en cuenta que la eliminación de este metal es tan lenta que su persistencia en un cuerpo llega a ser de unos 20 a 30 años [32].

Los límites permitidos para el cadmio se encuentran regulados por la Unión Europea (UE) por el reglamento (UE) N° 488/2014 [44] que es una modificación del reglamento (CE) N° 1881/2006 [16] donde se describen todos los límites legales de los metales pesados en los alimentos. El producto de comercialización con la mayor cantidad de cadmio posible corresponde, al igual que en el plomo, a productos marinos como las algas y moluscos desecados, con una concentración de 3,0 mg/kg peso fresco. Y los alimentos que menor concentración de cadmio admiten son los preparados para lactantes con un límite de 0,010 mg/kg peso fresco [44].

3.2.3.- Ácido okadaico

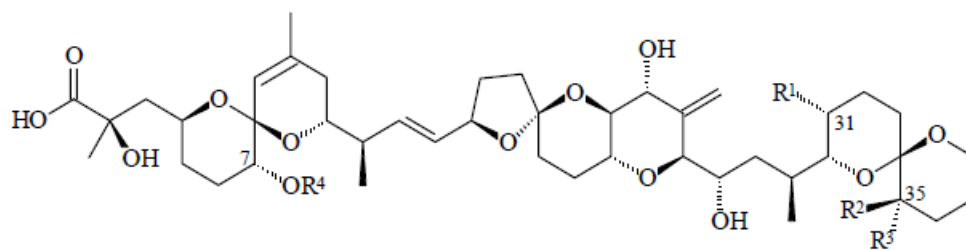
El ácido okadaico (OA) es una toxina que se produce de manera natural por dinoflagelados marinos, algas de plancton microscópicas del género *Dinophysis*. Ejemplos de especies que se han demostrado a día de hoy su capacidad para producir la toxina son las siguientes: *D. acuminata*, *D. acuta*, *D. fortii*, *D. mitra*, *D. norvegica*, *D. rotundata* y *D. tripos* [45]. Otras especies también capaces de producirlo pertenecen al género *Prorocentrum* (*P. concavum*, *P. lima* y *P. redfieldi*) [46]. Al conjunto de una gran población de fitoplancton de estos dinoflagelados se le conoce por marea roja [20].

El OA es una toxina que se acumula en moluscos bivalvos y pueden causar una intoxicación diarreica por su consumo (diarrhetic shellfish poisoning, DSP). La DSP consiste en una intoxicación que causa riesgos en la salud de los consumidores de

mariscos contaminados que han sido extraídos en aguas afectadas por el crecimiento de ciertos tipos de algas tóxicas [47,48].

El OA se acumula en el tejido hepático de peces depredadores y otros animales marinos por su alimentación de mariscos contaminados, pero es incierta la intoxicación de las personas por su presencia en la cadena alimentaria de los peces y animales marinos. En cambio, el consumo directo de los bivalvos contaminados puede causar diarrea, náuseas, vómitos y producir fuerte dolor abdominal. Los síntomas pueden ocurrir en un tiempo muy corto después de su ingestión. Estas intoxicaciones suceden debido a que el OA actúa como un potente inhibidor de la proteína fosfatasa serina / treonina, produciendo una acumulación de proteínas fosforiladas [49].

Las toxinas que causan las intoxicaciones diarreicas pueden ser ácidas, neutras y de compuestos sulfatados. El OA y las toxinas Dinofisistoxinas (DTX1, DTX2 y DTX3) pertenecen al primer grupo de toxinas ácidas. Son lipófilas y se acumulan en los tejidos adiposos de los moluscos [50]. Estudios sobre esta familia de toxinas determinan que el OA es el que mayor estabilidad térmica presenta, degradándose significativamente a partir de 120°C y a temperaturas de congelación, desde -20°C hasta -80°C, son altamente estables durante varios meses [51]. El potencial de toxicidad de la DTX1 es similar al OA, pero en cambio la DTX2 presenta menor toxicidad [47]. En la Fig. 3.1 se muestra la estructura química del Ácido Okadaico y las estructuras análogas de DTX1 y DTX2. La DTX3 son esterificaciones con un ácido graso saturado o insaturado de las demás toxinas, por lo tanto es un derivado del OA, DTX1 y DTX2 [52].



		Radical			
		R ¹	R ²	R ³	R ⁵
Toxina	OA	CH ₃	H	H	H
	DTX1	CH ₃	CH ₃	H	H
	DTX2	H	H	CH ₃	H
	DTX3	H/CH ₃	H/CH ₃	H/CH ₃	Ácido graso

Figura 3.1. Estructura química del OA y toxinas DTX1, DTX2 y DTX3.

Como se ha comentado anteriormente este tipo de contaminación marina se produce por el crecimiento excesivo de unas microalgas del género *Dinophysis* o *Prorocentrum* que puede ser causado por diferentes factores, como cambios climáticos, temperaturas, salinidad o turbulencias del agua [50,53]. También pueden ocurrir por exceso de nutrientes disueltos en el agua por fuentes del tipo antropológico o naturales [53]. Todos estos factores incontrolables hacen que la cantidad de células de algas productoras de la toxina pueden variar considerablemente a lo largo del año. Por ello, los niveles de biotoxinas acumuladas que puedan estar presentes en los moluscos, que se alimentan por un mecanismo de filtración de agua, pueden variar a lo largo del año. A lo largo de la historia se han realizado numerosos estudios poco exitosos para prevenir y controlar dichas mareas [45,54,55]. Hoy en día el control riguroso y constante de muestreo de los propios alimentos sigue siendo la forma más segura para garantizar la calidad del producto y la seguridad de los consumidores. Sin embargo, la posibilidad de que los alimentos contaminados entren en el mercado aún existe [54].

En Europa, según la regulación (EC) N° 853/2004 impuesta por el Parlamento Europeo, se permite la venta de los bivalvos con un contenido de biotoxinas marinas inferior al límite establecido de 160 µg de OA equivalente/kg de parte comestible [52]. No se

plantea solo un problema de salud pública, sino que también puede ser perjudicial para las actividades económicas en la industria de cultivo de moluscos.

La regulación de la Unión Europea, (EC) N° 854/2004 también requiere la implantación de programas de control, mediante monitorización, del contenido de biotoxinas marinas en las áreas de producción de mariscos [56]. Estos programas se utilizan para tomar decisiones sobre la apertura y el cierre de las bateas. La toma de decisiones con esta monitorización puede llevar a un aumento de la oferta del producto final en el mercado con una alta garantía de seguridad de los moluscos [47]. Además, esta regulación europea incide en el desarrollo de procedimientos de desintoxicación y control de las floraciones de algas tóxicas para mitigar las pérdidas económicas. No obstante, las empresas muestran dudas en conseguir una monitorización eficaz de las algas peligrosas, debido a las diferentes características de cada una de las especies contaminantes, señalan el elevado coste de las operaciones de control y el desconocimiento de los efectos del tratamiento sobre las propiedades de los productos a comercializar [19].

3.3.- Antecedentes bibliográficos

Diversos métodos electroquímicos han sido previamente desarrollados para un análisis preciso de los contaminantes de interés en el agua de mar que pueden generar un problema serio en la salud del consumidor. Son métodos que se han convertido en herramientas muy útiles para la determinación de concentraciones trazas.

Sin embargo, los cada vez más exigentes controles a los que está sometido la industria alimentaria para garantizar la seguridad alimentaria, hacen necesario el desarrollo de nuevos métodos para la determinación de sustancias contaminantes más sensibles y precisos, a la vez que económicos y rápidos.

3.3.1.- Plomo

El plomo es uno de los metales contaminantes del medio ambiente más estudiados electroquímicamente. Algunos de los trabajos, descritos en la bibliografía que se describen a continuación se basan en la medición de la señal directa de la oxidación y reducción del plomo con resultados óptimos.

Los estudios más previos de Suren y colaboradores midieron muestras de agua de mar mediante voltamperometría diferencial de impulso con un electrodo de gota colgante de mercurio. Las muestras son ajustadas a pH=7.7 donde se obtienen unas condiciones óptimas para un tiempo de acumulación de 60 s. Estas condiciones consiguen un rango lineal de 16 nM hasta 48 nM, con un límite de detección de 4.8 nM. [57].

Raquel Güell y colaboradores presentan un trabajo con una buena sensibilidad para detectar metales pesados con electrodos serigrafiados y utilizables al menos durante 10 días. El electrodo de trabajo usado en este estudio es de grafito modificado con una película de mercurio. Se utilizó la técnica de voltamperometría de onda cuadrada utilizando un tiempo de preconcentración de 120 s en muestras de agua de mar de diferentes lugares costeros y filtradas previamente. El rango lineal que se obtuvo es de 40 nM a 490 nM y el límite de detección fue de 8.7 nM [58].

En el caso de Wassana Yantasee y colaboradores consiguieron medir en agua de mar sin pretratamiento mediante voltamperometría de onda cuadrada, utilizando un electrodo de carbono vitrificado con monocapas autoensambladas de tiol sobre un soporte mesoporoso y Nafion. Esta vez, se consiguieron electrodos libres de mercurio con un límite de detección de 12 nM y necesitando una preconcentración de solo 60 s [59].

Zhaoshum Bi y colaboradores no midieron de manera directa en el agua de mar, sino que realizaron una digestión de 45 min con ultravioleta. Con este pretratamiento, la

muestra fue medida en una placa de mercurio donde se realizó una amalgama de plata para medir a continuación con onda cuadrada. Esta técnica ofreció un límite de detección de 12 nM utilizando un tiempo de deposición de 60 s [60].

Kunashegaran Hamsawahini y colaboradores consiguieron muy bajos límites de detección (0.5 nM) para la determinación del plomo. Estos autores utilizaron un electrodo de carbono reforzado con grafito y modificado electroquímicamente con óxido de grafeno electroreducido. Estudiaron varias técnicas y la voltamperometría de onda cuadrada ofreció los mejores resultados. Con esta técnica obtuvieron el rango lineal más bajo, desde 3 nM hasta 15 nM. La consecuencia de este trabajo fue la incapacidad para medir directamente la muestra de agua de mar sin ningún tipo de tratamiento. En este caso se tuvo que acidificar el medio con HCl (1M) [61].

Posteriormente Zinoubi y colaboradores consiguieron la misma sensibilidad usando un sensor electroquímico con voltamperometría de diferencial de impulsos y sin necesidad de acidificar el medio. El sensor consta de un electrodo de trabajo de carbono vitrificado que presenta una superficie modificada de celulosa nanofibrilada. Para conseguir mejorar la sensibilidad y optimizar el proceso aumentaron el tiempo de acumulación sobre el electrodo a 1200 s consiguiendo un límite de detección de 0.5 nM [62].

3.3.2.- Ácido okadaico

Se describen trabajos encontrados, previos a esta Tesis Doctoral, para la determinación del ácido okadaico que estudian métodos con bajos límites de detección y alta reproducibilidad.

El OA no tiene en su estructura grupos funcionales fácilmente oxidables o reducibles y no origina ninguna señal redox característica cuando se somete a un amplio barrido de potencial. Por tanto los métodos electroquímicos no miden de forma directa la oxidación-reducción de la molécula de esta toxina. Como consecuencia de este problema

se han desarrollado biosensores e inmunosensores electroquímicos para la determinación del OA con enzimas y anticuerpos. En los sensores enzimáticos se monitoriza el producto de una reacción y miden de manera indirecta el OA, por la inhibición que el analito ejerce en algunas enzimas.

Naoko Hamada-Sato y colaboradores propusieron un sensor amperométrico para la determinación del ácido okadaico. Se basaron en la inhibición de la proteína fosfatasa 2A (PP2A) por el ácido Okadacio. La concentración del ácido okadaico se determinó de manera indirecta por la cantidad de oxígeno consumido, que equivale a la cantidad de ion fosfato que se produce por la descomposición de una proteína y que es consumido posteriormente por el piruvato oxidasa inmovilizada en el electrodo. Consiguieron un rango lineal entre 0.1 – 4.5 µg/L pero únicamente se midió en solución buffer y no se pudo aplicar en muestras reales [63].

En el campo de la electroquímica se siguen investigando maneras diferentes de conseguir mejores resultados. En el caso de G. Volpe y colaboradores llevaron a cabo un biosensor que mide la detección de peróxido de oxígeno, que es el producto final de dos reacciones enzimáticas consecutivas. Las dos enzimas fueron inmovilizadas sobre la sonda de platino, donde la primera enzima, la fosfatasa 2A, es inhibida fuertemente por el ácido okadaico, lo que produce la disminución del producto de la reacción. A continuación, este producto es consumido por la siguiente enzima y da lugar al peróxido de oxígeno como producto final, cuya señal se mide. Consiguieron mediante la técnica electroquímica de amperometría un rango lineal de 30 ng/L hasta 250 ng/L, pero únicamente en solución buffer [64].

Más adelante, Akhtar Hayat y colaboradores, propusieron un nuevo inmunosensor. Utilizando electrodos serigrafiados sobre los que se inmovilizó del OA con perlas magnéticas sobre la superficie del electrodo de trabajo consiguieron realizar un inmunoensayo. La técnica electroquímica que se utilizó fue la voltamperometría diferencial de impulso. De esta manera se midieron muestras reales de mejillones en 60 min y presentaron límites de detección de 380 ng/L (472 pM) [65]. Un año después,

estos mismos autores investigaron sobre nuevos métodos de inmunosensores y técnicas de inmovilización diferentes para la medición en muestras reales de mejillones para el OA. En este caso la técnica de voltamperometría de diferencial de impulso se midió en una solución de ferri/ferrocianuro. En el inmunoensayo se midió la disminución de la señal del ferri/ferrocianuro en presencia del ácido okadaico, obteniendo con este sistema un límite de detección de 500 ng/L (621 pM). En comparación con el anterior trabajo se obtuvieron límites de detección comparables y similares, pero aportaron nuevos sistemas indirectos para la determinación de esta toxina [66].

Otro inmunoensayo fue estudiado por R. Domínguez y colaboradores. Se utilizó la técnica de inmovilización magnética, descrita y mencionada anteriormente por Akhtar Hayat y colaboradores, para aplicar sobre la superficie de un electrodo de trabajo de grafito. En este estudio con técnicas amperométricas se obtuvo un rango lineal de 190 – 25000 ng/L y un límite de detección de 150 ng/L (186 pM). Este sensor se validó en muestras reales de mejillones [67].

También se han reportado métodos de análisis diferentes a los métodos electroquímicos como es la cromatografía líquida, obteniendo los mejores límites de detección para el OA. Sin embargo, en comparación con los métodos electroquímicos es un método mucho más complejo, más lento y más costoso.

En los últimos años, se han llevado a cabo estudios para mejorar los límites de detección. Sevasti-Kiriaki Zervou y colaboradores realizaron un estudio basado en conseguir medir directamente en el agua de un lago la detección simultánea de varias clases de toxinas peligrosas. Para medir las muestras del agua del lago tratadas mediante extracción de fase sólida utilizaron la cromatografía líquida con espectrometría de masas. Consiguieron en el caso particular del OA un rango lineal entre 5 - 250 µg/L y un límite de detección de 10 ng/L (12.4 pM) [68].

Un año más tarde, Cristina Bosch-Orea y colaboradores obtuvieron un método de análisis para el agua de mar, centrada en los problemas de la industria alimentaria, con cromatografía líquida y un límite de detección de 0.3 ng/L (0.8 pM). En este estudio se

desarrolló el método para la detección de 10 tipos de biotoxinas marinas en agua de mar, una de ellas el OA. Para esta toxina se necesitó una filtración inicial de la muestra y un pretratamiento bastante tedioso, lo que implicaría una incapacidad como análisis rápidos, rutinarios o en continuo. Finalmente se consiguió un rango lineal entre 0.5 - 50 ng/L [69].

En esta tesis se buscan métodos de medición electroquímicos para medir directamente en agua de mar OA y que se puedan conseguir resultados tan satisfactorios como los que se obtienen con técnicas de cromatografías.

3.4.-Referencias

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4.- Fundamentos teóricos de la tecnología de membranas

4.1.- Tecnología de membranas

Las tecnologías de membranas son tecnologías limpias utilizadas para la separación de componentes en fluidos. Consisten en la permeación selectiva de uno o varios componentes a través de una membrana por acción de una fuerza impulsora. Las tecnologías de membranas tienen ventajas sobre otras técnicas de separación y purificación por requerir menor consumo de energía, un procesamiento sostenible en condiciones suaves de operación y presentar una gran simplicidad en la forma de operar [1]. Estos procesos dependen de la naturaleza de las membranas, la cual aporta características de filtrado específicas que se requieren en cada proceso. La naturaleza de la membrana depende de los materiales y del proceso utilizado en su fabricación, afectando a la carga superficial, a la morfología de la membrana, a la hidrofobicidad, y en su caso, al tamaño de los poros y a su distribución [2].

Una membrana es una barrera selectiva que separa dos fases fluidas, puede ser porosa o densa (sin poros) y permite la transferencia de determinados componentes de una fase a otra por acción de una fuerza impulsora. Esta fuerza impulsora, dependiente del proceso de separación, puede ser un gradiente de potencial químico y/o eléctrico. En la Fig. 4.1 se representa el diagrama de flujo de un proceso de separación con membranas. En un proceso convencional, una corriente continua que alimenta el módulo de membrana con flujo tangencial, que se escinde en dos corrientes fluidas que lo abandonan, la de retenido enriquecida en las especies que no son capaces de atravesar la membrana y la de permeado que contiene las especies capaces de permearla [3,4].

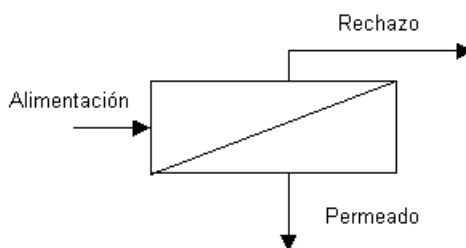


Figura 4.1. Diagrama de flujo de un proceso de separación con membrana.

La clasificación más habitual de las membranas atiende a su naturaleza. Las membranas pueden ser sintéticas y biológicas. Las biológicas son esenciales para la vida y pueden ser vivas y no vivas. Sus campos de aplicación están enfocados a los campos médicos y farmacéuticos. En general, las membranas sintéticas tienen mayor aplicación en el campo de la industria y en el uso de la filtración de agua.

Las membranas sintéticas se pueden clasificar a su vez en poliméricas u orgánicas y cerámicas o inorgánicas. Las membranas orgánicas son relativamente fáciles de fabricar, presentan un extenso rango de tamaño de poro y han sido ampliamente utilizadas. El inconveniente que presentan son las limitaciones en las condiciones de operación (pH, temperatura, presión, sensibilidad al cloro...).

Las membranas inorgánicas, al contrario que las orgánicas, destacan por su elevada resistencia mecánica y estabilidad química y térmica. Como consecuencia de sus propiedades, presentan amplios límites operacionales de temperatura, pH y presión, lo que repercute en una mayor vida útil [5]. Sus mayores limitaciones son la fragilidad ante golpes y vibraciones, y su mayor coste económico, lo que condiciona su disponibilidad en el mercado a los procesos más utilizados como la ultrafiltración (UF) y la microfiltración (MF) [3,6].

Aunque existen una variedad de procesos de separación con membranas, los más conocidos y ampliamente utilizados son los que necesitan como fuerza impulsora una diferencia de presión transmembranal. Entre ellos están la microfiltración (MF), ultrafiltración (UF), nanofiltración (NF) y osmosis inversa (RO). La separación alcanzada con estas membranas depende del tamaño de poro de la membrana y de su morfología, por lo que su uso dependerá de las necesidades específicas de separación que se requieran en cada caso. Los mecanismos que actúan en la separación de componentes, en cada uno de estos procesos, son de diferente naturaleza. Los principales mecanismos son el principio de exclusión por tamaños en el caso de membranas porosas y la diferencia de los distintos componentes en la solubilidad y difusividad en el caso de

membranas no porosas. Otros factores que pueden ser relevantes en la separación son las interacciones electrostáticas entre los solutos o con la membrana y la afinidad química [6]. En la siguiente figura se puede observar los rangos del tamaño de poro de las membranas con su respectivo proceso de aplicación.

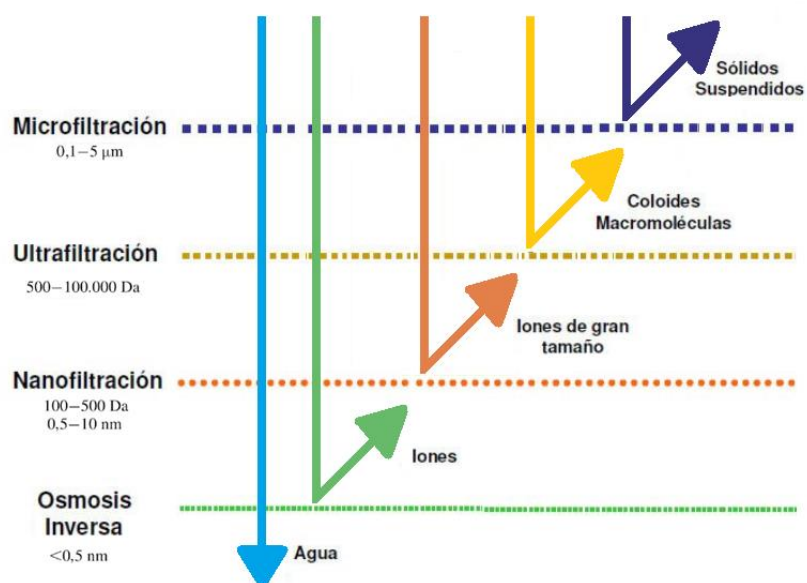


Figura 4.2. Representación de los rangos de aplicación de los procesos de separación con membranas impulsados por un gradiente de presión transmembranal.

Los procesos de separación con membranas presentan dos parámetros importantes a tener en cuenta: la eficiencia y productividad. La productividad está relacionada con la velocidad de permeación de los componentes a través de la membrana. La densidad de flujo de permeado referida a un componente, J_i , es la velocidad de transporte de materia de ese componente a través de la membrana, expresada por unidad de superficie de la membrana [6]. Cuando son varios los componentes que atraviesan la membrana, la densidad de flujo total de permeado, J_T es la suma de la de los diferentes componentes y se puede expresar de la siguiente manera:

$$J_T = \sum_i^n J_i \quad (1)$$

La eficiencia de separación está relacionada con la calidad del permeado como consecuencia del efecto de la membrana sobre cada componente. El rechazo de la membrana por un componente R_i , se determina por el grado de reducción en la concentración de dicho componente, calculado por la Ec. 2:

$$R_i = 1 - \frac{C_{p,i}}{C_{r,i}} \quad (2)$$

Donde $C_{p,i}$ y $C_{r,i}$ es la concentración del componente de interés en el permeado y en el retenido respectivamente.

En general, ambos parámetros J_i y R_i están afectados por la concentración, las características del medio, y por las condiciones de operación.

Los procesos con membrana pueden operar con flujo perpendicular a la membrana (dead-end flow) o con flujo tangencial (cross flow). En el primero, se produce la acumulación continua de solutos sobre la superficie de la membrana, llegando un momento en que el espesor de la torta generada impide de forma fehaciente el proceso de filtración. Por el contrario, el flujo tangencial limita el crecimiento de la torta sobre la membrana, de tal forma que durante largos periodos de tiempo se pueden alcanzar condiciones estables sobre la superficie de la membrana proporcionando flujos de permeado constantes.

4.2.- Polarización por concentración

La polarización por concentración es el fenómeno causado por la acumulación de solutos en la superficie de la membrana cuando se opera con flujo tangencial. Aunque es un fenómeno inherente a todos los procesos con membrana, su efecto es relevante en electrodiálisis y en los procesos impulsados por una diferencia de presión transmembranal, especialmente en microfiltración y ultrafiltración. En la cara del

retenido, las moléculas están sometidas a dos fuerzas, la proporcionada por la bomba de alimentación que induce un movimiento tangencial a la superficie de la membrana, y la proporcionada por la diferencia de presión transmembranal que produce un flujo convectivo de fluido hacia la membrana. Las moléculas que por distintas causas quedan retenidas por la membrana forman una capa estancada de solutos acumulados que, en condiciones de estado estacionario, alcanza un espesor constante. Esta capa estancada se la conoce como capa de polarización por concentración y supone una resistencia a la permeación que limita el flujo de permeado. También se la conoce como resistencia reversible, ya que su efecto se anula al cesar la fuerza impulsora para la permeación [6,7].

En la Fig. 4.3 se puede ver una descripción esquemática del proceso de polarización por concentración.

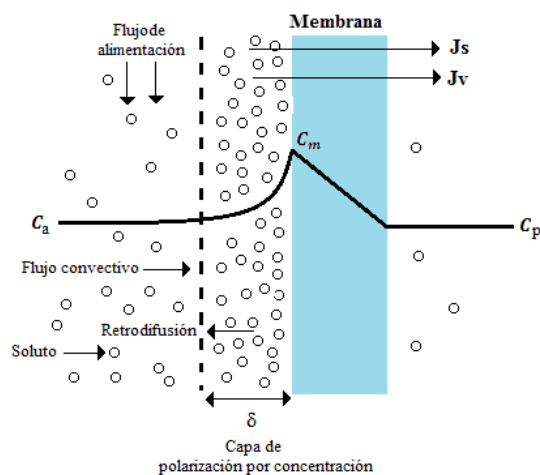


Figura 4.3. Descripción esquemática de la capa de polarización por concentración.

En el estado estacionario el espesor de la capa estancada o capa límite, y la densidad de flujo de permeado alcanza valores constantes. Esta situación se produce debido al hecho de que en la capa estancada se genera un flujo difusivo de materia hacia el seno del retenido como consecuencia de la existencia de un gradiente de concentración, expresado por la ecuación de Fick. El estado estacionario se alcanza cuando el flujo neto

de un soluto hacia la membrana, es decir el flujo convectivo hacia la membrana menos el flujo difusivo en sentido opuesto, es igual al flujo de permeado de dicho componente a través de la misma. El balance de materia en la capa de polarización queda expresado mediante la siguiente expresión:

$$J_v C + D \frac{dC}{dX} = J_v C_p \quad (3)$$

Donde J_v es la densidad de flujo y D es el coeficiente de difusión. La integración de la Ec. 3 entre los límites de la capa límite, $x=0$, $C=C_m$ y $x=\delta$, $C=C_p$, conduce a la Ec. 4:

$$J_v = K \cdot \text{Ln} \left[\frac{C_m - C_p}{C_r - C_p} \right] \quad (4)$$

La Ec. 4 representa la relación entre J_v y las concentraciones del componente de interés en la membrana (C_m), el permeado (C_p) y el retenido (C_r), siendo $K=D/d$ el coeficiente de transferencia de materia en la capa de polarización, que se puede calcular utilizando ecuaciones de correlación que relacionan K con las condiciones hidrodinámicas en la superficie de la membrana.

La acumulación de solutos en la capa de polarización conlleva a que el rechazo real o intrínseco ejercido por la membrana sea superior al valor observado (Ec. 2). Se define el rechazo real o intrínseco mediante la siguiente expresión:

$$R_i^* = 1 - \left(\frac{C_{p,i}}{C_{m,i}} \right) \quad (5)$$

$C_{m,i}$ es un valor difícil de determinar experimentalmente, pero puede ser estimado utilizando el modelo de polarización por concentración (Ec. 4).

La relación entre R_{obs} y R^* viene expresado por la Ec. 6

$$R^* = \left\{ 1 + \left[\frac{1 - R_{obs}}{R_{obs}} \right] \exp(-Pe) \right\}^{-1} \quad (6)$$

El número de Peclet expresa la relación entre el flujo convectivo y el flujo de retrodifusión $Pe = J \cdot \delta / D$. Bajo las condiciones donde la polarización por concentración no es importante, $R_{obs} = R^*$. Esta situación corresponde a baja presión transmembranal, elevada velocidad tangencial y solutos de pequeño tamaño donde $C_{m,i}$ está próximo a $C_{r,i}$. En el resto de las situaciones donde la polarización por concentración domina el comportamiento de la membrana, $R^* > R_{obs}$. En el diseño de las plantas industriales se deben de tener en cuenta todos estos aspectos.

En un proceso de filtración en membranas, la densidad de flujo de permeado, J_v , está relacionada con la fuerza impulsora del proceso, expresada como la diferencia entre la presión aplicada, ΔP , y la diferencia de presión osmótica a ambos lados de la membrana, $\Delta \pi$. Esta relación puede expresarse mediante la siguiente ecuación:

$$J_v = \frac{[\Delta P - \Delta \pi]}{\mu \cdot \sum R_i} = \frac{\Delta P - \Delta \pi}{\mu_T (R_m + R_p + R_b + R_f)} \quad (7)$$

La Ec. 7 representa un modelo de resistencias en serie, en donde se considera que la resistencia total (R_{tot}) a la permeación es la suma de todas las resistencias individuales que afectan al proceso, como son las resistencias impuesta por la membrana limpia (R_m), la capa de polarización por concentración (R_p), la torta formada sobre la superficie de la membrana (R_b), el bloqueo parcial de los poros o cualquier otro mecanismo de ensuciamiento irreversible como puede ser la adsorción en la superficie de los poros (R_f).

Cuando se trata de solutos de elevado peso molecular en soluciones diluidas, la diferencia de presión osmótica a ambos lados de la membrana es despreciable.

En el caso de filtración de agua, la ecuación anterior se puede simplificar como:

$$J_w = L_p \Delta P \quad (8)$$

L_p es la permeabilidad al agua y se calcula experimentalmente a partir de la pendiente que relaciona J_w con ΔP .

L_p se puede expresar matemáticamente de la siguiente manera:

$$L_p = \frac{1}{\mu \cdot R_m} \quad (9)$$

Las Ec. 8 y 9, donde μ es la viscosidad del agua y R_m es la resistencia hidráulica de la membrana, ayudan a explicar los comportamientos típicos observados en filtración. La resistencia de la membrana es constante y no depende de la composición de la alimentación, ni de la presión aplicada. Sin embargo, cuando hay solutos en disolución el comportamiento observado es totalmente diferente y se puede observar en la Fig. 4.4. En esta figura se puede observar que el flujo depende directamente de la presión, es decir, que cuando la presión es aumentada el flujo crece linealmente. En cambio cuando la disolución contiene solutos se llega a un valor límite donde el flujo de permeado deja de aumentar y la Ec. 7 no se cumple. A partir de este momento, el mecanismo que controla el proceso de filtración en membranas es la transferencia de materia en la capa de polarización. La resistencia impuesta por la capa de polarización por concentración depende de las condiciones hidrodinámicas sobre la membrana, velocidad de la alimentación, temperatura, y por la concentración de solutos en la alimentación. Si la presión permanece constante y se aumenta el flujo de alimentación, y/o las turbulencias, y/o la temperatura, el flujo límite de permeado aumentará. Por el contrario, el aumento de la concentración de solutos en la alimentación disminuirá el flujo límite de permeado [7].

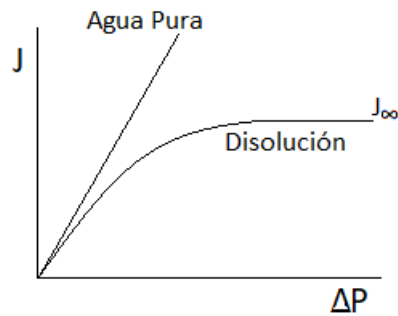


Figura 4.4. Representación del flujo como función de la presión aplicada para agua pura y una disolución.

4.3.- Ensuciamiento de la membrana

El ensuciamiento de la membrana es el principal causante de la disminución del flujo de permeado y la mayor limitación en el uso práctico de las tecnologías de membrana. El ensuciamiento puede deberse a la deposición de partículas retenidas, coloides, emulsiones, suspensiones, sales, etc sobre o dentro de la membrana. El ensuciamiento va produciendo una reducción continua del área activa de la membrana y no se puede eliminar simplemente con la parada del proceso de filtración, sino que requiere un proceso de limpieza, generalmente mediante el uso de reactivos químicos o biológicos. La consecuencia de esta reducción del área activa es la disminución en el flujo del permeado por debajo del flujo constante teórico de la membrana y el aumento de la energía consumida y los costes del proceso de filtración [6,7].

Los diferentes parámetros que pueden influir en el ensuciamiento de las membranas son la composición del fluido y la concentración de los solutos, el tipo de membrana, el tamaño de los poros y las características de la membrana como el material o la superficie activa. Los fenómenos de ensuciamientos conocidos son la adsorción, la interacción química con la membrana, la formación de la torta y el bloqueo total o parcial de los poros.

Generalmente, cuando se habla de ensuciamiento se hace referencia a cuatro tipos de mecanismo que se pueden observar. Estos mecanismos se conocen como el bloqueo completo, interno y parcial de los poros y la formación de la torta. El ensuciamiento interno de la membrana reducirá el paso del soluto a través de la membrana ya que sus poros se estrechan o se bloquean. Por lo tanto, en los procesos de separación con membranas es importante el estudio del ensuciamiento para llevar a cabo un uso inteligente de esta tecnología, procurar un ahorro de energía y un uso eficaz [6,8].

Para el estudio del ensuciamiento se han utilizado muchos parámetros con los que poder describir, predecir y entender el fenómeno de ensuciamiento como por ejemplo el índice de sedimentación, el índice de conexión, el índice de densidad del sedimento (SDI) y el índice modificado de ensuciamiento (MFI), uno de los más utilizados por su rapidez y sencillez es el SDI.

4.3.1.- Índice de densidad del sedimento (SDI)

Este índice SDI (silt density index) se basa en la medida de la velocidad a la cual una membrana de 0,45 μm de tamaño de poro se bloquea bajo condiciones de presión constante de 2 bar. La filtración se debe realizar en modo de paso único (“dead-end”) y es considerada una prueba empírica para caracterizar el potencial de ensuciamiento de una membrana por un fluido. Su valor suele estar referido a un tiempo de filtración de 15 min, y en este caso (SDI_{15}) se determina mediante la siguiente ecuación:

$$SDI = \frac{\left(1 - \frac{t_i}{t_f}\right) \cdot 100}{15} \quad (10)$$

Donde t_i representa el tiempo necesario para recoger los primeros 500 mL de permeado, y t_f el tiempo final necesario, una vez transcurridos 15 min de filtración desde el comienzo de la experiencia, para recoger otros 500 mL de permeado. Si $t_f \gg t_i$, el SDI

será alto y como consecuencia el potencial de ensuciamiento para el bloqueo de los poros también será alto [9].

El SDI se aplica de forma rutinaria en todo el mundo por ser un ensayo rápido, sencillo y barato, principalmente en el tratamiento de agua con membranas de osmosis inversa y de nanofiltración. Sin embargo, hay dudas de su fiabilidad en el tratamiento con membranas de ultrafiltración, ya que el agua filtrada no reúne la calidad esperada. Las dudas que surgen a cerca de este índice radican en la incapacidad de capturar partículas pequeñas menores de $0,45\ \mu\text{m}$ y que pueden constituir la principal causa del ensuciamiento de las membranas [9].

Además, esta prueba no se basa en ningún tipo de mecanismo de ensuciamiento, no se corrige con la temperatura, ni con la presión, ni con las propiedades de la membrana y no existe relación lineal entre el SDI y la concentración de la materia en suspensión [10].

4.3.2.- Índice de ensuciamiento modificado (MFI)

El MFI (modified fouling index) fue desarrollado por Schippers and Verdouw [11] y está basado en el mecanismo de filtración en torta. En la Fig. 4.5 se representa un proceso convencional de filtración en torta en donde se observan tres zonas diferenciadas. La primera corresponde a la etapa inicial en la cual se producen los fenómenos de adsorción y bloqueo parcial de los poros, simultáneamente también se establecen condiciones estables sobre el medio de filtración. La segunda etapa es una zona lineal donde se produce la filtración en condiciones estacionarias, es decir, la estructura de la torta porosa y su porosidad permanecen constante. Por último, se observa un perfil ascendente que se corresponde con un aumento rápido de la resistencia a la filtración asociada a la compresibilidad de la torta, es decir a la disminución de su porosidad por efecto de la presión al aumentar su espesor.

