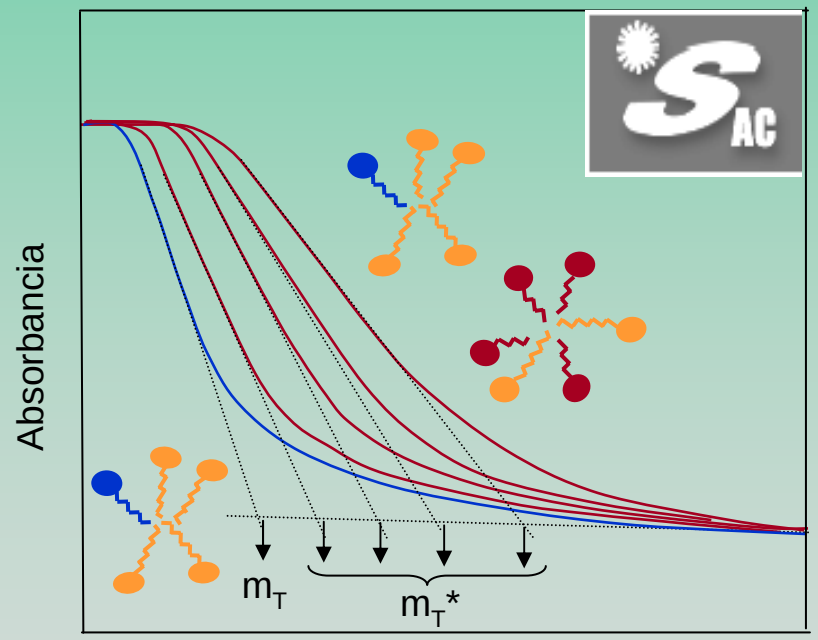
Universidad de Córdoba
Departamento de Química Analítica

NUEVOS MÉTODOS QUÍMICO-ANALÍTICOS BASADOS EN PROCESOS DE AGREGACIÓN MOLECULAR COMPETITIVA



Ana Maria Pedraza Vela
Córdoba, Mayo de 2009

TITULO: *Nuevos métodos químico-analíticos basados en procesos de agregación molecular competitiva.*

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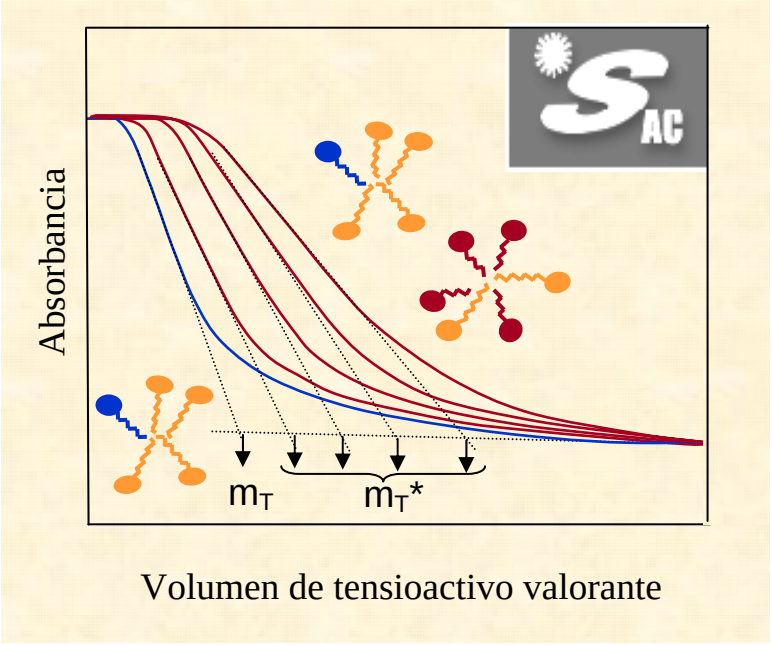

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NUEVOS MÉTODOS QUÍMICO-ANALÍTICOS BASADOS EN PROCESOS DE AGREGACIÓN MOLECULAR COMPETITIVA

LAS DIRECTORAS,

Fdo.: S. Rubio Bravo, Catedrática del Departamento de Química Analítica de la Universidad de Córdoba

Fdo.: M. D. Sicilia Criado, Profesora Titular del Departamento de Química Analítica de la Universidad de Córdoba

Trabajo presentado para optar al grado de Doctor en Ciencias, Sección Químicas

La doctoranda,

Fdo.: Ana Maria Pedraza Vela
Licenciada en Ciencias Químicas

Dª. SOLEDAD RUBIO BRAVO y MARIA DOLORES SICILIA CRIADO, Catedrática y Profesora Titular del Departamento de Química Analítica de la Universidad de Córdoba, en calidad de Directoras de la Tesis Doctoral presentada por la Licenciada en Ciencias Químicas, Dª. ANA MARIA PEDRAZA VELA.

CERTIFICAN: Que la citada Tesis Doctoral "*Nuevos métodos químico-analíticos basados en procesos de agregación molecular competitiva*" se ha realizado en los laboratorios del Departamento de Química Analítica de la Facultad de Ciencias de la Universidad de Córdoba y que, a nuestro juicio, reúne todos los requisitos exigidos a este tipo de trabajo.

Y para que conste y surta los efectos pertinentes, expiden el presente certificado en Córdoba, Abril de 2009.

Dª. S. Rubio Bravo

Dª. M. D. Sicilia Criado

Quiero agradecer al grupo de investigación “FQM 186 Química Analítica Fina y Ambiental “, la concesión de un contrato de investigación, que ha posibilitado mi dedicación a esta Tesis Doctoral durante cuatro años.

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OBJETO

El objetivo de esta Tesis Doctoral ha sido contribuir al desarrollo de los métodos basados en la medida de parámetros característicos de los sistemas supramoleculares, un campo de investigación iniciado recientemente y escasamente explorado, a pesar de su enorme potencial. Los trabajos de investigación aquí presentados se han centrado en la metodología de grado de enlace tensioactivo-colorante. El fundamento de esta metodología es la competencia que se establece entre un colorante y una sustancia química (analito) para interaccionar con un tensioactivo utilizado como reactivo, lo que causa una disminución en el valor del grado de enlace entre el tensioactivo reactivo y el colorante. Esta metodología fue inicialmente desarrollada por nuestro grupo de investigación para la determinación no específica de tensioactivos iónicos en muestras medioambientales, mostrando excelentes prestaciones analíticas. Los resultados obtenidos nos animaron a continuar con las investigaciones en esta área de trabajo con el fin de establecer las bases teóricas y prácticas de esta novedosa metodología, así como su idoneidad para la resolución de problemas analíticos reales en diferentes ámbitos de aplicación.

Con este objetivo genérico, las investigaciones se han orientado hacia la consecución de cuatro objetivos específicos:

1) Establecer los tipos de analitos que es posible determinar utilizando la metodología de grado de enlace tensioactivo-colorante. Para ello se han estudiado analitos con diferente estructura molecular y con capacidad para interaccionar de forma efectiva con tensioactivos. Los analitos investigados han sido:
    a) Compuestos anfifílicos. Además de tensioactivos iónicos, se han estudiado compuestos anfifílicos con estructura molecular muy diferente a la de éstos, concretamente drogas terapéuticas.
    b) Compuestos hidrotrópicos. Dentro de este grupo se han ensayado drogas terapéuticas y aditivos alimentarios con carácter hidrotrópico.

2) Estudiar los mecanismos a través de los que los diferentes tipos de analitos, compuestos anfifílicos e hidrotrópicos, ejercen su efecto sobre el grado de enlace tensioactivo reactivo-colorante. Con este fin, se ha investigado la capacidad de estos analitos para formar agregados mixtos con el tensioactivo reactivo.

3) Establecer las bases teóricas que permitan predecir cuál es el sistema tensioactivo-colorante más adecuado para resolver cada problema analítico concreto y qué variables experimentales se deben controlar para obtener buenas prestaciones analíticas utilizando la metodología objeto de estudio, fundamentalmente en términos de sensibilidad. Para alcanzar este objetivo, se ha estudiado la influencia de la estructura molecular del tensioactivo reactivo, del colorante y de los analitos en la sensibilidad obtenida, así como otras variables experimentales que pueden afectar a las interacciones tensioactivo reactivo-colorante y tensioactivo reactivo-analito.

4) Estudiar la aplicabilidad real de la metodología de grado de enlace tensioactivo-analito en diferentes ámbitos de aplicación: monitorización de la contaminación ambiental y control de la calidad de fármacos y de alimentos, mediante la comparación de las prestaciones de los métodos desarrollados con las proporcionadas por metodologías basadas en propiedades monoméricas de los analitos y su aplicación al análisis de una gran variedad de muestras reales.

Finalmente, esta Tesis Doctoral ha tenido como objetivo fundamental la formación de la doctoranda en tareas de investigación incluyendo, además del diseño, realización e interpretación de experimentos, la presentación de resultados para su difusión en artículos científicos y Congresos (Apéndices A y B de esta Memoria).

INTRODUCCIÓN

1. COMPUESTOS ANFIFÍLICOS

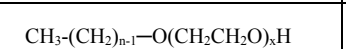
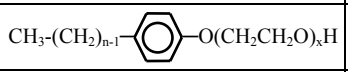
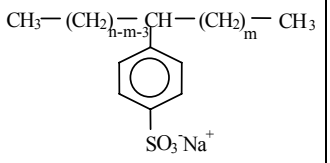
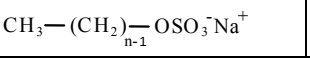
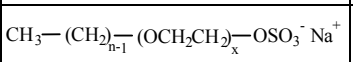
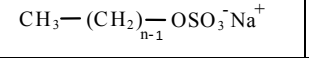
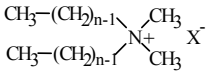
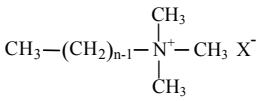
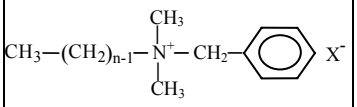
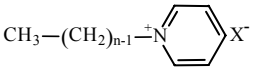
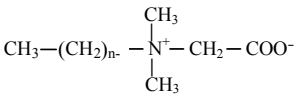
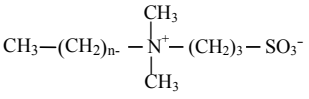
1.1. Estructura molecular

Los compuestos anfifílicos o anfipáticos, también denominados anfifilos, poseen dos regiones en sus moléculas, una hidrófila, denominada cabeza, y otra hidrófoba, denominada cola. La parte hidrófoba puede estar constituida por cadenas hidrocarbonadas, anillos hidrocarbonados saturados y anillos aromáticos, y la parte hidrófila por grupos polares de diferente naturaleza.

Atendiendo a su origen se diferencian dos tipos de compuestos anfifílicos: naturales y sintéticos. Un importante grupo de compuestos anfifílicos sintéticos es el constituido por los tensioactivos. Su región hidrófoba generalmente consiste en una o dos cadenas hidrocarbonadas lineales o ramificadas, frecuentemente saturadas, de 8 a 18 átomos de carbono. Esta región hidrófoba también puede incluir anillos aromáticos. Atendiendo a la naturaleza del grupo hidrófilo se diferencian cuatro tipos de tensioactivos: no iónicos, aniónicos, catiónicos y anfóteros. El número de compuestos con estructura molecular diferente dentro de cada uno de estos grupos de tensioactivos es muy elevado [1-3]. A modo de ejemplo, en la Tabla 1 se muestra la estructura química de diferentes tipos de tensioactivos. Finalmente, también existen tensioactivos sintéticos con dos grupos hidrófilos situados a ambos extremos de la cadena hidrocarbonada (tensioactivos bolaformes) [4] y con grupos hidrófobos constituidos por una o más cadenas perfluoroalquiladas C_nF_{2n+1} (fluorotensioactivos) [5].

Los compuestos anfifílicos naturales se obtienen directamente de una fuente natural como es el caso de los ácidos grasos, fosfolípidos, glicolípidos, lipopéctidos, lípidos fenólicos, etc. o se sintetizan a partir de una biomolécula obteniendo compuestos como los lipopolisacáridos, acilaminoácidos, etc. [6-11]. La estructura molecular de los denominados biotensioactivos o tensioactivos naturales, es muy variada. Así, por ejemplo, los ácidos grasos están constituidos

Tabla 1. Estructura química de diferentes tipos de tensioactivos sintéticos

TIPO	GRUPO ESTRUCTURAL	FÓRMULA MOLECULAR	n	x
NO IÓNICOS	Alquil etoxilatos		12-16	1-23
	Alquilfenol etoxilados		8, 9	1-23
ANIÓNICOS	Alquilbenceno sulfonatos		10-14	
	Alquil sulfonatos		10-18	
	Alquiletoxi sulfatos		12-18	2, 3
	Alquil sulfatos		10-18	
CATIÓNICOS	Sales de dialquildimetilamonio		12-18	
	Sales de alquiltrimetilamonio		12-16	
	Sales de alquildimetilbencilamonio		12-16	
	Sales de alquilpiridinio		12-16	
ANFÓTEROS	Alquilbetaínas		10-16	
	Alquilsulfobetaínas		10-16	

X^-: Cl^-, Br^-

por una cadena hidrocarbonada saturada o insaturada de entre 4 y 22 átomos de carbono unida a un grupo carboxílico [6]. La estructura de los fosfolípidos se muestra en la Figura 1 [7]. Estos biotensioactivos poseen un grupo glicerol unido a dos cadenas de ácido graso esterificado y a un grupo fosfato que a su vez, se une por enlace covalente a grupos polares colina, etanolamina, glicerol, serina o inositol. Otros lípidos anfifílicos: glicolípidos, lipopéctidos, lipopolisacáridos, lipoproteínas, acilaminoácidos y lípidos fenólicos están constituidos por cadenas hidrocarbonadas de ácido alquil carboxílico o alcoholes de cadena larga saturados o insaturados unidos a sacáridos (mono-, oligo- o polisacáridos), péptidos, proteínas, aminoácidos [8, 9] o fenoles [10].

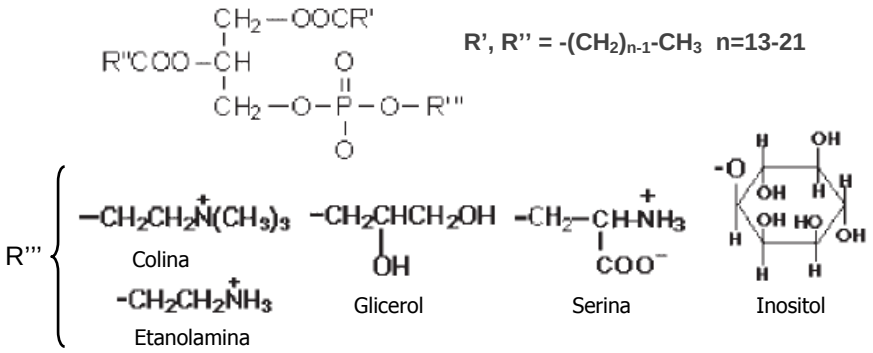
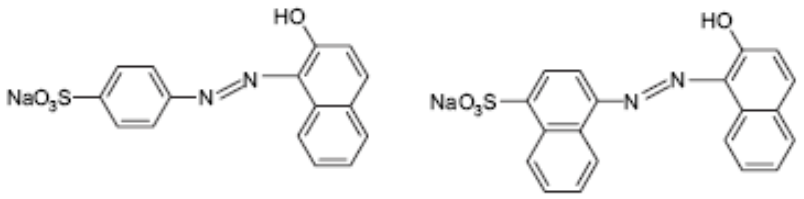


Figura 1. Estructura molecular de los fosfolípidos

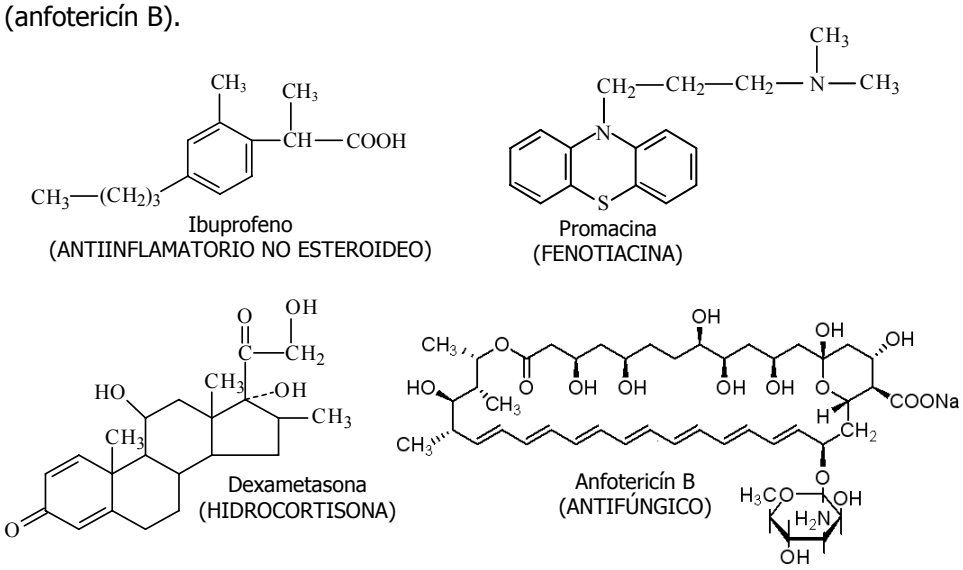
Otros compuestos con estructura molecular muy diferente a la de los tensioactivos sintéticos y naturales también tienen carácter anfifílico. Este es el caso de determinados colorantes, drogas terapéuticas y las sales biliares colato y deoxicolato sódico. Las sales biliares son anfifilos naturales constituidos por una superficie hidrófoba convexa (anillo esteroide) y una superficie hidrófila cóncava (grupos hidroxilo y grupos carboxílicos) [11]. Como colorantes anfifílicos podemos nombrar a las acridinas, cianinas, profirinas, colorantes tipo azo y esquarilio [12]. A modo de ejemplo, en la Figura 2 se muestra la estructura de dos colorantes anfifílicos tipo azo.



