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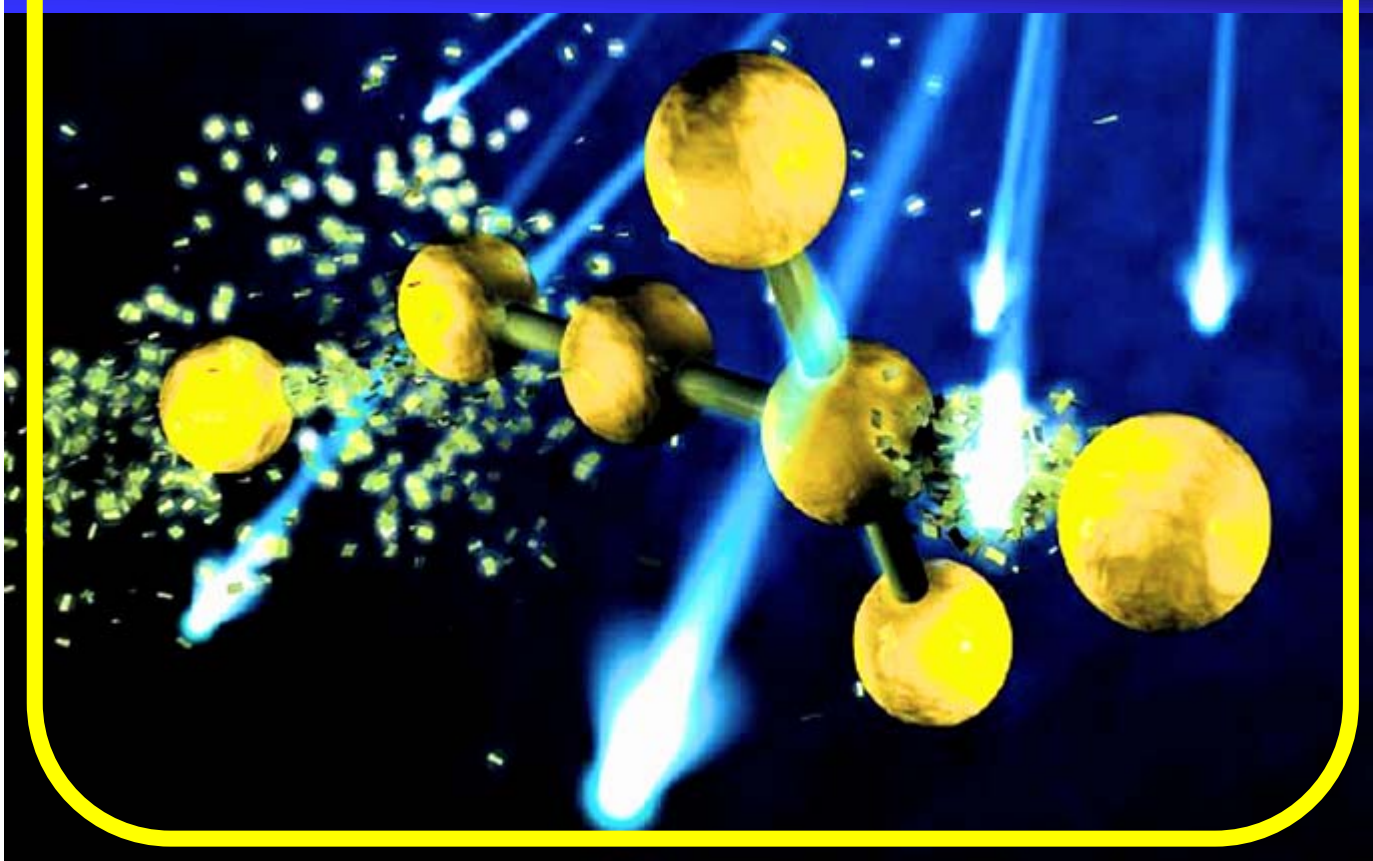
Seguimiento Cuantitativo de Procesos de Degradación de Contaminantes Orgánicos Fotosensibles Mediante Fluorescencia Molecular y Métodos de Análisis de Datos de Múltiples Vías

Tesis Doctoral
Marta V. Bosco
Tarragona, Octubre 2007



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Memoria presentada
por MARTA V. BOSCO
para optar al grado
de Doctor en Química
Tarragona, 2007

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Dra. **MARÍA SOLEDAD LARRECHI GARCÍA**, profesora Titular de Universidad del Departament de Química Analítica i Química Orgànica de la Facultat de Química de la Universitat Rovira i Virgili,

CERTIFICA: Que la presente memoria que tiene por título "SEGUIMIENTO CUANTITATIVO DE PROCESOS DE DEGRADACIÓN DE CONTAMINANTES ORGÁNICOS FOTOSENSIBLES MEDIANTE FLUORESCENCIA MOLECULAR Y MÉTODOS DE ANÁLISIS DE DATOS DE MÚLTIPLES VÍAS", ha sido realizada por **MARTA VERÓNICA BOSCO COSTA** bajo mi dirección en el Área de Química Analítica de esta Universidad y que todos los resultados presentados son fruto de las experiencias realizadas por dicho doctorando.

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Antes de ser hombres de ciencia, deberíamos ser hombres.

Albert Einstein

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Esta Tesis está dedicada con todo mi amor a mi familia: mis hermanos, Mary, Dany y Fer; a mi Abuela Modesta, a quien adoro con toda mi alma; a mi Mamá y a mi Papá…por darme la vida … porque gracias a ellos y todo el amor que me dan…. soy la personita que SOY… estando en el mismo lugar del mundo… o no… estamos SIEMPRE juntos

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haber propiciado siempre un clima de trabajo agradable. Gracias Xavier, Marisol, Pili, Itziar, Joan, Ricard, Jordi, Alicia, Mari, Enric, Santi, Jaume, Eulàlia, Ignacio, Cristina C., Marc, Cristina B., Verónica, Alberto, Esther, Pablo, Mariano,Paquita, Joe, Zayda, Néstor, Giselle, Gastón, Nicolás, Raquel, Rafa, Alí, Vanessa, Oleguer y Carolina.

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Meli.

Y como ya lo he mencionado en la dedicatoria, a mi familia, a quienes añoro terriblemente: mis hermanos, Dany, Fer, Mary y su maravillosa familia, Lucas y el intrépido Joaquín; a mi Abuela Modesta, a quien adoro con toda mi alma… y a mis Abuelos que ya no están aquí pero me acompañan y me cuidan desde el cielo, Abuelo Pedro, Lelita y Abuelo Tito; a mi Mamá y a mi Papá…por darme la vida y educarme con unos valores excepcionales… y aunque ya lo he dicho antes, nunca será suficiente mi agradecimiento… porque gracias a ellos y todo el amor que me dan… soy la personita que SOY… estando en el mismo lugar del mundo… o no… estamos SIEMPRE juntos

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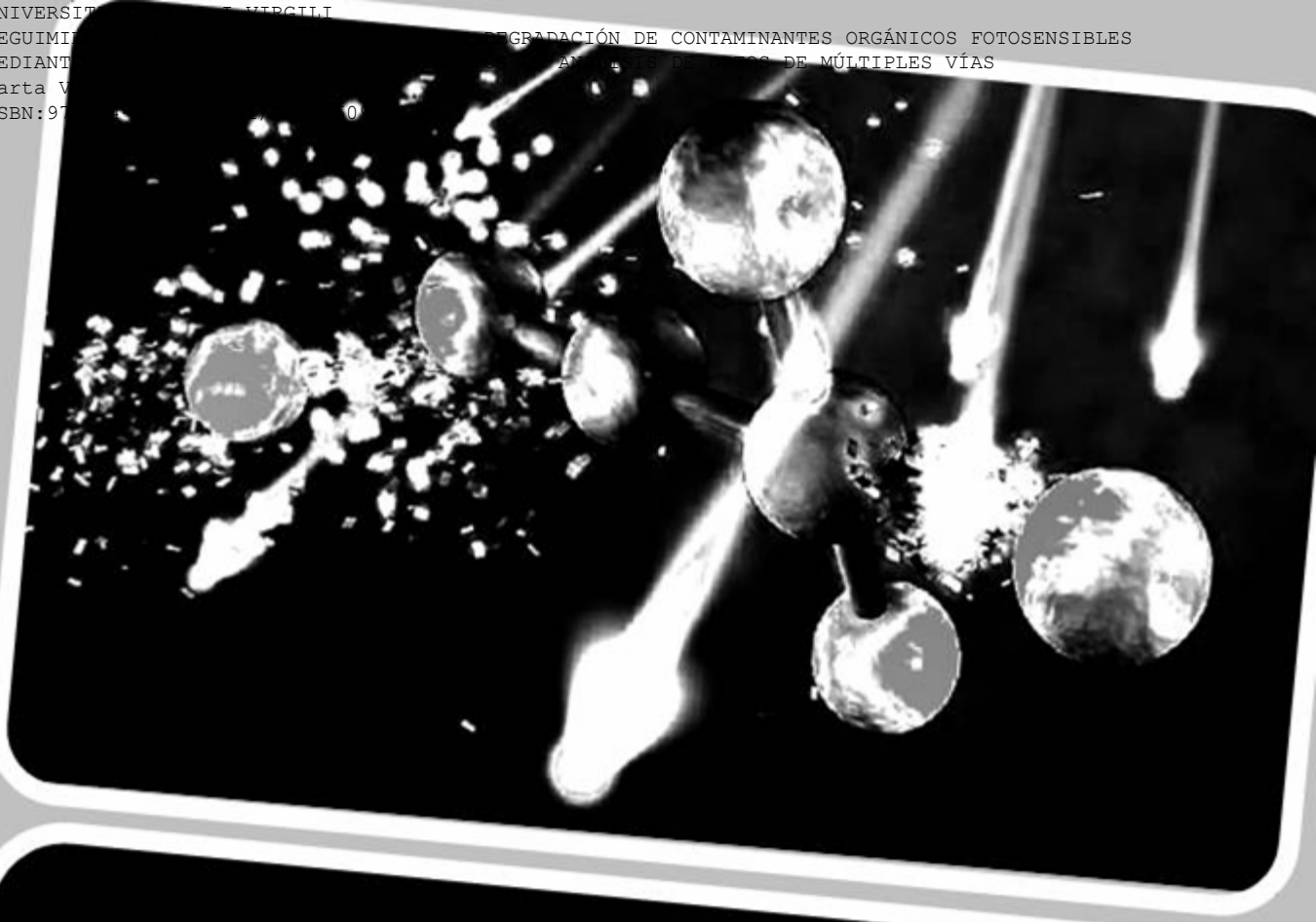

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ACN Acetonitrilo

ALS Mínimos cuadrados alternados

ANOVA Análisis de Varianza

ASTDR Agency for Toxics Substances and Disease Registry

BaA benzo[a]antraceno

BaP benzo[a]pireno

BkF benzo[k]fluoranteno

BLS Mínimos cuadrados bivariantes

DAD Detector de diodos integrados

Di(a,h)A dibenzo[a,h]antraceno

Df Grados de libertad

ECD Detector de captura de electrones

EEM Matrices de emisión-excitación

EM Espectrometría de masas

EPA Environmetal Protection Agency

Fit % Porcentaje de ajuste

FLD Detector de fluorescencia

GC Cromatografía gases

HPLC Cromatografía Líquida de Alta Eficacia

HPML Lámpara de vapor de mercurio de alta presión

IARC International Agency for Research on Cancer

IND Factor indicador de la función de Malinowski

IPA Isopropanol

IR Espectroscopía de infrarrojo

ITMS Detector de trampa de iones por espectrometria de masas

LC Cromatografía líquida

Lof Falta de ajuste

LPML Lámpara de vapor de mercurio de baja presión

MCR Métodos de resolución de curvas

N-PLS N Regresión PLS para datos de múltiples vías

PAHs Hidrocarburos policíclicos aromáticos

PARAFAC Análisis de factores paralelo

PCA Análisis de componentes principales

PLS Regresión por mínimos cuadrados parciales

PMT Tubo fotomultiplicador

R Sustrato

R * Estado excitado del sustrato

RE Error real o Desviación estándar residual

RMN Resonancia magnética nuclear

RMSEP Error medio del error de predicción

SVD Descomposición en valores singulares

TAO Tecnologías de procesos avanzados de oxidación

UV Radiación Ultravioleta

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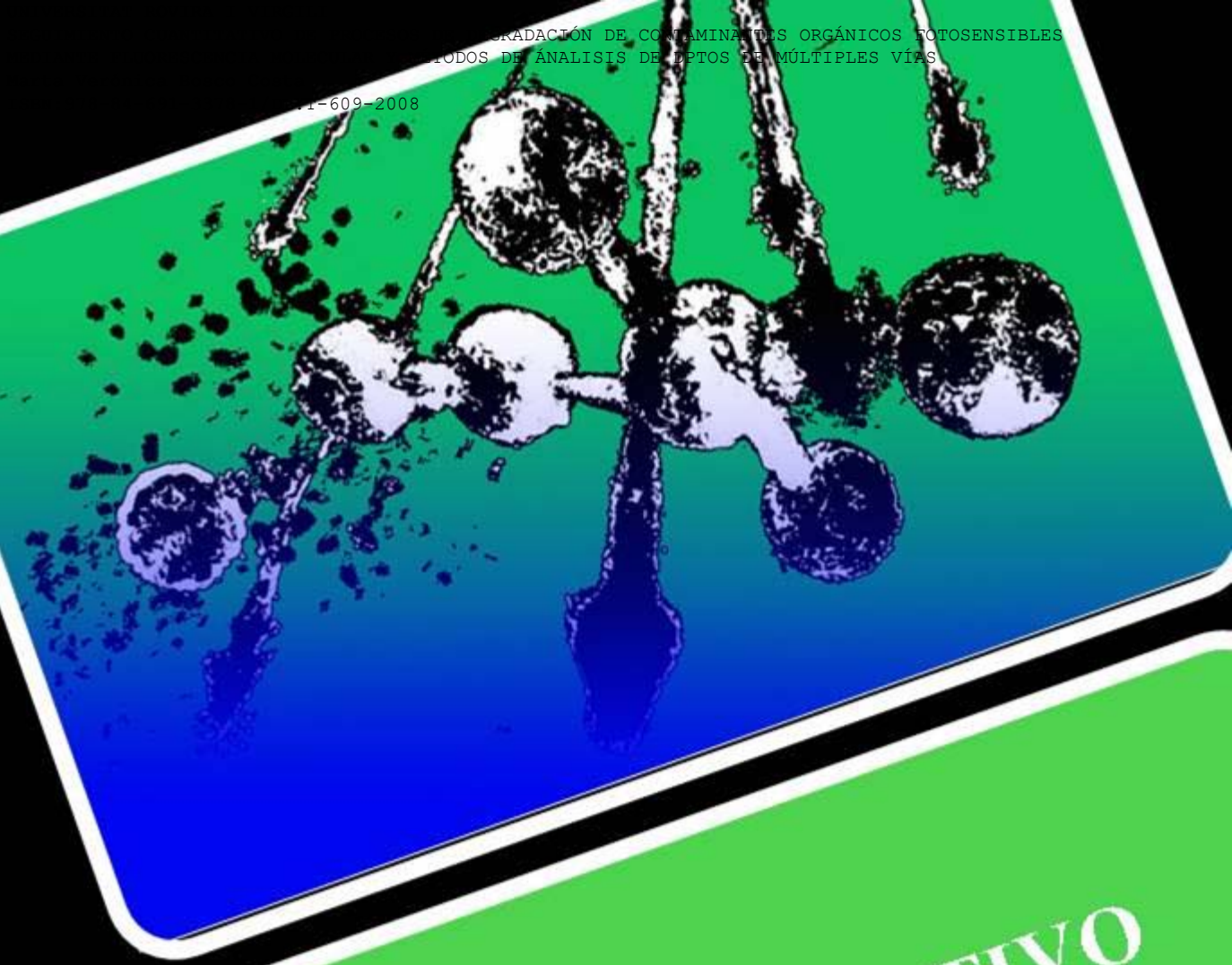

OBJETIVO

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OBJETIVO

En esta Tesis doctoral el objetivo general es el desarrollo de metodologías analíticas basadas en la aplicación de métodos de análisis de múltiples vías a datos de fluorescencia molecular de excitación-emisión obtenidos en el seguimiento de procesos de foto-degradación de contaminantes.

El interés de este estudio es obtener de forma rápida información útil en el desarrollo de tecnologías químicas basadas en procesos de degradación oxidativa inducidas por radiación ultravioleta.

Este objetivo general se ha centrado concretamente en:

- el seguimiento cuantitativo de reacciones de foto-degradación de hidrocarburos policíclicos aromáticos, y
- en el seguimiento cuantitativo de la degradación foto-catalítica del fenol

Alcanzar este objetivo general ha requerido:

1. Revisar la bibliografía actual con la finalidad de conocer los procesos de eliminación de contaminantes basados en procesos de oxidación inducidos por radiación ultravioleta, y las técnicas analíticas habitualmente empleadas.

2. Adquirir conocimientos apropiados para poder desarrollar experimentalmente los procesos de foto-degradación a nivel de laboratorio.

3. Conocer y utilizar correctamente la espectroscopia de fluorescencia molecular en su modalidad de excitación-emisión.

4. Planificar los experimentos utilizando técnicas de diseño de experimentos con la finalidad de analizar de forma conjunta el efecto de las variables que intervienen en los procesos en estudio.

5. Aplicar los métodos de resolución de curvas basados en el análisis de factores paralelos (PARAFAC) y en la optimización mediante mínimos cuadrados alternados (MCR-ALS), a medidas de excitación-emisión fluorescentes y determinar cuantitativamente los analitos involucrados en los procesos de foto-degradación en estudio.

6. Aplicar técnicas quimiométricas de calibración a datos de múltiples vías basado en mínimos cuadrado parciales (N-PLS), para desarrollar los modelos de calibración de los analitos de interés.

7. Comparar críticamente los resultados obtenidos utilizando los modelos desarrollados en los puntos 5 y 6.

8. Validar los resultados de las distintas metodologías de análisis de datos de múltiples vías desarrolladas a lo largo de la Tesis empleando HPLC como técnica de referencia.

Estos objetivos de generación, profundización y aplicación de conocimientos se complementan con las competencias a adquirir para ser un buen investigador. Entre ellas

podemos citar:

- la capacidad de contextualizar la investigación en el ámbito internacional del área de la especialidad,
- la habilidad para evaluar y sistematizar la bibliografía, con el objetivo de comprender los puntos clave del ámbito de conocimiento tratado, tanto desde un punto de vista disciplinar como multidisciplinar,
- la capacidad para analizar críticamente los cambios que se producen en el contexto de la disciplina,
- la aptitud para formular solicitudes de proyectos a los organismos adecuados, ya sea a la administración pública u otros organismos e instituciones, para gestionar de manera adecuada los recursos de la investigación, la propiedad intelectual y la comercialización de la innovación.

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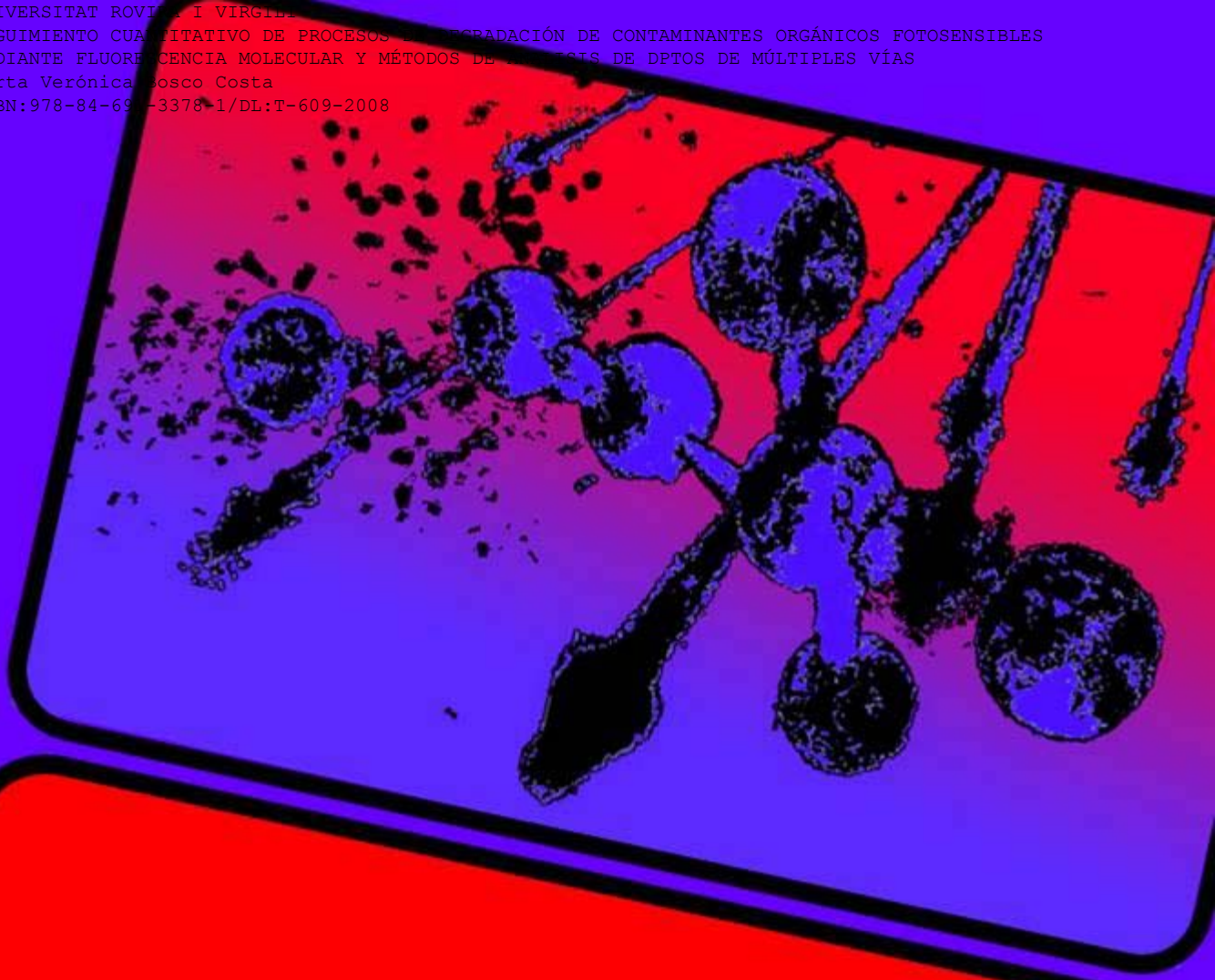

CAPÍTULO I

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Los hidrocarburos policíclicos aromáticos y el fenol forman parte de los contaminantes mas indeseables y nocivos para la salud humana, por ello la legislación mundial (EPA, Environmetal Protection Agency [1-2], IARC, The International Agency for Research on Cancer [3] y la ASTDR, Agency for Toxics Substances and Disease Registry [4]) es bastante estricta con las concentraciones permitidas de dichos contaminantes en el medio ambiente. El agua es uno de los medios naturales más controlados y en la actualidad existe gran interés en el desarrollo de tecnologías para la eliminación de contaminantes en ellas. Algunas de las más actuales, y que forman parte de las denominadas tecnologías de procesos avanzados de oxidación (TAO) [5-6], están basadas en procesos de foto-degradación química, donde la oxidación del contaminante está inducida por una fuente de radiación en ausencia de catalizador (foto-degradación propiamente dicha) o en presencia de un catalizador (degradación foto-catalítica).

Actualmente, desde el punto de vista del proceso de degradación oxidativa de los contaminantes a eliminar, los estudios se enfocan en:

- La evaluación de la eficacia en la eliminación de los compuestos

- La búsqueda de valores óptimos de las diferentes variables que afectan al proceso

- La cinética de degradación del/los compuesto/s [7-11] y en la detección de los intermedios de reacción, con el fin de establecer los mecanismos de degradación [7,12-18] y comprobar si tiene lugar la generación de productos de mayor toxicidad que la de los compuestos iniciales.

La información necesaria para procesar estos parámetros, se obtiene de la medida de las concentraciones de los compuestos que intervienen en el proceso de oxidación. Las técnicas instrumentales que, para ello se emplean de manera más habitual son:

- Para la evaluación de la eficacia del proceso, el análisis de carbono total (COT) [19-20] generado en la reacción, y que proporciona una medida del grado de mineralización del substrato.

- En la identificación de intermediarios, las técnicas cromatografías tanto de gases (GC) [14,21-23] como líquida (LC) [7-8,13-14,20-21,24-26] acopladas en algunos casos con la espectrometría de masas (EM) [27-28] son las más habitualmente empleadas. La espectroscopia de infrarrojo cercano (IR) puede proporcionar información "in situ" sobre reacciones que transcurren en solución acuosa [29-31] y también fuera del medio de reacción, cuando se analizan especies adsorbidas sobre el catalizador tras la reacción [32]. La espectroscopia de resonancia magnética nuclear (RMN) [31,33] y la

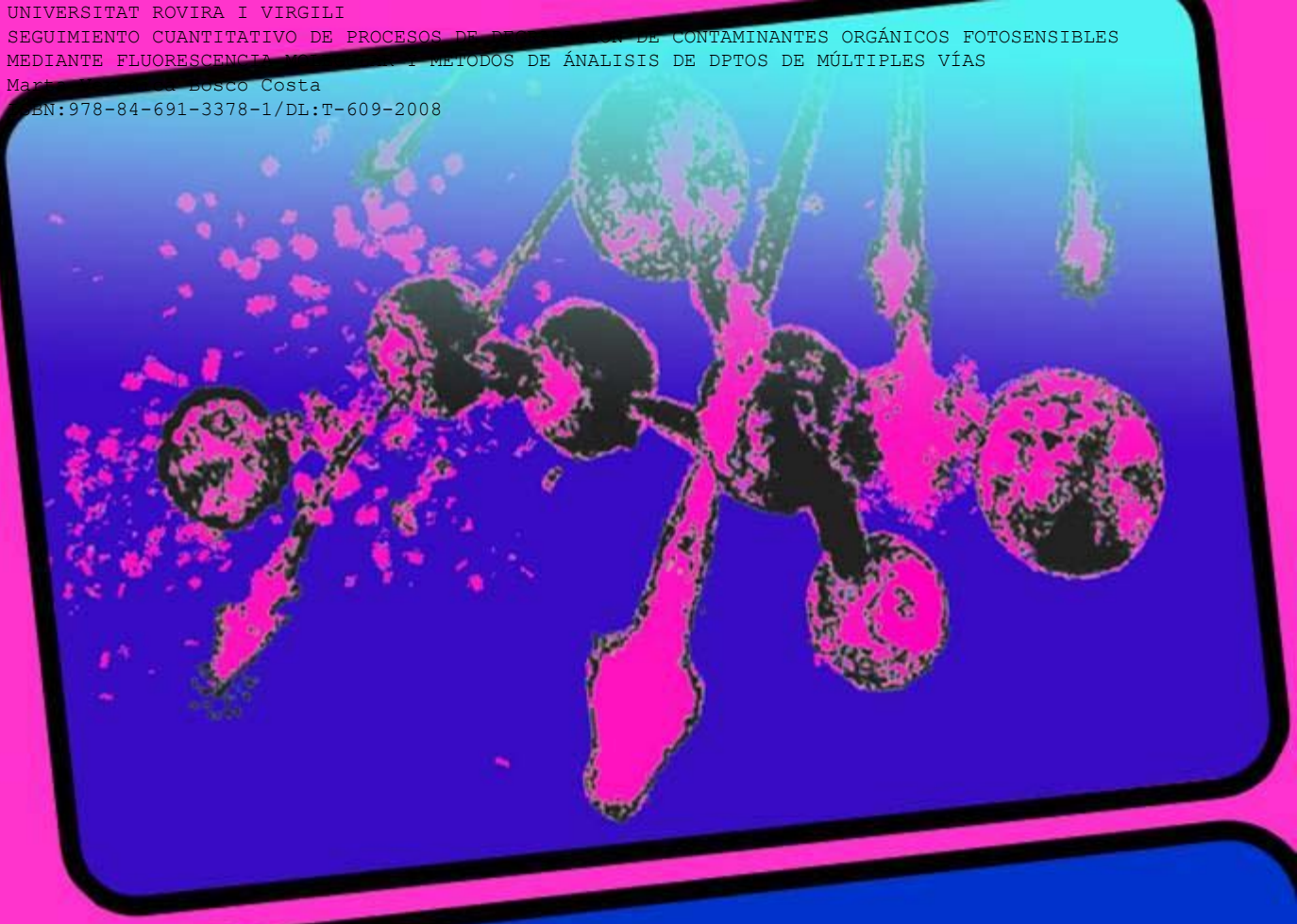
técnica de fluorescencia [24,34] también se han aplicado recientemente en el estudio "in situ" de las reacciones de degradación, con el fin de estudiar los mecanismos de reacción.

La espectrofluorimetría resulta una herramienta atractiva ya que debido a su elevada sensibilidad permite la detección de compuestos a niveles bajos de concentración. De hecho, es el detector habitualmente empleado cuando se trabaja en cromatografía líquida de alta resolución. El inconveniente que puede presentar, si no se ha hecho una separación previa de los compuestos presentes en la solución en estudio, es que tengan una señal espectral similar y/o a longitudes de onda coincidentes. Sin embargo, esta limitación se puede resolver o mejorar asociándola a metodologías quimiométricas, que permiten manejar la información contenida en todo el espectro y, por tanto, proporcionar mayor información.

En esta Tesis Doctoral, se aborda la aplicación de metodologías de análisis de datos de múltiples vías, a datos tridimensionales de fluorescencia molecular de excitación-emisión obtenidos a lo largo de una reacción, para obtener de forma rápida información de interés en el desarrollo de técnicas basadas en procesos de eliminación oxidativa de contaminantes orgánicos. Los datos registrados para cada muestra analizada constituyen una matriz o datos de segundo orden [35-40]. Ello nos permite trabajar con métodos de análisis multivariante que permiten analizar datos de múltiples vías como son el método de resolución de curvas basado en el análisis de factores paralelo (PARAFAC) [41-42] y el basado en la optimización por mínimos cuadrados (MCR-ALS) [43-44], así como el método de calibración multivariante en mínimos cuadrados parciales para datos de múltiples vías (N-PLS) [45-46]. La aplicación de estos métodos a datos de fluorescencia de excitación y emisión exige superar algunos problemas típicos de ellos como son las dispersiones Rayleigh y Raman.

Se afrontan dos casos en particular: la foto-degradación de una mezcla de hidrocarburos policíclicos aromáticos (benzo[a]antraceno, benzo[a]pireno, benzo[k]fluoranteno, dibenzo[a,h]antraceno) y la foto-catálisis del fenol. Para la planificación de los procesos en estudio, se han empleado técnicas de diseños de experimentos que permiten el análisis simultáneo de las variables que intervienen en ellos.

Las metodologías mencionadas, basadas en matrices de emisión-excitación (EEM) y técnicas de análisis de datos de múltiples vías, representan un recurso valioso que puede ser utilizado para el seguimiento cuantitativo de la concentración de los analitos implicados en un proceso de degradación y de esta forma evaluar parámetros cinéticos del proceso que sirvan para el análisis de las variables que intervienen en él. Sin embargo, desde el punto de vista cuantitativo, es necesario establecer la bondad de los resultados obtenidos mediante estos métodos. En esta Tesis, los resultados obtenidos de combinar espectroscopia de fluorescencia molecular con PARAFAC, MCR-ALS y N-PLS se comparan con los obtenidos mediante cromatografía líquida de alta resolución (HPLC), considerada como técnica de referencia para el seguimiento de este tipo de reacciones.



CAPÍTULO II

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2.1. Reacciones de Foto-degradación en medio acuoso.

2.1.1. Introducción

La fotólisis o foto-degradación primaria es el estudio de la degradación directa de ciertos compuestos bajo el efecto de la luz [6]. Los compuestos susceptibles de foto-degradarse absorben la radiación por sí mismos y son excitados electrónicamente sufriendo subsecuentes transformaciones. El conocimiento del comportamiento de estas sustancias frente a la radiación ultravioleta, y la formulación de su evolución es de gran importancia para el medio ambiente si éstas son contaminantes. El estudio de estos procesos, con el fin de elucidar los mecanismos de foto-degradación, está basado principalmente en técnicas de foto-degradación continua bajo irradiación simulada y el control de la formación de los foto-productos

La foto-catálisis heterogénea es el desarrollo y adaptación de un proceso de foto-degradación basado en la inclusión de un material semiconductor que iluminado con radiación ultravioleta, es capaz de degradar los principales contaminantes hasta los niveles de concentración aceptables según las normas. Una de sus ventajas es que no es selectiva y puede emplearse para tratar mezclas complejas de contaminantes [48].

Normalmente, en foto-catálisis heterogénea se utilizan óxidos semiconductores como catalizadores. Estos compuestos constituyen una amplia clase de materiales cuya conductividad eléctrica aumenta con la temperatura y es significativamente menor que la de los metales; las propiedades generales de estos materiales, han sido ampliamente descritas [49]. Por diversas razones, el proceso de tratamiento y/o purificación de

aguas mediante foto-catálisis heterogénea con dióxido de titanio como catalizador es, hoy por hoy, una de las aplicaciones fotoquímicas que más interés ha despertado entre la comunidad científica internacional.

2.1.2. Mecanismos de degradación

Fotólisis o foto-degradación primaria

En los sistemas acuosos los constituyentes más importantes son el agua, el oxígeno molecular y otras especies disueltas, eventualmente contaminantes que deben ser eliminados del sistema. Las reacciones de foto-oxidación sobre los contaminantes (sustrato, **R**) electrónicamente excitados implica en la mayoría de los casos una transferencia de electrones desde el estado excitado del sustrato (**R** *) al medio formando radicales [7,13]

$$\mathbf{R} + h\nu \rightarrow \mathbf{R}^{*+} + e^-_{(aq)}$$

$$\mathbf{R}^{*+} + e^-_{(aq)} + O_2 \rightarrow \mathbf{R}^{*+} + O_2^{\bullet -}$$

$$H_2O + h\nu \rightarrow OH^\bullet + H^+$$

Estos radicales son altamente reactivos frente a la mayoría de las moléculas orgánicas y muchas especies inorgánicas.

$$OH^\bullet + \mathbf{R}H \rightarrow \mathbf{R}^\bullet + H_2O$$

$$\mathbf{R}^\bullet + O_2 \rightarrow \mathbf{R}O_2^\bullet \rightarrow \rightarrow CO_2$$

$$OH^\bullet + \mathbf{R}X \rightarrow \mathbf{R}X^{\bullet +} + OH^-$$

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A través de reacciones de hidrogenación o de adición a dobles enlaces o a anillos aromáticos, se favorece la oxidación de sustratos mediante la formación de radicales libres que reaccionan fácilmente con el oxígeno disuelto, fragmentándose hasta alcanzar eventualmente la mineralización completa. De esta manera los compuestos orgánicos pueden oxidarse formando de dióxido de carbono, agua y ácidos inorgánicos [47].

Foto-catálisis

La foto-excitación con una energía igual o mayor que la del espacio entre las bandas de valencia (bv) y de conducción (bc) del catalizador, hace que un electrón de la banda de valencia sea promovido a la banda de conducción, generándose un hueco deficiente de electrones (h^+) en la primera banda [50]. Las especies foto-generadas pueden participar en reacciones redox con diversas especies químicas, ya que el hueco en la banda de valencia del catalizador es fuertemente oxidante y el electrón en la banda de conducción es moderadamente reductor (ver Figura 1)

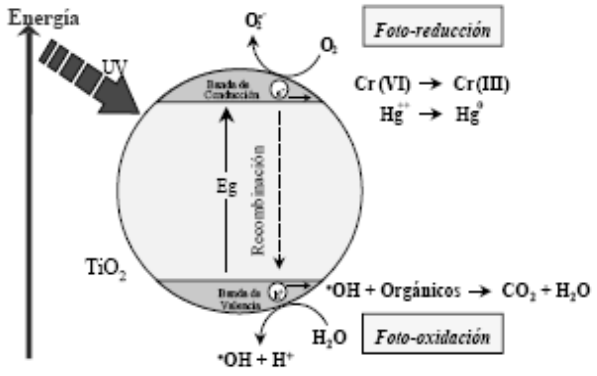

Fig. 1 Esquema del proceso de degradación solar foto-catalítica: la activación de la partícula de catalizador mediante luz resulta en la generación de radicales hidroxilo (OH•)

En estas circunstancias se generan radicales hidroxilo (·OH) que tienen un elevado potencial de oxidación y atacan cualquier sustancia orgánica que se encuentre en el medio, dando lugar a un proceso cuyo resultado es una progresiva ruptura de enlaces hasta concluir en compuestos como el dióxido de carbono, agua y simples ácidos inorgánicos diluidos como se ha mencionado anteriormente.

$$TiO_2 + h\nu \rightarrow e^-_{bc} + h^+_{bv}$$

$$h^+_{bv} + H_2O \rightarrow OH^\bullet + H^+$$

$$OH^\bullet + \mathbf{R}H \rightarrow \mathbf{R}^\bullet + H_2O$$

$$\mathbf{R}^\bullet + O_2 \rightarrow \mathbf{R}O_2^\bullet \rightarrow \rightarrow CO_2$$

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2.1.3. Hidrocarburos policíclicos aromáticos: Foto-degradación

La familia de los hidrocarburos policíclicos aromáticos (*polycyclic aromatic hydrocarbons*, PAHs) es un grupo de hidrocarburos que consisten en moléculas que contienen dos o más anillos aromáticos y con baja solubilidad en agua y elevada solubilidad en disolventes orgánicos. Se forman durante la incineración incompleta del carbón, el petróleo, el gas, la madera, la basura y otras sustancias orgánicas, como el tabaco y la carne asada al carbón [4]. Los PAHs se encuentran generalmente como mezclas complejas, no como compuestos simples. Estas sustancias se encuentran de forma natural en el medio ambiente.

Aunque existen más de 100 clases diferentes de PAHs solo 17 han sido clasificados por la Agencia de Protección Ambiental (EPA, *Environmetal Protection Agency)* como contaminantes principales [51].Los efectos de salud causados por cada uno de los PAHs individuales no son exactamente los mismos, su toxicidad suele estar asociada a su conversión fotoquímica a productos más tóxicos [52]. Varios PAHs, entre los que se incluyen benzo[a]antraceno, benzo[a]pireno, benzo[b]fluoranteno, benzo[j]fluoranteno, benzo[k]fluoranteno, criseno, dibenzo[a,h]antraceno e indeno[1,2,3-c,d]pireno, han causado tumores en los animales de laboratorio que han respirado estas sustancias, que las han consumido o que han entrado en contacto con las mismas a través de la piel durante largos períodos de tiempo. En la Figura 2 pueden verse la estructura de los PAHs estudiados en esta tesis.

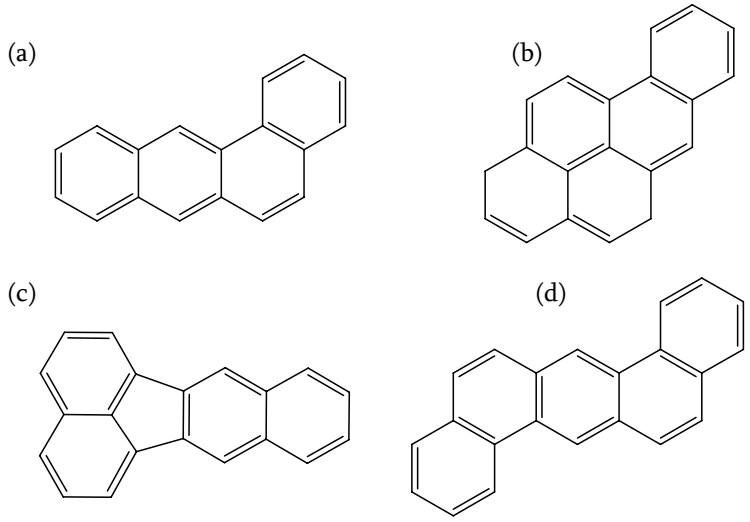

Fig. 2 (a) benzo[a]antraceno (BaA), (b) benzo[a]pireno (BaP), (c) benzo[k]fluoranteno (BkF), (d) dibenzo[a,h]antraceno (Di(a,h)A)

Los estudios realizados en los seres humanos demuestran que las personas expuestas a través de la respiración, el contacto con la piel o la ingestión a través de los alimentos durante largos períodos de tiempo de PAHs y otros compuestos, también pueden contraer cáncer [4]. Basándose en estos datos la EPA [2] y la IARC [3] han clasificado como probables contaminantes cancerígenos siguiendo el siguiente orden de toxicidad, comenzando con el más tóxico, con el benzo[a]antraceno (BaA), benzo[b]fluoranteno (BbF), benzo[k]fluoranteno (BkF), benzo[a]pireno (BaP), criseno y dibenzo[a,h]antraceno (Di(a,h)A).