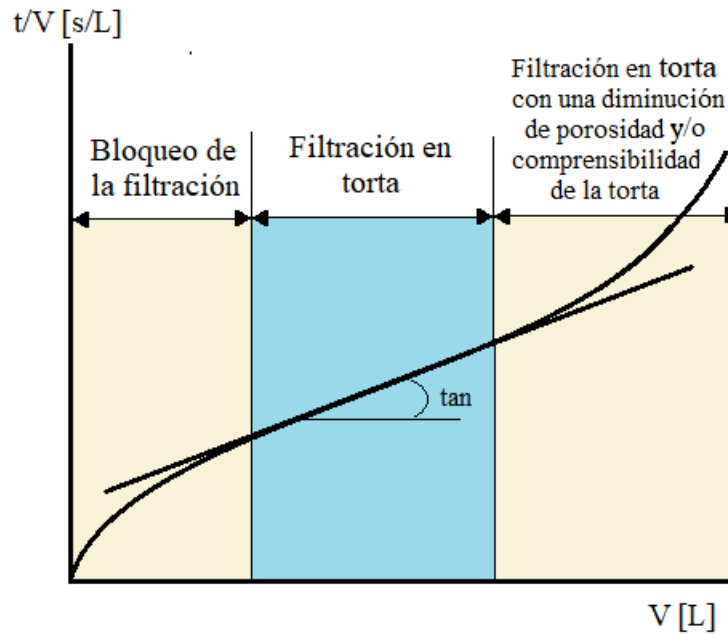


Figura 4.5. Filtración en torta. (Imagen tomada de Boerlage et al., 2003[12])

El MFI es un índice que representa el gradiente de la región lineal obtenida en una representación t/V frente a V en un proceso de filtración a PTM constante con una membrana de $0,45 \mu\text{m}$ en modo de filtración de paso único [11,12]. El MFI se calcula mediante la Ec. 11.

$$\frac{t}{V} = \frac{\mu_T R_m}{\Delta P \cdot A} + \frac{\mu_T I}{2\Delta P \cdot A^2} V \quad (11)$$

Donde, ΔP es la presión transmembranal, μ_T es la viscosidad del agua a temperatura T , A es el área activa de la superficie de la membrana, V es el volumen filtrado y R_m es la resistencia de la membrana a la permeación del agua limpia a temperatura T . El índice del potencial de ensuciamiento, I , es una función que depende del tamaño y naturaleza de las partículas y está directamente relacionado con su concentración en el agua según la expresión $I = \alpha \cdot C_b$ en el caso de que la torta sea incomprensible y $I = \alpha \cdot C_b \cdot (\Delta P)^w$ en el caso de que la torta sea comprensible. Se puede ver que depende de la resistencia específica de la torta (α), de la concentración de partículas en el agua de mar (C_b), y en

el caso de tortas comprensibles de la presión transmembranal elevada a un número que se corresponde con el factor de comprensibilidad (w) [12,13].

El MFI consigue solventar algunas deficiencias frente al SDI, pero es mucho más largo y costoso de calcular. Además, en determinadas situaciones se observó un ensuciamiento superior al arrojado por el MFI, debido a la afectación del ensuciamiento por la presencia de las partículas coloidales más pequeñas que no se consideran en el MFI [13].

El MFI-UF es una modificación del MFI basado en la filtración en torta con la incorporación en el modelo del ensuciamiento causado por las partículas más pequeñas, responsables frecuentemente del ensuciamiento de las membranas de microfiltración, y que son retenidas por una membrana de ultrafiltración. Para que en el MFI-UF se obtengan datos satisfactorios y de confianza se deben de cumplir unos requisitos. En primer lugar el MFI-UF debe estar estable por un tiempo y en segundo lugar el índice del MFI-UF debe presentar un comportamiento lineal con la concentración de partículas en la alimentación. Ambos requisitos contribuyen a la reproducibilidad del MFI-UF.

El MFI-UF se puede determinar gráficamente, representando (t/V) vs (V) y obteniéndose el valor de la pendiente en la región lineal, que corresponde al mecanismo de formación de la torta. El MFI-UF depende de la temperatura y la presión; por eso, en la siguiente expresión (Ec. 12) se utilizan parámetros para corregir el efecto de las condiciones experimentales para la presión transmembranal y la temperatura, en el valor del índice:

$$MFI - UF = \frac{\mu_0}{\mu} \frac{\Delta P}{\Delta P_0} \frac{d\left(\frac{t}{V}\right)}{d(V)} = \frac{\mu_0}{\mu} \frac{\Delta P}{\Delta P_0} \frac{\mu_T I}{2\Delta P \cdot A^2} \quad (12)$$

donde subíndice 0 se refiere a las condiciones estándar de 20 °C y 2 bar [13,14].

4.4.- Tipos de filtración

4.4.1.- Microfiltración

Los esfuerzos de investigación sobre la utilización de MF se han centrado en la eliminación de partículas coloidales, colorantes, materia orgánica y otros contaminantes de alto peso molecular procedentes de las corrientes de desecho [2]. Las membranas que se utilizan en estos procesos presentan un tamaño de poro entre 0.1 a 5 μm y trabajan con presiones bajas entre 0.1 - 2 bar [3].

Las membranas de MF más utilizadas son las poliméricas (poliamida, polisulfona, polipropileno...), aunque cada vez se utilizan más las inorgánicas, fundamentalmente de tipo cerámica (ZrO , TiO_2). Estas últimas membranas destacan por su mayor resistencia térmica y química, lo que les confiere mayor vida útil [2].

Actualmente, es un proceso que se aplica con éxito a gran escala para tratar partículas en suspensión pero la eficiencia del proceso se ve afectada por la deposición de partículas, la polarización por concentración y el ensuciamiento [15].

4.4.2.- Ultrafiltración

La UF utiliza membranas porosas, con un tamaño de poro entre 0.01 y 0.1 μm y, al igual que la MF, se realiza a baja presión (2 – 5 bar) [3].

Las membranas de UF pueden rechazar partículas coloidales, macromoléculas, biopolímeros y virus. La UF se ha utilizado ampliamente para el tratamiento de aguas residuales, la recuperación de surfactantes en soluciones de limpieza industrial, la separación de proteínas, la formación y concentración de emulsiones, la eliminación de grasa, y otras aplicaciones en el procesado de alimentos [2].

Las membranas de UF son fabricadas con materiales inorgánicos como las de MF (TiO_2 , Al_2O_3 , ZrO , etc, derivados de celulosa y típicos polímeros como el poliacrilonitrilo, polisulfonaamida, poliétersulfona, el polivinilideno, etc) [2].

4.4.3.- Nanofiltración

La NF es una tecnología de separación con membranas intermedias entre la ultrafiltración (UF) y la ósmosis inversa (OR). La NF tiene sus inicios en torno a 1970 con el desarrollo de la OR a baja presión (5 - 20 bar), pero ya en la década de 1980 se establece como un proceso con identidad propia y desarrollan sus primeras aplicaciones [16]. Las membranas utilizadas en este proceso tienen un corte molecular entre 0.001 - 0.01 μm , además poseen una ligera carga superficial que incrementa la selectividad a los diferentes iones. Aprovechando esta última propiedad, la NF se utiliza frecuentemente en la eliminación de compuestos iónicos, proporcionando flujos semejantes a los de la ósmosis inversa pero requiriendo menor presión. Es una técnica que se utiliza en numerosas aplicaciones como en el tratamiento de aguas, en la separación de iones y componentes de bajo peso molecular de muy diversa naturaleza en la industria alimentaria y en la industria farmacéutica [1,17–19].

La complejidad de las interacciones entre los solutos y la membrana justifica la necesidad de realizar una selección adecuada de la membrana de NF para cada situación particular y, por tanto, la necesidad de realizar una adecuada caracterización de la misma. El uso de modelos predictivos del rechazo de componentes es importante para la optimización de los procesos y para el cambio de escala.

Los mecanismos de transportes que intervienen en la permeación de componentes en NF pueden ser de diferente naturaleza. La primera opción que se estudió para determinar el transporte de iones a través de una membrana de NF se basa en la ecuación extendida de Nernst-Planck, mediante ecuaciones diferenciales para cada pareja de iones que debe resolverse con la doble condición de electro-neutralidad en los flujos y en la

concentración local. Sin embargo, este modelo contiene coeficientes que deben ser calculados usando otros modelos termodinámicos y cinéticos de exclusión de iones que representen convenientemente el transporte dentro de la membrana [20].

Una segunda opción es el modelo de nano-poros, que es una versión simplificada del conocido modelo de carga superficial (el principio de exclusión de Donnan). Este principio demuestra que las cargas eléctricas fijas en los poros de la membrana pueden dar lugar a rechazos de solutos cargados en los procesos de membrana impulsados por presión, como es el caso de la NF. Con el tiempo se le han incorporado nuevos parámetros, como por ejemplo los asociados a efectos estéricos y a las interacciones dieléctricas. Además, la descripción del equilibrio y el transporte en este modelo se basa en un gran número de parámetros experimentales que conllevan una elevada imprecisión [21–23].

El modelo de disolución-difusión (SDM) surge como una alternativa a los anteriores modelos, bajo la concepción de que el transporte de iones a través de la membrana se produce por un mecanismo de solución-difusión. El SDM se ha utilizado exitosamente para describir el transporte de iones en membranas de OR, pero no el de especies no cargadas como el boro en agua de mar [24,25]. El SDM ha sido aplicado posteriormente para describir también el transporte de iones a través de membranas en NF [26–28] mejorando la descripción dada por el modelo de nanoporos en la selectividad sulfato / cloruro [29]. Se ha demostrado que el SDM es aplicable a sales individuales y a solutos no electrolitos, incorporando con alguna modificación para considerar al efecto de la concentración. Sin embargo, en el caso de mezclas de electrolitos se deben tener en cuenta los acoplamientos entre los flujos transmembranales de los diferentes iones como consecuencia del campo eléctrico generado por el potencial de membrana. Además del acoplamiento entre los flujos en la membrana, existen también acoplamientos entre los flujos de los diferentes iones dentro de la capa de polarización por concentración, generada en la superficie de la membrana por efecto de la presión transmembranal aplicada. Todas estas consideraciones han sido incluidas en el modelo de película-

solución —difusión (SDFM). Por lo tanto, el SDFM asume que el transporte de soluto a través de la membrana se produce por un mecanismo combinado de solución — difusión y migración eléctrica, y en la capa de polarización por concentración, además de los mecanismos anteriormente citados, hay que considerar el transporte de materia por convección [20,30].

4.5.- Símbolos

Símbolos	Definición
A	Área de la superficie activa de la membrana
C_b	Concentración de partículas en el agua de mar
C_m	Concentración en la membrana
$C_{F,i}$	Concentración del compuesto de interés en la alimentación
$C_{P,i}$	Concentración del compuesto de interés en el permeado
C_R	Concentración del compuesto de interés en el rechazo
D	Coefficiente de difusión
I	Índice del potencial de ensuciamiento
J_i	Densidad de flujo del permeado a un componente
J_T	Densidad de flujo total
J_V	Densidad de flujo
J_w	Densidad de flujo del agua
L_p	Permeabilidad al agua de una membrana
R_i	Rechazo de cada componente
R_m	Resistencia hidráulica de la membrana
R^*	Resistencia intrínseca
R_T	Resistencia total es las suma de todas las resistencias del proceso
T	Temperatura
t_i	tiempo de filtración inicial
t_f	Tiempo de filtración final
V	Volumen total
w	Factor de comprensibilidad
Letras griegas	Definición
ΔP	Diferencia de la presión aplicada

$\Delta\pi$	Diferencia de presión osmótica a ambos lados de la membrana
μ	Viscosidad del agua
α	Resistencia específica de la torta
δ	Capa de polarización por concentración

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5.- Fundamentos teóricos de la electroquímica

5.1.- Métodos electroquímicos de análisis.

Un sensor químico es un dispositivo que transforma información química en una señal analítica útil sobre una especie química [1,2]. Los sensores químicos buscan presentar unas características ideales de sensibilidad, selectividad, estabilidad, precisión, facilidad de uso y bajo coste sobre especies de interés. Conseguir cumplir todas las características ideales son retos que se persiguen en química analítica para el desarrollo de sensores mejorados que presenten mejores prestaciones.

Entre los diferentes tipos de sensores, los electroquímicos tienen una especial relevancia en la determinación de la presencia y concentración de una o varias especies químicas en muestras problemáticas [1,3–5]. Su interés reside en su capacidad de proporcionar una información fiable sin interferencias generadas por otros componentes de la muestra. Además, presentan otras ventajas analíticas tales como la velocidad de análisis, la posibilidad de su miniaturización y su bajo coste. Las propiedades más destacables de estos sensores son la sensibilidad, la estabilidad y la selectividad que pueden alcanzarse mediante la optimización de las condiciones de trabajo como la temperatura, el pH, la presión y el entorno químico [5].

Las medidas electroquímicas, en que se basan este tipo de sensores, relacionan magnitudes eléctricas tales como intensidad, potencial o carga, con parámetros químicos de interés en un análisis químico [1,2]. El uso de este tipo de medidas en el área de la química analítica presentan un rango muy amplio de aplicación dentro del medio ambiente, en los controles de diferentes industrias o en los análisis relacionados con biología y medicina.

Las medidas electroquímicas se llevan a cabo en un montaje muy simple llamado celda electroquímica, que consta de al menos dos electrodos (conductores) y una disolución con el analito de interés (electrolito). Uno de los electrodos que se introducen en la celda electroquímica tiene un potencial constante, y se conoce como electrodo de

referencia, y el otro, llamado de trabajo o indicador responde al analito objeto de estudio. La superficie del electrodo constituye el punto de contacto o interfase entre el conductor iónico, la disolución, y el conductor electrónico, el electrodo [1]. El mecanismo más habitual de los sensores electroquímicos consiste en la medición de los cambios en las intensidades de corrientes y/o potenciales eléctricos que se producen como consecuencia de reacciones electrolíticas selectivas o interacciones químicas en la superficie del electrodo [3].

Los sensores electroquímicos se clasifican en potenciométricos, voltamperométricos, amperométricos o de impedancia dependiendo de la propiedad electroquímica que se quiera medir.

A continuación se describirán las técnicas voltamperométricas utilizadas en el desarrollo de este trabajo.

5.2.- Voltamperometría

La voltamperometría de barrido se basa en la variación controlada del potencial al que se somete al sistema, registrando la intensidad generada conforme se va produciendo la reacción electroquímica. En términos generales, los sensores voltamperométricos se basan en la aplicación de una señal de excitación en el electrodo de trabajo dentro de la celda electroquímica. La señal es un potencial variable que provoca una intensidad de corriente como respuesta característica de este método. La información proporcionada por la intensidad corresponde a la sustancia que se oxida y se reduce, además de aportar un valor de potencial característico del proceso de estudio, lugar donde aparece el pico característico en el voltamperograma.

El tipo de voltamperometría depende de la forma en que varía el voltaje de la fuente de alimentación y de las propiedades físicas y electroquímicas de la celda [6]. Existen diferentes técnicas voltamperométricas debido a la gran variedad de formas en las que se

puede generar las señales con las que sometemos el electrodo de trabajo. Por tanto, estas técnicas se clasifican según el tipo de función voltaje-tiempo aplicado, como la voltamperometría lineal, cíclica, diferencial de impulsos y de onda cuadrada.

5.2.1.- Voltamperometría cíclica

Es una técnica electroquímica fundamental que puede ser utilizada en primer lugar en un estudio electroanalítico para determinar información cualitativa y cuantitativa sobre el sistema electroquímico [6]. Presenta una capacidad destacable para proporcionar una información considerable sobre la termodinámica de los procesos redox, la cinética de las reacciones heterogéneas de transferencia de electrones y sobre reacciones químicas acopladas o procesos de adsorción [1,7].

La voltamperometría cíclica (CV) consiste en aplicar al electrodo de trabajo un potencial que cambia linealmente con el tiempo y consigue una señal de excitación triangular. Esta señal consiste en un barrido lineal desde el potencial inicial (E_i) hasta alcanzar un valor de potencial final (E_f), después la dirección de barrido se invierte y vuelve al potencial inicial. El número de ciclos que se realizan depende de la información que se quiera buscar [7]. En la Fig. 5.1 se muestra gráficamente la señal de potencial que se aplica en esta técnica frente al tiempo y el resultado de un voltamperograma habitual. Este se caracteriza por la presencia de dos picos relacionados con la oxidación y reducción característicos del analito y cuyo potencial nos da información del proceso de estudio.

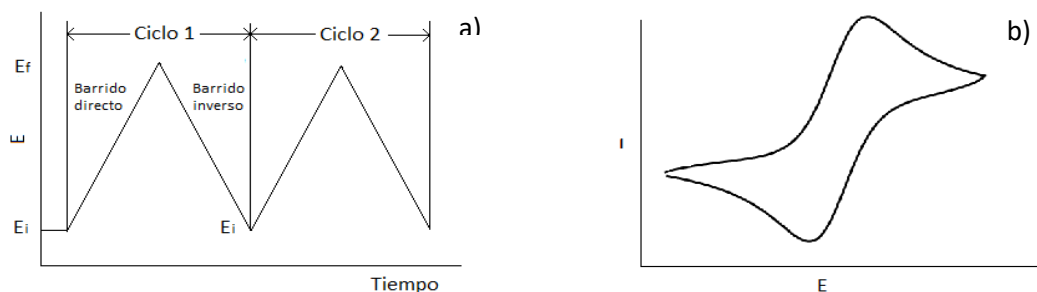


Figura 5.1. a) Potencial frente al tiempo de una señal triangular y b) voltamperograma de un ciclo obtenido del barrido de un potencial lineal.

5.2.2.- Voltamperometría diferencial de impulsos

Las técnicas voltamperométricas de pulsos se desarrollaron para mejorar los límites de detección de las técnicas voltamperométrica lineal y cíclica. La voltamperometría diferencial de impulsos (DPV) es una técnica muy sensible que se utiliza para medir trazas en análisis de especies orgánicas e inorgánicas.

En esta técnica se aplica al electrodo de trabajo pulsos superpuestos de potencial de magnitud fija sobre una rampa lineal. La corriente se muestrea dos veces, justo antes de aplicar el pulso y de nuevo una segunda vez al final de un tiempo suficientemente largo del pulso aplicado [4,8]. Se puede observar de forma esquemática en la Fig. 5.2 a como se aplican los pulsos de potencial frente al tiempo.

En esta técnica la diferencia de corriente que se muestrea se representa en función de la variación de potencial. El voltamperograma de la DPV, que se observa en la Fig. 5.2 b, presenta una señal en forma de pico cuya altura se corresponde con la intensidad de corriente y es directamente proporcional a la concentración del analito. La señal de respuesta está bien definida y proporciona una mejor información cuantitativa como consecuencia de la corrección de fondo y la eliminación de la intensidad capacitiva [8].

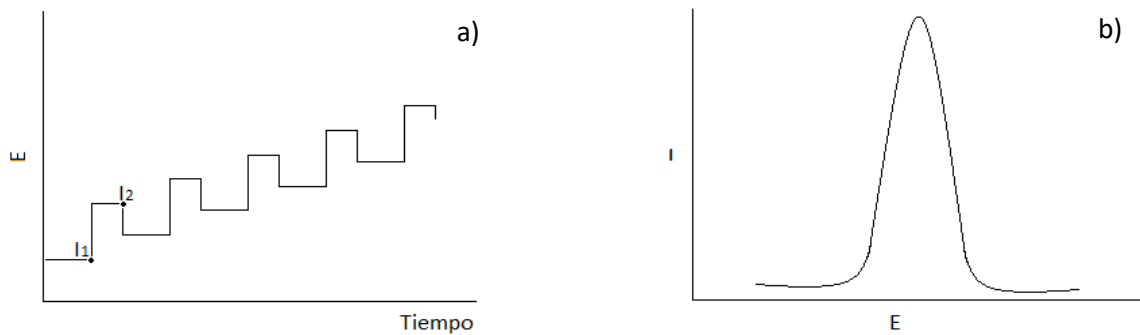


Figura 5.2. a) Pulsos de potencial aplicados en función del tiempo en una voltamperometría de diferencial de impulsos y b) Voltamperograma típico obtenido en DPV.

5.2.3.- Voltamperometría de onda cuadrada

En la voltamperometría de onda cuadrada (SWV) se aplica un potencial compuesto por ondas cuadradas superpuestas sobre una escalera de potencial (Fig. 5.3 a). La intensidad se muestrea dos veces durante cada ciclo completo de la onda cuadrada, es decir, primero se toma al final del impulso de ida y la segunda vez al final del impulso de vuelta [9].

Como la amplitud de modulación de la onda cuadrada es muy grande, los pulsos de vuelta provocan la reacción contraria del producto de los de ida. La diferencia entre las dos intensidades de corriente, tomadas en cada ciclo completo, se representa frente al potencial para dar lugar al voltamperograma típico de esta técnica. En la Fig. 5.3 b la señal obtenida tiene forma de pico simétrico y es proporcional a la concentración del analito [9,10].

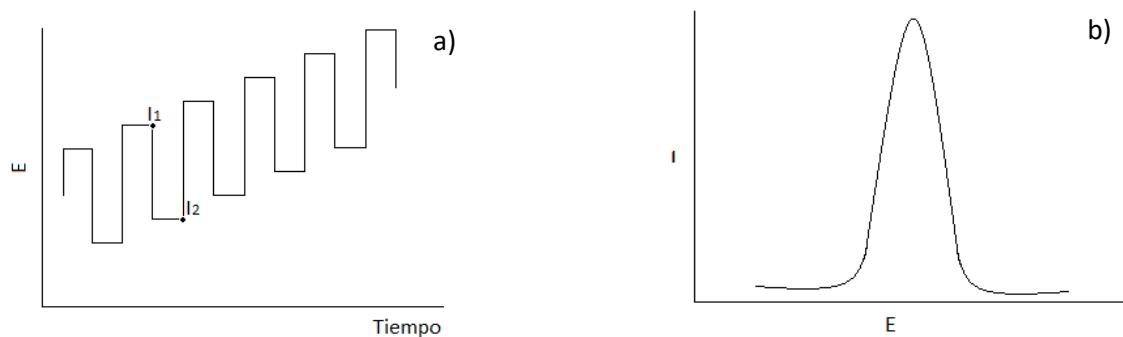


Figura 5.3. a) Pulsos de potencial aplicados en función del tiempo en una voltamperometría de onda cuadrada y b) Voltamperograma típico obtenido en SWV.

5.2.- Sensores serigrafiados

El uso de los sensores electroquímicos en nuevas y diferentes áreas ha requerido mejoras con respecto al uso de los electrodos sólidos convencionales. Por esta razón, estos sensores han buscado soluciones a problemas que se planteaban como por ejemplo la mala reproducibilidad para el desarrollo y comercialización de los electrodos, las tediosas tareas de limpiezas, el ajuste adecuado para diversas aplicaciones o la recuperación de la superficie tras su uso [11].

En los años 90, la aparición del uso de la tecnología de serigrafiado hizo frente a los problemas más destacados y se adaptó de manera rápida y precisa a la industria microelectrónica. Esta tecnología ha conseguido unos sensores muy reproducibles y de gran confianza, además de conseguir sensores desechables y con un bajo coste [12]. Como el uso de tecnologías capaces de medir “in situ” presenta un gran interés, la producción de sensores electroquímicos serigrafiados desechables de bajo coste y capaces de medir “in situ” ha experimentado un crecimiento enorme. Esta tecnología consigue sustituir a la tecnología convencional, de manera que se eliminan el uso de electrodos y celdas voluminosas y aparatosas [13–17].

Los sistemas electródicos serigrafiados (SPEs) son pequeñas tarjetas que normalmente están formados por tres electrodos impresos: trabajo, referencia y contraelectrodo que

entre sí forman un circuito eléctrico que a su vez es conectado a través de un circuito externo a un potencióstato [18]. Los electrodos son dispositivos que se producen por una impresión directa de diferentes tintas sobre diversos tipos de soportes (plásticos, cerámicas o telas). Los electrodos pueden ser impresos utilizando patrones diseñados por los analistas de acuerdo al propósito analítico que se busca. La flexibilidad y las diferentes tintas del diseño nos aportan grandes posibilidades en las geometrías de los electrodos y combinaciones de propiedades de diferentes materiales en una misma celda [18]. Estas posibilidades consiguen alta selectividad y sensibilidad para los sensores serigrafiados [11,15,19].

Los electrodos serigrafiados se imprimen capa por capa con tintas que se pueden dividir en conductoras y aislantes. Las tintas son depositadas sobre una superficie sólida con ayuda de una pantalla o malla que definen la geometría del sensor [19]. En primer lugar se depositan las tintas conductoras que están formadas por una resina en la que se dispersa un material conductor, como por ejemplo Ag/AgCl, oro, carbono, platino etc, para los distintos electrodos y vías. En el último lugar se depositan las tintas no conductoras que están hechas de materiales poliméricos o cerámicos, y forman la parte aislante del sensor, cuya finalidad es delimitar las áreas activas de los electrodos. Todas las tintas deben ser curadas a una temperatura y tiempo característicos, determinados por su composición. Los materiales utilizados como soportes para el electrodo serigrafiado deben ser inertes y de bajo coste económico para la producción de sensores rentables [18,20]. Las distintas etapas del proceso de fabricación en el caso de que el contraelectrodo y el electrodo de trabajo sean del mismo material se describen en la Fig. 5.4.

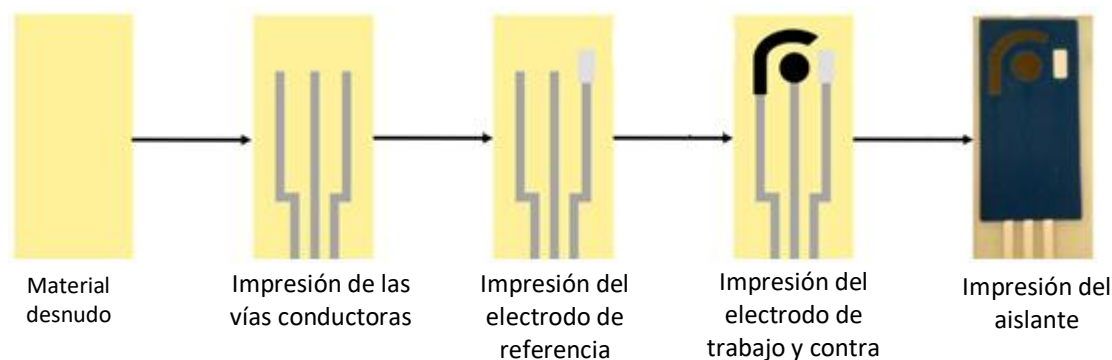


Figura 5.4. Esquema del proceso de la fabricación de un electrodo serigrafiado.

Gracias a todas las propiedades que poseen los electrodos serigrafiados han conseguido abrirse a numerosos campos de aplicación como en la medicina, en el medioambiente y en la industria [20–23].

La sociedad está en constante estado de crecimiento y desarrollo, por tanto es inevitable que se formen nuevas demandas para dispositivos clínicos o para aplicaciones en el campo industrial. Esto hace que esta tecnología en particular este en continuo crecimiento y en constante investigación [14].

5.2.1- Electrodo serigrafiados modificados

Una ventaja de los electrodos serigrafiados es la posibilidad de realizar modificaciones en el proceso de fabricación del electrodo, con una modificación de las tintas o sobre los electrodos una vez terminado el tratamiento de fabricación. Estos pequeños cambios consiguen propiedades esenciales que aumentan su rango de aplicación sin encarecer su coste. Las propiedades esenciales como el aumento de la selectividad y la sensibilidad del sistema sobre una especie de interés obtienen límites de detección muy bajos [18,24].

En la actualidad, varios tipos de nanomateriales son utilizados para mejorar las propiedades de construcción de los sensores y aumentar el rendimiento del sistema. En la fabricación de los SPEs se han conseguido grandes avances debido al uso de nanoestructuras como por ejemplo las nanopartículas, los nanotubos de carbono o las tintas con grafeno [18].

5.2.1.1.- Nanopartículas de oro

En los últimos años, los estudios de los materiales a nanoescala están presentando un alto crecimiento, particularmente con respecto a nanopartículas metálicas. El uso de nanopartículas para la modificación de los electrodos presenta una difusión mayor de transporte de masa, un área de superficie de trabajo mejorada, una selectividad mayor, una actividad catalítica, una mayor relación señal/ruido y unas propiedades ópticas únicas [25,26].

Los metales más utilizados en la síntesis de nanopartículas en la modificación de los SPEs son el oro y la plata, aunque también hay trabajos con nanopartículas con otros metales como el hierro, paladio, níquel, cobre, rutenio, iridio, etc. En el caso del oro hay muy buenos resultados por sus propiedades como material conductor y por su maleabilidad y ductilidad. Por tanto, el uso de nanopartículas de oro sobre la superficie del electrodo de trabajo en la modificación de SPEs es muy útil y eficaz, por la reducción de la cantidad necesaria de oro que ayuda en la disminución de los costes. Además de que las nanopartículas de oro son las nanopartículas metálicas más estables que presentan una gran habilidad de ensamblaje con múltiples tipos de materiales aportando mayor posibilidades de trabajo [26,27].

En la bibliografía se encuentran descritos diferentes métodos para la obtención de nanopartículas de oro sobre la superficie del electrodo. Los dos métodos más utilizados son la síntesis química mediante la reducción de NaBH_4 o citrato y la deposición

electroquímica. Esta última proporciona una alternativa fácil y rápida en la preparación del electrodo [15,26].

La electrodeposición consiste en la aplicación de una corriente eléctrica capaz de reducir los cationes de oro presentes en una disolución de HAuCl_4 que precipitan sobre la superficie del electrodo creando un recubrimiento. Este método es capaz de controlar el crecimiento de las nanopartículas, consiguiendo las propiedades más adecuadas para cada sistema [25,28,29].

5.2.1.2.- Película de óxido de grafeno

Desde el año 2004, el grafeno está ampliamente presente en las publicaciones científicas como consecuencia de un trabajo rerealizado por Geim y Novoselov, en el que se describe un método para la preparación de láminas individuales de grafeno [30].

El grafeno es considerado como nuevo material presentando superioridad sobre materiales tradicionales, por sus diferentes e interesantes propiedades mecánicas, térmicas y eléctricas. Las propiedades eléctricas están asociadas a la estructura bidimensional (2D) que presenta el grafeno y que ofrece una estabilidad a temperatura ambiente que le confiere propiedades electrónicas inusuales [30,31].

En el caso del óxido de grafito presenta una estructura donde los oxígenos expanden la distancia entre las capas, además de proporcionar hidrofiliidad. Como resultado de esta propiedad las capas oxidables se pueden exfoliar en el agua. Si las hojas exfoliadas contienen una o pocas capas de átomos de carbono como el grafeno, se denominan óxido de grafeno (GO) [31–33].

Las hojas de grafeno reducido se consideran un tipo de grafeno derivado químicamente. La propiedad más atractiva que presentan es la reducción de dichas hojas mediante la eliminación de los grupos que contienen oxígenos, de manera que se recupera la

estructura conjugada y es muy similar a las hojas de grafeno. Aunque no es correcto referirse a las formas reducidas (GO) como grafeno ya que las propiedades son totalmente diferentes debido a que la estructura del carbono realmente esta alterada [32,34]. El objetivo de la reducción es intentar conseguir materiales similares al grafeno para obtener las propiedades excepcionales eléctricas, térmicas y mecánicas [33].

Los GO posibilitan la disminución de los costos y tienen la propiedad de formar coloides acuosos estables para facilitar el ensamblaje de estructuras macroscópicas. Hay varias formas de producir la reducción de estas especies mediante la reducción térmica [35] y la reducción química [36]. En la reducción química se utilizan frecuentemente técnicas electroquímicas [32].

La electroreducción consiste en el uso de voltamperometría cíclica capaz de reducir el GO depositado sobre la superficie del electrodo de trabajo y formar un recubrimiento sobre la superficie. La reducción se puede lograr con un único ciclo y es un proceso irreversible. Esta técnica de reducción es rápida, sencilla y sin necesidad de utilizar agentes químicos. [33,37,38].

5.2.1.3.- Nanotubos de carbono

En la actualidad, varios tipos de nanomateriales se han utilizado para la modificación de la superficie de los electrodos y entre ellos se encuentran los nanotubos de carbono. Los nanotubos de carbono tienen propiedades físicas, eléctricas y químicas únicas. Los nanocompuestos basados en ellos han conseguido ampliar el campo de aplicación práctica [39–42].

Los nanotubos de carbono son láminas individuales de átomos de carbono enrolladas a la perfección, de solo unos pocos nanómetros de anchos [43]. Pueden estar compuestos por nanotubos de una sola capa (Single Wall Nanotube, SWNTs) o de varias capas (Multi Wall Nanotubes, MWNTs). Dependiendo de su estructura atómica, como el

ángulo de plegado o el diámetro del cilindro, los nanotubos pueden ser metálicos o semiconductores [42]. Por lo general, se organizan en un patrón regular que consiste en decenas o cientos de nanotubos en paralelos y en contacto entre sí [44].

Como consecuencia de las características únicas de estas estructuras, los nanotubos de carbono se utilizan para la modificación de la superficie del electrodo de trabajo y mejorar sus propiedades. Las propiedades electrónicas de los nanotubos de carbón tienen la capacidad de promover la transferencia de electrones cuando se usan como electrodos en reacciones químicas. Los sensores electroquímicos modificados con nanotubos ofrecen unos enfoques muy prometedores para el aumento de la respuesta electroquímica a los iones de metales pesados, superando de este modo los resultados obtenidos con otros electrodos de carbono [41,44].

Hay varios métodos para la modificación de los electrodos con nanotubos, pero una de las más simples consiste en la deposición de una gota sobre la superficie del electrodo. La película de nanotubos de carbono se ha creado una vez evaporada la gota a temperatura ambiente [45].

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6.- Application of the solution-diffusion-film model for the transfer of electrolytes and uncharged compounds in a nanofiltration membrane

En este capítulo se presenta un trabajo de validación del modelo de película –solución – difusión (SDFM) utilizando una planta piloto y un módulo de membranas en espiral con una membrana polimérica de 200 Da de límite de corte molecular. El SDFM describió correctamente el transporte de un único soluto, tanto sales como solutos neutros en solución, en concreto NaCl, KCl, MgCl₂, MgSO₄, Na₂SO₄, sacarosa, betaina ácido láctico y ácido cítrico, así como el de mezclas de electrolitos formadas por una sal dominante y otra sal en concentración a nivel traza.

El objetivos de este trabajo fue estudiar los principales fenómenos que acontecen en un proceso de filtración, (convección, difusión, polarización por concentración, potenciales eléctricos en la membrana y en la capa límite , flujos acoplados entre componentes, etc ...) y su repercusión más o menos significativa en el rechazo de componentes. El modelo SDFM que fue validado en este trabajo, es un modelo sencillo, capaz de explicar fenómenos complejos y cuantificar sus efectos en la permeación y el rechazo de componentes.

Este estudio se realizó de forma preliminar a la filtración de agua de mar y fue de gran utilidad en el diseño de experiencias y en la interpretación de resultados del trabajo que se describe en el siguiente capítulo.

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6.1.- Introduction

Nanofiltration (NF) is a pressure-based membrane separation technology which uses nanoporous membranes generally assumed to have a cut-off of 100–2000 Da [1]. These membranes are usually fabricated by depositing a thin polyamide film over a microporous polysulfone support layer reinforced by an underlying polymeric layer, which is usually made of a polyester web composite. The polyamide thin film shows an amphoteric behavior in which pH affects membrane performance by changing its potential, hydrophilicity and structure or chemistry [2].

NF membranes confer high selectivity in the separation of ions and low molecular weight compounds, operating with higher flux and lower pressures than reverse osmosis membranes. NF applications are of paramount importance and their uses worldwide have increased over the last 6 years. NF is applied especially in water, wastewater, and desalination treatments, with new applications in pharmaceuticals, biotechnology, food and non-aqueous separations, shedding light on the huge potential of NF for its future development. The detailed state-of-the-art can be consulted elsewhere [1]. Although NF requires a relatively low investment and has low operating and maintenance costs, membrane fouling is still a major drawback as it limits the operating competitiveness and cost effectiveness [3].

NF is a very complex process in which compound separation is affected by different properties such as the differences in size, modification of the Stokes radius, steric, electric and dielectric hindrance effects, and electroviscosity. Therefore, significant research efforts have been focused on developing and optimizing mathematical models that are able to predict NF membrane performance for compound separation. While the first black-box models were based on the thermodynamics of irreversible processes [4], they have since been replaced by more advanced models based on the extended Nernst–Planck equation, which provide information related to the properties of both the membrane and the process stream. These approaches involve mechanistic models, including the solution-diffusion model (SDM) and pore flow model (PFM). Among the

latter, the Donnan-steric-pore model (DSPM) takes into account the effect of finite pore size on both the diffusion and convection of species through narrow pores, and uses the equilibrium partitioning relationship to describe the distribution of species at the pore inlet and outlet. More recent models take into account dielectric exclusion by ion solvation phenomena. However, the DSPM and related models based on steric and electric exclusion mechanisms fail to describe the high rejections observed in the ionic solutions with divalent counterions [5]. In addition, many models require a knowledge of characteristic parameters, such as pore radius and membrane charge, that are not readily measured for nanoporous membranes.