Naranja Ácido 7 Rojo Ácido 88

Figura 2. Estructura molecular de colorantes anfifílicos tipo azo

En la Figura 3 se muestra la estructura molecular de algunas drogas anfifílicas sintéticas (ibuprofeno, promacina, dexametasona) y naturales (anfotericín B).



Ibuprofeno
(ANTIINFLAMATORIO NO ESTEROIDEO)

Promacina
(FENOTIACINA)

Figura 3. Estructura molecular de algunas drogas anfifílicas

Determinados antibióticos obtenidos directamente de fuentes naturales como los antibióticos peptídicos catiónicos, o sintetizados a partir de compuestos anfifílicos naturales como los antibióticos esteróides catiónicos, tienen carácter anfifílico facial [13]. Los grupos hidrófobos e hidrófilos de los compuestos anfifílicos faciales se sitúan en dos caras opuestas de la molécula. En la Figura 4 se muestra la estructura de uno de estos compuestos.

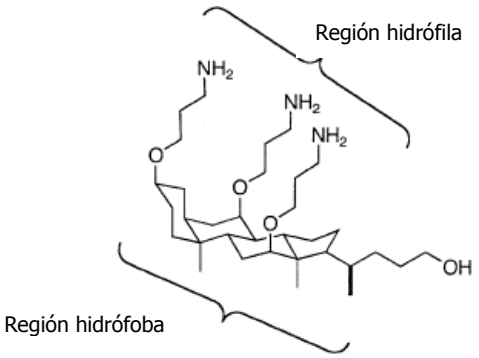


Figura 4. Estructura de un antibiótico esteroide anfifílico facial

1.2. Propiedades y aplicaciones

La estructura molecular de los compuestos anfifílicos les confiere dos importantes propiedades que los diferencian del resto de las sustancias químicas, su capacidad para:

a) Adsorberse en las interfases (gas-líquido, líquido-líquido y sólido-líquido), dando lugar a la formación de monocopas. Los anfifilos modifican la tensión superficial de las disoluciones que los contienen debido a su capacidad para adsorberse en la interfase líquido-aire.

b) Formar agregados supramoleculares (micelas, bicapas, vesículas, etc.) de dimensiones coloidales y estructura bien definida tanto en disolución acuosa como en medios apolares.

La mayoría de las aplicaciones industriales de los compuestos anfifílicos se basan en la primera de estas propiedades. Tensioactivos de diferente naturaleza se utilizan como agentes humectantes (adsorción en la interfase líquido-sólido), espumantes (interfase líquido-gas) y estabilizadores de emulsiones o microemulsiones (interfase líquido-líquido). El consumo mundial de tensioactivos supera los 12,5 millones de toneladas por año, con un crecimiento anual estimado de 500.000 toneladas [14]. De la producción total de tensioactivos, aproximadamente el 70% son tensioactivos aniónicos usados como agentes

espumantes en productos de limpieza industriales y doméstica y en productos de higiene personal. Los tensioactivos no iónicos, en segundo lugar en producción anual, se utilizan, junto con los tensioactivos aniónicos, en detergentes para lavadora y, con los tensioactivos catiónicos, en suavizantes para ropa y cabello. Los tensioactivos no iónicos también son ampliamente utilizados como humectantes y emulsionantes en la industria textil, papelera, de pinturas, etc. Finalmente, el principal ámbito de aplicación de los tensioactivos anfóteros se encuentra en la formulación de cosméticos, debido a la elevada tolerancia de la piel a estos compuestos.

Los tensioactivos también son ampliamente usados en la formulación de productos agroquímicos para mejorar la estabilidad, facilitar la aplicación y aumentar la eficacia agronómica de los principios activos. Así, por ejemplo, determinados tensioactivos no iónicos se utilizan para facilitar un mojado rápido y mayor cobertura con productos agroquímicos o para facilitar la penetración de plaguicidas químicos y bioplaguicidas en plantas [15]. Otros usos de los tensioactivos en agricultura y jardinería incluyen la restauración de suelos, mejora de la retención de agua y formulación de fertilizantes.

La industria alimentaria utiliza compuestos anfifílicos como conservantes [16, 17], antioxidantes [18] y para otorgar ciertas características a los productos elaborados [19]. Los más usados con este fin son los polisorbatos (tensioactivos no iónicos, nombres comerciales: Span y Tween) y la lecitina (fosfatidilcolina), utilizados como acondicionadores de la masa del pan y productos de bollería y como emulgentes en mantequillas, chocolates y productos precocinados.

Los compuestos anfifílicos tienen especial relevancia en la industria farmacéutica, ya que la mayoría de los principios activos tienen carácter anfifílico con el fin de facilitar su penetración en las células y favorecer su interacción con los centros receptores. Así, antibióticos como las penicilinas, cefalosporinas, sulfamidas, tetraciclinas, macrólidos, fluoroquinolonas, etc., antihistamínicos, antidepresivos tricíclicos, fenotiacinas, antiinflamatorios como los ácidos

fenámicos, propiónicos, etc., corticoides e incluso drogas terapéuticas usadas en tratamientos oncológicos, son anfifílicos.

Los antibióticos anfifílicos no sólo se usan en el tratamiento de infecciones humanas, sino también para diversos usos veterinarios y para aumentar la producción agrícola, ganadera y piscícola. Recientemente, se ha propuesto el uso de compuestos anfifílicos faciales con propiedades antibacterianas (antibióticos peptídicos y esteroides catiónicos) que actúan permeabilizando o rompiendo las membranas bacterianas [20, 21]. Estos antibióticos están especialmente indicados para el tratamiento de infecciones provocadas por bacterias resistentes a los antibióticos convencionales.

Fosfolípidos como la fosfatidilcolina y la miltefosina han demostrado su efectividad en el tratamiento de diferentes tipos de cáncer [22, 23] y derivados anfifílicos de la tetrasulfoftalocianina de aluminio se utilizan como fotosensibilizadores en terapia fotodinámica (PDT), un tratamiento curativo o paliativo anticáncer propuesto recientemente [24].

Una de las aplicaciones más novedosas de los compuestos anfifílicos en medicina es la preparación de fluidos sustitutos de la sangre a partir de fluorotensioactivos [25]. Las características de estos tensioactivos: estabilidad, gran capacidad para formar agregados y solubilizar gases, baja reactividad, fluidez, etc., los hacen especialmente adecuados para aplicaciones biomédicas [26, 27].

Finalmente, los compuestos anfifílicos también se utilizan como excipientes en la formulación de productos farmacéuticos, siendo especialmente útiles como estabilizantes [28], agentes solubilizantes [29] y para la obtención de sistemas de liberación controlada de los principios activos posterior a la administración de los medicamentos [30]. El principio activo es encapsulado en agregados moleculares, frecuentemente liposomas (agregados vesiculares de fosfolípidos), siendo liberado en el lugar del organismo o en el momento más adecuado para aumentar la eficacia del tratamiento. Los liposomas también son ampliamente

utilizados en cosmética para controlar la adsorción de ingredientes activos sobre la piel [31].

2. AGREGADOS SUPRAMOLECULARES DE COMPUESTOS ANFIFÍLICOS

2.1. Agregados de tensioactivo

Los tensioactivos forman agregados moleculares en disolución a concentraciones superiores a un determinado valor, denominado concentración crítica de agregación (cca). Los agregados formados pueden estar constituidos por un único tensioactivo, agregado simple, o por varios tensioactivos, agregados mixtos. A continuación comentaremos los diferentes factores que influyen en la morfología de los agregados simples y mixtos de tensioactivo formados y en el valor de la cca para la formación de dichos agregados.

2.1.1. *Morfología de los agregados*

La morfología de los agregados simples de tensioactivo depende, entre otros factores, de la estructura química del compuesto y de la naturaleza del medio en la que está disuelto. La formación de los agregados se produce mediante interacciones intermoleculares no covalentes: interacciones hidrofóbicas, formación de puentes de hidrógeno, etc., produciéndose en cada caso, el conjunto de interacciones que dan lugar a la estructura más estable.

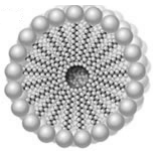
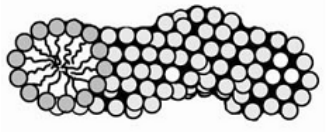
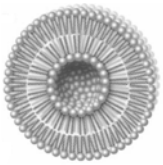
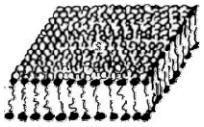
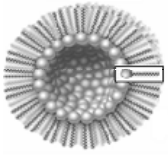
El parámetro de empaquetamiento crítico "P" [32] permite correlacionar la estructura del tensioactivo con la morfología del agregado formado en condiciones experimentales bien definidas. Este parámetro se calcula a partir de la expresión matemática:

$$P = \frac{\upsilon}{a_0 \, l_c} \qquad [1]$$

donde υ es el volumen del grupo o grupos hidrófobos del tensioactivo, a_0 el área de la sección transversal de su grupo cabeza en contacto con el disolvente por molécula de tensioactivo, y l_c es la longitud crítica del grupo hidrófobo, que es aproximadamente igual a la longitud efectiva máxima ($l_{máx}$) de dicho grupo.

En la Tabla 2 se muestran los tipos de agregados simples formados por tensioactivos en función del valor de P obtenido.

Tabla 2. Agregado formado en función del valor del parámetro de empaquetamiento crítico (P)

PARÁMETRO DE EMPAQUETAMIENTO CRÍTICO	AGREGADO	ESTRUCTURA DEL AGREGADO
P < 1/3	Micelas esféricas	
1/3 < P < 1/2	Micelas vermiformes	
1/2 < P <1	Vesículas	
P = 1	Bicapas	
P > 1	Micelas inversas	

En las micelas esféricas y vermiformes acuosas la región hidrófoba del tensioactivo se orienta hacia el interior del agregado para evitar el contacto directo con el agua, siendo las interacciones hidrofóbicas entre moléculas de tensioactivo las principales fuerzas causantes de la agregación. En las micelas inversas, formadas en disolventes apolares, es el grupo hidrofílico el que se orienta hacia el interior del agregado quedando el grupo hidrófobo en contacto con el disolvente. La bicapa consiste en una doble capa de moléculas de tensioactivo con la región hidrofóbica dirigida hacia el interior y la región hidrófila hacia el exterior de la lámina formada, siendo el espesor de dicha lámina el doble de la longitud máxima de la molécula. Finalmente, las vesículas son estructuras esféricas que contienen bicapas de tensioactivo y un núcleo acuoso.

La mayoría de los tensioactivos naturales y sintéticos con doble cadena hidrocarbonada y determinados tensioactivos con una única cadena hidrocarbonada ramificada [33] forman bicapas o vesículas en disolución acuosa, ya que el volumen de su región hidrófoba (υ) es muy elevado, lo que impide su empaquetamiento en estructuras micelares que mantengan un contacto adecuado del grupo cabeza con el agua (valor óptimo de a_0). Por el contrario, la mayoría de los tensioactivos con una única cadena hidrocarbonada lineal forman micelas.

Además de la estructura molecular del tensioactivo y la polaridad del medio, existen otros factores como la fuerza iónica, el pH y el procedimiento utilizado para la formación del agregado que influyen de forma decisiva en el tipo de agregado formado [34]. Determinados tensioactivos aniónicos como el tridecil-6-benceno sulfonato sódico forman micelas en disoluciones acuosas con bajo contenido en electrolitos y vesículas cuando la fuerza iónica es superior a 20 mM. Los ácidos alquilcarboxílicos forman micelas en disolución acuosa a valores de pH superiores a su pK_a aparente (pK_a de la molécula cuando se encuentra formando parte de una estructura [35]); sin embargo a valores de pH próximos a su pK_a aparente (valores en el intervalo 6-9 dependiendo de la longitud de la cadena hidrocarbonada) forman vesículas. En estas condiciones, el número de moléculas

de biotensioactivo protonadas y desprotonadas es aproximadamente igual y la agregación se produce mediante la formación de puentes de hidrógeno entre sus grupos carboxílico y carboxilato e interacciones hidrofóbicas entre sus cadenas hidrocarbonadas. Finalmente, un ejemplo de la influencia del procedimiento utilizado para la preparación del agregado lo encontramos en los liposomas, agregados que no se forman espontáneamente en disolución acuosa, sino que es necesario aplicar ultrasonidos a la disolución de fosfolípidos para estimular su formación o partiendo de una disolución de estos biotensioactivos en metanol o etanol llevar a cabo una dilución con agua.

Por último, un mismo tensioactivo puede formar agregados simples y mixtos con diferente morfología. Por ejemplo, el tensioactivo catiónico bromuro de didodecildimetilamonio (BrDDA), que en disolución acuosa forma vesículas simples, en presencia de concentraciones suficientemente elevadas de tensioactivos con una única cadena hidrocarbonada como el bromuro de dodeciltrimetilamonio, forma micelas mixtas constituidas por ambos tensioactivos [32]. Por el contrario, cuando se mezclan cantidades equimoleculares de determinados tensioactivos iónicos con única cadena hidrocarbonada y con carga opuesta (por ej. octilsulfato sódico y bromuro de hexadeciltrimetilamonio [36]) que individualmente forman agregados simples micelares, los agregados mixtos formados son vesículas.

2.1.2. Concentración crítica para la formación de agregados simples y mixtos

Factores que afectan a la cca para la formación de agregados simples

La concentración crítica para la formación de agregados simples de tensioactivo depende tanto de la estructura molecular del tensioactivo como de las condiciones experimentales en las que dichos agregados se forman. A continuación se discuten los factores con mayor influencia en el valor de cca obtenido en disolución acuosa:

a) Estructura del tensioactivo. La cca de un tensioactivo es tanto menor cuanto mayor es su hidrofobicidad, ya que las interacciones hidrofóbicas son fuerzas que favorecen el proceso de agregación. Así, para tensioactivos con el mismo grupo hidrófilo y cuyo grupo hidrófobo consiste en una cadena hidrocarbonada, la cca para la formación de micelas [concentración micelar crítica (cmc)] disminuye a medida que aumenta el número de átomos de carbono de dicha cadena hidrocarbonada (n_c) de acuerdo con la ecuación:

$$\text{Log cmc} = A - B\, n_c \qquad [2]$$

donde A y B son constantes que se determinan empíricamente. En la Tabla 3 se muestran los valores de A y B (constantes de Klevens) para diferentes tipos de tensioactivos obtenidas a temperaturas comprendidas entre 20 y 55 ºC [37].

Tabla 3. Constantes de Klevens para diferentes tipos de tensoactivos

TIPO DE TENSOACTIVO	TEMPERATURA (°C)	A	B
Alquilcarboxilatos sódicos	20	1,85	0,30
Alquilcarboxilatos potásicos	25	1,92	0,29
n-Alquil-1-sulfatos sódicos	45	1,42	0,30
n-Alquil-1-sulfonatos	40	1,59	0,29
p-n-Alquilbenenosulfonatos	55	1,68	0,29
n-Alquilamonio cloruros	25	1,25	0,27
n-Alquiltrimetilamonio bromuros	25	1,72	0,30
n-Alquilpiridinio bromuros	30	1,72	0,31

La naturaleza del grupo hidrófilo también influye en el valor de cca obtenido. Los tensioactivos iónicos tienen valores de cmc superiores a los de los tensioactivos no iónicos debido a que las interacciones electrostáticas repulsivas entre los grupos hidrófilos cargados desfavorecen el proceso de agregación.

b) *Fuerza iónica*. Un aumento de la fuerza iónica origina una disminución de la cca y un aumento en el número de agregación (número de moléculas de tensioactivo que forma el agregado), siendo dicho efecto más acusado para tensioactivo iónicos que para tensioactivos no iónicos. Cuando se eleva la fuerza iónica disminuyen las fuerzas repulsivas entre los grupos cabeza cargados de las moléculas de tensioactivo iónico, lo que favorece la agregación. Este efecto se ha descrito para la formación de micelas [37] y vesículas [38]. La cca de tensioactivos no iónicos y anfóteros se reduce en presencia de sales debido a una disminución de la solubilidad de la región hidrófoba del compuesto en disolución acuosa [37].

c) *Adición de disolventes orgánicos*. La adición de disolventes orgánicos como metanol, etanol o acetonitilo, a disoluciones acuosas de tensioactivos causa un aumento de su valor de cca que puede ser debido a: 1) un aumento de la hidratación del grupo hidrófilo como consecuencia de la ruptura de la estructura del agua, 2) una disminución de la constante dieléctrica de la disolución acuosa, lo que origina un aumento de las fuerzas electrostáticas repulsivas entre los grupos cabeza del tensioactivo o 3) un aumento en la solubilidad del tensioactivo.

d) *Temperatura*. La temperatura ejerce dos efectos contrapuestos en el proceso de agregación [37]: al aumentar la temperatura de la disolución de tensioactivo disminuye la hidratación del grupo hidrófilo, lo que favorece la formación de agregados; pero dicho aumento también produce la ruptura de la estructura del agua lo que desfavorece dicho proceso. El grado en que cada uno de estos efectos se produce determina la influencia de la temperatura en la cca. Para tensioactivos iónicos y no iónicos la cca disminuye al aumentar la temperatura hasta alcanzar un mínimo a partir del cual, nuevos incrementos en la temperatura originan un aumento en la cca. Este mínimo se alcanza a 25 ºC para tensioactivos iónicos y 50 ºC para tensioactivos no iónicos. La cca de tensioactivos anfóteros como las

alquilsulfobetaínas disminuye a medida que aumenta la temperatura entre 6 y 60 ºC.