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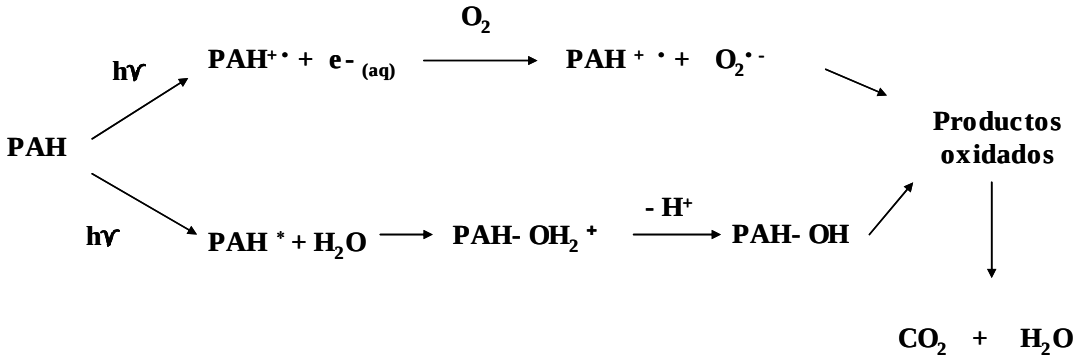
Mecanismo de foto-degradación de PAHs

Cuando los PAHs absorben radiación, pasan al estado excitado, y en soluciones acuosas son oxidados. A partir de las referencias encontradas [7,12-13,53] se puede observar el siguiente mecanismo de foto-degradación (Esquema 1)



Esquema 1. Mecanismo de reacción propuesto para los hidrocarburos aromáticos

2.1.4. Fenol: Foto-catálisis

Los fenoles son compuestos orgánicos de fórmula general ArOH, donde Ar corresponde a un compuesto aromático que puede tener sustituyentes. Además, difieren de los alcoholes en que tienen el grupo –OH directamente unido al anillo aromático.

En general, los fenoles se nombran como derivados del miembro mas sencillo de la familia, el fenol, aunque ocasionalmente se les denomina como hidroxicompuestos. En la Figura 3 se muestran los compuestos que se analizaron en esta tesis [54-55]. El fenol puro y los fenoles sustituidos suelen ser sólidos cristalinos, incoloros. No obstante, los fenoles experimentan una rápida oxidación a compuestos orgánicos de color y como resultado de ello, muchos fenoles son de color rosa o café debido a las impurezas presentes en la oxidación.

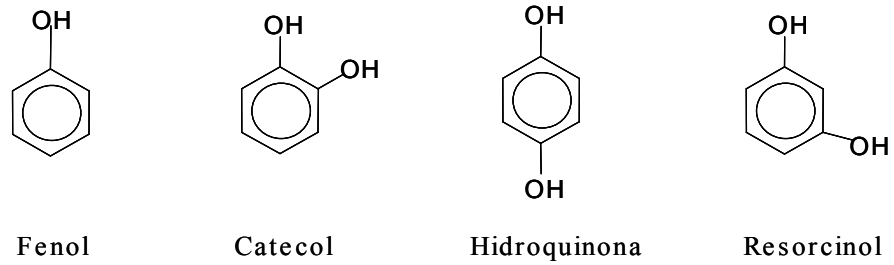


Fig. 3 Estructuras de los compuestos fenólicos utilizados en esta tesis

La contaminación de las aguas y del medio ambiente con compuestos fenólicos es un serio problema en la actualidad debido a su alta toxicidad y a su persistencia en el medio ambiente. Incluso en la lista de contaminantes publicada por la EPA en 1991, se ha

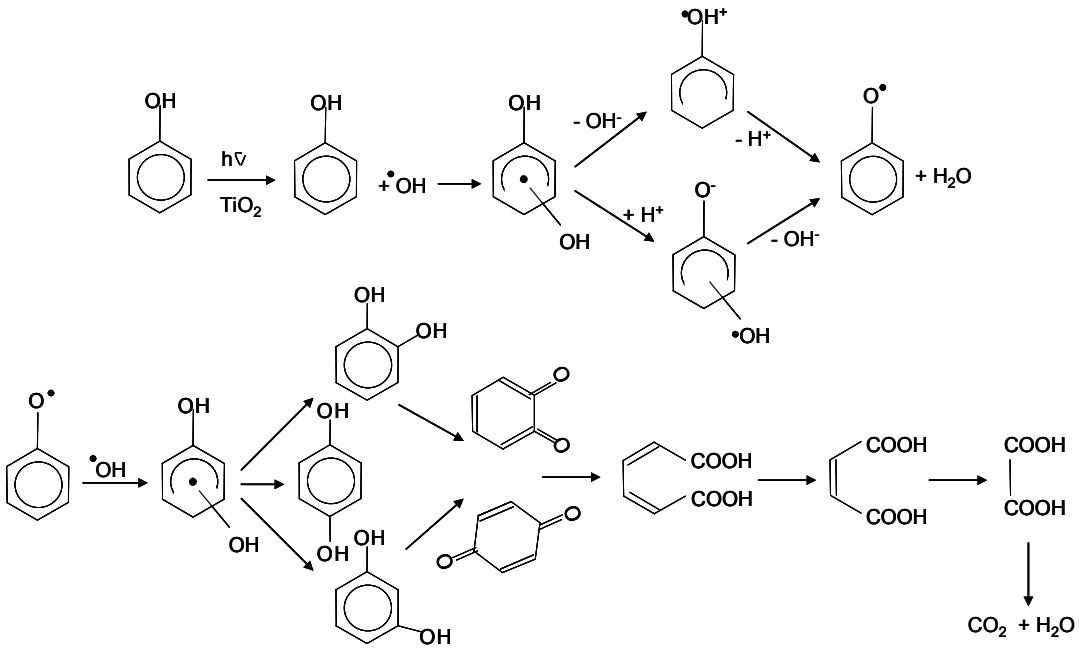
calificado a los compuestos fenólicos como químicos persistentes y bioacumulativos (PBT), y al fenol con puntuación más tóxica que sus derivados bi, tri y tetra-clorados [56].

El fenol es un compuesto muy corrosivo y puede producir quemaduras considerables al ponerse en contacto sobre la piel. La exposición a vapores del mismo también puede producir mareos, debilidad muscular y hasta convulsiones. El fenol ha sido citado como causante de envenenamiento crónico por exposición prolongada, aunque la IARC ha determinado que el fenol no es una sustancia clasificable como cancerígena para los humanos [57]. De acuerdo con la ATSDR la carga de fenoles máxima permitida en aguas interiores (lagos, ríos) y en peces contaminados, debe ser menor de 3.5mg/L [58] para el consumo humano.

Su presencia en agua potable incluso en bajas concentraciones (1-10 μg/L), genera clorofenoles durante el proceso de cloración, lo que produce un fuerte olor y sabor desagradable [59]. Los fenoles son extensamente usados en la producción o manufactura de una gran variedad de compuestos aromáticos, incluyendo explosivos, fertilizantes, pesticidas, carbón, pinturas, solventes, gomas, textiles, preparación de drogas, perfumes y plásticos y llegan al ecosistema a través de las descargas industriales, el desagüe municipal, los depósitos de gasolina, industrias del aceite y efluentes de destilerías [49,60]. También se forman durante la descomposición de sustancias húmicas, taninos, y ligninas [61].

Mecanismo de foto-catálisis del fenol:

El mecanismo para la foto-catálisis del fenol y sus reacciones subsecuentes ha sido propuesto por varios autores [14-18]. A partir de estas propuestas, en esta tesis se recoge el siguiente esquema general:



Esquema 2. Mineralización del fenol por foto-catálisis.

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2.2. Espectroscopia de fluorescencia molecular

La fluorescencia es un proceso de fotoluminiscencia en que los átomos o las moléculas son excitados por absorción de la radiación electromagnética. Las moléculas que han sido excitadas se relajan hacia el estado fundamental, liberando el exceso de energía en forma de fotones. Técnicamente hablando, es la emisión que resulta de la transición desde un estado singlete excitado al estado fundamental.

En la Figura 4, se ilustra el diagrama de energía de Jablonski [62] donde se presentan los procesos que implican a los estados excitados.

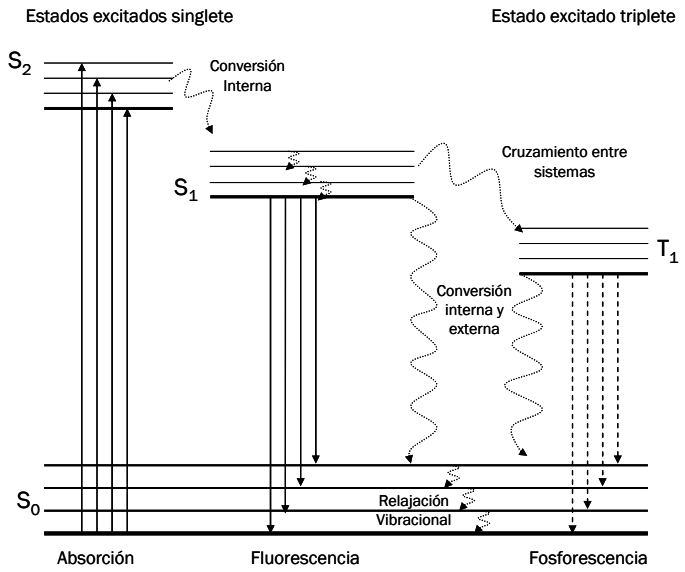

Figura 4. Diagrama de Jablonsky

Características de la emisión fluorescente

Una propiedad general de la fluorescencia es que los espectros de emisión son independientes de la longitud de onda de excitación, conociéndose este comportamiento como regla de Kasha [63], postulada por este autor en 1950.

Varios factores pueden afectar a la fluorescencia y pueden dividirse en tres grandes grupos: tipo de transición que se presenta, efectos estructurales y efectos ambientales. En este apartado se hace únicamente referencia a aquellos que tienen influencia sobre las condiciones de trabajo de los problemas abordados.

Efectos ambientales. La temperatura es un factor influyente pero no determinante de la eficacia de la emisión fluorescente. En general, produce un aumento de las colisiones entre moléculas y, por tanto, favorece los procesos de desactivación no radiante.

La naturaleza del disolvente, la viscosidad y polaridad del mismo, así como la capacidad de formar enlaces por puente hidrógeno, pueden afectar significativamente las características fluorescentes, ya que afectan directamente al estado excitado.

El pH de la disolución puede ejercer gran efecto sobre la fluorescencia, afectando tanto a la longitud de onda de emisión como la intensidad de ésta. Este comportamiento es debido a que el pH influye en la carga y en las formas resonantes del fluoróforo.

Efectos de dispersión

En fluorescencia, la dispersión Rayleigh ocurre cuando un electrón se excita a un nivel vibracional más alto que el nivel fundamental sin transición electrónica; en este caso la energía se conserva y un fotón de la misma energía se reemite dentro de 10^{-15} s, mientras que el electrón, vuelve a su estado original [64]. Este proceso, en general, es causado por las moléculas de soluto que oscilan a la misma frecuencia que la radiación incidente, y por eso emiten a la misma longitud de onda de la radiación incidente [65]. La dispersión de Rayleigh ocurre a todas las longitudes de onda y este problema es mayor cuando los espectros de absorción y emisión de los fluoróforos están a longitudes de onda muy cercana [64].

Esta dispersión no proporciona ninguna información acerca de las características de fluorescencia de la muestra y, en lo posible, debe ser disminuida o eliminada. En fluorescencia de emisión-excitación, su efecto produce una línea diagonal a través del espectro total. Esta línea aparece siempre en las longitudes de onda de emisión iguales a las longitudes de onda de excitación (Rayleigh de primer orden), aunque también puede presentarse de emisiones obtenidas a longitudes de onda alrededor dos veces de la longitud de onda de excitación (Rayleigh segundo orden). Estas señales fluorescentes, no siempre tienen la misma magnitud y no corresponden al fluoróforo en sí mismo, por lo tanto, no es proporcional a la concentración de éste.

La banda de dispersión Raman aparece en el espectro de fluorescencia a una frecuencia constante debida a la radiación de excitación que es dependiente del solvente de la solución. La banda Raman es mucho más débil que la dispersión Rayleigh, pero llegan a ser significativas cuando se utilizan fuentes de alta intensidad o cuando las soluciones son muy diluidas.

2.3. Técnicas quimiométricas

2.3.1. Introducción

Actualmente, la disponibilidad de métodos instrumentales que permiten obtener datos en función de dos o más variables controladas, junto con la incorporación de sistemas informáticos de gran capacidad para procesar datos han hecho posible el desarrollo de métodos analíticos automatizados, generando gran cantidad de datos en tiempos muy reducidos. La extracción de información relevante de esos datos para la resolución de un determinado problema químico, requiere la utilización de herramientas matemáticas y estadísticas. La disciplina química que emplea el conjunto de estas técnicas se denomina Quimiometria [66].

En esta tesis se han empleado técnicas quimiométricas para el análisis de datos de múltiples vías con la finalidad de:

- Cuantificar e identificar todos los compuestos implicados en procesos de foto-degradación aprovechando las ventajas que ofrecen los métodos de resolución de curvas (MCR), análisis paralelo de factores paralelos (PARAFAC) y la calibración por mínimos cuadrados parciales para múltiples modos (N-PLS)

- Analizar de forma simultánea las variables que influyen en los procesos de foto-degradación utilizando técnicas de diseño de experimentos.

El desarrollo y aplicación de métodos de análisis de múltiples vías [67-70] permite

la determinación simultánea de la concentración de diversos componentes en muestras complejas. Estos métodos minimizan o eliminan el tratamiento previo de la muestra, reduciendo notablemente el tiempo de análisis, lo cual constituye una importante ventaja para los análisis de rutina o de control sobre un elevado número de muestras.

2.3.2. Estructuras de los datos

La señal analítica registrada en el espectrofluorímetro ha sido el espectro de emisión-excitación en un intervalo de longitudes de onda y conforma una matriz **X** de datos ($J \times K$) (datos de segundo orden), EEM (*Emission-Excitation Matrix*).

Un conjunto de datos de segundo orden, obtenidos del análisis de distintas muestras se pueden agrupar para formar una estructura de tres vías ($I \times J \times K$) (Figura 5), formando un cubo de datos $\underline{\mathbf{X}}$.

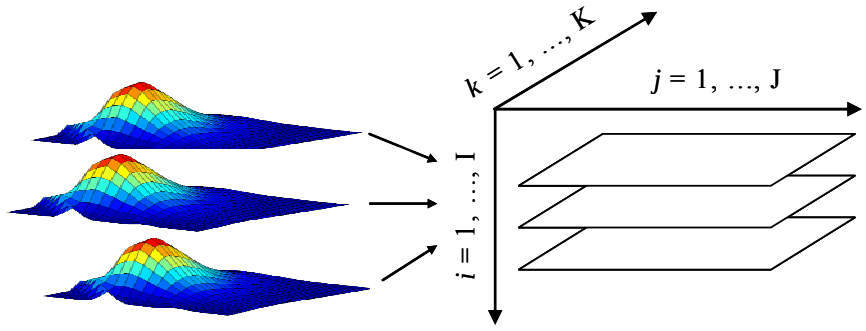

Fig. 5. Estructura de tres vías

Las matrices de datos EEM de cada muestra medida también se pueden alinear para obtener una matriz aumentada de datos [73]. Las matrices aumentadas se pueden hacer tanto en el modo emisión como en el modo excitación. Aumentando en el modo excitación, que es el utilizado en esta tesis, las matrices se colocan una sobre otra, y las longitudes de onda de excitación se mantienen en la misma columna (Figura 6(a)).

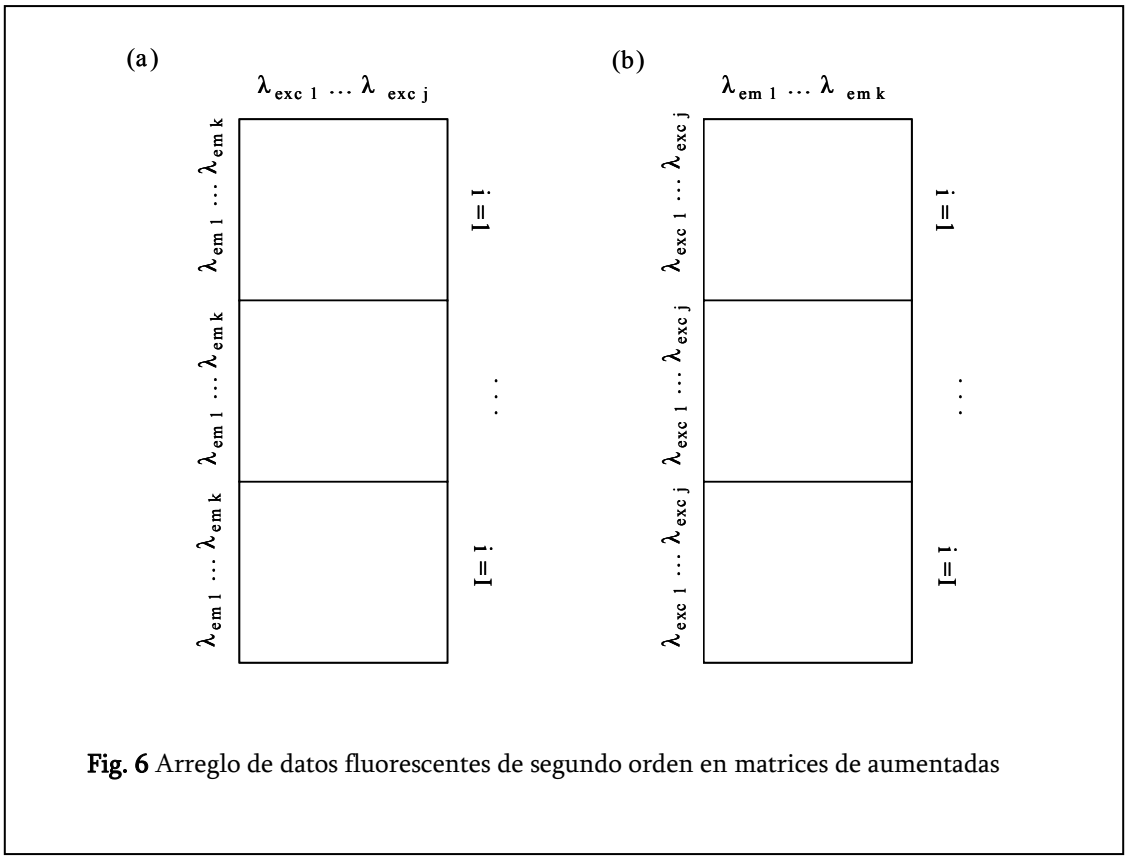

Fig. 6 Arreglo de datos fluorescentes de segundo orden en matrices de aumentadas

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2.3.3. Pretratamiento de datos

Algunos algoritmos utilizados en esta tesis, resuelven con mayor eficacia datos estrictamente trilineales, tales como PARAFAC, lo cual constituye una limitación cuando se analizan datos que presentan desviaciones de la trilinealidad. Un ejemplo de esto es la presencia de fenómenos como las dispersiones Rayleigh y/o Raman (Figura 7) [64,74-75] por lo que es necesario eliminarlos o reducirlos tanto como sea posible.

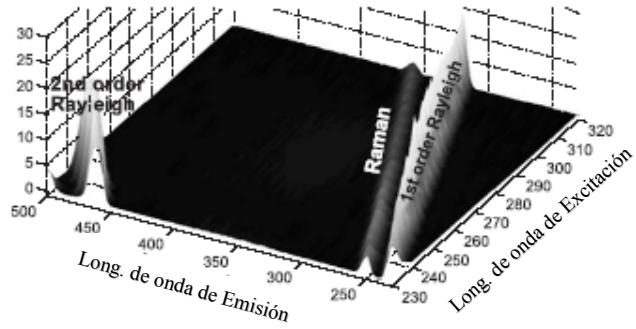

Fig. 7. Muestra de agua donde se ven claramente los efectos de las dispersiones.

La dispersión Raman es debida al solvente y puede ser completamente eliminada sustrayendo el espectro del solvente al espectro de la muestra [64]. El efecto de la dispersión Rayleigh se puede eliminar utilizando estrategias de ponderación de coeficientes (*weighting elements*) [65,74,76], insertando Valores desconocidos (*missing values*) [77-79] o colocando valor cero (*insertion of zeros*) [77] a la zona en cuestión.

Ponderación de coeficientes (Weighting elements)

Como se explica anteriormente, la señal debida a la dispersión Rayleigh ocasiona la pérdida de la trilinealidad de los datos experimentales y por lo tanto, PARAFAC no puede modelarlos correctamente. Una de las soluciones a este problema es la construcción de un "cubo ponderado" $\underline{\mathbf{W}}_{ijk}$, (Figura 8). Este tipo de ponderación, es de asignación binaria [74], otorgando valores de 0 o 1 a los elementos del cubo.

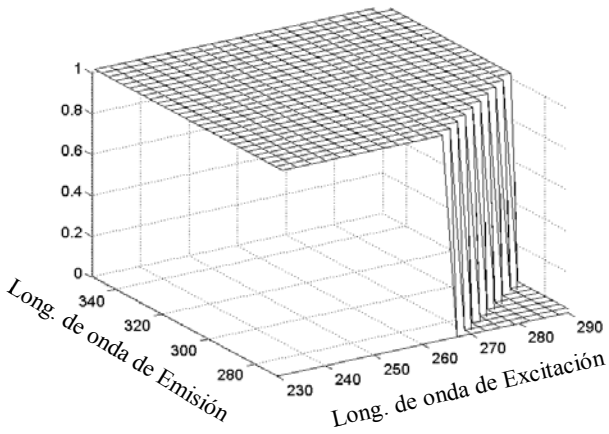

Fig. 8 Matriz de datos ponderados $\mathbf{W}_{ijk}$

Al área que presenta la dispersión Rayleigh se le adjudica un valor 0, mientras que el resto del cubo tiene un valor de 1. Este cubo es multiplicado por el cubo de datos experimentales a modelar, y se optimiza mediante el algoritmo ALS.

$$\sum_{ijk} (\underline{\mathbf{W}}_{ijk} (\underline{\mathbf{X}}_{ijk} - \sum_{f=1}^{F} \mathbf{A}_{if} \mathbf{B}_{jf} \mathbf{C}_{kf})^2$$

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Valores desconocidos /Inserción de ceros (Missing values / insertion of zeros)

Otra alternativa para la eliminación de los efectos de dispersión es reemplazar la zona problemática por ceros o, por valores "desconocidos". El algoritmo que se utiliza para asignar estos valores es el descrito por R. Bro [42]. El ancho de banda a eliminar, es elegido por inspección visual de los datos a fin de que se mantenga la señal [79]. En la Figura 9, se muestra el espectro de emisión-excitación después de la eliminación de la dispersión Rayleigh utilizando *missing values.*

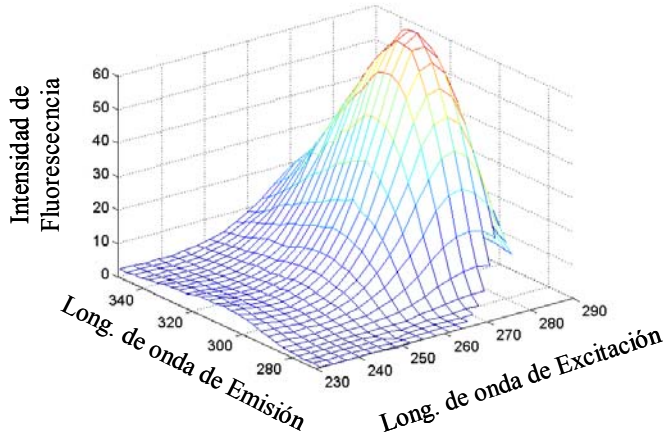

Fig. 9 Espectro de emisión-excitación luego de la eliminación de la dispersión Rayleigh utilizando *missing values*

2.3.4. Técnicas de análisis de datos de múltiples vías

2.3.4.1. Tucker3 y análisis de factores paralelos (PARAFAC)

El modelo Tucker3 [80] reduce los datos de tres vías de **X** ($I \times J \times K$) a tres matrices: **A**, **B** y **C** (véase la Ecuación 1 y Figura 10). Así como el cubo residual **E**, también se obtiene un cubo base – el cubo **G** -, que da las relaciones entre los tres modos. El modelo Tucker3 [81,82] se puede considerar como una extensión del PCA bilineal para el caso multidimensional.

$$x_{ijk} = \sum_{p=1}^{P}\sum_{q=1}^{Q}\sum_{r=1}^{R} a_{ip} b_{jq} c_{kr} g_{pqr} + e_{ijk} \qquad \text{(Ec. 1)}$$

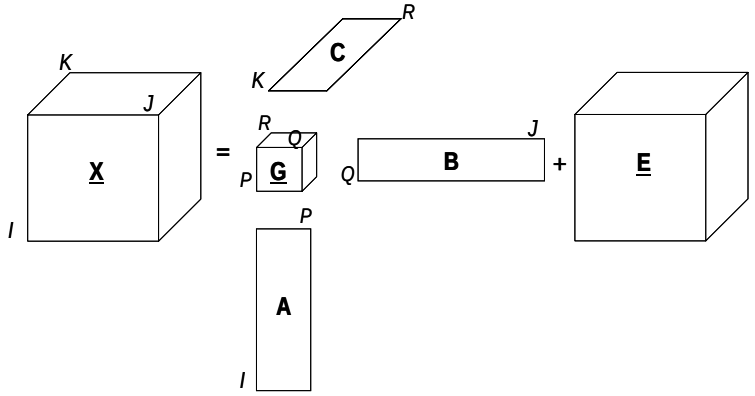

Fig. 10. Descomposición Tucker de **X** en la suma de las matrices **A**, **B** y **C**. **E** es el cubo de los residuales y **G** es el cubo base

Una propiedad importante del modelo Tucker es que no requiere que el número de factores de **A**, **B** y **C** sean los mismos. Los vectores *P*, *Q* y *R* no tienen que ser

necesariamente los mismos y pueden interactuar; éste es el significado explícito de una matriz no-diagonal $\underline{\mathbf{G}}$, aunque una interpretación física de estas interacciones puede no ser evidente.

Cuando $\underline{\mathbf{G}}$ es un cubo identidad, es decir un cubo del tamaño $R \times R \times R$ con unos en la superdiagonal y ceros en el resto de posiciones, es equivalente al modelo PARAFAC [41]. PARAFAC es un método multi-vías o multidireccional que tiene su origen en la psicometría [83-84] que habitualmente es utilizado como modelo trilineal cuando se aplica a datos fluorescentes. Específicamente la intensidad de emisión de un fluoróforo en soluciones o suspensiones diluidas es linealmente proporcional a su concentración, a pesar de verse bastante influenciados por los factores ambientales.

Conceptualmente, se puede entender como una generalización del PCA bilineal [41,85-86] Una de las ventajas principales de este modelo es la unicidad de su solución, obtenida mediante optimización por mínimos cuadrados alternados (*Alternating least squares*, ALS). Durante este proceso se pueden imponer restricciones [42,87] a las soluciones para que estas resulten más fáciles de interpretar o se ajusten mejor a las características físico-químicas del sistema estudiado.

PARAFAC descompone la estructura de tres vías $\underline{\mathbf{X}}$ en tríadas, dando las matrices $\mathbf{A}$, $\mathbf{B}$ y $\mathbf{C}$ siguiendo el modelo estructural reprensado en la Ecuación 2 y gráficamente en la Figura 11.

Las explicaciones detalladas del modelo, del algoritmo y de los usos se pueden encontrar en la literatura [41-42].

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$$x_{ijk} = \sum_{f=1}^{F} a_{if}\, b_{jf}\, c_{kf} + e_{ijk} \qquad \text{(Ec. 2)}$$

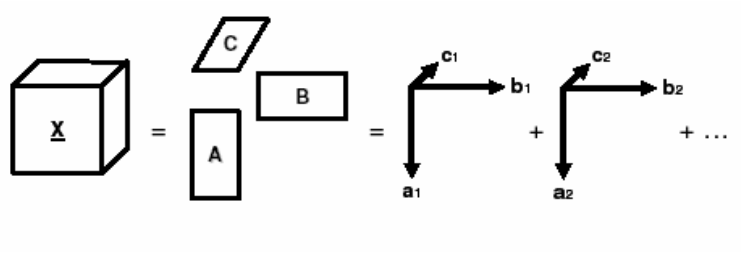

Fig. 11. Representación de un modelo PARAFAC.

Cuando se trabaja con datos de fluorescencia de excitación emisión para I muestras, la intensidad de fluorescencia de emisión en una muestra *i* medida a una longitud de onda de emisión *j* y excitada a una longitud de onda *k* se expresar como la Ecuación 2. El parámetro b_{jf} está relacionado con la intensidad de fluorescencia relativa del fluoróforo *f* a una longitud de onda de emisión *j*. El parámetro c_{kf} está relacionado con el coeficiente de extinción del fluoróforo *f* a una longitud de onda de excitación *k* y la concentración relativa del analito *f* en la muestra *i* es a_{if}, y que constituyen los elementos en las matrices **A**, **B** y **C**.

Utilizando el producto Khatri-Rao el modelo estructural también puede formularse como se ve en la Ecuación 3

$$\mathbf{X}^{(I\times JK)} = \mathbf{A}\,(\mathbf{C} \mid \otimes \mid \mathbf{B})^{T} + \mathbf{E}^{(I\times JK)} \qquad \text{(Ec.3)}$$

donde $\mathbf{X}^{(I\times JK)}$ es el desdoblamiento del cubo de datos $\underline{\mathbf{X}}$ *(I×J×K)*. Esta forma se acerca claramente al modelo PCA y el problema se puede resolver como un modelo de dos vías y encontrar la solución por mínimos cuadrados alternados (ALS).

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Para el modelo PARAFAC, y teniendo en cuenta la Ecuación 3 donde $\mathbf{Z} = (\mathbf{C} \mid \otimes \mid \mathbf{B})$, la solución a este problema está dada por:

$$\mathbf{A} = \mathbf{X}\,(\mathbf{Z}^{T})^{+} = \mathbf{X}^{(I\times JK)}\mathbf{Z}(\mathbf{Z}^{T}\,\mathbf{Z})^{-1} \qquad \text{(Ec 4)}$$

Después de dar los valores iniciales para **B** y **C**, se obtiene una estima de **Z** y la primera estima de **A** se calcula con la Ecuación 4. El modelo itera generando una estima de la matriz **B** y a partir de la primera estima de **A** y, ahora considerando $\mathbf{Z} = (\mathbf{C} \mid \otimes \mid \mathbf{A})$, se estima **B** a partir de

$$\mathbf{B} = \mathbf{X}^{(J\times IK)}\mathbf{Z}(\mathbf{Z}^{T}\,\mathbf{Z})^{-1} \qquad \text{(Ec 5)}$$

Del mismo modo partir de las estimas calculadas para **A** y **B**, y considerando $\mathbf{Z} = (\mathbf{B} \mid \otimes \mid \mathbf{A})$, se calcula una estima de **C** a partir de

$$\mathbf{C} = \mathbf{X}^{(K\times IJ)}\mathbf{Z}(\mathbf{Z}^{T}\,\mathbf{Z})^{-1} \qquad \text{(Ec 6)}$$

dando lugar al proceso de optimización que finaliza cuando el cambio relativo en el ajuste es pequeño.

En el caso abordado en esta Tesis, la espectrofluorimetría de EEM, las dos matrices **B** y **C** contienen la información acerca de los perfiles de excitación y de emisión de los compuestos activos y se utilizan para la identificación cualitativa de la especie fluorescente en las muestras (propósito de la resolución). Los datos para la cuantificación de los fluoróforos se obtienen de los valores de la matriz **A**, que son valores relativos a las concentraciones de estos compuestos. Las concentraciones absolutas se pueden obtener

de una regresión de los valores de **A** en función de la concentración conocida del analito de interés en la/s muestra/s de calibración.

Una vez determinado el modelo, puede ser utilizado para predecir la concentración de los compuestos en nuevas muestras. El valor de **A** de estas nuevas muestras se calcula simplemente aplicando las ecuaciones 4, 5 y 6 con la matriz **Z** del modelo y los nuevos datos de la muestra, $\mathbf{U}_{nueva}$.

Otra estrategia para predecir muestras es incluirlas en el modelo PARAFAC inicial. De esta manera, se recupera una matriz **A** con los valores relativos a las concentraciones de la/s muestra/s de calibración y las muestras a predecir. En este caso, las muestras se consideran simultáneamente para calcular los parámetros del modelo. Esto fuerza el modelo encontrar una solución que explique lo mejor posible todas las variaciones. Este procedimiento puede incluir muestras alejadas del modelo "ideal" y conducir a un ajuste global más pobre, pero en ausencia de muestras anómalas, y realizando menor cantidad de pasos para el cálculo, los resultados pueden no diferir demasiado de los obtenidos sin incluir las muestras a predecir.

2.3.4.2. Resolución Multivariante de Curvas- Optimización mediante Mínimos Cuadrados Alternados (MCR-ALS)

MCR-ALS [43,88-89] es un método de resolución de curvas muy versátil que puede extenderse al análisis simultáneo de varias matrices de datos. En contraste con PARAFAC, MCR-ALS es capaz de utilizar datos de segundo orden no lineales, es decir estructuras trilineales y no-trilineales.

La estrategia para realizar MCR-ALS para un conjunto de datos tridimensionales, es ordenar las distintas matrices de datos en matrices aumentadas tal como se describe en la sección 2.3.3. El método asume la linealidad de los datos (Ley de Beer-Lambert), la cual asume la aditividad de la fluorescencia para las especies activas y las matrices aumentadas en el modo excitación (modo-columnas) puede modelarse con la siguiente ecuación:

$$\mathbf{D}_{aug}^{ex} = \mathbf{Y}_{aug}\mathbf{X}^{T} + \mathbf{E}_{aug} \qquad \text{(Ec. 7)}$$

donde $\mathbf{D}_{aug}^{ex}$ es la matriz de datos aumentada en el modo excitación, $\mathbf{Y}_{aug}$ es la matriz aumentada de los espectros de emisión, $\mathbf{X}^{T}$ es la transpuesta de la matriz de los espectros de excitación y $\mathbf{E}_{aug}$ es la matriz de los residuales. La información cuantitativa se encuentra contenida en las intensidades relativas de los espectros de emisión $\mathbf{Y}_{aug}$.

La descomposición de las matrices aumentadas con MCR-ALS fue realizado utilizando restricciones de no negatividad ya que los espectros de emisión y excitación deben ser siempre positivos. La aplicación de MCR-ALS involucra los siguientes pasos: (i) determinación del número de especies presentes en el sistema mediante el análisis de valores singulares (svd); (ii) obtener una estima inicial de los espectros de excitación a

partir de los espectros de los analitos puros; (iii) optimización mediante mínimos cuadrados alternados sujetos a la restricción anteriormente mencionada.

La concentración relativa de un compuesto en particular ($\mathbf{C}_{unk}$) en esta tesis, se calcula haciendo el cociente de las áreas debajo de los espectros resueltos de emisión del analito en la matriz desconocida ($\mathbf{A}_{unk}$) y el área de los espectros resueltos del emisión del mismo compuesto en otra matriz de datos incluida en el mismo análisis simultáneo ($\mathbf{A}_{stand}$):

$$\mathbf{C}_{unk} = (\mathbf{A}_{unk} / \mathbf{A}_{std})\, \mathbf{C}_{std} \qquad \text{(Ec. 8)}$$

Como las muestras de predicción han sido utilizadas junto con las muestras de calibrado (calibrado con un solo punto) para construir el modelo, las primeras tienen tanta influencia como las segundas en el proceso iterativo.

2.3.4.3. Regresión por mínimos cuadrados parciales multi-lineal (N-PLS)

El modelo PLS multi-lineal o N-PLS [45] es una extensión del modelo ordinario, donde el modelo es ajustado describiendo la covarianza de las variables dependientes (*y*) e independientes (*x*). Las variables independientes se modelan de modo que se enfatiza en la variación que es especialmente relevante para predecir las variables dependientes.

La terminología general de N-PLS depende del orden de los datos: el prefijo griego indica el orden de **X** y de un sufijo árabe después de que PLS indica el orden de **Y**. En tri-PLS1, por lo tanto, cada muestra de calibración es caracterizada por una matriz $\mathbf{X}_i$ de dimensiones ($J \times$ K), para construir un cubo de datos $\underline{\mathbf{X}}$ de dimensiones $I \times J \times K$, y para cada muestra hay un valor de mesurando conocido tal que se prediga su valor mediante variables independientes. Estos valores se recogen en una matriz general $\underline{\mathbf{Y}}$ de las dimensiones $I \times L \times M$. Cuando los mesurando son concentraciones, se recogen en un vector $I \times 1$ llamado **y**. Si más de una variable es encontrada, éstas son recogidas en una matriz **Y** de dimensiones $I \times L$, donde L es el número de analitos (tri-PLS2, tri-PLS3, etc.).

Cuando estamos en presencia de más de una variable dependiente, todos los vectores $\mathbf{y}_i$ pueden ser modelados simultáneamente (como en PLS2) y cada variable dependiente puede ser modelada con el algoritmo PLS1.

La principal característica del algoritmo N-PLS es que produce vectores de valores, en el sentido trilineal, que tienen la máxima covarianza con la parte no explicada de la variable dependiente. Para tri-PLS (L, L ≥ 2), una descomposición trilineal de $\underline{\mathbf{X}}$ e $\underline{\mathbf{Y}}$ se consigue como en la Figura 12. Para ambos conjuntos de datos, dependientes e

independientes, se asume que están centrados en el modo *I*, es decir, en el modo columna.

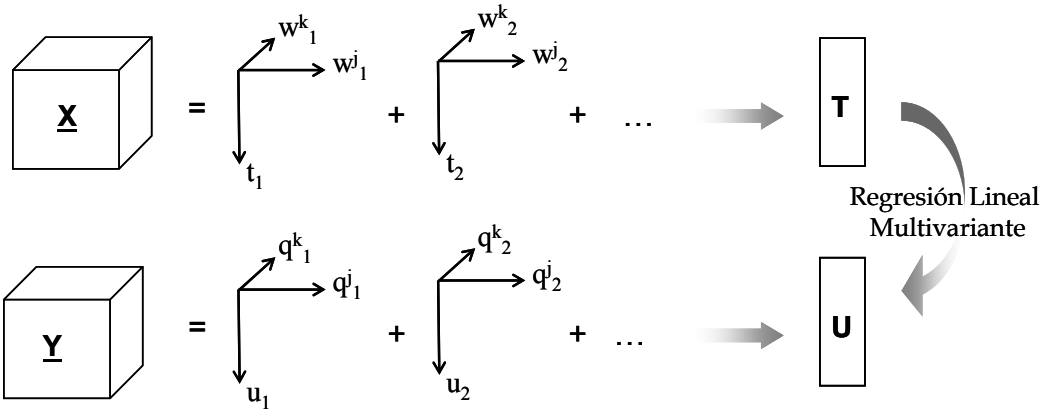

Fig. 12. Esquema general de una descomposición trilineal N-PLS.