The SDM has appeared as the best alternative to the other models over the past 20 years because of its simplicity [2,6,7]. This model can be applied for a single salt (ionic species) and non-electrolyte transfer in reverse osmosis [8] and NF [9], provided the unstirred layer on the membrane surface (also known as the stagnant layer) can be neglected. However, in the case of electrolyte mixtures, a coupling is observed between the transmembrane fluxes of different ions as the membrane potential generates an electric field. When the unstirred layer on the membrane surface is significant, coupling is also observed between the fluxes of the different ions within it. These observations have been incorporated into the SDM to obtain the solution-diffusion-film model (SDFM) [10], in which individual ions (not ion pairs) reach the membrane at varying speeds and then migrate through the membrane active layer in a manner whereby the negative charge of an anion is balanced by the positive charge of an accompanying cation. The SDFM assumes that solute (uncharged species or individual ions in the case of an electrolyte) transport occurs via diffusion and electric migration through both the membrane and the unstirred layer, in which convection also occurs. The SDFM has been used to successfully describe the rejection of non-electrolytes and their mixtures, as well as single electrolyte rejection in NF processes. However, in the case of electrolyte mixtures, the electric fields arising promote the coupling between fluxes of different ions, and the analytical solution to the SDFM requires the knowledge of the electrical

field distribution through the membrane and the unstirred layer, which are difficult to obtain.

The aim of this work was to evaluate the performance of a NF membrane for different charged and uncharged solutes by measuring their observable rejections and permeate fluxes under different applied pressures using a pilot plant. Likewise, we aimed to check the validity of the SDFM in electrolyte mixtures formed by a dominant salt and trace ions to analyze the information generated by the model, in order to understand the NF membrane transport mechanisms regarding ion retention and the permeability dependence with the composition of the medium.

6.2.- Experimental

6.2.1.- Chemicals

Sodium chloride (98%, Scharlau), potassium chloride (98%, Panreac), magnesium chloride (98%, Panreac), anhydrous magnesium sulfate (97%, Panreac), anhydrous sodium sulfate (97%, Panreac), anhydrous betaine (98%, Fluka), sucrose (98%, Scharlau), citric acid (99.5%, Sigma), and lactic acid (90%, Fluka) were used as supplied. Aqueous solutions of the above reagents were prepared by weighing out the exact amount of each component on an analytical balance (Sartorius, accurate to ± 0.0001 g). Ultrapure deionized type II water (Wasserlab Ecomatic 3L DP50L) was used to prepare all solutions.

Phosphoric acid (85%, Aldrich), potassium dihydrogen phosphate (99.5%, Merck), maleic acid (99%, Fluka), methanol (HPLC grade, HiPerSolv Chromanorm), and nitric acid (65%, EMSURE grade, Merck) were used for the analytical methods.

Solutions containing single solutes, either electrolytes or non-electrolytes (NaCl, KCl, Na₂SO₄, MgSO₄, MgCl₂, sucrose, betaine, lactic acid, and citric acid) were used in the initial experiments. Subsequently, several experiments were performed using different electrolyte mixtures containing a dominant salt (NaCl, Na₂SO₄, MgSO₄, MgCl₂) and

trace ions (Na^+ , SO_4^{2-} , Cl^- , Mg^{2+}). The molar concentration of single solutes was between 0.05–0.08 mol/L, whereas in the mixtures it was about 0.10 mol/L for the dominant salt. Trace ion concentrations were less than 2% of the dominant salt concentration. Table 6.1 shows the composition and pH of all solutions used in this work, whereas the properties of ions and solutes are displayed in Table 6.2.

Table 6.1. Composition and pH of aqueous solutions used in the NF experiments.

Single Solutes			Electrolyte mixtures	
Solutes	Concentration (mol/L)	pH (± 0.01)	Trace solutes (Dominant salt)	Concentration (mol/L) (± 0.0001) (d)
NaCl	0.060 \pm 0.001 (a)	7.40	MgSO ₄ (NaCl)	0.0021 (0.090)
KCl	0.055 \pm 0.002 (a)	7.66	NaCl (MgCl ₂)	0.0018 (0.101)
MgCl ₂	0.051 \pm 0.001 (a)	7.23	NaCl (MgSO ₄)	0.0023 (0.112)
MgSO ₄	0.067 \pm 0.001 (a)	7.35	NaCl (Na ₂ SO ₄)	0.0021 (0.098)
Na ₂ SO ₄	0.060 \pm 0.002 (a)	7.50		
Citric acid	0.068 \pm 0.001 (b)	2.39		
Lactic acid	0.089 \pm 0.001 (b)	2.21		
Betaine	0.085 \pm 0.001 (b)	7.72		
Sucrose	0.050 \pm 0.001 (c)	7.82		

Concentration measured by (a) conductivity, (b) HPLC, (c) polarimetry, and (d) techniques described in Section “Analytical methods” for each ion.

Table 6.2. Properties of ions and solutes: molecular weight, Stokes radius and diffusion coefficients in aqueous solution.

Ions/Solutes	Molecular weight (g/mol)	Stokes radius (nm)	Diffusion coefficient $\cdot 10^{10}$ (m ² /s)
Na ⁺	22.99	0.184 (a)	13.3 (a)
K ⁺	39.10	0.133 (b)	18.3 (b)
Mg ²⁺	24.30	0.348 (b)	7.1 (b)
SO ₄ ²⁻	96.10	0.230 (a)	10.6 (a)
Cl ⁻	35.45	0.121 (a)	20.3 (a)
Citric acid	192.12	0.350 (d)	6.5 (d)
Lactic acid	90.08	0.230 (a)	10.6 (a)
Betaine	117.15	0.310 (c)	3.0 (c)
Sucrose	342.30	0.460 (c)	1.8 (c)

(d) Umpuch et al. [11], (b) Somrani et al. [12], (c) Giacobello et al. [13], (d) Muller and Stokes [14].

6.2.2.- Analytical methods

The salt concentration in single electrolyte aqueous solutions (NaCl, KCl, Na₂SO₄, MgSO₄, MgCl₂) was determined by conductivity measurements using a Crison GLP 31 conductivity-meter, with an analytical error of $\pm 0.1 \mu\text{S/cm}$. The pH was measured using a Crison GLP 22 pH-meter with an analytical error of ± 0.01 pH units.

For aqueous solutions containing electrolyte mixtures, the concentration of each ion was measured as follows: i) Cl⁻ and SO₄²⁻ concentrations were determined by photometry using a Hach DR 2800 spectrophotometer, ii) Na⁺ concentration was determined by atomic emission spectroscopy using a Perkin Elmer 3300 flame photometer, and iii) Mg²⁺ concentration was measured by atomic absorption spectroscopy with the aforementioned Perkin Elmer apparatus at a wavelength of 202.6 nm, using a 5 vol.% aqueous nitric acid solution as both blank solution and sample solvent. Analytical errors were $\pm 2 \text{ g/m}^3$, $\pm 0.1 \text{ g/m}^3$, and $\pm 0.1 \text{ g/m}^3$ for each technique, respectively.

The sucrose content was measured by polarimetry at 880 nm using an Anton Paar MCP 500 Sucromat polarimeter with a precision of ± 0.01 °Z (about $\pm 0.045 \text{ g/L}$) and a reproducibility of ± 0.01 °Z [15,16].

Total concentrations of betaine, lactic acid, and citric acid in the solutions were determined by high performance liquid chromatography using a HPLC Shimadzu following the method described in previous studies [15–17].

6.2.3.- Experimental set-up

A thin film composite NF membrane (ATF2540-LS05-S, PTI Advanced Filtration) with 200 Da cut-off was used in the experiments. This is a spiral-wound module with a length of 101.6 cm and an internal diameter of 6.35 cm, with a spacer of 0.165 cm (37% porosity) and a total area of 1.7 m^2 . The membrane layer consists of polypiperazine amide supported on polystyrene.

Nanofiltration experiments were carried out in a pilot plant manufactured by Iberlact S.L. (Fig. 6.1). This pilot plant is equipped with two vertical multistage high-pressure centrifugal pumps (SV series, Lowara) (B 01 and B 02), a 20 L feed tank (BTD), electromagnetic flowmeter (FT 01), flow control valves (VB), a retentate flow control valve (PCVM), pressure (PT) and temperature (TT) transducers, and a heat exchanger (I01). A control panel with a display allows the values of the operating variables can be visualized.

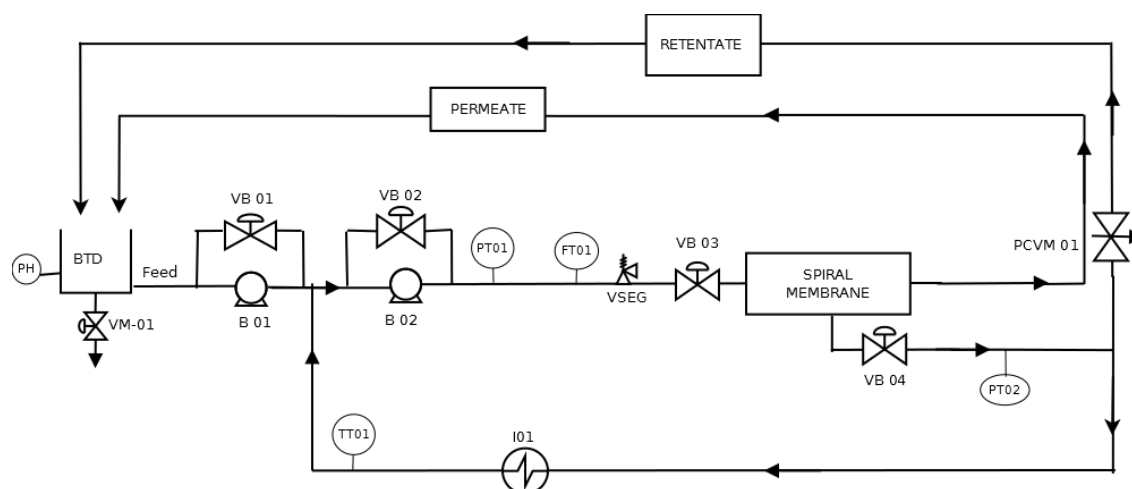


Figure 6.1. Flowchart of the nanofiltration pilot plant.

NF experiments were carried out in total recycle mode (i.e. both retentate and permeate were returned to the feed tank) at constant pH, temperature, feed flow rate and feed concentration. Experiments were initially performed with deionized water as feed under several operating conditions (pressure and feed flow rate).

Feed aqueous solution (10 L) with the composition shown in Table 6.1 was prepared and added to the feed tank. Before each experiment the feed was recirculated at low pressure for a short time and a sample was taken from the feed tank to measure the real concentration of the solutes in the feed solution, taking into account the dead volume of the plant (about 5 L). NF experiments were then performed at a feed flow rate of 1050 ± 50 L/h, 27 ± 2 °C, and varying the operating pressure from 9 to 17 bar, using only the first pump (B 01). As mentioned above, experiments were performed in total recycle

mode, gradually increasing the pressure. Steady-state operation was reached 15 min after each operating pressure was fixed, at which point the values of the feed flow rate, temperature, pressure and pH shown on the display were recorded. Permeate flux was measured 5 times and retentate and permeate samples were taken at each pressure.

After each NF run, the membrane was rinsed several times with deionized water at 45 °C, with a feed flow rate of 4200 L/h and a pressure of 16 bar. A chemical treatment (basic cleaning using Iberlact B-20 detergent) was required in the case of non-electrolytes. Finally, permeate flux was measured with deionized water at 25 °C and several pressures to check membrane cleaning.

6.2.4.- Data processing

The fundamentals and assumptions inherent to SDFM lead to the analytical solution given by Yaroshchuk et al. [10] for electrolyte mixtures under the limiting condition of trace ions in the presence of a single dominant salt, as explained briefly in Appendix in order to clarify the data treatment performed in this work according to the protocol established by Pages et al. [18]. The steps involved are described below.

First, permeate flux values, J_v , and observable rejection of single solutes, or dominant salt for the electrolyte mixtures, $R_s^{(obs)} = 1 - (c_s^{(P)}/c_s^{(F)})$, were fitted to Eq. 3 in order to obtain their diffusion permeability in the membrane and stagnant layer, P_s and $P_s^{(\delta)}$, respectively, as fitting parameters. Eq. 3 represents the SDFM for a single solute and is obtained by combining Eqs. 1 and 2, which are derived from Eqs. A.10 and A.23 of Appendix for the transfer of a single solute or dominant salt (in the limiting case of a single dominant salt and any number of trace ions) in the membrane and stagnant layers, respectively.

$$R_s^{(int)} = \frac{(J_v/P_s)}{1 + (J_v/P_s)} \quad (1)$$

$$\frac{1 - R_s^{(obs)}}{R_s^{(obs)}} = \frac{1 - R_s^{(int)}}{R_s^{(int)}} \exp(J_v / P_s^{(\delta)}) \quad (2)$$

$$R_s^{(obs)} = \frac{(J_v / P_s) \exp(-J_v / P_s^{(\delta)})}{1 + (J_v / P_s) \exp(-J_v / P_s^{(\delta)})} \quad (3)$$

The solute or dominant salt diffusion permeability in the membrane and stagnant layer are defined by Eqs. 4 and 5, respectively.

$$P_s = \frac{D_s}{L} \quad (4)$$

$$P_s^{(\delta)} = \frac{D_s^{(\delta)}}{\delta} \quad (5)$$

For electrolyte mixtures, the diffusion coefficient of the dominant salt in the stagnant layer, $D_s^{(\delta)}$, was assumed to be equal to the value for the single salt in aqueous solution. This was calculated using Eq. A.27 in Appendix, applying the ion diffusion coefficients, $D_{\pm}^{(\delta)}$, shown in Table 6.2.

Secondly, the intrinsic rejection of the individual solute or dominant salt, $R_s^{(int)}$, was calculated using Eq. 1 and the P_s parameter obtained previously. The intrinsic rejection of each trace ion, $R_t^{(int)}$, as expressed by Eq. 6, was obtained by solving Eq. A.24 in Appendix A using the Maple 17 software.

$$R_t^{(int)} = 1 - \frac{c_t^{(p)}}{c_t^{(m)}} \quad (6)$$

Thirdly, the reciprocal transmembrane passage of dominant salt, f_s , and trace ion, f_t , were calculated using Eqs. 7 and 8, respectively, and fitted to Eq. 9 in order to obtain the fitting parameters b and K, as defined by Eqs. 10 and 11 (Eqs. A.11–A.15 in Appendix).

$$f_s = \frac{c_s^{(m)}}{c_s^{(p)}} = \frac{1}{1 - R_s^{(int)}} \quad (7)$$

$$f_t = \frac{c_t^{(m)}}{c_t^{(p)}} = \frac{1}{1 - R_t^{(\text{int})}} \quad (8)$$

$$f_t = (f_s)^b + K \frac{f_s - (f_s)^b}{1 - b} \quad (9)$$

$$b = Z_t \theta_m \quad (10)$$

$$K = \frac{D_s}{D_t} \quad (11)$$

Finally, the membrane permeability for individual ions of the dominant salt, P_{\pm} , and trace ions, P_t , were calculated using Eqs. 12 and 13, respectively.

$$P_{\pm} = \frac{P_s}{1 - \left(\frac{Z_{\pm}}{Z_t} \right)^b} \quad (12)$$

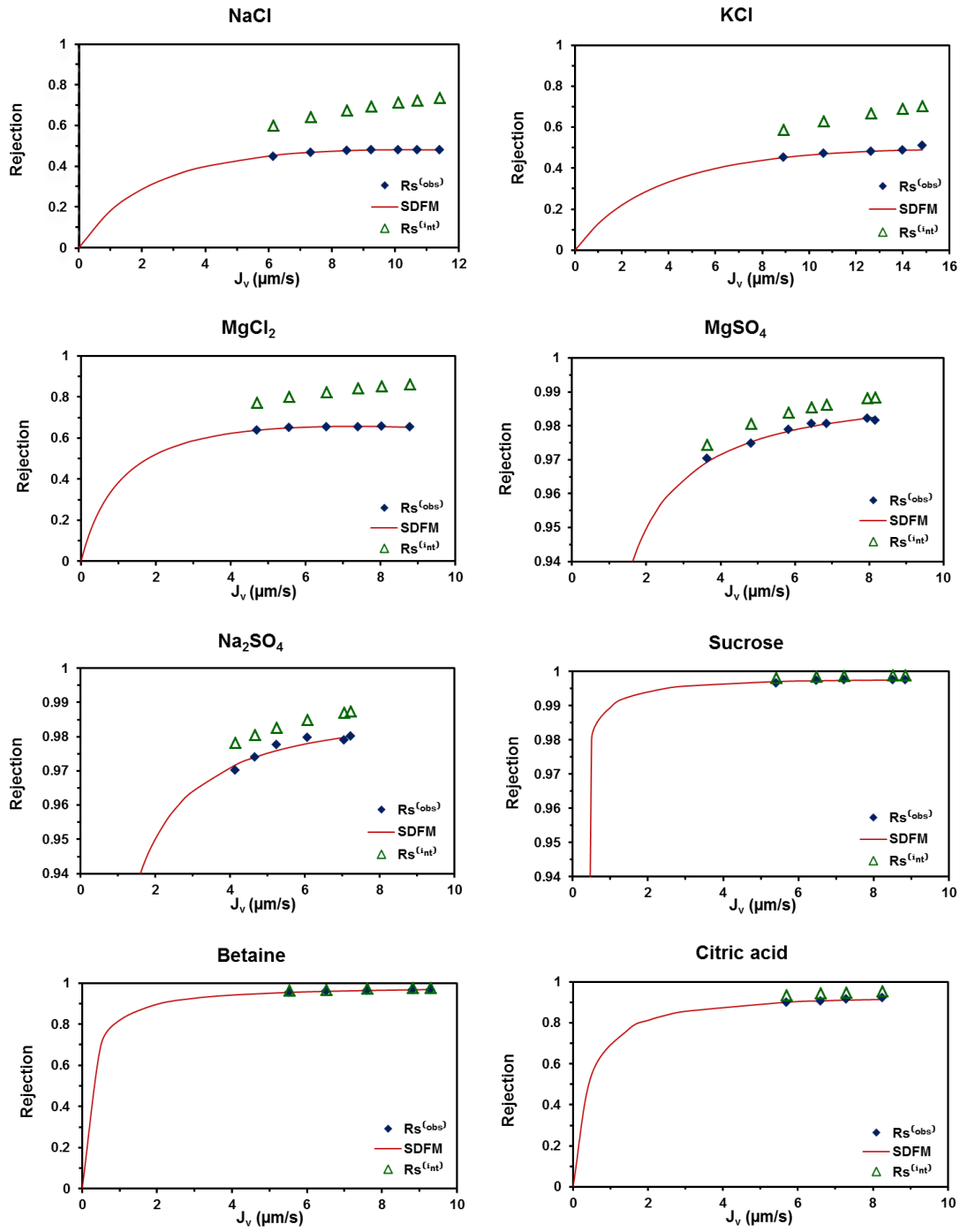
$$P_t = \frac{P_s}{K} \quad (13)$$

6.3.- Results and discussion

6.3.1.- Rejection of single solutes

The first experiments were run with single solute aqueous solutions, as shown in Table 6.1.

Fig. 6.2 depicts the observable rejections (experimental and calculated using Eq. 3), along with the intrinsic rejection calculated using Eq. 1. The fitting parameters P_S and $P_S^{(\delta)}$ for Eq. 3 are shown in Table 6.3. They were obtained using the solver.xlm software and the General Reduced Gradient (GRC) nonlinear algorithm for minimizing differences between the experimental and calculated observable rejections.



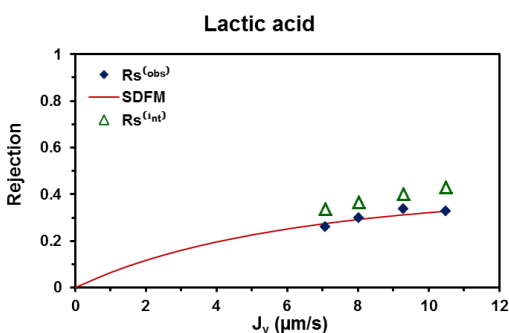


Figure 6.2. Observable rejection of single solutes (filled symbols: experimental values; continuous line: values calculated using Eq. 3, and intrinsic rejections (open symbols) calculated using Eq. 1.

The differences between the observable and intrinsic rejections shown in Fig. 6.2 indicate the existence of an unstirred layer at the membrane surface. The observable rejection sequence for salts was: $\text{Na}_2\text{SO}_4 \approx \text{MgSO}_4 > \text{MgCl}_2 > \text{KCl} \approx \text{NaCl}$, in accordance with the literature [18–20]. Our findings indicate that the nanofiltration membrane tested presents a negative surface charge because anion valence mainly determines solute rejection, i.e. divalent SO_4^{2-} anions are strongly rejected by the membrane whereas monovalent Cl^- anions show lower rejections. The observable rejection sequence for non-electrolyte solutes was: sucrose $>$ betaine \approx citric acid $>$ lactic acid.

Table 6.3 shows that $P_S < P_S^{(\delta)}$ in all systems, thus indicating that solute transfer in the membrane by solution-diffusion is slower than in the unstirred layer, where convection also occurs. As expected, the smaller the observable rejection, the higher its membrane permeability. Our findings are in accordance with the properties shown in Table 6.2: the smaller the molecular weight and Stokes radius, the higher the membrane permeability.

Table 6.3. Membrane permeabilities, P_s , and unstirred layer permeabilities, $P_s^{(\delta)}$, for single solutes, obtained as fitting parameters for Eq. 3.

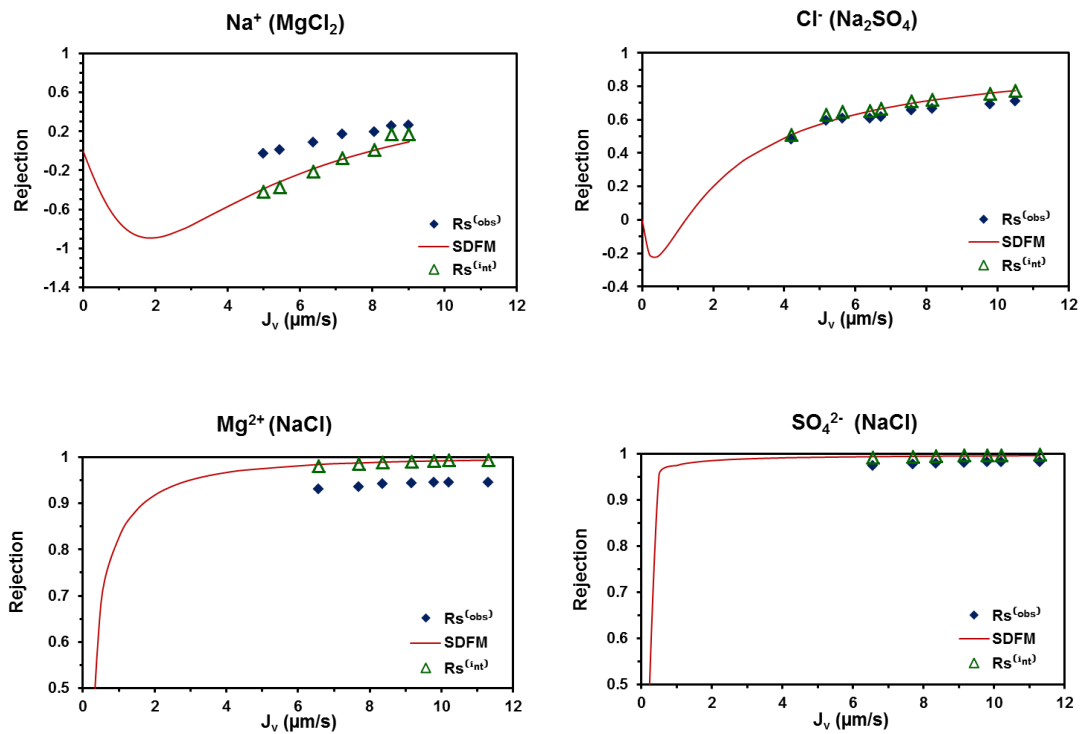
Single solute	P_s ($\mu\text{m/s}$)	$P_s^{(\delta)}$ ($\mu\text{m/s}$)
NaCl	4.076	10.300
KCl	6.283	16.446
MgCl ₂	1.396	7.264
MgSO ₄	0.095	19.598
Na ₂ SO ₄	0.092	15.580
Citric acid	0.391	12.308
Lactic acid	13.895	23.916
Betaine	0.211	26.108
Sucrose	0.009	10.871

Under identical fluid-dynamic conditions on the membrane, unstirred layer permeabilities should follow their diffusion coefficients in the bulk solution, thus meaning that $P_s^{(\delta)}$ for lactic acid and citric acid should be higher than that for betaine. These differences can be attributed to the partial ionization of the organic acids. At the experimental pH of 2.21 (see Table 1), 2.19% of the initial lactic acid ($\text{pK}_a = 3.86$ [21]) is present as anionic species that could be rejected by the negatively charged membrane. The same can be said for citric acid ($\text{pK}_{a1} = 3.13$, $\text{pK}_{a2} = 4.47$ and $\text{pK}_{a3} = 6.43$ [15]), with 15.69% thereof being present as monovalent anionic species at the experimental pH of 2.39. The experimental pH for betaine was somewhat higher than its pK_a of 1.81 [22], thus meaning that 100% of this compound is present as zwitterionic species. In the case of salts, deviations in permeability from the predicted behavior can be attributed to the non-ideality of solutions or ion-complexation effects, as indicated elsewhere [18].

Deviations between experimental and SDFM-calculated values for the observable rejection of single solutes used in this work are shown in Appendix (Figs. A.1 and A.2, and Table A.1).

6.3.2.- Rejection of electrolyte mixtures

Experiments with electrolyte mixtures were performed as described in Section “Experimental set-up” under the limiting conditions of one dominant salt and one or two trace ions, as shown in Table 6.1. The observable and intrinsic rejections of trace ions in the presence of the respective dominant salt are shown in Fig. 6.3. The fitting parameters b and K of Eq. 9, shown in Table 6.4, were calculated using the solver.xlm software with the GRC nonlinear algorithm. The resulting values were used to estimate intrinsic trace ion rejection, as depicted by the continuous lines in Fig. 6.3.



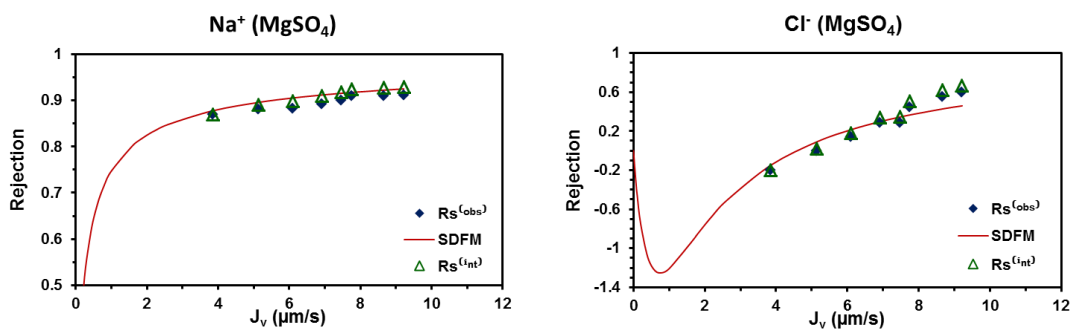


Figure 6.3. Observable rejections (filled symbols) and intrinsic rejections (open symbols) of trace ions vs. permeate flux in the presence of a dominant salt (in round brackets). Continuous lines depict the intrinsic rejections estimated using Eq. 9 with the values for b and K shown in Table 6.4.

Table 6.4. SDFM parameters obtained by fitting of data to Eq. 9.

Trace ion (Dominant salt)	b	K
Na^+ (MgCl_2)	-1.774	0.401
Mg^{2+} (NaCl)	1.918 ⁽¹⁾	13.890
Na^+ (MgSO_4)	0.561 ⁽²⁾	0.001
SO_4^{2-} (NaCl)	-1.919 ⁽¹⁾	190.100
Cl^- (Na_2SO_4)	-0.260	0.050
Cl^- (MgSO_4)	-0.571 ⁽²⁾	0.020

(1) and (2) refer to trace ions with the same charge but opposite signs in the presence of the same dominant salt.

As shown in Table 6.4, the absolute value of parameter b is equal for the trace ions SO_4^{2-} and Mg^{2+} in the presence of the same dominant salt NaCl . Moreover, in presence of the same dominant salt MgSO_4 the trace ions Na^+ and Cl^- also have almost the same absolute value of parameter b . These findings are in accordance with Eq. 14, which can be obtained from Eq. 12. As such, Eq. 14 reflects that trace ions with the same charge but opposite sign have an identical absolute value of parameter b in the presence of the same dominant salt.

$$b = Z_t \frac{(P_+ - P_-)}{Z_+ P_+ - Z_- P_-} \quad (14)$$

Negative rejections for some trace ions, specifically Cl^- (MgSO_4), Na^+ (MgCl_2) and Cl^- (Na_2SO_4), can be seen in Fig. 6.3. This phenomenon reflects a fast permeation of trace monovalent ions due to electric field formation when the permeabilities of the cations and anions from the dominant salts are different. The effects of these electric fields are more marked for trace ions in electrolyte mixtures. The negative rejection of Na^+ trace ion in the presence of the dominant salt MgCl_2 is due to the lower permeability of Mg^{2+} ion with respect to Cl^- , which means that the generated electric field accelerates Na^+ permeation and makes its rejection negative in order to achieve stoichiometric conditions [11]. Furthermore, for the dominant salt MgSO_4 the membrane permeability for SO_4^{2-} is lower than that for Mg^{2+} , thus meaning that the generated electric field accelerates Cl^- trace ion permeation and yielding negative rejections. As expected, the Na^+ trace ion with the same dominant salt MgSO_4 presents positive rejections. A similar behavior is observed when the dominant salt is Na_2SO_4 : the membrane permeability for Na^+ is higher than that for SO_4^{2-} , therefore negative rejections are obtained for Cl^- . It should be noted that Mg^{2+} and SO_4^{2-} ions did not present negative rejections because of their low membrane permeabilities, as it was expected due to their high observable rejections.

Fig. 6.4 shows the transmembrane passage of trace ions as a function of transmembrane passage of the dominant salt, using the SDFM fitting parameters b and K shown in Table 6.4. This figure shows that SDFM correctly describes permeation differences for a same trace ion when mixed with different dominant salts.

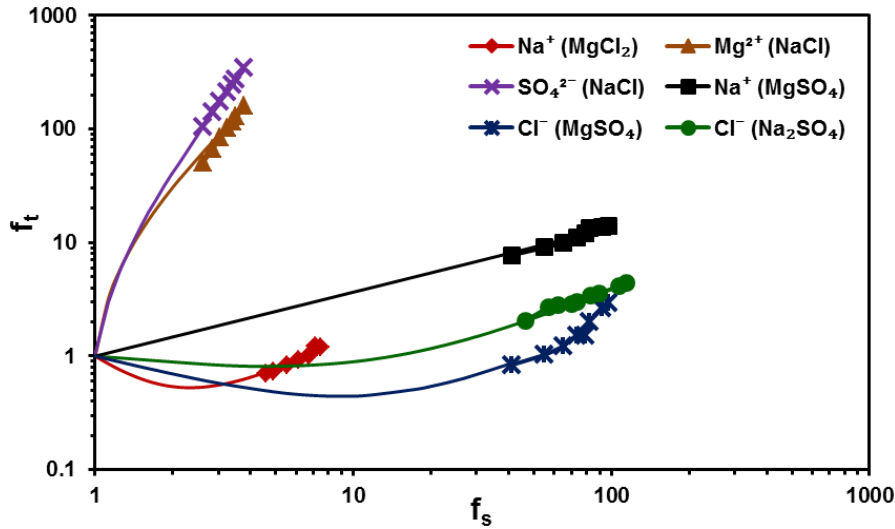


Figure 6.4. Reciprocal transmembrane passage of trace ions vs. the reciprocal transmembrane passage of dominant salts (in round brackets). The symbols are data calculated from experimental values using Eqs. 7 and 8, and the solid lines are data estimated by the SDFM using Eq. 9 and parameters b and K from Table 6.4.

The membrane permeabilities for the individual ions in the electrolyte mixtures are shown in Table 6.5. They were calculated using Eqs. 12 and 13 together with the SDFM parameters b and K from Table 6.4. It can be seen that the membrane permeability for Na^+ ion is significantly higher than that for Cl^- ion when they are components of the dominant salt NaCl , thus revealing the negative surface charge of the NF membrane, as discussed previously. The permeability for Na^+ ion in the presence of the dominant salt MgCl_2 shows anomalously low values, possibly due to its very low concentration (see Table 6.1). When the dominant salt is MgCl_2 , the Na^+ ion has a b value of -1.774 (Table 6.4), which means that, using Eq. 14, the permeability of the dominant ion Cl^- is higher than that for the dominant ion Mg^{2+} ($P_+ < P_-$), as shown in Table 6.5, and hence Na^+ trace ion permeation is accelerated by the electric fields. This results in negative rejections, as seen from Fig. 6.3, and greater Na^+ permeability than shown in Table 6.5.

Table 6.5. Membrane permeability, P_{\pm} , for individual ions of the dominant salt (calculated using Eq. 12), and trace ions P_t (calculated using Eq. 13) in electrolyte mixtures, using SDFM parameters b and K from Table 6.4.

Dominant salt	P_{\pm} or P_t ($\mu\text{m/s}$)			
	Na^+	Mg^{2+}	SO_4^{2-}	Cl^-
Na_2SO_4	0.12	–	0.060	2.020
MgCl_2	>>3.487	0.307	–	1.803
NaCl	95.920	0.300	0.020	2.070
MgSO_4	73.490	0.680	0.040	3.330

6.4.- Conclusions

The characterization of a 200 Da cut-off NF membrane has been performed on a pilot-plant scale by measuring the flux-dependent observable rejections of single solutes (NaCl , KCl , MgCl_2 , MgSO_4 , Na_2SO_4 , sucrose, betaine, lactic acid, and citric acid) at pressures similar to those used in industrial plants. The observable rejection sequence for single electrolytes was: $\text{Na}_2\text{SO}_4 \approx \text{MgSO}_4 > \text{MgCl}_2 > \text{KCl} \approx \text{NaCl}$. This result shows that the NF membrane presents a negative surface charge as the anion valence mainly determines the solute rejection. The observed rejection sequence for non-electrolytes was: sucrose > betaine \approx citric acid > lactic acid.

The SDFM was applied, and good agreement was observed between experimental and model results, thus meaning that the model can be considered suitable for simulating the NF process in the treatment of single solutes for the concentration range tested. Intrinsic rejections calculated by SDFM were higher than those observed, which indicates the presence of a concentration polarization layer on the membrane surface, this layer being more significant for solutes with high permeability. Membrane permeability for all single solutes was lower than that in the stagnant layer, thus indicating that membrane

transfer by solution-diffusion of the solute is slower than that in the unstirred layer, where convection also occurs. Membrane permeability values are inversely related to their molecular weights and Stokes radius, as these parameters affect the membrane transport of solutes. The permeability of the stagnant layer should follow the bulk solute diffusion coefficients; hence, permeability should be higher for lactic acid and citric acid than for betaine. Differences were attributed to the partial ionization of organic acids at the experimental pH. In the case of single electrolytes, deviations can be attributed to the non-ideality of solutions or ion complexation effects.

Electrolyte mixtures formed by a dominant salt and trace ions were also tested in order to study the coupling between permeate fluxes of ions. The SDFM correctly describes differences in permeation for a same trace ion when mixed with different dominant salts. The SDFM assumes coupling between ion fluxes is caused by the electric fields generated in the membrane and the unstirred layer, which can even lead to negative rejections for some trace ions. Negative rejections for Cl^- trace ion in the presence of MgSO_4 and Na_2SO_4 as dominant salts, and for Na^+ trace ion in the presence of MgCl_2 as dominant salt, were correctly explained by the SDFM.

The use of SDFM presented in this work could be useful for evaluating commercial NF membrane performance and for NF process optimization for separation of compounds, including trace ions, of paramount importance in several industrial applications.