Concentración crítica para la formación de agregados mixtos

De acuerdo con el modelo de separación de fases [40], la concentración crítica de agregación para una mezcla de tensioactivos (C^*) varía en función de la fracción molar de cada uno de ellos en disolución (α_i) de acuerdo con la expresión matemática:

$$\frac{1}{C^*} = \sum_{i=1}^{n} \frac{\alpha_i}{f_i \, C_i} \qquad [3]$$

donde C_i es la cca del compuesto anfifílico "*i*" y f_i es el factor de actividad, un parámetro que expresa la contribución de las interacciones entre las moléculas de tensioactivo dentro del agregado. Aplicando el modelo de disolución regular a una mezcla binaria de tensioactivos [40]:

$$f_1 = e^{\delta x_2^2} \qquad [4]$$

$$f_2 = e^{\delta x_1^2} \qquad [5]$$

En estas expresiones, x_1 y x_2 son las fracciones molares de los tensioactivos 1 y 2 en el agregado mixto y δ es un parámetro adimensional que representa la diferencia entre las interacciones que se producen entre las moléculas de tensioactivo en los agregados simples y mixtos.

$$\delta = \frac{N \, (W_{11} + W_{22} - 2W_{12})}{RT} \qquad [6]$$

N es el número de Avogadro, W_{11} y W_{22}, las energías de interacción entre moléculas de tensioactivo en los agregados formados por un único compuesto y W_{12} la energía de interacción entre los anfifilos 1 y 2 en el agregado mixto.

Uno de los factores que más influyen en el valor de δ obtenido para un determinado agregado mixto es la naturaleza de los tensioactivos que

constituyen dicho agregado. Así, en el caso de micelas mixtas, el valor de δ se aproxima a cero y los factores de actividad se aproximan a 1 para mezclas de tensioactivos con grupos hidrófilos de la misma naturaleza (por ejemplo no iónico-no iónico). Sin embargo, para mezclas de tensioactivos con grupos hidrófilos de diferente naturaleza, el valor de δ es inferior a cero y los factores de actividad inferiores a 1. Las micelas iónicas-no iónicas presentan valores de δ que varía entre -1,0 y -5,0, siendo menores para mezclas de tensioactivos no iónicos-aniónicos que para no iónicos-catiónicos. Las interacciones más fuertes y, por lo tanto, los valores de δ y de factor de actividad más bajos se producen en micelas mixtas formadas por tensioactivos con carga opuesta (los valores de δ obtenidos para micelas aniónicas-catiónicas son inferiores a -10). Finalmente, el comportamiento de las micelas anfóteras-aniónicas es similar al observado para micelas aniónicas-catiónicas; sin embargo las interacciones son mucho más débiles entre tensioactivos anfóteros y catiónicos y entre tensioactivos anfóteros y no iónicos.

Puesto que C^* es directamente proporcional a los valores de f_i [ec. 3], la cca de agregados mixtos formados por tensioactivos con grupos hidrófilos de diferente naturaleza será siempre inferior a los valores de cca para la formación de los correspondientes agregados simples. La disminución del valor de C^* con respecto a la cca del agregado simple será tanto más acusada cuanto mayor sea la energía de interacción entre las moléculas de tensioactivo dentro del agregado mixto, o lo que es lo mismo, cuanto más negativo sea el valor de δ.

La longitud de la cadena hidrocarbonada de los tensioactivos que constituyen el agregado mixto también puede influir en el valor de δ obtenido. Así, por ejemplo, en micelas aniónicas-catiónicas, un aumento en la longitud de la cadena hidrocarbonada de ambos tensioactivos da lugar a valores del parámetro δ más negativos.

Finalmente, variables experimentales como la fuerza iónica y la temperatura ejercen su efecto en la formación de agregados mixtos disminuyendo la energía

de interacción entre moléculas de tensioactivo de diferente naturaleza causando un aumento del valor de δ y por lo tanto, del valor de *C**.

2.2. Agregados de otros compuestos anfifílicos

Otros compuestos anfifílicos con estructura molecular muy diferente a la de los tensioactivos, como colorantes, drogas terapéuticas, sales biliares, ect., forman, al igual que éstos, agregados moleculares en disolución.

Los colorantes forman agregados simples en disolución acuosa cuando su concentración alcanza un determinado valor que, con frecuencia, se denomina concentración micelar crítica [41]. La morfología de estos agregados es muy diferente a la de los agregados formados por tensioactivos, pero, al igual que en éstos, las interacciones hidrofóbicas son las principales responsables de la agregación. En disoluciones concentradas de colorantes se forman dos tipos de agregados: J-agregados, caracterizados por un desplazamiento batocrómico de la longitud de onda de máxima absorción del colorante respecto a la obtenida cuando éste se encuentra en forma monomérica, y H-agregados caracterizados por un desplazamiento hipsocrómico de dicha longitud de onda [42]. Estos agregados se forman a partir de estructuras unidimensionales con diferentes morfologías (cintas, tubos, etc.) dependiendo de la estructura molecular del colorante [43], que se ordenan en el espacio para formar agregados bidimensionales (Figura 5).

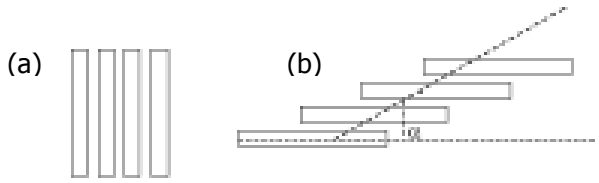


Figura 5. Esquema de la estructura bidimensional de (a) H-agregados y (b) J-agregados de colorante

Las drogas terapéuticas anfifílicas forman micelas de bajo número de agregación en disolución acuosa. Fenotiazinas como clorpromazina, promazina, prometazina y tioridazina forman micelas con geometría aproximadamente esférica con un número de agregación de 10 mediante un proceso de apilamiento ("staking") vertical de los monómeros de droga [44]. También se ha descrito la formación de micelas de antidepresivos tricíclicos [45-47], penicilinas [48, 49] antiinflamatorios no esteroideos [50], antihistamínicos, β-bloqueantes y otras drogas anfifílicas [51]. La concentración micelar crítica para la formación de micelas de diferentes tipos de drogas se muestra en la Tabla 4.

Tabla 4. Concentración micelar crítica (cmc) de diferentes drogas terapéuticas en disolución acuosa a 25ºC

DROGA	cmc (mM)	DROGA	cmc (mM)
Antidepresivos tricíclicos		***Antiinflamatorios***	
Impramine	51[a]	Ibuprofeno	180[f]
Clomipramina	23[a]	***Antihistamínicos***	
Amitriptilina	25[b]	Difenhidrina	122[g]
Nortriptilina	20[b]	***β-bloqueantes***	
Desipramina	35[b]	Propranolol	124[g]
Doxepina	69[c]	Alprenolol	100[g]
Butriptilina	49[c]	***Anestésicos locales***	
Antibióticos		Lidocaína	195[g]
Cloxacilina	93[d]	Tetracaína	38[g]
Dicloxacilina	86[d]	***Relajantes musculares***	
Penicilina V	40[e]	Orfenadrina	10[g]

Referencias: [a][45], [b][46], [c][47], [d][48], [e][49], [f][50], [g][51]

Las investigaciones realizadas sobre el efecto de la temperatura en el valor de cmc de drogas terapéuticas han demostrado que muchas de estas drogas (por ej. imipramina, clomipramina [45], cloxacilina, dicloxacilina [48] y penicilina V [49]) se comportan de forma similar a los tensioactivos; la cmc disminuye al

aumentar la temperatura hasta alcanzar un mínimo a 25 ºC, a partir del cual, nuevos incrementos en la temperatura originan un aumento en la cmc. Existen algunas excepciones como es el caso de la butriptilina, cuya cmc aumenta ligeramente a medida que aumenta la temperatura de la disolución entre 10 y 40 ºC [47].

Las sales biliares forman dos tipos de agregados en disolución y, por lo tanto poseen dos valores de cmc [11]. La primera cmc corresponde a la formación de agregados helicoidales constituidos por dos monómeros en el caso del colato sódico y tres en el caso del deoxicolato sódico [52] (cmc_1 del deoxicolato sódico = 10 mM [11]) y la segunda a la formación de estructuras cilíndricas constituidas por agregados helicoidales (cmc_2 del deoxicolato sódico = 60 mM [11]).

Los compuestos anfifílicos con estructura molecular diferente a la de los tensioactivos también tienen capacidad para formar agregados mixtos. Se han realizado numerosos estudios sobre la agregación en mezclas binarias de tensioactivo y otros compuestos anfifílicos de diferente naturaleza: colorantes [41,53-64], drogas terapéuticas [51, 65-70], sales biliares [71-74], clorofila [75], complejos [76] y otros compuestos orgánicos [77]. A continuación se discutirán los aspectos más relevantes de la agregación en mezclas binarias tensioactivo-colorante y tensioactivo-droga anfifílica por ser de especial interés para las investigaciones realizadas en esta Tesis Doctoral.

2.3. Agregados mixtos colorante-tensioactivo

Los colorantes forman agregados mixtos con tensioactivos iónicos, no iónicos y anfóteros. La adición de un tensioactivo iónico en concentración inferior a su cca a una disolución acuosa de colorante con carga opuesta y concentración del mismo orden a la del tensioactivo puede dar lugar a la formación de un agregado coloidal o formar una sal insoluble, dependiendo fundamentalmente de la estructura molecular del colorante. Así, las disoluciones de los tensioactivos

catiónicos bromuro de 1-carbetoxipenta-deciltrimetilamonio [53] y bromuro de cetilpiridinio (BrCP) [54] forman agregados mixtos con los colorantes aniónicos rojo fenol y el ácido 8-hidroxiquinolein-5-sulfónico, respectivamente. Sin embargo, estos mismos tensioactivos precipitan en presencia de otros colorantes como el azul de bromofenol y el verde de bromocresol en el caso del bromuro de 1-carbetoxipentadecil-trimetilamonio [53], y el rojo de bromopirogalol mediante su interacción con BrCP [55]. En cualquier caso, las mezclas de tensioactivo y colorante que precipitan como sales insolubles también pueden dar lugar a la formación de agregados mixtos colorante-tensioactivo cuando la concentración de éste último alcanza un valor ligeramente inferior a su cmc, produciéndose entonces la redisolución del precipitado.

En lo que se refiere a los agregados mixtos de colorante con tensioactivos no iónicos, anfóteros o iónicos de igual signo, como ejemplo citaremos la formación de micelas mixtas de remazol F3B o amarillo brillante GNL (colorantes aniónicos) y dodecilsulfato sódico (SDS, tensioactivo aniónico), octilfenol-nonaoxietilen éter (tensioactivo no iónico) o dodecildimetilamoniopropiosulfato (tensioactivo anfótero) [41].

Los modelos desarrollados para explicar el comportamiento de mezclas de tensioactivos en disolución acuosa son igualmente válidos para mezclas de tensioactivo-colorante. El modelo de disolución regular se ha aplicado con éxito a mezclas de un colorante aniónico con un tensioactivo aniónico, no iónico o anfótero, siendo éste último tipo de tensioactivo para el que se obtuvieron los valores más negativos del parámetro δ [41]. No obstante, las energías de interacción más elevadas se producen en agregados mixtos de tensioactivo y colorante con carga opuesta. Así, el colorante aniónico Coomassie Azul Brillante G (CABG) forma agregados mixtos con diferentes tensioactivos catiónicos a concentraciones de éstos inferiores a sus respectivas concentraciones micelares críticas en 2 o 3 órdenes de magnitud [56]. Las interacciones electrostáticas entre los grupos hidrófilos de las moléculas de colorante y tensioactivo son especialmente fuertes para colorantes que poseen grupos $-SO_3^-$ ya que la carga

negativa se encuentra localizada en el grupo iónico y no deslocalizada entre éste y el anillo aromático como ocurre en colorantes con grupos $–COO^-$. Las interacciones hidrofóbicas también son determinantes en la formación de agregados de colorante y tensioactivo con carga opuesta, aumentando la energía de la interacción a medida que aumenta la hidrofobicidad de ambos compuestos anfifílicos [57, 58].

El proceso de formación de agregados mixtos de tensioactivo y colorante con carga opuesta se inicia con la formación de un par iónico [57, 59-64]. Simončič y col. [57, 63, 64] proponen un modelo mononuclear para la formación de agregados de tensioactivo y colorante de carga opuesta en disoluciones diluidas, en el que el colorante es el elemento central con el que interaccionan moléculas de tensioactivo para formar agregados de diferente estequiometría a medida que aumenta la concentración de tensioactivo en disolución. Para un colorante aniónico (C^-) y un tensioactivo catiónico T^+:

$$C^- + T^+ \leftrightarrow CT$$
$$CT + T^+ \leftrightarrow CT_2^+$$
$$\cdots\cdots$$
$$CT_{n-1}^{+(n-2)} + T^+ \leftrightarrow CT_n^{+(n-1)}$$

El grado de enlace tensioactivo-colorante, β_C, parámetro que representa el número de moléculas de tensioactivo enlazadas a cada molécula de colorante en el agregado mixto, viene dado por:

$$\beta_C = \frac{m_T - m_{T,M}}{m_C} \qquad [7]$$

donde m_T es la concentración total de tensioactivo, $m_{T,M}$ la concentración de tensioactivo en forma monomérica y m_C la concentración total de colorante.

2.4. Agregados mixtos droga anfifílica-tensioactivo

Las mezclas acuosas de droga anfifílica y tensioactivo se comportan de forma similar a las mezclas de tensioactivos. Las drogas y los tensioactivos de la misma naturaleza generalmente forman micelas mixtas. Por ejemplo, se ha descrito la formación de micelas mixtas del tensioactivo catiónico bromuro de dodeciltrimetilamonio (BrDTA) y amitriptilina (droga catiónica) [65]. Sin embargo, la adición de un tensioactivo iónico a una disolución de droga con carga opuesta puede dar lugar a la formación de micelas o vesículas mixtas, dependiendo de la estructura molecular, la fracción molar y la concentración total de ambos compuestos anfifílicos en disolución. Así, el tensioactivo catiónico bromuro de tetradeciltrimetilamonio (BrTTA) y el tensioactivo aniónico SDS forman micelas mixtas con la droga aniónica ibuprofeno y la droga catiónica lidocaína, respectivamente, independientemente de la fracción molar de droga y tensioactivo en disolución [66]. Sin embargo, con otras drogas catiónicas como la orfenadrina [66], imipramina, difenhidramina y tetracaína [67], el SDS forma tanto vesículas como micelas, dependiendo el tipo de agregado formado de la fracción molar del SDS en la disolución y la concentración total [SDS]+[droga]. Finalmente, los ácidos dodecanóico y decanóico forman vesículas con numerosas drogas catiónicas: aprenolol, amitriptilina, difenhidramina, lidocaína, orfenadrina, propranolol y tetracaína, a fracciones molares de biotensioactivo que varían en función del ácido alquilcarboxílico y la droga de que se trate, siendo estos valores siempre superiores a 0,4 [51].

El modelo de disolución regular también es válido para mezclas de tensioactivos y drogas de igual [65] o diferente [68-70] naturaleza. Como en el caso de mezclas de tensioactivos y de tensioactivos y colorantes, las mayores energías de interacción se producen cuando el tensioactivo y la droga que forman el agregado mixto tienen carga opuesta, produciéndose importantes disminuciones de la cca del agregado mixto con respecto a la de los agregados simples. Este es el caso de los agregados mixtos formados por SDS con fenotiacinas [68], antihistaminas [69] y antidepresivos tricíclicos [70]. En la

Figura 6 se muestra la variación de la cca para la formación de agregados mixtos de SDS y prometacina, promacina, clorpromazina o tioridacina en función de la fracción molar de droga en la disolución acuosa.