El objetivo del algoritmo es descomponer el cubo $\underline{\mathbf{X}}$ en tríadas. Una tríada consiste en un vector de puntuaciones (**t**) y otros dos vectores de coeficientes o pesos, uno en el segundo modo llamado $\mathbf{w}^J$ ($J \times 1$) y el otro en el tercer modo llamado $\mathbf{w}^K$ ($K \times 1$).

Reordenando los datos del cubo $\underline{\mathbf{X}}$, en su forma matricial de datos independientes $\mathbf{X}$ ($I \times JK$) e $\mathbf{Y}$ es un arreglo matricial de datos dependientes ($I \times LM$), el modelo N-PLS descompone estas matrices como:

$$\underset{(I\times JK)}{\mathbf{X}} = \underset{(I\times F)}{\mathbf{T}} \cdot \underset{(JK\times F)}{\mathbf{W}^T} = \mathbf{T}(\mathbf{W}^K \mid \otimes \mid \mathbf{W}^J)^T + \mathbf{E}_X \qquad \text{(Ec. 9)}$$

$$\underset{(I\times LM)}{\mathbf{Y}} = \underset{(I\times F)}{\mathbf{U}} \cdot \underset{(LM\times F)}{\mathbf{Q}^T} = \mathbf{U}(\mathbf{Q}^L \mid \otimes \mid \mathbf{Q}^M)^T + \mathbf{E}_Y \qquad \text{(Ec. 10)}$$

donde los vectores de puntuaciones de $\mathbf{X}$ e $\mathbf{Y}$ son denominados $\mathbf{T}$ y $\mathbf{U}$ respectivamente, y los vectores de coeficientes son llamados $\mathbf{W}$ y $\mathbf{Q}$ el superíndice J o K y L o M, respectivamente, son utilizados para especificar a que modo corresponde cada vector. El

modelo encuentra el conjunto de vectores $\mathbf{w}^J$, $\mathbf{w}^K$, $\mathbf{q}^L$ y $\mathbf{q}^M$ tal que **t** y **u**, tengan la máxima covarianza. Estos valores son determinados sucesivamente siguiendo este criterio hasta que el número de componentes calculado sea el adecuado. La validación cruzada (*cross-validation*) puede ser utilizada como criterio para determinar el número de componentes en el modelo final. Los valores son relacionados teniendo en cuenta la relación interna:

$$\underset{(I\times F)}{\mathbf{U}} = \underset{(I\times F)}{\mathbf{T}} \cdot \underset{(F\times F)}{\mathbf{B}} + \mathbf{E}_U \qquad \text{(Ec. 11)}$$

La matriz de regresión **B** debe ser calculada con todos los vectores **t** calculados, los actuales y los previos, hasta que **T** tenga propiedades de no ortogonalidad.

El modelo N-PLS es único en el sentido de que consiste en estimar modelos sucesivos de un componente. Sin embargo, a pesar de la unicidad de este caso, rara vez se puede recuperar los espectros correspondientes a los analitos puros (las matrices **W** y **Q**) porque las asunciones del modelo no reflejan ningún modelo fundamental o teórico. Aquí el objetivo es predecir **Y**.

La variable dependiente de una nueva muestra $\mathbf{r}^T_{unk}$ ($1\times JK$) es predicha de la siguiente forma (esto también puede ser aplicado a un nuevo conjunto de muestras $\mathbf{X}_{unk}$). A partir del modelo de **X** con la Ecuación 9, se puede determinar **t**. Con la Ecuación 11, se predicen los valores de la matriz **Y** y a partir del modelo de **Y** (Ecuación 10) se predice $\mathbf{y}^T_{pred}$:

$$\mathbf{r}^T_{unk} = \mathbf{t}^T_{unk} \cdot \mathbf{W}^T \rightarrow \mathbf{t}^T_{unk} = \mathbf{r}^T_{unk} \cdot (\mathbf{W}^T)^+ \qquad \text{(Ec. 12)}$$

$$\mathbf{u}^T_{unk} = \mathbf{t}^T_{unk} \cdot \mathbf{B} \qquad \text{(Ec. 13)}$$

$$\mathbf{y}^T_{pred} = \mathbf{u}^T_{unk} \cdot \mathbf{Q}^T \qquad \text{(Ec. 14)}$$

Si solo se quiere obtener la predicción de **Y**, es posible obtener un conjunto de coeficientes de regresión que estén directamente relacionados con la matriz centrada/escalada **X** y con la matriz centrada/escalada **Y**:

$$\mathbf{B}_{PLS} = (\mathbf{W}^T)^+ \mathbf{B}\, \mathbf{Q}^T \qquad \text{(Ec. 15)}$$

Por consiguiente, para una nueva muestra $\mathbf{r}^T_{unk}$ la variable predicha **Y** es:

$$\mathbf{y}^T_{pred} = \mathbf{r}^T_{unk} \,.\, \mathbf{B}_{PLS} \qquad \text{(Ec. 16)}$$

Como ventajas que ofrece N-PLS se puede decir que, a diferencia de PARAFAC, el rango puede ser superior al número de especies fluorescentes; el algoritmo es más rápido debido a la poca cantidad de parámetros a calcular y al hecho de que el problema se reduce a una descomposición de valores propios; la incorporación de las variables dependientes en la descomposición de las variables independientes, podría estabilizar el modelo de predicción [45].

La ventaja de usar tri-PLS en lugar de métodos de desdoblamiento en matrices es doble. Un modelo trilinear cumple el principio de parsimonia, es decir, es más simple y por lo tanto más fácil interpretar y será menos azaroso ya que la información a través de todos los órdenes es utilizada para la descomposición.

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2.3.5. Diseño de experimentos.

El diseño de experimentos, proporciona herramientas para

- la planificación sistemática de una experimentación, con el menor número de experimentos posibles obtener la máxima información [90-91].
- El análisis de la influencia de las variables experimentales en una respuesta de interés
- optimizar la respuesta y
- modelar esta respuesta dentro del dominio experimental en estudio.

En esta tesis doctoral, se han aplicado técnicas de diseños de experimentos para planificar la experimentación y para analizar la influencia de las variables que afectan a los procesos de foto-degradación en estudio.

2.3.5.1 Diseño factorial completo

Un diseño factorial completo es una disposición experimental que consiste en realizar todas las combinaciones posibles entre los diversos factores y sus niveles. En general, un diseño de *k* factores con *m* niveles contiene un número de experimentos m^k. Cuando el número de factores es muy grande, el diseño se hace muy costoso ya que el número de experiencias es muy elevado, por lo que normalmente se utiliza en casos de que el número de factores es relativamente bajo.

El diseño factorial completo, permite analizar los efectos de los factores principales (A, B, C… *k*) sobre una determinada respuesta, y también si existe efecto de interacción entre los factores. Los efectos miden la influencia que tiene en la respuesta un cambio en el valor de las variables y la interacción de los factores en la respuesta. Existe interacción cuando el efecto de un factor es diferente a distintos niveles de otro(s) factor(es).

La influencia de un factor A en la respuesta y en un diseño experimental se puede calcular como:

$$b_A = \frac{\sum Y(+) + \sum Y(-)}{n} \qquad \text{(Ec.17)}$$

donde $\sum Y(+)$ y $\sum Y(-)$ son la suma de las respuestas donde el factor A se sitúa al nivel alto (+1) y nivel bajo (–1) respectivamente, y *n* es el número de veces que cada factor esta en el nivel (+1) o (–1). La Ecuación 17 puede ser aplicada tanto para los efectos

de los factores principales como para los efectos de las interacciones.

Existen varias herramientas para estudiar la importancia de los efectos estimados [92]. Estas herramientas estadísticas pueden clasificarse como: (i) de interpretación visual o (ii) ANOVA.

(i) Diagrama de Pareto: exhibe un histograma de frecuencias donde la longitud de cada barra es proporcional al valor absoluto del efecto estandarizado.

(ii) ANOVA: o análisis de la varianza, que sirve para decidir que factores y que interacciones son significativas a partir de los resultados obtenidos del cociente entre la variabilidad en la respuesta seleccionada (para cada efecto) respecto al error residual (error experimental).

2.3.5.2. Diseño de mezclas:

En un experimento de mezclas, los factores independientes son proporciones de diversos componentes de una mezcla. Hay diseños estándares de mezclas tales como el Simplex-Lattice y el Simplex-Lattice centrado.

La distinción principal entre los diseños de mezclas y los experimentos de variables independientes es que las variables o los componentes de entrada son cantidades proporcionales no negativas de la mezcla, y si están expresados como fracciones de la mezcla, deben sumar uno.

Diseños Simplex-Lattice

Un diseño {q,m} Simplex –Lattice [93] para q componentes consiste de un conjunto de puntos definido siguiendo las coordenadas siguientes:
Las proporciones asumidas por cada componente toman m +1 valores igualmente espaciados entre 0 y 1.

$$x_i = 0, 1/m, 2/m, \ldots, 1 \text{ for } i = 1, 2, \ldots, q$$

Y todas las posibles combinaciones de las proporciones de esta ecuación.

Cada una de las proporciones en la mezcla es un número fraccionario donde la suma de las fracciones es igual a uno. Cuando se grafican estos diseños, los puntos están distribuidos simétricamente con respectos a los vértices y a los lados del Simplex.

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El número de combinaciones posibles que pertenecen a un diseño {*q, m*} Simplex-Lattice esta dado por la Ecuación 18 [94]

$$\left(\frac{q+m-1}{m}\right)=\frac{(q+m-1)!}{m!(q-1)!} \qquad \text{(Ec. 18)}$$

En la Tabla 1 se muestra el número de puntos en un diseño {*q, m*} Simplex-Lattice para valores de *q* y *m* desde $3 \le q \le 10$, $1 \le m \le 4$.

Grado del modelo *m*	Numero de componentes							
	q=3	4	5	6	7	8	9	10
1	3	4	5	6	7	8	9	10
2	6	10	15	21	28	36	45	55
3	10	20	35	56	84	120	165	220
4	15	35	70	126	210	330	495	715

Tabla 1. Número de puntos en un diseño {q, m} Simplex-Lattice para valores de q y m desde $3 \le q \le 10$, $1 \le m \le 4$.

En esta tesis se utilizará un diseño de mezclas {*4,3*} Simplex-Lattice que consta de 20 puntos como se puede ver en la Figura 13

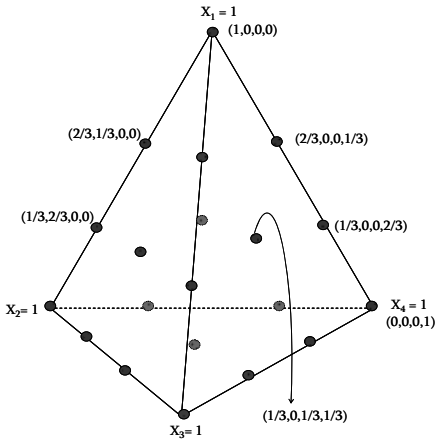


Fig.13. Representación gráfica de un diseño de mezclas {*4,3*} Simplex-Lattice

2.4. Cálculos cinéticos

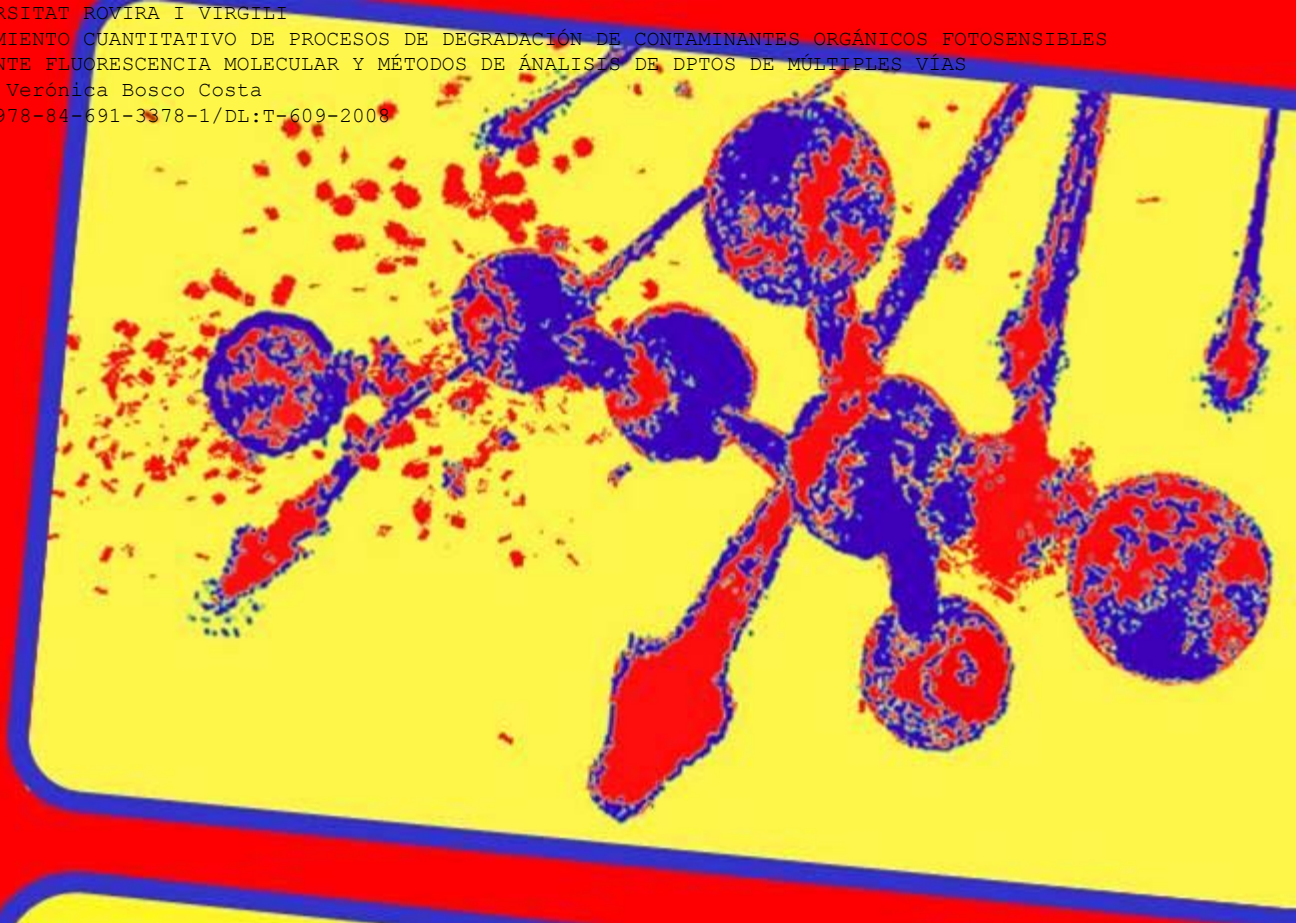
Los procesos de degradación estudiados en esta tesis doctoral, se ajustan normalmente a una cinética de pseudo-primer orden [7-9] siguiendo la Ecuación 19

$$\ln\frac{C_0}{C_i} = kt \qquad \text{(Ec. 19)}$$

donde C_0 y C_i son las concentraciones a tiempo cero y a tiempo t respectivamente, y k es la constante de velocidad.

El tiempo de vida media de cada analito $t_{1/2}$ es calculado derivando la Ecuación 20 y reemplazando C_i por $C_0/2$:

$$t_{1/2} = \ln\frac{2}{k} \qquad \text{(Ec. 20)}$$



CAPÍTULO III

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3.1. Foto-degradación de hidrocarburos policíclicos aromáticos: benzo[a]antraceno, benzo[a]pireno, benzo[k]fluoranteno, dibenzo[a,h]antraceno

3.1.1. Tipo de reactor

Uno de los procesos de mineralización en estudio es la foto-degradación de benzo[a]antraceno (BaA), benzo[a]pireno (BaP), benzo[k]fluoranteno (BkF), dibenzo[a,h]antraceno (Di(a,h)A). Este proceso fue estudiado por varios autores [7,12-13,53] y su mecanismo de reacción ha sido descrito en la sección 2.1.2.El proceso de foto-degradación se realizó en un reactor anular cilíndrico, como el de la Figura 14.

El mismo, consiste en un tubo de inmersión de cuarzo (2.5 cm d.i., y 38 cm de largo) que sostiene una lámpara de vapor de mercurio de baja presión (LPML) de 15 W (Heraeus Noblelight, Alemania) o una lámpara de vapor de mercurio de alta presión (HPML) de 47 W (Heraeus Noblelight, Alemania) dependiendo del experimento realizado. La fuente de luz emite en la zona ultravioleta y tiene un máximo de emisión a 254 nm para la LPML y un máximo predominante de 366 nm para HPML. Cuando se utilizó la lámpara de alta presión el tubo de inmersión fue colocado dentro de una camisa de enfriamiento de cuarzo. El tubo de cuarzo se coloca en un reactor externo de Pyrex (0.7 l de capacidad) el cual fue cubierto con papel de aluminio para no perder radiación.

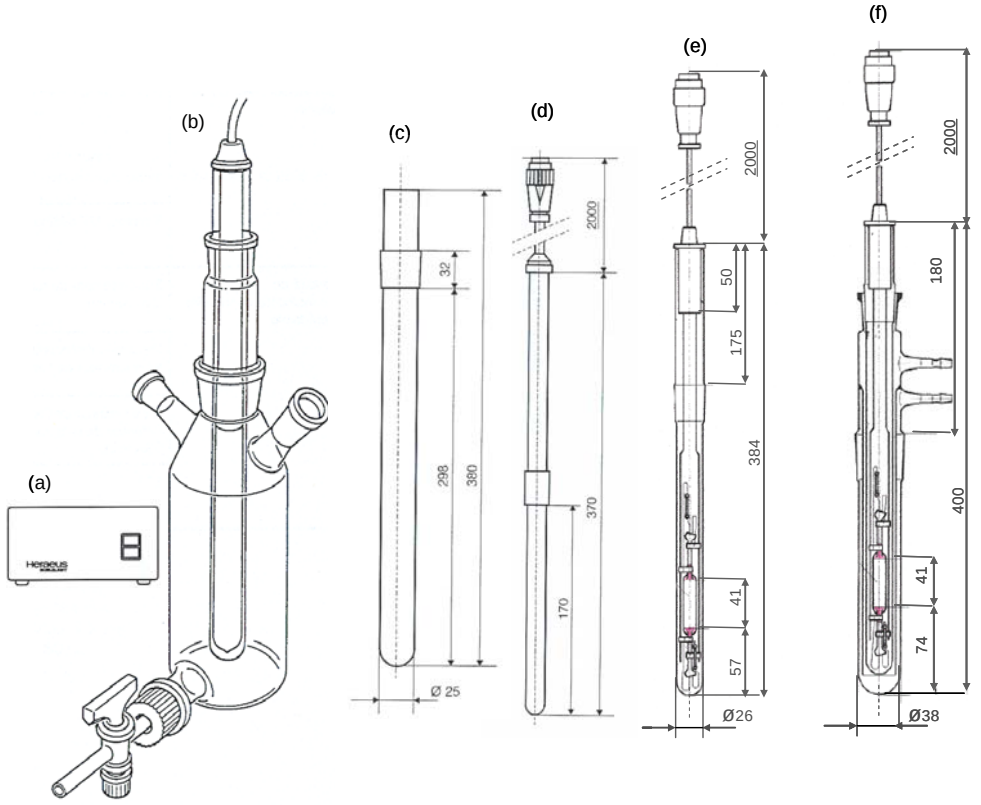

Fig. 14 Reactor empleado para la degradación, a) fuente de energía, b) reactor externo, c) tubo de inmersión de cuarzo, d) lámpara de vapor de mercurio de baja presión (LMPL); e) lámpara de vapor de mercurio de alta presión (HPML); f) HPML y camisa de enfriamiento de cuarzo.

3.1.2. Preparación de las muestras

Se realizaron ocho experimentos de foto-degradación siguiendo un diseño factorial completo 2^3[95]. Las variables elegidas para estudiar a dos niveles fueron pH (2.5

y 11), solvente (Etanol, C_2H_5OH, y Metanol, CH_3OH) y tipo de radiación, lámpara UV (LPML y HPML) dando como matriz de experimentos la Tabla 2

Experimento	Solvente	pH	Lámpara
1	C_2H_5OH	2.5	LPML
2	CH_3OH	2.5	LPML
3	C_2H_5OH	11	LPML
4	CH_3OH	11	LPML
5	C_2H_5OH	2.5	HPML
6	CH_3OH	2.5	HPML
7	C_2H_5OH	11	HPML
8	CH_3OH	11	HPML

Tabla 2. Diseño factorial completo 2^3 utilizado

Las soluciones estándar de todos los compuestos fueron realizadas pesando una cantidad apropiada de los reactivos puros de Dib(a,h)A, 97%, BkF, 98%, B(a)A, 99%, y B(a)P, 97%, disolviéndolos en acetonitrilo (ACN).

Para realizar las mezclas a foto-degradar, se agregó la cantidad adecuada de solución estándar de todos los analitos tal que resulte una solución de concentración inicial de 100 μg L^{-1} de cada uno ellos. La relación de solventes empleada fue Agua: Alcohol: ACN, 50:40:10. El ACN se ha agregado para minimizar la adsorción de los PAHs en las paredes del reactor y el volumen total de la mezcla fue de 500 ml.

El pH inicial de la mezcla reaccionante fue ajustado con una solución de hidróxido de sodio 5M o con ácido fosfórico (Aldrich 85%), dependiendo del tipo de experimento realizado. Para verificarlo, se utilizó un pH-metro Orion.

Las reacciones se llevaron a cabo a temperatura ambiente y agitando continuamente a lo largo de toda la reacción (120 min). Se ha tomado muestra cada 2

minutos durante los primeros 30 minutos, y luego cada diez minutos hasta llegar a los 120 minutos de reacción tal que cada experimento consta de 25 muestras.

Las muestras -de aproximadamente 5 ml- se tomaron a través de la válvula de muestreo que posee el reactor y se guardaron hasta el momento de realizar las medidas instrumentales correspondientes en condiciones de oscuridad.

3.1.3. Procedimiento de la medida de fluorescencia

Los datos espectrofluorimétricos fueron adquiridos en un espectrómetro Aminco -Bowman Series 2 (SLM Aminco, Rochester, NY, E.E.U.U.) como el de la Figura 15 equipado con una lámpara de xenón continua (150W) y un tubo fotomultiplicador (PMT) como detector. Para la adquisición espectral, el instrumento fue conectado a una tarjeta GPIB y a un ordenador PC Pentium que posee el programa AB2 versión 1.40.

Fig. 15 Espectrofluorímetro Aminco -Bowman Series 2

Los espectros tridimensionales de emisión-excitación fueron recogidos desde 360 a 450 nm para las longitudes de onda de emisión y desde 230 a 300 nm para las longitudes

de onda de excitación, en ambos casos registrados cada 3 nm. La velocidad de barrido del monocromador se mantuvo a 30 nms^{-1} y el ancho de banda se fijó a 4 nm tanto para la excitación como para la emisión. Todas las medidas se realizaron en una celda de cuarzo de 10 mm a 590V.

Los espectros fluorescentes fueron exportados desde el software del instrumento en formato ASCII al MATLAB [96] donde se procesó esa información con la subrutina adecuada.

3.1.4. Medida cromatográfica

Los experimentos realizados en cromatografía líquida fueron diseñados con la finalidad de utilizar esta técnica como referencia para comparar estadísticamente los resultados obtenidos con los alcanzados utilizando los métodos de análisis de datos de múltiples vías.

La preparación de la reacción y el procedimiento para llevar a cabo la toma de muestras se realizó de igual manera que para el experimento 1 explicado en el apartado 3.1.2. (ver Tabla 2).

La cromatografía líquida de alta resolución (HPLC) se llevó a cabo en fase inversa en un cromatógrafo Agilent Series 1100, equipado con un detector de fluorescencia (FLD) para la monitorización de las especies eluídas. La separación cromatográfica se realizó en una columna analítica Tracer PAH C-18 para fase inversa suministrada por Teknokroma,

con dimensiones de 250 mm de largo y 4 mm de diámetro interno, y rellena con partículas de 5 μm de diámetro. Para la inyección directa de la muestra se utilizó un bucle de 20 μL.

El análisis por HPLC se realizó en gradiente de elución de acetonitrilo/agua (ambos solventes de grado HPLC), con una velocidad de flujo de 1,5 mL min^{-1}. El gradiente comenzó con una mezcla de 75 % (v/v) de acetonitrilo y 25 % de agua, la cual se fue modificando de manera continua hasta llegar a un 95% de acetonitrilo, al cabo de 23 minutos. Con el fin de aumentar la sensibilidad del detector, se empleó un programa de longitudes de onda donde se seleccionó la longitud de onda de excitación y emisión óptimas para cada analito (ver Tabla 3).

Tiempo (min)	Longitud de onda (nm)		Analito
	Excitación	Emisión	
0	286	387	BaA
14	295	410	BkF/BaP
19.50	296	396	DibA

Tabla 3. Condiciones del detector empleadas para la determinación de los PAHs

Los datos cromatográficos fueron recogidos por el software HP Chemstation versión A.06.01.

La concentración de las especies estudiadas se calculó por interpolación de las áreas obtenidas para los correspondientes picos cromatográficos en las rectas de calibrado construidas a partir de soluciones estándar de los compuestos puros.

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3.2. Foto-catálisis del fenol

3.2.1. Tipo de reactor

El proceso de degradación foto-catalítica de fenol utilizando TiO_2 como catalizador, fue estudiado por varios autores [14-18] y su mecanismo de reacción ha sido descrito en la sección 2.1.3.

El proceso de foto-catálisis se realizó en un reactor anular cilíndrico, como el de la Figura 14. (ver sección 3.1.1.). La lámpara de vapor de mercurio empleada para la degradación de estos compuestos fue la de baja presión (LPML) de 15 W. La fuente de luz emite en la zona ultravioleta y tiene un máximo de emisión a 254 nm.

3.2.2. Muestras empleadas

3.2.2.1. Muestras de reacción a 1000 ppm

Se preparó una suspensión (o lechada) mezclando una solución de fenol de 1000 ppm con 0.3 g/100 ml de TiO_2. Antes de la encender la lámpara y comenzar la foto-degradación, se agitó uniformemente durante 15 minutos en la oscuridad, utilizando un agitador magnético. El volumen total de la suspensión fue de 500 ml. El pH inicial de la mezcla reaccionante fue ajustado a 2 con $HClO_4$.

La reacción se llevó a cabo a temperatura ambiente y agitando continuamente a lo largo de toda la reacción (1500 minutos).

Se tomaron muestras de aproximadamente 3 ml a través de la válvula de muestreo que posee el reactor. Las partículas de catalizador fueron eliminadas filtrando la muestra recogida con una membrana Millipore de 0.45 μm, manteniendo las soluciones filtradas en condiciones de oscuridad.

Para las medidas fluorimétricas, se tomaron 125 μl de cada alícuota de solución filtrada, y se llevó a 25 ml con una solución al 1% de ácido acético, con el objeto de asegurar que las especies en solución están su forma molecular, ya que esta forma es la que posee propiedades fluorescentes.

3.2.2.2. Mezclas de diseño {4,3} Simplex-Lattice

Con el propósito de poder evaluar la capacidad de las metodologías de análisis de datos de múltiples vías de resolver y cuantificar mezclas de estos analitos, se preparó un conjunto de 20 mezclas siguiendo un diseño {4,3} Simplex-Lattice [94]. Se prepararon soluciones estándar de 5 μg ml^{-1} para cada analito puro (fenol, catecol, hidroquinona y resorsinol).

Este diseño fue realizado teniendo en cuenta la relación de concentración existente entre las especies durante el proceso de degradación [14,97], así como también el intervalo de linealidad de las medidas fluorescentes.

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El conjunto de mezclas fue preparado por duplicado, para evaluar el error de predicción. La Tabla 4 refleja de forma detallada el contenido de las muestras preparadas.

Muestras [a]	Fenol (μg ml $^{-1}$)	Hidroquinona (μg ml $^{-1}$)	Catecol (μg ml $^{-1}$)	Resorcinol (μg ml $^{-1}$)
Fenol	5.00	0.00	0.00	0.00
Hidroquinona	0.00	5.00	0.00	0.00
Catecol	0.00	0.00	5.00	0.00
Resorcinol	0.00	0.00	0.00	5.00
S1	5.00	0.05	0.05	0.05
S2	3.00	0.05	0.25	0.05
S3	1.50	0.05	0.75	0.05
S4	0.05	0.05	1.00	0.05
S5	3.00	0.25	0.05	0.05
S6	1.50	0.75	0.05	0.05
S7	0.05	1.00	0.05	0.05
S8	3.00	0.05	0.05	0.25
S9	1.50	0.05	0.05	0.75
S10	0.05	0.05	0.05	1.00
S11	0.05	0.25	0.05	0.75
S12	0.05	0.75	0.05	0.25
S13	0.05	0.75	0.25	0.05
S14	0.05	0.25	0.75	0.05
S15	0.05	0.05	0.75	0.25
S16	0.05	0.05	0.25	0.75
S17	0.05	0.25	0.25	0.25
S18	1.50	0.25	0.05	0.25
S19	1.50	0.25	0.25	0.05
S20	1.50	0.05	0.25	0.25

[a] Todas las muestras en 1% (v/v) de ácido acético.

Tabla 4 Concentración de los analitos en los estándares individuales y en las mezclas sintéticas siguiendo un diseño {4,3} Simplex-Lattice.

3.2.2.3. Muestras para el diseño de experimentos

Para poder analizar tanto el efecto de algunos de los factores que pueden tener relevancia en la degradación foto-catalítica del fenol como si existen interacciones entre los mismos, se realizó un diseño factorial completo 2^3. Las variables a tener en cuenta fueron estudiadas a dos niveles: pH (2.5 y 10), naturaleza del catalizador (ZnO y TiO_2) y concentración del substrato, es decir, concentración de fenol (20 y100 μg L^{-1}). La Figura 16 es una representación de este diseño

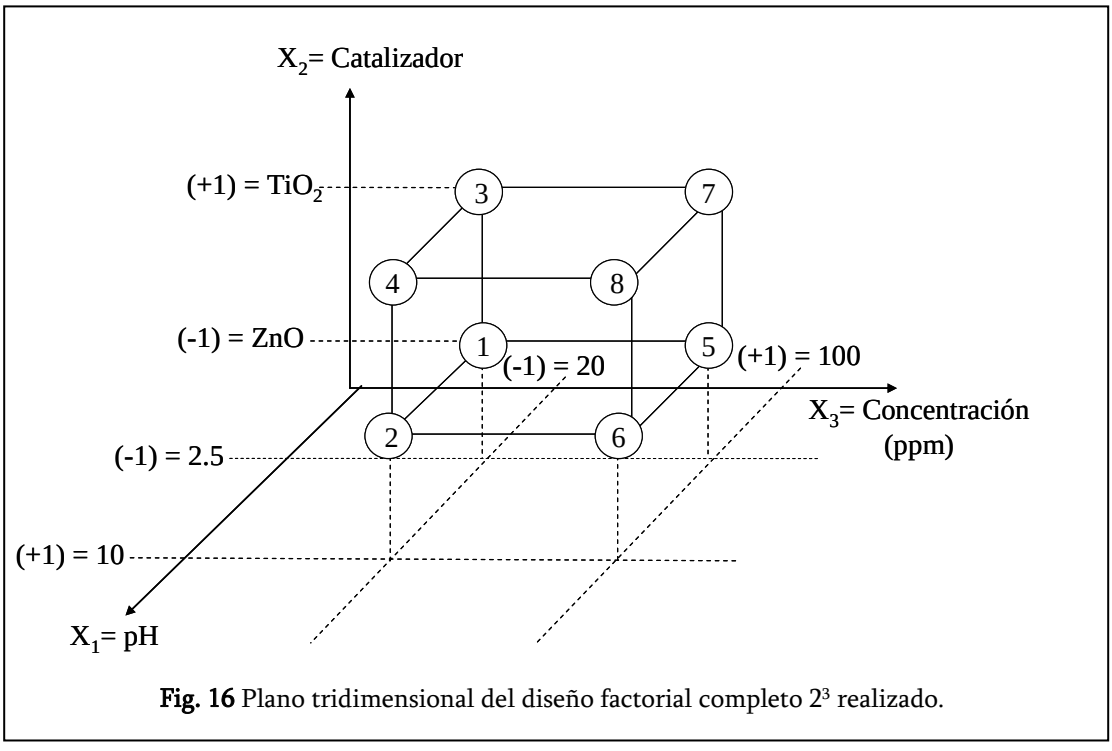


Fig. 16 Plano tridimensional del diseño factorial completo 2^3 realizado.

Se prepararon soluciones patrón pesando cantidades de reactivo adecuada y se disolvió en agua millipore. Todas las soluciones fueron preparadas en medio ácido al 1 %

(v/v) con ácido acético, de modo que los analitos permanecieran en la forma molecular, y almacenadas en botellas oscuras a 4ºC. En estas condiciones, dichas soluciones se mantienen estables por un mes. Las soluciones de trabajo fueron preparadas por dilución de las soluciones stock. El TiO_2 (99.8%) en su forma anatase, y ZnO (99.9%) fueron utilizados sin tratamiento previo adicional.

Las degradaciones se llevaron a cabo en un reactor cilíndrico como el descrito en la sección 3.1.1. Se tomaron muestras de aproximadamente 5 ml a través de la válvula de muestreo que posee el reactor. Las partículas de catalizador fueron eliminadas filtrando la muestra recogida con una membrana Millipore de 0.45 μm, manteniendo las soluciones filtradas en condiciones de oscuridad.

Antes de realizar las medidas de fluorescencia las alícuotas de solución filtrada fueron diluidas con solución acética al 1 %, tal que la concentración a tiempo = 0 min fuera de 5 ppm de fenol.

3.2.3. Procedimiento de la medida de fluorescencia

Los datos espectrofluorimétricos fueron adquiridos en un espectrómetro Aminco -Bowman Series 2 como el descrito en la sección 3.1.3.

Todas las soluciones (estándares individuales, mezclas sintéticas, muestras y solvente) fueron medidas en las mismas condiciones. Los espectros tridimensionales de emisión-excitación fueron recogidos desde 270 a 350 nm para las longitudes de onda de

emisión y desde 231 a 291 nm para las longitudes de onda de excitación, en ambos casos registrados cada 3 nm. La velocidad de barrido del monocromador se mantuvo a 30 nms^{-1} y el ancho de banda se fijó a 4 nm tanto para la excitación como para la emisión. Todas las medidas se realizaron en una celda de cuarzo de 10 mm a 620V.

3.2.4. Análisis cromatográfico

La cromatografía líquida de alta resolución (HPLC) se llevó a cabo en fase inversa en un cromatógrafo Agilent Series 1100, equipado con un detector de fluorescencia (FLD) para la monitorización de las especies eluídas.

La separación cromatográfica (incluyendo rectas de calibrado) se realizó teniendo en cuenta la metodología oficial propuesta por Health Canada para la determinación de compuestos fenólicos [98].

La columna analítica utilizada fue una Tracer C-18 para fase inversa provista por Teknokroma, con dimensiones de 250 mm de largo y 4 mm de diámetro interno, y rellena con partículas de 5 μm de diámetro. Para la inyección directa de la muestra se utilizó un bucle de 20 μL. La columna se mantuvo a temperatura ambiente.

Para la fase móvil se utilizó un programa de elución y para la detección se empleó un programa de longitudes de onda como los descritos en la Tabla 5

Tiempo (min)	Composición fase móvil		
	%A	%B	%C
0	100	0	0
5	100	0	0
10	75	25	0
17	0	100	0
19	0	0	100
22	0	0	100
24	95	0	5
	Longitud de onda (nm)		
	Excitación	Emisión	
0	304	338	
5	274	298	

Tabla 5. Programa de elución y de longitudes de onda utilizado para la determinación de los fenoles en estudio

El solvente A consistía en una solución 1 % acetonitrilo, 1 % ácido acético, 1% isopropanol; el solvente B: 28 % acetonitrilo, 1 % ácido acético, 1 % isopropanol; y solvente C acetonitrilo, todos ellos filtrados y desgasificados. La velocidad de flujo fue de 1,5 mL min^{-1}.

Los datos cromatográficos fueron recogidos por el software HP Chemstation versión A.06.01.

La concentración de las especies estudiadas se calculó por interpolación de las áreas obtenidas para los correspondientes picos cromatográficos en las rectas de calibrado construidas a partir de soluciones estándar de los compuestos puros.

3.3. Software empleado

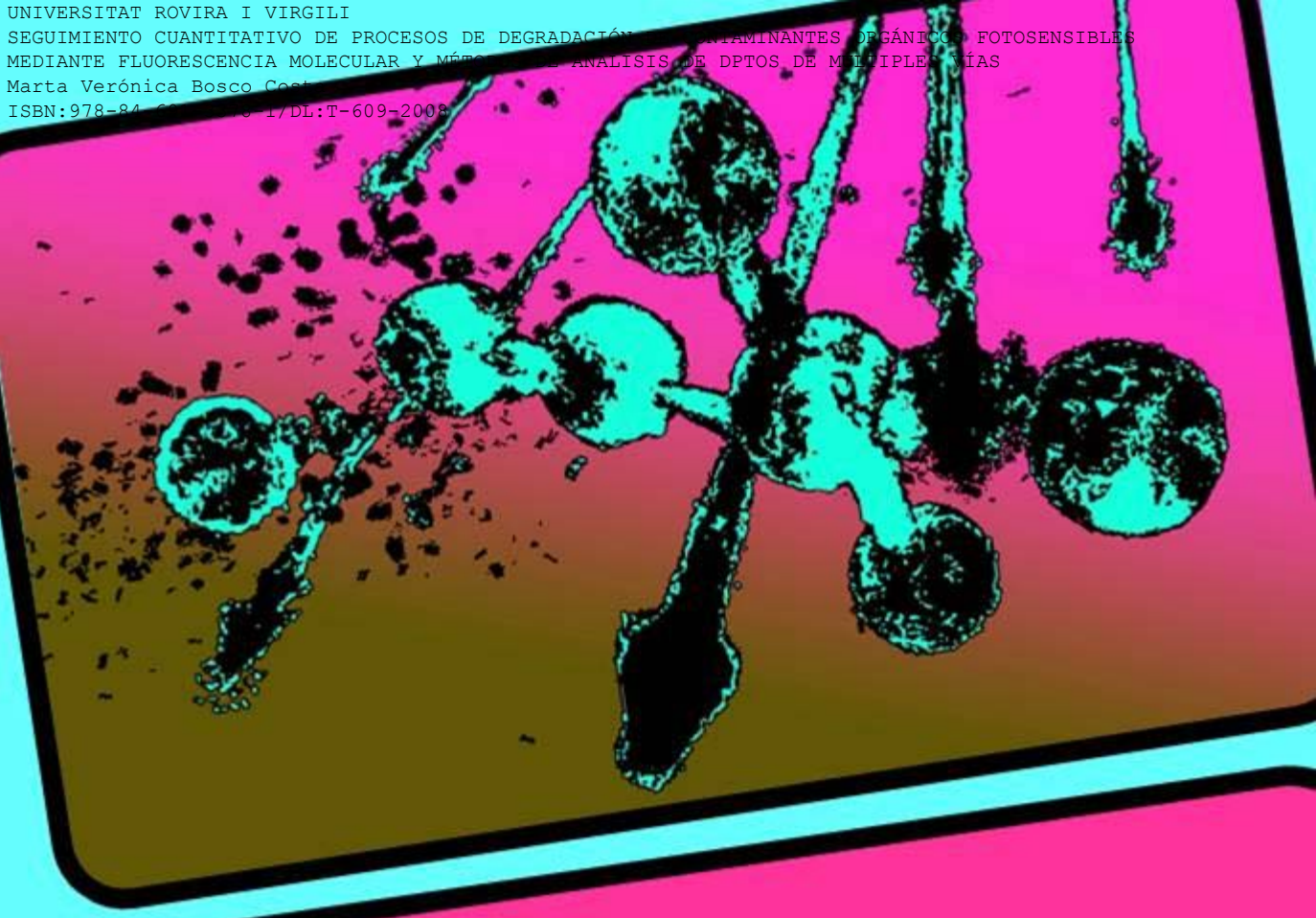
Todos los tratamientos quimiométricos realizados en esta Tesis se llevaron a cabo empleando el programa MATLAB® [96], en su versión 6.1.