6.5.- Appendix

This appendix describes the analytical solution developed by Yaroshchuk et al. [10] for application of the solution-diffusion-film model (SDFM) to electrolyte mixtures under the limiting condition of trace ions in the presence of a single dominant salt, assuming that the membrane properties are unaffected by the presence of trace ions. Although the model is described in detail elsewhere [10], this appendix briefly describes the fundamentals and assumptions made to SDFM to arrive at the analytical solution. The

following explanations are an attempt to clarify the data treatment performed in this work, as described in section 6.2.4 of this chapter.

The SDFM considers two premises for the permeation of solutes through the membrane: independence between solute and solvent convective fluxes, and the existence of an ideal virtual solution in thermodynamic equilibrium with an infinitely small volume within the membrane, as described by Spiegler and Kedem [23]. Under these premises, the transmembrane flux of one solute or ion inside the membrane is produced by two driving forces: the concentration gradient and the electrostatic potential gradient generated by the different mobility of ions within the membrane. This can be represented by the following equation:

$$J_i = -D_i \left(\frac{dc_i}{dx} + Z_i c_i \frac{d\phi}{dx} \right) \quad (\text{A.1})$$

where J_i is the transmembrane i -th solute flux, ϕ is the dimensionless virtual electrostatic potential in the virtual solution whose virtual solute concentration is c_i , D_i is the effective diffusion coefficient of the i -th solute in the membrane, which includes the equilibrium partition of solute between the membrane and the virtual solution, Z_i is the ion charge number, and x is the spatial coordinate scaled on the membrane thickness with the origin at the upstream membrane surface.

The SDFM assumes that the solute is transported by diffusion, electric migration and convection within the stagnant layer. Accordingly, the solute flux in the stagnant layer is described by the following transport equation:

$$J_i = -D_i^{(\delta)} \left(\frac{dc_i}{dx} + Z_i c_i \frac{d\phi}{dx} \right) + c_i J_v \quad (\text{A.2})$$

where $D_i^{(\delta)}$ is the effective diffusion coefficient of solute in the unstirred layer, c_i is its local concentration, φ is the dimensionless local electrostatic potential in the unstirred layer, and J_v is the permeate flux.

Analytical solutions for this model can be achieved in the case of uncharged solutes (single and mixtures) and a single electrolyte by solving Eqs. A.1 and A.2 simultaneously under steady-state conditions. For electrolyte mixtures, the electrostatic potential distribution generated by the presence of all ions is unknown, therefore an analytical solution is unattainable.

Under steady-state conditions, the transmembrane solute flux is independent of x coordinate and is assumed to be constant. Therefore, $J_i = c_i^{(p)} J_v$, and the dimensionless local and transmembrane differences of electric potential can be written as $\Delta\varphi(x) = \varphi(x) - \varphi(0)$ and $\Delta\varphi_m = \varphi(L) - \varphi(0)$, respectively. Accordingly, Eq. A.1 can be rewritten as a differential equation and integrated over the membrane thickness to yield:

$$\frac{c_i^{(m)}}{c_i^{(p)}} = \exp(Z_i \Delta\varphi_m) + J_v \int_0^L \frac{\exp(Z_i \Delta\varphi(x))}{D_i} dx \quad (\text{A.3})$$

where $c_i^{(m)}$ and $c_i^{(p)}$ are the solute concentration at the membrane surface and in permeate, respectively, and L is the membrane thickness. This equation shows that it is necessary to know the electric potential distribution across the membrane, which depends on all ion distributions, in order for it to be used directly. In the limiting case of electrolyte mixtures formed by a single dominant salt and any number of trace ions, the zero current condition in the membrane can be applied exclusively to the dominant salt ions, that is:

$$I = F(Z_+ J_+ + Z_- J_-) = 0 \quad (\text{A.4})$$

This condition, along with the electrical neutrality condition in the virtual solution, allows the following relationship between the electric potential gradient and the dominant salt concentration gradient to be established:

$$\frac{d\varphi}{dx} = -\theta_m \frac{d \ln(c_s)}{dx} \quad (\text{A.5})$$

where θ_m is the dominant salt transport coefficient in the membrane, which depends on its local concentration. Accordingly, Eq. A.3 can then be written in terms of the following equations for a single solute or dominant salt, and any of the trace ions, respectively:

$$c_s^{(p)} L = \frac{1}{J_v} \int_{c_s^{(p)}}^{c_s^{(m)}} D_s dc_s \quad (\text{A.6})$$

$$\frac{c_t^{(m)}}{c_t^{(p)}} = \exp\left(Z_t \int_{c_s^{(p)}}^{c_s^{(m)}} \theta_m d \ln(c_s)\right) + \frac{1}{c_s^{(p)}} \int_{c_s^{(p)}}^{c_s^{(m)}} \frac{D_s}{D_t} \exp\left(Z_t \int_{c_s^{(p)}}^{c_s^{(m)}} \theta_m d \ln(c_s)\right) dc_s \quad (\text{A.7})$$

In these equations the membrane transport coefficient, θ_m , and the diffusion coefficients of the dominant salt, D_s , and trace ion, D_t , depend on the virtual dominant salt concentration. Hence, if these dependences are specified, Eqs. A.6 and A.7 can be used to obtain the dependence of dominant salt and trace ion rejection on the permeate flux.

At this point, the model assumes that the three membrane transport coefficients (θ_m , D_s and D_t) are independent of dominant salt concentration. The coefficients θ_m and D_s for the dominant salt are defined by Eqs. A.8 and A.9, where D_{\pm} and Z_{\pm} are the effective diffusion coefficients and charge numbers of cations and anions of the dominant salt, respectively.

$$\theta_m = \frac{D_+ - D_-}{Z_+ D_+ - Z_- D_-} \quad (\text{A.8})$$

$$D_s = \frac{(Z_+ - Z_-)D_+D_-}{Z_+D_+ - Z_-D_-} \quad (\text{A.9})$$

Accordingly, Eqs. A.6 and A.7 take the following simple forms:

$$f_s = 1 + \frac{J_v L}{D_s} \quad (\text{A.10})$$

$$f_t = (f_s)^b + K \frac{f_s - (f_s)^b}{1-b} \quad (\text{A.11})$$

where f_s and f_t are the reciprocal transmembrane passage of dominant salt and trace ion, defined by Eqs. A.12 and A.13, respectively, and b and K are coefficients defined by Eqs. A.14 and A.15.

$$f_s = \frac{c_s^{(m)}}{c_s^{(p)}} = \frac{1}{1 - R_s^{(int)}} \quad (\text{A.12})$$

$$f_t = \frac{c_t^{(m)}}{c_t^{(p)}} = \frac{1}{1 - R_t^{(int)}} \quad (\text{A.13})$$

$$b = Z_t \theta_m \quad (\text{A.14})$$

$$K = \frac{D_s}{D_t} \quad (\text{A.15})$$

$R_s^{(int)}$ and $R_t^{(int)}$ are the intrinsic rejections of the dominant salt and trace ion, respectively, expressed as:

$$R_s^{(int)} = 1 - \frac{c_s^{(p)}}{c_s^{(m)}} \quad (\text{A.16})$$

$$R_t^{(int)} = 1 - \frac{c_t^{(p)}}{c_t^{(m)}} \quad (\text{A.17})$$

Eqs. A.10 and A.11 describe the dependence of dominant salt and trace ion intrinsic rejection on permeate flux. However, in order to use the experimental concentrations on both sides of the membrane, it is necessary to relate the intrinsic rejections to the observable ones when concentration polarization occurs. To do that, a similar treatment to that performed for Eq. A.1 can be applied to Eq. A.2 for mass transfer in the stagnant layer. Under steady-state conditions, and assuming that the diffusion coefficient of each solute through the stagnant layer is constant, and that the electric potential difference can be written as $\Delta\varphi(\xi) = \varphi(\xi) - \varphi(0)$, Eq. A.2 can be rewritten as a differential equation and integrated through the stagnant layer thickness to yield the following equation:

$$\frac{c_i^{(m)}}{c_i^{(F)}} = \exp(Pe_i - Z_i\Delta\varphi(1)) \left[1 - \frac{c_i^{(p)}}{c_i^{(F)}} Pe_i \int_0^1 \exp(-Pe_i\xi + Z_i\Delta\varphi(\xi)) dx \right] \quad (\text{A.18})$$

where $c_i^{(F)}$ is the solute concentration in feed, Pe_i is the dimensionless Péclet number of solute (dominant salt or trace ion), and ξ is the dimensionless coordinate, both defined as:

$$Pe_i = \frac{J_v \delta}{D_i^{(\delta)}} \quad (\text{A.19})$$

$$\xi = \frac{x}{\delta} \quad (\text{A.20})$$

with δ being the stagnant layer thickness.

In Eq. A.18, the electrostatic potential distribution in the stagnant layer depends on the distribution of all ionic species, which is unknown for mixtures of electrolytes.

However, in the limiting case of a single dominant salt and any number of trace ions, the zero current condition in the stagnant layer can be applied exclusively to the dominant salt. Furthermore, if local electroneutrality within the stagnant layer is assumed, the following relationship between electrostatic potential and dominant salt concentration is obtained:

$$\frac{d\varphi}{d\xi} = -\theta^{(\delta)} \frac{d \ln(c_s)}{d\xi} \quad (\text{A.21})$$

where $\theta^{(\delta)}$ is the dominant salt transport coefficient in the stagnant layer, defined as:

$$\theta^{(\delta)} = \frac{D_+^{(\delta)} - D_-^{(\delta)}}{Z_+ D_+^{(\delta)} - Z_- D_-^{(\delta)}} \quad (\text{A.22})$$

with $D_{\pm}^{(\delta)}$ being the diffusion coefficient of cation and anion of the dominant salt in the stagnant layer.

The integration of Eq. A.21 and its substitution into Eq. A.18 for the case of a single dominant salt allows the following equation to be obtained:

$$\frac{c_s(\xi)}{c_s^{(F)}} = 1 + R_s^{(obs)} (\exp(Pe_s) - 1) \quad (\text{A.23})$$

Substitution of Eq. A.23 into Eq. A.18 leads to the following equation for the trace ions, where the auxiliary variable $y = c_i \exp(Z_i \varphi)$ has been used:

$$\frac{c_i^{(m)}}{c_i^{(F)}} = \exp(Pe_i) \left[1 + R_s^{(obs)} (\exp(Pe_s) - 1)^{b^{(\delta)}} \right] \left[1 - (1 - R_t^{(obs)}) \int_{\exp(-Pe_i)}^1 \frac{dy}{\left[1 + R_s^{(obs)} (y^{-\alpha} - 1) \right]^{b^{(\delta)}}} \right] \quad (\text{A.24})$$

where the observable rejections of dominant salt and trace ion, diffusion coefficient of dominant salt for its application in the Péclet dimensionless number, and parameters α and $b^{(\delta)}$ are defined as follows:

$$R_s^{(obs)} = 1 - \frac{c_s^{(p)}}{c_s^{(F)}} \quad (\text{A.25})$$

$$R_t^{(obs)} = 1 - \frac{c_t^{(p)}}{c_t^{(F)}} \quad (\text{A.26})$$

$$D_s^{(\delta)} = \frac{(Z_+ - Z_-)D_+^{(\delta)}D_-^{(\delta)}}{Z_+D_+^{(\delta)} - Z_-D_-^{(\delta)}} \quad (\text{A.27})$$

$$b^{(\delta)} = Z_t \theta^{(\delta)} \quad (\text{A.28})$$

$$\alpha = \frac{D_t^{(\delta)}}{D_s^{(\delta)}} \quad (\text{A.29})$$

Eq. A.24 shows the relationship between the stagnant layer rejection of trace and dominant ions due to the electrical coupling between them, which in turn depends on the difference between the diffusion coefficients of the dominant salt ions in the stagnant layer, as reflected in parameter $\theta^{(\delta)}$ defined in Eq. A.22.

Eq. A.24 allows the trace ion intrinsic rejection to be calculated, and hence parameters b and K for trace ions to be estimated, as fitting parameters of Eq. A.11.

Figs. A.1 and A.2 show the agreement between the experimental and SDM calculated values for the observable rejection of single solutes used in this work. In general, deviations calculated by Eq. A.30 are lower than 0.7%, with the exception of one value

for NaCl and another for KCl, as shown in Table A.1. The greatest deviations (6–7.5%) are for lactic acid, probably due to the fraction of species dissociated which are negatively charged and could be rejected by the negatively charged membrane, as it is mentioned in section 6.4.1 of this chapter concerning Fig. 6.2. Standard deviations of the experimental observable rejection ($\pm\sigma_{\text{exp}}$) in Table A.1 show acceptable values, revealing the use of suitable techniques for solute concentration measurements.

$$\text{Error\%} = \frac{R_{SDFM}^{obs} - R_{exp}^{obs}}{R_{exp}^{obs}} \times 100 \quad (\text{A.30})$$

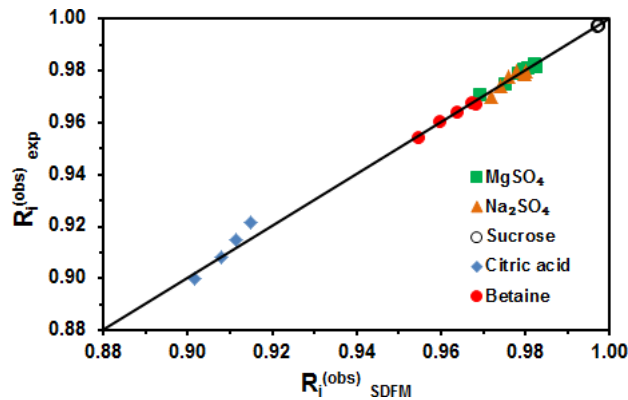


Figure A.1. Plot of the experimental observable rejections vs. those estimated by SDFM for the following single solutes: $MgSO_4$, Na_2SO_4 , sucrose, citric acid, and betaine.

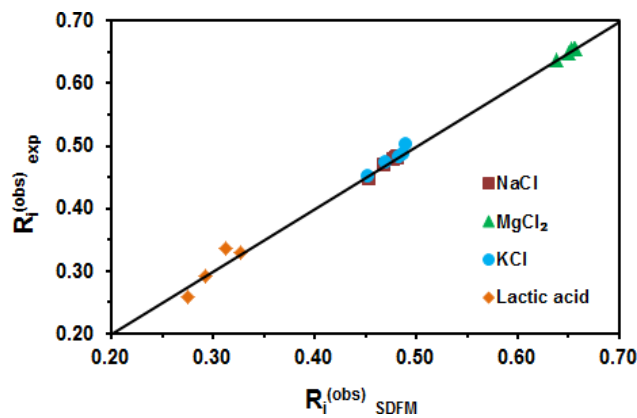


Figure A.2. Plot of the experimental observable rejection vs. those estimated by SDFM for the following single solutes: NaCl, $MgCl_2$, KCl, and lactic acid.

Table A.1. Experimental values of observable rejection ($Ri^{(obs)}_{exp}$) and its standard deviation ($\pm\sigma_{exp}$), observable rejection calculated by the SDFM ($Ri^{(obs)}_{SDFM}$), and error calculated by Eq. (A.30).

Solute	$Ri^{(obs)}_{exp}$	$\pm\sigma_{exp}$	$Ri^{(obs)}_{SDFM}$	Error (%)
NaCl	0.447	0.002	0.453	1.450
	0.469	0.007	0.469	0.000
	0.478	0.005	0.477	0.210
	0.480	0.003	0.480	0.017
	0.482	0.034	0.482	0.098
	0.481	0.005	0.482	0.121
	0.480	0.004	0.480	0.027
MgCl ₂	0.638	0.004	0.638	0.036
	0.649	0.002	0.649	0.009
	0.655	0.007	0.656	0.182
	0.655	0.003	0.657	0.278
	0.656	0.003	0.656	0.001
MgSO ₄	0.654	0.001	0.652	0.292
	0.970	0.014	0.969	0.116
	0.975	0.005	0.975	0.038
	0.979	0.005	0.978	0.052
	0.981	0.002	0.980	0.079
	0.981	0.001	0.981	0.012
Na ₂ SO ₄	0.982	0.001	0.982	0.000
	0.982	0.001	0.983	0.098
	0.970	0.001	0.972	0.161
	0.974	0.004	0.974	0.000
	0.978	0.002	0.976	0.172
	0.980	0.000	0.978	0.182
KCl	0.979	0.001	0.980	0.084
	0.980	0.000	0.980	0.000
	0.452	0.011	0.452	0.000
	0.472	0.064	0.470	0.544
	0.483	0.004	0.483	0.000
Sucrose	0.487	0.032	0.488	0.040
	0.502	0.023	0.489	2.588
	0.997	0.000	0.997	0.042
	0.997	0.001	0.997	0.015
	0.997	0.001	0.997	0.007
	0.997	0.001	0.998	0.003
	0.998	0.001	0.998	0.000

Citric acid	0.900	0.005	0.901	0.200
	0.908	0.003	0.908	0.000
	0.915	0.012	0.911	0.396
	0.921	0.001	0.915	0.681
Betaine	0.954	0.006	0.955	0.074
	0.960	0.005	0.960	0.023
	0.964	0.007	0.964	0.000
	0.968	0.005	0.967	0.005
	0.967	0.005	0.969	0.149
Lactic acid	0.259	0.011	0.275	6.202
	0.291	0.002	0.292	0.373
	0.337	0.008	0.312	7.523
	0.329	0.004	0.328	0.428

6.6.- Symbols

Symbols	Full name
b	Coefficient defined by Eq. 14
$b^{(\delta)}$	Parameter defined by Eq. 28
c_i	Virtual solute concentration (mol/L)
$c_i^{(m)}$	Solute concentration at the membrane surface (mol/L)
$c_i^{(p)}$	Solute concentration in the permeate (mol/L)
$c_i^{(F)}$	Solute concentration in the feed (mol/L)
D_i	Effective diffusion coefficient of solute in the membrane (m^2/s)
$D_i^{(\delta)}$	Diffusion coefficient of solute in the unstirred layer (m^2/s)
D_s	Effective diffusion coefficient of dominant salt in the membrane (m^2/s), defined by Eq. A.9
$D_s^{(\delta)}$	Diffusion coefficient of dominant salt in the unstirred layer (m^2/s), defined by Eq. A.27
F	Faraday constant (C/mol)
f_s	Reciprocal transmembrane passage of dominant salt defined by Eq. 7
f_t	Reciprocal transmembrane passage of trace ion defined by Eq. 8
I	Electric current density (A/m^2)
J_i	Transmembrane solute flux ($mol/m^2 s$)
J_+/J_-	Transmembrane flux of cations/anions ($mol/m^2 s$)
J_v	Permeate flux (m/s)
K	Coefficient defined by Eq. 11
L	Membrane thickness (m)
$P_s^{(\delta)}$	Diffusion permeability of solute in the stagnant layer (m/s) defined by Eq. 5

P_s	Diffusion permeability of solute in the membrane (m/s) defined by Eq. 4
P_{\pm}	Membrane permeability to individual ions of dominant salt (m/s) defined by Eq. 12
P_t	Membrane permeability to trace ion (m/s) defined by Eq. A.36
Pe_i	Dimensionless Péclet number of solute
$R_i^{(obs)}$	Observable rejection of solute
$R_i^{(int)}$	Intrinsic rejection of solute
x	Spatial coordinate with the origin at the upstream membrane surface (m)
y	Auxiliary variable in Eq. A.24, defined as $y = c_i \exp(Z_i \varphi)$
Z_i	Ion charge number
α	Parameter defined by Eq. A.29
δ	Stagnant layer thickness (m)
θ_{μ}	Dominant salt transport coefficient in the membrane, defined by Eq. A.8
$\theta^{(\delta)}$	Dominant salt transport coefficient in the stagnant layer, defined by Eq. A.22
ξ	Dimensionless spatial coordinate defined by Eq. A.20
φ	Dimensionless local electrostatic potential
$\Delta\varphi(x)$	Dimensionless local electric potential difference
Subscripts	
i	Species
s	Dominant salt or uncharged solute
t	Trace ion
\pm	Anion or cation of the dominant salt.

6.7.- References

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7.- Study of ceramic membrane behavior for okadaic acid and heavy metal determination in filtered seawater

Como se comentó al inicio de esta memoria, el trabajo que se presenta en este capítulo se orienta al diseño de un sistema de alerta temprana para la detección de contaminantes en el agua de mar que rodea las bateas de cultivo de mejillón. En este contexto, se planteó estudiar un sistema de filtración con membranas con el objetivo de proteger el dispositivo analítico de agentes naturales que pudieran afectar su seguridad (mareas, algas, peces, etc., en el caso de estar sumergido); y/o de la limpieza de la medida analítica para la eliminación de interferentes.

En este trabajo se describe el comportamiento de varias membranas cerámicas de MF y UF en la filtración del agua de mar, en concreto de las bateas situadas en la ría de Pontevedra. La velocidad y el nivel de ensuciamiento alcanzado en las diferentes membranas ensayadas se evaluaron utilizando el índice de ensuciamiento modificado aplicado a membranas de UF (MFI-UF). La calidad en los permeados fue mayor en UF que en MF como se deduce de la evaluación del grado de reducción alcanzado en varios parámetros de calidad como turbidez, alcalinidad, demanda química de oxígeno (DQO) y contenido de clorofila. Los rechazos de las membranas al Pb^{2+} y al ácido okadaico (OA), principal toxina que arrojan determinadas algas tóxicas, se midieron bajo diferentes condiciones de pH y presión. Las mediciones se realizaron en diferentes tiempos de filtración, antes y después de la formación de una torta estable sobre la superficie de la membrana. Los resultados indican que los iones pesados Pb^{2+} y Cd^{+2} , cuando están presentes en concentración traza son rechazados, fundamentalmente, por interacciones electrostáticas con la carga de la membrana hasta alcanzarse la saturación. Se observó que cuando el pH fue más bajo que el punto isoeléctrico de las membranas, los rechazos de estos iones fueron nulos. De forma diferente, el rechazo de OA se produce fundamentalmente por un mecanismo de retención en la torta formada sobre la superficie de la membrana. El rechazo de OA dependió del tamaño del poro de la membrana y de la presión aplicada. Como consecuencia del carácter compresible de la torta, se obtuvieron rechazos negativos de OA bajo condiciones específicas de filtración.

Este trabajo ha sido presentado en el sexto congreso de química “EuCheMS” en Sevilla (2016), y ha sido publicado en Journal of Environmental Management 232, (2019), 564-573.

7.1.- Introduction

Pollution in seawater is caused by anthropic actions in urban, rural and industrial areas and by natural causes such as the effects of bloom-forming algae. Toxins and some heavy metal ions accumulate in marine organisms, particularly bivalve mollusks. They can eventually reach dangerous levels that threaten the life of marine flora and fauna, causing serious disorders in anyone who consumes them [1].

The genera *Dinophysis* and *Prorocentrum* of the dinoflagellate species produce the dangerous okadaic acid (OA) group toxins. Okadaic acid is a highly water insoluble organic compound with a molecular weight of 804 g/mol. Shortly after their ingestion, these toxins, also known as diarrhetic shellfish poisons (DPS), can cause diarrhea, nausea, vomiting, and abdominal pain in humans [2,3].

It is well known that heavy metal ions such as Pb^{2+} , Cd^{2+} and Hg^{2+} are highly toxic and can also cause poisoning. The main compounds to which they bind in seawater are chlorides, carbonates and sulfates, but they can also bind to other species such as humic acids and organic matter [4–6]. However, routine analytical testing of shellfish toxicity levels, to guarantee consumer health, involves expensive and time-consuming analytical techniques. So, one of the current challenges in research is the design of tools for the analysis of the seawater surrounding shellfish that will rapidly detect the presence of toxins. The detection of pollutants in seawater requires the use of analytical techniques with very low detection limits ($<10^{-10}$ mol/l). To do so, seawater samples are generally pretreated with membrane technologies, in order to separate possible interferents such as particles in suspension and organic matter.

Microfiltration (MF) and ultrafiltration (UF) are pressure-based membrane separation technologies that use porous membranes for permeation and separation of components. In recent years, the use of MF and UF low-pressure processes as raw-seawater pretreatment in reverse osmosis processes have steadily grown in popularity [7–12].

However, the main hurdle that membrane processes have to overcome is fouling caused by Natural Organic Materials (NOM). NOM are mainly composed of humic acids and extracellular and intracellular Algal Organic Matter (AOM) that intensifies during seaweed blooms. Extracellular matter can reach 80% of the overall AOM, which is mainly composed of polysaccharides, nucleic acids, lipids, amino acids, and other organic acids. These substances have the potential to cause significant irreversible organic fouling. Besides, the presence of divalent salts in high concentrations significantly increases the fouling potential of organic matter [13]. Periodic membrane cleaning is required, to maintain acceptable values of permeation flux and effluent quality, which increases the process cost and reduces the life of the membranes [14]. Ceramic membranes have been chosen for this study, because of their higher chemical resistance and longer shelf life than organic membranes.

Nowadays, a number of tests are used to measure the rates at which membrane filters become plugged. The most successful tests are the Silt Density Index (SDI) [15,16] and the Modified Fouling Index (MFI) [17]. Both are used as quick tests to quantify membrane fouling through a 0.45 μm microfilter in dead-end flow under constant pressure. Unlike SDI, which is not based on any fouling mechanism and has no linear model for particle concentration, MFI is based on a cake-filtration mechanism and can be used to model flux decline in membrane systems [18]. MFI applied to UF membranes is referred to as MFI-UF and considers particle sizes smaller than 0.45 μm that are the main cause of membrane fouling [18,19]

Despite the existence of different models of membrane-fouling mechanisms published in the literature, many of them are based on the evaluation over time of parameters that are difficult to measure. Other simpler models that are easier to apply, such as the Hermia models [20–24], have been widely criticized for their inability to discriminate between different mechanisms and for relying on unrealistic assumptions [25,26]. In this study, the MFI-UF values, under cake filtration conditions, were used to provide guidance when selecting both the membrane and the membrane cleaning methods. Although

several membrane-fouling mechanisms often occurred simultaneously at the beginning of the filtration process, cake formation permitted flux stabilization.

This paper aims to study the performance of several MF and UF ceramic membranes used for the filtration of seawater surrounding the mussel rafts of the Pontevedra estuary in Galicia (Spain). Spain is the third largest global supplier of mussels and the main supplier to the European market, with 98% of its production from Galicia, a region of northwestern Spain [27]. In this region, the closure of mussel rafts due to toxic episodes of bloom-forming algae costs the industry millions of Euros each year. An early warning system, based on routine measurements of seawater, would therefore help the industry to reduce economic losses during the mussel harvest. In this study, MFI-UF was used to compare the effect of different seawater samples on the same membrane and, in contrast, the effect of the same seawater samples on different membranes. Also, based on MFI-UF profiles, observable rejection of Pb^{2+} , Cd^{2+} and okadaic acid were measured at different permeate sampling times, in order to elucidate the membrane fouling state effect on component rejections. The effect of the membrane on different quality parameters was also analyzed. The main control parameters were turbidity, alkalinity, pH, conductivity, chlorophyll content, and chemical oxygen demand (COD), which were measured in feed and permeates.

7.2.- Theory and background

7.2.1.- Modified fouling index (MFI)

MFI was developed by Schippers and Verdouw [15], based on the cake filtration mechanism.

$$\frac{t}{V} = \frac{\mu_T R_m}{\Delta PA} + \frac{\mu_T I}{2\Delta PA^2} V \quad (1)$$

where, ΔP is the transmembrane pressure; μ_T is the water viscosity at temperature T; A is the area of the membrane; V is the filtrate volume; and, R_m is the membrane resistance to clean-water permeation at temperature T. The index I is a function of the size and nature of the particles and is directly correlated with their concentration in the seawater by the following expressions: $I = \alpha \cdot C_b$ for incompressible cake and $I = \alpha \cdot C_b \cdot (\Delta P)^w$ for compressible cake; where, α is the specific resistance of the cake; w the compressibility factor; and, C_b , the concentration of particles in the feed water. As previously mentioned, MFI was extended to UF membranes and expressed as MFI-UF [18,19]. MFI-UF is graphically determined as the slope of the linear region of minimum slope, which corresponds to the cake filtration mechanism, from the plot of (t/V) vs (V) . MFI-UF depends on temperature and pressure. As all experiments in this work were carried out at 20° C but at different pressure, the following expression was used, in order to correct the effects of different experimental transmembrane pressures:

$$MFI-UF = \frac{\Delta P}{\Delta P_0} \frac{d\left(\frac{t}{V}\right)}{d(V)} = \frac{\Delta P}{\Delta P_0} \frac{\mu_T I}{2\Delta P A^2} \quad (2)$$

where, the subscript 0 refers to the standard conditions of 20 °C, and 2 bar.

7.3.- Materials and methods

7.3.1.- Experimental set-up

The experiments were performed in a Spirlab filtration cell using TAMI flat-disc ceramic membranes, with an active layer of TiO₂ and ZrO₂ for the MF and the UF membranes, respectively, both supported on a 90 mm diameter piece of titania with an effective area of 56.3 cm². The feed tank was a jacketed tank (15 L) in which the seawater was held at a constant temperature and stirred at 375 rpm. Seawater was fed into the filtration cell by a Masterflex peristaltic pump at a prefixed flow rate and

pressure. The system was also equipped with a rotameter and a pressure gauge, both placed at the inlet of the filtration cell. Most of the experiments, except those specifically mentioned in the text, were performed in dead-end flow, at 20 °C, and with a transmembrane pressure (TMP) of between 0.6 — 2.0 bar. The cumulative weight of filtered seawater was recorded over time using an electronic balance (Sartorius, accurate to ± 0.0001 g). Fig. 7.1 depicts the experimental set up. Table 7.1 shows the membrane characteristics and experimental conditions used in the seawater filtration. Some experiments were performed with the addition of Pb^{2+} and Cd^{2+} in the seawater feed, as specified in Table 7.1. The use of an activated charcoal (AC) bed on the membrane was also investigated (exps. 16 and 17).

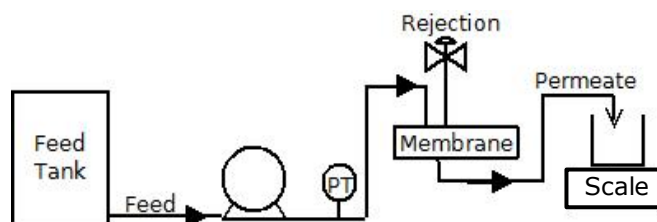


Figure 7.1. Schematic diagram of dead-end filtration system.

Table 7.1. Conditions of seawater filtration experiments. All experiments were carried out at 20°C. The same letter refers to the same seawater sample (same place and date of collection). The same number between brackets (*) refers to the same membrane from among those used with the same pore size. L_p represents membrane permeability at 20 °C.

Exp.	Membrane (*)	Feed	$L_p \cdot 10^5$ (m/s bar)	TMP (bar)
1	150 kDa (1)	a	9.42	1.90
2	150 kDa (2)	b	9.39	1.70
3	150 kDa (2)	c + Pb^{2+} + Cd^{2+} added	9.39	1.85
4	150 kDa (2)	d	9.39	1.70

*Determinación de contaminantes del mar en aguas de bateas de cultivo de mejillón:
desarrollo de sensores electroquímicos y estudios de filtración con membranas*

5	150 kDa (3)	j	6.91	0.80
6	150 kDa (3)	j	6.91	1.00
7	150 kDa (3)	j	6.91	1.50
8	300kDa (1)	a	10.36	1.70
9	300kDa (1)	f	10.36	1.75
10	300kDa (2)	j	7.52	0.80
11	300kDa (2)	j	7.52	1.00
12	300kDa (2)	j	7.52	1.50
13	0.14 µm (1)	a	24.65	2.00
14	0.14 µm (2)	f	23.64	1.90
15	0.2 µm (2)	b	30.24	1.90
16	0.2 µm (1)+AC bed	b	32.84	1.70
17	0.2 µm (1)+AC bed	c + Pb ²⁺ + Cd ²⁺ added	32.84	1.40
18	0.2 µm (1)	c + Pb ²⁺ + Cd ²⁺ added	32.84	1.90
19	0.2 µm (3)	d	29.51	1.90
20	0.2 µm (3)	h	29.51	1.00
21	0.2 µm (3)	h + HCl added (pH = 2)	29.51	1.00
22	0.45µm (1)	g	54.90	1.80
23	0.45µm (2)	e	42.56	1.70
24	0.45µm (2)	i + Pb ²⁺ added	42.56	0.60
25	0.45µm (2)	i + Pb ²⁺ added	42.56	1.20
26	0.45µm (2)	i + Pb ²⁺ added	42.56	1.80
27	0.45µm (2)	d + Pb ²⁺ added	42.56	0.80
28	0.45µm (2)	h	42.56	1.00
29	0.45µm (2)	h + HCl added (pH = 2)	42.56	1.00
30	0.8µm (1)	e	64.74	1.70
31	0.8µm (1)	i + Pb ²⁺ added	64.74	0.60
32	0.8µm (1)	i + Pb ²⁺ added	64.74	1.20
33	0.8µm (1)	i + Pb ²⁺ added	64.74	1.80
34	0.8µm (1)	h	64.74	1.00

35	0.8µm (1)	h + HCl added (pH = 2)	64.74	1.00
36	1.4µm (1)	e	90.67	1.70

The membranes were firstly characterized with deionized water at different TMP to determine their water permeability at 20 °C. Previously, each membrane had been rinsed with deionized water at 2 bar for 5 h. The results are depicted in Fig. 7.2 showing a correlative increase in membrane permeability with the pore size, as shown in Table 7.1.

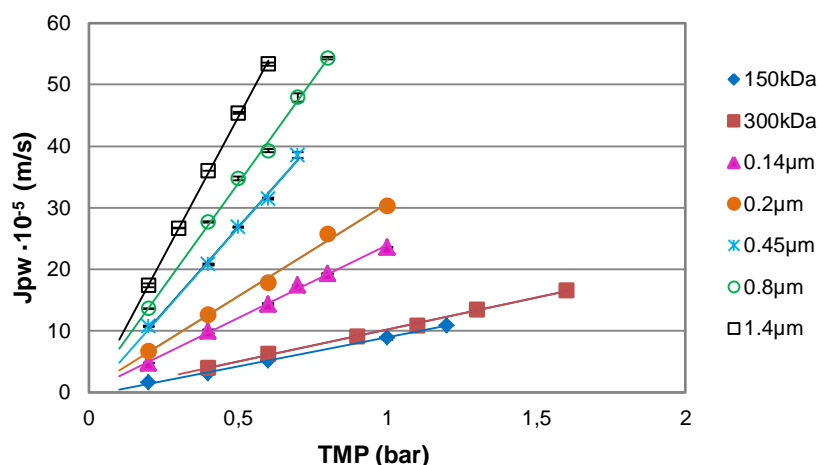


Figure 7.2. Water permeability of the membranes at 20 °C

After each run, several cleaning cycles were applied. Each cycle consisted in backwashing with 500 mg/L NaClO 1 L at 0.5 bar, rinsing with deionized water 1 L in a single pass, cleaning for 2 h with 500 mg/L NaClO in total recirculation at 0.2 bar, and finally rinsing with deionized water 1 L in a single pass, followed by total recirculation of deionized water for 2 h at 0.2 bar. The degree of cleaning reached after each cycle was evaluated by the clean water flux (CWF) defined by Eq. 3:

$$CWF = \frac{\mu_T}{\mu_{20}} \cdot \frac{Q_T}{A \cdot \Delta P} \tag{3}$$

where, Q_T is the clean water flow (m^3/s) at temperature T . CWF was measured after each cycle and normalized in relation to the one previously obtained with deionized water and the new membrane before it was used (CWF^N).

7.3.2.- Chemicals

Sodium hypochlorite (14% Cl_2 , GPR Rectapur), and ultrapure deionized water type II (Wasserlab Ecomatic 3L DP50L) were used in the membrane cleaning cycles. Sulfuric acid (95%, GPR Rectapur), nitric acid (65%, Merck), cadmium and lead standard solutions suitable for atomic absorption spectrometry (Panreac), bromocresol green-methyl red indicator (Panreac), acetone (99.5%, Fluka), potassium dichromate (95.5%, Sigma-Aldrich), silver-ion catalyst (Scharlau), ferrous ammonium sulphate (Probus) and ferroin indicator (Panreac) were used for the different analytical methods. Norit powdered activated charcoal (Norit 97876, Sigma-Aldrich) was used in the experiments with the AC bed on the membrane surface, and as a cleaning agent. Lead (II) nitrate (98%, Panreac), cadmium nitrate tetrahydrate (98%, Sigma- Aldrich), and okadaic acid potassium salt (98%, Alfa Aesar) were used to study membrane rejection of both the heavy metals and the okadaic acid.