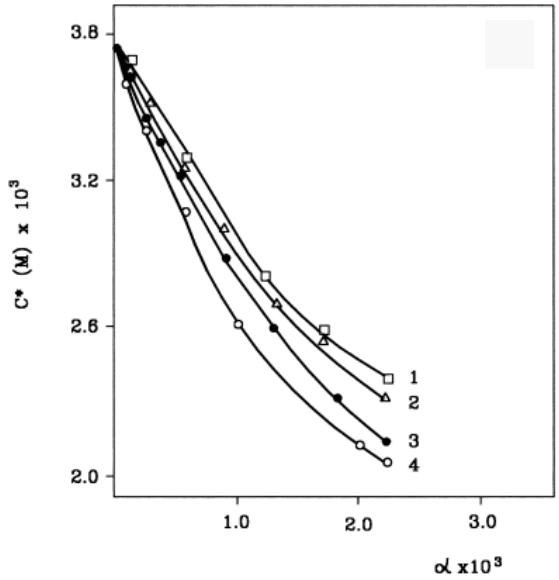


Figura 6. Variación de la cca de agregados mixtos de SDS y diferentes fenotiazinas: (1) prometazina, (2) promazina, (3) clorpromazina y (4) tioridazina, en función de la fracción molar de fenotiazina

2.5. Agregación en mezclas ternarias colorante-tensioactivo-anfifilo

La adición de determinadas sustancias químicas (por ej. compuestos anfifílicos) a disoluciones de tensioactivo iónico y colorante con carga opuesta puede originar una disminución del grado de enlace tensioactivo-colorante, siempre que la sustancia química adicionada compita con el colorante para interaccionar con el tensioactivo [57, 64, 78-80]. En presencia de dicha sustancia química, el grado de enlace tensioactivo-colorante, β_C, viene dado por [57]:

$$\beta_C = \frac{m_T - m_{T,M} - \beta_S \, m_S}{m_C} \qquad [8]$$

donde m_T, $m_{T,M}$ y m_C tienen el mismo significado que en la ec. [7], m_S es la concentración de sustancia química adicionada a la disolución acuosa y β_S es el grado de enlace tensioactivo-sustancia química.

La diferencia entre el valor de β_C obtenido para una determinada concentración de tensioactivo y colorante en presencia y ausencia de la sustancia química varía en función de β_S y m_S (comparar la ec. [7] en la sección 2.3 con la ec. [8]).

Hasta la realización de esta Tesis, las únicas sustancias químicas para las que se había descrito este efecto eran tensioactivos no iónicos e iónicos. La adición de un tensioactivo no iónico a concentraciones superiores a su cmc a disoluciones de colorante y tensioactivo iónico con carga opuesta origina una disminución del parámetro β_C debida a la formación de agregados mixtos tensioactivo iónico-tensioactivo no iónico [57, 64, 78, 79]. Estudios realizados en mezclas ternarias de colorante aniónico (Naranja ácido 7 o Rojo ácido 88), tensioactivo catiónico (BrDTA o hexadeciltrimetil- amonio) y diferentes tensioactivos no iónicos: Triton X-100, Brij 35, Brij 30, Brij 56, Brij 58, Brij 700 o Tween 20, han demostrado que la estructura molecular del tensioactivo no iónico influye de forma decisiva en el efecto que dicho tensioactivo ejerce en la formación de agregados tensioactivo catiónico-colorante aniónico [78, 79]. A medida que aumenta el número de unidades oxietileno en el tensioactivo no iónico aumenta la carga parcial negativa en los enlaces –O y, por lo tanto, las interacciones electrostáticas atractivas entre el tensioactivo no iónico y el tensioactivo catiónico, lo que da lugar a una disminución más acusada de las interacciones tensioactivo catiónico-colorante aniónico.

Por otra parte, tensioactivos aniónicos del tipo alquilbenceno sulfonato, alquilsulfonato y alquilsulfato disminuyen el grado de enlace entre el tensioactivo catiónico BrDDA y el colorante aniónico CABG debido a la competencia que se establece entre el colorante y los tensioactivos aniónicos para formar agregados mixtos con BrDDA [80, 81]. Se han calculado valores de grado de enlace tensioactivo aniónico-BrDDA que varían entre 4,3 y 6,9 dependiendo del

tensioactivo aniónico de que se trate [81]. Para tensioactivos aniónicos pertenecientes al mismo grupo estructural, el grado de enlace aumenta a medida que aumenta la longitud de su cadena hidrocarbonada debido a un aumento de las interacciones hidrofóbicas BrDDA-tensioactivo aniónico.

3. COMPUESTOS HIDROTRÓPICOS

3.1. Estructura molecular

Los compuestos hidrotrópicos, también denominados hidrótropos, al igual que los compuestos anfifílicos, poseen dos regiones en su molécula, una hidrófoba y otra hidrófila, pero su región hidrófoba tiene un tamaño inferior a la de las moléculas anfifílicas. La estructura molecular de estos compuestos es muy variada. En la Figura 7 se muestran algunos ejemplos de compuestos hidrotrópicos.

Son compuestos hidrotrópicos [82-84] :

a) Determinados compuestos aromáticos, como por ejemplo: sulfonatos (benceno sulfonato, benceno disulfonato y p-hidroxibencenosulfonato sódico), alquilbenceno sulfonatos (p-tolueno, p-xileno y cumeno sulfonato sódico), carboxilatos (salicilato, acetilsalicilato, benzoato, p-amino benzoato y 4-piridinocarboxilato sódico), alcoholes (resorcinol, catecol, pirogalol y β-naftoles), alcaloides (cafeína y nicotina) y aminas (procaína).

b) Compuestos orgánicos no aromáticos como ácido ascórbico y etanol.

c) Tensioactivos con un número de átomos de carbono en su cadena o cadenas hidrocarbonadas igual o inferior a cuatro: alquil carboxilatos, alquilmonoglicol sulfatos y haluros de tetraalquilamonio.

d) Determinados aminoácidos y derivados de aminoácidos como el ácido glutámico y la N-cocoil-N-metil-β-alanina.

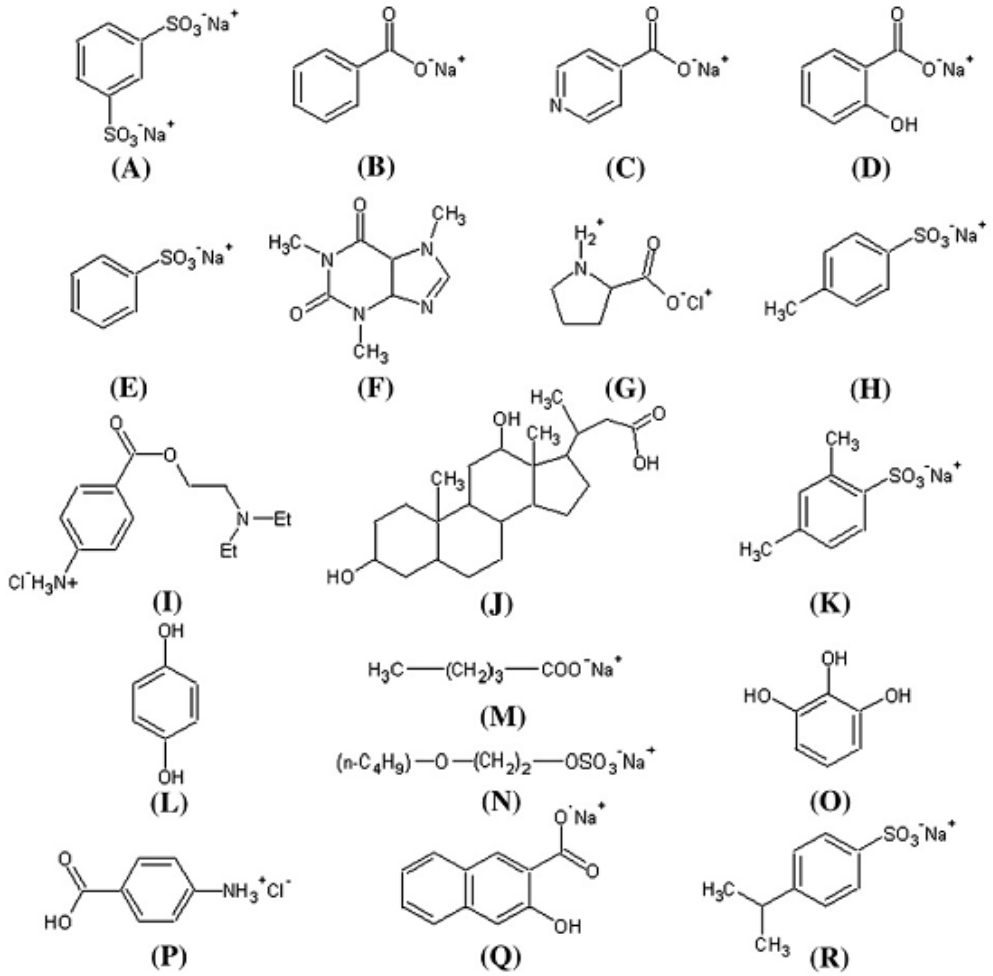


Figura 7. Estructura molecular de los hidrótropos: (A) 1,3-bencenodisulfonato sódico, (B) benzoato sódico, (C) 4-piridinocarboxilato sódico, (D) salicilato sódico, (E) bencenosulfonato sódico, (F) cafeína, (G) prolina, (H) p-tolueno sulfonato sódico, (I) procaína, (J) ácido pregnano-20-3,12-dihidroxi carboxílico, (K) p-xileno sulfonato sódico, (L) resorcinol, (M) n-pentanoato sódico, (N) butil monoglicol sulfato sódico, (O) pirogalol, (P) ácido 4-aminobenzóico, (Q) 3-hidroxi-2-naftoato sódico, (R) cumeno sulfonato sódico

3.2. Propiedades de los hidrótropos. Capacidad de agregación

La propiedad más característica de los compuestos hidrotrópicos es su capacidad para aumentar la solubilidad de los compuestos orgánicos en agua, con frecuencia en varios órdenes de magnitud, propiedad en la que se basan la mayoría de las aplicaciones industriales de estos compuestos. Los mecanismos propuestos para explicar este efecto son [82-85]: la formación de complejos hidrótropo-compuesto orgánico de elevada solubilidad, la modificación de la estructura del agua y la solubilización del compuesto orgánico en agregados de hidrótopo. El mecanismo a través del cual ejerce su efecto cada hidrótropo depende fundamentalmente de su estructura molecular y de su concentración. En la mayoría de los casos la máxima capacidad de solubilización se alcanza a concentraciones de hidrótropo a la que existen agregados en disolución.

Los compuestos hidrotrópicos, al igual que los compuestos anfifílicos, disminuyen la tensión superficial del agua, si bien dicha disminución es menos acusada que en el caso de los anfifilos. Así, por ejemplo, la tensión superficial del agua disminuye desde 72 mN m^{-1} hasta 40 mN m^{-1} en presencia de una concentración de p-tolueno sulfonato sódico de 0,25 M [85]. A mayores concentraciones de hidrótropo la tensión superficial permanece constante, lo que se ha interpretado como una evidencia de la asociación de moléculas de hidrótropo en disolución.

Tabla 5. Concentración mínima de hidrótropo (CMH) para la formación de agregados de diferentes compuestos hidrotrópicos

HIDRÓTROPO	CMH (M)	HIDRÓTROPO	CMH (M)
2,4-dihidroxi benzoato sódico	0,12	Salicilato sódico	1,05
Butil monoglucosulfato sódico	0,70	Prolina	0,75
p-Tolueno sulfonato sódico	[a]0,25	Pirogalol	1,05
Xileno sulfonato sódico	0,40	Catecol	0,8
Cumeno sulfonato sódico	0,10	Resorcinol	0,8

Datos obtenidos de la Ref.[83]; [a][85]

De forma similar a los anfifilos, se requiere una concentración mínima de compuesto hidrotrópico para que se formen agregados. Esta concentración se denomina concentración mínima de hidrótopo (CMH) [86, 87]. En la Tabla 5 se muestran los valores de CMH para diferentes compuestos hidrotrópicos. En general, la CMH es muy superior a la cca de los anfifilos, aunque puede reducirse mediante la adición de compuestos orgánicos de reducido tamaño como n-alcoholes [88].

La estructura de los agregados formados por hidrótopos no se conoce con exactitud, si bien existe la certeza de que las asociaciones moleculares de compuestos hidrotrópicos son más abiertas y menos organizadas que las constituidas por compuestos anfifílicos. Esto se debe al reducido tamaño de la región apolar de los hidrótropos, lo que reduce las interacciones hidrofóbicas entre moléculas con respecto a las que se produce en agregados de anfifilos. Según estudios realizados con rayos X [86], los alquilbenceno sulfonatos, p-ter-butilbenceno, cumeno, p-tolueno y 3,4-dimetilbenceno sulfonato sódico, forman asociaciones planas mediante la interacción de los extremos opuestos al grupo polar de los anillos aromáticos de las moléculas de hidrótopo. Las superficies hidrófobas formadas interaccionan entre sí estructurándose en capas. Sólo en el caso del p-tolueno sulfonato sódico se produce un cierto solapamiento lateral de los anillos aromáticos, aunque las interacciones laterales son más débiles que las interacciones entre capas. Por otra parte, de acuerdo con los resultados obtenidos mediante la técnica de dispersión de neutrones de pequeño ángulo (SANS) [87], el butilbenceno sulfonato forma agregados elipsoidales a concentraciones aproximadamente ocho veces superior a su CMH mediante el apilamiento de los anillos aromáticos de las moléculas de hidrótropo.

3.3. Efecto de los hidrótopos en la agregación de tensioactivos

Los compuestos hidrotrópicos modifican el comportamiento de los tensioactivos en disolución acuosa, influyendo en su capacidad de agregación. La

adición de un hidrótropo a disoluciones de tensioactivo puede originar cambios en:

a) *La temperatura mínima para la coacervación de tensioactivos no iónicos* [punto de nube, "cloud point (CP)" en terminología anglosajona]. Se pueden distinguir dos grupos de hidrótropos [82]: 1) Los que aumentan el CP debido a que originan la ruptura de la estructura del agua, lo que desfavorece las interacciones hidrofóbicas entre moléculas de tensioactivo y, por lo tanto, la formación de agregados. Pertenecen a este grupo la nicotinamida, los bromuros de tetrapropil y tetrabutil amonio [84], el salicilato sódico [89], el xileno sulfonato sódico, p-tolueno sulfonato sódico y clorobenceno sulfonato sódico [90]. 2) Los que disminuyen el CP debido a que interaccionan más efectivamente con el agua que los tensioactivos, lo que origina una disminución de la solvatación de las moléculas de tensioactivo y favorece su agregación. La prolina, el pirogalol y el resorcinol se encuentran en este grupo [89].

b) *La concentración micelar crítica (cmc)*. El efecto que la adición de hidrótopos ejerce en el valor de la cmc depende de la naturaleza del compuesto hidrotrópico y del tensioactivo. Los hidrótopos iónicos disminuyen la cmc de tensioactivos iónicos de carga opuesta debido a que neutralizan la carga del grupo cabeza de las moléculas de tensioactivo que forman el agregado, lo que evita que se produzcan fuerzas electrostáticas repulsivas entre ellos y favorece la micelización. Así, por ejemplo, se ha descrito la disminución de la cmc de diferentes tensioactivos catiónicos de alquilpiridinio y alquiltrimetilamonio en presencia de los hidrótropos aniónicos salicilato [89], xileno sulfonato, p-tolueno sulfonato y clorobenceno sulfonato [90] sódico. Sin embargo, los hidrótropos iónicos apenas influyen en el valor de cmc obtenido para tensioactivos iónicos de igual carga [90]. Por otra parte, la cmc de tensioactivos catiónicos también disminuye en presencia de hidrótropos aromáticos no iónicos como el pirogalol, el resorcinol y la prolina [89]. En este caso, interacciones π-catión

entre el anillo aromático del hidrótropo y el grupo cabeza del tensioactivo, son probablemente las responsables de la disminución del valor de la cmc. Por último, la cmc de determinados tensioactivos no iónicos apenas se ve afectada por la adición de hidrótropos, como es el caso del Tritón X-102 [90]. Sin embargo la cmc de otros tensioactivos no iónicos como el dodecil-6-etoxilato aumenta de forma apreciable en presencia de determinados hidrótropos (por ej. p-tolueno sulfonato sódico), siendo dicho aumento proporcional a la concentración de hidrótropo en disolución [85].

c) *La morfología y el tamaño de los agregados*. La adición de un hidrótropo iónico a una disolución de micelas iónicas con carga opuesta puede originar un aumento del grado de empaquetamiento de las moléculas de tensioactivo en el agregado debido a la neutralización de su carga, produciéndose, bien un crecimiento axial de la micela (transformación de micela esférica en vermiforme) [83, 91-93] o la transición de agregado micelar a vesícula [94]. El número de agregación de determinados tensioactivos iónicos aumenta de forma espectacular en presencia de hidrótopos de carga opuesta, dependiendo dicho aumento de la relación molar tensioactivo/hidrótopo en disolución. Así, el número de agregación del tensioactivo aniónico SDS aumenta desde 79 a 721 en presencia del hidrótropo catiónico p-toluidina adicionado en una relación molar hidrótropo/tensioactivo de 0,6 [94]. Finalmente, los hidrótopos permiten obtener vesículas de tamaño controlado modificando la relación tensioactivo/hidrótropo. Así, por ejemplo, el tamaño medio de liposomas de lecitina aumenta linealmente al aumentar la relación molar lecitina/xileno sulfonato sódico [95].