Los espectros fluorescentes fueron exportados desde el software del instrumento (AB2 versión 1.40) en formato ASCII compatibles con el formato utilizado por MATLAB.

Las subrutinas empleadas para aplicar PARAFAC y N-PLS fueron proporcionadas por el Department of Dairy and Food Science de la Univeridad de Copenhague las cuales están disponibles en forma gratuita en Internet [99].

Las subrutinas utilizadas para aplicar MCR-ALS fueron suministradas por el Grupo de Quimiometría y Equilibrio en Solución de la Universidad de Barcelona. Las mismas se pueden obtener de forma gratuita en Internet [100].

Para procesar la información estadística relacionada con el diseño de experimentos se utilizó el programa estadístico MINITAB [101].



CAPÍTULO IV

UNIVERSITAT ROVIRA I VIRGILI
SEGUIMIENTO CUANTITATIVO DE PROCESOS DE DEGRADACIÓN DE CONTAMINANTES ORGÁNICOS FOTOSENSIBLES MEDIANTE FLUORESCENCIA MOLECULAR Y MÉTODOS DE ÁNALISIS DE DPTOS DE MÚLTIPLES VÍAS
Marta Verónica Bosco Costa
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4.1. Seguimiento cuantitativo de reacciones de foto-degradación de hidrocarburos policíclicos aromáticos mediante espectrofluorimetría de excitación-emisión y métodos de resolución de curvas.

4.1.1. Introducción

En esta sección se discuten los resultados relacionados con la aplicación de los métodos de resolución de curvas basados en el análisis paralelo del factor (PARAFAC) y en la optimización mediante mínimos cuadrados alternados (MCR-ALS) a los datos de fluorescencia de emisión excitación obtenidos en el seguimiento cuantitativo de las reacciones de foto-degradación de mezclas sintéticas de benzo[a]antraceno (BaA), benzo[a]pireno (BaP), benzo[k]fluoranteno (BkF), dibenzo[a,h]antraceno (DibA) siguiendo la metodología experimental descrita en el apartado 3.1.

Parámetros habituales que permiten evaluar la eficacia de un proceso de foto-degradación son: el tiempo de inducción necesario para que comience la reacción química, la constante de velocidad del proceso y el tiempo de vida media del contaminante. Esta información se obtiene del seguimiento cuantitativo de la concentración de los analitos que se degradan.

Los parámetros cinéticos mencionados están relacionados directamente con las variables de trabajo como son: la radiación incidente, la acidez del medio, la concentración del sustrato o la presencia de catalizador. En la Sección 4.1.2. se analiza de forma simultánea, utilizando un diseño factorial completo a dos niveles, la influencia de la fuente de radiación UV utilizada, el pH del medio y el solvente utilizado,

considerando como variable respuesta la vida media de los analitos. Este parámetro para cada una de las experiencias, apartado 3.1.2., se ha calculado a partir de las constantes cinéticas derivadas de las curvas de concentración de cada analito obtenidas mediante PARAFAC, asumiendo que los compuestos se degradan siguiendo una cinética de pseudo-primer orden.

Los resultados encontrados permitieron constatar la interacción de las tres variables experimentales y la importancia del análisis conjunto de sus efectos sobre el proceso para la selección de las condiciones óptimas de trabajo. En el dominio experimental en estudio dentro de esta tesis se encontró que la degradación es más rápida cuando se trabaja a pH ácido utilizando etanol como solvente e irradiando con una lámpara de baja presión de mercurio.

Tanto al aplicar PARAFAC como MCR-ALS el número de factores significativos fue de cuatro, uno por cada analito presente en la muestra. La bondad de los espectros recuperados en ambos casos fue evaluada utilizando el criterio de similaridad entre el espectro recuperado por el modelo y el espectro de cada analito puro. Estos valores siempre fueron cercanos a 1.

En las condiciones óptimas del proceso de degradación, los valores de concentración calculados por aplicación de PARAFAC y MCR-ALS se confrontaron con los obtenidos mediante cromatografía líquida de alta resolución (HPLC) y calibración univariante (sección 4.1.3.).

Cuando los valores de concentración obtenidos en el proceso de degradación mediante PARAFAC o MCR-ALS aplicados a datos EEM fueron representados frente a los obtenidos mediante HPLC se obtuvo una línea de regresión mediante mínimos

cuadrados, con pendiente próxima a 1 e intercesión en el origen próxima a 0. El test conjunto de la pendiente y la ordenada en el origen para corroborar la ausencia de sesgo fue superado para todos los analitos.

Las características que ofrece la combinación de fluorescencia de emisión-excitación con PARAFAC y MCR-ALS hacen de las estrategias propuestas una buena alternativa para la monitorización cuantitativa de las reacciones de degradación de estos contaminantes, ya que los resultados obtenidos son estadísticamente comparables con las metodologías tradicionales.

4.1.2. Simultaneous analysis of the photocatalytic degradation of polycyclic aromatic hydrocarbons using three-dimensional excitation-emission matrix fluorescence and parallel factor analysis

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) may be photochemically degraded. Monitoring of degradation process of PAHs is carried out by traditional methods which normally imply time-consuming procedures that do not allow the chemical process to be analyzed in real time. In the present study, photodegradation kinetics of dibenz[a,h]anthracene, benz[a]anthracene, benz[a]pyrene and benz[k]fluorantene were investigated in aqueous solutions under different conditions. A 2^3 factorial design was used for optimizing the degradation process.

Fluorescence spectroscopy is a fast, cheap and sensitive analytical method, attractive for use in conjunction with chemometric methods; in this case three-way

analytical methodology based on fluorescence excitation-emission matrix (EEM) and parallel factor analysis (PARAFAC) was employed. A four-factor PARAFAC model made it possible to resolve the species presents in the degradation mixture and quantify the relative concentration of the analytes throughout the degradation. Several different parameters, such as core consistency, % of Fit and correlation coefficients between recovered and reference spectra were employed to determine the suitable number of factors for the PARAFAC model. This new methodology allows us to determine satisfactorily the PAHs concentration during the photodegradation in mixtures of arbitrary composition, representing an interesting alternative to the conventional techniques normally used for the monitoring of degradation reactions.

Keywords: polycyclic aromatic hydrocarbons; PARAFAC model; photodegradation; optimization; excitation-emission matrix.

1. Introduction

The analysis of polycyclic aromatic hydrocarbons (PAHs) is of considerable importance because of their detrimental effect on human metabolism and the ecosystem in general [1]. PAHs enter the environment from natural and artificial sources [2] and both soil and water can be contaminated with PAHs in a variety of ways (through industrial and municipal waste, or rain water) [3]. For this reason, considerable effort has been made to develop and improve the chemical processes that destroy pollutants in general and PAHs in particular. Photodegradation is a particularly important methodology in this field, in which major progress is being made in the oxidative methods for degrading organic compounds dissolved or dispersed in aquatic media [4,5].

PAHs in complex mixtures are usually determined by gas chromatography (GC) [6] or high resolution liquid chromatography (HPLC) with either UV-visible diode array detectors (DAD) [5,7,8] or fluorescence detectors [9].

Several authors have recently described the use of full excitation-emission fluorescence spectra (EEM: excitation-emission matrix) for identifying and quantifying PAHs in aqueous solutions in combination with the additional information resources provided by the power of such chemometric tools as parallel factor analysis (PARAFAC) [10] or partial least squares regression (PLSR) [11]. These techniques make it possible to perform a complete analysis of complex mixtures of PAHs without a previous, time-consuming separation. This is of major importance in the optimization stage of variables that affect a chemical process (such as photodegradation). However, in the bibliography consulted, we were unable to find any references on how to use it to monitor analytes in a process of this sort. Recently, our research group used this methodology to monitor and quantitatively determine the analytes involved in the process of photodegradation of phenol, using TiO_2 as a catalyst and applying PARAFAC to excitation-emission flourescence data recorded throughout the reaction [12].
What is more, the conditions in which the photodegradation processes are carried out are usually univariately optimized [6,8,13-20], not being able to simultaneously analyze the possible interaction of the variables to be optimized is also considerable additional experimental effort.

In the present paper, the photodegradation conditions of a mixture of PHAs are approached in a simultaneous fashion. The factorial design is full and the EEM spectra are recorded throughout the process being studied. The analytes are quantified with the

curve resolution method PARAFAC, which is much quicker than the usual working procedures.

In this paper we present the results of the photolysis of a mixture of four analytes: dibenz[a,h]anthracene (Dib[a,h]A), benz[a]anthracene (B[a]A), benz[a]pyrene (B[a]P) and benz[k]fluorantene (B[k]F) and three variables: UV light responsible for the photodegradation, pH, and solvents. Eight experiments were carried out using a full factorial two-level design (2^3).

The results of establishing the PARAFAC model from the recorded fluorescent data (EEM) for the set of eight experiments are discussed. The results are also analyzed in kinetic terms: for example, induction time, % of degradation, degradation constant and half life of each analyte in different conditions.

Parallel factor analysis

Excitation-emission fluorescent measurements can provide a three-way data set, in which each sample gives rise to a data matrix. A series of data matrices obtained for multiple samples make up a data cube **X**. The PARAFAC algorithm [10,21] decomposes the data cube **X** into **A**, **B** and **C** loading matrices, and generates a trilinear model that minimizes the sum of the squares of the residuals:

$$\mathbf{X}_{ijk} = \sum_{f=1}^{F} \mathbf{A}_{fi}\, \mathbf{B}_{fj}\, \mathbf{C}_{fk} + \mathbf{E}_{ijk} \qquad (1)$$

where each element of $\mathbf{X}_{ijk}$ is the fluorescence intensity of sample k at emission wavelength i and excitation wavelength j, and F is the number of factors used to build the model. The f columns in **A** are the pure emission spectra predicted for the analytes, the f columns in **B** are the pure excitation spectra of the various species and the f columns in **C** contain the information about the concentration of each compound in the samples. The residual cube $\mathbf{E}_{ijk}$ contains the unmodeled information.

Ideally, PARAFAC should be able to find the pure emission and excitation spectra of each compound in a mixture when the appropriate number of F components for the model is selected. Alternating least squares (ALS) is used to find the solution to eq. (1). During this process, restrictions can be imposed on the solutions [21,22] so that they can be interpreted more easily or so that they fit the physical and chemical characteristics of the system under study. One of the advantages of PARAFAC is the uniqueness of the solution [10,21,22], because the problems of rotational ambiguity typical of bilinear models do not arise.

A crucial stage in the development of the model is the determination of the number of components (F). There are various criteria for evaluating F; the percentage of fit (Fit %) [23] and the core consistency test (%) [22,24] which provide a measure of the variability of the experimental data reflected by the model. Values close to one hundred in both parameters are desirable.

To evaluate the quality of the retrieved profiles, we used the criterion of similarity (correlation coefficient) (r), and compared the true spectra (if there were any) with the spectra obtained from the PARAFAC algorithm. A value of r=1 indicates total coincidence.

Kinetic calculations

The kinetic constants of photodegradation processes, assuming that they normally fit pseudo first order kinetics [5,8,19], and the half lives of the analytes are calculated using equations (2) and (3)

$$\ln \frac{C_0}{C_i} = kt \qquad (2)$$

where C_0 and C_i are the concentrations at time zero and time t, respectively, and k is the velocity constant.

The half-life time of each analyte $t_{1/2}$ is calculated by deriving equation 2 and replacing C_i with $C_0/2$:

$$t_{1/2} = \ln \frac{2}{k} \qquad (3)$$

2. Materials and methods

2.1. Materials

All the reagents used were of analytical quality. The PAHs dibenz[a,h]anthracene (Dib[a,h]A, 97%), benz[k]fluorantene (B[k]F, 98%) benz[a]anthracene (B[a]A, 99%) and benz[a]pyrene (B[a]P, 97%) were obtained from Aldrich. The solutions of pure analytes (100 μg L^{-1}) were produced by weighing the appropriate amount of reagent and dissolving it in Millipore water. The pH was adjusted with 5M sodium hydroxide solution (Aldrich 97%) or phosphoric acid (Aldrich 85%), depending on the

experimental design. The solvents used were methanol (MetOH), ethanol (EtOH) and acetonitrile (ACN). They were obtained from Merck and were HPLC quality.

2.2. Instrumental

The photodegration studies were carried out in a cylindrical annular batch reactor. The reactor consists of an immersion quartz tube (2.5 cm i.d., and 38 cm long) which holds a low pressure mercury vapor lamp (LPML) of 15 W (Heraeus Noblelight, Germany) or a high pressure mercury vapour lamp (HPML) of 47 W (Heraeus Noblelight, Germany) depending on the experimental design. The light source emitted by the LPML is predominantly at 254 nm, and for HPML it is predominantly at 366 nm. When we used the HPML, the immersion tube was covered by a cooling quartz jacket. The quartz tube was placed in a Pyrex glass outer reactor (0.7 l capacity). An Orion pH-meter was used to check the pH of the reacting mixture.

The spectrofluorimetric data were acquired on an Aminco–Bowman Series 2 Luminescence spectrometer (SLM Aminco, Rochester, NY, USA) equipped with a 150W continuous xenon lamp and a PMT detector.

2.3. Photolyisis experiments

The eight photodegradation experiments were carried out following a 2^3 full factorial experimental design [25]. The chosen variables were studied on two levels: the

variables used were pH (2.5 and 11), solvent (EtOH and MetOH) and UV lamp (LPML and HPML). (Table 1)

Experiment		pH	Lamp
1	EtOH	2.5	LPML
2	MetOH	2.5	LPML
3	EtOH	11	LPML
4	MetOH	11	LPML
5	EtOH	2.5	HPML
6	MetOH	2.5	HPML
7	EtOH	11	HPML
8	MetOH	11	HPML

Table 1. 2^3 full factorial experimental design

The degradations were carried out in a cylindrical reactor, such as the one described in section 3.2, and with the lamp, pH and solvent for each experiment. Each experiment consisted of a mixture of the four analytes (Dib[a,h]A, B[k]F, B[a]A y B[a]P) at a concentration of 100 μg L^{-1} and a solvent volume ratio of Water :Alcohol :ACN, 50:40:10. The ACN was added to minimize the adsorption of the PHAs on the walls of the reactor. The total volume of the solution was 500 ml. The photodegradation was carried out at ambient temperature with stirring throughout the reaction (120 min).

Five-millilitre samples were taken through the reactor's sampling valve and the filtered solutions were kept in darkness. In the first 30 minutes, samples were taken every 2 minutes, and then every 10 minutes up to the 120 minutes of reaction time. The sampling scheme produces 25 samples for experiment.

2.4. Measurements

All solutions (individual standards, samples and solvent) were measured in the same conditions. Three-dimensional excitation-emission spectra were recorded between 360 and 450 nm in the emission domain and between 230 and 300 nm in the excitation domain, both at regular steps of 3 nm. The scanning rate of the monochromators was maintained at 30 nms^{-1}. The excitation and emission monochromator slit widths were set to 4 nm. All measurements were made in a 10mm quartz cell at 590V. The fluorescence spectra were exported in ASCII format from the instrument software to MATLAB [26]. This information was then processed with the PARAFAC algorithm, obtained from the N-way toolbox [27].

2.5. Procedure for establishing the model

The procedure used to apply PARAFAC is described below:

- Absorption-emission spectra (31×24) were acquired of the pure analytes at a concentration of 100 μg L^{-1} so as to obtain the reference spectra for the different species studied. An individual data cube was constructed for each analyte from the excitation-emission matrices, and one-factor PARAFAC was applied with a non-negativity constraint. The normalized spectral profiles obtained in this way were used as the pure spectra to calculate the correlation with the spectra recovered by PARAFAC when mixtures of these compounds were analyzed.

- We applied PARAFAC with non-negativity constraints to the data cube for the samples of all the photo degradation experiments (cube of 200×31×24). To select the most suitable model, we used the parameters of % Fit, core consistency and correlation coefficients (*r*) between the spectra used as references and those retrieved by PARAFAC.

3. Results and discussion

By way of example, Figure 1 shows the EEM data matrix for experiment 1 at time zero and the EEM matrices of each analyte at 100 ppb.

The signal recorded is not specific to any analyte in any zone of the spectrum. The values of the emission and excitation correlation coefficients among the analytes are always higher than 0.7, which means that there is considerable overlapping between the spectra. The time required to obtain this signal is less than 2 minutes.

Table 2 shows the results of the PARAFAC models with various factors on the three-dimensional data matrix (200 x 31 x 24).

Nº of factors (F)	1	2	3	4	5
%Fit	97.47	99.03	99.58	99.88	99.93
Core Consistency	100	98.59	96.91	92.69	-1265

Table 2. % Fit and core consistency for PARAFAC models using 1–5 factors

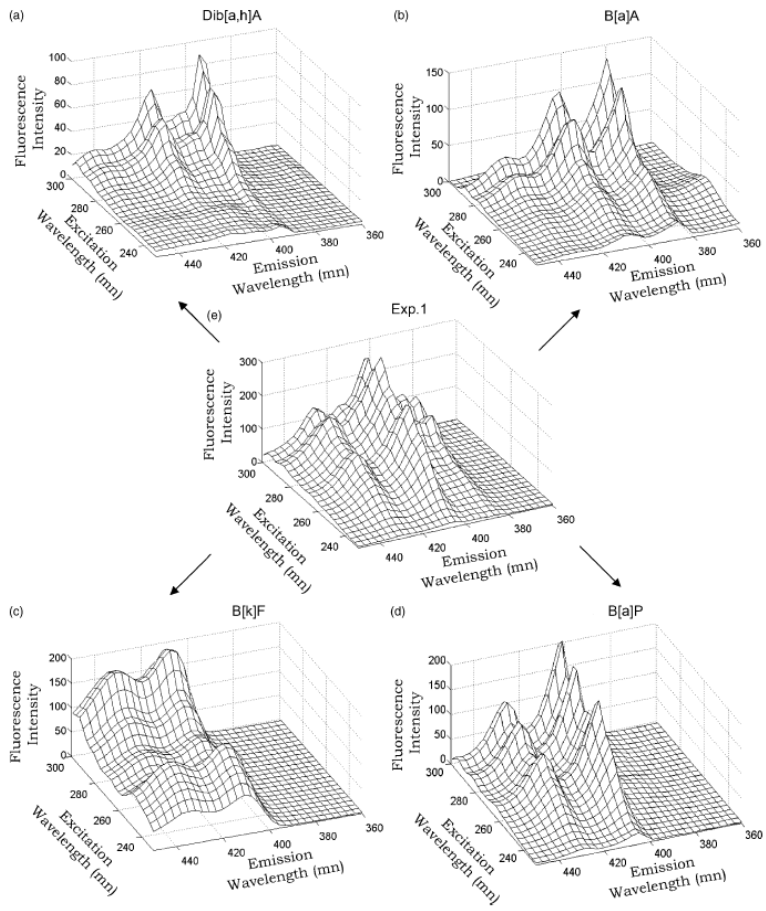

Figure 1. Signal recorded for: (a)Dib[a,h]A, (b)B[a]A, (c)B[k]F and (d)B[a]P at 100 μL^{-1} and (e) for experiment 1 (Exp.1) in which there is a mixture of all the analytes

It can be seen that as the number of factors increases, the percentage of fit also increases, and that there is no significant difference between the value of four (99.88%) or five factors (99.93%). For five factors, the core consistency is negative, which indicates that the model is overfitted and unstable [22,28]. This agrees with the expected result, where each factor represents the contribution of each analyte to the recorded signal, and

confirms that if there are secondary products in the degradation process, they are not sufficiently fluorescent to generate variability in the signal [5].

The high values of the correlation coefficient (Table 3) between the pure spectra of each analyte and the emission and excitation spectra obtained by applying the four-factor PARAFAC model suggest that the spectra retrieved by PARAFAC are representative of the analytes in the samples.

Correlation coefficients	BaA	BaP	BkF	DibA
$r_{Emission}$	0.9972	0.9934	0.9998	0.9956
$r_{Excitation}$	0.9986	0.9990	0.9960	0.9913

Table 3. Correlation coefficients for the emission and excitation spectra for the four-factor PARAFAC model

Fig. 2 shows the relative values of the concentration of each analyte calculated using the PARAFAC model developed, throughout the experiment and for all experimental conditions. They are calculated by means of a linear relationship between the value of the **C** coefficients at time zero and the corresponding value at each time. These values are fitted to a pseudo first-order kinetics (Eq. (2)), except for Dib[a,h]A, B[a]A and B[k]F in experiment 1 (Fig. 3). The data that do not follow pseudo first-order kinetics have not been included in Fig. 3. It can be seen that, for Dib[a,h]A (Fig. 2a) there is no induction time in any case. In all of the conditions tested, Dib[a,h]A began to degrade from the beginning, and until 40 minutes there were no significant differences between the different experiments.

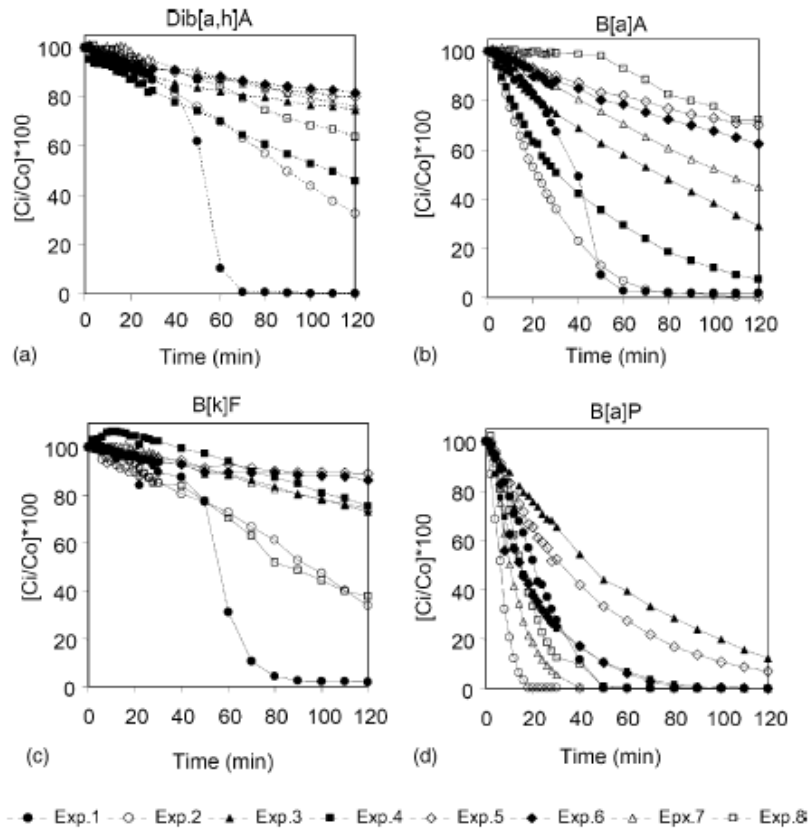

Figure 2. Degradation %: (a)Dib[a,h]A, (b)B[a]A, (c)B[k]F and (d)B[a]P for the various experiments

From this point on, Dib[a,h]A degrades at different rates in each of the experiments and, as can be seen from Fig. 3a, these data perfectly fit a pseudo first-order kinetics for all the experiments. The only exception is experiment 1, in which after 40 minutes there is a sharp change in the slope, and the analyte is totally consumed after 70 minutes.

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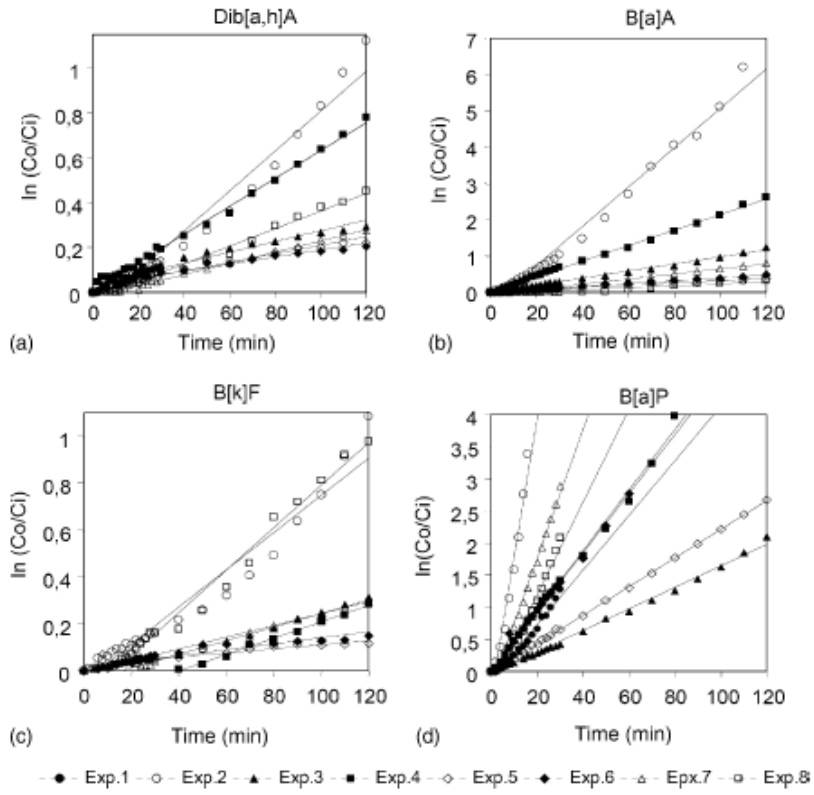

Figure 3. Pseudo first order kinetic plot of photolysis of: (a)Dib[a,h]A, (b)B[a]A, (c)B[k]F and(d)B[a]P for the experiments

Similar comments can be made for B[a]A and B[k]F (Fig. 2b and 2c). Once again, in the conditions of experiment 1, the data do not behave as habitual pseudo first-order kinetics in these cases. A detailed analysis of the results in Fig. 2b shows that, at pH 11 and with MetOH as solvent and the HPML (Exp. 8), there is an induction period of 50 minutes, after which there is a process of slow degradation. Like Dib[a,h]A, in all of these experiments these data fit a pseudo first-order kinetics (Fig. 3b), although the slopes are very different.

The B[k]F (Fig. 2c) has a relatively long induction period (50 minutes) when working at pH 11, with EtOH as solvent and low-pressure mercury lamps (Exp. 4). Finally, the B[a]P presents no induction times in any experiments. The results in all cases fit pseudo first-order kinetics (Fig. 3d). The degradation is total for all the experiments, except 3 and 5. Even in the worst conditions, the efficacy of the degradation is greater than that of the other analytes.

Table 4 shows the pseudo-first order rate constants and half-life time of all the analytes. In general, it can be seen that the values of the kinetic rate constants for B[a] P are higher than the values corresponding to the other analytes. On the other hand, when low-pressure mercury lamp is used (see Exp 1, 2, 3 and 4), the half-life times are lower for the four analytes.

To evaluate the influence of the each experimental variable, Figure 4 shows the value of the half-life times of each analyte for each experiment. For Dib[a,h]A, B[a]A and B[k]F, in experiment 1, the value was calculated using the concentration values that were fitted to a pseudo first-order kinetics in Figure 2 (between 40 and 90 min for Dib[a,h]A, 30 and 70 min for B[a]A, and from 40 and 100 min for B[k]F).

An overall analysis of the results shows that: For Dib[a,h]A, B[a]A and B[k]F the values of the degradation times are lower when the pH is 2.5, the solvent is EtOH and the radiation source is the low-pressure mercury lamp (Exp. 1). For B[a]P, they are lower when the pH is 2.5, the solvent is MeOH and the radiation source is the low-pressure mercury lamp (Exp. 2) .

	Experiment	k (s^{-1})	$t_{1/2}$(min)
Dib[a,h]A	1	-	-
	2	8.90E-3	5.42
	3	2.50E-3	6.68
	4	6.20E-3	5.78
	5	1.90E-3	6.96
	6	1.60E-3	7.13
	7	2.50E-3	6.68
	8	3.90E-3	6.24
B[a]A	1	-	-
	2	54.3E-3	3.61
	3	9.81E-3	5.32
	4	21.50E-3	4.53
	5	3.02E-3	6.50
	6	4.00E-3	6.21
	7	7.05E-3	5.65
	8	2.93E-3	6.54
B[k]F	1	-	-
	2	8.00E-3	5.52
	3	2.50E-3	6.68
	4	3.50E-3	6.35
	5	0.90E-3	7.71
	6	1.30E-3	7.34
	7	2.90E-3	6.54
	8	9.10E-3	5.39
B[a]P	1	42.75E-3	3.85
	2	214.45E-3	2.23
	3	16.04E-3	4.83
	4	47.22E-3	3.75
	5	22.48E-3	4.49
	6	46.03E-3	3.77
	7	100.38E-3	2.99
	8	72.15E-3	3.32

Table 4. Apparent constants (k) and half-life time ($t_{1/2}$) of the analytes for the different degradation conditions

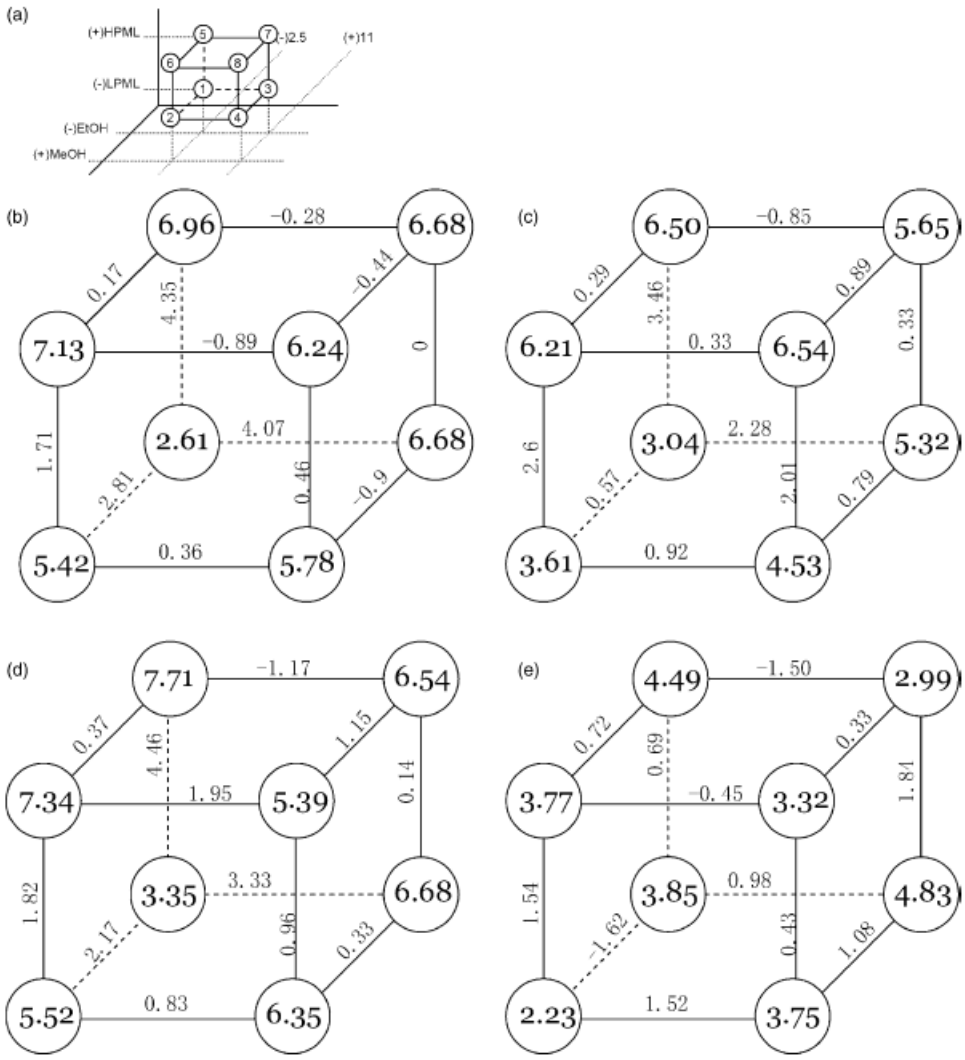

Figure 4. Half-life time ($t_{1/2}$) of the analytes corresponding to the 2^3 experimental design, (b)Dib[a,h]A, (c)B[a]A, (d)B[k]F and (e)B[a]P

The quantitative analysis of the increases in the values of $t_{1/2}$ between the different experiments on each edge of the cube (Fig.4) makes it possible to analyze the effect each experimental variable (pH, solvent and type of lamp) has on $t_{1/2}$, when we

move from one level (sign -, fig a) to another level (sign +, fig a). Thus it can be seen that for all the analytes (Fig.4 b-e) these increases depend on the levels of the other experimental variables. For example, for Di[a,h]A, the effect of pH is 4.07 in ethanol and 0.36 in metanol when it is used with a low-pressure mercury lamp, and -0.28 and -0.89 when it is used with a high-pressure mercury lamp. The fact that these increases are not constant is indicative of a correlation between the three experimental variables. The same conclusion can be drawn if we analyze the values of the increases associated with the other edges of the cube. Therefore, the individual analysis of the effect each variable has on the value of the half-life time makes no sense.

The results discussed above corroborate the importance of simultaneously analysing all the variables if the aim is to optimize the conditions of a photodegradation process.

That natural waters are not usually polluted by just one analyte suggests that it is logical to search for an overall optimum that will lead to maximum degradation of all pollutants in the shortest possible time. Therefore, in this study, the optimum conditions are those used in experiment 1 (pH = 2.5, low pressure mercury lamp and ethanol as solvent) which completely degrade all pollutants in 100 minutes, even though the B[a]P degrades faster for Exp. 2, 7 and 8.

4. Conclusions

The curve resolution method PARAFAC can be successfully applied to excitation-emission spectra matrices to determine dibenz[a,h]anthracene,

benz[a]anthracene, benz[a]pyrene and benz[k]fluorantene throughout the degradation process in the aqueous medium. Its rapidity means that it can be extremely useful when optimizing a process for degrading these analytes.

The strategy used simultaneously analysed the variables normally involved in the photodegradation processes of these pollutants: pH, solvent and UV radiation source. This made it possible to select an overall optimum for all the analytes being studied.

From the data obtained by this methodology, the kinetic parameters of photodegradation reactions can be easily derived for each analyte in the different conditions: for example, the degradation constant and the half-life time. The analytical methodology described can be easily automatized, which means that it is a good alternative to the habitual methods of analysis in photodegradation processes.

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4.1.3. PARAFAC and MCR-ALS applied to the quantitative monitoring of the photodegradation process of polycyclic aromatic hydrocarbons using three dimensional excitation emission fluorescent spectra. Comparative results with HPLC.

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Abstract

Two methods were developed for the simultaneous quantitative monitoring of photodegradation process of dibenz[a,h]anthracene (DibA), benz[a]anthracene (BaA), benz[a]pyrene (BaP) and benz[k]fluorantene (BkF) using excitation–emission fluorescence spectroscopy. Parallel factor analysis (PARAFAC) and multivariate curve resolution-alternating least squares (MCR-ALS) were satisfactory applied to the data obtained during this process. The results achieved were statistically compared by means of the joint interval test of slope and intercept, with the data obtained using the reference methodology, high performance liquid chromatography method (HPLC). There are not significant differences between the methodologies proposed and the standard one,

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and may be a good alternative to the traditional methods of analysis for monitoring the degradation of these pollutants.

Keywords: polycyclic aromatic hydrocarbons; PARAFAC; MCR-ALS; photodegradation; excitation-emission matrix.

1. Introduction

Polycyclic aromatic hydrocarbons (PAH) are one of the largest groups of chemical carcinogens and mutagens [1]. Demand for determining trace concentrations of these substances and removing them from the natural environment is constantly increasing [2].

Several methods based on the chemical destruction of very stable PAHs have been proposed and tested. Photodegradation is a particularly important methodology in this field, in which major progress is being made in oxidative methods for degrading organic compounds dissolved or dispersed in aquatic media [3,4]. The effectiveness of these methods is evaluated in terms of the time that the polluting agent takes to disappear, which is verified by analytical determination throughout the photodegradation process.

PAHs in complex mixtures are usually determined by gas chromatography (GC) [5] or high resolution liquid chromatography (HPLC) with either UV-visible diode array detectors (DAD) [4, 6,7] or fluorescence detectors [8]. Several authors have recently described the use of full excitation-emission fluorescence spectra (EEM: excitation-

emission matrix) for identifying and quantifying PAHs in aqueous solutions in combination with the additional information resources provided by such powerful chemometric tools as parallel factor analysis (PARAFAC) [9] or partial least squares regression (PLSR) [10].

One problem that is hindering the wider application of fluorescence spectroscopy for environmental monitoring is the intrinsic lack of selectivity in excitation and emission fluorescence measurements and several studies have discussed how best to increase the selectivity of these methods [11,12]. Recently several authors have argued that data sets obtained from kinetic experiments help to increase the selectivity of spectroscopic determinations in the application of PARAFAC model [13].

The ability to analyze multicomponent mixtures without a previous separation is attractive in the study, at laboratory level, of the optimization of the catalytic process. It would allow of fast form to evaluate the effectiveness of a photodegradation process.

In this paper, we present the results of monitoring the photolysis process of a mixture of four analytes: dibenz[a,h]anthracene (DibA), benz[a]anthracene (BaA), benz[a]pyrene (BaP) and benz[k]fluorantene (BkF). PARAFAC [14,15] and MCR-ALS [16] were applied to the emission-excitation data collected. These results were compared with the ones obtained with HPLC. They were also analyzed in kinetic terms and the % of degradation, the degradation constant and the half life of each analyte, all habitual parameters in this area of study, were determined.

In the bibliographic review that we made, we found no studies similar to this one. Previous studies on calibration with fluorescence excitation-emission matrices of PAHs and multivariate curve resolution methods have used prediction error as the most

common means of measuring whether these methodologies were successful. A simultaneous study, using the two analytical techniques, HPLC with univariate calibration, and fluorescence with curve resolution methods, is necessary if their advantages and disadvantages are to be evaluated. It, may be useful for drafting a protocol for using these methodologies in the quantitative monitoring of photodegradation processes. The ability of these methodologies in more complicated situations where, interference effects by other PAHs that could be presented will be reason of study for future works.

2. Experimental

2.1. Materials

All the reagents used were of analytical quality. The PAHs dibenz[a,h]anthracene (DibA, 97%), benz[k]fluorantene (BkF, 98%) benz[a]anthracene (BaA, 99%) and benz[a]pyrene (BaP, 97%) were obtained from Aldrich. The standard solutions were prepared by weighing the appropriate amount of reagent and dissolving it in acetonitrile (ACN). The pH was adjusted with phosphoric acid (Aldrich 85%). The solvents used for the photodegradation were ethanol (EtOH), acetonitrile (ACN) and Milli-Q quality water. They were obtained from Merck and were HPLC quality.

2.2. Instrumental

The photodegradation studies were carried out in a cylindrical annular batch

reactor. The reactor consisted of an immersion quartz tube (2.5 cm i.d., and 38 cm long) which holds a low pressure mercury vapor lamp (LPML) of 15 W (Heraeus Noblelight, Germany). The light source emitted by the LPML is predominantly at 254 nm. The quartz tube was placed in a Pyrex glass outer reactor (0.7 l capacity). An Orion pH-meter was used to check the pH of the reacting mixture.

The spectrofluorimetric data were acquired on an Aminco–Bowman Series 2 Luminescence spectrometer (SLM Aminco, Rochester, NY, USA) equipped with a 150W continuous xenon lamp and a PMT detector.

HPLC analyses were performed on an Agilent 1100 Series Fluorescence Detector (Germany) equipped with a programmable fluorescence detector. Chromatographic data were collected and recorded on an HP Chemstation version A.06.01.

2.3. Photolysis experiments

The degradations were carried out in a cylindrical reactor, such as the one described in section 2.2. The degradation mixture consisted of a solution containing the four analytes (DibA, BkF, BaA and BaP) at a concentration of 100 μg L –1, thesolvent volume ratio of Water:Alcohol:ACN was 50:40:10 and the pH was 2.5. The ACN was added to minimize the adsorption of the PHAs on the walls of the reactor. The total volume of the solution was 500 ml. The photodegradation was carried out at ambient temperature with stirring throughout the reaction (120 min).