7.3.3.- Analytical methods

Several control parameters such as pH, conductivity, turbidity, alkalinity, chemical oxygen demand (COD), and chlorophyll content were measured in both the seawater feed and the permeates. The effect of membrane filtration on permeate quality was determined by the degree of reduction of each control parameter, R_i , calculated by:

$$R_i = 1 - \frac{C_{i(P)}}{C_{i(F)}} \quad (4)$$

where, C_i refers to the control parameter value, and subscripts P and F to permeate and seawater feed, respectively.

Conductivity was measured with a Crison GLP 31 conductivity-meter, at an analytical error of $\pm 0.1 \mu\text{S}/\text{cm}$. The pH was measured using a Crison GLP 22 pH-meter with an analytical error of ± 0.01 pH units. Turbidity was measured at 850 nm with an infrared-emitting diode using a TN-100/T-100 portable turbidimeter (Eutech Instruments), with an error of ± 0.01 NTU.

Alkalinity was measured by titration with 0.02 N sulfuric acid using bromocresol green-methyl red indicator [28].

Chlorophyll was measured by spectrophotometric methods [13,29] using the equations proposed by Jeffrey, S. W. & Humphrey, G. F. [30]. Briefly, samples were filtrated through glass microfiber filters of $0.7 \mu\text{m}$ (Milipore), pigment was extracted with 99% acetone, stored at -20°C in the dark for at least 10 h, centrifuged (3000 rpm for 10 min) and, finally, measured at 664, 647, 630, and 750 nm wavelengths (Hitachi U-2000 spectrophotometer).

The COD was analyzed by sample reduction with $\text{K}_2\text{Cr}_2\text{O}_7$ and sulfuric acids using a silver ions catalyst, heated under reflux for 2 h at 200°C , and, finally, titrated with ferrous ammonium sulfate (FAS) and ferroin as an indicator [31].

OA was measured by the test for the detection of the OA-toxins group supplied by Zeulab S.L. with a detection limit of $1.20 \cdot 10^{-11}$ M. This test is based on phosphatase activity inhibition by the OA-toxins group.

Pb^{2+} and Cd^{2+} were analyzed by Induction Coupled Plasma with Mass Spectrometry (ICP-Mass) with a detection limit of $1.24 \cdot 10^{-10}$ M.

7.4.- Results and discussion

Fig. 7.3 shows the decrease of the normalized permeate flux (J_p/J_w) over filtration time. It shows a much greater decrease in permeate flux for MF membranes than for UF ones, which reveals a much more severe fouling in the former. Values of CWF^N after each cleaning cycle are shown in Fig. 7.4. Since the fouling varied with the seawater quality, the study was performed by comparing experiments working with the same seawater sample. From Fig. 7.4a, it is observed that the 0.14 μm MF membrane needed at least four cycles for recovery of 90% permeability, while only two cleaning cycles were needed for the UF membranes. Fig. 7.4b shows no significant differences between the fouling level and the pore size in the MF membranes, however it reveals that five cleaning cycles were needed for 90% permeability recovery. According to other works [32–35], the complete recovery of $CWF^N = 100\%$ was not possible. Fig. 7.4c shows that experiments working with an AC bed on the 0.2 μm MF membrane surface (exps. 16 and 17) yielded CWF^N higher than 92% after only one cleaning cycle. Comparison of experiments 17 with the AC bed, and 18 without the AC bed, both with the same seawater and the 0.2 μm membrane, yielded CWF^N higher than 90% after the 1st and 4th cleaning cycle, respectively. Reduction of the permeate flux was very low in the experiment with the AC bed as shown in Fig. 7.3. The AC bed adsorbed the organic compounds, probably low molecular weight solutes that could be absorbed on the walls of the large pores of the MF membranes, before they reached the membrane.

We also used AC as a cleaning agent for the MF membranes. In these experiments, the membranes were removed from the filtration cell and immersed for 24 h in a glass flask containing an aqueous dispersion of AC. Next, the membrane was put back in the filtration cell and conventional cleaning cycles were applied. Fig. 7.4d shows a comparison of expts. 24, 25, and 26 with the 0.45 μm membrane and the same seawater, but at different TMP, with two of the three experiments cleaned with AC. The results yielded values of $CWF^N > 90\%$ after the 3rd cycle using AC ($CWF^N = 90.06\%$ for exp. 24 at 0.6 bar), after the 4th cycle without using AC ($CWF^N = 90.01\%$ for exp. 25 at 1.2

bar), and after the 5th cycle using AC ($CWF^N = 96.90\%$ for exp. 26 at 1.8 bar). The results reflected more severe membrane fouling as the TMP increased, but they gave no conclusive indication of the convenience of using AC as a cleaning agent. In Fig. 7.4e, a comparison between exps. 31 (cleaned with AC), 32 (cleaned without AC), and 33 (cleaned with HCl instead of AC) for the 0.8 μm membrane at different TMP, yielded $CWF^N > 90\%$ after the 4th cycle for exps. 31 and 32, disregarding the use of AC. However, only three cleaning cycles were needed in exp. 33, despite the fact that the TMP was higher in this experiment, revealing that HCl is a good membrane cleaning agent. Finally, experiments performed at different pHs (20, 21, 28, 29, 34, 35) are compared in Fig. 7.4f. No conclusions were reached on the effect of the pH level, however increased fouling with pore size was observed. An effect that was not observed in Fig. 7.4b with MF membranes, but it should be taken account that both figures corresponded to different seawater samples (samples e and h) with different degrees of dirtiness.

The AC cleaning effect was analyzed by the contact angle measurement on the membrane surface. Fig. 7.5 shows comparative results of three membranes: a new 0.2 μm membrane after rinsing with water, the same membrane after seawater filtration followed by two cleaning cycles, and finally the same membrane after seawater filtration followed by immersion for 24 h in an AC water dispersion. Cleaning with AC was observed to yield similar contact angles to those of the new membrane. In contrast, after two cleaning cycles ($CWF^N > 95\%$), several regions with different contact angles were observed ('dirty' and 'clean' regions shown in Fig. 7.5), although they were all higher than those for the new membrane. These results indicated that the AC cleaned the surface of the membrane, but had no effect on the fouling in the pores, which is relevant in the microfiltration membranes, according to the above results.

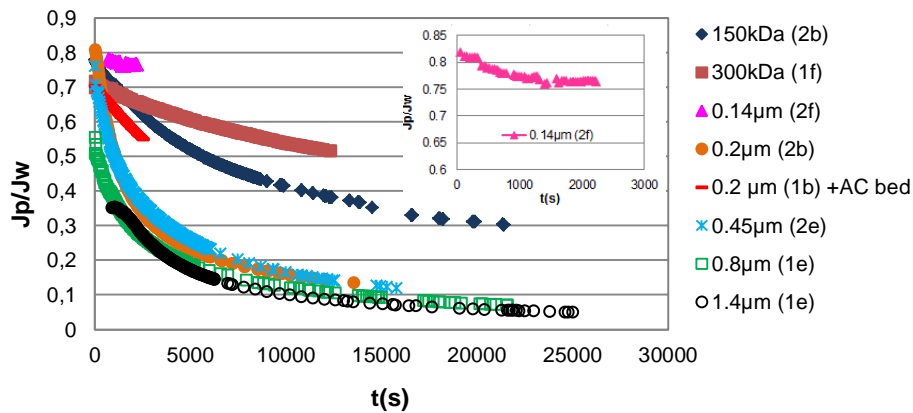
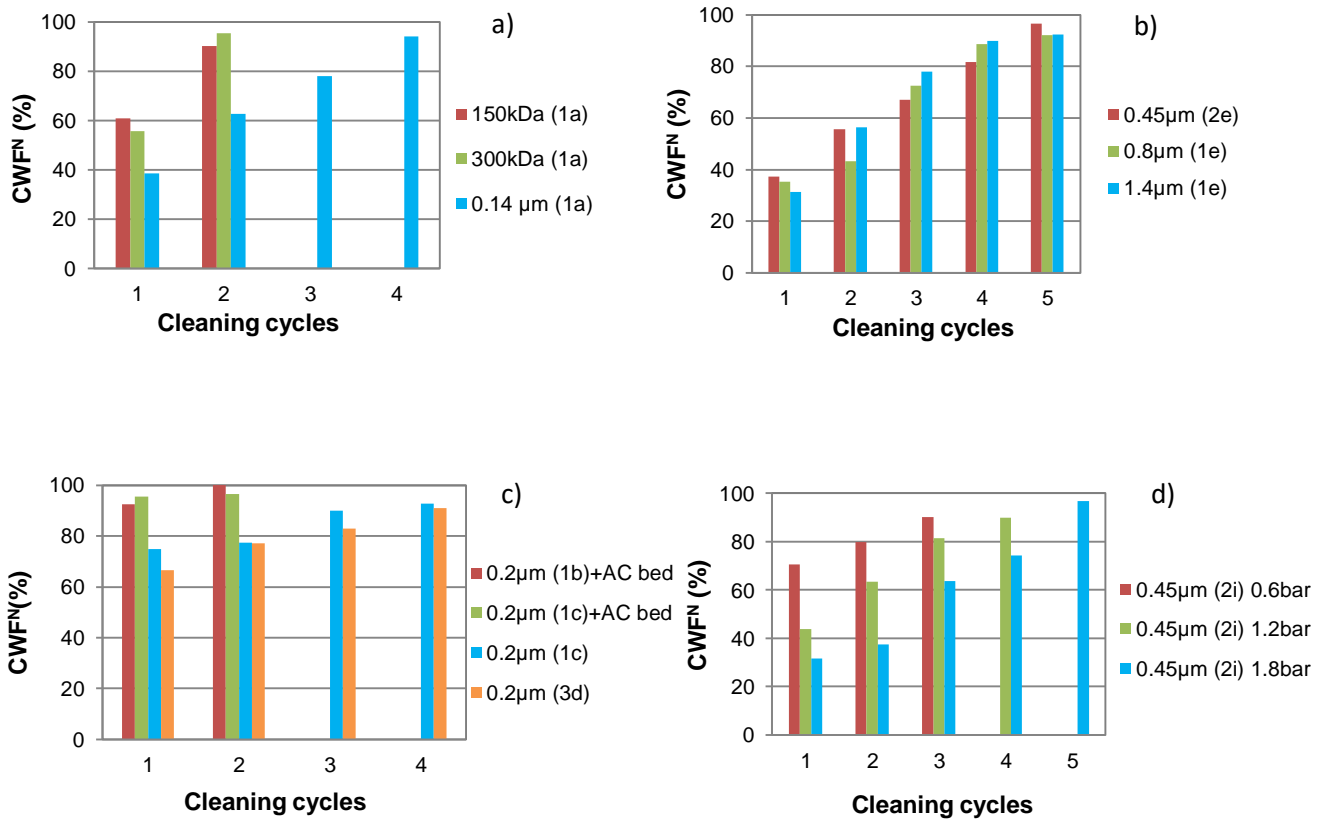


Figure 7.3. Permeate flux normalized over time. In the legend, between brackets, the same letter refers to the same seawater sample (same place and date of collection) and the same number refers to the same membrane from among those used with the same pore size, according to Table 7.1.



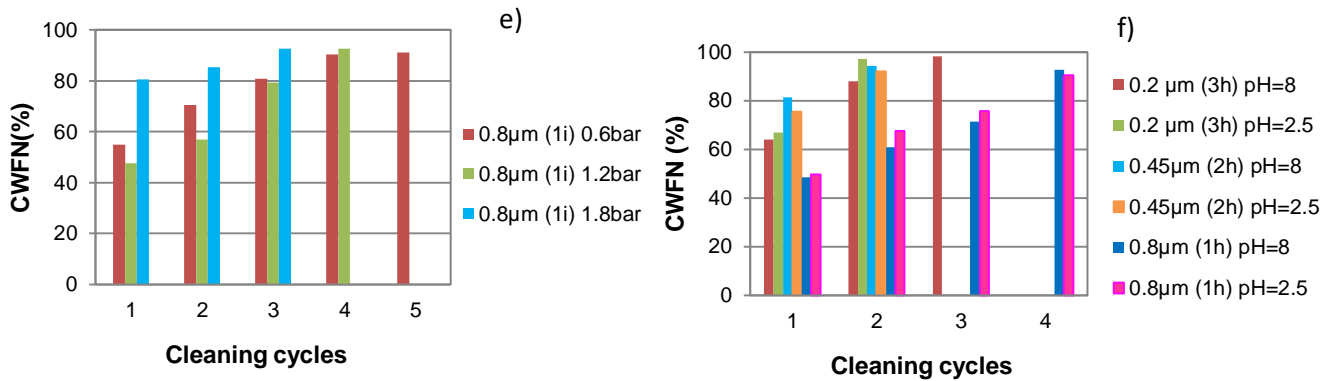


Figure 7.4. Normalized clean water flux (CWF^N (%)) obtained after each cleaning cycle for experiments: a) several UF and MF membranes with the same seawater sample; b) different pore size MF membranes and the same seawater sample; c) 0.2 μm membrane with and without an activated charcoal (AC) bed; d) 0.45 μm membrane at different TMP; e) 0.8 μm membrane at different TMP; and, f) experiments with pH=2.5 and pH=0.8 in several MF membranes.

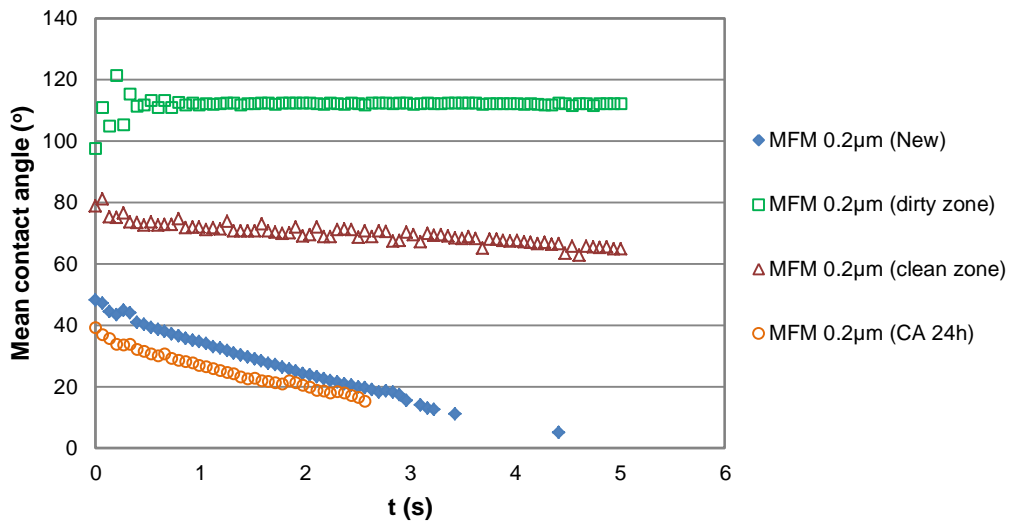


Figure 7.5: Comparison of the contact angles in a 0.2 μm membrane surface: new membrane, membrane used and cleaned by two cleaning cycles (clean and dirty zones), and membrane used and cleaned by immersion for 24 h in activated charcoal water dispersion.

7.4.1.- MFI-UF

The Modified Fouling Indexes (MFI-UF) were estimated by Eq. 2 and are depicted in Fig. 7.6.

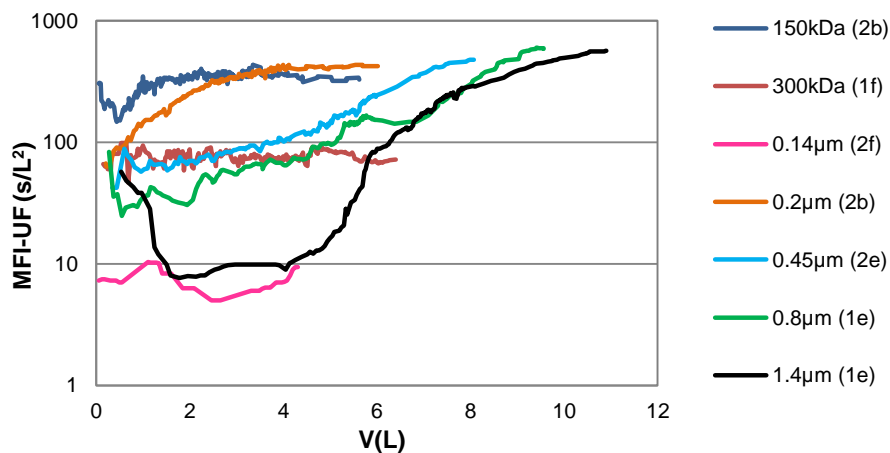


Figure 7.6. MFI-UF vs. the cumulative permeate volume for seawater filtration with different membranes.

MFI-UF is an indicator of the membrane fouling speed, reaching constant values when a stable cake layer is formed on the membrane surface. From that moment, MFI-UF can be used to quantify the fouling level of different membranes and will help to discriminate the membrane selection. As expected, larger cumulative permeate volumes were required with the MF than with the UF membranes to achieve stable MFI-UF values. As observed in Fig. 7.6, for experiments with the same seawater (same letter in the legend) MF membranes reached constant MFI-UF values higher than those of UF (0.2 μm (2b) versus 150 kDa (2b)), which indicated higher levels of fouling in the former. However, the comparison of MFI-UF values of 300 kDa and 0.14 μm membranes can yield incorrect results, because the seawater sample (sample f) in those experiments was not as dirty as the other samples and the time of experimentation with the 0.14 μm membrane was insufficient to reach stable cake. Fig. 7.7 shows the filtration curves according to Eq. 1. In this figure, two regions are observed, a curved region followed by a straight region corresponding to cake filtration. The critical point between

both regions matches the beginning of the stable MFI-UF region in Fig. 7.6. It can be seen that the membranes required a higher volume of filtering for stable formation of caking as the pore size increased.

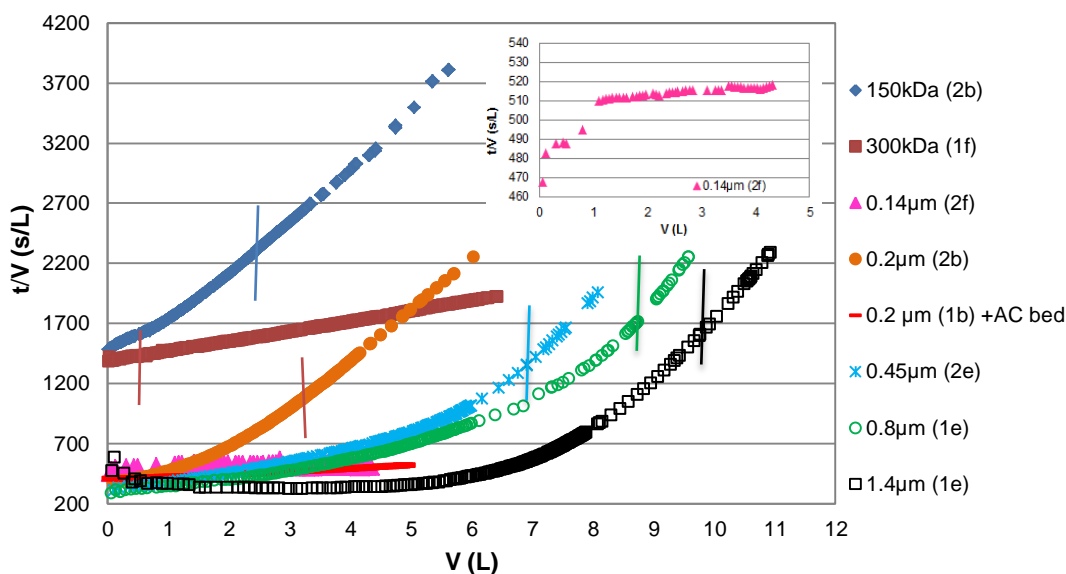


Figure 7.7. Plot of t/v vs the cumulative permeate volume showing the filtration curves for membranes with different pore sizes.

The AC bed effect is compared in Fig. 7.8 with other experiments using the same seawater. As expected, the constant MFI-UF value was lower and was reached faster with the 150 kDa UF membrane than with the 0.2 μm MF membrane. Besides, a very low MFI-UF value was observed for the AC bed experiment, because of its protector effect on the membrane. The AC bed adsorbed organic compounds that caused membrane fouling, with no resistance to the permeate flux. This result corroborates the advantages of working with an AC bed on MF membranes that facilitates the rapid formation of a porous cake and decreases membrane fouling, resulting in shorter cleaning treatment. However, other effects, such as component rejections, must be analyzed for the purpose of this work.

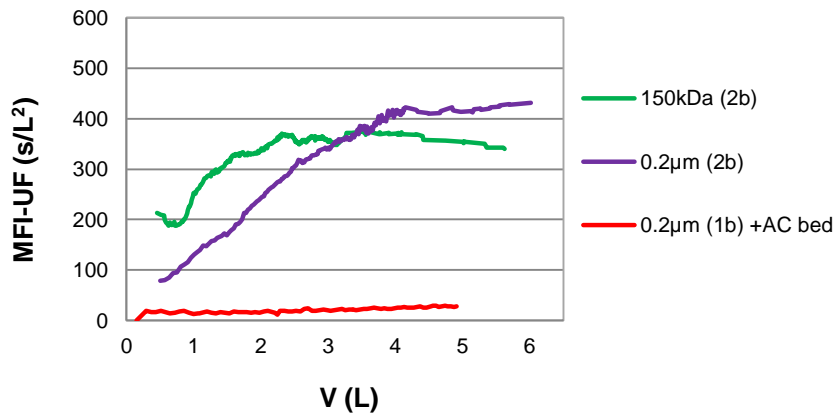
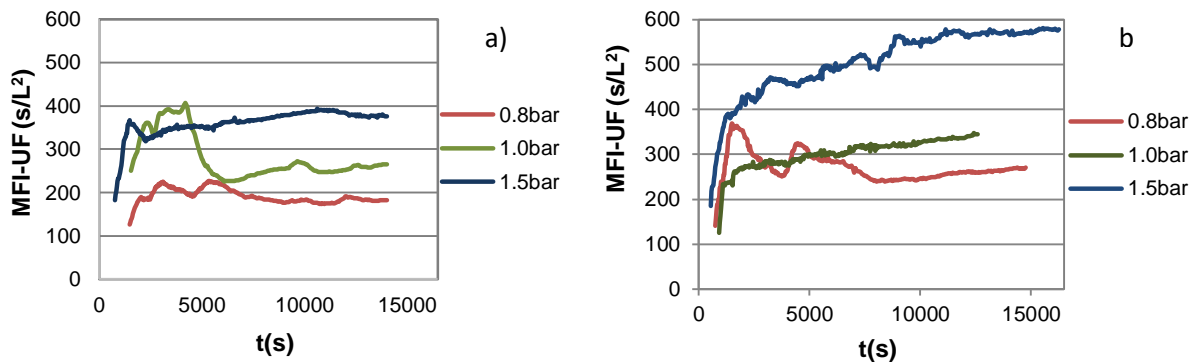


Figure 7.8. MFI-UF vs. the cumulative permeate volume for seawater filtration with different membranes: 150 kDa, 0.2 µm, and 0.2 µm with an activated charcoal bed.

The effect of the pressure on the cake is shown in Fig. 7.9. Significant differences of MFI —UF values with the TMP are observed for both UF and MF membranes, revealing that the cake is compressible [19]. Moreover, as it is shown in Fig. 7.9d, MFI-UF values were significantly larger (about ten times) in the 0.8 µm membrane than in the 0.45 µm (Fig. 7.9c), for all the TMP tested; which can be due to a higher particles concentration in the cake on the 0.8 µm membrane, as the filtered volume to reach a MFI UF constant value is also higher. The compressibility factor was determined from a log plot of the fouling index (I calculated with Eq. 2) and the TMP of each experiment. Fig. 7.10 shows a cake compressibility factor well above zero (between 2 and 3).



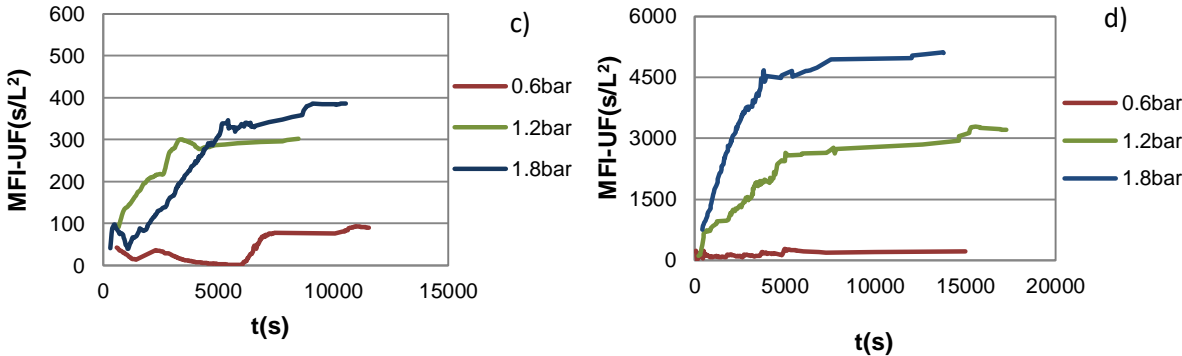


Figure 7.9. Effect of the TMP on the MFI-UF for seawater dead-end mode filtration at 20 °C by the following membranes: a) 150 kDa (exps. 5, 6 and 7) b) 300 kDa (exps. 10, 11 and 12) c) 0.45 µm (exps. 24, 25 and 26) and d) 0.8 µm (exps. 31, 32 and 33).

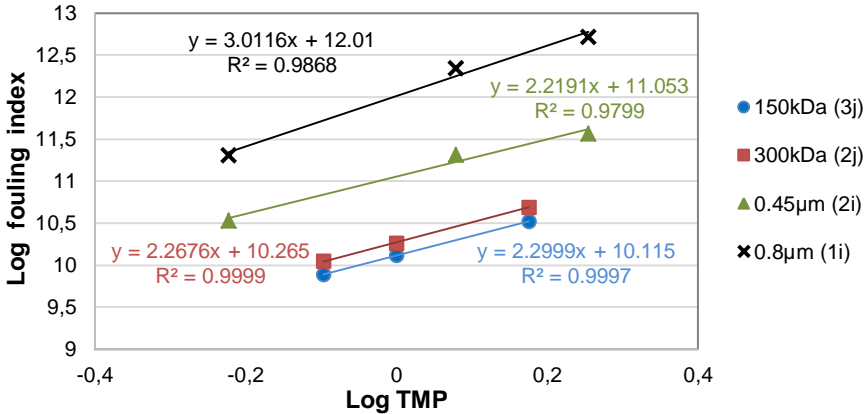


Figure 7.10. Log fouling index (*I* in Eq. 2) vs. the log of transmembrane pressures.

7.4.2.- Effect of the membrane type on permeate quality

The main parameters affected by the filtration processes were turbidity, alkalinity, chemical oxygen demand (COD), and chlorophyll content, while pH and conductivity hardly varied. The permeates under analysis corresponded to samples collected at the end of the filtration time, after cake formation. The reduction of the control parameters is shown in Fig. 7.11. The results are in agreement with those found in the literature,

pointing to increased quality of the permeates due to cake formation on the membrane [22, 35–39].

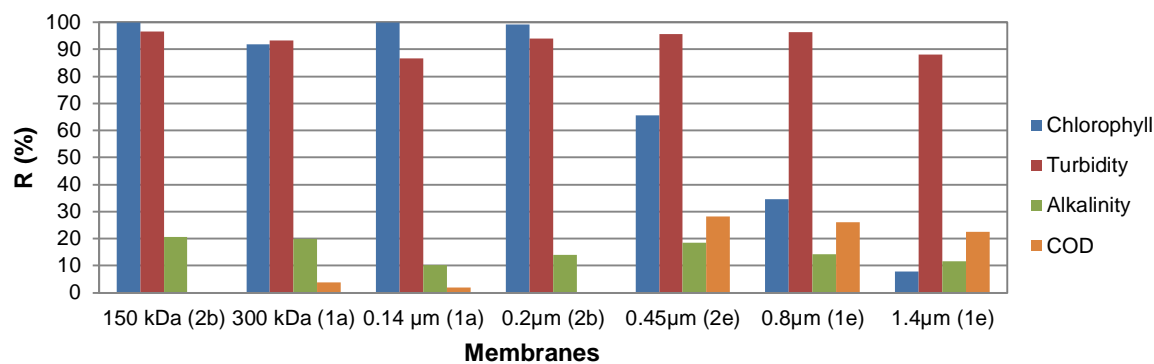


Figure 7.11. *Reduction of chlorophyll content, turbidity, alkalinity and COD in seawater filtration with several MF and UF ceramic membranes.*

A comparison of experiments with the same seawater (150 kDa and 0.2 μm; 300 kDa and 0.14 μm; and 0.45, 0.8 and 1.4 μm) showed higher reduction degrees as the membrane pore size decreased. Reduction of turbidity was higher than 85% for all the membranes, whilst chlorophyll content reduction was significantly lower in the MF than in the UF membranes. Reductions in COD and alkalinity in the former were lower than 30% and 20%, respectively.

7.4.3.- Effect of filtration conditions on heavy metal rejection

Three dead-end filtration experiments were conducted on seawater (feed c) to which Pb^{2+} and Cd^{2+} had been added, in order to study membrane rejection to heavy metals, using membranes of 150 kDa, 0.2 μm, and 0.2 μm with an AC bed. Permeate samples were collected at the end of the filtration time, after stable cake formation. The results are shown in Table 7.2: a 2-4% rejection of Pb^{2+} and Cd^{2+} ions by the 150 kDa membrane, and null rejection by the 0.2 μm ones, with and without the AC bed. The results confirmed the suitability of these membranes, especially the 0.2 μm MF

membrane with the AC bed due to low fouling, for the detection of heavy metals in their permeates when the heavy metals content in the seawater was of a molar order. However, the high-heavy metal content could mask the interactions between the metallic ions and ceramic membranes, so the following experiments were performed at trace level with unmodified seawater (no addition of metals), using 0.45; 0.8 and 1.4 μm MF membranes. Based on the results in Table 7.2 showing very similar rejections of Pb^{2+} and Cd^{2+} , only Pb^{2+} is analyzed in the following experiments. The results are shown in Table 7.3.

Table 7.2. Rejection of Pb^{2+} and Cd^{2+} ions at the cake filtration period (permeate final) by three membranes: 150 kDa, 0.2 μm , and 0.2 μm with AC bed. Feed corresponds to the same seawater (sample c) to which Pb^{2+} and Cd^{2+} were added. Experiments in dead-end mode filtration are specified in Table 7.1. SD: Standard Deviation.

Exp	Membrane	Sample	Pb^{2+} (M) $\cdot 10^5$	SD (M) $\cdot 10^7$	Rejection Pb^{2+} (%)	Cd^{2+} (M) $\cdot 10^6$	SD (M) $\cdot 10^7$	Rejection Cd^{2+} (%)
3	150 Kda (2)	Feed	4.05	4.29	2.44	8.77	1.24	4.25
		P(final)	3.95	11.3		8.39	2.48	
17	0.2 μm (1) +AC	Feed	4.13	4.29	0	9.60	3.80	0
		P(final)	4.15	7.43		9.64	10.2	
18	0.2 μm (1)	Feed	4.05	4.29	0	7.98	6.26	0
		P(final)	4.05	8.58		8.68	6.26	

The interpretation of the results shown in Table 7.3 means giving consideration to the state of the membrane at the particular moment of filtration, i.e. the electric charge of the membrane and the fouling level that takes place. Permeate samples collected in the final period, after cake formation, yielded rejections of Pb^{2+} in the range of 52–70% for the three membranes, however, rejection of Pb^{2+} was higher in the permeate bulk, i.e. the volume of permeate accumulated throughout the experiment. These results and the others shown below indicate that most of the Pb^{2+} ions were retained in the period prior to cake formation, due to electrostatic interactions with the negatively charged membrane, which continues to have an effect on Pb^{2+} trace ions even during the cake

filtration period. Such results indicated that the negative charge of the membrane was the main effect that controlled the rejection rates of heavy metals in trace concentrations. The time needed for the saturation of membrane active sites where the rejection would be practically null was not determined in this study, as seawater samples were not available for long-term experiments.

Table 7.3. *Rejection of Pb²⁺ ions by several MF membranes. Feed: real seawater with Pb²⁺ ions at trace level (19.8 ·10⁻⁹ M of Pb²⁺). Experiments in dead-end mode filtration, at 20 °C and 1.7 bar TMP. Permeate samples were collected during the cake filtration period (final) and from permeate bulk (bulk) when filtration ended. SD: Standard Deviation.*

Exp.	pH	Membrane	Permeate Pb ²⁺ (M) ·10 ⁹	SD (M) ×10 ⁹	Rejection of Pb ²⁺ (%)
23	7.744	0.45 μm (2)	8.61	3.30	56.51 (final)
30	7.567	0.8 μm (1)	9.59	3.04	52.05 (final)
36	7.536	1.4 μm (1)	6.01	2.54	69.76 (final)
36	7.455	1.4 μm (1)	0.55	0.17	97.57 (bulk)

The effect of membrane charge was studied using synthetic seawater (formulation given in [40]) to which 120·10⁻⁹ M of Pb²⁺ had been added and the pH modified by the addition of 0.1M HCl. Assays were performed with a 0.45 μm MF membrane and a 300 kDa UF membrane. An additional assay using the 0.45 μm membrane on real seawater was performed, to which Pb²⁺ (120·10⁻⁹ M) had also been added after analytically checking its absence in the seawater sample. Experiments were performed by filtering 1 L of feed in the dead-end filtration mode at a pressure of 2 bar. Fig. 7.12 shows the significant effect of the membrane charge on the rejection of Pb²⁺. A similar behavior

was observed in the two membranes both on synthetic seawater (Fig. 7.12a) and on real seawater (Fig. 7.12b). At a pH greater than the isoelectric point (IP), the membranes became negatively charged and electrostatic attraction forces caused the retention of Pb^{2+} ions and membrane fouling. At $pH < IP$, the membranes were positively charged and exerted repulsive forces towards the positively charged ions. However, as filtration was running in dead-end flow mode, forcing permeation through the membrane because the retentate valve was totally closed, the passage of Pb^{2+} ions through the positively charged membrane pores occurred without any retention, at a sufficiently low pH, as shown in Fig. 7.12a. The IP of the membranes had a net charge of zero and there was no electrostatic effect on the retention of ions in solution. IP values, corresponding to 50% rejections of Pb^{2+} of 3.5 and 4.5 for the ZrO_2 UF membrane and the TiO_2 MF membrane, respectively, can be observed in Fig. 7.12a.

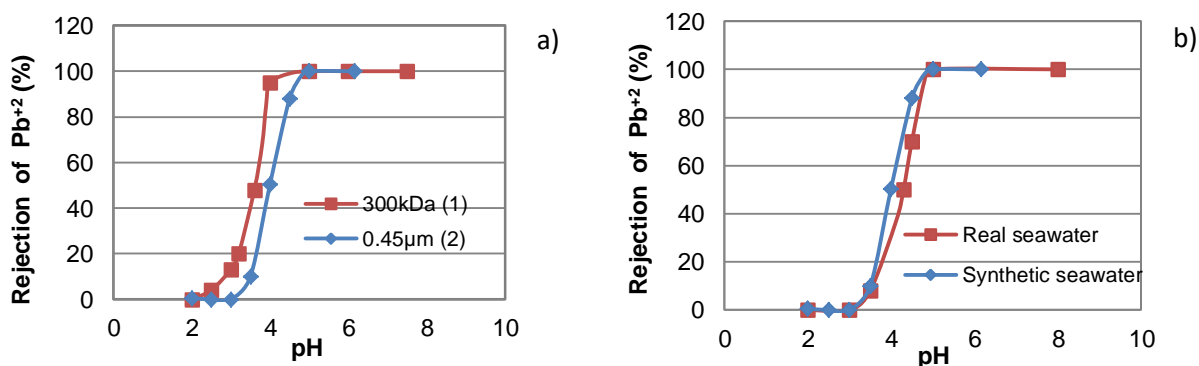


Figure 7.12. Effect of the pH on the rejection of Pb^{2+} ions by 0.45 μm and 300 kDa ceramic membranes. Experiments in dead-end mode filtration, at 20 °C and 2 bar. A) Synthetic seawater with $120 \cdot 10^{-9}$ M of Pb^{2+} added, b): Comparative values for the 0.45 μm membrane on synthetic and real seawater, both with $120 \cdot 10^{-9}$ M of Pb^{2+} added.