4. MÉTODOS BASADOS EN LA MEDIDA DE PARÁMETROS DE AGREGACIÓN

Estos métodos analíticos se basan en la influencia que determinados sustancias químicas (analitos) ejercen sobre parámetros característicos de

agregados supramoleculares de compuestos anfifílicos. El primer método basado en la medida de parámetros de agregación, el método de agregados mixtos, fue desarrollado por nuestro grupo de investigación a mediados de los 90 [96]. A día de hoy, el fundamento teórico y el campo de aplicación de este método ha sido establecido y su utilidad para la determinación de compuestos anfifílicos de diferente naturaleza demostrada [39, 56, 68-70, 96-102]. Más recientemente, se han desarrollado diferentes métodos basados en agregación competitiva que utilizan medidas fotométricas [80, 103] o volumétricas (método de grado de enlace tensioactivo-colorante [81]) como señal analítica. A continuación comentaremos los aspectos teóricos y prácticos más relevantes de estos métodos.

4.1. Método de agregados mixtos

4.1.1. *Fundamento del método*

Este método se basa en la variación que se produce en la concentración crítica de agregación de mezclas de compuestos anfifílicos (C*) en función de la concentración de cada uno de los anfifilos en disolución (ec. [3]). Sobre la base teórica del modelo de separación de fases [40], se ha deducido una expresión matemática [97] que permite obtener calibraciones lineales para la determinación de compuestos anfifílicos:

$$1 - \frac{C_T^M}{C_T} = \frac{1}{f_A\, C_A} C_A^M \qquad [9]$$

donde C_T y C_A representan la cca del tensioactivo que se añade a la muestra para formar agregados mixtos con el analito y la cca del analito, respectivamente, C_T^M es la concentración de tensioactivo en forma monomérica en la disolución y C_A^M y f_A la concentración de analito en la muestra y su factor de actividad en el agregado mixto, respectivamente.

La curva de calibrado se construye representando el parámetro 1-(C_T^M/C_T) frente a C_A^M. El intervalo de linealidad para la determinación del analito anfifílico se obtiene para concentraciones del mismo a las que f_A se mantiene constante.

Los parámetros C_T^M y C_T se obtienen mediante valoración usando el tensioactivo como reactivo valorante y un colorante como sonda fotométrica. En la Figura 8 se muestran curvas de valoración típicas obtenidas en ausencia (curva 1) y en presencia de diferentes concentraciones de analito (curvas 2-4). El cálculo de C_T^M y C_T se realiza fácilmente a partir de los volúmenes de tensioactivo consumido en las valoraciones en ausencia (V_T) y presencia (V_T^M) de analito.

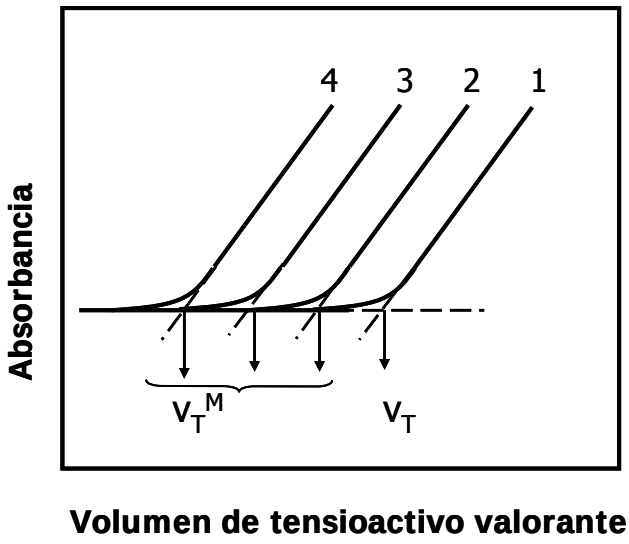


Figura 8. Curvas de valoración típicas del método de agregados mixtos obtenidas en (1) ausencia y (2-4) presencia de diferentes concentraciones de analito

4.1.2. *Características analíticas*

La sensibilidad del método de agregados mixtos, expresada como pendiente de la recta de calibrado, depende de la cca del analito anfifílico (C_A) y de su

factor de actividad en el agregado (f_A). La sensibilidad aumenta a medida que disminuye el valor de ambos parámetros. Por lo tanto, este método es especialmente adecuado para la determinación de compuestos anfifílicos con bajos valores de cca, como es el caso de los tensioactivos no iónicos (cca = 10^{-4}-10^{-5} M). Con el fin de mejorar la sensibilidad para la determinación de anfifilos con valores de cca elevados, es decir anfifilos iónicos (cca = 10^{-1}-10^{-3} M), se han propuesto diferentes estrategias:

1) Realizar las medidas en un medio con elevada fuerza iónica, lo que origina una importante disminución de la cca de los analitos [98].

2) Formar agregados premicelares inducidos por colorante; la cca para la formación de estos agregados es entre 3 y 4 órdenes de magnitud inferior a la cca para la formación de micelas [56, 68].

3) Seleccionar un tensioactivo valorante adecuado. Los valorantes que proporcionan mayor sensibilidad para la determinación de anfifilos iónicos son tensioactivos iónicos con carga opuesta a la del analito [68-70, 98-100], ya que los valores del parámetro δ más negativos se obtienen para agregados catiónicos-aniónicos. También se ha obtenido una sensibilidad elevada para la determinación de anfifilos catiónicos utilizando Triton X-100 como tensioactivo valorante [99] debido a las fuertes interacciones π-catión que se producen entre el anillo aromático del tensioactivo no iónico y el grupo hidrófilo de los analitos.

En lo que se refiere a la selectividad del método de agregados mixtos, la metodología se basa en una propiedad general de los compuestos anfifílicos y por lo tanto, no es selectiva para un determinado anfifilo. Los compuestos anfifílicos interferentes presentes en la muestra deben, por tanto, eliminarse en una etapa previa a la determinación. Sin embargo, no se producen interferencias debidas a compuestos orgánicos no anfifílicos a concentraciones relativamente altas [98], ya que su solubilización en los agregados apenas afecta a la cca. Finalmente, las interferencias debidas a sales inorgánicas se evitan ajustando la fuerza iónica del medio de valoración.

Otras características analíticas destacables de esta metodología son versatilidad, simplicidad y bajo coste.

4.1.3. *Aplicaciones*

Las aplicaciones de la metodología de agregados mixtos se han centrado en la determinación de tensioactivos en muestras medioambientales [100, 101] y productos formulados [100, 102], aditivos en alimentos [39] y drogas anfifílicas en fármacos [68-70]. La concentración total de tensioactivos de la misma naturaleza (no iónicos, aniónicos o catiónicos) es un parámetro de interés para el control de la calidad de productos formulados y un parámetro indicativo de la contaminación de aguas naturales y residuales. Las respuestas obtenidas para diferentes tensioactivos de la misma naturaleza mediante el método de agregados mixtos son aditivas e independientes del peso molecular del tensioactivo, lo que permite obtener medidas exactas de estos parámetros globales. De los métodos desarrollados para el control de calidad de productos formulados, alimentos y fármacos cabe destacar la simplicidad del tratamiento de muestra, ya que sólo se requiere la disolución de los analitos en un disolvente adecuado (por ej. agua destilada, etanol, etc.).

4.2. Metodologías basadas en agregación competitiva

4.2.1. *Métodos fotométricos*

Estos métodos se basan en la medida de la absorbancia de una disolución que contiene un colorante y un tensioactivo utilizado como reactivo, en ausencia y en presencia de un analito anfifílico. En ausencia del analito, el tensioactivo reactivo y el colorante interaccionan, lo que origina un cambio en la absorbancia medida para el colorante. La adición del analito a la disolución de tensioactivo reactivo y analito origina nuevos cambios en la absorbancia medida para el

colorante, debido a la competencia que se establece entre el analito y el colorante para interaccionar con el tensioactivo reactivo.

Se han desarrollado dos métodos basados en agregación competitiva y medidas de absorbancia. El primero propone la determinación no específica de tensioactivos catiónicos de dialquildimetilamonio basada en la medida de la absorbancia del colorante aniónico CABG en presencia del tensioactivo aniónico SDS [80]. El CABG forma agregados mixtos con los analitos mediante interacciones electrostáticas e hidrofóbicas, lo que origina una disminución en la absorbancia del colorante. La adición de SDS, usado como tensioactivo reactivo, causa una disminución del grado de enlace analito-colorante que se traduce en un aumento de la absorbancia medida para el colorante. El método propuesto permite determinar tensioactivos de alquildimetilamonio en aguas residuales a concentraciones del orden de los μg L^{-1}. La elevada sensibilidad del método se debe a que los agregados mixtos analito-SDS se forman a concentraciones de los tensioactivos de dialquildimetilamonio muy bajas, tres órdenes de magnitud inferiores a sus respectivos valores de cca. Además, el método es muy selectivo; otros tensioactivos catiónicos de amonio cuaternario y de piridinio y tensioactivos aniónicos y no iónicos no interfieren en la determinación.

El segundo método, desarrollado muy recientemente [103], propone la determinación de la droga catiónica gemfibrozil basada en la medida de la absorbancia del colorante aniónico Eriocromo Azul Negro R (EANR) en presencia del tensioactivo catiónico BrDDA [103]. La absorbancia de las disoluciones de EANR y BrDDA disminuye en presencia de gemfibrozil, debido a la competencia que se establece entre colorante y droga para interaccionar con el tensioactivo. El intervalo de linealidad del método es de 0,15-6,0 mg L^{-1} y ha sido aplicado a la determinación de gemfibrozil en fármacos.

4.2.2. *Método volumétrico*

El parámetro de medida utilizado por el método de grado de enlace tensioactivo-colorante para la cuantificación de los analitos es la diferencia entre

la cantidad de un tensioactivo iónico necesaria para alcanzar un determinado grado de enlace entre el tensioactivo y un colorante de carga opuesta, en ausencia y presencia de analito. Este parámetro se obtiene a partir de medidas volumétricas utilizando al propio colorante como sonda fotométrica. Esta metodología se ha aplicado a la determinación no específica de tensioactivos aniónicos en aguas residuales basándose en la disminución que estos tensioactivos causan en el grado de enlace entre el tensioactivo catiónico BrDDA y el colorante aniónico CABG [81]. Las respuestas obtenidas para los distintos tensioactivos aniónicos son aditivas e independientes del peso molecular del tensioactivo. El método desarrollado permite determinar concentraciones totales de tensioactivos aniónicos en el intervalo 0,3-3,0 mg L^{-1} sin la interferencia de tensioactivos no iónicos presentes en las muestras.

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PARTE EXPERIMENTAL

MÉTODO DE GRADO DE ENLACE TENSIOACTIVO – COLORANTE

MÉTODO DE GRADO DE ENLACE TENSIOACTIVO-COLORANTE

La disminución que se produce en el grado de enlace entre un colorante y un tensioactivo con carga opuesta en presencia de determinadas sustancias químicas puede utilizarse con fines analíticos. Considerando que la sustancia química que origina la disminución del grado de enlace entre el tensioactivo, usado como reactivo, y el colorante es el analito cuya concentración se desea determinar y basándonos en la ecuación [8] de la Introducción de esta Memoria, el valor de β_C en presencia de dicho analito vendrá dado por:

$$\beta_C = \frac{m_T^* - m_{T,M} - \beta_A\, m_A}{m_C} \qquad [9]$$

donde m_T^* es la cantidad de tensioactivo reactivo necesaria para alcanzar el mismo grado de enlace en presencia y ausencia de analito, β_A el grado de enlace tensioactivo-analito y m_A la concentración de analito.

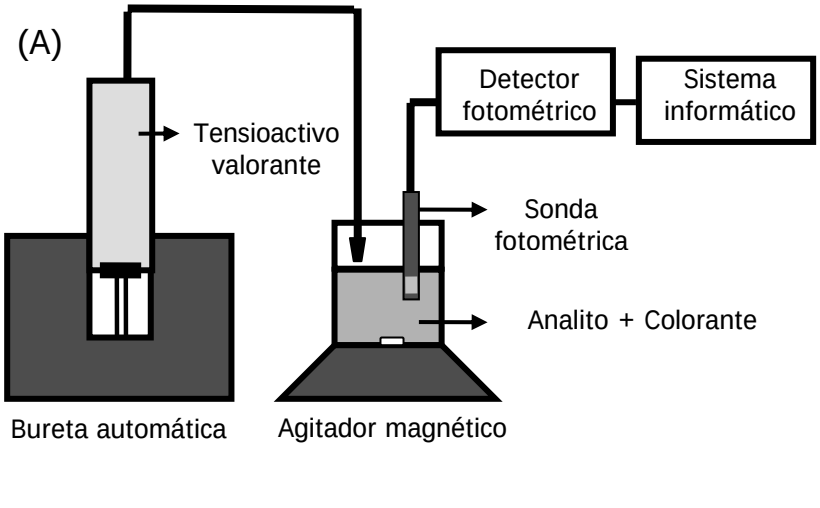
Asumiendo que la concentración de tensioactivo reactivo en forma monomérica ($m_{T,M}$) no depende de la concentración de analito presente en la disolución acuosa, la ecuación [7] de la Introducción de esta Memoria y la ecuación [9] pueden combinarse para obtener:

$$m_T^* - m_T = \beta_A\, m_A \qquad [10]$$

La curva de calibrado se construye representando el parámetro m_T^*-m_T frente a m_A. El intervalo de linealidad para la determinación del analito se corresponde con concentraciones del mismo a las que β_A se mantiene constante.

Los parámetros m_T^* *y* m_T se obtienen mediante valoración usando el tensioactivo reactivo como valorante y midiendo los cambios de absorbancia del colorante debidos a la formación de agregados mixtos tensioactivo reactivo-colorante. El tensioactivo reactivo y el colorante usados en los métodos desarrollados en esta Tesis han sido el BrDDA y el CABG, respectivamente. El

sistema de medida utilizado, un valorador 794 Basic Titrino de Metrohm, se muestra en la Figura 1.



(B)

Figura 1. (A) Esquema del sistema de medida usado para la implementación del método de grado de enlace tensioactivo-colorante y (B) fotografía del mismo

En el vaso de valoración se coloca una disolución acuosa que contiene el colorante y la muestra con el analito o analitos a determinar y en ella, se introduce la sonda fotométrica que permitirá medir los cambios de absorbancia del colorante a medida que desde la bureta automática, se adicione el

tensioactivo valorante. La disolución se agita de forma continua para su homogenización. La absorbancia medida a una longitud de onda adecuada para los diferentes volúmenes de valorante añadido son tomadas y tratadas por un sistema informático que consiste en un ordenador personal equipado con un micropocesador Pentium 4, un sistema operativo Microsoft Windows XP y el programa de Metrohm TiNet 2.5 Light. En la Figura 2 se muestran varias curvas de valoración típicas obtenidas en ausencia (curva 1) y en presencia de diferentes concentraciones de analito (curvas 2-4). El cálculo de m_T^* y m_T se realiza a partir de los volúmenes de tensioactivo consumido en las valoraciones en presencia (V_T^*) y ausencia (V_T) de analito, conocida la concentración de tensioactivo reactivo en la disolución valorante y el volumen inicial de disolución en el vaso de valoración (25 mL). Los volúmenes consumidos en las valoraciones son automáticamente obtenidos por la aplicación informática.

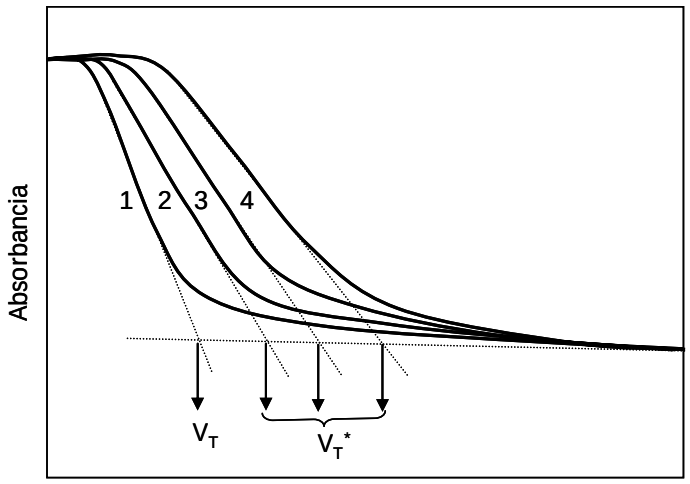


Figura 2. Variación de la absorbancia del colorante CABG en función de la concentración de DDABr en (1) ausencia de analito y (2-4) presencia de diferentes concentraciones de analito

PARTE I

DETERMINACIÓN DE COMPUESTOS ANFIFÍLICOS

CAPÍTULO 1

DETERMINACIÓN NO ESPECÍFICA DE TENSIOACTIVOS

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Assessment of the surfactant-dye binding degree method as an alternative to the methylene blue method for the determination of anionic surfactants in aqueous environmental samples

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The surfactant to dye binding degree (SDBD) method is proposed for the routine monitoring of anionic surfactants in aqueous environmental samples and their analytical features compared with those provided by the standard methylene blue (MB) method. This new analytical approach is based on the effect that anionic surfactants exert on the binding degree of the cationic surfactant didodecyldimethylammonium bromide (DDABr) to the anionic dye Coomassie Brilliant Blue G (CBBG). The formation of DDABr-CBBG aggregates is monitored photometrically. The analytical applicability of the proposed method was demonstrated by determining anionic surfactants in tap, river and reservoir water, and raw and treated sewage. The mean recoveries obtained ranged between 99 and 101%. The SDBD method offers important advantages over the classical MB method: it is more sensitive, selective, precise, simple and rapid; the analytical response is independent of the molecular structure of the anionic surfactants, and the volume of sample required for analysis and the consumption of organic solvents are significantly reduced.