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Five-millilitre samples were taken through the reactor's sampling valve and the filtered solutions were kept in darkness. In the first 30 minutes, samples were taken every 2 minutes, and then every 10 minutes up to the 120 minutes of reaction time. A total of 25 samples were taken throughout the experiment.

2.4. Measurements

All solutions (individual standards and samples) were measured in the same conditions. Three-dimensional excitation-emission spectra were recorded between 360 and 450 nm in the emission domain and between 230 and 297 nm in the excitation domain, both at regular steps of 3 nm. The scanning rate of the monochromators was maintained at 30 nms–1. The excitation and emission monochromator slit widths were set to 4 nm. All measurements were made in a 10 mm quartz cell at 590V. The fluorescence spectra were exported in ASCII format from the instrument software to MATLAB [17]. This information was then processed with the PARAFAC algorithm, obtained from the N-way toolbox [18] and with the MCR-ALS algorithm [19].

The analyses with HPLC were carried out with a 250 x 4 mm Tracer PAH C-18 reverse-phase column from Teknokroma (Barcelona, Spain) with a particle size of 5 μm. A 20 μl loop was used to directly inject the sample. A linear elution gradient programme was performed from 75% (v/v) ACN in water to 95 % for 23 min at a flow rate of 1.5 ml min-1. For more sensitive detection of the PAHs, optimum excitation and emission wavelengths had to be used for each component and a wavelength detection program was developed for this purpose (Table 1).

Time (min)	Wavelength (nm)		Analyte
	Excitation	Emission	
0	286	387	BaA
14	295	410	BkF/BaP
19.50	296	396	DibA

Table 1. Fluorescence detection conditions used to analyse PAHs

3. Results and discussion

By way of example, Fig. 1 shows the EEM data matrix at time zero and the EEM matrices of each analyte at 100 ppb.

The time required to obtain this signal is less than 2 minutes. The signal recorded is not specific to any analyte in any zone of the spectrum. The sensitivities of each compound at the same concentration were very different: the most sensitive analyte was BkF, then BaP, BaA and DibA. The spectra -overlap and the maximum peaks of absorbance are straits. For this reason, when HPLC uses a fluorescent detector the wavelength must be changed during the elution chromatographic process.

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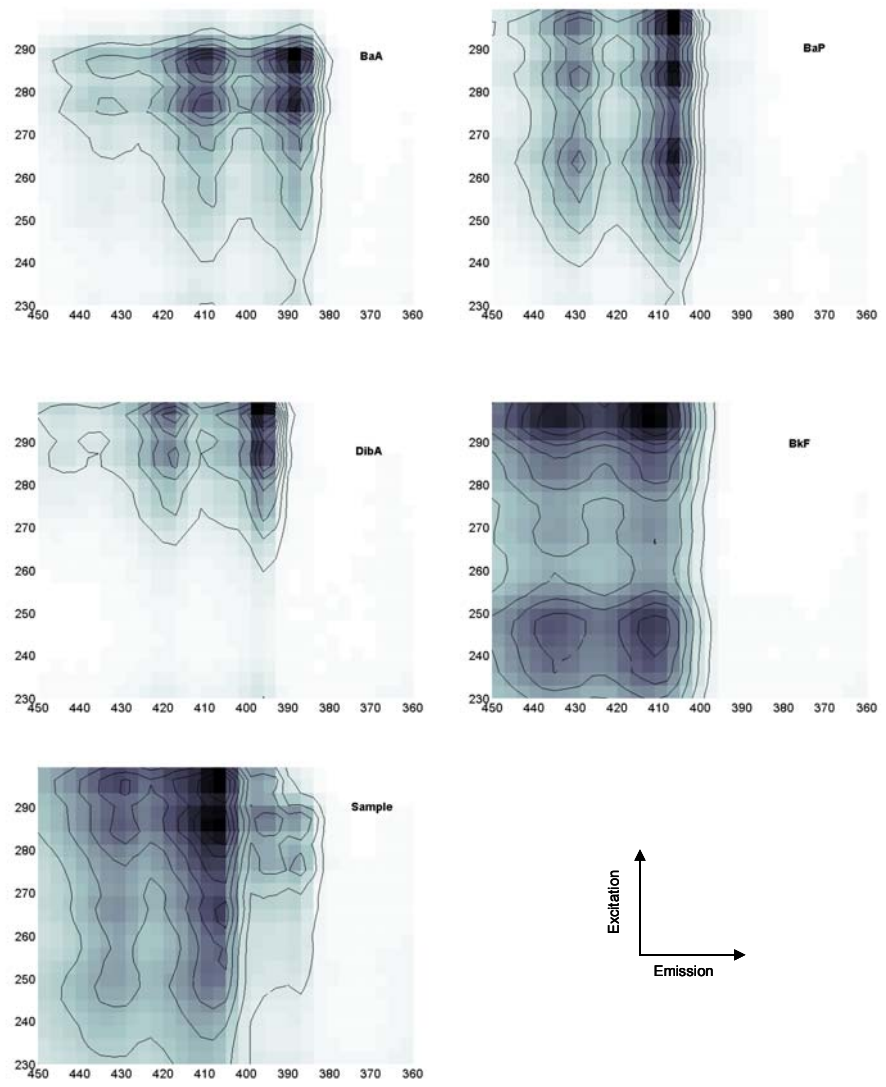

Figure 1. EEM matrices for BaA, BkF, DibA and BaP at 100 ppb, and the EEM data matrix of a sample at time zero of the photoreaction

The differences and the simililarities of the spectra are evaluated by the value of the correlation coefficients (r) [20]. A value of r = 1 indicates that the spectra are completely overlapped. Table 2 shows the emission and excitation correlation

coefficients (r) between the pure analytes of 100 ppb.

	B[a]A (Ex/Em)	Dib[a,h]A (Ex/Em)	B[a]P (Ex/Em)
Dib[a,h]A	0.7973/0.8273		
B[a]P	0.8649/0.7212	0.8962/0.6891	
B[k]F	0.7484/0.6911	0.8650/0.7153	0.8951/0.8879

Table 2. Emission and excitation correlation coefficients (r) between the analytes

Then, we applied PARAFAC with non-negativity constraints to the data cube for the samples of all the photodegradation experiments.

A four factor model gave us a % fit of 99.88 and a core consistency of 92.69 (Table 3). This information indicates that intermediate fluorescent compounds do not appear in the process of photodegradation.

Nº of factors (F)	1	2	3	4	5
%Fit	97.47	99.03	99.58	99.88	99.93
Core Consistency	100	98.59	96.91	92.69	-1265

Table 3. % Fit and core consistency for PARAFAC models using 1–5 factors

Fig. 2 shows the excitation and emission spectra obtained by PARAFAC and the pure spectra.

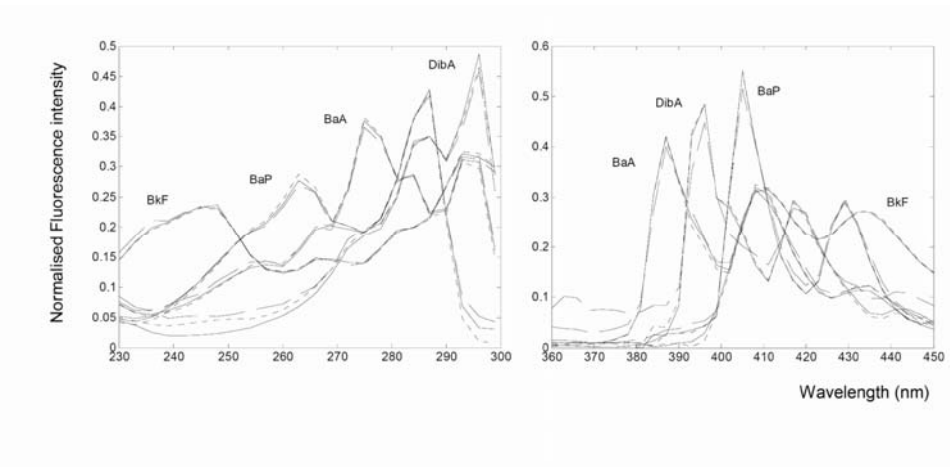

Figure 2. Excitation and emission spectra obtained by PARAFAC (solid line), MCR-ALS (dotted lines) and the pure spectra of the analytes (broken lines)

The high values of the correlation coefficients (Table 4-A) between the pure spectra of each analyte (Fig.2 broken lines) and the emission and excitation spectra obtained by applying the four-factor PARAFAC model (Figure 2, solid line) suggest that the spectra retrieved by PARAFAC are representative of the analytes in the samples.

(A) PARAFAC Correlation coefficients	B[a]A	B[a]P	B[k]F	Dib[a,h]A
$r_{Emission}$	0.9972	0.9934	0.9998	0.9956
$r_{Excitation}$	0.9986	0.9990	0.9960	0.9913
(B) MCR-ALS Correlation coefficients	**B[a]A**	**B[a]P**	**B[k]F**	**Dib[a,h]A**
$r_{Emission}$	0.9990	0.9999	0.9996	0.9957
$r_{Excitation}$	0.9956	0.9995	0.9983	0.9881

Table 4. Correlation coefficients for the emission and excitation spectra for the four-factor PARAFAC (A) and MCR-ALS (B) model

Fig. 3 shows the relative values of the concentration of each analyte calculated using the PARAFAC model developed, throughout the experiment. They are calculated by means of a linear relationship between the value of the score at time zero and the corresponding value at each time for each analyte [21].

For applied MCR-ALS, the EEM were arranged in an excitation-column-wise augmented data matrix [16] built with the 25 photodegradation samples and the standard matrices (each matrix consists of 31 emission vectors and 24 excitation vectors). The number of chemical species in this EEM was first estimated by singular value decomposition, since it was assumed that chemical components were associated with the largest singular values. Once again, four chemical species were found in the rank analysis.

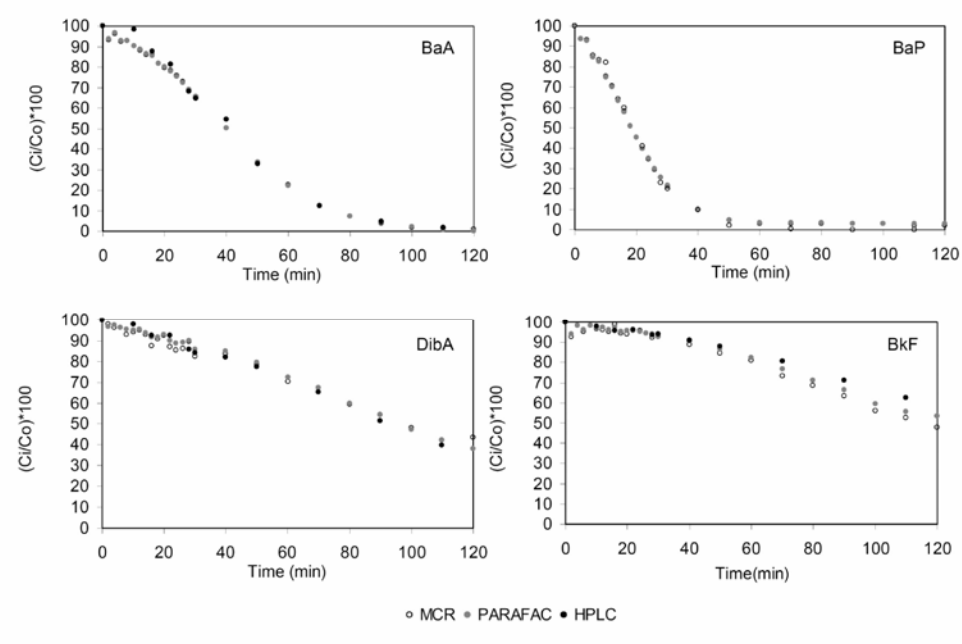

Figure 3. Relative concentration of each analyte calculated using PARAFAC (grey points), MCR-ALS (white points) and HPLC (black points), throughout the experiment

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The MCR-ALS method used non-negativity for both spectral modes, a three-way data structure (equal shape and synchronization) and non-normalization constraints in. Excitation spectra were used as initial estimates. The highest percentage of variance explained (r^2) was 99.94. Table 4-B shows the correlation coefficients between the excitation and emission spectra obtained (Fig 2 dotted lines) and the pure spectra (Fig.2 broken lines) of the analytes at 100 ppb. For the purposes of this calculation, the emission spectrum obtained by MCR-ALS was considered to be the one corresponding to a photodegradation time of zero.

Fig. 3 shows the relative values of the concentration of each analyte calculated using the MCR-ALS methodology. The quantification was performed by comparing the areas below the emission spectra of the analyte in the standard and in each sample obtained during the photodegradation process [16].

The chromatograms of some of the samples taken during the irradiations are presented in Fig. 4. The intensities of the peaks of the analytes of interest were observed to decrease with time, and no other peaks appeared to denote the presence of any intermediates. PAH concentrations were determined from a calibration curve of fluorescence peak area versus concentration of PAH injected onto the HPLC. The correlation coefficients for each calibration line were 0.9984 for BaP, 0.9959 for BaA, 0.9975 for BkF and 0.9969 for DibA. The data found have been represented in Fig.3.

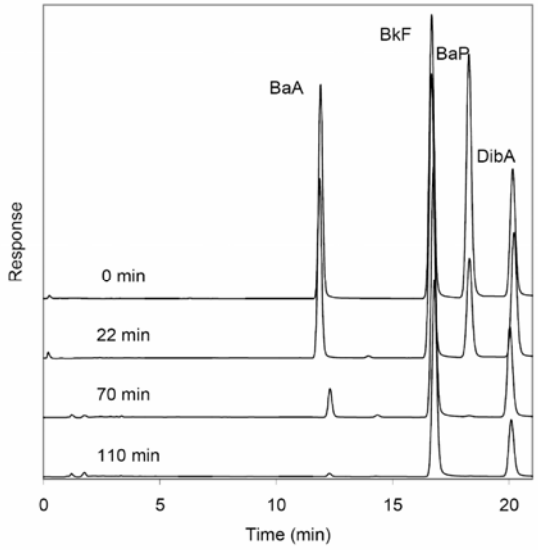

Figure 4. The chromatograms of some of the samples taken at 0 min, 22 min, 70 min and 110 min of the photodegradation

As can be seen in Fig. 3 BkF that shows a difference between the three methods, although it can be seen that the results found with PARAFAC and MCR-ALS are more similar to one another than the results obtained by HPLC.

The presences of bias for both methods, regressed against to HPLC, were studied by means of the joint interval test of slope and intercept (Fig. 5). It is possible to see that the results obtained with PARAFAC present not significant differences at a significance level of α = 5 %, while MCR-ALS (Fig.5 (b)) is comparable at a significance level of α = 1%.

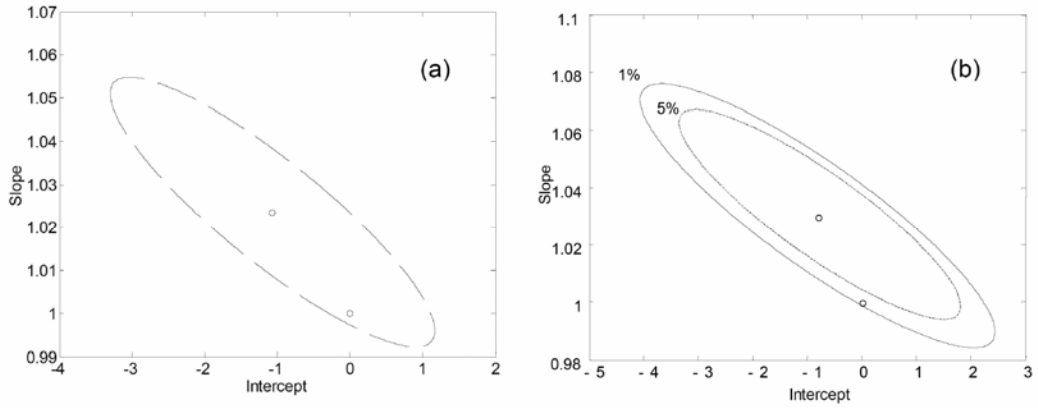

Figure 5. Joint interval test for the slope and the intercept, (a) for PARAFAC, (b) MCR-ALS.

In photodegradation studies the rate constants and the half-lives of analytes are used to measure the success of the process and these values are listed in Table 5 for the three methods. They were calculated assuming that the degradation normally fits pseudo-first order kinetics [4,7,22].

It can be observed that the values of *k* found by the three methods are of the same order of magnitude. When the absolute differences between the times that the analyte concentration takes to decrease by half ($t_{1/2}$) are analysed, it is observed that the greatest differences are for BaP and BkF, but are in no case higher than the 0.33 minutes that we can assume to not be significant.

	PARAFAC		MCR-ALS		HPLC	
	$*10^{-3}k$ (s^{-1})	$t_{1/2}$(min)	$*10^{-3}k$ (s^{-1})	$t_{1/2}$(min)	$*10^{-3}k$ (s^{-1})	$t_{1/2}$(min)
BaP	61.1	3.49	61.7	3.48	75.0	3.28
BaA	33.8	4.09	37.5	3.98	39.4	4.08
DibA	7.6	5.57	7.1	5.64	8.2	5.49
BkF	5.1	5.97	5.8	5.84	4.2	6.17

Table 5. PAH photodegradation rate constants (k) and half-lives ($t_{1/2}$) calculated on the basis of analytical data

A bibliographic review of the values of k and $t_{1/2}$ [4,22-24] for these analytes in similar conditions shows results that agree with the ones that we have found here. So, fluorescence in conjunction with MCR-ALS or PARAFAC was successfully used to monitor the decomposition of PAHs in dilute aqueous solutions and the results are similar to the ones obtained by means of HPLC in a situation as the considered.

A more extensive discussion about the goodness of this methodology, in comparison with HPLC, it would require another experimental work that allows to analyze the effect of the different concentrations of the analytes in the resolution of them. This can be another interesting work that could have own body.

4. Conclusions

The results obtained by our methodology present no significant differences with the habitual methodology and it may be a good alternative to the traditional methods of analysis for monitoring the degradation of pollutants. The analysis times are very short and no solvents are used so no residues are generated.

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PARAFAC and MCR-ALS can be successfully applied to excitation-emission spectra matrices to semiquantitatively determine the concentration of BaA, BaP, DibA and BkF throughout the degradation process in the aqueous medium . This should be useful for establishing a protocol for using these analytical methodologies to monitor the degradation reactions of fluorescent analytes in a situation as the considered .

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4.2. Seguimiento cuantitativo de reacciones de foto-catálisis del fenol mediante espectrofluorimetría de emisión-excitación y análisis de datos de múltiples vías

4.2.1. Introducción

La reacción de foto-degradación del fenol constituye un caso mas complicado que el de la sección anterior, ya que:

- en el proceso de foto-degradación en medio acuoso aparecen especies químicas intermedias (sus dihidroxiderivados) que presentan estructuras químicas muy semejantes y por tanto, espectros fluorescentes similares.

- el intervalo de longitudes de onda de excitación y de emisión, del fenol y sus intermediarios se solapan originando señales de dispersión (dispersión Rayleigh), que deben de ser eliminadas, antes de la aplicación de algunos de los métodos en estudio, concretamente PARAFAC.

- en esta zona espectral se observa la dispersión Raman característica del agua.

- Desde el punto de la catálisis, esta reacción se suele llevar a cabo en presencia de un catalizador, y la naturaleza del mismo juega un papel importante en el proceso de degradación. La utilización de catalizador conlleva la necesidad de filtrarlo para poder hacer la medida analítica, y este proceso físico se ve fuertemente afectado por la acidez de las condiciones de la solución reaccionante.

En esta sección se discuten los resultados relacionados con:

- La aplicación de los métodos de resolución de curvas, PARAFAC y MCR-ALS
- La aplicación del método de calibración multivariante para datos de múltiples vías, N-PLS

En la evaluación de la capacidad de resolución y cuantificación de los métodos de resolución de curvas, PARAFAC y MCR-ALS, para el seguimiento cuantitativo de la degradación foto-catalítica del fenol, se trabajó con el conjunto de mezclas sintéticas diseñadas siguiendo un diseño {4,3} Simplex-Lattice para mezclas cuaternarias y descrito en el apartado 3.2. Se analiza tanto el error global de predicción como el error a diferentes niveles de concentración de los analitos implicados en el proceso de foto-degradación en estudio (Sección 4.2.3.).

Se aplicó a la cuantificación de la foto-degradación del fenol en unas condiciones experimentales determinadas.

En el estudio de este proceso, utilizando un diseño factorial completo a dos niveles (Sección 4.2.4.) se evalúa el efecto cuantitativo de tipo de catalizador utilizado el pH del medio y la concentración inicial del fenol en la constante de velocidad del proceso de foto degradación. La constante de velocidad fue calculada a partir de las curvas de concentraciones obtenidas utilizando PARAFAC asumiendo que el fenol se degrada en todas las condiciones siguiendo una cinética de pseudo-primer orden.

Los resultados del error de predicción global de los modelos empleados permiten concluir que con ambos métodos, PARAFAC y MCR-ALS, se puede realizar un seguimiento cuantitativo de la concentración del fenol y de la concentración de sus intermediarios, catecol, resorcionol e hidroquinona en un proceso de foto-degradación a partir de sus espectros fluorescentes de excitación-emisión.

Un análisis detallado de los resultados permite observar que el fenol y la hidroquinona se resuelven perfectamente y se obtienen errores bajos a los diferentes niveles de concentración analizados mientras que catecol y el resorcinol que están fuertemente solapados presentan errores significativamente mayores.

El análisis de los efectos de las variables implicadas en el proceso de degradación en estudio: tipo de catalizador, pH del medio y concentración del sustrato en la constante de velocidad de la reacción permitió concluir que todas ellas interaccionan entre sí y la importancia de sus efectos resultó significativa a un nivel de confianza del 95% cuando se realizó un test estadístico ANOVA.

Siguiendo el objetivo planteado en esta tesis de desarrollar metodologías analíticas rápidas basadas en datos de fluorescencia de excitación-emisión y técnicas de análisis de múltiples vías, en la Sección 4.2.5. se llevó a cabo el estudio de los resultados que se pueden conseguir al trabajar con otra técnica de análisis de datos de múltiples vías, como es mínimos cuadrado parciales para datos de múltiples vías (N-PLS), en particular tri-PLS1, con el propósito de analizar si los errores encontrados fundamentalmente para el catecol y resorcinol eran susceptibles de mejora.

Los valores encontrados de aplicar este método y los anteriormente descritos a un nuevo conjunto de mezclas representativas del proceso en estudio fueron comparados en

términos del error global cometido.

Para finalizar, en esta misma sección, los resultados obtenidos aplicando todas las metodologías de análisis de datos de múltiples vías consideradas han sido validadas utilizando HPLC como técnica de referencia.

A partir de los resultados encontrados en los distintos apartados de este capítulo, hemos corroborado que la utilización conjunta de la espectrofluorimetría de datos EEM y la aplicación de diferentes métodos de análisis de datos de múltiples vías, ha resultado ser una opción válida y atractiva para la monitorización cuantitativa de la evolución de la degradación foto-catalítica del fenol y sus intermediarios.

4.2.2. Determination of phenol in the presence of its principal degradation products in water during a TiO_2-photocatalytic degradation process by three-dimensional excitation–emission matrix fluorescence and parallel factor analysis

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Abstract

This paper describes a simple and rapid way of monitoring a photocatalytic degradation of phenol in aqueous suspensions of TiO_2. A three-way analytical methodology based on fluorescence excitation-emission matrix (EEM) and parallel factor analysis (PARAFAC) was developed to resolve the species present in the reaction mixture and quantify the concentration of phenol and its principal degradation products throughout the degradation. Parameters such as core consistency, Fit % and correlation coefficients between recovered and pure spectra were used to determine the appropriate number of factors for the PARAFAC model. The accuracy of the model was evaluated by the root mean square error of prediction (RMSEP). Using a four-factor PARAFAC

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model, phenol, hydroquinone, resorcinol and catechol, were satisfactorily determined. The proposed method is an interesting alternative to the traditional techniques normally used for monitoring degradation reactions.

Keywords: Phenol; PARAFAC model; Photodegradation reactions; Fluorescence; Excitation–emission matrix

Introduction

Photocatalytic degradation as a tool for treating water containing industrial pollutants is of considerable current interest in the field of catalysis, and many studies have been made of the development of catalysts and the most appropriate conditions for effectively carrying out the process [1-3]. For the effectiveness to be evaluated, the concentration of the analytes involved in the degradation process needs to be quantitatively determined. The habitual methods of analysis usually involve time-consuming analytical procedures, which cannot analyze the chemical process in real time; for example, extraction and/or preconcentration, followed by such separation processes as gas chromatography (GC) [4,5] or high performance liquid chromatography (HPLC) [5,6].

Many of the pollutants that can be degraded photochemically are phenolic compounds. Phenol, in particular, is a compound that is widely used in the chemical industry for various purposes [7] and as a result it is found in a wide range of effluents and has a direct or indirect effect on the ecosystem [6]. Its photodegradation is well documented from the catalytic point of view [4-6,8,9]. Through the process of degradation, phenol decomposes completely to CO_2 and H_2O following a mechanism

of hydroxylation of the aromatic ring [5-7]. Hydroquinone, catechol and resorcinol are the habitual intermediates although in lower concentrations than phenol.

One of the attractive characteristics of phenolic compounds is that they present natural fluorescence. This means that they can be determined by molecular fluorescence, which is highly sensitive and has moderate selectivity. However, in complex mixtures, spectral overlapping is often a serious drawback and separation techniques must be used before univariate spectrofluorimetric techniques can be used. To avoid extremely time-consuming separation techniques, chemometric methods that can resolve and identify species present in complex mixtures have been developed using multivariate information [8]. Multivariate calibration techniques such as partial least squares (PLS) assume that there is a linear relationship between concentration and fluorescent intensity and have been successful at the quantitative analysis of multicomponents when the signals were considerably overlapped [10]. However, these techniques require the use of reference methods and a high number of samples.

The application of parallel factor analysis (PARAFAC) to data matrices consisting of excitation-emission fluorescence spectra (EEM) has satisfactorily resolved complex mixtures of several analytes [11,12]. These multivariate spectral resolution methods make it possible to discriminate accurately and reliably the analyte signal in the presence of unknown interferents and non-calibrated spectral interferences [11,13]. The main applications are the determination of pesticides [11,14], pharmaceutical compounds [15,16] and natural products [17,18]. Four-way PARAFAC applications have recently been reported [13,17,19] also with good results.

The aim of the present study is to develop an analytical methodology to quantitatively determine the analytes involved in the process of the photodegradation of

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phenol using TiO_2 as the catalyst and applying PARAFAC to excitation-emission fluorescence data recorded throughout the reaction.

The number of components for the PARAFAC model was selected by analyzing the parameters of Fit % and core consistency. The analytes were identified by analyzing the correlation between the emission and excitation spectra recovered by PARAFAC for each compound and the corresponding pure spectra. The predictive ability of the PARAFAC models was evaluated with the root mean square error of prediction (RMSEP) [14]. The method developed is a quick and straightforward way of monitoring the phenol degradation reaction and it simultaneously makes it possible to quantify the concentration of phenol and its degradation products.

The use of this analytical methodology to study phenol degradation is a practical example that has not been documented among the applications of PARAFAC to spectroscopic data in the course of a chemical reaction. The strategy considered is an attractive alternative to the normally used method (HPLC), not only because it saves time, but also because it can be easily automated, which means that the process can almost be measured *in situ*.

2. Theory

Excitation-emission fluorescent measurements can provide a three-way data set, in which each sample gives rise to a data matrix. A series of data matrices obtained for multiple samples make up a data cube $\underline{\mathbf{X}}$. The PARAFAC algorithm [20-22] decomposes the data cube $\underline{\mathbf{X}}$ into $\mathbf{A}$, $\mathbf{B}$ and $\mathbf{C}$ loading matrices, and generates a trilinear model that

minimizes the sum of the squares of the residuals:

$$\underline{\mathbf{X}}_{ijk} = \sum_{f=1}^{F} \mathbf{A}_{fi}\, \mathbf{B}_{fj}\, \mathbf{C}_{fk} + \underline{\mathbf{E}}_{ijk} \qquad (1)$$

where each element of $\underline{\mathbf{X}}_{ijk}$ is the fluorescence intensity of sample k at emission wavelength i and excitation wavelength j, and F is the number of factors used to build the model. The f columns in $\mathbf{A}$ are the pure emission spectra predicted for the analytes, the f columns in $\mathbf{B}$ are the pure excitation spectra of the various species and the f columns in $\mathbf{C}$ contain the information relative to the concentration of each compound in the samples. The residual cube $\underline{\mathbf{E}}_{ijk}$ contains the unmodeled information.

Ideally, PARAFAC should be able to find the pure emission and excitation spectra of each compound in a mixture when the appropriate number of F components for the model is selected. Alternating least squares (ALS) is used to find the solution to Eq. (1). During this process, restrictions can be imposed on the solutions [20,22] so that they can be interpreted more easily or so that they fit the physical and chemical characteristics of the system under study. One of the advantages of PARAFAC is the uniqueness of the solution [20-22], because the problems of rotational ambiguity typical of bilinear models do not arise. The trilinear data make it possible to assume that the vectors of the loading matrices, obtained after the factor decomposition indicated in Eq. (1), are linearly independent and, therefore, the solution is unique [20]. The fact that PARAFAC only resolves trilinear structures is a drawback when data are analyzed which have deviations in their trilinearity. One example of this is the presence of such phenomena as Rayleigh and/or Raman scatterings, which can cause some mathematical problems in the decomposition [11,23] so they must be removed or reduced as much as possible. Raman scattering is due to the solvent and can be completely eliminated by

removing the solvent spectrum from the sample spectrum [24]. The effect of the Rayleigh scattering can be removed using strategies such as *weighting elements* [11] or inserting *missing values* [12,22]. In this study we have used the latter with the algorithms described by R. Bro [22].

A crucial stage in the development of the model is the determination of the number of components (F). There are various criteria for evaluating F; the percentage of fit (Fit %):

$$\text{Fit \%} = 100 \times \left(1 - \frac{\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K} e_{ijk}^2}{\sum_{i=1}^{I}\sum_{j=1}^{J}\sum_{k=1}^{K} x_{ijk}^2} \right) \qquad (2)$$

where e_{ijk} is the residual of the ijkth element in cube $\underline{\mathbf{E}}$ (Eq.1) and x_{ijk} the corresponding data element in cube $\underline{\mathbf{X}}$ (Eq.1). The Fit % provides a measure of the variability of the experimental data reflected by the model [12]. PARAFAC decomposition was also assessed by the core consistency test (%) defined by

$$\text{Core consistency (\%)} = 100 \times \left(\frac{1 - \sum_{d=1}^{F}\sum_{e=1}^{F}\sum_{f=1}^{F} \left(g_{def} - t_{def} \right)^2}{\sum_{d=1}^{F}\sum_{e=1}^{F}\sum_{f=1}^{F} t_{def}^2} \right) \qquad (3)$$

where g_{def} and t_{def}, in the Tucker model [22], denote the elements of the calculated core and of the intrinsic super-diagonal core, respectively, and F the number of factors in the model. If g_{def} is equal to t_{def}, the core consistency is perfect and has a value of unity

(100%). A core consistency that is close to zero or even negative means that the model is not valid [25]. The appropriate number of factors is accessed by the model with the highest number of factors and a valid value of the core consistency diagnostic test. The joint analysis of all of this criteria makes it possible to select the most appropriate number of components for the model.

One way to evaluate the quality of the recovered profiles is to use the criterion of similarity (correlation coefficient), and compare the true spectra (if there are any) with the spectra obtained from the PARAFAC algorithm, in accordance with the following equation:

$$r = \cos\gamma = \frac{\mathbf{s_i^T \hat{s}_i}}{\|\mathbf{s_i}\| \cdot \|\mathbf{\hat{s}_i}\|} \qquad (4)$$

where γ is the angle defined by the vectors associated with the recovered spectra ($\hat{s}_i$) (any *f* columns in **A** or **B**) and the pure spectra (s_i) of the studied compound.
In the calibration stage, a linear relation is established between the values of the matrix of loadings **C** and the concentration of the standards of each analyte. This relation is used to predict the concentration of each analyte in future samples.

The accuracy of the model was calculated by the root mean squares error of prediction (RMSEP):

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{m}(x_i - \hat{x}_i)^2}{m}} \qquad (5)$$

where x_i and $\hat{x}_i$ are the measured and predicted concentrations of each analyte in the ith prediction sample, and m is the number of prediction samples.

3. Experimental

3.1. Chemicals

All reagents were of analytical grade: phenol, catechol, hydroquinone, and resorcinol were used as received from Sigma-Aldrich (99%) and glacial acetic acid (100%) from Merck. Stock solutions were prepared by weighing the appropriate amounts of the reagents and dissolving them in millipore water. All solutions were prepared in an acidic medium of 1 % (v/v) acetic acid, so that the analytes remained in the molecular form, and stored in dark bottles at 4ºC. They remained stable for one week. Working solutions were prepared by diluting stock solutions with millipore water, maintaining a concentration of 1% (v/v) acetic acid. TiO_2 in the anatase form (99.8%), (Sigma-Aldrich), was used as the photocatalyst without further treatment.

3.2. *Apparatus*

The photodegration studies were carried out in a cylindrical annular batch reactor. The reactor consisted of an immersion quartz tube (2.5 cm i.d., and 38 cm in length) which held a low pressure mercury vapor lamp (LPML) of 15 W (Heraeus Noblelight, Germany). The light source emitted predominantly at 254 nm. The quartz tube was placed in a Pyrex glass outer reactor (with a capacity of 0.7 l). An Orion pH-

meter was used to verify the suitable pH of the reacting mixture.

The spectrofluorimetric data were acquired on an Aminco–Bowman Series 2 Luminescence spectrometer (SLM Aminco, Rochester, NY, USA) equipped with a 150W continuous xenon lamp and a PMT detector. The instrument was interfaced by a GPIB card and driver with a PC Pentium microcomputer provided with the AB2 software version 1.40, for spectral acquisition.

3.3. *Reaction and procedure*

The process of mineralizing phenol according to Mylonas et al [5] can be seen in Scheme 1, where the $OH^{\cdot}$ radicals generated [26] (see Scheme 1 (a)) mainly attack in the *para*, and *ortho* positions and generate hydroquinone and catechol as principal intermediate products before mineralization to CO_2 and H_2O. However, the *meta* position is less favored by the electrophilic nature of the $OH^{\cdot}$ radicals, so resorcinol is produced at trace level [5,7].

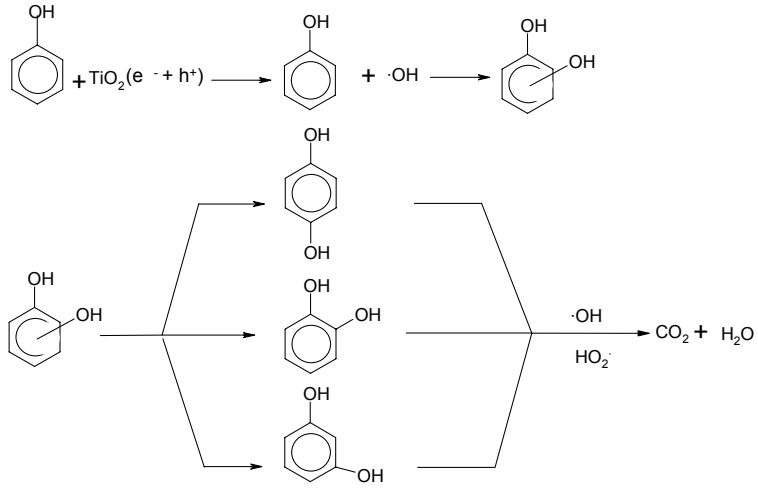
a)

$TiO_2 + h\nu \rightarrow TiO_2 (e^- + h^+)$

$TiO_2 (e^- + h^+) + H_2O \rightarrow TiO_2 (e^-) + OH^{\cdot} + H^+$

$TiO_2 (h^+) + OH^- \rightarrow TiO_2 + OH^{\cdot}$

b)



Scheme 1. a) Formation of $OH^{\cdot}$ radicals, b) degradation mechanism due to the $OH^{\cdot}$ radical attack

In the experiments, a solution of phenol of 1000 μg ml^{-1} was used. The suspension was prepared by mixing the phenolic solution with 0.3g l^{-1} of TiO_2; it was stirred uniformly using a magnetic stirrer for 15 min in the dark before the photodegradation started. The volume of the suspension was 500 ml. The reaction was carried out at room temperature, and stirring was continued throughout the reaction time (1500 min). The initial pH was adjusted to 2 with $HClO_4$.

Samples of around to 3 ml were collected from a sample valve throughout the reaction. The catalyst particles were removed by filtering with a 0.45 μm Millipore

membrane. All the solutions were wrapped and kept in the dark. Before the fluorescence was measured, 125 µl of the filtered sample solutions were diluted to 25 ml with 1% acetic acid. Individual standards of 5 µg ml^{-1} of each one of the pure analytes (phenol, cathecol, hydroquinone and resorcinol) were prepared.

A set of 20 mixtures was also prepared following a {4,3} Simplex-Lattice design [27] for quaternary mixtures so that the ability of the model to resolve and quantify these analytes can be evaluated. The set of mixtures was prepared in duplicate. The design took into account the relation between the concentrations of the species during the degradation process and the linearity interval of the fluorescent measurements. Table 1 shows the content of the samples prepared in detail.

Samples [a]	Phenol (µg ml^{-1})	Hydroquinone (µg ml^{-1})	Catechol (µg ml^{-1})	Resorcinol (µg ml^{-1})
Phenol	5.00	0.00	0.00	0.00
Hydroquinone	0.00	5.00	0.00	0.00
Catechol	0.00	0.00	5.00	0.00
Resorcinol	0.00	0.00	0.00	5.00
S1	5.00	0.05	0.05	0.05
S2	3.00	0.05	0.25	0.05
S3	1.50	0.05	0.75	0.05
S4	0.05	0.05	1.00	0.05
S5	3.00	0.25	0.05	0.05
S6	1.50	0.75	0.05	0.05
S7	0.05	1.00	0.05	0.05
S8	3.00	0.05	0.05	0.25
S9	1.50	0.05	0.05	0.75
S10	0.05	0.05	0.05	1.00
S11	0.05	0.25	0.05	0.75
S12	0.05	0.75	0.05	0.25
S13	0.05	0.75	0.25	0.05
S14	0.05	0.25	0.75	0.05
S15	0.05	0.05	0.75	0.25
S16	0.05	0.05	0.25	0.75
S17	0.05	0.25	0.25	0.25
S18	1.50	0.25	0.05	0.25
S19	1.50	0.25	0.25	0.05
S20	1.50	0.05	0.25	0.25

[a] All samples in 1% (v/v) of acetic acid

Table 1. Concentration of the analytes in the mixtures and individual standards, following a {4,3}Simplex-Lattice design

3.4. *Measurements*

All solutions (individual standards, mixtures, samples and solvent) were measured in the same conditions. Three-dimensional excitation-emission spectra were recorded from 270 to 350 nm in the emission domain and from 231 to 291 nm in the excitation domain, both at regular steps of 3 nm. The scanning rate of the monochromators was maintained at 30 nm s^{-1}. The excitation and emission monochromator slit widths were set to 4 nm. All measurements were performed in a 10 mm quartz cell at 620 V.

The fluorescence spectra were exported in ASCII format from the AB2 software to MATLAB [28] and, then manipulated with the PARAFAC algorithm obtained from the N-way toolbox [29].

3.5. *Procedure for establishing the model*

The procedure used to apply PARAFAC is described below:

- Pretreatment of the recorded data: (a) The effect of Raman scattering was eliminated by removing the fluorescent signal of the solvent for all the spectra. (b) The effect of Rayleigh scattering was eliminated using the *missing values* strategy [12,22]. The bandwidth was chosen visually so that the removal of the banded scatter could be manually optimized and the signal maintained. The removal of the signal means that the elements where the scatter occurred were considered to be missing. In the

modelling of the data below, these elements were not used and so did not affect the fitted parameters [30].