The contribution of cake to Pb^{2+} rejection was analyzed with the results shown in Table 7.4, performed in dead-end mode filtration on seawater with Pb^{2+} added in trace concentrations, using the 0.45 and 0.8 μm MF membranes, at 20 °C and TMP of 0.6, 1.2 and 1.8 bar. Permeate samples were taken at the beginning (after 30 min) and at the end

of the process (after the cake layer formation). The results indicated only very slightly higher Pb^{2+} rejection during the final period than at the initial period of filtration (about 3%, with the exception of exp. 24 with a 20% difference), which shows that the cake layer also contributed, but to a much lower extent than the membrane charge, to Pb^{2+} rejection at trace level.

7.4.4.- Effect of filtration conditions on okadaic acid rejection

Rejection rates of OA by several membranes at different pressures are depicted in Fig. 7.13. Permeate samples were collected in the final period and at the beginning (30 min elapsed) of all the experiments with the MF membranes, (data shown in Table 7.4). Positive rejections of OA were obtained at low pressure and negative rejections at high pressure due to cake compressibility. Moreover, significant differences of OA rejections were obtained according to the moment at which the permeate sample were collected, as is shown in Fig. 7.13 c and d. These results indicated that OA rejection depended on the membrane pore size and cake height at the particular moment of the filtration process. A detailed study with MF membranes at low pressures revealed that the OA rejection was independent of the time at which the permeate samples were collected and was lower with the 0.45 μm membrane than with the 0.8 μm membrane. At 1.2 bar TMP, both MF membranes showed significant differences between OA rejection measured at the beginning and at the end of the filtration period, which were even more pronounced at 1.8 bar TMP, with negative rejections in the final filtration period (and high values in the MFI-UF). This fact reveals that OA retention mainly occurred in the compressible cake, which accumulated OA until reaching a level at which it was released by the effects of pressure. The dynamic morphology of caking was described in Akhondi et al. (2015) i.e. [22], its structure (thickness, porosity, roughness etc.) and its composition both change over time, within reasonable values, but with constant global resistance to the permeate flux throughout the duration of the cake filtration mechanism. Cake compression leads to the modification of its morphology and the release of OA due to the rupture of bonds under pressure.

Table 7.4. Effect of the TMP on the rejection of Pb^{2+} and okadaic acid (OA) by the 0.45 and 0.8 μm MF membranes. Experiments in dead-end mode filtration at 20 °C Feed: seawater with natural content of OA and Pb^{2+} added. SD: Standard Deviation.

Membrane	Exp	Sample	PTM (bar)	Pb^{+2} (M) $\cdot 10^9$	SD (M) $\cdot 10^9$	Rejection of Pb^{+2} (%)	OA (M) $\cdot 10^{10}$	SD (M) $\cdot 10^{10}$	Rejection of OA (%)
0.45 μm (2)	24	Feed (i)	0.6	108.84	10.13	-	6.02	0.25	-
		P(30min)		35.33	1.12	67.54	3.77	0.65	37.37
		P(final)		7.40	1.51	93.20	3.99	0.47	33.80
	25	Feed (i)	1.2	170.63	13.49	-	6.02	0.25	-
		P(30min)		8.72	3.42	94.89	3.91	0.42	35.04
		P(final)		5.33	0.93	96.88	2.21	0.08	63.31
	26	Feed (i)	1.8	112.89	16.08	-	6.02	0.25	-
		P(30min)		15.15	5.02	86.58	3.71	0.22	38.50
		P(final)		9.29	1.04	91.77	6.47	0.54	-7.41
0.8 μm (1)	31	Feed (i)	0.6	175.13	20.21	-	6.02	0.25	-
		P(30min)		19.24	3.52	89.01	2.85	0.23	52.77
		P(final)		16.52	2.71	90.57	2.93	0.44	51.31
	32	Feed (i)	1.2	124.10	33.20	-	6.02	0.25	-
		P(30min)		13.28	2.38	89.30	3.28	0.93	45.50
		P(final)		5.40	1.29	95.65	4.22	0.95	29.92
	33	Feed (i)	1.8	127.76	18.19	-	6.02	0.25	-
		P(30min)		20.37	2.63	84.06	3.40	0.25	43.52
		P(final)		8.71	0.79	93.18	10.40	0.91	-72.75

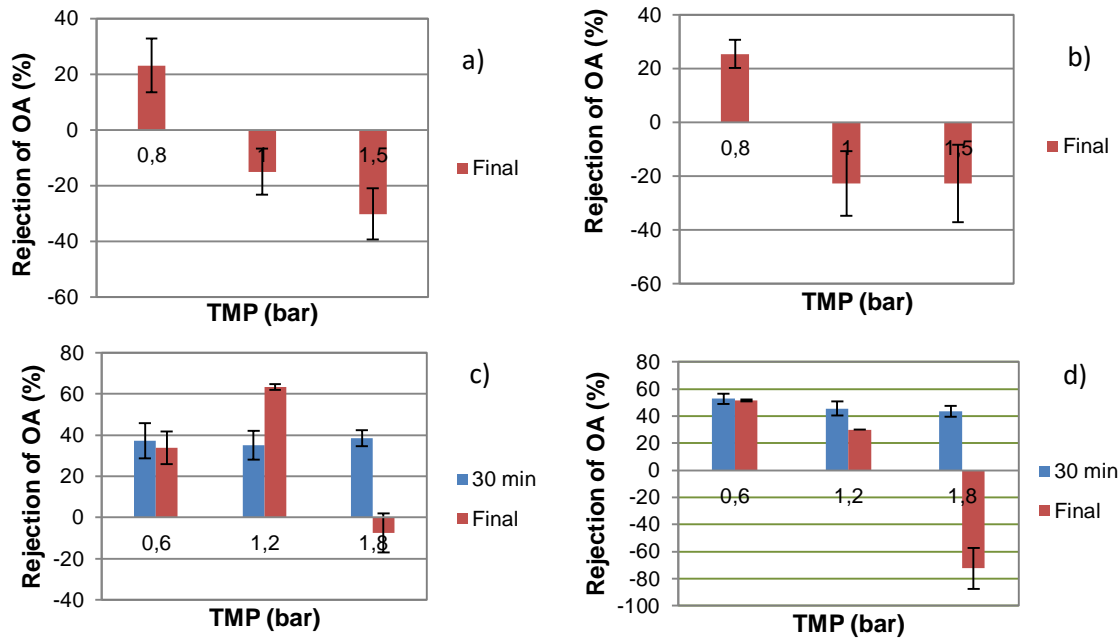


Figure 7.13. Rejection of okadaic acid by the following membranes: a) 150 kDa (exps. 5, 6 and 7) b) 300 kDa (exps. 10, 11 and 12) c) 0.45 μm (exps. 24, 25 and 26) and d) 0.8 μm (exps. 31, 32 and 33). Experiments in dead-end mode filtration at 20° C and at different pressures.

Table 7.5 shows OA rejections by several membranes on seawater samples with different concentrations of OA. All the permeates corresponded to samples collected at the final filtration period, with the exception of exp. 27 for which permeate was also collected at the beginning (30 min elapsed). The results showed that OA rejection was similar for experiments with the 150 kDa and 0.2 μm membranes and the same seawater (exps. 2 and 15, and exps. 4 and 19), but depended on the dirtiness of the seawater and its OA content, as the higher the OA concentration in the seawater, the lower the rejection rate. Moreover, it is remarkable that the 0.45 μm membrane showed high OA rejection rates (about 30% for exp. 23), despite the TMP of 1.7 bar. This result is significantly different from the expected one (negative rejection at 1.8 bar TMP in exp. 26 of Table 7.4), and can be attributed to insufficient pressure for OA release, taking into account the differences in seawater quality in both experiments. Table 7.5 also shows negative rejections for exp. 30 and 36, which are in accordance with those of

Table 7.4, thereby corroborating the OA released from the compressible cake under pressure.

The data of exp. 27 at a low TMP corroborated the retention of OA in appreciable amounts (40%) at the beginning of the filtration time, but it is also inferred that OA was mainly retained in the cake, because the rejection in the final period (80%) was higher than at the beginning. It should be noted that OA is at trace level in the seawater and can be significantly linked to the few particles and NOM retained by the membrane at the start of filtration process.

Table 7.5. *Rejection of okadaic acid (OA) by several MF and UF ceramic membranes. Experiments in dead-end mode filtration at the indicated PTM and at 20 °C. All permeates were taken at the end of the filtration period after cake formation, with the exception of exp. 27 for which permeates were taken at the beginning and at the end of the final filtration period, as specified.*

Exp.	Membrane	TMP (bar)	Sample	OA (M) · 10 ¹¹	SD (M) · 10 ¹¹	OA rejection (%)
-	-	-	Feed (b)	12.70	4.65	-
2	150 kDa (2)	1.7	P(final)	7.21	1.26	31.17
15	0.2 µm (2)	1.9	P(final)	9.01	2.01	29.24
16	0,2 µm (1) + AC bed	1.7	P(final)	8.76	1.02	43.38
-	-	-	Feed (d)	53.20	5.62	-
4	150 kDa (2)	1.7	P(final)	42.30	4.23	20.46
19	0.2 µm (3)	1.9	P(final)	43.00	3.69	19.09
-	-	-	Feed (e)	8.70	1.32	-
23	0.45 µm (2)	1.7	P(final)	6.21	1.01	28.57
30	0.8 µm (1)	1.7	P(final)	13.70	2.36	-57.14
36	1.4 µm (1)	1.7	P(final)	11.20	2.84	-68.57
-	-	-	Feed (d)	53.20	5.62	-
27	0.45 µm (2)	0.8	P(30 min)	32.01	3.00	39.83
			P(final)	28.25	0.14	46.89

The comparison between expts. 15 and 16 in Table 7.5 shows higher rejection rates of the latter, due to OA adsorption in the AC bed on the membrane surface. Adsorption of OA on AC was confirmed by supplementary equilibrium experiments (not shown) through direct contact of seawater (sample d) with AC, yielding 93.25% OA adsorption. These results invalidated the initial proposal to work with an AC bed on the membrane surface.

Additional experiments, to check the effect of the membrane on OA retention, were performed using a solution of $5 \cdot 10^{-9}$ M OA in bi-distilled water and the 0.45 and 0.8 μm MF membranes. Experiments were run in total recirculating mode at 0.8 bar TMP. Rejections of about $10 \pm 5\%$ were obtained for the two membranes. These results might be due to the supra-molecular structures formation or precipitates of OA, as adsorption of OA on strongly hydrophilic membranes, such as the used in this work, is unlikely. According to some works [38-41], adsorption is not to be expected for hydrophilic membranes, unless the solute and the membrane have opposite charges. It may therefore be concluded that OA retention will be negligible in the absence of cake. Finally, the effect of the membrane charge on OA retention was checked in expts. 20–21, 28–29, and 34–35 using the 0.2, 0.45 and 0.8 μm MF membranes and the same seawater sample at its natural pH (pH=8), and acidified with HCl additions (pH=2.5). Experiments were performed in dead-end mode under 1 bar TMP. Permeates were collected at the end, when the cake layer had formed. At the natural pH, the OA was mainly in an anionic form (pKa=3.1, estimated using program [42] (MarvinSketch, 2017)), and could undergo electrostatic repulsion with the negatively charged membrane. However, Fig. 7.14 shows OA rejections that are independent of the pH and therefore independent of the membrane charge.

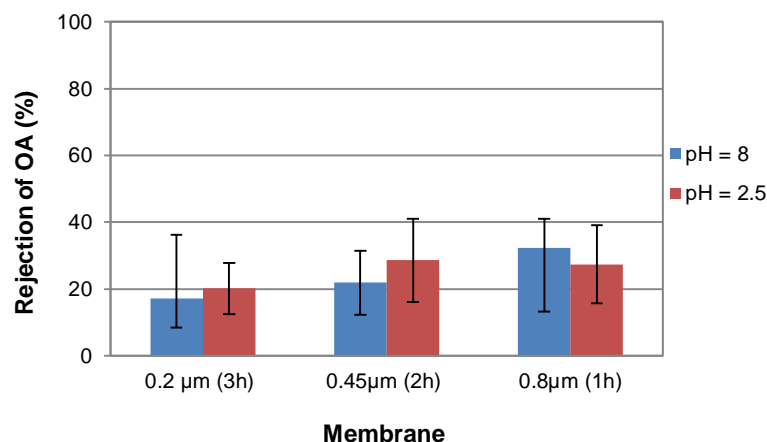


Figure 7.14. Rejection of okadaic acid by several MF membranes in seawater samples with different pH (exps. 20-21, 28-29, and 34-35 of Table 1 under 1 bar TMP).

7.5.- Conclusions

In this study, the performance of several TiO₂ microfiltration (MF) and ZrO₂ ultrafiltration (UF) membranes, have been described in relation to seawater filtration in which okadaic acid (OA) and heavy metal ions (Pb²⁺ was selected as model) can be present at trace levels. The following relevant conclusions were obtained:

The MFI-UF index, to the extent that it reaches stable values under cake filtering mechanisms, has shown itself to be a useful tool for the comparison of membrane fouling levels. UF membranes have a lower MFI-UF than MF, requiring fewer cleaning cycles for permeability recovery. The MFI-UF values varied with the pressure indicating that cake is compressible with a compressibility factor of between 2 and 3.

Rejection of OA was negligible in the absence of cake, nevertheless it depended on the cake height, and transmembrane pressure. OA retention was due to hydrophobic interactions with particles and NOM on the membrane surface. Rejection of OA was low in experiments with low MFI-UF, so it is advisable to use low pressure UF. Cake

compressibility was relevant in the 0.8 μm MF membranes at a TMP higher than 1.7 bar, resulting in negative rejections which provides the accumulation of OA in permeate and therefore facilitates its detection in seawater. The results have shed light on the design of a submerged filter for the seawater filtration surrounding mussel rafts. The use of this type of filters will allow the detection of OA at trace levels and could act as an early warning system for the detection of the risk of toxic episodes directly related to the presence of OA.

Rejection of Pb^{2+} was independent of the membrane type. It was verified that the negative charge of the membrane is the main effect that controls the retention of Pb^{2+} ions when they are in trace levels. In this regard, zero rejections of Pb^{2+} were obtained when the pH of the samples was lower than the isoelectric point of the membranes. However, membrane rejection of Pb^{2+} depended on its concentration, being negligible in the molar order range, because saturation of the membrane charge occurs.

7.6.- Symbols

Symbols	Full name
A	Area of the membrane (m^2)
C_b	Concentration of particles in the feedwater (kg/m^3)
$C_{i(F)}$	Solute concentration in the feed (mol/L)
$C_{i(p)}$	Solute concentration in the permeate (mol/L)
CWF	Compared with the initial measurements with the new membrane ($\text{L}/\text{m}^2\text{h}$)
CWF^N	Ratio of both yields the normalized clean water flux (%)
I	Fouling index in Eq. 2 (m^{-2})
J_w	Water flux in the clean membrane (m/s)
K	Phenomenological coefficient for dead-end filtration ($(\text{s}/\text{L})^{n-2}$)
L_p	Membrane permeability ($\text{m}/\text{s bar}$)
n	Characteristic model constant (dimensionless)
ΔP	Transmembrane pressure (bar)
Q_T	Clean water flow at temperature T (L/h)
R_i	Rejection of solute (%)
t	Filtration time (s)

V	Permeate volume (L)
w	Compressibility factor
μ_T	Water viscosity at temperature T (Pa·s)
α	Specific resistance of the cake (m/kg)

7.7.- References

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*8.- Comparison of backing materials of screen printed electrochemical sensors
for direct determination of the sub-nanomolar concentration of lead in
seawater*

En la introducción se han descrito los efectos perjudiciales de la presencia de plomo en el agua de mar. Concretamente, su detección al nivel subnanomolar es muy importantes para la industria del mejillón, por el efecto tóxico que produce en las personas el consumo de este molusco contaminado con plomo.

En este capítulo, se presenta un método electroquímico con voltamperometría de redisolución anódica con sensores desechables serigrafados para la determinación de Pb en concentraciones de niveles subnanomolares.

El rendimiento del sensor se optimizó para la determinación de Pb a través de varias modificaciones de superficie, mediante el uso de nanotubos de carbono de pared simple, óxido de grafeno electroreducido y nanopartículas de oro. La deposición de nanopartículas de oro electrogeneradas de varios tamaños sobre la superficie del electrodo de trabajo se estudiaron con microscopía electrónica de barrido e indicaron que los mejores resultados fueron observados cuando el tamaño de las nanopartículas esféricas sobre el electrodo de trabajo era de 100 nm. La modificación de los electrodos de trabajo con nanopartículas de grafeno y oro permitió la determinación de Pb^{2+} en agua de mar sin modificar el pH de la muestra. El límite de detección obtenido fue de $3.21 \cdot 10^{-10}$ M.

Los sistemas de electrodos se imprimieron sobre materiales de soporte tanto rígidos como textiles, para observar la influencia de esos materiales en el rendimiento final del sensor.

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8.1.- Introduction

The presence of pollutants in the environment has increased in recent times, as a consequence of industrial development, caused mainly by factory waste discharged as effluent into seawater, degrading the environmental quality of coastal waters [1,2]. It is a serious issue for flora and fauna in contaminated ecosystems. Some of the heavy metal pollutants, among which lead (Pb), present high toxicity, low biodegradability and accumulate in the bodies of living beings.

In addition to causing anemia and deformities in marine animals [3] the presence of Pb can also jeopardize mussel-farm production and result in economic losses. Although mussels have a detoxification mechanism, the consumption of these bivalves can produce Pb intoxication in humans, because Pb can accumulate in bones, kidneys, muscles, and the brain, as well as causing damage to the immune, reproductive, and central nervous systems [4–6].

European Directive (2013/39/EU) states that the average annual value of lead concentration in seawater is 1.3 µg/L.

Several analytical techniques have been employed for the determination of sub-nanomolar Pb concentrations in natural samples, and the presence of other heavy metals in freshwater rivers and lakes and in seawater [7]. Spectrometry techniques are widely used in the detection of heavy metals. These techniques include flame atomic absorption spectrometry (FAAS) [8], electrothermal atomic absorption spectrometry (ETAAS) [9], graphite furnace atomic absorption spectrometry (GFAAS) [10–12], inductively coupled plasma atomic emission spectrometry (ICP-AES) [13], inductively coupled plasma mass spectrometry (ICP-MS) [14,15] and atomic fluorescence spectrometry (AFS) [16]. There is increasing interest in the use of electrochemical techniques for determining trace concentrations of pollutants, particularly the use of stripping voltammetry techniques, due to their high sensitivity, that are commonly used for quantitative analysis of

electroactive species, such as heavy metal ions [17]. High metal traces have usually been studied with mercury-based electrodes, such as hanging mercury drop electrodes [18] and mercury film electrodes [19]. That choice is due to their high sensitivity, reproducibility and high hydrogen overpotential of the mercury electrodes, as well as the possibility of amalgam formation with heavy metals [20]. However, these electrode types are now replaced by mercury-free electrodes, because of the high toxicity of that element. Studies have focused on the use of gold electrodes [21], glassy carbon electrodes [22], silver electrodes [23], and modifications of the electrode surface by bismuth-coated [24], metal nanoparticles [25], and carbon nanostructures [26], such as graphene [27] and carbon nanotubes [28].

There is a growing need in the industrial sector to find a simple system for on-site determination of analytes [21]. Such a system would circumvent sample transportation and thereby avoid the risk of possible contamination. One of the most suitable ways to achieve that effect is through the use of screen printed electrodes (SPEs), due to their highly simplified reproducibility in serial production and inherent characteristics, such as miniaturization, and their low cost, making them a preferred choice [29]. Their small size and the integrated system of three electrodes in a single piece make SPEs ideal devices for on-site electrochemical determination when coupled to a portable potentiostat. In addition, flexible materials and textile fabrics been introduced as backing materials have over recent years in the development of screen printing systems, for the production of electrochemical sensors as usable wearable devices [30,31] and energy storage devices [32,33].

Determination of Pb in seawater has also been intensely studied using anodic stripping voltammetry (ASV) and several methods have been developed using different electrodes. However, to the best of our knowledge, to date there are only a few studies on ASV detection of Pb without a conditioning process or pH adjustment of the samples in a matrix as complex as seawater (Table 8.1). Considering the recent interest in the development of wearable sensors, the main objectives of this paper are, firstly, to study

the influence of backing materials in sensor performances and, second, to determine the best modification of an electrode surface from among all those performed in this study for improving in situ Pb determination in seawater.

8.2.- Experimental

8.2.1- Reagents and solutions

All the chemicals were of analytical grade and were used as received without further purification. High-quality water (Milli Q A10 system, Millipore, Bedford, USA) was employed for preparing all solutions.

Pb(NO₃)₂, HCl and H₂SO₄ were supplied by Merck (Darmstadt, Germany). AuCl₃·3H₂O, single-walled carbon nanotubes (SWCNT) and graphene oxide dispersion in water (4 g/L) and 1,2-dichloroethane (DCE) were purchased from Sigma-Aldrich (Steinheim, Germany).

C10903P14 (carbon/graphite ink) and D2071120D1 (dielectric ink) were purchased from Gwent Electronic Materials (Torfaen, U.K). Electrodag 6037 SS (Ag/AgCl ink), and Electrodag 418 (Ag ink) were supplied by Acheson Colloiden (Scheemda, Netherlands).

Gore-Tex® Pro-Shell Nylon Liner and 2-Layer Gore-Tex® Paclite® Shell Ripstop Nylon fabrics were supplied by Rockywoods Fabrics, LLC (Loveland USA).

500 µm thickness polyester film (PMX981) was supplied by HiFi Industrial film (Dardilly, France).

Table 8.1. Summary of analytical characteristics of Pb traces determination in seawater samples.

Sample	Electrochemical technique	Preconcentration time	LOD	Electrode material	Reference
Seawater	SWASV	120 s	8.7 nM	Graphite screen, printed electrode modified with mercury film	[34]
Seawater	SWV	60 s	12 nM	Glassy carbon modified with thiol alfa ssembled mono-layers on mesoporous supports and Nafion	[35]
Seawater	SPASV	240 s	0.5 nM	Glassy carbon electrode modified with nanofibrillated cellulose	[36]
Acidified seawater up to 1 M HCl	SWV	-	0.5 nM	Graphite reinforced carbon electrode modified with electroreduced graphene oxide	[37]
Acidified UV-SW	SWASV	60 s	12 pM	Vibrating silver amalgam with Hg microwire electrode	[38]
Seawater	DPAdASV	60 s	4.8 nM	HMDE	[39]
Seawater	DPASV	300 s	0.321 nM	AuNP/ERGO/SPCE	This work

SWASV square wave anodic stripping voltammetry, SWV square wave voltammetry, UV-SW ultraviolet digested seawater, DPAdASV differential pulse adsorptive anodic stripping voltammetry, HMDE hanging mercury drop electrode, DPASV differential pulse anodic stripping voltammetry.

8.2.2.- Instrumentation

Electrochemical measurements were taken with a PalmSens® portable electrochemical potentiostat (PalmSens® Instruments BV, Houten, The Netherlands) controlled by PS Trace software to register the data. All measurements were performed at room temperature and refer to data collected with screen printed Ag/AgCl pseudoreference electrodes.

Inductively coupled plasma mass spectrometry analyses were carried out with ICP-MS Agilent Serie 7500 (Agilent Technologies, California, U.S.A).

Solution pH was measured with an HI 221 pH meter (Hanna Instruments, USA).

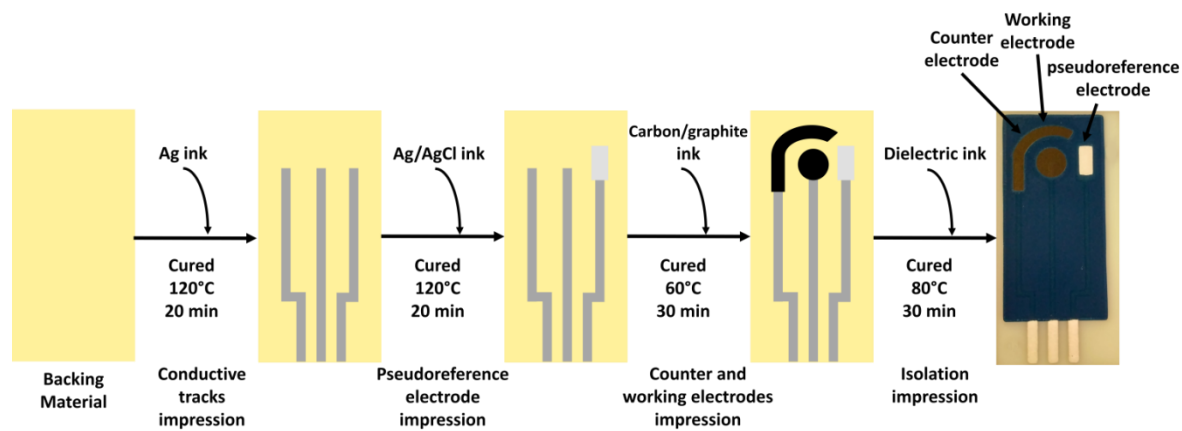
Screen printed carbon electrodes (SPCEs) were fabricated on a DEK 248 screen printer (DEK, Weymouth, UK).

8.3.- Methods

8.3.1.- Sensor manufacturing

The SPCEs used in this study were produced in the laboratory by sequential deposition of different commercial inks, following the procedures described in a previous work [40]. The screen-printing process followed the steps shown in (Scheme 8.1). First, a silver ink (Electrodag 418) was printed on the support materials and the conductive tracks were defined. Then, Ag/AgCl ink (Electrodag 6037 SS) was printed and cured to define pseudoreference electrode. Subsequently, carbon/graphite ink (C10903P14) was used to print the counter and the working electrode. Finally, printing with a dielectric ink insulated the conductive circuit and defined the geometrical area of the electrodes (geometrical working area, 12.56 mm²). The electrodes were printed on the backing materials: polyester film and two textile fabrics. Fabrics based on Gore-Tex® were selected for this purpose, because it is suitable as an electrodic support to develop

screen-printed sensors, due to its inherent properties as a waterproof, inert, and resistant material.



Scheme 8.1. *Scheme of the screen-printed process step by step.*

8.3.2.- Single-walled carbon nanotubes modified SPCE (SWCNT/SPCE) preparation

The working electrode surface was modified with a thin film of SWCNT. A volume of 5 μL of stock solution was drop-casted on the working electrode surface and the solvent was left to evaporate at room temperature. The stock solution was prepared by dispersing 0.5 mg of SWCNT in 100 mL of 1,2-dichloroethane and then ultrasonically blending the solution for thirty minutes, to obtain a homogenized dispersion before coating onto the electrode surface.

8.3.3.- Electroreduced graphene oxide modified SPCE (ERGO/SPCE) preparation

The graphene film was generated by electroreduction of grapheme oxide. A commercial solution of graphene oxide was diluted down to 0.2 g/L. The diluted mixture was subjected to ultrasonication for one hour. Once the solution was well-dispersed, 5 μL of

the dispersed solution was deposited onto the electrode surface by drop-casting and it was left to dry at room temperature. Then the graphene oxide modified working electrode surface was electroreduced by cyclic voltammetry scanning in a potential range from -0.20 V to -1.50 V at 0.05 V/s for 5 cycles in 0.10 M KNO_3 solution. Finally, the electrodes were rinsed in water. This graphene generation method was selected, because it needs no special chemical agents and can be produced directly on the electrode surface [41, 42].

8.3.3.1.- AuNP/SPCE, AuNP/SWCNT/SPCE and AuNP /ERGO/SPCE preparation

The gold nanoparticles were electrogenerated in a drop of 200 μL 0.50 M H_2SO_4 containing $1 \cdot 10^{-3}$ M or $5 \cdot 10^{-3}$ M AuCl_3 and placed onto the bare electrode surface, or onto an electrode surface previously coated with SWCNT or ERGO. This process involved a chronoamperometry experiment in which a potential of $+0.18$ V was applied over a certain period of time, in which, the nanoparticles nucleate and evolve increasing in size. Finally, the electrodic system was carefully washed with deionized water.

8.3.4.- Techniques and experimental conditions for lead determination

Two electrochemical methods, square wave anodic stripping voltammetry (SWASV) and differential pulse anodic stripping voltammetry (DPASV), were used for lead determination.

Measurements were performed in a cell containing 5 mL of sample and the electrochemical parameters were optimized, in order to obtain the best sensitivity for Pb in both methods. Stirring conditions were used during the deposition step while the voltammetric signal was captured in quiescent solution.

8.3.4.1.- SWASV measurement conditions

The SWASV measurements were performed in acid media using SPCE without any modification. Pb was electrodeposited under stirring conditions at a potential of -0.80 V for 300 s on the electrode surface in a solution containing PbNO_3 in 4.9 mL deionized water and 0.1 mL

HCl 1 M. The anodic stripping was carried out in a potential range from -1.20 V to -0.20 V under a scan rate of 0.03 V/s. SW amplitude = 0.025 V, SW frequency = 100 Hz. Step potential = 0.003 V.

8.3.4.2.- DPASV measurement conditions

In the case of the DPASV experiments, the working SPCEs were modified with AuNP (AuNP/SPCE), AuNP and SWCNT (AuNP/SWCNT/ SPCE) or Au and ERGO (AuNP/ERGO/SPCE).

The DPASV measurements were performed in acid media, at AuNP/ SPCE. Pb was electrodeposited at a potential of -0.70 V for 300 s on the electrode surface in a solution containing PbNO_3 in 4.9 mL deionized water and 0.1 mL HCl 1 M.

The DPASV measurements were performed in 0.6M NaCl at AuNP/SWCNT/SPCE and at AuNP/ERGO/SPCE. Pb was electrodeposited under stirring conditions at a potential of -0.70 V for 300 s by the reduction of Pb^{2+} on the electrode surface in 5 mL of a 0.6M NaCl solution containing PbNO_3 .

After electrodeposition step, the anodic stripping was performed in a potential range between -0.70 V and 0.00 V (pulse potential = 0.028 V, pulse time = 0.01 s, scan rate = 0.03 V/s and step potential = 0.003 V).

8.4.- Results and discussion

Pb determination was performed with anodic stripping voltammetry, using both differential pulse and square wave techniques. With the aim of developing wearable sensors, the electrodic systems were screen-printed on 2 fabric supports, Gore-Tex® Pro-Shell Nylon Liner and 2-Layer Gore-Tex® Paclite® Shell Ripstop Nylon. Sensor performances built with these backing materials was compared with the performance of sensors that used a rigid polyester film support. To do so, SEM analyses of the different electrode surfaces were performed (Section 8.4.1). Then, the analytical characteristics of the sub-nanomolar Pb determination were determined using different working electrodes, support materials, and two electrochemical techniques: SWASV and DPASV (Sections 8.4.2 and 8.4.3). Finally, Pb²⁺ levels in spiked seawater samples were determined (Section 8.4.4).

8.4.1.- SEM characterization of working electrode surfaces

SEM images of the different working electrodes surfaces were analyzed. Fig. 8.1 a, b and c show the surface of the bare working SPCE on the three support materials under study. The roughness of the electrode surface is visibly caused by the graphite flakes present in the carbon ink. Similar features can be observed on the working electrode surfaces modified with SWCNT (Fig. 8.1 d, e and f). However, the thick ERGO film (Fig. 8.1 g, h and i) smooths over the roughness of the bare electrode surface. As can be observed, electrode surfaces apparently are similar on the three backing materials, both in the case of the bare surface and in the different modifications.

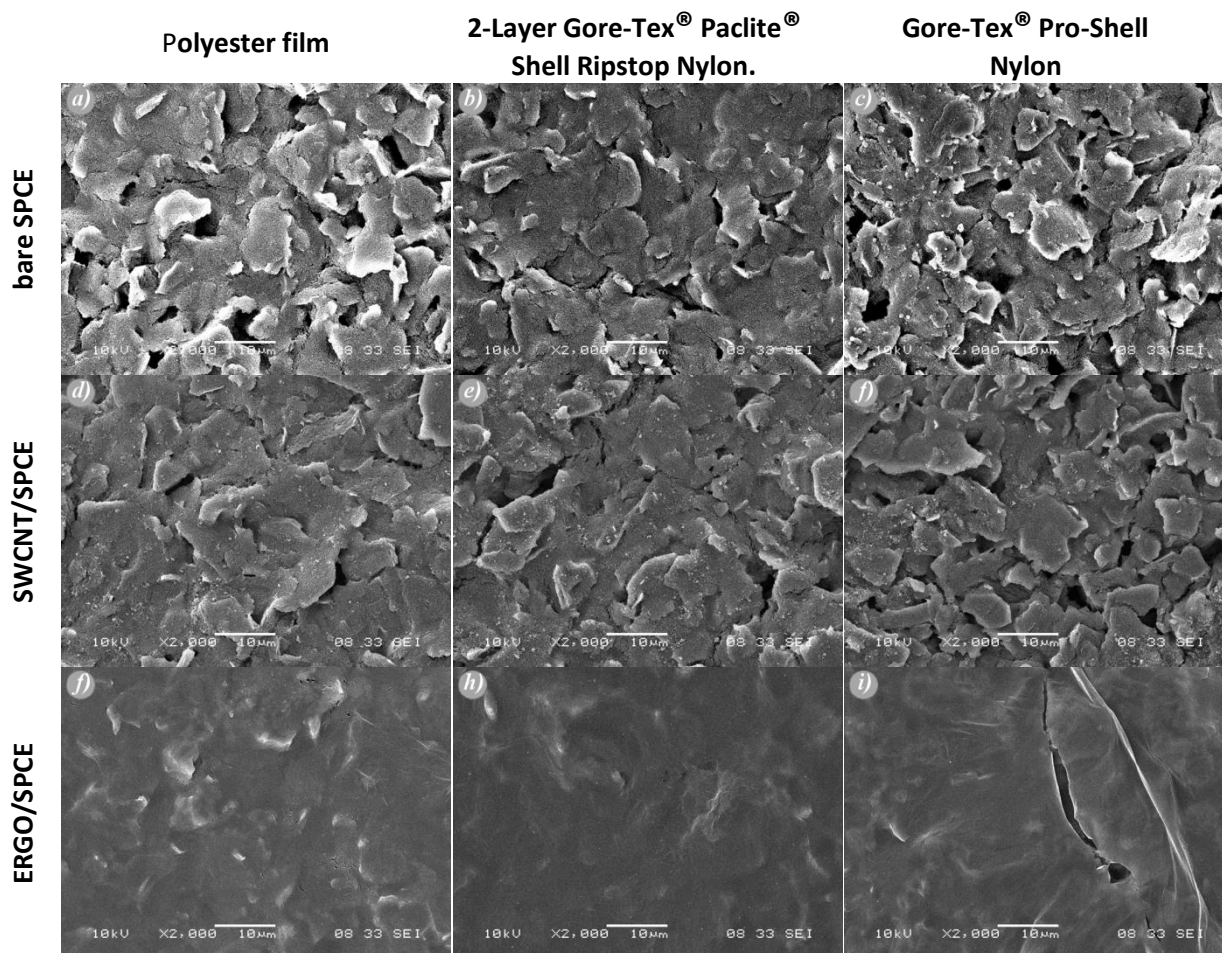


Figure 8.1. Scanning electron micrographs (2000 \times magnification). Bare surface of the screen-printed electrodes coated with the supporting materials of the electrode surfaces: a) polyester film, b) 2-Layer Gore-Tex $^{\text{®}}$ Paclite $^{\text{®}}$ Shell Ripstop Nylon, c) Gore-Tex $^{\text{®}}$ Pro-Shell Nylon. Electrode surface modified with SWCNT coated with the supporting materials of the electrode surfaces: d) polyester film, e) 2-Layer Gore-Tex $^{\text{®}}$ Paclite $^{\text{®}}$ Shell Ripstop Nylon, f) Gore-Tex $^{\text{®}}$ Pro-Shell Nylon. Electrode surface modified with ERGO with the supporting materials of the electrode surfaces coated with d) polyester film, e) 2-Layer Gore-Tex $^{\text{®}}$ Paclite $^{\text{®}}$ Shell Ripstop Nylon, f) Gore-Tex $^{\text{®}}$ Pro-Shell Nylon.

Fig. 8.2 shows the scanning electron micrographs of the AuNP/SPCE, the AuNP/SWCNT/SPCE and the AuNP/ERGO/SPCE surfaces of the three different backing materials. The nanoparticles generated on the bare electrode, Fig. 8.2 a, b and c, were bigger than on the two previously coated sensors.