Keywords: Anionic surfactants; Aqueous environmental samples; Dye-surfactant aggregates; Competitive aggregation

1. Introduction

The high consumption of surfactants throughout the world (more than 15 million tonnes/year [1]), together with their toxicity and negative effect on the self-purification capability of surface waters, make these synthetic organic compounds one of the main environmental concerns. Anionic surfactants are the most widely used (in Europe they comprise about 65% of all surfactants manufactured) mainly due to their good detergency at low temperature in neutral solutions. Within the anionic surfactant group, linear alkylbenzene sulphonates (LAS) are the most important class, followed by alkylethoxy sulphates (AES), alkyl sulphates (AS) and alkyl sulphonates (ASo) (chemical structures are given in Figure 1). All of them are commercialized as complex mixtures of homologues; the number of carbon atoms in the alkyl chain ranges in the intervals 10-14 for LAS [2,3], 12-18 for AES [4] and 10-18 for AS and ASo [5,6]. Each LAS and ASo homologue consists of different positional isomers, each one defined by the carbon atom to which the benzene sulphonate or sulphonate group is attached. In AES, the average number of ethylene oxide units generally varies between 2 and 3 [4].

As a result of their general use as industrial cleaners and household detergents (LAS and ASo), and personal care products (AES and AS), anionic surfactants are released into the environment through both domestic and industrial wastewater. In spite of the high biodegradability of these active surface substances (at least a 90% biodegradability is required for anionic surfactants being marketed [7]) and the high efficiency of wastewater treatment plants to remove them [8,9], anionic surfactants are present in many aquatic ecosystems [10-14].

The routine monitoring of anionic surfactants in environmental samples is usually performed by measuring their total concentration by non-specific methods. Specific methods based on chromatographic techniques [11, 15-19] are not suitable for routine anionic surfactant determinations because, although they are more selective and accurate than the non-specific approaches, they are not

versatile enough to quantify, in a single run, the high number of structurally different anionic surfactants present in environmental samples. Besides, they require a long time for sample preparation.

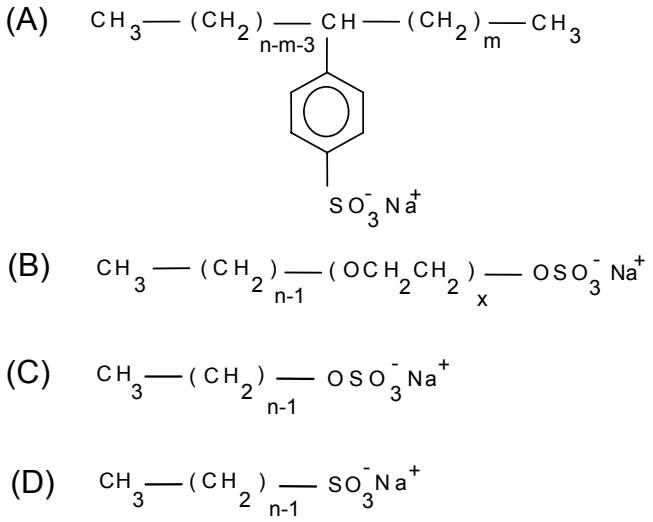

Fig. 1. Chemical structures of anionic surfactants: (A) alkylbenzene sulphonates, (B) alkylethoxy sulphates, (C) alkyl sulphates and (E) alkyl sulphonates.

Non-specific methods are generally based on the photometric measurement of a solvent-extractable ion pair formed between the anionic surfactant and a colored cationic reagent [mainly Methylene Blue (MB)]. The MB approach has been long applied as a standard method [2] and it is the official method in Europe for the determination of anionic surfactants in water [20]. Nevertheless, it exhibits serious drawbacks: 1) Due to the low extractability of MB-anionic surfactant ion pairs, a triple extraction using chloroform as organic solvent is recommended [2]. 2) Numerous substances normally present in environmental samples interfere. Thus, both organic and inorganic anionic compounds may transfer MB to the chloroform phase causing positive interferences. Aqueous washing of the chloroform extracts is recommended to remove these

interferences [2], the effectiveness of the backwash step being highly dependent on the extractability of the anionic interference-MB ion pairs formed. Since most of the interferent compounds are not completely removed, materials determined by the MB method are designated as methylene blue active substances (MBAS). On the other hand, cationic substances yield negative interferences as a result of their competition with MB to form ion pairs with anionic surfactants. Also colored materials extractable into chloroform interfere. Because of the lack of selectivity of the MB method, the analysis of complex samples such as wastewaters requires the extraction of anionic surfactants by gas stripping and evaporation of 220 mL of ethyl acetate [2] prior to the MB analysis. 3) The MB method needs a large amount of sample (100-400 mL) and a large quantity of chloroform (100 mL each measurement). 4) The standard procedure is tedious (Fig. 2) and time-consuming.

This work deals with the evaluation of the surfactant to dye binding degree (SDBD) method [21] for the routine determination of total anionic surfactants in aqueous environmental samples. For this purpose, a complete procedure was developed and applied to tap, natural and waste water samples, and, the analytical features of the proposed approach were compared with those provided by the standard MB method. The SDBD method is based on the photometric detection of surfactant reactant-dye aggregates, with a determined binding degree, in the absence and presence of amphiphilic analytes. The formation of these aggregates is monitored by recording the change of the absorbance of the dye as a function of the amount of surfactant reactant added to the titration vessel. The increment in the amount of titrant required in the presence of analyte is due to the formation of competitive surfactant reactant-analyte aggregates. Performance qualities of this new methodology in terms of sensitivity, analytical response, which is independent of the molecular structure of analytes, simplicity and rapidity, make it promising as a useful tool for the routine anionic surfactant monitoring.

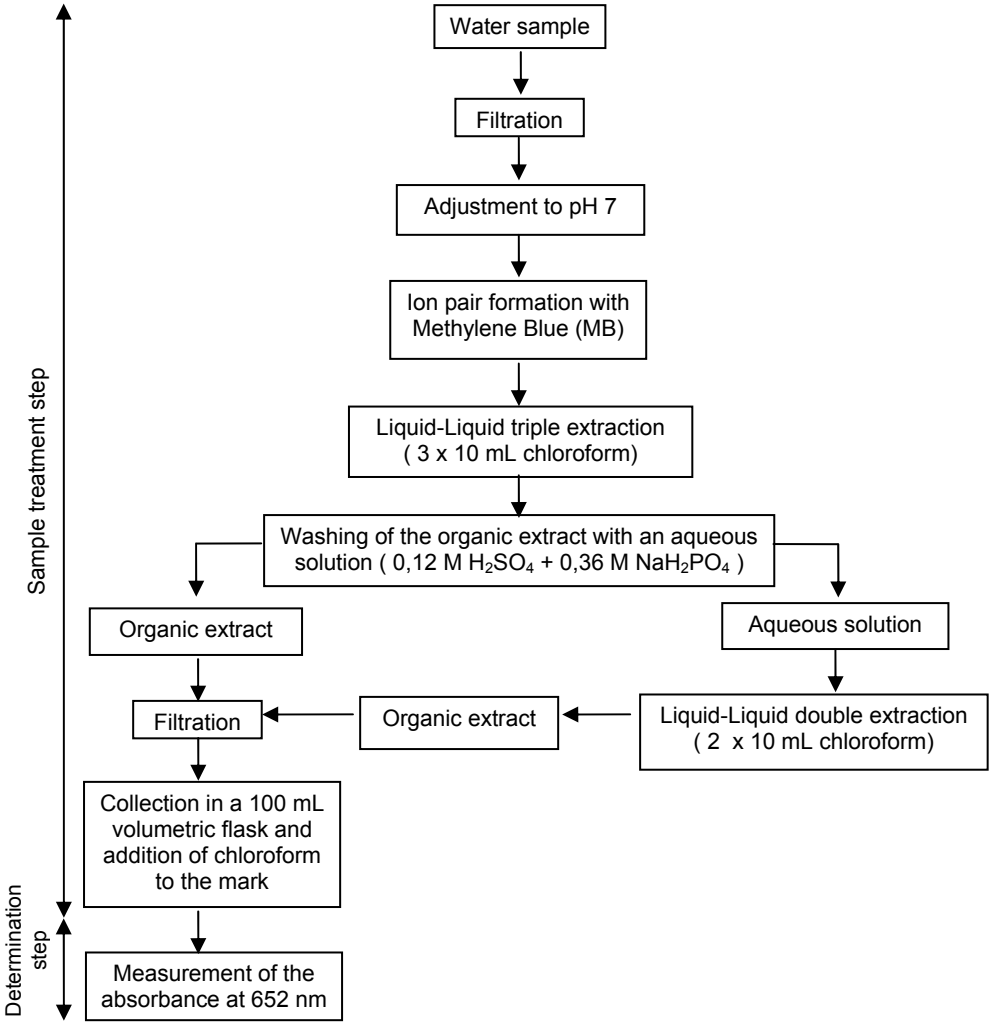

Fig. 2. Steps required in the determination of anionic surfactants by the MB method.

2. Experimental

2.1. Apparatus

A Metrohm 794 Basic Titrino titrator (Herisau, Switzerland) equipped with a 20-mL autoburet, a fan stirrer and a titration vessel was used for titrations. The detection unit was a Metrohm 662 spectrophotometer furnished with an immersion probe (1-cm path length). The instrument control and data processing were performed using a computer made up of a Pentium 4 processor, a Microsoft Windows XP operating system and a Metrohm TiNet 2.5 Light software.

2.2. Reagents and solutions

Highest-grade commercially available reagents were used throughout, without further purification. A 0.14 mM aqueous solution of Coomassie Brilliant Blue G (CBBG, Sigma Chemical CO., St. Louis, MO, USA) was prepared by dissolving 0.125 g of the reagent in 1 L of distilled water with sonication for 15 min. This solution was prepared at least 24 h prior to use and was stable for one month. The buffer solution used consisted of 0.25 M triethanolamine (TEA) with the pH adjusted to 7.0 with HCl. A 1.0 mM aqueous solution of didodecyldimethylammonium bromide (DDABr, Fluka Chemie GmgH, Buchs, Germany) was also prepared. Methanol was obtained from Panreac (Sevilla, Spain).

Aqueous solutions (25% w/v) of the pure linear alkylbenzene sulphonates (LAS) sodium decylbenzene sulphonate (SDeBS), sodium undecylbenzene sulphonate (SUBS) and sodium dodecylbenzene sulphonate (SDBS), and an aqueous LAS mixture (LAS-M, total LAS concentration = 20% w/v) containing 4.7% C12, 88.8% C13 and 6.5% C14, were kindly supplied by Petresa (San Roque, Spain). The commercial LAS, Petrelab P-550, and AES, EMAL 270E, were kindly supplied by Massó and Carol (Barcelona, Spain) and Kao Corporation (Barcelona, Spain), respectively. The proportional composition of the different homologues in Petrelab P-550 is: 9.6% C10, 38.1% C11, 31.3% C12, 19.1% C13 and 1 % C14, and, in EMAL 270E: 73% C_{12} and 27% C_{14} (both homologues with two

ethylene oxide units). AS and ASo pure standards were supplied by Merck (Darmstadt, Germany): sodium decyl sulphate (SDeS) and sodium hexadecyl sulphate (SHS); Aldrich Chemie GmbH & Co KG (Steinheim, Germany): sodium dodecyl sulphate (SDS), sodium tetradecyl sulphate (STS) and sodium octadecyl sulphate (SOS); and Fluka Chemie GmgH (Buchs, Switzerland): sodium decane sulphonate (SDeSo), sodium dodecane sulphonate (SDSo), sodium tetradecane sulphonate (STSo) and sodium hexadecane sulphonate (SHSo). Stock solutions (100 mg L^{-1}) of these anionic surfactants were prepared in distilled water and remained stable for at least one month.

2.3. Sample Collection and Preservation

Raw sewage and final effluent samples were obtained from the Mengibar wastewater treatment plant (Jaen, Spain) in March 2006. Natural water samples were taken from the Guadajoz River and the Navallana Swamp in the south of Spain in February 2006. They were collected in brown glass bottles containing formaldehyde (final concentration 1% w/w) as preservative, filtered through 0.45 µm HNWP nylon filters (Millipore, Bedford, MA, USA) to remove suspended solids, and stored at 4ºC until analysis.

2.4. Solid Phase Extraction (SPE)

Reverse phase (RP) C_{18} columns (Varian 04302, 500 mg, Harbor City, CA, USA) were used to extract and purify samples. Extractions were performed on a SPE vacuum manifold (Varian VAC Elut SPS 24). RP C_{18} columns were preconditioned by passing sequentially 10 mL of methanol and 10 mL of distilled water at a rate of 10 mL min^{-1}. A volume (100-250 mL) of water sample containing 7.5-80 µg of total anionic surfactants was passed through the extraction column at a rate of less than 10 mL min^{-1} by adjusting the vacuum to ca. 50 KPa. Afterwards, the cartridge was rinsed with 5 mL of distilled water and 20 mL of methanol-water (40-60) and dried under vacuum for 5 min. Anionic surfactants were eluted from the car-

tridge with 2 mL of methanol at a rate of 1 mL min^{-1} by adjusting the vacuum to ca. 10 KPa. The sample extract was evaporated to dryness under a stream of nitrogen at room temperature and the dried residue was redissolved in 5 mL of distilled water. The whole sample was then analyzed by the SDBS method.

2.5. Surfactant to Dye Binding Degree-Based Procedure

In a 25-mL volumetric flask were placed, in sequence, 3.6 mL of 0.14 mM CBBG solution, 12 mL of 0.25 M TEA buffer (pH=7.0), an aliquot of standard or treated sample containing between 7.5 and 80 µg of anionic surfactants, and distilled water to the mark. This solution was placed in a 50-mL titration vessel and titrated with 1.0 mM DDABr delivered from the autoburet at a rate of 10 mL min^{-1}. The stirring rate was set at 700 rpm. Titration curves were obtained by recording the absorbance at 580 nm as a function of the titrant volume.

2.6. Calculations

Determination of anionic surfactants concentration was based on the equation [21]: $m_S^* - m_S = \beta_A m_A$ where m_S and m_S^* are the amount of DDABr, expressed as a molar concentration, need to obtain a given DDABr-CBBG binding degree, in the absence and the presence of anionic surfactant, respectively; m_A is the concentration of analyte and β_A is the DDABr-anionic surfactant binding degree. Linear calibrations were obtained (see Table 1) by plotting the parameter $m_S^* - m_S$ as a function of the concentration of anionic surfactant. The m_S^* and m_S values were calculated from the volumes of DDABr consumed in titrations performed in the absence (V_S) and presence (V_S^*) of anionic surfactant, respectively. Because a nearly uniform response was obtained for analytes with different molecular structure, any anionic surfactant could be used as a standard for quantification. Typical titration curves for different concentrations of a hypothetical anionic surfactant are shown in Fig. 3.

Table 1

Calibration parameters for the determination of anionic surfactants using the SDBD method

Anionic Surfactant	n[a]	Detection Limit[b] (µg)	Linear Range[c] (µg)	Intercept ± s[d] (10^{-5} M)	Slope ± s[d] (10^{-7} M µg^{-1})	r[e]	S_{yx}[f] (10^{-6})
Alkylbenzene sulphonates (LAS)							
SDeBS	10	2.0	7.5 - 80	0.04 ± 0.03	8.6 ± 0.2	0.9992	0.47
SUBS	11	2.0	7.5 - 80	0.01 ± 0.03	8.2 ± 0.2	0.9990	0.53
SDBS	12	2.0	7.5 - 80	0.03 ± 0.02	7.8 ± 0.1	0.9994	0.40
LAS–M[g]	13	2.0	7.5 - 80	0.02 ± 0.03	8.4 ± 0.2	0.9992	0.47
Petrelab P-550[g]	11.5	2.0	7.5 - 80	0.03 ± 0.02	8.1 ± 0.1	0.9997	0.29
Alkylethoxy sulphates (AES)							
EMAL 270E[h]	12.5	2.0	7.5-80	0.03±0.03	8.1±0.1	0.9991	0.49
Alkyl sulphates (AS)							
SDeS	10	2.0	7.5 - 80	0.05 ± 0.04	8.3 ± 0.2	0.998	0.74
SDS	12	2.0	7.5 - 80	0.03 ± 0.02	8.6 ± 0.1	0.9994	0.43
STS	14	2.0	7.5 - 80	0.1 ± 0.1	8.4 ± 0.2	0.996	1.03
SHS	16	2.0	7.5 - 80	0.03 ± 0.03	8.1 ± 0.1	0.9993	0.44
SOS	18	2.0	7.5 - 80	0.06 ± 0.04	8.0 ±0.2	0.998	0.58
Alkyl sulphonates (ASo)							
SDeSo	10	2.0	7.5 - 80	0.04 ± 0.04	7.8 ± 0.2	0.998	0.68
SDSo	12	2.0	7.5 - 80	0.1 ± 0.1	8.2 ± 0.3	0.996	1.05
STSo	14	2.0	7.5 - 80	0.1 ± 0.1	8.5 ± 0.3	0.997	0.96
SHSo	16	2.0	7.5 - 80	0.06 ± 0.04	8.1 ± 0.3	0.997	0.53

[a]Number of carbon atoms in the hydrocarbon chain; for LAS and AES mixtures, average number of carbon atoms.