- The absorption-emission spectra of the pure analytes were collected and they were all unimodal. The one-factor PARAFAC model with non-negativity and unimodality constraints was applied to the matrices containing the excitation-emission spectra for each analyte. The normalized spectral profiles obtained in this way were used like the pure spectra (s_i in Eq. (4)) to calculate the correlation with the spectra recovered by PARAFAC ($\hat{s}_i$ in Eq.(4)) when mixtures of these compounds were analyzed.

- PARAFAC with non-negativity and unimodality constraints was applied to the data matrix consisting of the four standards of 5 μg ml^{-1}, the 20 mixtures (see Table 1) and the photodegradation samples so that the ability of the model to resolve could be evaluated. The 20 mixtures were included in the data cube for the sole purpose of evaluating the prediction error Eq. (5) when it was quantified with the standards of 5 μg ml^{-1}. The most appropriate model was selected using the models' parameters, Fit % (Eq. (2)), core consistency (Eq. (3)) and the correlation coefficients between the spectra recovered by PARAFAC and the pure spectra (Eq. (4)).

4. Results and Discussion

Figure 1 shows the fluorescence spectra for the 5 μg ml^{-1} solutions of each of the standards.

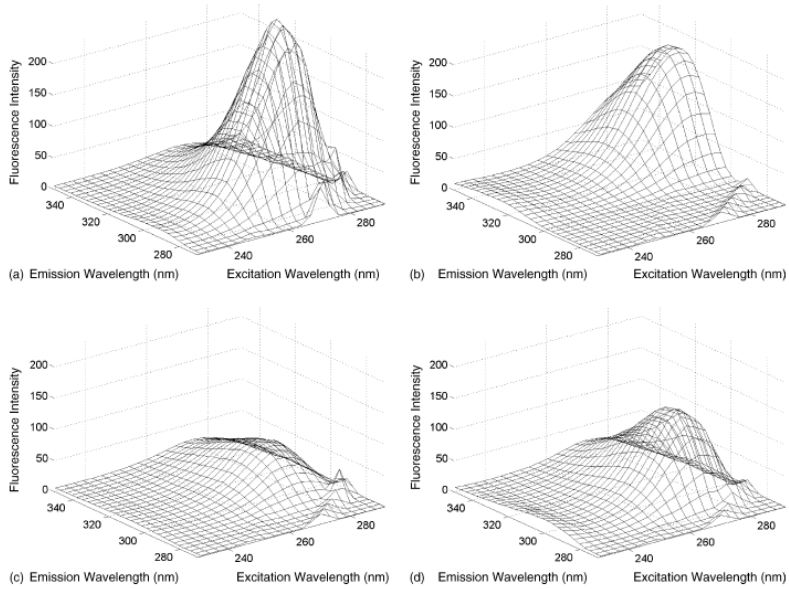


Figure 1. Excitation-emission fluorescence spectra (a) phenol, (b) hydroquinone, (c) catechol and (d) resorcinol at a concentration of 5 μg ml^{-1}

A detailed analysis of these spectra shows that phenol and hydroquinone (Fig. 1 a & b) are more sensitive than catechol and resorcinol (Fig. 1 c & d). The excitation and emission maxima for hydroquinone (λ_{em} = 328 nm y λ_{ex} = 288 nm) are significantly different from the maxima of the other three analytes (λ_{em} = 294 nm and λ_{ex} = 270 nm for phenol, λ_{em} = 311 nm and λ_{ex} = 276 nm for catechol, λ_{em} = 302 nm and λ_{ex} = 274 nm for resorcinol) which are similar to one another.

The calculated correlation coefficients among the pure spectra of the analytes (for both excitation and emission spectra) are shown in Table 2.

	Phenol (Ex/Em)	Hydroquinone (Ex/Em)	Resorcinol (Ex/Em)
Hydroquinone (Ex/Em)	0.5205 / 0.3314		
Resorcinol (Ex/Em)	0.9570 / 0.9261	0.6634 / 0.6038	
Catechol (Ex/Em)	0.9074 / 0.7718	0.7661 / 0.7899	0.9847 / 0.9527

Table 2. Correlation coefficients among pure spectra (excitation and emission) for the analytes under study.

A detailed analysis makes it possible to observe that the hydroquinone spectrum significantly differs from the spectra of the other analytes and that there is a moderated correlation between the spectrum of phenol and the spectra of catechol and resorcinol. On the other hand, the spectra of catechol and resorcinol are highly correlated.

In conjunction with the fact that in the photodegradation reaction studied the hydroquinone, catechol and resorcinol are produced in lower concentrations than phenol; this suggests that, *a priori*, it will be more difficult to resolve the individual contributions of catechol and resorcinol than the contribution of hydroquinone, because they have highly correlated spectra.

In an attempt to preserve the trilinear structure of the experimental data, we eliminated the sources of variability present in the spectra that were not due to the variation in the concentration of the analytes. This was done by applying the data pretreatments described above (see section 3.5). Fig. 2 illustrates the effect that the pretreatment had on the spectra.

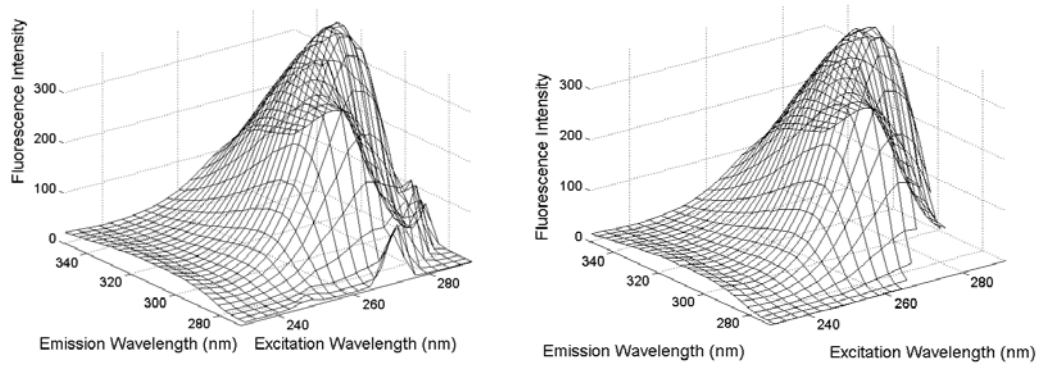


Figure 2. Excitation-emission fluorescence data for a mixture of the four analytes. On the left (a) are the data without pretreatment and on the right (b) are the data after the effects of Raman and Rayleigh scattering have been eliminated

To evaluate PARAFAC's ability to resolve the studied system, several data sets were considered: data set (a) consisted of the four individual standards of 5 μg ml^{-1}, data set (b) the four individual standards of 5 μg ml^{-1} and the twenty mixtures, and data set (c) which included the spectra corresponding to the four individual standards of 5 μg ml^{-1} and the degradation samples. The spectra of the pure standards were included in the data cubes to improve PARAFAC's capacity to reliably recover the spectra of analytes that are not very selective and which are highly correlated with each other [14].

This analysis made it possible to study the effect of spectral overlap in mixtures (comparison between data sets (a) and (b)) and the effect of the correlation of the species in the time direction (comparison between data sets (b) and (c)) in the PARAFAC results. Table 3 shows the Fit % and core consistency obtained for data sets (a), (b) and (c) when PARAFAC was applied using 1to 4 number of factors.

Nº of Factors (F)	Data Set (a)		Data Set (b)		Data Set (c)		Data Set (d)	
	% Fit	C. C.	% Fit	C. C.	% Fit	C. C.	% Fit	C. C.
1	70.59	100	90.49	100	93.93	100	95.11	100
2	97.14	100	99.04	100	99.34	100	99.48	99.98
3	99.93	99.92	99.97	99.78	99.98	99.77	99.97	99.68
4	99.99	84.92	99.99	-35.55	99.90	46.39	99.98	-22.00

Table 3. Fit % and core consistency (C. C.) in PARAFAC models using 1to 4 factors for data sets (a), (b), (c) and (d) (See text)

The Fit % calculated for three and four factors were high and similar for all data sets. When a model with four factors was applied, a high core consistency value was obtained for data set (a). This was to be expected because the spectral information analyzed corresponded to the pure individual standards. However, the core consistency for the data set (b) was negative, which indicates that, when mixtures of the four analytes were analyzed, spectral overlap considerably affected the PARAFAC results. This fact agrees with the correlation coefficients shown in Table 2. The value of core consistency obtained for data set (c) was considerably lower than the value obtained for the model with three factors, but better than the core consistency obtained with four factors for data set (b).

The value was probably improved because in the degradation samples the amounts of resorcinol produced throughout the time was at trace level [5,7] and then the contribution of this analyte could be neglected. Therefore, the spectral overlap in the degradation samples was smaller.

In order to quantify the concentration of phenol and its intermediates throughout the photodegradation process, a data set (d) was constructed with the four individual standards, the twenty mixtures and the degradation samples. The twenty mixtures (prepared in duplicate) were included in the model to calculate the RMESP with the standards of 5 μg ml^{-1}. To select the most appropriate number of factors, PARAFAC models with different number of factors were applied (see Table 3). It can be seen that as the number of factors increases, the Fit % also increases although there is no significant difference between the value for three factors (99.97%) or four factors (99.98%). It is to be expected that the optimum result would correspond to a PARAFAC model of four factors, each of which represents the contribution of one analyte. However, the core consistency value is considerably different when there are three factors (98.21) or four (-22.00), and the final value could indicate an unstable model with an overfit of the data [13, 19].

In an attempt to identify the spectra obtained with PARAFAC, they were compared with the pure spectra for each analyte (see section 3.5) using the correlation coefficient (r) Eq. (4)). Table 4 shows the correlation values obtained for the PARAFAC models with different numbers of factors. It is clear that when one factor is used, the spectrum recovered is that of phenol. When two factors were used, the first spectrum recovered is highly correlated with the pure spectrum of phenol while the other one corresponds to the spectrum of hydroquinone. When the three-factor model was used, the spectra recovered for phenol and hydroquinone have high correlation coefficients. The problem lies in the difficulty to assign the third factor to resorcinol or catechol; although the correlation value is slightly better when it is attributed to resorcinol (r_{em}= 0.9959; r_{ex}= 0.9985) and not catechol (r_{em}= 0.9762; r_{ex}= 0.9945). When a four-factor model

is used, the correlation coefficients indicate that the spectra of the four analytes are recovered correctly (see Table 4).

Nº of factors	Phenol	Hydroquinone	Resorcinol	Catechol
Emission spectra				
1	0.9979			
2	0.9995	0.9969		
3	0.9999	0.9999	0.9959	
4	0.9999	0.9999	0.9995	0.9999
Excitation spectra				
1	0.9918			
2	0.9976	0.9975		
3	0.9999	0.9998	0.9985	
4	0.9999	0.9998	0.9995	0.9998

Table 4. Correlation values of the emission and excitation spectra

Also, the RMSEP was investigated in order to evaluate the predictive capacity of PARAFAC models (with 1 to 4 factors) for data set (d) was analyzed. With this aim, the mixtures were quantitatively analyzed by relating the score of each pure standard of to the score of each analyte in each sample. In turn, the RMSEP for the different models helped to choose the correct number of factors. Table 5 shows the prediction errors obtained for each analyte.

Nº of factors	RMSEP (%)			
	Phenol	Hydroquinone	Resorcinol	Catechol
1	27.10	-	-	-
2	21.38	13.26	-	-
3	5.61	4.23	29.23	-
4	5.22	3.89	9.00	16.78

Table 5. Prediction errors for PARAFAC models using 1 to 4 factors

The prediction error decreases as the number of factors increases. There were no significantly differences between RMSEP for three and four factor for phenol and hydroquinone. On the other hand, the RMSEP for resorcinol was considerably improved when applied the model with four factors. The RMSEP for catechol was relatively high in comparison with the errors obtained for the other analytes.

Once we had analyzed the values of Fit %, core consistency, the correlation coefficients between the pure spectra and the spectra recovered by PARAFAC (both emission and excitation spectra) and the values of RMSEP, we proposed a model with four factors for the determination of phenol and its degradation products. In this model the first factor was assigned to phenol, the second to hydroquinone, the third to resorcinol and the fourth to catechol.

The selected model made it possible to monitor the photocatalytic degradation of phenol. The concentration profiles obtained for phenol, hydroquinone and catechol are

shown in Fig. 3 and 4. No significant quantities of resorcinol were detected throughout the process.

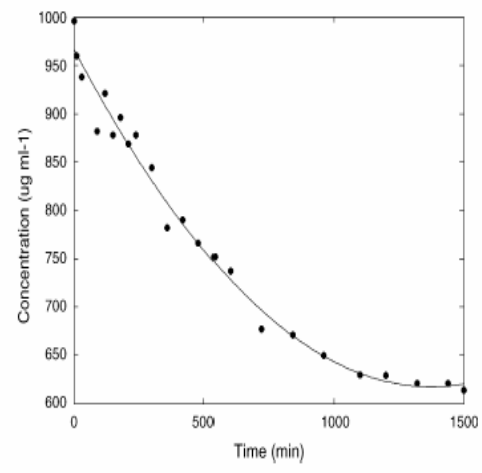


Figure 3. Variation in phenol concentration throughout the photodegradation of phenol using TiO_2 as catalyst

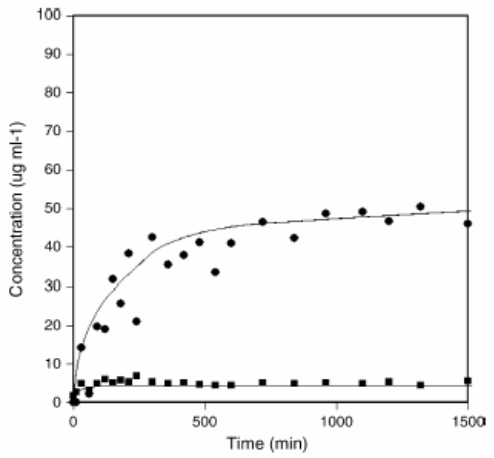


Figure 4. Variation in hydroquinone and catechol concentration throughout the photodegradation of phenol using TiO_2 as catalyst

The obtained concentration values agree, in order of magnitude, with those in the bibliography obtained by HPLC [5,7]. As phenol degrades, dihydroxy phenols appear and, in turn, degrade until mineralization is complete. The concentration of hydroquinone remained constant after two hours of reaction, while the concentration of catechol was almost constant after 5 hours (see Fig. 4). This suggests that hydroquinone and catechol are formed at the same velocity as they degrade to quinones, which are not fluorescent.

The analytical method developed is faster than HPLC, which is normally used to monitor this sort of reaction. The new methodology makes it possible to obtain valuable information for the future optimization of the degradation process. This is not within the scope of the present paper and shall be studied in future work.

5. Conclusions

PARAFAC is a good tool that can be applied to spectrofluorimetric excitation-emission data to monitor phenol degradation reactions. The strategy we used resolved phenol and its degradation products throughout the photodegradation process.

The quantitative analysis of a set of mixtures gave satisfactory results and made it possible to unequivocally quantify the four analytes which take part in the degradation process with low prediction errors. Calibration with PARAFAC requires very few samples and enables quantitative determinations to be made in the presence of uncalibrated interferents.

The methodology is a good alternative to the traditional methods of analysis for monitoring the degradation of pollutants and the analysis times are very short.
The new methodology makes it possible to obtain valuable information for the future optimization of the degradation process. Extrapolating this strategy to natural samples with complex matrices requires further optimization of the PARAFAC model. This is a topic for future research.

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4.2.3. Resolution of phenol, and its di-hydroxyderivative mixtures by excitation–emission fluorescence using MCR-ALS. Application to the quantitative monitoring of phenol photodegradation

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Abstract

The photodegradation of phenol using TiO_2 as catalyst was studied and monitored by fluorescence excitation–emission matrix (EEM). Hydroquinone, catechol and resorcinol were the dihydroxyderivative intermediates although in lower concentrations than phenol. The data were analyzed using a three-way multivariate curve resolution alternating least squares method (MCR-ALS) and augmented matrices. The procedure was assessed using synthetic samples prepared with a {4,3} Simplex-lattice design that considered a representative range of analyte concentrations. The results were analyzed in terms of overall RMSEP for the overall data set. A detailed study was made of how the analytes behaved at each concentration level and how the concentration of

the other species affected the process. The method was used to quantify phenol in photodegradation samples with an overall prediction error of 5.37%. The conversion values were fitted to pseudo first-order kinetics and the apparent rate constant was calculated to be $-4.9\ 10^{-4}$ min $^{-1} \pm 5.2\ 10^{-5}$ min^{-1}.

Keywords: Phenol; MCR-ALS; Photodegradation reactions; Fluorescence; Excitation–emission matrix

1. Introduction

Phenol is widely used in the chemical industry for various purposes [1]. It is found in a wide range of effluents and has a direct or indirect effect on the ecosystem [2]. Photodegradation is one of the most effective advanced oxidation processes for treating organic pollutants such as phenol [2-5]. Through the process of degradation, phenol completely decomposes to CO_2 and H_2O following a mechanism of hydroxylation of the aromatic ring [1, 2, 6]. Hydroquinone, catechol and resorcinol are the habitual intermediates although in lower concentrations than phenol. The habitual methods of analysis usually involve time-consuming analytical procedures: for example, extraction and/or preconcentration, followed by such separation processes as gas chromatography (GC) [6, 7] or high performance liquid chromatography (HPLC) [2].

Phenolic compounds can be determined by molecular fluorescence, which is highly sensitive and has moderate selectivity. However, in complex mixtures, spectral overlapping is often a serious drawback and separation techniques must be used before univariate spectrofluorimetric techniques can be used.

Collecting a two-dimensional total fluorescence spectrum, known as an excitation–emission matrix (EEM) spectrum, increases selectivity. In EEM spectroscopy a total fluorescence spectrum is obtained by systematically varying the excitation and emission wavelengths and collecting the resulting $I \times J$ data matrix [8].

The principal advantage of EEM is that it can use potent, multiway deconvolution methods on all the available data to increase the information content extracted from a data set. This makes it possible to develop instrumentation that can be applied in real time. In this field, R. D. JiJi *et al.* have developed a battery of instrumental methods that use fluorescence spectroscopy and EEM for accurate remote, in-situ, and field screening for pesticides and PAHs in the environment [9, 10]. Nowadays, developing catalysts that can be used to degrade organic polluting agents and optimizing these processes is a subject of particular interest in the field of catalysis. The degradation kinetics of a polluting agent and the yield of the degradation process require the polluting agent to be quantitatively determined. UV-visible spectrophotometry with univariate calibration is the common method for analysing organic pollutants, but this methodology requires previous sample pretreatment. In the particular case of phenol photodegradation, the analytical signal is largely caused by phenol but its hydroxyderivateives —hydroquinone, catechol and resorcinol— also make a small contribution [1, 6, 11]. Obtaining the results in this way, then, can lead to erroneous conclusions about the reality of the process, particularly as far as quantification is concerned.

The present study is part of our research group's general objective, which is to develop analytical methods based on EEM and curve resolution methods, and to find practical uses for quantitative, in-situ monitoring of photodegradation reactions of organic pollutant agents such as phenol.

Parallel factor analysis (PARAFAC) [12-14] is one of several methods for deconvoluting multiway data and has been used by our research group to analyse phenol [11]. It assumes an underlying trilinear structure, and yields a single solution. However, the single profiles obtained are not necessarily the true profiles if the data set is not trilinear. This study discusses the ability of another method for deconvoluting multiway data: Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) [15-17]. This method has some important features that differentiate it from other three-way data analysis methods. Firstly, it can be used for three-way data with different data structures: trilinear and not trilinear. Methods which do not assume trilinearity may provide more ambiguous solutions, although they are more flexible at modelling the profile shape. Secondly, it has a simple algorithmic implementation based on matrix inversion. Thirdly, eigenvalue-eigenvector decomposition of the experimental data matrix is used to determine the number of independent contributions. And finally, it means that several constraints can be simply applied during the ALS optimization with increasing reliability of the solutions obtained [18].

In the present study, synthetic mixtures were prepared with a {4,3} Simplex-lattice design [19] for quaternary mixtures so that they represented the concentration intervals of the problem being studied. First, the ability of MCR-ALS to resolve highly overlapped spectra was evaluated in terms of overall prediction error (root mean square error of prediction, RMSEP) for each analyte, and the effect of the analyte concentrations in the mixtures on the error. Next, the phenol in the photodegradation process was quantified using TiO_2 as catalyst. The results were fitted to a pseudo first order kinetics so that the rate constant could be calculated.

The study of these concentration mixtures and the use of phenol degradation is a practical example that has not yet been documented among the applications of MCR-ALS to spectroscopic data.

2. Background

2. 1. Application of MCR-ALS to EEM data

MCR is based on a linear model that assumes the response additivity of all fluorescent components in the samples measured [20]. MCR-ALS is a flexible method which can be used to analyze: (a) a single data matrix containing data recorded throughout an individual experiment, (b) a column-wise augmented data matrix containing data recorded over several experiments or techniques, (c) a row-wise data matrix, and (d) a simultaneous column and row-wise augmented data matrix. In excitation-emission fluorescence data, all the data matrices for the EEM spectra of each measured sample can be aligned to one another to obtain an augmented data matrix [21].

Both excitation-wise and emission-wise matrix augmentations are possible in fluorescence. In the excitation-wise augmentation used here, the matrices are put on top of one other and the common excitation wavelengths are kept in the same column. Using this resolution method, the excitation-wise (column wise) augmented data matrices are modeled with the equation:

$$\mathbf{D}_{aug}^{ex} = \mathbf{Y}_{aug}\mathbf{X}^{T} + \mathbf{E}_{aug} \qquad (1)$$

where $\mathbf{D}_{aug}^{ex}$ is the excitation-wise augmented response data matrix, $\mathbf{Y}_{aug}$ is the augmented matrix of emission spectra, $\mathbf{X}^T$ is the transposed matrix of excitation spectra and $\mathbf{E}_{aug}$ is the matrix of residuals. The quantitative information is contained in the relative intensities of the emission spectra $\mathbf{Y}_{aug}$.

2.1.2. Rank analysis

Before the alternating least squares iterative process begins, the number of compounds in a particular experiment (matrix) is estimated. This is usually done by Principal Component Analysis (PCA) [22] or Singular Value Decomposition (SVD) [23,24]. It is assumed that the variance explained by chemical compounds is larger than noise variance and, therefore, that only larger principal components will sufficiently explain the experimental data.

In this study, we estimated the rank analysis of the data matrices (augmented matrices) by SVD and used the indicator proposed by Malinowski [25] to determine the number of significant values. This indicator is based on an empirical function called the factor indicator function (IND) defined as

$$IND = \frac{RE}{(c-n)^2} \qquad (2)$$

where c is the number of columns of the data matrix and n is the number of factors. RE is the real error (or residual standard deviation) defined by

$$RE = \left(\frac{\sum_{j=n+1}^{c} \lambda_j^0}{r\,(c-n)} \right)^{1/2} \qquad (3)$$

where λ_j^0 is the value of the eigenvalue.

2.1.3. ALS optimization

The ALS optimization began by using initial estimates. This can be done in many different ways. It is sensible to start with the best possible estimates available (i.e. the component spectra if they are known). In this case, these initial estimates of the fluorescence excitation spectra were chosen by analysing the excitation spectra with the maximum fluorescence of the standard matrices of the analytes and choosing the most representative spectra of each one. These estimates were initially used to calculate the emission spectra as:

$$\mathbf{Y_{aug}} = \mathbf{D_{aug}}\,((\mathbf{X}^T\,\mathbf{X})^{-1}\mathbf{X}^T) \qquad (4)$$

From this new emission spectra matrix $\mathbf{Y_{aug}}$, the excitation spectra were updated using the equation:

$$\mathbf{X}^T = ((\mathbf{Y_{aug}}\,\mathbf{Y_{aug}}^T)^{-1}\,\mathbf{Y_{aug}})\,\mathbf{D_{aug}} \qquad (5)$$

These two steps were repeated until convergence was achieved.

The constraints applied to get physically meaningful solutions during the ALS optimization were: (a) the excitation and emission spectra had to be non-negative, (b) there had to be a correspondence between common species in the different data matrices, (c) the excitation spectrum of each species had to be the same in all matrices where that species was present and (d) the emission spectrum shape of each species had to be the same in all runs where that species was present.

The relative concentrations of a particular species were simply obtained from the quotient between the areas below the emission spectra of the analyte in the standard and in the unknown sample:

$$C_{unk} = \left(\frac{A_{unk}}{A_{std}} \right) C_{std} \qquad (6)$$

where C_{unk} and C_{std} were the concentrations of the analyte in the unknown and standard samples, respectively; and A_{unk} and A_{std} were the areas below the excitation or emission spectra profiles in the unknown and in the standard samples, respectively.
The overall prediction error was calculated by the root mean squares error of prediction (RMSEP):

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{m} (C_{i\,true} - \hat{C}_{i\,calc.})^2}{m}} \qquad (7)$$

where $C_{i\,true}$ and $\hat{C}_{i\,calc.}$ were the true and calculated concentrations of each analyte in the

*i*th prediction sample, and *m* is the number of prediction samples.

2.1.2 Kinetic calculations

The kinetic constants of photodegradation processes, assuming that they normally fit pseudo first order kinetics [5, 8], and the half lives of the analytes are calculated using equations (8):

$$\ln \frac{C_0}{C_i} = kt \qquad (8)$$

where C_0 and C_i (calculated by MCR-ALS) are the concentrations at time zero and time *t*, respectively, and *k* is the velocity constant.

3. Experimental

3.1. *Chemicals*

All reagents were of analytical grade: phenol, catechol, hydroquinone, and resorcinol were used as received from Sigma-Aldrich (99%) and glacial acetic acid (100%) was provided by Merck. Stock solutions were prepared by weighing the appropriate amounts of the reagents and dissolving them in Millipore water. All solutions were prepared in an acidic medium of 1 % (v/v) acetic acid, so the analytes remained in molecular form, and were stored in dark bottles at 4ºC. They remained stable for one

week. Working solutions were prepared by diluting stock solutions with Millipore water, and maintaining a concentration of 1% (v/v) acetic acid. TiO_2 in the anatase form (99.8%) (Sigma-Aldrich) was used as the photocatalyst without further treatment.

3.2. Samples

Individual standards of 5 ppm of each analyte were prepared in aqueous solution. A set of 20 mixtures was also prepared following a {4,3} Simplex-Lattice design for quaternary mixtures so that the ability of the model to resolve and quantify these analytes could be evaluated.

The set of mixtures was prepared in duplicate. The design took into account the relation between the concentrations of the species during the degradation process and the linearity interval of the fluorescent measurements. Figure 1 shows the content of the samples prepared in detail.

The photodegradation process was similar to one used in a previous study [11] as can be seen in Scheme 1. A total of 23 samples corresponding to the photodegradation of phenol (Scheme 2) were analyzed.

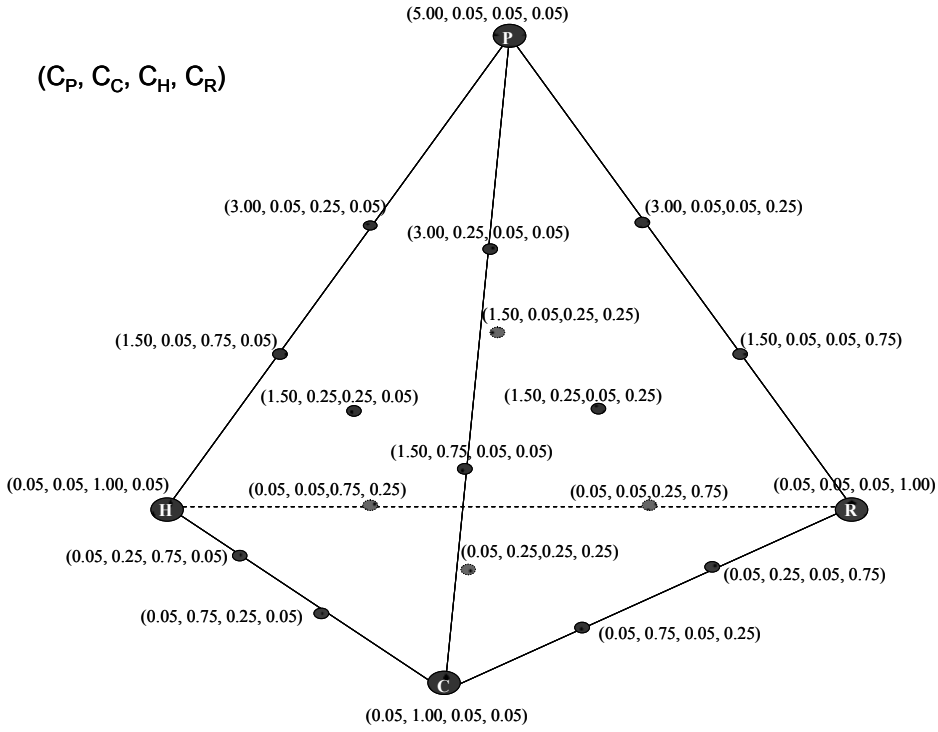
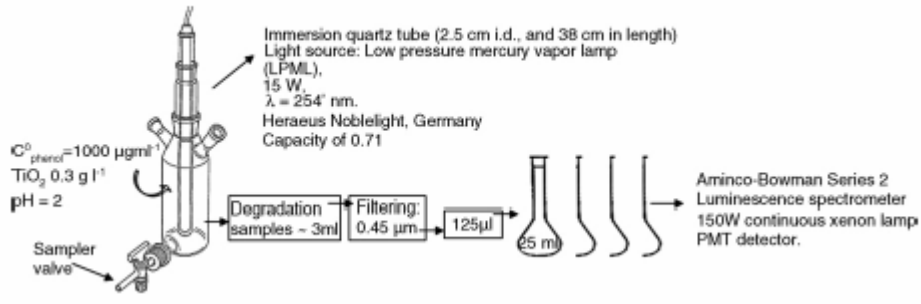


Fig.1. Set of samples prepared following a {4,3} Simplex-Lattice design. Concentration values of phenol (P), hydroquinone (H), catechol (C) and resorcinol (R) in ppm

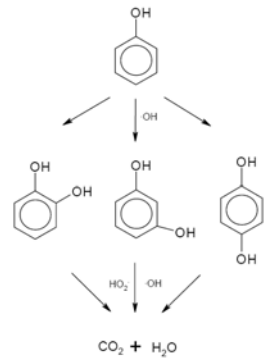
3.3. *Measurements*

The spectrofluorimetric data were acquired on an Aminco–Bowman Series 2 Luminescence spectrometer (SLM Aminco, Rochester, NY, USA) equipped with a 150W continuous xenon lamp and a PMT detector. The instrument was interfaced by a GPIB card and driver with a PC Pentium microcomputer provided with the AB2 software version 1.40 for spectral acquisition.



Scheme 1. Photodegradation process

All solutions (individual standards, mixtures, samples and solvent) were measured in the same conditions. Three-dimensional excitation-emission spectra were recorded from 270 to 350 nm in the emission domain and from 231 to 291 nm in the excitation domain, both at regular steps of 3 nm. The scanning rate of the monochromators was maintained at 30 nm s^{-1}. The excitation and emission monochromator slit widths were set to 4 nm. All measurements were made in a 10 mm quartz cell at 620 V.



Scheme 2. Process of mineralizing phenol

4. Data set and data analysis

A data matrix of 28 x 21 in size was obtained for each sample analyzed. The augmented data matrix was arranged by setting one matrix on top of the other and keeping the common excitation wavelengths in the same column (excitation-wise).

Four data sets are involved in this study: a) augmented matrix **A** (112x21) built with the four individual standards, b) augmented matrix **B** (1120x21) built with the synthetic samples, c) augmented matrix **C** (1232x21), built with the four individual standards and synthetic samples, d) augmented matrix **D** (1876x21), built with the four individual standards, the synthetic samples and the photodegradation samples. The fluorescence spectra were exported in ASCII format from the AB2 software to MATLAB [22, 23].

5. Results and discussion

Fig. 2 shows, as an example, the emission and excitation spectra for the particular maximum of each analyte obtained from a 5 ppm sample of phenol, catechol, resorcinol and hydroquinone. Only the excitation and emission maxima for hydroquinone (λ_{em} = 328 nm y λ_{ex} = 288 nm) are significantly different from the maxima of the other three analytes (λ_{em} = 294 nm and λ_{ex} = 270 nm for phenol, λ_{em} = 311 nm and λ_{ex} = 276 nm for catechol, λ_{em} = 302 nm and λ_{ex} = 274 nm for resorcinol) and the emission and excitation spectra of catechol and resorcinol are embedded in the spectrum of phenol. It should also be pointed out that the analytical technique is more sensitive to variation in phenol concentration than to variation in any other analyte; it is least sensitive to catechol.

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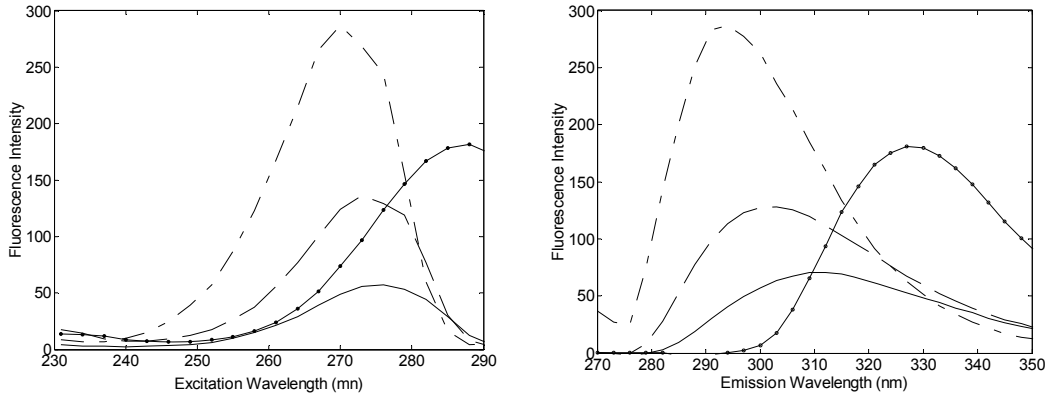

Figure 2. Excitation (231 to 291) nm and emission (270 to 350 nm) spectra obtained for 5 ppm phenol (— - —), catechol (—), resorcinol (— —) and hydroquinone (—◦—). Scanning rate: 30 nm s^{-1}; monochromator slit widths: 4 nm; 620 V.

Therefore, it is very difficult to mathematically resolve these components by individually analysing a mixture of these analytes at the same concentration [24].

As can be seen in Fig.3, the EEM spectra for a mixture of the four analytes present a diagonal signal (270-290 nm in both modes) which rarely provides any additional chemical information. More specifically, it is in the corner and is produced by the first order Rayleigh scatter. This dispersion does not have any chemical meaning, and takes place at all wavelengths when the excitation and emission fluorescence radiation are close together [25]. This Rayleigh scattering becomes more pronounced for less sensitive analytes such as catechol and resorcinol and is very difficult to eliminate by blank subtraction because the intensity of the dispersion is not constant for all the measured samples. However, blank subtraction can minimize Rayleigh scattering quite considerably.

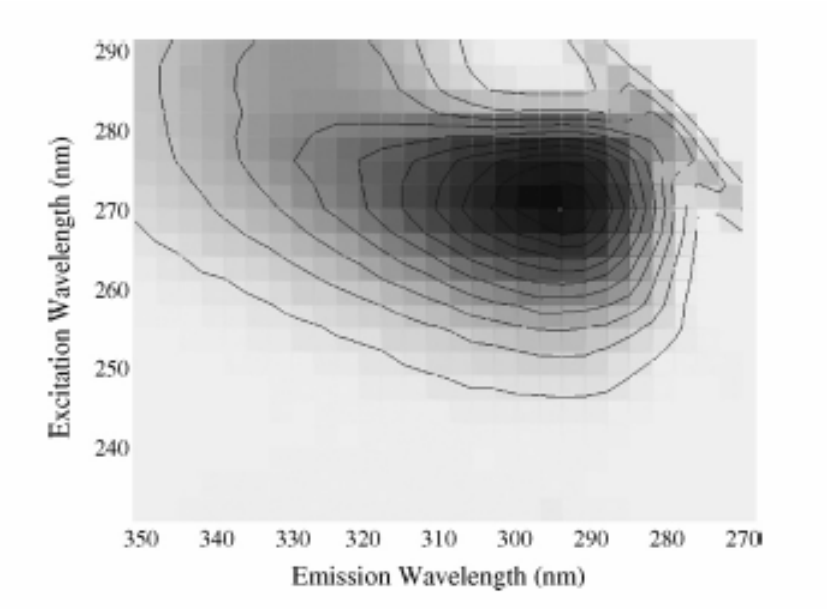

Figure 3. EEM spectra of a mixture of the four analytes recorded from 270 to 350 nm in the emission domain and from 231 to 291 nm in the excitation domain (steps of 3 nm); scanning rate: 30 nm s^{-1}; monochromator slit widths: 4 nm; 620 V.

The effects of the Raman scattering were minimized by subtracting the mean blank from all spectra.

Table 1 shows the results of the rank analysis of augmented matrices by singular value decomposition. As can be seen by visual inspection, for matrix **A** the rank is lower than the total number of absorbing species presents (apparent rank deficiency).

Singular values	Data matrix			
	A	B	C	D
s1	2267.2	4425.2	4955.0	7087.6
s2	995.5	615.8	1237.5	1262.8
s3	218.9	349.4	355.6	357.4
s4	47.2	242.0	293.9	303.8
s5	39.9	115.1	198.7	204.6
s6	32.3	85.2	88.9	92.4
s7	21.3	40.7	60.2	71.5
s8	16.0	32.4	40.0	45.6
s9	12.3	28.8	34.3	39.5
s10	9.6	24.9	31.6	38.5

Table 1. Results of rank analysis of augmented matrices by singularvalue decomposition

This matrix contains the four analytes, at the same concentration, and only three eigenvalues seem to have significant values. This can be attributed to the fact that the sensitivity of phenol is higher than that of catechol and resorcinol at the same concentration, and their spectra are embedded within the phenol spectrum in both the emission and excitation modes (see Fig. 2). In a previous study [11] in which we worked with the same data set (i.e. a cube built by PARAFAC with the four individual standards) we found four significant contributions. However, in the rank analysis of data matrices **B**, **C** and **D**, where the fluorescence intensities are for different concentrations (and concentration combinations) of the four analytes, the number of significant contributions difficulties not so easy to decide only after visual inspection.

Table 2 gives the results of the indicator function proposed by Malinowski [25] for the augmented matrices. The indicator function gives a minimum value for the number of independently detected chemical species or chemical rank. Five significant contributions were found for data matrices **B**, **C** and **D**. Four were interpreted as the variability yield by the chemical rank (i.e. for the absorbing species present in the data

analysis). The other contributions were attributed to the Rayleigh scattering. Here, the rank deficiency is overcome since we are working with data matrices with different concentrations and combinations of the analytes.

Singular values	Data matrix							
	A		**B**		**C**		**D**	
	RE	**IND(x 10^{-3})**	**RE**	**IND(x 10^{-3})**	**RE**	**IND(x 10^{-3})**	**RE**	**IND(x 10^{-3})**
s1	0.798	1.996	0.426	1.066	0.268	0.669	0.273	0.683
s2	0.450	1.248	0.323	0.894	0.193	0.534	0.199	0.552
s3	0.325	1.003	0.274	0.846	0.166	0.514	0.174	0.537
s4	0.295	1.021	0.227	0.784	0.139	0.479	0.147	0.509
s5	0.265	1.035	0.192	0.752	0.115	0.448	0.125	0.486
s6	0.236	1.049	0.171	0.759	0.103	0.456	0.114	0.506
s7	0.215	1.096	0.157	0.803	0.094	0.478	0.104	0.532
s8	0.197	1.164	0.147	0.872	0.087	0.517	0.098	0.581
s9	0.181	1.257	0.137	0.952	0.081	0.564	0.092	0.642
s10	0.167	1.382	0.126	1.043	0.074	0.614	0.085	0.704

Table 2. Real error (RE) and indicator function (IND) [25] values for the singular values of the augmented data matrices

From this first analysis, we concluded that for this particular problem, the quantitative determination of phenol in the presence of its di-hydroxyderivative, the MCR-ALS application requires four factors.