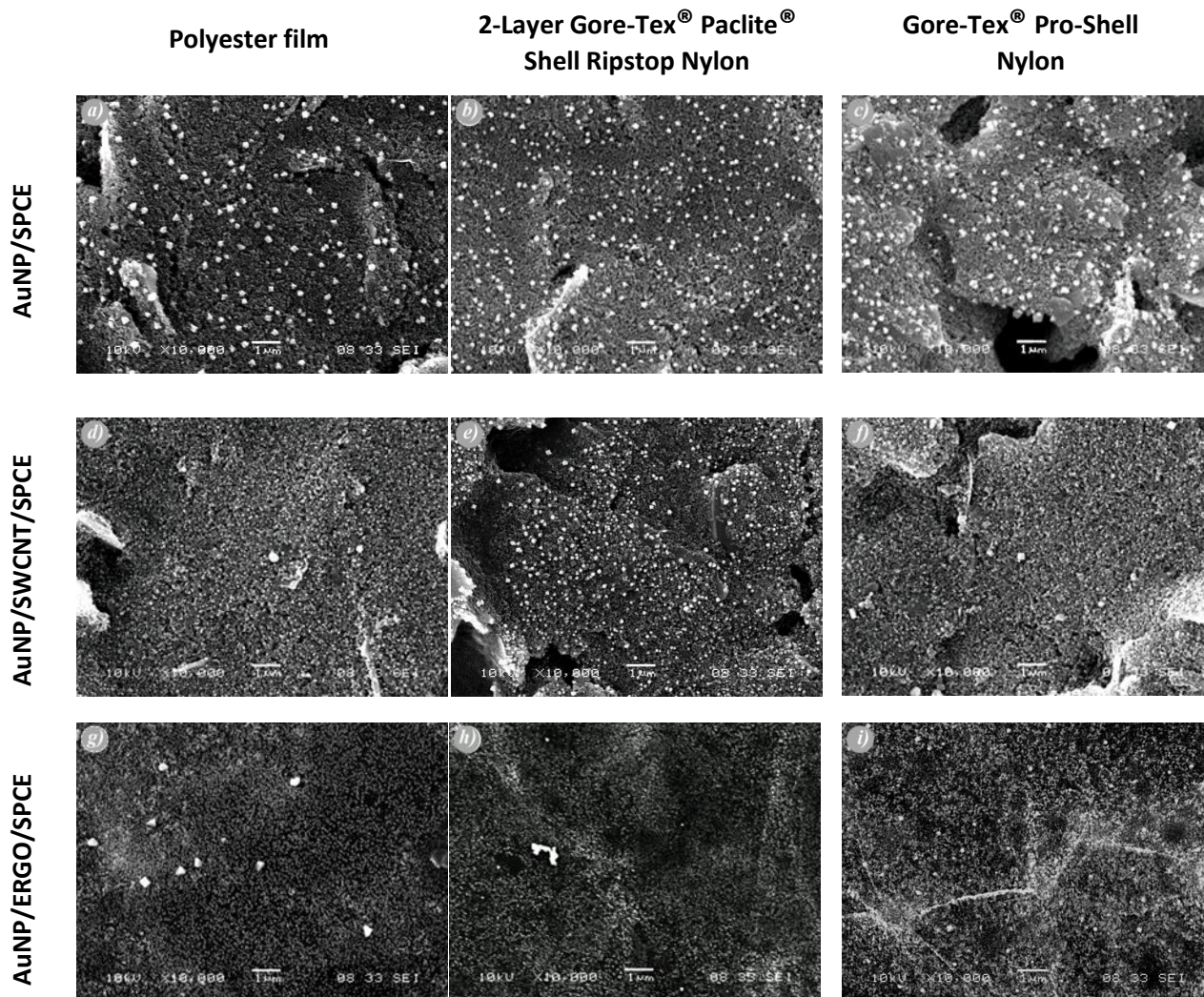


Figure 8.2. Scanning electron micrographs ($10.000\times$ magnification) of the electrode surfaces. a) AuNP/SPCE, b) AuNP/SWCNT/SPCE and c) AuNP/ERGO/SPCE on $500\ \mu\text{m}$ thickness polyester film. d) AuNP/SPCE, e) AuNP/SWCNT/SPCE and f) AuNP/ERGO/SPCE on 2-Layer Gore-Tex® Paclite® Shell Ripstop Nylon backpacking. G) AuNP/SPCE, h) AuNP/SWCNT/SPCE and i) AuNP/ERGO/SPCE on Gore-Tex® Pro-Shell Nylon.

The small diameter of the SWCNT, 1–2 nm [43] and its low concentration make it impossible to observe the SWCNT in the SEM images. However, micrographs of the AuNP/SWCNT/SPCE surfaces (Fig. 8.2 d, e and f) show differences in the structure of

deposited AuNP, depending on the three support materials in use. It can be seen that the AuNP generated on this surface (SWCNT/SPCE) were smaller than those synthesized on the bare electrode surface (SPCE), due to the presence of SWCNT. Polyester film and Gore-Tex Pro-Shell Nylon Liner backed electrodes showed nanoparticles of a smaller size than the AuNP present on 2-Layer Gore-Tex® Paclite® Shell Ripstop Nylon fabric. So, taking into account the results obtained in Section 8.4.3, we could suggest that the nanoparticles of a bigger size yielded worse results for Pb determination than the presence of smaller AuNP.

AuNP/ERGO/SPCE surface images (Fig. 8.2 g, h and i), show the electrode surfaces completely covered by the ERGO film. Their AuNP are mostly small size and homogeneously dispersed on the ERGO coating. Nevertheless, differences exist between the backing materials. When polyester film was used as support material, big nanoparticles of around 150–200 nm of diameter appeared in addition to small AuNP.

The effect of the nanoparticle size was also studied, by analyzing different plating times and the concentration of the precursor gold solution. This behavior was observed in each of the graphene modified electrodes with the different backing materials. Hence, the selection of 2-Layer Gore-Tex® Paclite® Shell Ripstop Nylon supporting material as proof of concept. Fig. 8.3 shows different types of AuNP on the electrode surfaces, varying in accordance with the electrochemical plating time and Au salt concentration selected for AuNP electrosynthesis. At a deposition time of 100 s (Fig. 8.3 a), small nanoparticles (around 100 nm) covering the electrode surface were observed. Over lengthier deposition times (Fig. 8.3 b), Au was continuously generated and the size of the nanoparticles and nanoparticle density grew to form aggregates, restricting the availability of active sites. In addition, larger geometrical metal structures can appear over longer times (Fig. 8.3 c). This effect was also studied with AuNP electrogenerated from a more concentrate gold solution ($5 \cdot 10^{-3}$ M AuCl₃). In this case, the AuNP were of a larger size than the AuNP synthesized over the same length of time from a more diluted gold solution (Fig. 8.3 d, e and f) and they appear in aggregate form. Fig. 8.3 f shows the gold deposition of the most concentrated solution at the longest deposition

time, where the nanoparticle aggregates have increased so much that they are forming a continuous film, resulting in a pauperization of the electron transfer, due to the reduction of the active surface area. Thus, considering the above results, a $1 \cdot 10^{-3}$ M AuCl_3 solution and a deposition time of 100 s were used for Pb determination in this work.

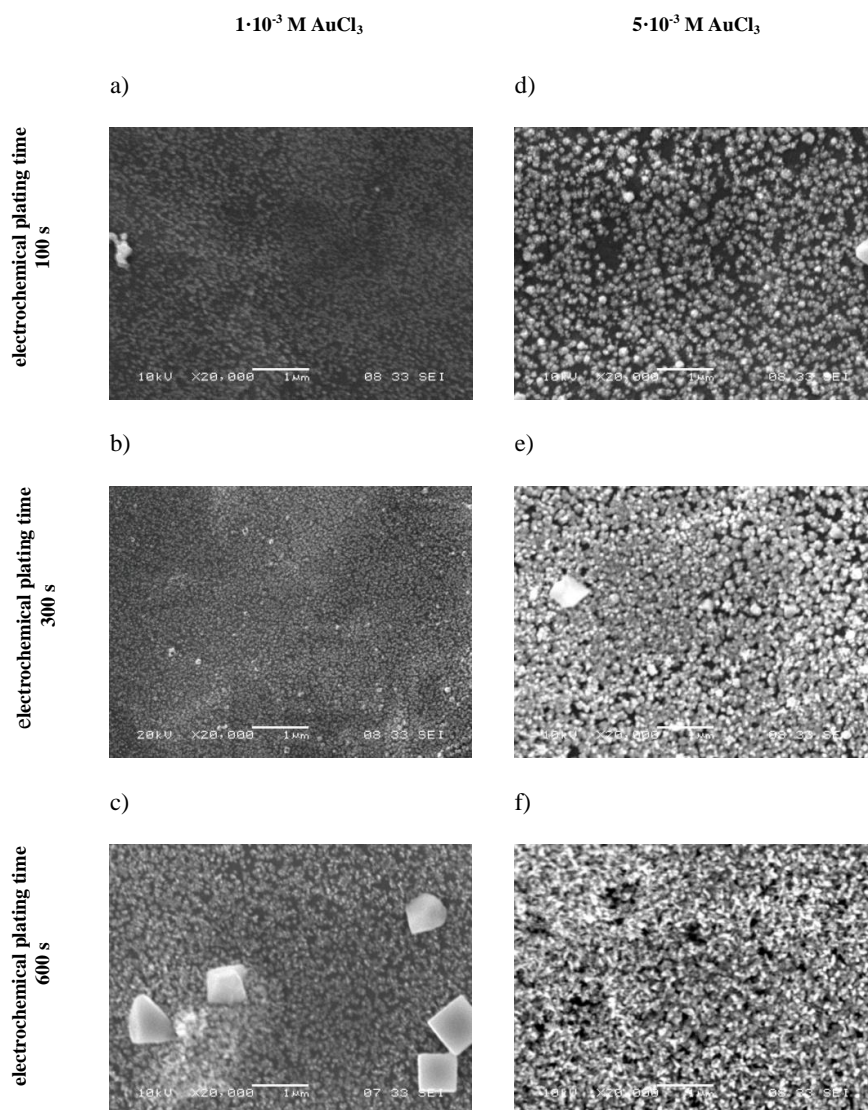


Figure 3. Scanning electron micrographs ($20.000\times$ magnification) of gold nanoparticles electrogenerated on ERGO/SPCE surfaces from a $1 \cdot 10^{-3}$ M AuCl_3 solution during a) 100 s, b) 300 s, and c) 600 s, and gold nanoparticles electrogenerated on ERGO/SPCE surfaces from a $5 \cdot 10^{-3}$ M AuCl_3 solution for c) 100 s, d) 300 s and e) 600 s. 2-Layer Gore-Tex® Paclite® Shell Ripstop Nylon supporting material.

8.4.2.- Analytical characteristics for the determination of sub-nanomolar concentration of Pb by SWASV

SWASV scans of a Pb^{2+} acid solution revealed a well-defined oxidation peak of about -0.48 V for SPCE on the three different substrates (Polyester film, 2-Layer Gore-Tex® Paclite® Shell Ripstop Nylon and Gore-Tex® Pro-Shell Nylon Liner). The voltammetric peak is strongly dependent on pH, and signals of poor analytical were observed for pH values higher than 2.

So, three different sensors were developed using the three substrates. Several calibration curves were recorded for the characterization of the precision of each sensor. Precision was calculated in terms of reproducibility, considering the relative standard deviation (RSD) of the slopes of three calibration curves by using different electrodes. Table 8.2 shows these results as well as the calibration range and the limit of detection for each of the three sensors, which was calculated according to $3\sigma/m$ (where σ is the standard deviation of the blank solution and m is the slope of the calibration curves).

As can be seen in Table 8.2, the capability of the sensor is highly dependent on the substrate employed, as the polyester film backed sensor provides the best reproducibility. Furthermore, differences were observed between the two Gore-Tex® supports; while 2-Layer Gore-Tex® Paclite® Shell Ripstop Nylon showed suitable RSD values and limits of detection for use in a wearable sensor capable of detecting the subnanomolar Pb concentration, Gore-Tex® Pro-Shell Nylon Liner showed inappropriate parameters for accurate detection.

Table 8.2. Comparison of analytical characteristics of Pb determination in different media by SWASV using sensors printed on different support materials. $E_{deposition}$: -0.80 V, deposition time: 300 s.

Media	Backing Material	Concentration Range ($\cdot 10^{-9}$ M)	RSD % (n = 3)	LOD ($\cdot 10^{-9}$ M)	Sensitivity (nA/nM)
0.02 M HCl	500 μ m thickness polyester	1-6	2.5	0.032 \pm 0.006	424.03
	2-Layer Gore-Tex® Paclite® Shell Ripstop Nylon	2-12	6.2	0.799 \pm 0.120	54.68
	Gore-Tex® Pro-Shell Nylon Liner	2-12	24.8	5.811 \pm 1.534	180.88

8.4.3.- Analytical characteristics for the determination of sub-nanomolar Pb concentration by DPASV

The DPASV scans also revealed a well-defined oxidation peak of -0.48 V at the AuNP/SPCE over the three different substrates (Polyester film, 2-Layer Gore-Tex® Paclite® Shell Ripstop Nylon, Gore-Tex® Pro-Shell Nylon Liner). Likewise than SWASV analysis, three different sensors have been developed using the three substrates.

The focus of this study is on sensors that are capable of directly detecting the sub-nanomolar Pb concentration in seawater, so no acid medium was also tested. An enhancement of the signal is for that purpose necessary to determine the sub-nanomolar Pb concentration, since the amounts of free Pb^{2+} cations are lower at higher pH, due to their lower solubility, as chlorocomplexes [44] and lead hydroxide [45,46] can be formed. With that aim in sight, the electrode surfaces were modified with AuNP, to improve the performance conditions of the SPE for heavy metal trace determination. In addition to AuNP deposition, two different modifications of the SPCE surface prior to AuNP deposition were applied, to heighten the sensitivity of the DPASV experiments.

On the one hand, SPCE was modified with SWCNT casting on the electrode surface. On the other, SPCE was modified with ERGO. Carbon nanotubes and ERGO were selected to improve sensor sensitivity for direct seawater measurements, due to their remarkable mechanical, electrical, thermal and optical properties [43,47–49].

Taking into account the high salinity of the seawater samples, mainly due to NaCl, the performance of the AuNP/SWCNT/SPCE and the AuNP/ERGO/SPCE-based sensors was studied using a 0.6M NaCl solution that functioned as the supporting electrolyte in this first approach to the analysis of seawater samples.

As in the SWASV analysis, the procedures developed, using the sensors based on the three different substrates, were characterized by precision in terms of reproducibility.

Table 8.3 shows the results, the calibration range, and the sensitivity, for each of the three sensors. Better sensitivity can be seen in the polyester film than in the AuNP/SPCE-based sensor backed with fabric materials, explained by the better conductivity of the electrodes in the rigid material. This modification showed good parameters for Pb detection, nevertheless the analysis has to be performed in an acid media.

The calibration curves for the AuNP/SWCNT/SPCE-based sensors performed in NaCl media using the three backing materials in a calibration range from $2 \cdot 10^{-9}$ M to $30 \cdot 10^{-9}$ M yielded different results. While the performance in the 500 μm thickness polyester film provided good values for sensitivity and reproducibility (Table 8.3), the two fabric materials showed poorer results, suggesting that those sensors are not suitable for the determination of sub-nanomolar Pb concentrations.

Sensors based on AuNP/ERGO/SPCE printed on the three different supports were also analyzed in NaCl solution within a calibration Pb^{2+} range between $2 \cdot 10^{-9}$ M and $14 \cdot 10^{-9}$ M. From the results obtained with these sensors, summarized in Table 8.3, it can be concluded that the sensors modified with ERGO showed suitable values of

reproducibility in terms of RSD and better sensitivity than those modified with SWCNT to the evaluation of the sub-nanomolar Pb^{2+} concentration.

Table 8.3. Comparison of analytical characteristics of Pb determination in different media by DPASV using sensors printed on different support materials.

Media	Electrode	Backing material	Concentration Range ($\cdot 10^{-9}$ M)	RSD % (n = 3)	Sensitivity
0.02 M HCl	AuNP/SPCE	500 μ m thickness polyester	2-10	6.0	97.43
		2-Layer Gore-Tex [®] Paclite [®] Shell Ripstop Nylon	20-120	7.4	21.02
		Gore-Tex [®] Pro-Shell Nylon Liner	20-120	2.2	36.63
0.6 M NaCl	AuNP/SWCNT/SPCE	500 μ m thickness polyester	4-38	8.16	72.128
		2-Layer Gore-Tex [®] Paclite [®] Shell Ripstop Nylon	2-50	6.84	16.325
		Gore-Tex [®] Pro-Shell Nylon Liner	2-60	18.22	52.041
	AuNP/ERGO/SPCE	500 μ m thickness polyester	2-14	5.74	157.85
		2-Layer Gore-Tex [®] Paclite [®] Shell Ripstop Nylon	2-14	8.2	86.92
		Gore-Tex [®] Pro-Shell Nylon Liner	6-18	7.5	107.25

AuNP gold nanoparticles, SPCE screen-printed carbon electrode, SWCNT single-walled carbon nanotubes, ERGO electroreduced graphene oxide.

As can be observed in Tables 8.2 and 8.3, it is also remarkable that the sensitivity of the sensor depends on its screen-printed support material. In general, the SPE systems based on textile support showed a lower sensitivity than the rigid polyester support. The adhesion of inks on the fabric materials was weaker than on the polyester film during the screen-printing process, probably due to the hydrophobic properties of Goretex. In consequence, the conductivity of the fabric sensors was lower than the conductivity of

the polyester screen-printed sensors and the fabric sensors therefore tended to be of lower sensitivity.

8.4.4.- Analysis of lead traces in seawater samples

Similarly as in Sections 8.4.2 and 8.4.3, the electrochemical determination of traces of lead in seawater has been carried out by SWASV and DPASV using different working electrodes (SPCE, AuNP/SWCNT/SPCE, AuNP/ERGO/SPCE) and backing materials (Polyester film, 2-Layer Gore-Tex® Paclite® Shell Ripstop Nylon, Gore-Tex® Pro-Shell Nylon Liner).

Seawater samples were collected from mussel farms located on the Pontevedra coast, in the north-west of Spain. The samples were subsequently stored in high density polyethylene bottles in a freezer until the analysis. Inductively coupled plasma mass spectrometry analysis demonstrated that the lead levels of the samples were not over 0.06 µg/L. Thus, in order to explore the feasibility of the Pb determination in seawater samples under the concentration allowed by legislation (2013/39/UE), spiked samples were analyzed and SWASV and DPASV calibration curves were constructed.

Using SWASV, the electrochemical determinations of lead were performed in an electrochemical cell containing 4.9 mL of seawater sample acidified with 0.1 mL HCl (1 M). Increasing concentrations of Pb²⁺ were added to this solution and its calibration curves recorded, Fig. 8.4 a. Deposition time and deposition potential values used were the same as those used previously (Section 8.3.4.1). Table 8.4 shows the calibration parameters obtained on seawater samples and the comparison using different backing materials. The results can be seen to agree with those in the acid medium solution; the sensor printed on polyester film providing the highest sensitivity values.

DPASV determination of Pb²⁺ was performed under the same experimental conditions described in Section 8.3.4.2. Since the modification with SWCNT provided imprecise results for the textile sensor, those textile sensors were not tested in the seawater study, unlike the AuNP/ SWCNT/SPCE polyester-based sensor. The modification based on

ERGO (AuNP/ERGO/SPCE) was employed for the direct determination of Pb in seawater with the three backing materials, providing good results.

To do so, 5 mL aliquots of the same seawater samples, (Pontevedra coast, Spain) were studied. Increasing concentrations of Pb^{2+} were added to each aliquot and after the stripping process, differential pulse voltammograms were recorded on the AuNP/ERGO/SPCE-based sensors. Fig. 8.4 b and c show the voltammograms, obtained with the successive additions of Pb^{2+} to both the rigid and the textile backed AuNP/ERGO/SPCE-based sensors in seawater. From the values of Table 8.4, it can be seen that the polyester film and the Gore-Tex® Pro-Shell Nylon liner provided good sensitivities for Pb^{2+} detection in seawater. However, the sensor printed on the Gore-Tex® Pro-Shell Nylon Liner was considered to be the most suitable sensor, because it presented the best RSD values, providing a calculated limit of detection for Pb^{2+} in seawater of $3.21 \cdot 10^{-10} \pm 3.4 \cdot 10^{-11}$ M.

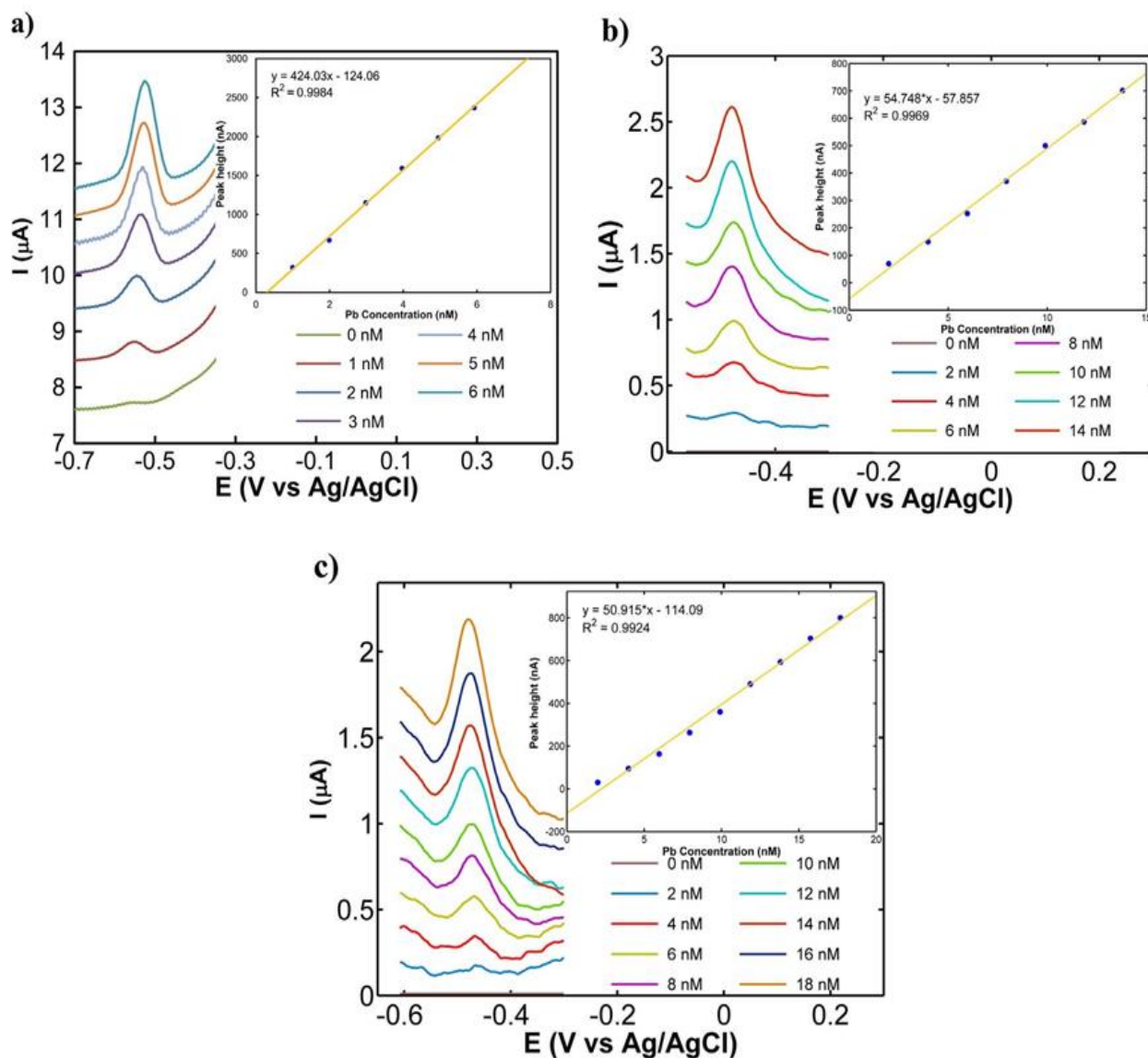


Figure 8.4. a) Square wave anodic stripping voltammogram recorded at SPCE sensor on polyester film in acidified seawater with increasing levels of Pb²⁺. Differential pulse anodic stripping voltammograms corrected by subtracting the blanks recorded for increasing levels of Pb²⁺ in seawater using AuNP/ERGO/SPCE screen-printed sensors on b) polyester film and c) Gore-Tex® Pro-Shell Nylon Liner. Insets show the corresponding calibration curves.

Table 8.4. Analytical characteristics of Pb determination in seawater by SWASV and DPASV using sensors printed on different support materials.

Media	Electrode	Backing Material	Concentration Range ($\cdot 10^{-9}$ M)	RSD% (n = 3)	Detection Limit ($\cdot 10^{-9}$ M)	Sensitivity
SWASV						
Deposition Potential: -0.8 V		Deposition time: 300 s				
Acidified seawater	AuNP /SPCE	500 μ m thickness polyester	2–16	3.4	0.172 \pm 0.014	317.26
		2-Layer Gore-Tex® Paclite® Shell	2–12	9.2	1.350 \pm 0.295	172.59
		Ripstop Nylon				
		Gore-Tex® Pro-Shell Nylon Liner	2–12	15.2	3.592 \pm 0.521	104.99
DPASV						
Deposition Potential: -0.7 V		Deposition time: 300 s				
Seawater	AuNP/SWCNT/SPCE	500 μ m thickness polyester	2– 30	4.64		52.135
	AuNP/ERGO/SPCE	500 μ m thickness polyester	2– 14	10.2	2.102 \pm 0.341	54.748
		2-Layer Gore-Tex® Paclite® Shell	6– 16	14.2	3.070 \pm 1.184	26.24
		Ripstop Nylon				
		Gore-Tex® Pro-Shell Nylon Liner	2– 18	4.2	0.321 \pm 0.034	50.915

AuNP gold nanoparticles, SPCE screen-printed carbon electrode, SWCNT single-walled carbon nanotubes, ERGO electroreduced graphene oxide.

Finally, a test sample of seawater doped with an amount of 0.8 µg/L of Pb²⁺, selected so that it was under the average annual value for lead established in European Directive (2013/39/UE), was checked by both methods, in an evaluation of the calibrations curves that yielded the recovery values shown in Table 8.5. In the case of the seawater samples analyzed with SWASV, they were acidified with HCl to be in agreement with the SWASV study performed above, for this reason the intensity is about one order of magnitude higher than in the results obtained for the DPASV technique used to analyze the untreated seawater samples.

Table 8.5. Recovery (%) and RSD for 0.8 µg/L Pb²⁺ spiked in acidified seawater (SWASV) and in untreated seawater (DPASV).

Method	Added Concentration (µg/L)	Intensity (nA)	Calculated concentration (µg/L)	Recovery %	RSD Recovery (n = 3)
SWASV	0.8	902	0.87	92.46	7.11
	0.8	1081	0.85	106.56	
	0.8	982	0.79	98.76	
DPASV	0.8	109	0.87	109.54	7.57
	0.8	78	0.75	94.32	
	0.8	90	0.80	100.21	

SWASV experiments were performed using SPCE with polyester film as support material in acidified seawater.

DPASV experiments were performed using AuNP/ERGO/SPCE with Gore-Tex® Pro-Shell Nylon Liner as support material in seawater.

8.5.- Conclusions

The direct determination of the sub-nanomolar Pb concentration in seawater samples, without further preparations, by anodic stripping analysis has been demonstrated using screen-printed sensors on different backing materials.

The best results have been achieved by the AuNP/ERGO/SPCEbased sensors. Although this sensor showed different behaviors for each backing material, no great differences have been observed between the rigid polyester film materials and the Gore-Tex® Pro-Shell Nylon Liner textile fabrics, while maintaining the sensor properties without major changes in sensitivity in the analysis of the seawater samples. It has also been observed that a homogeneous distribution of high density small size nanoparticles (around 100 nm) provided better Pb determination than bigger AuNP.

The research reported in this paper has shown that the proposed sensors could be used in different fields such as nanomolar Pb detection in seawater, and textile screen-printing of electrodes, displaying a promising initial approach to the development of a low cost, fully integrated portable/wearable sensor for use by professionals working in environments that might contain Pb levels as high as those found in the petroleum and mining industries, and the diving activity of submariners and underwater welders in waters potentially contaminated by heavy metals.

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9.- Sensor system based on flexible screen-printed electrodes for electrochemical detection of okadaic acid in seawater

En este capítulo se aborda la puesta a punto de un método analítico para la determinación del ácido okadaico directamente en agua de mar.

La monitorización del OA, en el agua de mar puede servir como un sistema de alerta temprana para la prevención de posibles efectos negativos en la industria alimentaria y en la salud humana en general. Se ha desarrollado un sistema de sensor desechable utilizando electrodos serigrafados para la detección electroquímica de dicha toxina.

El método descrito está basado en la inhibición de la proteína fosfatasa tipo 2A por el OA, evaluando la actividad enzimática utilizando diferentes sustratos de la enzima: fosfato de p-nitrofenilo, el fosfato de 4-metilumbeliferilo y el fosfato de fenilo. Todos los sustratos han mostrado su utilidad para la determinación del OA, proporcionando buenos límites de detección, alrededor de $2,7 \cdot 10^{-12}$ M. El método propuesto en este capítulo se ha aplicado con éxito en la determinación de OA directamente en agua de mar.

Los sistemas de electrodos se imprimieron en diferentes materiales de soporte rígido y textil para observar cómo pueden influir en el rendimiento final del sensor.

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9.1.- Introduction

Okadaic acid (OA) is a marine lipophilic toxin produced by toxicogenic dinoflagellates, mainly belonging to the genera *Dinophysis* and *Prorocentrum* [1], which may accumulate in the digestive glands of shellfish when they feed on these kinds of microalgae [2,3]. Although OA and its dinophysistoxin derivatives produce no harmful effect in shellfish, it is the major diarrhetic shellfish poisoning (DSP) toxin in humans, causing gastrointestinal troubles such as diarrhea, nausea, vomiting and abdominal pain. The toxicity mechanism of OA and of all DSP toxins lies in blocking the active sites of the enzymes and therefore the consequent inhibition of serine/threonine protein phosphatases type 1 (PP1) and type 2A (PP2A), resulting in an over-phosphorylation of proteins in cells [4–6]. Human intoxication by DSP can have a disastrous effect on the shellfish industry resulting in huge financial losses [7]. Early detection of these toxins in shellfish tissue, or even directly in the seawater they inhabit prior to collection, could be key to minimizing the risk of collecting infected shellfish. In this regard, the European Commission Regulation EU No 786/2013 establishes a maximum permitted concentration of 160 µg of OA equivalents per kg of live bivalve molluscs for human consumption [8].

Several methods for OA detection have been reported in an effort to protect people from the potential health risks of eating contaminated shellfish. One of the most commonly used methods for years has been mouse bioassay (MBA) [9,10]. However, due to the ethical concerns surrounding animal testing and the poor specificity of this method, new methods have been developed in recent years.

Immunological assays such as the enzyme-linked immunosorbent assay (ELISA) have been used due to their high sensitivity and stability, since they rely on the structure of the toxin for detecting the interaction between antibodies and toxins [11]. Immunosensors follow the main principle as that of ELISA methods, linking antibodies to the electrode surface and obtaining the OA signal through electrochemical detection

[12–15]. Eissa and co-workers developed an immunosensor based on a screen-printed electrode modified with graphene on which OA antibodies were covalently immobilized, providing a limit of detection (LOD) of 19 ng/L [16]. Dominguez developed another immunosensor with an LOD of 150 ng/L under flow conditions [17].

Chromatographic techniques have also been widely used in the detection of DSP toxins, often coupled with other techniques to improve detection. Such techniques include liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) [18], ion spray mass spectrometry [19], dual solid phase extraction coupled with liquid chromatography-tandem mass spectrometry (SPE-LC-MS/MS) for the simultaneous determination of multi-class cyanobacterial and algal toxins in water [20], high performance liquid chromatography (HPLC) [21], and liquid chromatography-linked protein phosphatase bioassay [22].

Serine/threonine protein phosphatase inhibition has commonly been detected using colorimetric and fluorometric techniques by monitoring changes of absorbance or fluorescence between the substrate and the product of the enzymatic reaction [23–25]. The most common protein phosphatase used are PP1 and PP2A, due to the high inhibition capability of OA on these enzymes.

The use of electrochemical techniques with screen-printed electrodes presents a promising alternative to other costly techniques, offering many advantages such as low costs, inherent miniaturization and portability, simplifying the measurement process. In fact, electrochemical biosensors have also been used to monitor protein phosphatase inhibitions [26–30].

In this study, an electrochemical sensor system was developed based on the detection of the product of the PP2A enzymatic reaction in the presence of OA with different substrates: p-nitrophenyl phosphate (p- NPP), 4-methylumbelliferyl phosphate (4-MUP) and phenyl phosphate (PP). The proposed method is a very simple and sensitive

procedure, detecting a concentration of $2.7 \cdot 10^{-12}$ M (2.17 ng/L). To the best of our knowledge, this value is less than the lowest values obtained by other electrochemical methods mentioned in the bibliography, even those that use immunosensors (Table 9.1). Only some chromatographic techniques coupled with other techniques such as LC-HRMS reported by Bosch-Orea [18] are able to provide a better limit of detection (0.3 ng/L). However, they require tedious sample treatments and expensive equipment, and have only been used to detect OA in bivalve samples but not in seawater, which is a considerably more difficult matrix to directly analyze using chromatographic techniques.

9.2.- Experimental

9.2.1.- Reagents and materials

All chemicals used in this study were of analytical-reagent grade or above and were used as received without further purification. Highquality water (Milli Q A10 system, Millipore, Bedford, USA) was employed for preparing all solutions.

OA in ethanol solution was purchased from Cayman (Michigan, USA), sodium chloride was provided by Panreac (Barcelona, Spain), and p-nitrophenol (p-NP), 4-methylumbelliferone (4-MU), p-NPP, 4- MUP, phenol and PP were all obtained from Sigma-Aldrich (St. Louis, MO, USA).

Lyophilized Protein Phosphatase A from human blood cells obtained from Zeulab (Zaragoza, Spain) was rehydrated according to the procedure describe by Smienk [31], by adding 2 mL phosphate buffer solution and mixing gently at 60 rpm for 60 min at room temperature on a New Brunswick Scientific orbital shaker (Endfield, CT, USA).

Table 9.1. Summary of analytical methods employed for OA detection in real samples.

Method	Detection Technique	Linear range	LOD	Applicability	Reference
LC	HRMS	0.5 – 50 ng/L	0.3 ng/L	Filtered seawater	18
SPE-LC	MS/MS	5 – 250 µg/L	10 ng/L	Lake water	20
Electrochemical biosensor	Amperometry	30 – 250 ng/L	–	Buffer solution	27
Electrochemical immunosensor	SWV	≤5 µg/L	19 ng/L	Mussels	16
Electrochemical immunosensor	Amperometry	0.19 – 25 µg/L	150 ng/L	Mussels	17
Electrochemical biosensor	Amperometry	0.1– 4.0 µg/L	100 ng/L	Buffer solution	34
Electrochemical immunosensor	DPV	–	380 ng/L	Mussels	35
Electrochemical immunosensor	DPV	0.78 – 500 µg/L	500 ng/L	Mussels	36
Electrochemical biosensor	DPV	0.41 – 56.3 ng/L	2.17 ng/L	seawater	This work

LC liquid chromatography, HRMS high resolution mass spectrometry, SPCE-LC solid extraction phase coupled with liquid chromatography, MS/MS tandem mass spectrometry, SWV square wave voltammetry, DPV differential pulse voltammetry.

Metal salts solutions for the synthetic sea water preparation and interferences study were prepared with MgCl_2 , CaCl_2 and $\text{Cd}(\text{NO}_3)_2$ provided by Panreac (Barcelona, Spain), and $\text{Zn}(\text{NO}_3)_2$, CuSO_4 , $\text{Pb}(\text{NO}_3)_2$ and HgSO_4 purchased from Merck (Darmstadt, Germany). KCl was provided by Scharlau (Barcelona, Spain), NaHCO_3 was obtained from Fluka (Steinheim, Germany), and KBr and SrCl_2 were purchased from Merck (Darmstadt, Germany).

Synthetic seawater was prepared according to the procedure that can be found in [32], by dissolving 23.926 g of NaCl , 4.008 g of Na_2SO_4 , 0.026 g of H_3BO_3 , 10.831 g of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 1.518 g of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.677 g of KCl , 0.196 g of NaHCO_3 , 0.098 g of KBr and 0.140 g/L of SrCl_2 in 1 L of high-quality water.

Seawater samples were collected from mussel farms located in the Pontevedra coast, Spain. They were stored at $-20\text{ }^\circ\text{C}$ in high-density polyethylene bottles until analysis.