[b]Calculated as 3-fold the standard deviation of m_S.

[c]Quantification limit calculated as 10-fold the standard deviation of m_S.

[d]Standard deviation.

[e]Correlation coefficient.

[f]Standard error of the estimate (six different concentrations).

[g]LAS mixtures (see composition in Section 2).

[h]AES mixture (see composition in Section 2).

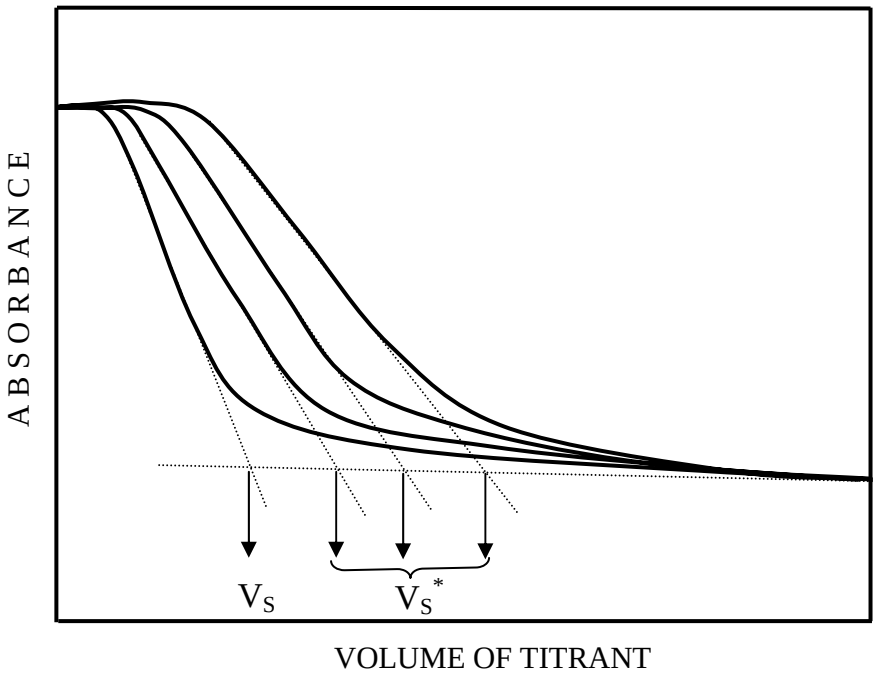

Fig. 3. Typical titration curves for different concentrations of a hypothetical anionic surfactant.

3. Results and discussion

3.1. Assessment of the SDBD method for the quantification of anionic surfactants in aqueous environmental samples

Fig. 4 shows the proposed procedure for the determination of the total concentration of anionic surfactants in aqueous environmental samples. To establish the feasibility of this procedure both the treatment and measurement steps were assessed. The measurement step was evaluated by running calibration graphs for pure anionic surfactants belonging to different structural groups (linear alkylbenzene sulphonates, alkyl sulphates and alkane sulphonates) with hydrocarbon chain lengths comprised between 10 and 18 carbon atoms. A LAS mixture with a high proportion of the C13 homologue (88.8%, LAS-M) and the commercial LAS, Petrelab P550, were also

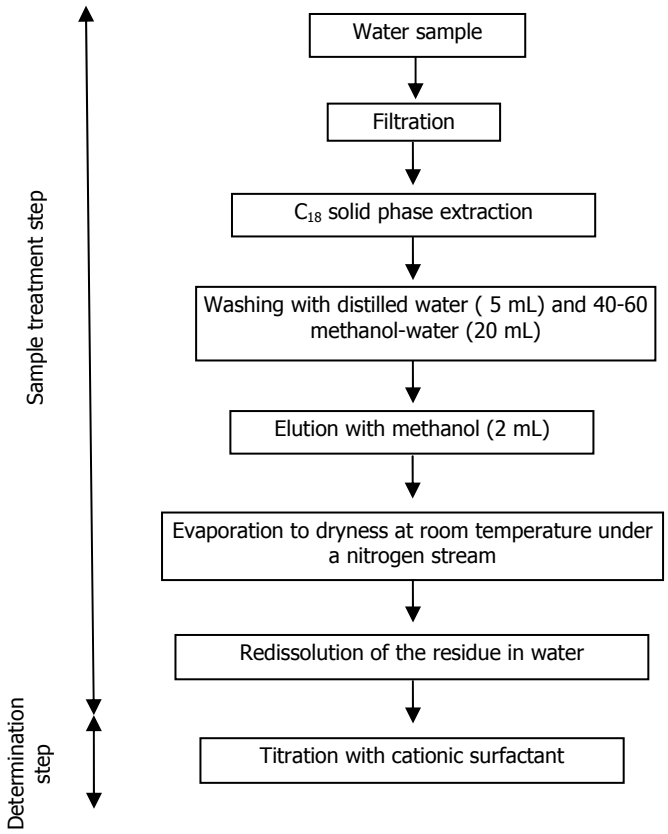

Fig. 4. Steps required in the determination of anionic surfactants by the SDBD method.

essayed. Since no pure alkyl ethoxysulphates were available, the commercial AES, EMAL 270E, containing the C_{12} and C_{14} homologues, both with two ethylene oxide units, was tested. The results obtained are shown in Table 1. A nearly uniform response on a weight basis was obtained for all the surfactants tested [the relative standard deviation (RSD) for the slope values was ca. 3.1%], therefore, the SDBD method permits the accurate determination of the total concentration of anionic surfactants, independently of the proportional composition of the different anionic surfactants in the sample and the analyte used as a standard for calibration.

The sample treatment step (Fig. 4)

was evaluated from recovery studies. The complete procedure was applied to different non-spiked and spiked aqueous samples: tap, river and reservoir water, and raw sewage and final effluent from a wastewater treatment plant (sampling locations are given in the Experimental section). The anionic surfactant concentrations found in the samples analyzed were lower than the maximum tolerated limit established for drinking water (0.2 mg L^{-1} [22]) except for the raw sewage sample (Table 2). Spiking was performed by adding SDBS at concentrations between 0.1 and 0.5 mg L^{-1}. The mean recoveries obtained for SDBS were between 99 and 101% (Table 2), which indicated that the surfactant was not adsorbed on the nylon filter during the filtration step, and it was quantitatively retained on the C_{18} extraction column and quantitatively eluted from it with 2 ml of methanol. No effect of the matrix composition on the recovery achieved was observed. To assess the capability of the proposed SPE procedure to quantitatively extract anionic surfac-tants belonging to different structural groups (LAS, AES, AS and ASo) and having different hydrocarbon chain lengths (C10-C18), 100 and 250 mL distilled water containing 2 µg (20 and 8 µg L^{-1}, respectively) of each of the anionic surfactant investigated (for AES, 2 µg of EMAL 270E) were passed through the C_{18} column, eluted with methanol and determined by the SDBD method. The efficiency of the SPE procedure was inde-pendent on both the structure of the surfactants and the volume of sample; mean recoveries of 102±3 % and 104±4 %, based on duplicate analyses, were obtained for 100 and 250 mL aqueous solutions, respectively.

3.2. Comparative study of the SDBD and the MB methods

The analytical features of the SDBD and the MB methods were comparatively investigated in order to determine the advantages and limitations of the first one to be used as an alternative to the classical photometric method in environmental analysis.

Table 2

Recovery of SDBS from aqueous samples by the SDBD method

Sample	Concentration added (mg L^{-1})	Concentration found[a] $\pm$ s[b] (mg L^{-1})	Recovery $\pm$ s[b] (%)
Tap water[c]	0	0.030 $\pm$ 0.001	
	0.1	0.131 $\pm$ 0.002	101 $\pm$ 2
	0.2	0.229 $\pm$ 0.003	99 $\pm$ 1
River water[d]	0	0.132 $\pm$ 0.004	
	0.2	0.336 $\pm$ 0.005	101 $\pm$ 2
	0.5	0.634 $\pm$ 0.003	100 $\pm$ 1
Reservoir water[c]	0	0.046 $\pm$ 0.001	
	0.1	0.146 $\pm$ 0.002	99 $\pm$ 1
	0.2	0.245 $\pm$ 0.002	99 $\pm$ 1
Raw sewage[d]	0	0.367 $\pm$ 0.005	
	0.1	0.465 $\pm$ 0.004	99 $\pm$ 1
	0.4	0.763 $\pm$ 0.006	99 $\pm$ 1
Sewage effluent[c]	0	0.044 $\pm$ 0.001	
	0.1	0.143 $\pm$ 0.002	99 $\pm$ 1
	0.2	0.243 $\pm$ 0.001	99 $\pm$ 1

[a]Mean of three independent determinations
[b]Standard deviation
[c]Sample volume = 250 mL;
[d]Sample volume = 100 mL

3.2.1. Analytical responses.

The differences in the analytical responses obtained for surfactants with different molecular structures are a common source of error in the determination of the total amount of surfactants in environmental samples using non-specific methods. In order to evaluate the magnitude of this error for the determination of anionic surfactants using the MB method, calibration curves were run for the surfactants under study.

Calibration parameters obtained on a weight basis, the usual way to express anionic surfactant amount in environmental samples, are shown in Table 3. The slope values of the calibration graphs obtained for LAS and AS were independent of the alkyl chain length, but those obtained for ASo increased as the number of carbon atoms in the alkyl chain did. We did not find a clear reason for this behavior. The analytical responses obtained for surfactants bearing different hydrophilic groups (e.g. compare responses for AS and ASo, Table 3) and structures in the hydrocarbon chains (e.g. compare responses for AES and AS or LAS and ASo, Table 3) were also different. These results prove that, on the contrary to the SDBD method (Table 1), the MB one does not provide accurate results in the determination of anionic surfactants in environmental samples; the magnitude of the systematic error obtained using this method will mainly depend on the surfactant used as a standard for calibration and the composition of the sample, which is unknown and highly variable.

3.2.2. Sensitivity

Detection and quantification limits calculated for anionic surfactants using the SDBD method were between 3 and 9 fold lower than those obtained by the MB one (see Tables 1 and 3). So, the volume of sample required to determine anionic surfactants can be significantly reduced. Thus, to determine 0.2 mg L^{-1} of anionic surfactants, the minimum volumes of sample need to apply the MB and the SDBD methods were 175 and 38 ml, respectively.

Table 3

Calibration parameters for the determination of anionic surfactants using the MB method

Anionic Surfactant	n[a]	Detection Limit[b](μg)	Linear Range[c] (μg)	Intercept ± s[d] (10^{-5})	Slope ± s[d] (10^{-7} μg^{-1})	r[e]	S_{yx}[f] (10^{-6})
Alkylbenzene sulphonates (LAS)							
SDeBS	10	10.0	35 - 300	0.002 ± 0.003	2.41 ± 0.02	0.9997	0.63
SUBS	11	10.0	35 - 300	0.01 ± 0.01	2.43 ± 0.04	0.9993	1.10
SDBS	12	10.0	35 - 300	-0.007 ± 0.003	2.51 ± 0.02	0.99991	0.46
LAS–M[g]	13	10.0	35 - 300	0.003 ± 0.0003	2.43 ± 0.02	0.9998	0.57
Petrelab P-550[g]	11.5	10.0	35 - 300	0.01 ± 0.01	2.34 ± 0.09	0.997	1.51
Alkylethoxy sulphates (AES)							
EMAL 270E[h]	12.5	17.0	55 - 300	- 0.006±0.004	1.58 ± 0.03	0.9993	0.76
Alkyl sulphates (AS)							
SDeS	10	9.0	30 - 300	0.01 ± 0.01	2.98 ± 0.06	0.998	1.14
SDS	12	9.0	30 - 300	0.005 ± 0.008	2.89 ± 0.07	0.998	1.22
STS	14	9.0	30 - 300	0.02 ± 0.01	2.96 ± 0.06	0.998	1.73
SHS	16	9.0	30 - 300	0.01 ± 0.01	2.90 ± 0.08	0.998	1.62
SOS	18	9.0	30 - 300	- 0.003 ± 0.003	2.89 ± 0.03	0.9998	0.42
Alkyl sulphonates (ASo)							
SDeSo	10	19.0	65 - 300	0.004 ± 0.004	1.39 ± 0.07	0.997	0.57
SDSo	12	10.0	35 - 300	0.01 ± 0.01	2.54 ± 0.07	0.997	1.35
STSo	14	9.0	30 - 300	0.004 ± 0.004	3.13 ± 0.03	0.9996	0.65
SHSo	16	6.0	20 - 300	- 0.01 ± 0.01	4.17 ± 0.09	0.998	1.62

[a]Number of carbon atoms in the hydrocarbon chain; for LAS and AES mixtures, average number of carbon atoms.

[b]Calculated as 3-fold the standard deviation of measurements corresponding to blank solutions (distilled water containing no analyte).

[c]Quantification limit calculated as 10-fold the standard deviation of measurements corresponding to blank solutions.

[d]Standard deviation.

[e]Correlation coefficient.

[f]Standard error of the estimate (six different concentrations).

[g]LAS mixtures (see composition in Section 2).

[h]AES mixture (see composition in Section 2).

3.2.3. Selectivity

The effect of possible interferents from environmental aqueous matrices on the determination of anionic surfactants by using the MB and the SDBD methods was studied by using the whole analytical procedure (Figs. 2 and 4, respectively). The foreign species tested were inorganic cations and anions and organic natural and pollutant substances. SDBS was used as a representative anionic surfactant to perform this study. A given compound was considered not to interfere with the determination if the mixture of interference plus analyte yielded a signal comprised in the range $S_x \pm s$, where S_x is the signal provided by the analyte in the absence of interferent and s is the standard deviation of the method. Results obtained are shown in Table 4.

Any of the foreign species tested interfered on the determination of anionic surfactants by the SDBD method at the maximum ratio of interferent to analyte tested. Most of these species were effectively removed by the sample treatment step (i.e. inorganic ions and polar organic compounds).

By using the MB method, positive interferences due to a number of inorganic anions (those specified in Table 4 except sulphide) and organic compounds such as phenol and humic acid, and negative interferences due to iron(III), sulphide, albumin and Triton X-100 were observed. Sulphide reacts with MB to form a colorless reduction product, its interference being of special concern in raw and primary treated wastewater samples where sulphides are often present. Among positive interferences, those due to thiocyanate, iodide and humic acid were the most important. At the working pH 7, carboxylic groups in humic acid were deprotonated and strongly interacted with MB, increasing the amount of MB transferred to the organic phase. At concentrations higher than 5 mg L^{-1}, humic acid precipitated in the chloroform phase. Taking into account the low mole ratio of humic acid to anionic surfactant tolerated by the MB method (Table 4) and that this organic substance is generally present in natural waters at concentrations in

Table 4

Effect of foreign species on the determination of SDBS by the SDBD and the MB methods, expressed as tolerated mole ratio of foreign species to SDBS

Foreign species	SDBD[a]	MB[b]
Inorganic cations		
NH_4^+	> 20000	> 20000
K^+	> 20000	> 20000
Na^+	> 20000	> 20000
Ca^{2+}	> 20000	> 20000
Mg^{2+}	> 20000	> 20000
Fe^{3*}	> 20000	15000
Inorganic anions		
HCO_3^-	> 20000	> 20000
NO_3^-	> 20000	> 20000
NO_2^-	> 20000	2000
PO_4^{3-}	> 20000	15000
SO_3^{2-}	> 20000	15000
SO_4^{2-}	> 20000	2000
S^{2-}	> 20000	2000
CN^-	> 20000	> 20000
SCN^-	> 20000	0.1
F^-	> 20000	> 20000
Cl^-	> 20000	> 20000
Br^-	> 20000	10000
I^-	> 20000	1
Organic compounds		
Aniline	> 20000	>20000
Phenol	> 20000	10000
Triton X-100[c]	> 100	40
Humic acid[c]	> 100	2
Albumin[c]	> 100	1

Volume of sample= 100 mL.
[a]25 µg SDBS.
[b]100 µg SDBS.
[c]Tolerated weight ratio.

the interval 1-20 mg L^{-1} [23, 24], interference from this specie could be expected when this type of samples is analyzed by the standard method. Experiments performed by applying the SDBD method to aqueous solutions containing 0.25 mg L^{-1} SDBS and humic acid at concentrations up to 25 mg L^{-1} demonstrated that this substance was removed from aqueous solutions by the proposed SPE procedure (for experimental conditions see the Experimental Section).