Therefore, the MCR-ALS method was applied to augmented matrix **C** to evaluate the prediction error (to quantify the analytes properly we needed a simultaneous analysis that included the four standard data matrices in addition to the mixtures). Non-negativity and unimodality constraints were applied in excitation and emission modes. In this particular case we used an excitation-wise augmented data matrix with excitation spectra as initial estimates that we had obtained by individually analysing standard matrices and choosing the most representative spectra of each analyte (see Section 2).

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The optimal solution with four components was obtained in the 7th iteration, with a fitting error (lack of fit, lof) at the optimum of 12.71 %. The percent of variance explained at the optimum was 98.38%. The recoveries of the emission spectra were measured as the dissimilarity between the ALS recovered spectrum and the true spectrum (those obtained from the matrices of the pure analytes). In all cases the recoveries were good: the dissimilarities were 0.0451 for phenol, 0.0263 for hydroquinone, 0.1108 for catechol and 0.0722 for resorcinol. They were higher than those found by PARAFAC [11]: 0.0045 for phenol, 0.0026 for hydroquinone, 0.0118 for catechol and 0.0132 for resorcinol. This is because the method used strictly trilinear data and because it is better to use a dissimilarity measure such as the *sin* of the angle between two vectors than the *cos* of the angle between vectors since, for very similar profiles, the discrimination power is greater. Dissimilarity equal to 0.1 means a correlation equal to 0.995 and dissimilarity equal to 0.01 means a correlation equal to 0.9999.

In the quantification step, the overall prediction errors (Eq. 5) for each analyte were: 5.37 % for phenol, 4.67 % for hydroquinone, 28.52 % for catechol and 13.24 % for resorcinol. The percent of variance explained by PARAFAC for a model with four factors was 99.98% [11]. The RMSEPs for phenol were practically the same (5,22 %) and slightly better for hydroquinone (3.84 %), catechol (16.78 %) and resorcinol (9 %). We consider that these results are comparable since the determination of these analytes by other techniques such as high performance liquid chromatography (HPLC) gave coefficients of variation of 10.4 % for phenol, 14.9 % for hydroquinone, 14.9 % for catechol and 70.5 % for resorcinol [30].

It should also be pointed out that the main advantage of MCR-ALS is that PARAFAC needs to pre-treat the data matrix because the data need to be strictly

trilinear. So, before PARAFAC is used, the sources of variability present in the spectra that are not caused by variation in the concentrations of the analytes need to be eliminated. PARAFAC needs the experimental data to preserve their trilinear structure if it is to work correctly. MCR-ALS, however, only requires the data set *to tend* to trilinearity, but it is not a hard condition. In our case, when we minimized the scatter effect by blank subtraction, results were good.

Table 1 and Table 2, then, show that the relative concentration between the analytes is very influential when evaluating the resolution possibilities of EEM and MCR-ALS. Therefore, we believe that a detailed analysis of the results should be made considering the different concentration levels of each analyte.

Fig. 4 (a-c) represents the values of the error in each analyzed sample following the design of Figure 1. To facilitate the interpretation of the results, the tetrahedron in Fig.1 has been turned, so that the planes that contain the same concentration value of the analyte being studied can be viewed more easily.

The error values were calculated using a variation coefficient for each concentration level. Fig. 4a shows the results for the quantification of phenol in which the major error was when its concentration in the mixtures was 0.05 ppm, and the error was never above 10%. This conclusion is independent of the concentration values of the other analytes. Fig. 4b shows the results for hydroquinone. At low concentrations the error is very large but at overall RMSEP this error was not observed.

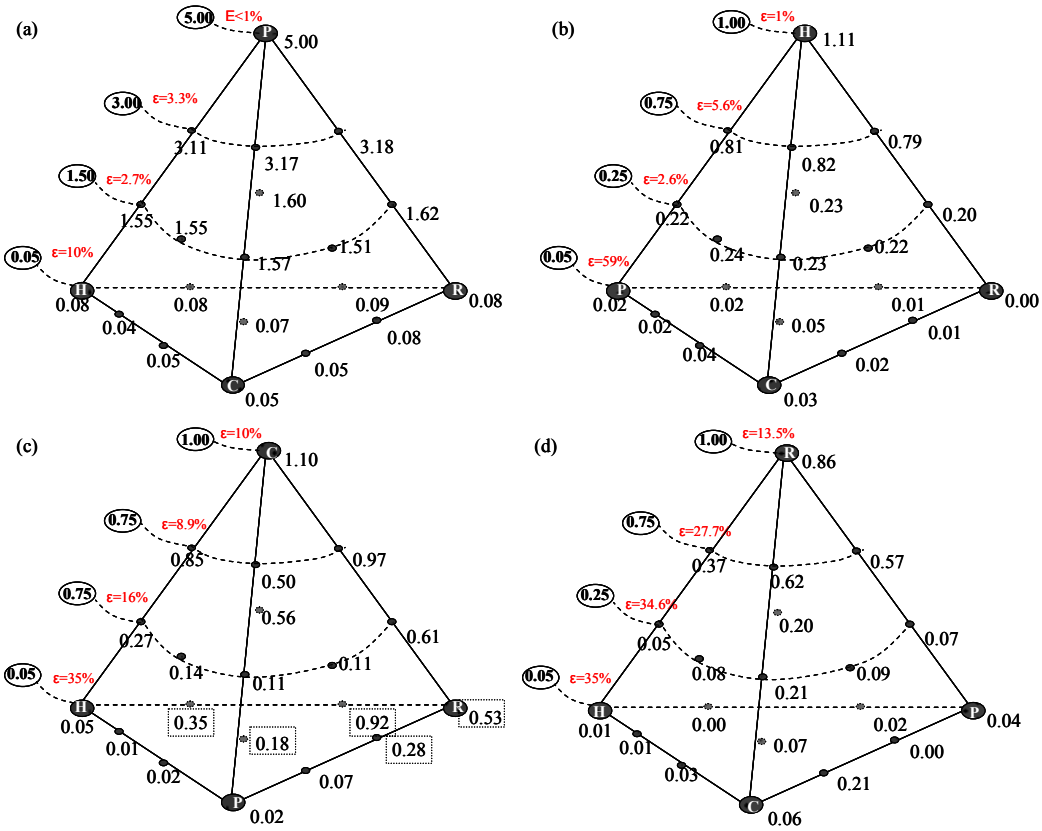

Fig. 4 Average values (ppm) found by MCR-ALS for phenol (P), hydroquinone (H), catechol (C) and resorcinol (R) for the 20 samples prepared in duplicate. Real concentration values (ppm) are surrounded by a solid line

The values for catechol and resorcinol are shown in Fig. 4c and d, respectively. As can be seen for catechol, the error is always above 9%. At low concentrations and when the resorcinol concentration in the mixture is increased, the error is above 100%. Errors are only lower than 10% if there is no resorcinol in the mixture and its concentration is higher than 0.25 ppm. Error values are always high for resorcinol. This analysis shows that phenol is the only analyte that can be quantified with an acceptable

error and hydroquinone can only be quantified if the concentration is higher than 0.05 ppm.

As the purpose of this work is to follow the quantitative monitoring of the photodegradation of phenol, a model with four factors was applied to matrix **D**. The results are shown in Fig. 5. These values were fitted to pseudo first-order kinetics (Eq. (6)). The data was fitted using weighted least squares considering the error committed at each concentration.

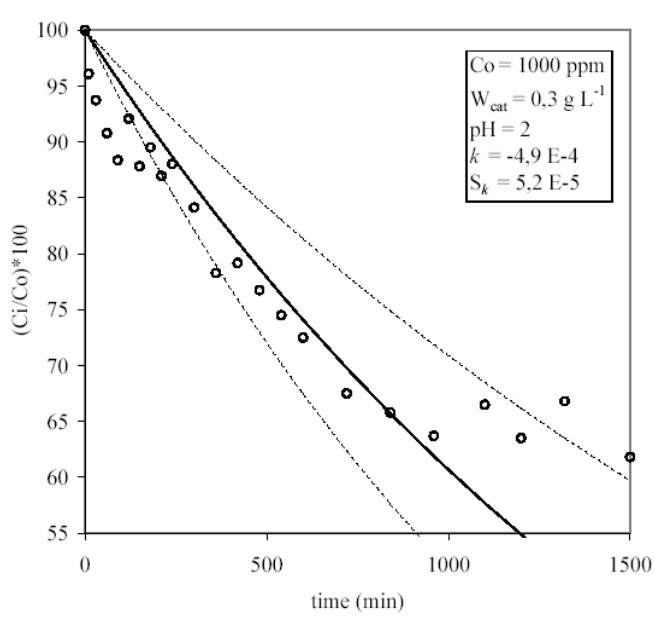

Fig. 5 Variation of phenol throughout the photodegradation of phenol (circle); fitted concentrations according to the calculated apparent rate constant (solid line); confidence bands related to the estimated error for the apparent rate constant (broken lines).

It could be assumed that in the interval of values in which we have measured the degradation samples the error was between 1 and 3 %. The value of slope, representative

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of the apparent rate constant, was of -4.9 10^{-4} min $^{-1}$ and his standard deviation of 5.2 10^{-5} min^{-1}. This value could be considered an estimation of the error for the apparent rate constant. As can be seen (Fig. 5) the values obtained by MCR-ALS for the photodegradation are in acceptable agreement with the calculated rate constant (solid line). Indeed, most of the values are within the confidence bands related to the estimated error for the apparent rate constant (broken lines). However, the later values (from 1100 to 1500 min) show a little deviation from the solid line. One explanation for this fact is that, from the catalytic point of view, the photocatalytic degradation did not work in optimal conditions. Nevertheless the objective of this work is to show the advantages of the developed analytical method rather than to optimize the appropriate conditions of the photodegradation.

Figure 6 shows the degradation concentration values obtained for hydroquinone and catechol by MCR-ALS.

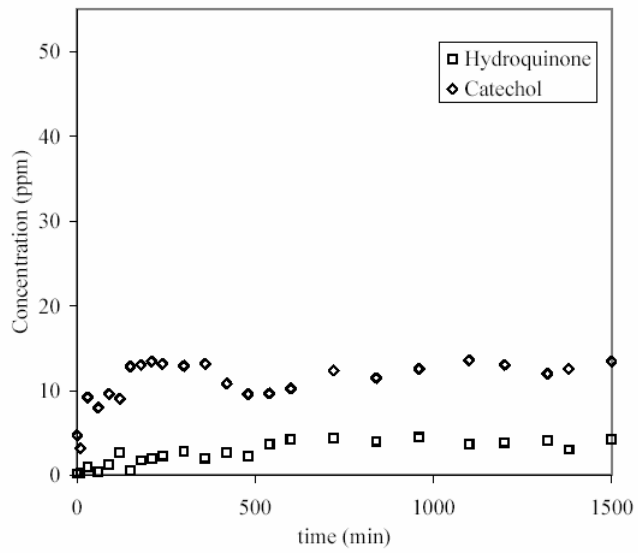

Fig. 6 Variation in hydroquinone and catechol concentration (calculated by MCR-ALS) throughout the photodegradation of phenol

These results are slightly different to those obtained by PARAFAC in a previous work [11], but the kinetic behaviour is conserved.

The analytical method developed is faster than HPLC, which is normally used to monitor this sort of reaction. This methodology makes it possible to obtain rapidly and valuable information for the future optimization of the degradation process.

6. Conclusions

EEM and MCR-ALS are good tools for quantitatively monitoring the degradation of phenol.

It has been shown that, for quantification to be correct, the contribution of the hydroxyderivatives of phenol must be separated. Hydroquinone can be quantified when concentrations are higher than 0.05 ppm. Catechol and resorcinol can be differentiated from phenol and hydroquinone but their quantification errors are very high.

In the light of the results, this methodology could be a fast, cheap alternative for the quantitative monitoring of the phenol photodegradation process.

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4.2.4. Rapid and quantitative evaluation of the kinetic parameters of the photocatalytic degradation of phenol using experimental design techniques and parallel factor analysis of excitation-emission matrix fluorescence spectra

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Sumitted

Abstract

A 2^3 full factorial design was used to analyse the effect of pH, the nature of the catalyst and the concentration of the substrate on the photodegradation reaction of phenol. Parallel factor analysis (PARAFAC) was applied to the data obtained from monitoring the process in the various experimental conditions by means of excitation-emission fluorescence, and was used to quantitatively determine the value of the concentration of phenol and of the intermediates produced: catechol, resorcinol and hydroquinone. This study worked directly with the reaction samples generated in the photocatalytic process itself. The value of the rate constant found for each experiment on the basis of this data was used as response variable in the process of multivariate analysis

of the effect of the variables studied.

It was observed that the three variables considered have a statistically significant effect on constant rate at the 95 % confidence level. The significance of these effects has been corroborated using an ANOVA test.

The chemical interpretation of the coefficients value found for the effects can contribute to analyze the influence of the variables in degradation process. The proposed methodology that combines experimental design and multivariate techniques as PARAFAC supposes a new rapid alternative for the study the different conditions that can be to influences in the chemical process.

Introduction

There is considerable interest nowadays in studying the degradation of toxic compounds by heterogeneous photocatalysis. The studies basically consist of monitoring the degradation kinetics of the compounds and detecting the reaction intermediates, which may generate products that are more toxic than the initial reagents. This is of particular interest when the mineralization is not complete. With this purpose, it is habitual to study the effect of some reaction variables on the kinetics of the processes since it has been reported that the pH determines the surface load of the catalyst and the chemical form of the substrate, and that the intensity of the radiation determines the efficiency of the degradation [1-7]. Other important factors are the concentration of the substrate and the catalyst [6] and the presence of substrates that can compete for the same active sites on the surface of the catalyst [8-9]. The most commonly used

instrumental techniques in the quantitative study of the degradation of compounds are gas and liquid chromatography [10-12], which are costly in terms of the analysis time required. The effect of each variable on the reaction kinetics is not independent, but for the sake of simplicity most research articles that have been published carry out a univariate analysis of how each variable under study affects the kinetics, which often gives limited information about the process. Some studies in the bibliography [13-17] adopt a multivariate approach to how variables affect the kinetics of photocatalytic reactions of compounds that are of environmental interest.

These studies [14-17], which are an advance on univariate analysis, analyse realistic approximations obtained results from few experimental data provided by high precision liquid chromatography diode array detector (HPLC-DAD) [14,17], chromatography with electron capture detection (GC-ECD) and gas chromatography with ion trap mass spectrometric detection (GC-ITMS) [15] LC-MS-MS [16] on the degradation process of the analytes under study. These analytical methodologies need previous preconcentrations of the photodegradation process and the analytes in study. Nevertheless theses analyses have a high economic cost and a lot of time consuming.

This paper applies the curve resolution method parallel factor analysis (PARAFAC) [18] to data obtained from monitoring the whole process of photodegradation of phenol in natural waters by excitation-emission fluorescence and quickly determines the concentration of all the compounds involved in the process. The ability of PARAFAC to separate the compounds that are normally generated in these processes—phenol, catechol, hydroquinone and resorcinol—has already been discussed by our research group in previous articles [19-20]. In these articles, the PARAFAC model found with sets of synthetic samples of the analytes under study was used to determine the concentrations in degradation processes. The present study works directly with

the reaction samples generated in the photocatalytic process itself. The rate constant found using these data, for each experiment, is used as a response variable in the multivariate analysis of the effect of pH, concentration and nature of the catalyst using a 2^3 full factorial design [21-22]. As yet, no other study of this type has been made. It makes it possible to analyse a large set of experimental data and facilitates the joint interpretation of all the variables that intervene in processes of this type.

Experimental

Reagents

All reagents were of analytical grade: phenol, catechol, hydroquinone, and resorcinol were used as received from Sigma-Aldrich (99%) and glacial acetic acid (100%) from Merck. Stock solutions were prepared by weighing appropriate amounts of the reagents and dissolving them in Millipore water. All solutions were prepared in an acidic medium of 1 % (v/v) acetic acid, so that the analytes remained in the molecular form, and stored in dark bottles at 4ºC. They remained stable for one month. Working solutions were prepared by diluting stock solutions with Millipore water. Concentration was kept at 1% (v/v) acetic acid.

TiO_2 (99.8%), in the anatase form (Sigma-Aldrich), and ZnO (99.9%) were used as photocatalysts without further treatment.

Apparatus

The photodegration studies were carried out in a cylindrical annular batch reactor. The reactor consists of an immersion quartz tube (2.5 cm i.d., and 38 cm long) which contained a low pressure mercury vapor lamp (LPML) of 15 W (Heraeus Noblelight, Germany). The light source emitted predominantly at 254 nm. The quartz tube was placed in a Pyrex glass outer reactor (0.7 l in capacity).

An Orion pH-meter was used to verify the pH of the reacting mixtures.

The spectrofluorimetric data were acquired on an Aminco–Bowman Series 2 Luminescence spectrometer (SLM Aminco, Rochester, NY, USA) equipped with a 150W continuous xenon lamp and a PMT detector. The instrument was interfaced by a GPIB card and driver with a PC Pentium microcomputer provided with the AB2 software version 1.40, for spectral acquisition.

Photocatalytic experiments

The eight photodegradation experiments were carried out following a 2^3 full factorial experimental design. The chosen variables were studied on two levels: the variables used were pH (2.5 and 10), catalyst (ZnO and TiO_2) and substrate concentration, i.e. phenol concentration (20 and 100 μg L^{-1}).

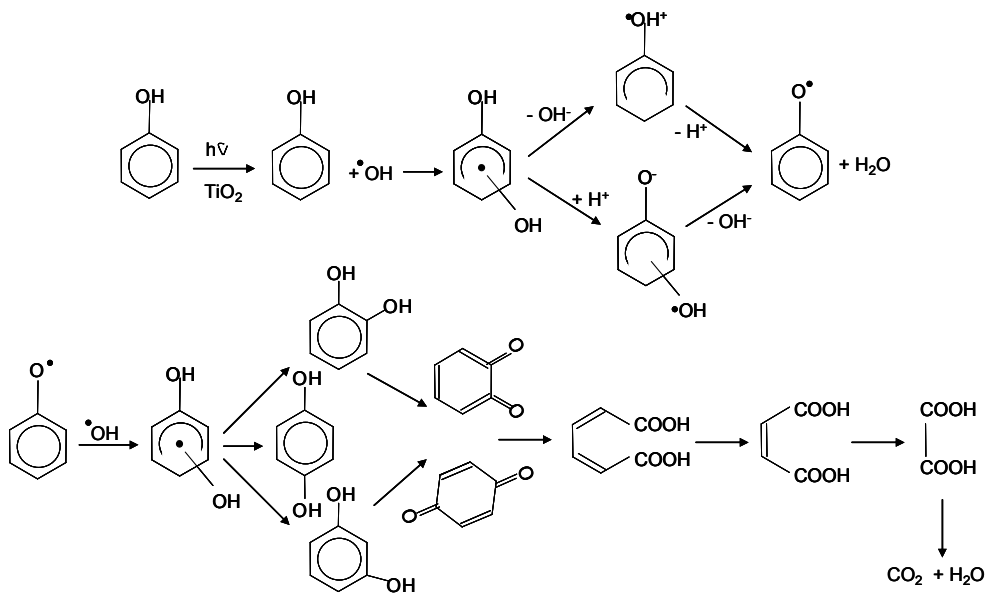
The degradations were carried out in a cylindrical reactor, such as the one described in the section above, and with the pH, catalyst and substrate concentration for

each experiment. The total volume of the solution was 500 ml. The photodegradation was carried out at ambient temperature with stirring throughout the reaction.

Five-millilitre samples were taken through the reactor's sampling valve and the filtered solutions were kept in darkness. In all the experiments the samples were extracted every five minutes from t =0 minutes at t = 90 minutes. In experiences 1, 2, 3 and 4, them every 10 minutes, until t = 180 minutes, moment at which a null fluorescent signal was observed. A total of 28 samples were gathered in each experiment. Experience 1 has been realised three times in order to obtain the experimental error. In experience 5, the extractions every 10 minutes were carried out until t = 270 minutes. A total of 37 samples were extracted. For experiences 6, 7 and 8, in that it was observed that the fluorescent diminution was slowly along the time, from t = 100 minutes, and until the end of the photocatalytic degradation t = 460 minutes, the extractions were made every 20 minutes. A total of 38 samples were extracted.

Before the fluorescence was measured, appropriate amounts of the filtered sample solutions were diluted to 10 ml with 1% acetic acid.

The mechanism for the photolysis of phenol and subsequent reactions (Scheme 1) has been proposed for many authors [23-26].



Scheme 1. Proposed mechanism of phenol photodegradation

Standard samples

Individual standards of 5 ppm of each analyte (phenol, catechol, hydroquinone and resorcinol) were prepared in aqueous solution by diluting stock solutions with Millipore water, and keeping a concentration of 1% (v/v) acetic acid.

Measurements

All solutions (individual standards, samples and solvent) were measured in the same conditions. Three-dimensional excitation-emission spectra were recorded from 270 to 350 nm in the emission domain and from 231 to 291 nm in the excitation domain,

both at regular steps of 3 nm. The scanning rate of the monochromators was maintained at 30 nm s^{-1}. The excitation and emission monochromator slit widths were set to 4 nm. All measurements were made in a 10 mm quartz cell at 620 V.

Data matrix

For each experiment a data cube **D** whit the corresponding emission-excitation spectra for each sample and the spectra of the four individual standards were arranged. The dimensions of the cube in experiment 1,2,3,4: **D** (32 x 28 x 21); **D** (41 x 28 x 21) in experiment 5, and **D** (42 x 28 x 21) in experiments 6, 7 and 8.

Data processing

The fluorescence spectra were exported in ASCII format from the instrument software to MATLAB [27]. This information was then processed with the PARAFAC algorithm, obtained from the N-way toolbox [28]. A complete description of pre-treatment for the data cube (Raman and Rayleigh scattering) and the PARAFAC process (applied constraint) it has already been described for the authors [19].

For processed statistical information relating with the experimental design the data were treated with MINITAB program [29].

5. Results and discussion

By way of example, Figure 1 shows the EEM spectra for phenol in experiment 5 at time zero and at time 10, 120 and 180 min. It can be seen that the maximum decreases to λ_{em} = 294 nm and λ_{ex} = 270 nm, which is characteristic of phenol, at time zero and other maxima appear at λ_{em} = 328 nm and λ_{ex} = 288 nm, which indicate the presence of hydroquinone. Catechol and resorcinol present maxima at λem = 311 -302 nm and λ_{ex} = 276-274 nm respectively, and are therefore considerable overlapped with the phenol and hydroquinone signals.

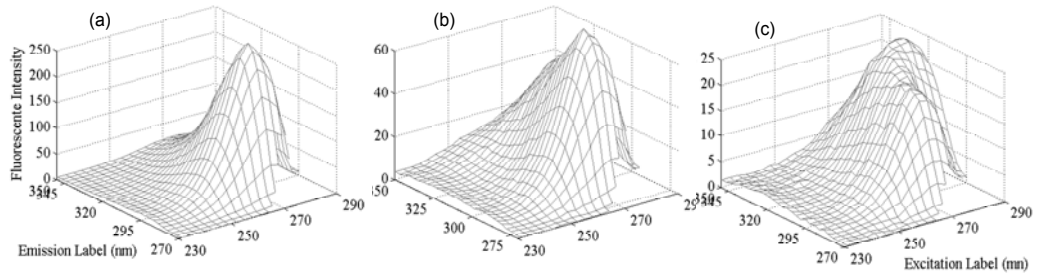


Figure1. EEM fluorescence spectra for experiment five at 10 (a), 120 (b) & 180 min(c)

Table 1 shows the number of factors, % of fit and the core consistency, obtained for each PARAFAC model, i.e. for each experiment.

Nº experiment	% Fit	Nº of Factor	Core Consistency
1	99.96	5	-22.90
2	99.98	5	-16.35
3	99.96	5	-22.21
4	99.71	6	-41.13
5	99.98	5	43.69
6	99.98	5	71.28
7	99.97	5	42.81
8	99.94	6	-44.33

Table 1. Number of factors obtained by PARAFAC in each experiment, % of fit and core consistency of their corresponding models

The necessary number of factors has been extracted until recovering the profiles of the analytes of interest. Due to the important spectral overlapping of the analytes spectra in study, the core consistencies distance of the ideal value of 100 [19]. The % of fit is always higher than 99 % which indicates that practically all the information contained in the experimental data has been collected.

For most of the experiments, the numbers of significant components were five, except in experiments 4 and 8 in which there were six. Four sources of variability can be attributed to the contribution of the four analytes that are expected from the same degradation reaction (see scheme 1). In experiments 4 and 8, there was one more source of variability that was produced by the particular dispersion that was caused by the medium when working with TiO_2 as the catalyst at a pH of 10. At this pH, the particles of TiO_2 are very small and the suspension coagulates, which prevents it from being completely filtered [3] and, when the fluorescence produced by the resulting solution is measured, they are one more contribution to the analytical signal.

By an example in the Figure 2 it showed the shape of the resolved PARAFAC spectra in the experiment 8.

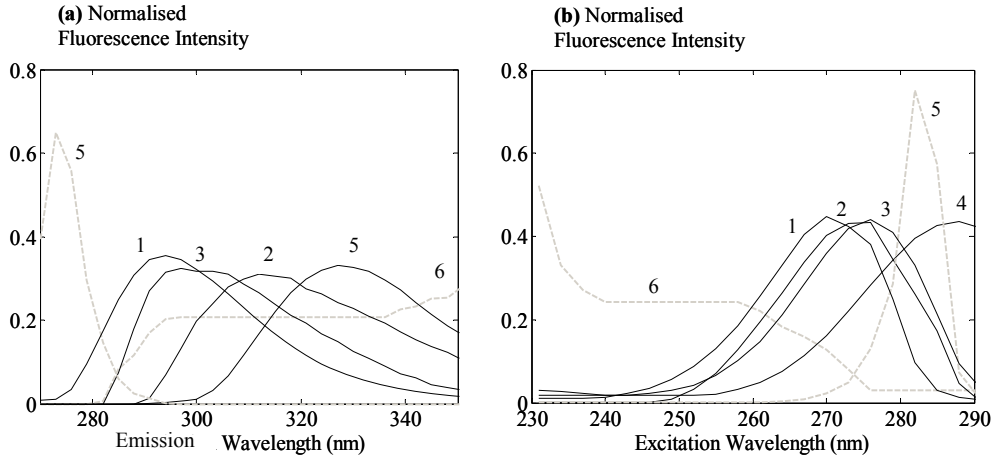


Figure 2. (a) Excitation and (b) emission spectra recovered by PARAFAC model; solid lines: phenol (1), catechol (2), resorcinol (3) and hydroquinone; broken lines: spectra attributed the particles of TiO_2 not completely filtered (5) and a possible interferential spectrum (6)

As it can be seen, in addition to the spectral signals due to the four analytes of interest, a typical signal of dispersion attributed to particles in solution (see above) it can be observed. The other signal does not have a defined spectral form, and is similar in all the models and non attributable to any analyte of interest, behaves like a possible interferente. Theses contributions must be separated if the analytes of interest—resorcinol, catechol, hydroquinone and phenol—are to be correctly quantified. It should be pointed out that it is important to separate this contribution from that produced by the analytes of interest because each signal makes a contribution to the total

signal. Therefore, if it is not correctly separated, it will be quantified as part of the analytes.

The correlation between PARAFAC recovered spectra and the true emission and excitation spectra for the analytes were measured as the dissimilarity coefficient (the sin of the angle between the recovered profile and the true profile [30] (Table 2).

Disimilarity coefficient		Exp 1	Exp 2	Exp 3	Exp 4	Exp 5	Exp 6	Exp 7	Exp 8
Phenol	emission	0.0056	0.0060	0.0061	0.0216	0.0047	0.0052	0.0049	0.0096
	excitation	0.0089	0.0087	0.0062	0.0242	0.0150	0.0113	0.0072	0.0118
Hydroquinone	emission	0.0195	0.0195	0.0192	0.0213	0.0187	0.0198	0.0196	0.0212
	excitation	0.0150	0.0150	0.0152	0.0150	0.0162	0.0154	0.0150	0.0175
Catechol	Emisión	0.0140	0.0161	0.0189	0.0957	0.0387	0.0199	0.0179	0.1453
	excitation	0.0246	0.216	0.0169	0.0740	0.1253	0.0123	0.0134	0.1174
Resorcinol	emission	0.0314	0.0323	0.303	0.1530	0.328	0.380	0.0321	0.1004
	excitation	0.0276	0.0281	0.0272	0.0696	0.0646	0.0302	0.0278	0.0952

Table 2. Dissimilarities between recovered and true profiles for phenol, hydroquinone, catechol and resorcinol for each experiments measured by the sin of the angle between them

As in previous studies, the recoveries for phenol and hydroquinone were good in all cases. A dissimilarity of 0.1 meant a correlation of 0.995 and a dissimilarity of 0.01 meant a correlation of 0.999. Catechol and resorcinol, which are the analytes whose fluorescence spectra are most similar but least sensitive, are the most difficult to separate, although the results are always acceptable [19,20].

Fig. 3 shows, in all experimental conditions, the relative values of the phenol concentration throughout the experiment calculated with the PARAFAC model

developed.

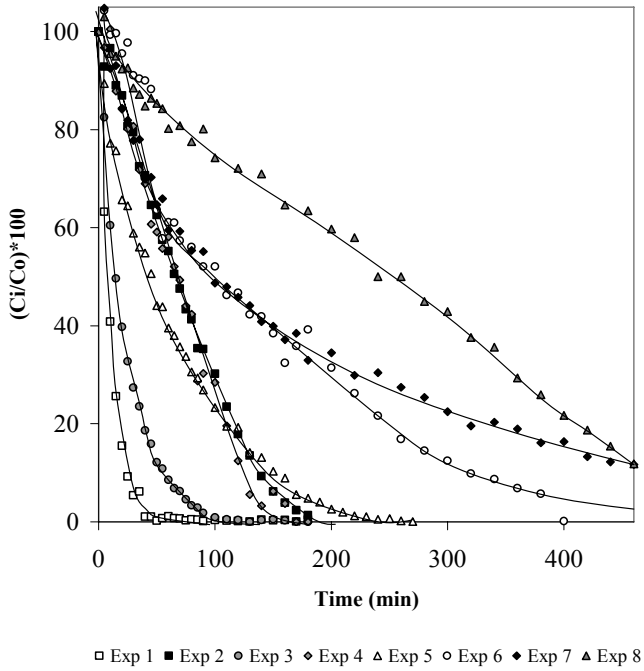


Figure 3. Degradation (%) of phenol during the eight experiments obtained using the corresponding PARAFAC model

They are calculated by means of a linear relationship between the value of the score coefficients at time zero and the corresponding value at each time. It can be seen that there is no induction time in any case and that it is clearly a pseudo first order kinetic. It can be seen that, in general, the degradation has finished, although at low concentrations (experiments 1-4), the time is shorter. These values are fitted to a pseudo first-order kinetics (ln (Co/Ci) = *k*.t) to calculate the rate constant (Table 3). An initial analysis of these results shows that the phenol degradation rate was greatest when the pH was acidic and the catalyst was ZnO.

Experiment	*k*
1	0.1116
2	0.0144
3	0.0443
4	0.0154
5	0.0143
6	0.0073
7	0.0040
8	0.0027

Table 3. Apparent constant (k) of the phenol for the different degradation conditions

Figure 4 shows the concentrations of the intermediate compounds, hydroquinone, catechol and resorcinol. They were calculated by means of a linear relationship between the score coefficients for a standard of 5 μg L –1 of each analyte and the corresponding score value at each time.

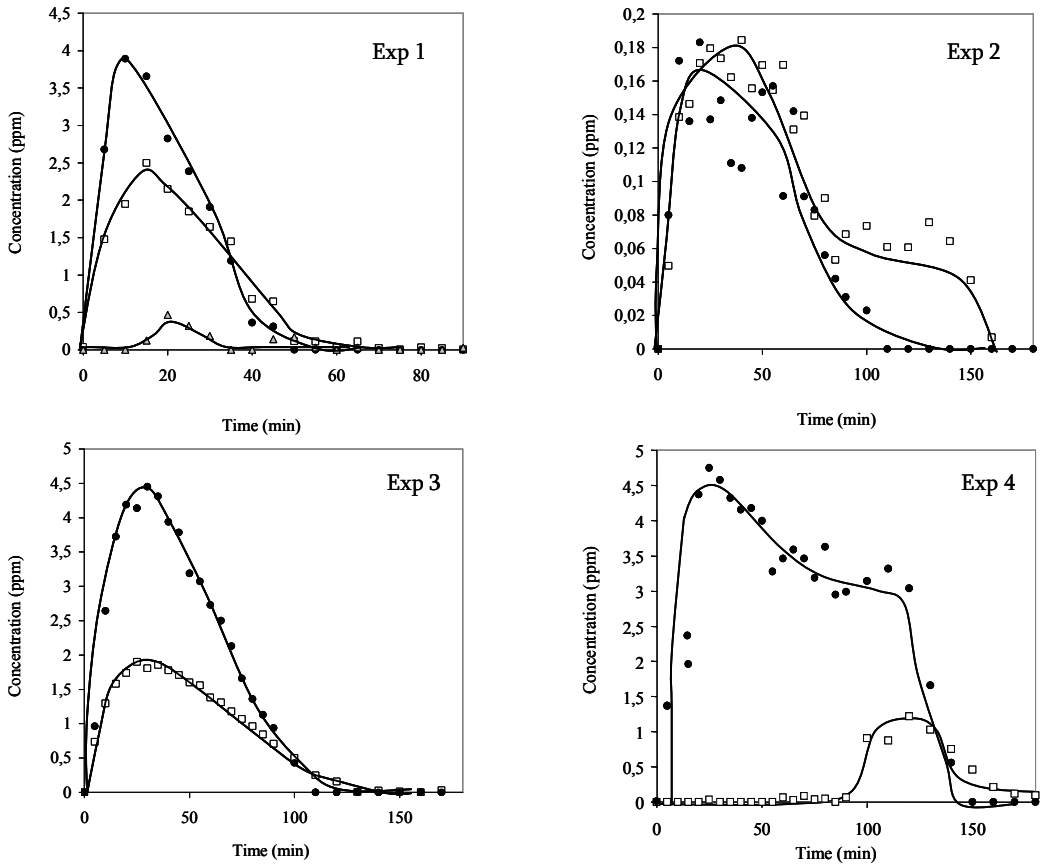


Figure 4. Degradation (%) of intermediate compounds, resorcinol, catechol, and hydroquinone for the 1-4 experiments obtained using the corresponding PARAFAC model

An analysis of these results shows that the evolution of these intermediates is characterised by the working conditions. The intermediates are not always the same ones and the concentrations are often different. Likewise, the compounds do not always appear and disappear at the same time in all the experiments. It should also be pointed out that, although mineralization is generally total, at the end of the process in experiment 8 hydroquinone and resorcinol seem to persist.

Respect to the evolution in the time of the signals in experiment 4 and 8 it is observed that they are constants (not shown).

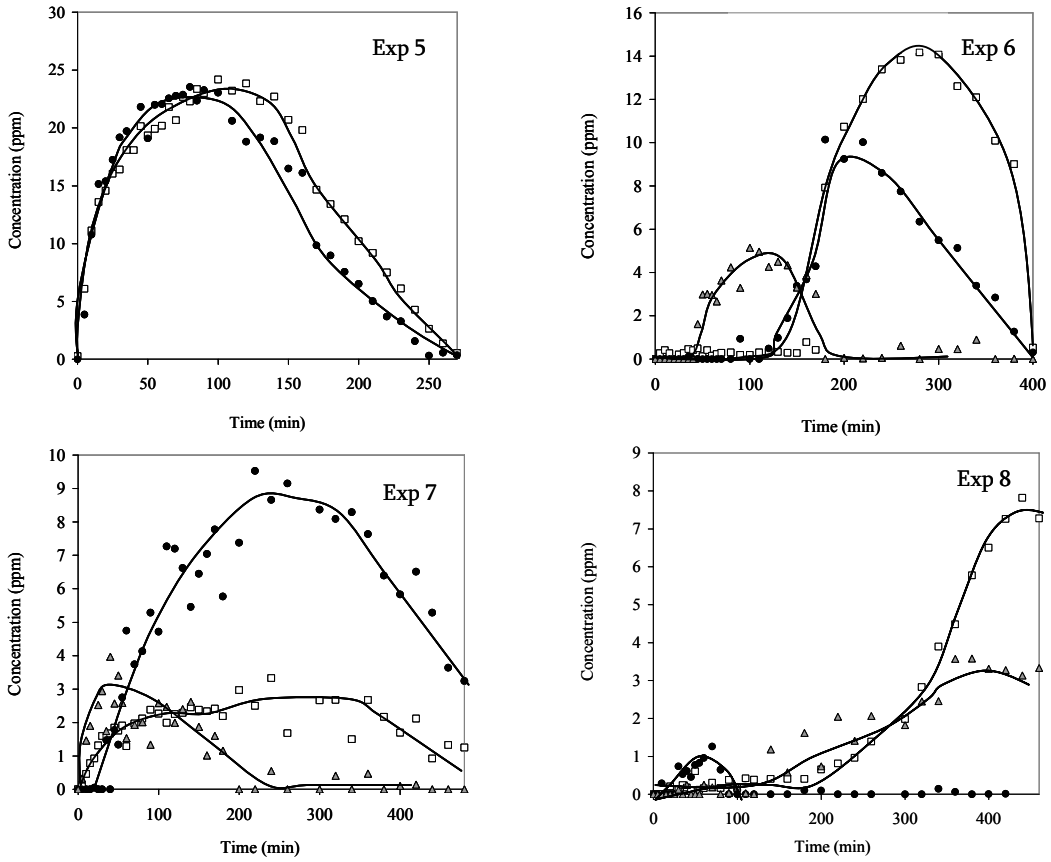


Figure 4. Degradation (%) of intermediate compounds, resorcinol, catechol, and hydroquinone for the 5-8 experiments obtained using the corresponding PARAFAC model

To evaluate how each experimental variable influences the process of phenol photodegradation in different conditions, the rate constant is considered as the response variable.