C10903P14 (carbon/graphite ink) and D2071120D1 (dielectric ink) were purchased from Gwent Electronic Materials (Torfaen, U.K). Electrodag 6037 SS (Ag/AgCl ink) and Electrodag 418 (Ag ink) were supplied by Acheson Colloiden (Scheemda, Netherlands).

9.2.2.- Screen-printed carbon electrode (SPCE) fabrication

Home made SPEs, used in this work, consist of a carbon working electrode (area, 12.56 mm^2), a carbon counter electrode and a Ag/AgCl reference as a pseudoreference electrode. Ag ink was used in conductive paths of SPEs. These electrodes were produced on a DEK 248 screenprinter (DEK, Weymouth, UK) by sequential deposition of different commercial inks according to the procedure described in a previous work [33]. The electrodes were printed using three different backing materials (BM): a $500\text{ }\mu\text{m}$ thickness polyester film (PMX981) provided by HiFi Industrial film (Dardilly, France), a Gore-Tex® Pro-Shell Nylon Liner and a 2-Layer Gore-Tex® Paclite® Shell Ripstop,

both purchased from Rockywoods Fabrics, LLC (Lovelan, USA). These will be referred to hereinafter as backing material 1 (BM1), backing material 2 (BM2) and backing material 3 (BM3), respectively.

9.2.3.- Instrumentation and measurements

Electrochemical measurements were performed with a PalmSens® portable electrochemical potentiostat (PalmSens® Instruments BV, Houten, The Netherlands) controlled by PS Trace software to collect the data register. All measurements were carried out at room temperature using a screen printed Ag/AgCl pseudoreference electrode.

The electrochemical measurements were performed by differential pulse voltammetry (DPV) from 0.00 V to +1.20 V at 0.030 V/s, applying a potential pulse of 0.028 V with a time pulse of 0.010 s in a 100 µL drop cast onto the electrodic screen-printed system. Blanks of 0.6 M NaCl were carried out between measures to ensure that no substance is adsorbed on the electrode surface.

LC liquid chromatography, HRMS high resolution mass spectrometry, SPCE-LC solid extraction phase coupled with liquid chromatography, MS/MS tandem mass spectrometry, SWV square wave voltammetry, DPV differential pulse voltammetry.

9.2.4.- Enzyme incubation

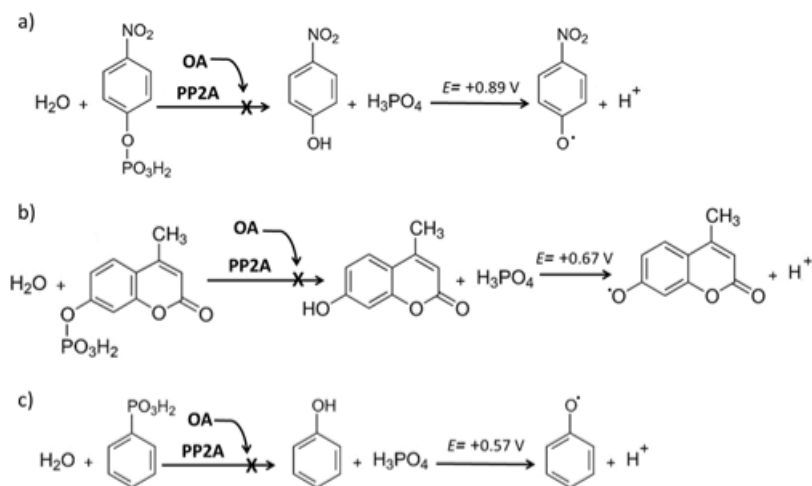
Aliquots of 750 µL were prepared containing either $1 \cdot 10^{-2}$ M, $1 \cdot 10^{-3}$ M or $1.5 \cdot 10^{-2}$ M of p-NPP, 4-MUP and PP substrate respectively, 50 µL of enzyme solution (1.73 mU), and OA in a range from $5 \cdot 10^{-13}$ M to $7 \cdot 10^{-1}$ M for spiked measurements. Lastly, the final volume was made up with 0.6M NaCl solution or synthetic seawater. In the case of the real seawater samples, 750 µL aliquots contained the same amount of substrate and enzyme solution as described above, and 300 µL of the seawater sample. The final

volume was also made up with 0.6M NaCl. The aliquots were incubated for 30 min at 37 °C in an oven and then cooled down to 0 °C to stop the enzymatic reaction until the electrochemical analysis.

9.3.- Results and discussion

9.3.1.- Optimization of the experimental parameters

The determination of OA is based on the electrochemical detection of products of enzymatic dephosphorylation (p-NP, 4-MU and phenol), since the presence of OA implies a decrease in the concentration of these products due to the enzymatic inhibition that it produces (Scheme 9.1).



Scheme 9.1. Enzymatic reaction of the substrates a) p-NPP, b) 4-MUP and c) PP with PP2A enzyme followed by the corresponding electrochemical oxidation of the products.

The inhibition by OA was calculated as shown in Eq. 1 below.

$$\% \text{Inhibition} = \frac{I_0 - I_i}{I_0} \times 100 \quad (1)$$

Where I_0 represents the oxidation peak current of the control sample, that is, the product of the enzymatic reaction without the addition of OA, I_i means the oxidation peak current of the product of the enzymatic reaction in the presence of the selected concentration of OA.

The precision of the measurements was calculated in terms of reproducibility considering the relative standard deviation (RSD) of the slopes of three calibration curves. From each calibration curve, LOD has been calculated according to $3\sigma/m$ (where σ is the standard deviation of the blank, and m represents the slope of the calibration curve) [37,38]. In this case, σ is the estimated standard deviation of the % inhibition caused by the lowest concentration that was detected.

The enzymatic products p-NP, 4-MU and phenol were firstly studied using cyclic voltammetry between -1.50 V and $+1.50$ V. The 4-MU product exhibits an irreversible oxidation peak at around $+0.67$ V (vs screen-printed pseudoreference Ag/AgCl electrode) due to the oxidation of its hydroxyl group. However, p-NP and phenol show both oxidation and reduction peaks. The oxidation peaks of p-NP and phenol appear at around $+0.89$ V and $+0.57$ V respectively, corresponding to the oxidation of the hydroxyl group of each molecule. The reduction peak of p-NPP at around -0.89 V is related to the reduction of the nitro group. In the case of p-NP, although it exhibits two electrochemical peaks, the reduction peak is observed both in the substrate and in the product. Therefore, the oxidation peak has been selected for the DPV study since it is only present in the enzymatic product, with the aim of reducing the possible interferences that the unreacted substrate may cause.

Incubation time and incubation temperature were optimized to obtain the optimum electrochemical signal. An incubation temperature range from $+25$ °C to $+90$ °C was checked; obtaining the most suitable operating temperature so that the enzyme transforms the substrate to product at $+37$ °C. The electrochemical signal increases from

+25 °C to +37 °C, where a maximum signal intensity is achieved, with enzyme activity then starting to decrease, indicating that the optimal temperature is +37 °C.

The enzyme/substrate ratio was also examined and the amount of PP2A and the concentration of substrate for p-NPP, 4-MUP and PP were optimized. Different concentrations of substrate from $1 \cdot 10^{-4}$ M to $5 \cdot 10^{-2}$ M and different amounts of buffered enzyme solution from 25 μ L to 200 μ L in a total volume of 750 μ L were studied. The optimized values were 50 μ L of enzyme PP2A and a substrate concentration of $1 \cdot 10^{-2}$ M, $1 \cdot 10^{-3}$ M and $1.5 \cdot 10^{-2}$ M for p-NPP, 4-MUP and PP, respectively.

9.3.2.- Proof of concept

Under the optimized parameters, a first electrochemical study was carried out in a 0.6M NaCl solution. This electrolyte solution has been selected as proof of concept to carry out the electrochemical measurements, due to the high concentration of NaCl in seawater. With this aim, calibration curves of 0.6M NaCl samples spiked with OA were performed in a linear range from $5 \cdot 10^{-13}$ M to $7 \cdot 10^{-11}$ M of OA for p-NPP and 4-MUP, and from $5 \cdot 10^{-12}$ M to $7 \cdot 10^{-11}$ M of OA for PP, as it cannot detect $5 \cdot 10^{-13}$ M of OA. The analyses were performed in the three electrodic systems with different backing materials. Fig. 9.1 shows DPV curves after the incubation process with different concentrations of OA, where current peaks appear centered at +0.89 V (Fig. 9.1.a), at around +0.67 V (Fig. 9.1 b) and at around +0.57 V (Fig. 9.1 c), which correspond to the oxidation of p-NP, 4-MU and phenol respectively. As can be observed, the height of the oxidation peak decreases as OA concentration increases for each product, confirming the inhibition suffered by PP2A in the presence of OA. As can be seen in Table 9.2, the developed sensor system is able to detect an OA concentration in the picomolar range using any of the studied substrates, displaying good reproducibility with RSD values of less than 10%. The use of one support material in place of another does not provide great differences in terms of LOD. However, it was observed that BM1 provides a lower RSD

value, which could be explained by the better reproducibility of the electrode surfaces printed on rigid material compared to the flexible fabric material BM2 and BM3.

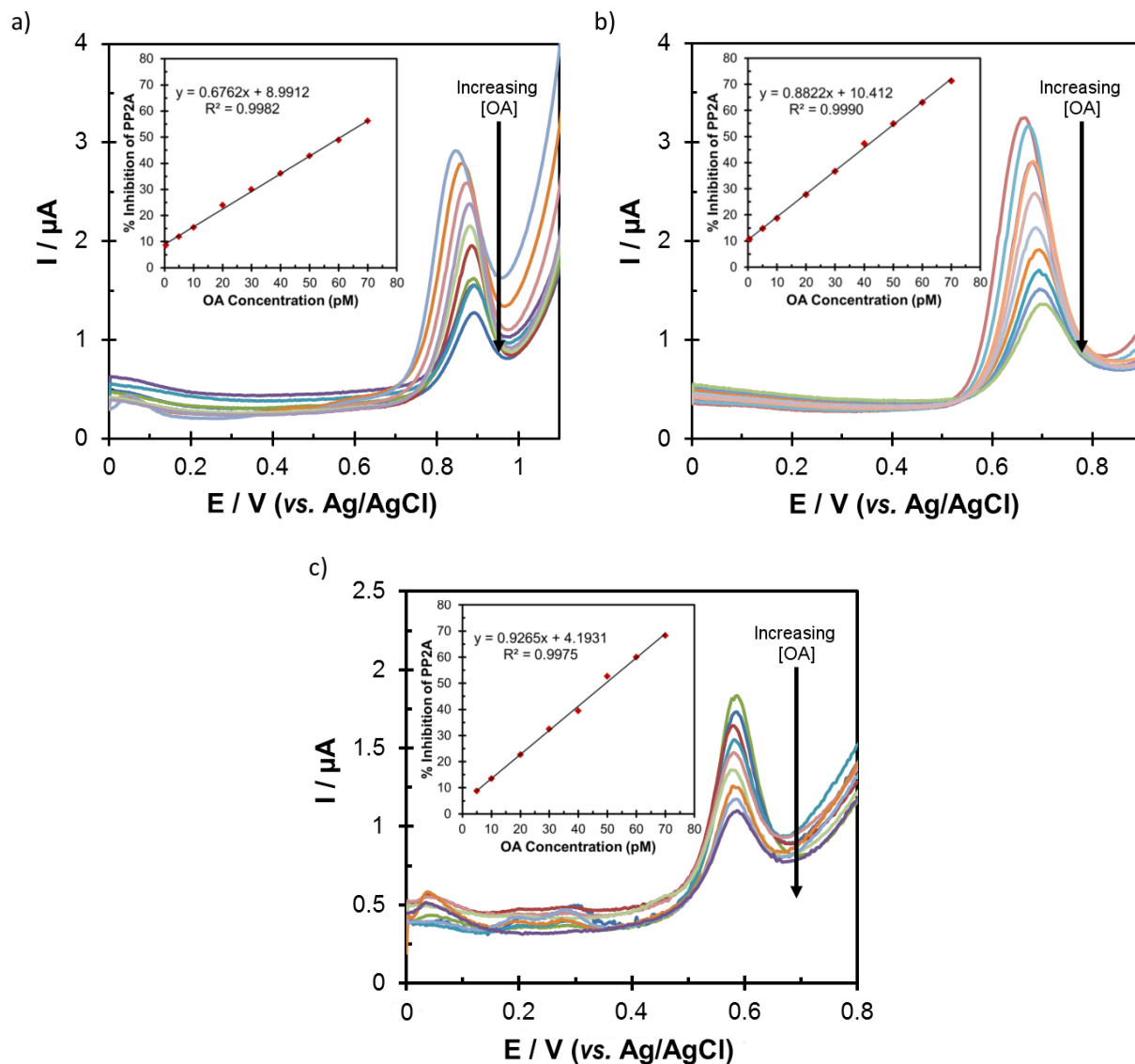


Figure 9.1. Differential pulse voltammogram of PP2A enzymatic reaction products a) *p*-NP, b) 4-MU and c) Phenol after incubation in presence different concentration of OA. Insets show the corresponding calibration curves of PP2A inhibition rates versus OA concentration (0.5–70.0 pM in a) and b) and 5.0–70.0 pM in c)) in 0.6M NaCl.

Table 9.2. Parameters obtained from calibration curves for the OA inhibition of PP2A enzyme versus OA concentration in 0.6M NaCl solution.

Substrate	Backing material	IC50 (pM)	Calibration Range (pM)	Sensitivity (nM/nA) (n = 3)	RSD (%)	LOD (pM) (n=3)	SLOD (pM)
p-NPP	BM1	86.1	3.7 – 70.0	555.6	2.6	3.7	0.1
p-NPP	BM2	69.4	6.9 – 70.0	614.8	9.4	6.9	0.7
p-NPP	BM3	60.6	3.3 – 70.0	676.8	8.1	3.3	0.3
4-MUP	BM1	40.7	5.2 – 70.0	976.8	6.9	5.2	0.4
4-MUP	BM2	45.8	3.3 – 70.0	1013.0	9.1	3.3	0.3
4-MUP	BM3	44.9	2.7 – 70.0	882.2	8.2	2.7	0.2
PP	BM1	39.2	7.6 – 70.0	795.9	4.0	7.6	0.3
PP	BM2	40.8	8.8 – 70.0	800.1	4.7	8.8	0.4
PP	BM3	49.4	6.3 – 70.0	926.5	5.1	6.3	0.3

9.3.3.- OA detection in synthetic seawater

After observing the results obtained in the 0.6M NaCl solution, a closer approach was taken in synthetic seawater to emulate real-life conditions and check the possible matrix effect that can take place in a more complex system. As in the previous case, several samples with synthetic seawater, PP2A and p-NNP, 4-MUP or PP containing spiked OA in a range from $5 \cdot 10^{-13}$ M to $7 \cdot 10^{-11}$ M were incubated and used to perform the calibration curves with the three different BMs. The results obtained show that the synthetic seawater does not produce a significant matrix effect, providing similar RSD and LOD values, as shown in table 9.3.

Table 9.3. *Parameters obtained from calibration curves for the OA inhibition of PP2A enzyme versus OA concentration in synthetic seawater solution.*

Substrate	Backing material	IC50 (pM)	Calibration Range (pM)	Sensitivity (nM/nA) (n = 3)	RSD (%)	LOD (pM) (n=3)	SLOD (pM)
p-NPP	BM1	60.8	6.3 – 70.0	688.8	8.5	6.3	0.5
p-NPP	BM2	44.8	3.2 – 70.0	923.4	1.4	3.2	0.1
p-NPP	BM3	49.7	8.3 – 70.0	954.7	4.2	8.3	0.3
4-MUP	BM1	53.1	6.1 – 70.0	781.6	8.7	6.1	0.5
4-MUP	BM2	47.1	6.5 – 70.0	742.4	6.9	6.5	0.4
4-MUP	BM3	45.8	7.7 – 70.0	918.5	6.8	7.7	0.5
PP	BM1	83.9	7.0 – 70.0	593.8	6.3	7.0	0.4
PP	BM2	60.8	6.3 – 70.0	809.3	4.0	6.3	0.3
PP	BM3	55.0	8.0 – 70.0	905.8	8.7	8.0	0.7

9.3.4.- Study of the inhibition type

The inhibitory effect of OA on different enzyme-substrate complexes was examined through the kinetics parameter of the Lineweaver-Burk plots, Fig. 9.2. For that, three regression curves were performed for each substrate; one without OA, one with $5 \cdot 10^{-11}$ M OA, and the last one in the presence of $7 \cdot 10^{-11}$ M OA. Lineweaver-Burk plots showed that OA produces a mixed inhibition, indicating that these substrates bind at a different active site than OA, which may result in their inhibition through changes in the three-dimensional shape of the enzyme, decreasing its activity. This is in keeping with other studies referenced in the bibliography that also support the claim that OA produces a mixed inhibition on the PP2A enzyme [4,6,39]. KM values were calculated, resulting in the following order: $KM \text{ p-NPP} > KM \text{ PP} > KM \text{ 4-MUP}$. These results suggest that 4-MUP is the substrate with the most affinity for PP2A, while p-NPP has the least affinity for it among the three substrates studied.

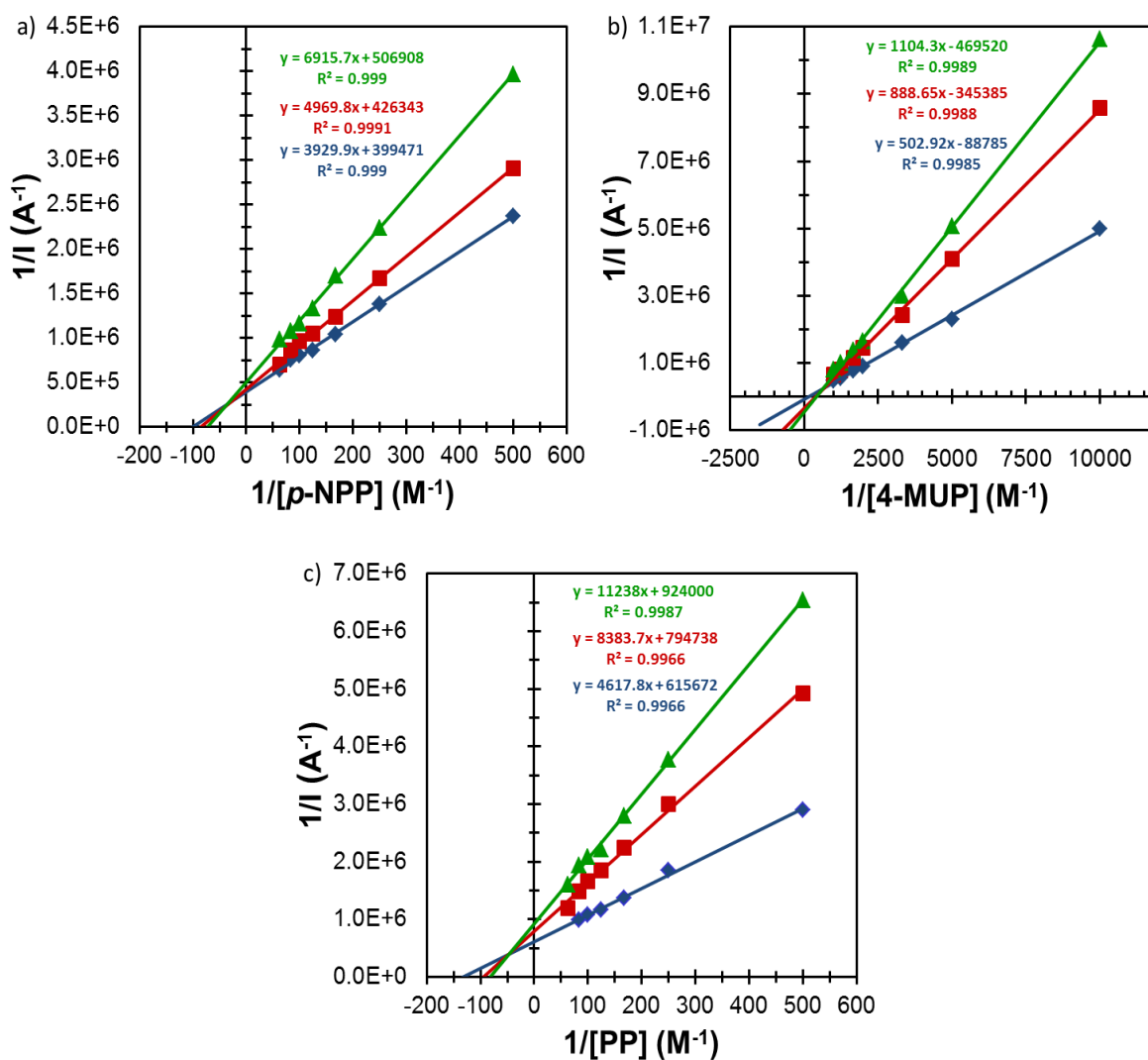


Figure 9.2. Lineweaver-Burk plot of the inhibitory effect of OA in the enzymatic reaction of: a) PP2A / p-NPP, b) PP2A / 4-MUP y c) PP2A / PP whitout OA (blue line), with $5 \cdot 10^{-11}$ M OA (red line) and $7 \cdot 10^{-11}$ M OA (green line).

9.3.5.- Interference study

Seawater is a complex matrix where metal ions of oligo-elements or heavy metal contaminants may be present, and they may also induce the inhibition of PP2A. Fig. 9.3 compares the inhibition- of different divalent cations with that caused by OA. The

results indicate that a concentration of $1 \cdot 10^{-6}$ M of mercury or copper ions produce a strong inhibition of the enzymatic dephosphorylation of PP2A, similar to the inhibition produced by $7 \cdot 10^{-11}$ M of OA. However, the inhibition is not observable at lower concentrations of $1 \cdot 10^{-8}$ M of these cations. On the other hand, Mg^{2+} , Ca^{2+} , Pb^{2+} , Cd^{2+} and Zn^{2+} exhibit either only a small sign of inhibition or none at all. In fact, some of the cations exhibit a slight increment of the enzymatic products, which may be due to the catalytical and central role that metal ions play in the activation of a water molecule for the dephosphorylation reaction [40]. Furthermore, the purification and long-term storage of PP2A may render the enzyme metal dependent to maintain the activity [41], broadening its substrate specificity [42]. It should also be noted that when p-NPP is employed as the substrate, the inhibition by metal ions is generally higher than when 4-MUP is used. This may be explained by the specificity between enzyme and substrate, showing that PP2A has a higher affinity for 4-MUP than p-NPP.

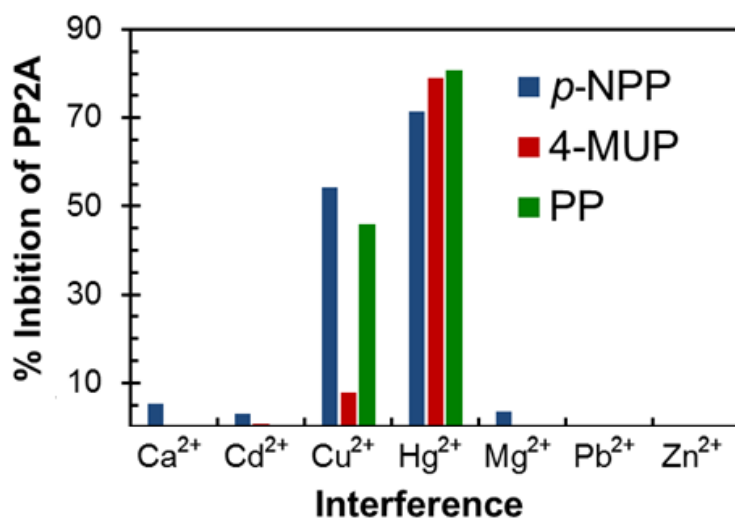


Figure 9.3. Results of the interference study of the inhibition of PP2A activity by different divalent metal ions added in a concentration of $1 \cdot 10^{-6}$ M.

9.3.6.- OA detection in real seawater samples

Two seawater samples collected on the same day from the Atlantic Ocean off the Galician coast during red tide were analysed, in order to determine the OA present in this water. The collected samples were incubated with the enzyme and the substrate, and measured in triplicate using DPV for each of the three substrates. Given that the tested BMs do not offer any significant differences, the samples were measured using only BM1 as backing material of the electrodic system. The intensity of the current (peak height) allowed for the determination of OA in the problem samples using the previous calibration curves performed, obtaining the results shown in Table 9.4. The samples were also analysed using the ELISA [31] method, providing a concentration of OA ≤ 50 p.M. This demonstrates that all three substrates exhibit good capability in the determination of OA.

Table 9.4. OA concentration measured in seawater samples, with the different substrates.

Substrate	Seawater Sample 1	Seawater Sample 2
p-NPP	49 \pm 4.3 pM	48 \pm 10.2 pM
4-MUP	48 \pm 5.0 pM	47 \pm 4.8 pM
PP	48 \pm 6.8 pM	50 \pm 5.0 pM

9.4.- Conclusions

In this work, an electrochemical method using disposable electrodes was developed based on the inhibition of the PP2A enzyme by okadaic acid (OA), and applied to the detection of this marine diarrhetic shellfish poisoning toxin in seawater. P-nitrophenyl phosphate, phenyl phosphate and 4-methylumbelliferyl phosphate demonstrated to be appropriate substrates to monitoring the inhibition by measuring the intensity decrease

of the oxidation peak of their enzymatic reaction products. Okadaic acid was seen to behave as a mixed inhibitor in the presence of the three substrates.

The method uses only a small amount of enzyme and is highly sensitive, simple and rapid. It has proven to be capable of detecting low OA concentrations ($\text{LOD} = 2.7 \cdot 10^{-12}$ M) in seawater samples that have not received any treatment. An interference study was also performed, showing that metals ions present in seawater do not interfere with the determination of OA.

The influence of the backing material of the electrodic system was also studied, observing that flexible textile supports offer results comparable to those obtained with rigid supports.

The method allows the determination of OA at concentration levels of 10^{-11} M in seawater, the aim of which is to provide an early warning method to prevent the contamination of mussels with OA.

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Las conclusiones globales de esta Tesis Doctoral se describen a continuación en este último apartado.

- 1- El modelo de SDFM se aplicó exitosamente a la NF de soluciones acuosas de un solo componente (NaCl, KCl, MgCl₂, MgSO₄, Na₂SO₄, sacarosa, betaína ácido láctico y ácido cítrico) y a mezclas de electrolitos formadas por una sal dominante y otra en concentración traza. La estrecha coincidencia entre los rechazos observados y los calculados por el modelo ponen de manifiesto la fortaleza del SDFM como modelo de simulación del proceso de NF. Se puede considerar que el modelo SDFM es una herramienta sencilla y útil para describir correctamente los mecanismos complejos que intervienen en los procesos de filtración con membranas y por tanto para su simulación y optimización.
- 2- Las diferencias obtenidas entre los rechazos observados y los intrínsecos, calculados por el SDFM evidencian la existencia de una capa de polarización por concentración en la superficie de la membrana, con un efecto más significativo en la NF de solutos con mayor permeabilidad.
- 3- Se observó que la permeabilidad de la membrana para los solutos individuales es menor que la permeabilidad en la capa estancada. Esto indica que el transporte en la membrana por difusión es más lento que en la capa estancada, hecho debido a un efecto sinérgico con la difusión de materia causado por el mecanismo de transporte convectivo en la capa límite.
- 4- Los rechazos observados de las sales individuales siguieron la siguiente secuencia: Na₂SO₄ \approx MgSO₄ > MgCl₂ > KCl \approx NaCl. Este comportamiento revela la existencia de cargas negativas en la superficie de las membranas ya que es la valencia del anión el factor determinante del rechazo de las sales individuales en solución acuosa.

- 5- En el caso de solutos no electrolitos, los rechazo observados siguieron la siguiente secuencia: sacarosa > betaína \approx ácido cítrico > ácido láctico.
- 6- Las permeabilidades en la membrana de los solutos individuales fueron inversamente proporcionales a su peso molecular y al radio de Stokes, ya que según el SDFM, éstos parámetros afectan significativamente el transporte en la membrana mediante un mecanismo de solución – difusión.
- 7- La permeabilidad en la capa estancada debería ser, según el SDFM, directamente proporcional al valor del coeficiente de difusión del soluto en solución, y por tanto la permeabilidad debería ser mayor para los ácidos láctico y cítrico que para la betaína. Las desviaciones del modelo son atribuidas a la parcial disociación de los ácidos orgánicos al pH alcanzado en la disolución experimental, lo que justifica que la fracción ionizada de los ácidos orgánicos sea rechazada por repulsiones electrostáticas con la membrana. En el caso de la betaína el pH experimental fue mayor que el pK_a , por lo que se encuentra totalmente en su forma anfotérica, lo que justifica que presentara una permeabilidad mayor en la capa de polarización por concentración que la de los ácidos orgánicos.
- 8- En el caso de mezclas de electrolitos, se observaron diferencias significativas en la permeación de un mismo ion traza en presencia de diferentes sales dominantes. Estas diferencias se deben atribuir, según el SDFM, al acoplamiento entre los flujos de los iones que se transportan, el cual está causado por la generación de campos eléctricos en la membrana y en la capa de polarización por concentración.
- 9- El modelo SDFM explica correctamente el rechazo negativo del ion traza Cl^- en presencia de diferentes sales dominantes ($MgSO_4$ y Na_2SO_4) y del ion Na^+ en presencia de $MgCl_2$ como sal dominante.

- 10- Se ha demostrado que el índice MFI-UF, siempre que se determine bajo condiciones que garanticen un mecanismo de filtración en torta, es una herramienta útil para comparar los niveles de ensuciamiento que alcanzan diferentes membranas de MF y UF.
- 11- Las membranas de UF de ZrO_2 presentan menor índice MFI-UF que las membranas de MF de TiO_2 en la filtración de agua de mar, por lo que estas últimas requieren mayor esfuerzo de limpieza para recuperar su permeabilidad.
- 12- La variación de los índices de ensuciamiento con la presión de trabajo indica que la torta formada sobre la superficie de las membranas es comprensible, con un factor de compresibilidad entre 2 y 3.
- 13- El rechazo del OA por las membranas dependió de la altura de la torta formada sobre su superficie y de la presión aplicada. Su retención se debe principalmente a las interacciones hidrofóbicas con las partículas NOM que se concentran sobre la superficie de la membrana.
- 14- Se ha demostrado que trabajar con membranas de UF y a baja presión, son las mejores condiciones para conseguir los objetivos del proyecto en su orientación de una máxima retención de materia coloidal y un mínimo rechazo del OA.
- 15- Cuando se trabaja a presiones altas (≥ 1.7 bar) y con membranas de MF de ≥ 0.45 μm de tamaño de poro, se obtuvieron rechazos negativos del AO. En estas condiciones se produce una acumulación de OA en el permeado. Estas condiciones pueden ser de utilidad en la detección de la toxina cuando se encuentra a concentración muy baja en el agua de mar, y por tanto en el diseño de un sistema de alerta temprana.

- 16- Los rechazos del Pb^{2+} con membranas cerámicas de UF y MF son significativos cuando su concentración está a nivel de traza. Esto se debe a que el principal mecanismo de retención del ion Pb^{2+} es de tipo electrostático por interacciones con la membrana, cargada negativamente al pH del agua de mar. El rechazo de las membranas cerámicas al ion Pb^{2+} es independiente del tamaño del poro de la membrana y de la presión aplicada, pero depende de su concentración, siendo despreciable a concentraciones de orden molar, una vez saturada la carga de la membrana.
- 17- La calidad en los permeados fue mayor en UF que en MF como se deduce de la evaluación del grado de reducción alcanzado de varios parámetros de calidad como turbidez, alcalinidad, demanda química de oxígeno (DQO) y contenido de clorofila
- 18- Las técnicas electroquímicas y concretamente las voltamperométricas, utilizando sistemas electródicos serigrafiados, han demostrado ser muy eficaces para la determinación de contaminantes marinos como el ácido okadaico y plomo al nivel de trazas en el agua de mar.
- 19- La determinación de plomo en agua de mar acidificando previamente la muestra, es posible con el uso de la voltamperometría de onda cuadrada de redisolución anódica (SWSAV) utilizando un electrodo de trabajo serigrafiado de carbono. La reproducibilidad del método, en términos del RSD de las pendientes asociadas a las rectas de calibración, en el intervalo de concentraciones de $1 \cdot 10^{-9}$ a $6 \cdot 10^{-9}$ M, es 2.5% ($n=3$). La capacidad de detección de $0.032 \pm 0.006 \cdot 10^{-9}$ M
- 20- Los análisis realizados mediante voltamperometría están muy influenciados por la modificación de la superficie del electrodo y del soporte de impresión.

- 21- Las condiciones óptimas para depositar electroquímicamente las nanopartículas de oro (AuNPs) sobre la superficie de un electrodo de trabajo se consiguieron mediante cronoamperometría aplicando un potencial de +0.18V vs Ag/AgCl durante 100s con una gota de disolución de $1 \cdot 10^{-3}$ M AuCl₃.
- 22- El sensor obtenido mediante la modificación del electrodo de trabajo serigrafiado de carbono con grafeno y nanopartículas de oro, AuNP/ERGO/SPCE, permite la determinación de plomo mediante voltamperometría diferencial de impulsos de redisolución anódica (DPASV) al nivel trazas directamente en muestras de agua de mar sin tratamiento previo.
- 23- El sensor basado en la utilización de AuNP/ERGO/SPCE como electrodo de trabajo, impreso sobre Gore-Tex® Pro-Shell Nylon Liner y utilizando la técnica DPASV, muestra los mejores resultados. La precisión, en términos del RSD de las pendientes asociadas a la rectas de calibración es 4.2% (n=3), en el rango concentraciones entre $2 \cdot 10^{-9}$ M y $10 \cdot 10^{-9}$ M y la capacidad de detección es $0.321 \pm 0.034 \cdot 10^{-9}$ M.
- 24- Los métodos propuestos para la determinación del plomo fueron satisfactoriamente validados mediante ensayos de recuperación.
- 25- Es posible llevar a cabo la determinación de OA por la inhibición que ejerce sobre la enzima PP2A. El sensor desarrollado para su determinación mide la variación de concentración de un producto que se obtiene de la reacción enzimática PP2A con un sustrato, cuando ambos se incuban en presencia de OA.

- 26- La optimización de las variables experimentales influyentes en la determinación del OA para el proceso de incubación conduce a las siguientes condiciones óptimas de trabajo: 37°C durante 30 min con 50 µl de la disolución de enzima.
- 27- La utilización del método desarrollado para medir OA mediante voltamperometría diferencial de impulso, empleando p-NPP, 4-MUP y PP como sustratos de la reacción enzimática, posibilitó rangos de medida en el intervalo de concentraciones comprendido entre $5 \cdot 10^{-13}$ M y $7 \cdot 10^{-11}$ M. Las reproducibilidades fueron menores del 10% (n=3), en términos de RSD de las rectas asociadas a las respectivas rectas de calibración en todos los casos.
- 28- Los diferentes soportes estudiados para la impresión de electrodos serigrafados para la optimización de la determinación del OA fueron BM1, BM2 y BM3, siendo el soporte BM1 el que aporta mejores RSDs de las pendientes asociadas a las rectas de calibración respectivas, frente a los otros materiales.
- 29- En el estudio de interferentes como Ca^{2+} , Cd^{2+} , Cu^{2+} , Hg^{2+} , Mg^{2+} , Pb^{2+} y Zn^{2+} , se pudo observar una gran inhibición de la enzima PP2A en el caso del Hg^{2+} y del Cu^{2+} a $1 \cdot 10^{-6}$ M. Sin embargo, a las concentraciones que se encuentran los iones en el agua de mar, inferiores a $1 \cdot 10^{-8}$ M, no se ha observado ningún tipo de interferencia para la determinación de OA.
- 30- La viabilidad del sensor impreso en BM1, como soporte, ha sido demostrada mediante ensayos de recuperación de 50 pM de OA en muestras reales de agua de mar, utilizando los diferentes sustratos.

- 31- El sensor electroquímico permite determinar OA en muestras reales de agua de mar en un rango que es lineal desde 0.41 ng/L hasta 56.3 ng/L, obteniendo una capacidad de detección de 2.17 ng/L.

- 32- El sensor desarrollado en este trabajo para el OA permite una determinación más sensible, simple y rápida que los descritos en bibliografía.

- 33- Los sensores desarrollados para la determinación del plomo y OA permiten su monitorización, estableciendo un sistema de alerta temprana de la contaminación de mejillones en el agua de mar, posibilitando su prevención.