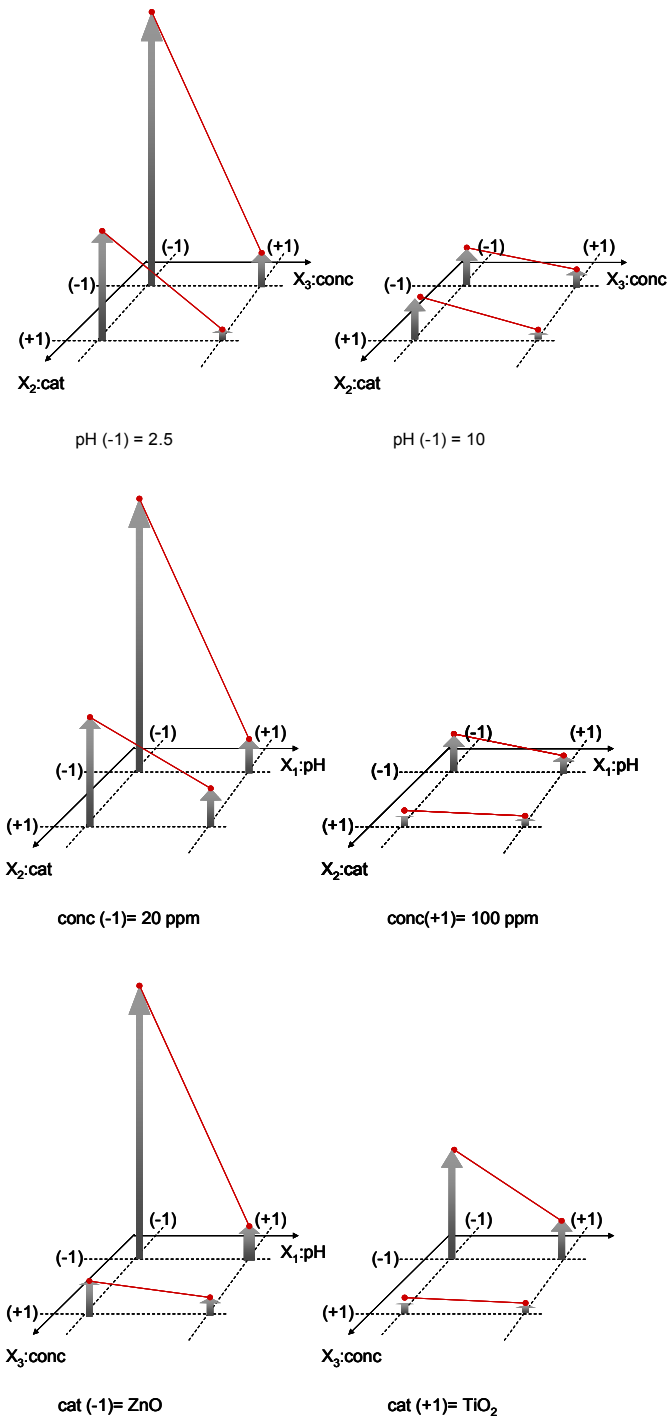


Figure 5. Graph of the main and interaction effects of each variable on the rate constant

The main and interaction effects of each of the variables on the photolysis rate constant are represented in Fig.5. In order to evaluate the main effects, proportional arrows to the values of rate constant were elevated. To evaluate the interactions the superior ends of these arrows were connected in direction (-1) to (+1). From these plots it can be inferred that there is interaction between the three variables considered: pH, nature of the catalyst and substrate concentration, because these lines are not parallel. The differences found between the values of the constants when experimenting on their lowest (-1) and highest (+1) level depend on the value considered for the other variables.

The significance of these effects has been evaluated using an ANOVA test (Table 4).

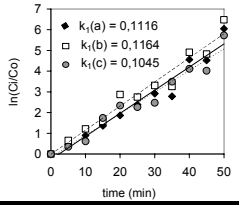
Source	Regression coeffs.	Sum Square Effect	Df	Mean Square	F-Ratio
MAIN EFFECTS					
Independent term	0,0273568				
A: ph	-0,0174068	0,00749954	1	0,00749954	209,23
B: Cat	-0,0107568	0,00311102	1	0,00311102	86,79
C: conc	-0,0202818	0,00540293	1	0,00540293	150,74
INTERACTIONS					
AB	0,00985682	0,00137085	1	0,00137085	38,25
AC	0,0153318	0,00228552	1	0,00228552	63,76
BC	0,00703182	0,000435129	1	0,000435129	12,14
RESIDUAL		7,16867E-05	2	3,58433E-05	
				F table α=0,05(1,2)	**38,51**
				F table α=0,1(1,2)	**18,51**

Table 4. ANOVA table for testing the significance of the effects

As can be seen in picture in Table 4, the experimental error has been calculated employing the constant rate determinate when experience 1 was repeated three times. Assuming that the random error is constant in all the performed experiences, this value has been employed for comparing the associated variances to the calculated effects.

From the F-ratios determinate we can infer that for an $\alpha = 0.05$ the associated variance to the main effects and the interaction between pH and the concentration, significantly defer from the experimental error. Therefore these factors have a statistically significant effect on the constant rate at the 95 % confidence level. It can be observed that for an $\alpha = 0.10$ the interaction between pH and the catalyst also have a statistically significant effect on *k*.

Analyzing the coefficients found for the effects may help to chemically interpret the influence that the experimental variables have on the degradation process under study. The negative value of the pH coefficient indicates a decrease in the degradation rate of the phenol at basic pH. It is well known that pH has a notable effect on the degradation rate of compounds [10,25,31]. At pHs lower than the isoelectric point of the catalysts, the surface charge is positive and at higher pHs it is negative, which hinders the adsorption of the compounds to be degraded since they are anionic species and they delay the formation of OH radicals. Therefore, the photocatalytic degradation decreases. This provides an explanation for the fact that the intermediate compounds appear later when a basic pH is used and that they persist for longer throughout the process. Likewise, the negative value of the coefficient relative to catalyst also indicates a decrease in the degradation rate when TiO_2 is used instead of ZnO. The photocatalytic process itself begins on the surface of the catalyst and in heterogeneous photocatalysis, TiO_2 is the most commonly used catalyst [2,10,32,33], which seems to contradict what has

been found in this study. In this respect, it should be pointed out that the pH used in the experiments favoured the compounds being adsorbed on the surface of the ZnO catalyst (isoelectric point 8.0) and not on the surface of TiO_2 (isoelectric point 6.25) [9]. Since F-ratio for interaction effect between the pH and the catalyst is bigger than the Ftab for $\alpha = 0.1$, this interaction have a statistically significant effect and the individual variables cannot be interpreted in isolation.

The negative value of the substrate concentration coefficient also indicates a decrease in the degradation rate of phenol when concentrations are higher. One possible explanation is that, at higher concentrations, competition for the active sites of the catalyst increases which leads to lower rate constants when, as is the case, the amount of catalyst is not increased when the substrate concentration is varied.

The coefficient values associated with the interaction terms between every two variables quantitatively reflect qualitative changes in the photodegradation rate induced by parameters of interaction, which are difficult to interpret chemically. It should be pointed out that the highest values were obtained for the interaction effects in which the pH takes part, which is in agreement withe the previous analysis.

Conclusions

Analytical methodologies based on excitation-emission fluorescence monitoring and the use of multivariate methods such as PARAFAC rapidly determine the concentration of the compounds of interest and evaluate the photodegradation process of phenol in the presence of other interferences, with no need for a previous separation. PARAFAC quickly provides values for the kinetic constants of a high number of real

samples.

Experimental design and multivariate analysis of the value of rate constants allows the simultaneous analysis of the variables that influence the degradation process as well as the interaction among them. In this way, the relative and absolute contribution of each parameter can be interpreted both qualitatively and quantitatively.

Beyond the results discussed, the work methodology can be used as a protocol for studying photocatalytic processes that involve fluorescent analytes.

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4.2.5. N-PLS vs. PARAFAC or MCR-ALS to quantitative determination of phenols in representative mixtures of a phenol photocatalytic degradation process using emission-excitation fluorescence. Validation of the results using HPLC as reference technique

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Submitted

Abstract

This paper reports a comparison of the results obtained by Multi-linear partial Least Squares (N-PLS) vs. Parallel factor analysis (PARAFAC) and Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) to quantitative determination of phenols in representatives' mixtures phenol photocatalytic degradation process using emission excitation fluorescence data matrices (EEM).

Sixty-four samples of mixtures of phenol, catechol, resorcinol and hydroquinone were prepared following a Simplex-lattice design.

Root mean square error of prediction (RMSEP) was used as measure of the overall

error obtained with each method. The error using EEM/N-PLS is always similar to or lowers than the values obtained by EEM/PARAFAC and EEM/ MCR-ALS.

The validation of the results obtained by the three multi-way methodologies using high performance liquid chromatography (HPLC) as a reference method is reported. The trueness of the results has been evaluated in terms of lack of bias. Using Bivariate Least Squares (BLS) and the joint confident interval test, the absence of bias in the EEM/PARAFAC, EEM/MCR-ALS and EEM/N-PLS methods has been assessed for the analytes, except for the values obtained using MCR-ALS for catechol and for those obtained using PARAFAC for resorcinol which do not represent a good fit vs. HPLC.

In our opinion all multi-way techniques should represent useful tools to quantitative determination of phenols in the representative mixtures of a phenol photocatalytic degradation process.

Introduction

Multi-way methods have been widely used to determine analytes of environmental interest [1] or to quantitatively monitor chemical processes related to the destruction of pollutants [2-3] such as processes of chemical photodegradation [4-6]. In this field, one of the most widely studied pollutants is phenol [7-12]. In its photodegradation, phenol decomposes completely to CO_2 and H_2O following a mechanism of hydroxylation of the aromatic ring. Hydroquinone, catechol and resorcinol are the habitual intermediates although in lower concentrations than phenol [8-10]. In previous studies[2-3] the possibility of correctly quantifying phenol in the presence of its hydroxyderivatives, catechol, resorcinol and hydroquinone has been

demonstrated using methods of curves resolution such as PARAFAC [13] or MCR-ALS [14-15], but the errors committed in the quantification of catechol and resorcinol always presented major errors. Nevertheless the errors for hydroquinone were relatively low when its mean concentrations were superior to 0.05 ppm.

The catechol, resorcinol and hydroquinone are analytes as polluting as phenol, and since the general objective of the investigation group is to develop analytical methods based on EEM and multi-way analysis to find practical uses such as quantitative in-situ monitoring of photodegradation reactions of organic pollutants agents, the present work consider the analysis of the results obtained by applying to another multi-way technique such as N-PLS [16-17].

Also, the results already obtained, were helpful in order to estimate the kinetic rate constant of the phenol in a photodegradation process and evaluate the optimal conditions to do this process, although no validation studies were carried out for the applied methods [2-3]. For this reason, the other important aim of the present paper is to assess the validity of the values of concentration obtained by all methods, PARAFAC, MCR-ALS and N-PLS.

In the present paper, the performance criteria analyzed are trueness (i.e. verification of the presence of method bias), and precision (intermediate precision). The bias of an analytical method can be determined by comparing the response of the method under validation with a value obtained by reference method [18] with smaller bias indicating greater trueness. In the case under study, the three methods (EEM/PARAFAC, EEM/MCR-ALS and EEM/N-PLS) were tested vs. HPLC using BLS, since this technique could be considered appropriate because it takes into account errors in both axes [19].

The concentration values obtained for a set of 20 mixtures were prepared for triplicate in intermediate precision conditions and were used to calculate the error at each concentration level in terms of the standard deviations. In this way, when the values of the method under validation were regressed on the results obtained by the reference method, a straight line was expected. If the slope and the intercept of this regression line do not differ significantly from one and zero, respectively, the results produced by both methods will not be statistically different at a given level of significance α. Therefore, in order to test whether there are significant differences between the regression coefficients and the theoretical values of zero in the intercept and one in the slope, the trueness was assessed by applying the joint confidence interval test for the slope and the intercept [20].

The mixtures of the analytes under study were prepared in triplicate and following a {4,3} Simplex-Lattice design for quaternary mixtures [21]. The design took into account the relation between the concentrations of the species during a phenol degradation process and the linearity interval of the fluorescent measurements.

This paper presents the results of the successful validation of multi-way methods using HPLC as reference technique, since they enable the fast and reliable to quantitative determination of phenols in representative mixtures of phenol photocatalytic degradation.

Experimental

Reagents

All reagents were of analytical grade. Phenol, catechol, hydroquinone and resorcinol were used as received from Sigma-Aldrich (99%) and glacial acetic acid (100%) from Merck. Stock solutions were prepared by weighing appropriate amounts of the reagents and dissolving them in Millipore water. All solutions were prepared in an acidic medium of 1 % (v/v) acetic acid, so that the analytes remained in the molecular form, and stored in dark bottles at 4ºC. They remained stable for one month. Working solutions were prepared by diluting stock solutions with Millipore water, maintaining a concentration of 1% (v/v) acetic acid.

Instrumentation

The spectrofluorimetric data were acquired on an Aminco–Bowman Series 2 Luminescence spectrometer (SLM Aminco, Rochester, NY, USA) equipped with a 150W continuous xenon lamp and a PMT detector.

HPLC analyses were performed on an Agilent 1100 Series Fluorescence Detector (Germany) equipped with a programmable fluorescence detector. Chromatographic data were collected and recorded on an HP Chemstation, version A.06.01.

Samples

Individual standards of 5 mgL^{-1} of each analyte were prepared in aqueous solution, in an acidic medium of 1 % (v/v) acetic acid (See above). A set of 20 mixtures was also prepared following a {4, 3} Simplex-Lattice design for quaternary mixtures so that the ability of the models to resolve and quantify these analytes could be evaluated. The set of mixtures was prepared in triplicate. The design took into account the relation between the concentrations of the species during a degradation process and the linearity interval of the fluorescent measurements.

HPLC Calibration samples

For quantification, calibration standards (0.025-7 mg l^{-1}) were prepared in accordance with the general conditions proposed by Health Canada for determining phenolic compounds [22].

Spectrofluorimetry measurements

All solutions (individual standards, mixtures, samples and solvent) were measured in the same conditions. Three-dimensional excitation-emission spectra were recorded between 270 and 350 nm in the emission domain and between 231 and 291 nm in the excitation domain, both at regular steps of 3 nm. The scanning rate of the

monochromators was maintained at 30 nm s^{-1}. The excitation and emission monochromator slit widths were set to 4 nm. All measurements were made in a 10 mm quartz cell at 620 V. The fluorescence spectra were exported in ASCII format from the instrument software to MATLAB [23]. This information was then processed with the PARAFAC and N-PLS, obtained from the N-way toolbox [24], and with the MCR-ALS algorithm [25].

HPLC-FLD analysis

The analyses with HPLC were carried out with a 250 x 4 mm Tracer C-18 reverse-phase column from Teknokroma (Barcelona, Spain) with a particle size of 5 μm. A 20 μl loop was used to directly inject the sample. The column was kept at room temperature. A mobile phase gradient programme was performed (Table 1(a)) using Solvent A (1 % acetonitrile, 1 % acetic acid, 1% IPA filter and degas); Solvent B: 28 % acetonitrile, 1 % acetic acid, 1 % IPA filter and degas) and Solvent C (acetonitrile). The flow rate was 1.5 ml min^{-1}.

a) Time (min)	Composition		
	%A	%B	%C
0	100	0	0
5	100	0	0
10	75	25	0
17	0	100	0
19	0	0	100
22	0	0	100
24	95	0	5

b) Time (min)	Wavelength (nm)	
	Excitation	Emission
0	304	338
5	274	298

Table 1. Mobile phase gradient and wavelength profile

For more sensitive detection of the analytes, optimum excitation and emission wavelengths had to be used for each component and a wavelength detection program was developed for this purpose (Table 1 (b)).

5. Results and discussion

Figure 1 shows the fluorescence, emission and excitation spectra of the synthetic samples 1, 3 & 7 prepared of Simplex-Lattice design (sample 1: 5 mg l^{-1} of phenol and 0.05 mg l^{-1} of the other analytes; sample 3: 5 mg l^{-1} of phenol, 0.25 mg l^{-1} of catechol and 0.05 mg $l^{-1}$of hydroquinone and resorcinol; sample 7: 0.05 mg l^{-1} of phenol, 1 mg l^{-1} of hydroquinone and 0.05 mg $l^{-1}$of catechol and resorcinol).

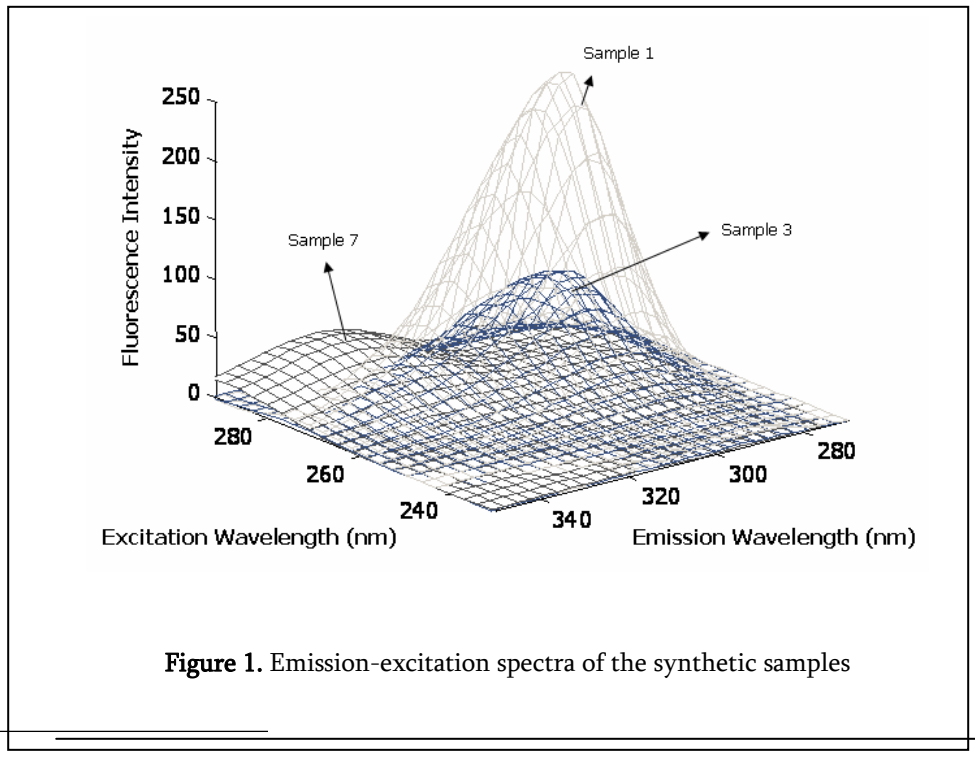

Figure 1. Emission-excitation spectra of the synthetic samples

prepared 1, 3 & 7

At first sight, there seem only to be two maxima, of very different intensities, corresponding to the wavelengths characteristic of phenol (λexcitation = 270nm, λ emission = 294 nm) and hydroquinone (λ excitation = 288nm, λ emission = 328 nm). The maxima for catechol and resorcinol (λ excitation = 276nm, λ emission = 311 nm; and λexcitation = 274nm, λ emission =302 nm, respectively) were totally overlapped with the phenol signal, which gives some idea about the strong overlapping of the signals recorded in the process.

In table 2 the results of applied tri-PLS1 to a matrix of 64x28x21 of the spectral data recorded for the whole set of samples plus four individual standards are shown.

N-PLS	Nº Comp.	%Variance	
		Spectral Matrix	Sample conc. Matrix
Phenol	4	99.64	99.63
Hydroquinone	4	99.79	99.22
Catechol	7	99,.73	99.92
Resorcinol	6	99.91	99.00

Table 2. N-PLS parameters

As samples concentration matrix, was used the values of the expected concentrations for each analyte in the 64 samples. A common procedure for selecting the number of PLS factors is to monitor the average prediction error for an independent test set. The optimum number of factors selected is the one for which RMSEP is either a minimum or reaches a plateau [26]. The number of optimum components was four for phenol and hydroquinone, seven for catechol and six for resorcinol.

The tri-PLS1 models considered always retain a high percentage of variance, higher than 99%, in both the **X** cube (spectral cube) and the **Y** matrix (sample concentrations matrix). The error percentages indicated for each analyte were calculated by cross validation [27].

In order to do a comparison PARAFAC was applied to the same **X** cube data and MCR-ALS was applied to a **X** matrix of 1792x21 obtained after arranged the spectral data ((64x28)x21). The spectral profiles of each of the analytes of interest were recovered on the first four factors and the quantification was carried out using the score found for the 5 ppm sample as a reference. The overall error committed with these methods for the analytes was of the same order of magnitude as that found in previous studies [3]. The results of the overall error obtained by all multi-way methods and the overall error obtained by HPLC and univariate calibration as reference method are shown in Table 3.

	Overall Error (%)			
	PARAFAC	MCR-ALS	N-PLS	HPLC
Phenol	6.08	6.29	6.95	10.7
Hydroquinone	6.37	6.38	6.12	9.41
Catechol	7.64	24.35	10.27	7.56
Resorcinol	23.64	15.22	7.42	2.64

Table 3. Overall Error (%) of multi-way models and HPLC

An initial analysis of the overall errors found with the three methods of multi-way analysis seems to show that there are no appreciable differences for the phenol and the hydroquinone but that there are for catechol and resorcinol. For resorcinol, the errors obtained by N-PLS are remarkably smaller than those obtained by PARAFAC or

MCR-ALS. In the case of catechol, the errors obtained by N-PLS are slightly greater to those by PARAFAC and significantly lower than those obtained by means of MCR-ALS. As expected, the errors found by means of N-PLS are lower than those found with the methods of curves resolution, since in this case the calibrated model is constructed taking account the values of concentration of the analytes in the calibration samples. The advantage of this algorithm is accentuated in the case of resorcinol and catechol, where those analytes are strongly overlapped.

In order to assess the trueness of the results found with the different methodologies, Figure 2 shows a superposition of the mean values at each concentration obtained by EEM/PARAFAC, EEM/MCR-ALS, EEM/N-PLS and HPLC vs. the theoretical values in the mixtures.

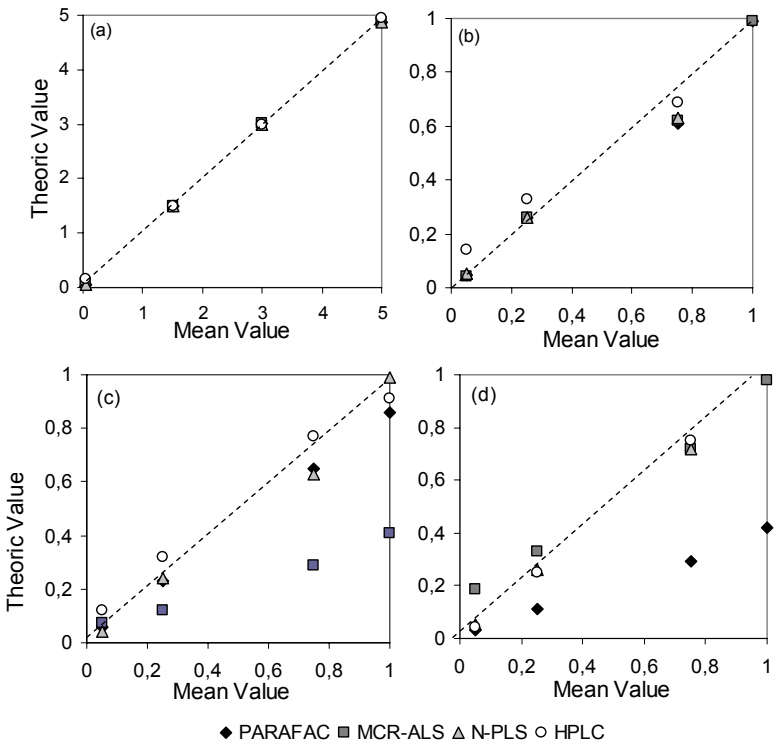

Figure 2. Representation of the mean values for each concentration obtained by

EEM/PARAFAC, EEM/MCR-ALS, EEM/N-PLS and HPLC vs. the theoretical values

A priori, this figure demonstrates that the correspondences between the values obtained by all techniques for phenol and hydroquinone were good. However, good results were obtained for catechol by EEM/PARAFAC, EEM/N-PLS and HPLC. For resorcinol the correspondences were good for EEM/MCR-ALS and EEM/N-PLS.

From these results and as it is to be expected, when the values of concentration obtained by the three multi-way methods were plotted vs. the values of concentration obtained by HPLC, straight regression lines were obtained by BLS technique (not shown). In all case the slope were near to one and the intercept near to zero, except for the values obtained using MCR-ALS for catechol ($y = 2.55 x - 0.04$) and for the values obtained using PARAFAC for resorcinol ($y = 2.57x - 0.03$) . Except for the two last cases, the statistical joint interval test of slope and intercept was used to analyse the presence of bias.

If there are no significant differences between the results obtained by both techniques (multi-way vs. HPLC), the value one for the slope and zero for the intercept should fall within the elliptical confidence region centred on the values of slope and intercept of the obtained straight regression lines. The results show (Figure 3) that in all techniques for phenol there was not a significant statistical difference between the concentration profiles obtained by both techniques, considering that $\alpha = 0.05$ as the overall significance level.

UNIVERSITAT ROVIRA I VIRGILI
SEGUIMIENTO CUANTITATIVO DE PROCESOS DE DEGRADACIÓN DE CONTAMINANTES ORGÁNICOS FOTOSENSIBLES MEDIANTE FLUORESCENCIA MOLECULAR Y MÉTODOS DE ÁNALISIS DE DPTOS DE MÚLTIPLES VÍAS
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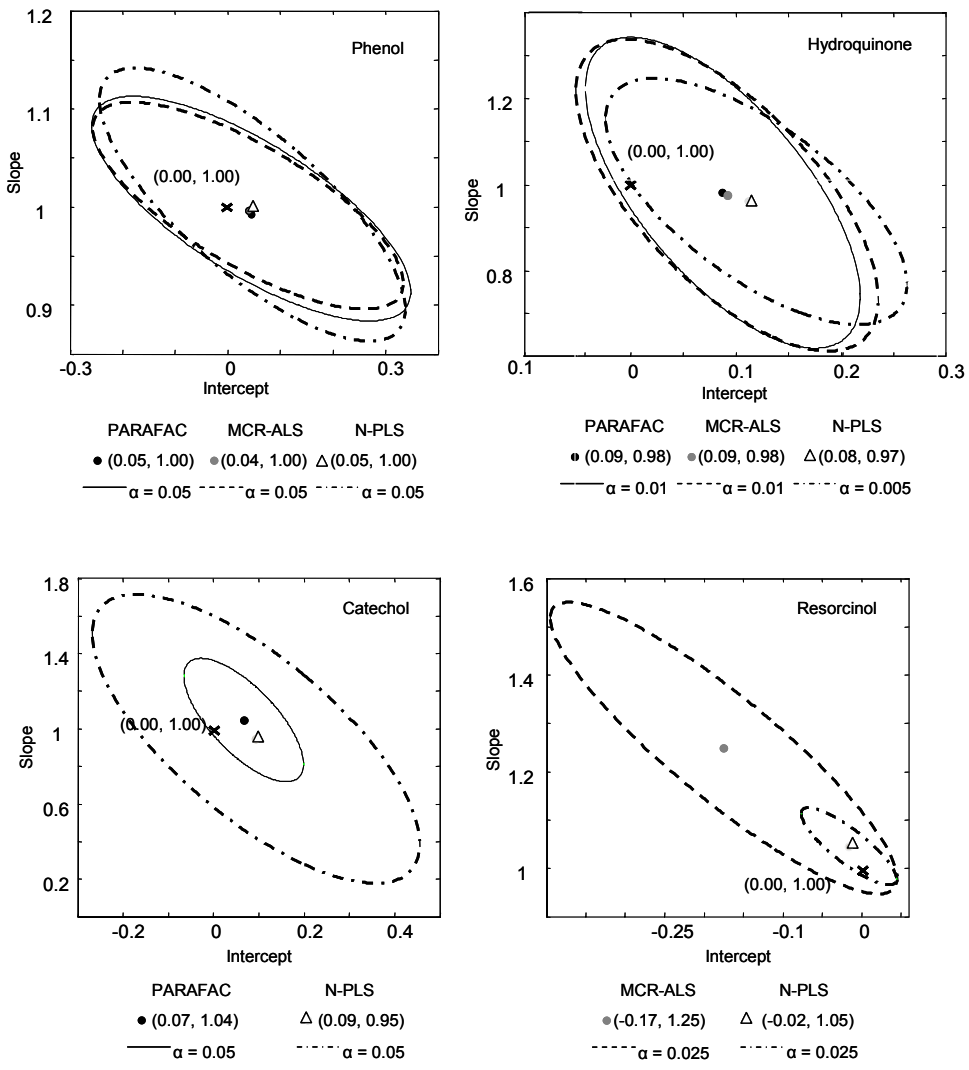

Figures 3. Joint confidence region for the BLS regression coefficients (○) represents the estimated a^ and b^ valued and (X) corresponds to the theoretical value of zero intercept and unity slope.

In the case of hydroquinone, and for the calculated concentration values using

PARAFAC and MCR-ALS, the test was successful considering that $\alpha = 0.01$ and using N-PLS considering that $\alpha = 0.0\ 01$. The values of slope and intercept for catechol do not show statistical difference of ideal value (1, 0) with $\alpha = 0.05$ and $\alpha = 0.025$ for PARAFAC and N-PLS respectively. For resorcinol, the test was successful $\alpha = 0.025$ for N-PLS and also for MCR-ALS.

Conclusions

The errors obtained in the quantification of the concentration of representative mixtures of a photocatalytic process of phenol using EEM/N-PLS, were always similar to or lower than the values obtained by EEM/PARAFAC and EEM/ MCR-ALS. Significant improvements have been found for the determination of catechol with respect to the values obtained by MCR-ALS. For resorcinol, the values obtained by N-PLS were better than those obtained by PARAFAC and MCR-ALS.

The results obtained from all multi-way techniques have been validated. Using Bivariate Least Squares and the joint confident interval test, the absence of bias in the EEM/PARAFAC, EEM/MCR-ALS and EEM/N-PLS methods have been assessed for the analytes, except for the values obtained using MCR-ALS for catechol and for those using PARAFAC for resorcinol, since they did not represent a good fit vs. HPLC.

Of the methodologies discussed, the quickest are EEM with PARAFAC or MCR-ALS, because calibration can be done with a single reference pattern, whereas EEM with N-PLS requires a reference value for each one of the calibration samples used in the

model and these models should be established with a relatively high number of samples, however the errors are lower.

All multi-way techniques represent useful tools to the quantitative monitoring of a photocatalytic degradation process of phenol.

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CUANTITATIVO DE PROCESOS
FLUORESCENCIA MOLECULAR Y MÉTODOS DE ANÁLI
ónica Bosco Costa
84-691-3378-1/DL:T-609-2008

CAPÍTULO V

UNIVERSITAT ROVIRA I VIRGILI
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CONCLUSIONES

Durante el transcurso de esta Tesis ha sido posible profundizar en la problemática de las reacciones de foto-degradación de contaminantes orgánicos y en la posibilidad de su seguimiento cuantitativo utilizando la espectroscopia de fluorescencia molecular de excitación-emisión, junto con técnicas quimiométricas, más específicamente, con metodologías de análisis de datos de múltiples vías.

La rapidez en la obtención de la información que aportan estas metodologías es de gran interés en el campo en estudio cuando se combinan con técnicas de diseños de experimentos para el análisis simultáneo de las variables que intervienen en los procesos de foto-degradación.

La utilización conjunta de la espectroscopia de fluorescencia molecular en su modalidad de excitación-emisión (EEM) y los métodos de resolución de curvas, PARAFAC y MCR- ALS han resultado ser una opción válida y atractiva para el seguimiento cuantitativo de las reacciones de foto-degradación de una mezcla de hidrocarburos policíclicos aromáticos (benzo[a]antraceno, benzo[a]pireno, benzo[k]fluoranteno, dibenzo[a,h]antraceno.

El error obtenido en la cuantificación de la concentración de las mezclas representativas del proceso foto-catalítico del fenol utilizando N-PLS ha sido siempre

similar o más bajo que los valores obtenidos mediante PARAFAC y MCR-ALS. Mejoras significativas se han encontrado para la determinación del catecol respecto a los valores encontrados por MCR-ALS y para el resorcinol tanto cuando se trabaja con PARAFAC como con MCR-ALS.

De las metodologías discutidas, las más rápidas son EEM con PARAFAC y MCR-ALS, debido a que la calibración se puede hacer con un solo patrón de referencia, mientras que EEM con N-PLS requiere un número relativamente alto de patrones de referencia para la calibración del modelo representativo de las muestras a analizar.

El seguimiento de las reacciones estudiadas mediante EEM y métodos de análisis de datos de múltiples vías proporciona en general resultados comparables a los obtenidos mediante la cromatografía líquida de alta resolución.

De los resultados encontrados se concluye que estas metodologías de gran potencial para el estudio de las condiciones óptimas en las que se deben realizar las reacciones de destrucción de contaminantes orgánicos hacen posible extraer de forma rápida, información relacionada con la influencia de las variables en el proceso en estudio, la cinética del proceso y la existencia de posibles intermediarios.

En un futuro, pensando en la aplicación de estas metodologías para el seguimiento en línea de un proceso de eliminación de contaminantes en una planta industrial, sería necesario experimentar con muestras representativas de estas plantas.

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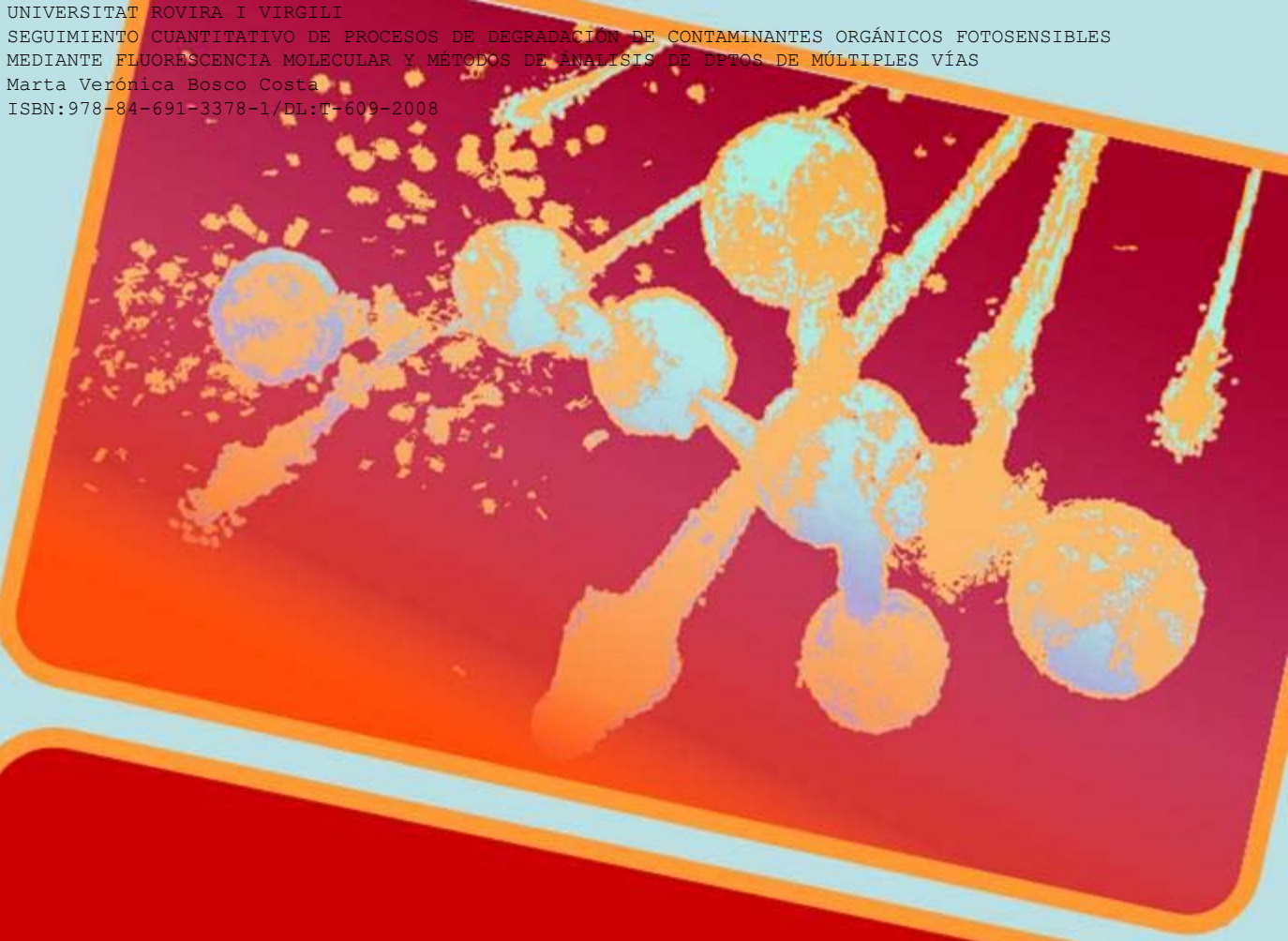

ANEXO

UNIVERSITAT ROVIRA I VIRGILI
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Autores: Marta V. Bosco and M. Soledad Larrechi

Título: "PARAFAC and MCR-ALS applied to the quantitative monitoring of the photodegradation process of polycyclic aromatic hydrocarbons using three-dimensional excitation emission fluorescent spectra: Comparative results with HPLC"

Revista: Talanta, Volume 71, Issue 4, 15 March 2007, Pages 1703-1709

Received 6 April 2006; revised 31 July 2006; accepted 1 August 2006. Available online 22 December 2006

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ANEXO

Autores: M. Bosco, M.P. Callao and M.S. Larrechi

Título: "Resolution of phenol, and its di-hydroxyderivative mixtures by excitation–emission fluorescence using MCR-ALS: Application to the quantitative monitoring of phenol photodegradation"

Revista: Talanta, Volume 72, Issue 2, 30 April 2007, Pages 800-807

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Autores: Marta Bosco, M. Soledad Larrechi

Título: "N-PLS vs. PARAFAC or MCR-ALS to quantitative determination of phenols in representative mixtures of a phenol photocatalytic degradation process using emission-excitation fluorescence. Validation of the results using HPLC as reference technique"

Revista: Submited- Talanta

Autores: Marta Bosco, M. Soledad Larrechi

Título: "Rapid and quantitative evaluation of the kinetic parameters of the photocatalytic degradation of phenol using experimental design techniques and parallel factor analysis of excitation-emission matrix fluorescence spectra"

Revista: Submited- Analytical Bioanalytical Chemistry

CONTRIBUCIONES A CONGRESOS

Autores: Bosco, M. V., Garrido, M. y Larrechi, M.S.

Título: "Spectrofluorimetric determination of phenols in cigarette mainstream smoke using parallel factor analysis"

Tipo de participación: Presentación de comunicación; Póster

Congreso: Euroanalysis XIII- 2003

Lugar de celebración: Salamanca (ESPAÑA) **Año:** 2003

Autores: M.V. Bosco, M. Garrido, M.S. Larrechi

Título: "Seguimiento cuantitativo de reacciones de fotodegradación de contaminantes orgánicos en aguas, utilizando datos fluorescentes tridimensionales y análisis factorial paralelo"

Tipo de participación: Presentación de comunicación, Póster

Congreso: 1er Workshop de la Xarxa Catalana de Quimiometría

Lugar de celebración: Barcelona (ESPAÑA) **Año:** 2005

Autores: M.V. Bosco, M.S. Larrechi

Título: "Photolytic decomposition of polycyclic aromatic hydrocarbons (PAHs) in aqueous solutions detected by three-dimensional excitation-emission matrix fluorescence and parallel factor analysis"

Tipo de participación: Presentación de comunicación, Póster

Congreso: 11 Jornadas de Análisis Instrumental (JAI)

Lugar de celebración: Barcelona (ESPAÑA) **Año:** 2005

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ANEXO

Autores: M.V. Bosco, I. Ruisanchez, M.S. Larrechi

Título: "Excitation-emission fluorescence using MCR-ALS. Application to the quantitative monitoring of the photodegradation of phenol"

Tipo de participación: Presentación de comunicación, Póster

Congreso: Reunión Nacional de espectroscopia

Lugar de celebración: Ciudad Real (ESPAÑA) **Año:** 2006

Autores: M.V. Bosco, M.S. Larrechi

Título: "Three dimensional emission-excitation fluorescence and N-PLS for the quantitative monitoring of ZnO-photocatalytic degradation of phenol "

Tipo de participación: Presentación de comunicación, Póster

Congreso: The 9th Internacional Symposium on Kinetics in Analytical Chemistry

Lugar de celebración: Marrakech (MOROCCO) **Año:** 2006

Autores: M.V. Bosco, M.S. Larrechi

Título: "Rapid monitoring of phenol photodegradation process. Quantitative determination of phenol, catechol and its isomers."

Tipo de participación: Presentación de comunicación, Póster

Congreso: 1º Encuentro de Jóvenes Investigadores en Quimiometría

Lugar de celebración: Tarragona (ESPAÑA) **Año:** 2006

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ANEXO

Autores: M.V. Bosco, M.S. Larrechi

Título: "Rapid and quantitative evaluation of the kinetic parameters of the photocatalytic degradation of phenol using experimental design techniques and four-way parallel factor analysis of excitation-emission matrix fluorescence spectra"

Tipo de participación: Presentación de comunicación, Póster

Congreso: Colloquium Chemometricum Mediterraneum

Lugar de celebración: Saint Maximin La Saint-Baume (FRANCIA) **Año:** 2007

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