3.2.4. Precision

The precision of the SDBD and the MB methods was evaluated in terms of repeatability and reproducibility using SDBS as a model analyte. Because of the different sensitivities of these methods for the determination of anionic surfactants (section 3.2.2), the amounts of SDBS selected were different and they were about in the middle of the linear ranges of the corresponding calibrations curves. Repeatability studies were performed by applying the two whole procedures (Fig.s 2 and 4) to six 100 mL-standard solutions. To assess the precision between assays, repetitions of the analyses were carried out in three different days. The SDBD method surpassed the MB one in terms of precision (compare relative standard deviations obtained for both methods in Table 5) as a result of its simplicity. It should be noticed that the between assay standard deviation obtained for the SDBD method hardly increased compared with that obtained within assays, as a result of the robustness of the proposed method. On the contrary, the dispersion of the results obtained by using the MB method significantly increased when analyses were performed in different days.

3.2.5. Other practical aspect

In routine analysis, practical aspects such as sample throughput and organic solvent consumption are key parameters to reduce costs. The volume of organic solvent consumed and the time spent by the SDBD and MB methods for both calibration and analysis were estimated. For calibration, measurements from six different standard solutions (included a blank solution containing no analyte)

Table 5

Precision of the SDBD and the MB methods for the determination of SDBS

	Within assay precision			Between assay precision		
Method	$\bar{x}$ [a] (µg)	s[b]	RSD[c] (%)	$\bar{x}$ [d] (µg)	s[b]	RSD[c] (%)
SDBD	25.2	0.6	2.5	25.2	0.7	2.8
MB	103	6	5.8	103	9	8.7

Volume of sample = 100 mL.
[a]Average of 6 independent determinations.
[b]Standard deviation.
[c]Relative standard deviation.
[d]Average of 18 independent determinations performed during three days, six repetitions each day.

Table 6.

Analysis time and organic solvent consumption using the SDBD and the MB methods

	Time (min)		Organic solvent used	
Method	Calibration[a]	Analysis[b]	Calibration[a]	Analysis[b]
SDBD	60	60	No organic solvent used	Methanol (60 mL)
MB	180	90	Chloroform (600 mL)	Chloroform (300 mL)

[a]Six different standard solutions.
[b]Three independent determinations (sample volume = 100 mL).

were performed. For analysis, three independent determinations from 100 mL-raw sewage samples were carried out. Table 6 shows the results obtained. The time to obtain the calibration curve by the SDBD method was three times lower than that spent by the MB method. The main reason is that calibration in the MB method requires applying the whole procedure, including the six liquid-liquid extractions (Fig. 2), to each standard solution. For the SDBD method, mixing of reagents and analyte, and titration with DDABr are the only operations performed (measurement step in Fig. 4). Analyses of real samples also were more rapid using the SPE-SDBD procedure. Finally, the consumption of organic solvent was greatly reduced when the proposed method was applied to the analysis of environmental samples compared with that required by the MB method.

4. Conclusions

The advantageous analytical features of the proposed method for the determination of the total concentration of anionic surfactants in aqueous environmental samples make it a suitable alternative to the classical Methylene Blue (MB) method. The approach based on C_{18} solid phase extraction (SPE) and determination by the surfactant to dye binding degree (SDBD) method, surpasses to the MB one in terms of:

1) Accuracy. On the contrary of the MB method, the SDBD approach provides responses independent of molecular structures of surfactants permitting the accurate determination of anionic surfactants whatever their proportional composition in the sample. Systematic errors, which magnitude will depend on both the surfactant used for calibration and the anionic surface active substances present in samples will be obtained, however, by using the MB method.

2) Selectivity. The improved selectivity of the proposed method compared with that of the MB method is a result of both the high efficiency of the proposed SPE procedure to remove interferent species and the ability of the aggregation parameter-based method to preferably respond to ionic amphiphilic compounds bearing

opposite charge to that of the dye used as reactant.

3) Sensitivity. Detection limits are decreased by factors between 3 and 9 by using the proposed method.

4) Precision. Because of the simplicity of the proposed method, the uncertainty of the analytical procedure is highly reduced with respect to that of the MB method, where the high number of liquid-liquid extraction performed (six) highly contributes to increase result dispersion.

5) Other analytical features. Due to the higher sensitivity of the SDBD method, the volume of sample required for anionic surfactant determination is lower than that need to apply the MB method. The analysis time and the consumption of organic solvent are also reduced.

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CAPÍTULO 2

DETERMINACIÓN DE DROGAS ANFIFÍLICAS

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Surfactant-dye binding degree method for the determination of amphiphilic drugs

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The surfactant-dye binding degree (SDBD) method was extended to the determination of amphiphilic drugs. This new methodology was based on the effect of amphiphilic compounds on the degree of binding of a surfactant to dye molecules. The dye induces the formation of surfactant aggregates and allows this process to be monitored from changes in their spectral features. The interaction between the anionic dye Coomassie Brilliant Blue G (CBBG) and the cationic surfactant didodecyldimethylammonium bromide (DDABr) was used for the sensitive determination of phenamic acids (meclophenamic, mephenamic, fluphenamic and niflumic acid), non-steroidal anti-inflammatory drugs. The addition of phenamic acid to the dye-surfactant mixture resulted in the formation of drug-DDABr aggregates with well-defined stoichiometries (between 1:1 and 1:3) and, hence, in decreased interactions between the dye and the cationic surfactant. Mixtures of drug-surfactant were demonstrated to behave as those made up of pure surfactant, and, therefore, the expression previously derived for determining surfactants could be used to quantify amphiphilic drugs. The proposed method permitted the determination of phenamic acids at the mg L^{-1} level with the precision required for quality control (the relative standard deviation for 7 mg L^{-1} of meclophenamic acid was 1.1%). Pharmaceutical preparations were analysed directly after dissolution of the samples in ethanol.

Keywords: surfactant-dye binding degree method, dye-induced surfactant aggregates, phenamic acids, pharmaceuticals.

1. Introduction

Amphiphiles play an essential role in the existence of life and living processes and are widely used in the industry, agriculture, medicine, pharmacology, etc [1,2]. All supramolecular assemblies produced by spontaneous association of amphiphiles in aqueous media are characterised by parameters such as the critical aggregation concentration (cmc for micelles, cvc for vesicles, etc), the aggregation number, shape, etc, and therefore these parameters can provide the basis for the development of analytical methodologies of general use in amphiphile determinations, independently on the structure and physical properties of these molecules.

Several years ago, we proposed the mixed aggregate (MA) method [3], which is based on the measurement of the critical micelle concentration (cmc) of mixed premicellar and micellar aggregates, to determine amphiphiles. At this point, the principles that support the MA method have been established and the strategies devised for increasing sensitivity, selectivity and precision have been discussed [4]. The suitability of this method to determine the total amount of non-ionic [3,5], cationic [6-8] or anionic [9] surfactants (parameters of interest in environment and consumer products), additives in foodstuffs [10], drugs in pharmaceuticals [11-13], etc, has been proved, and procedures based on cmc measurements have been developed that notably improve the analytical features of those based on conventional measurements.

Recently, we have extended the use of aggregation parameters of supramolecular assemblies in the determination of amphiphiles with the development of a new approach, based on the measurement of the degree of binding of a surfactant to dye molecules, that induce the formation of mixed aggregates (the surfactant to dye binding degree (SDBD) method [14]). A common feature of the MA and SDBD methods is the need to produce mixed aggregates constituted by, at least, three components (a dye acting as probe and, in many systems, as inductor of the aggregate, a

surfactant reagent and one/several analytes). Although no considered as an essential requirement, the amphiphilic character of the dye and analytes will maximise hydrophobic and ionic/polar interactions between components in the mixed aggregate and, as a result, more sensitive methods will be achieved. Since the measurement parameter is different in both methods (i.e. cmc and binding degree) different analytical benefits are expected from them, and in fact, these differences were made clear in the first application of the SDBS method [14], which involved the determination of anionic surfactants. The selectivity obtained by the SDBS method dramatically increased compared to that achieved by the MA method [9] and this allowed the determination of anionic surfactants in sewage samples without the interference of non-ionic ones. Therefore, it is interesting to establish the performance of this new approach in different applications in order to establish its real scope.

This work deals with the application of the SDBS method to the determination of drugs. Most of therapeutic drugs are selected or designed to be amphiphilic, they posses discrete hydrophobic (e.g. aromatic groups) and hydrophilic (often ionised groups, e.g. carboxylic acids and nitrogen-containing groups) regions in their molecular structure, in order to penetrate cells and tissues and to favour interaction of drug molecules with receptor sites. As a result of their amphiphilic character, drugs show surface activity and self-assembly ability in a manner similar to those exhibited by surfactants [15]. Drugs also interact with surfactant molecules to form mixed aggregates [11-13, 16-20] and therefore they are expected to strongly interact with the surfactant reagent in order to modify the degree of binding between the surfactant and dye.

Phenamic acids, non-steroidal anti-inflammatory drugs derivatives of diphenylamine-2-carboxylic acid, were selected for this study. These compounds are widely used as potent analgesic and antiinflammatory agents in the treatment of osteo-arthritis, rheumatoid arthritis and other painful musculoskeletal disorders, the most common ones

being fluphenamic, meclophenamic, mephenamic and niflumic acid. The anionic dye Coomassie Brilliant Blue G (CBBG) was used as photometric probe and as inductor of the formation of didodecyldimethylammonium bromide (DDABr) aggregates, that was used as reagent. The fit of the chemical system to the mathematical expression previously derived for calibration of analyte surfactants [14] was investigated, the interactions between DDABr and phenamates were determined, variables affecting the SDBS method were optimised and its feasibility for quality control of these drugs in pharmaceutical preparations was assessed.

2. Experimental

2.1. Apparatus

The equipment used for titrations consisted of a Metrohm 794 Basic Titrino titrator furnished with a 20-mL autoburette, a fan stirrer and a titration vessel. The detection unit was a Metrohm 662 spectrophotometer equipped with an inmersion probe (1-cm path length). The control of the temperature for optimisation studies was performed using a Deward titration vessel termostated by means of circulating water (temperature uncertainty = $\pm$0.1 ºC). The instrument control and data processing was performed using a computer made up of a Pentium 4 processor, a Microsoft Windows XP operating system and a Metrohm TiNet 2.5 Light software. Chemical interactions between components in mixed aggregates were studied using a Hitachi U-2000 Spectrophotometer.

2.2. Reagents and solutions

Commercially available highest-grade reagents were used throughout, without further purification. A 1.4×10^{-4} M aqueous solution of Coomassie Brilliant Blue G (CBBG, Sigma) was prepared by dissolving 0.125 g of the reagent in 1 L of distilled water with sonication for 15 min. This solution was prepared at least 24 h prior to use and was stable for one month. The buffer solution used consisted of 0.1M KH_2PO_4 (pH= 5.9). A 1.0×10^{-3} M aqueous solution of didodecyldimethylammonium bromide (DDABr,

Fluka) was also prepared. Stock solutions of phenamic acids (2 g L^{-1}) [meclophenamic, mephenamic, niflumic (Sigma) and fluphenamic (Aldrich) acid] were prepared in ethanol. These solutions were stable for two weeks.

2.3. Procedure

To a 25-mL volumetric flask were added, in sequence, 4.3 mL of 1.4 x 10^{-4} M CBBG solution, 12.5 mL of 0.1 M phosphate buffer (pH=5.9), volumes of standard or treated pharmaceutical sample solutions to give a final concentration of phenamic acid between about 1.5 and 15 mg L^{-1}, and distilled water to the mark. This solution was placed in a 50-mL titration vessel and titrated with 1.0 x 10^{-3} M DDABr, added from the burette at a rate of 10 mL min^{-1}. The stirring rate was set at 700 rpm. Titration curves were obtained by recording the absorbance at 610 nm as a function of the titrant volume.

2.4. Calculations

Calibrations were based on the mathematical expression previously derived for the determination of surfactants [14]. Briefly, the binding of the surfactant reagent DDABr (S^+) to the dye CBBG (D^-) is assumed to occur through successive aggregates ($DS_n^{+(n-1)}$) which are formed after a DDABr critical aggregation concentration (cac). The cationic surfactant-dye binding degree (β_D) is given by $\beta_D = (m_S - m_{S,M})/m_D$ where m_S and m_D are the total concentrations of surfactant and dye in the aqueous surfactant-dye mixture, respectively, and $m_{S,M}$ is the concentration of surfactant in monomeric form. The analytes phenamic acids (A^-) interact with DDABr in the presence of CBBG forming also successive aggregates ($AS_n^{+(n-1)}$, binding degree $\beta_A = m_s/m_A$) which decrease the value of β_D according to $\beta_D = (m_S^* - m_{S,M} - \beta_A m_A)/m_D$. The parameter m_S^* is the surfactant reagent concentration necessary to reach the same β_D value in the presence of phenamic acid than that obtained in its absence, and m_A is the analyte concentration. Combination of both equations gives:

$$m_S^* - m_S = \beta_A m_A \quad (1)$$

from which calibrations for phenamic

acids are obtained by plotting the parameter $m_S^*-m_S$ as a function of m_A. The values of m_S^* and m_S are determined graphically from the intercept of the extrapolated straight line before and after the end-point of the titration curves obtained in the presence and the absence of phenamic acid, respectively.

2.5. Determination of phenamic acids in pharmaceutical preparations

The pharmaceutical samples analysed were both liquids (oral solutions) and solids (capsules, gels and suppositories). Liquid formulations were analysed by dilution with ethanol without further treatment. The resulting solution contained about 1 g L^{-1} of phenamic acid. When solid formulations were analysed, an amount of sample containing about 100 mg of phenamic acid was accurately weighed and dissolved in about 80 mL of ethanol. Magnetic stirring for 30 min and slight heating (about 30°C) was used to aid dissolution of samples. If any insoluble material was present in the solution after this treatment, it was removed by filtration and washed several times with ethanol. Dissolved samples were diluted to 100 mL with ethanol and aliquots of them analysed as described above. The analysis of formulations containing flufenamic acid, required to remove the additive contribution of salicylic acid to the analytical signal from the nominal concentration specified in the sample.

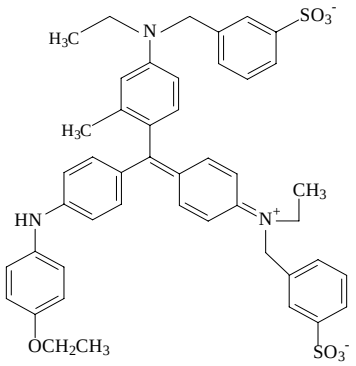
3. Results and discussion

3.1. Study of interactions in the dye-surfactant-phenamic acids chemical system

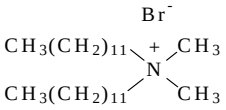
Fig. 1 shows the structure of the components making up the chemical system under study, namely, the dye (A), the surfactant reagent (B) and target analytes (C). In order to apply the SDBD method to the determination of phenamic acids, several requirements should be fulfilled. Thus, the dye should not interact with analytes and have appropriate spectral characteristics, which should be modified by interaction with the surfactant reagent. On the other hand, surfactant reagent-analytes

interactions should be strong enough to modify the degree of binding of the surfactant reagent to the dye. Therefore, the charge and hydrophobicity of analytes will condition the selection of both the dye and the surfactant reagent.

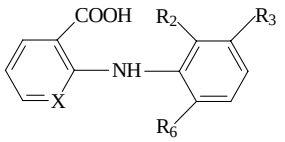
(A)



(B)



(C)



Drug	X	R	R	R
Meclophenamic	C	C	CH	C
Mephenamic	C	CH	CH	H
Fluphenamic	C	H	CF	H
Niflumic	N	H	CF	H

Fig.1. Structures of (A) CBBG, (B) DDABr and (C) phenamic acids.

In the system under study, CBBG has been previously known [14] to interact with the cationic surfactant DDABr through ionic (sulphonate-ammonium groups) and hydrophobic (aromatic and alkyl groups) interacttions, producing aggregates of well-defined stoichiometries above a DDABr threshold value, named the critical aggregation concentration (cac). Since the cac value is highly dependent on experimental conditions (e.g. CBBG concentration, pH, ionic strength, etc.), we calculated it under the experimental conditions recommended for phenamic acids determination (see section 2.3). The cac was found to be (3.8±0.4) x 10^{-5} M. The CBBG spectral changes induced by the presence of DDABr (compare curves 1 and 3 in Fig. 2A) constitutes the basis of the use of the dye as a photometric probe.

No evidence of interaction between

phenamate anion and CBBG was obtained from comparison of CBBG spectra in the absence (curve 1 in Fig. 2A) and presence (curve 2 in Fig. 2A) of meclophenamate anion at concentrations within the interval of analytical interest (micromolar level). This behaviour can be explained on the basis of the repulsive electrostatic forces between sulphonate and carboxylate (the pKa for phenamic acids ranges between 3.6 and 4.7 and the working pH is 5.9) ionic groups.

The addition of phenamic acid to the DDABr-CBBG aqueous mixture caused further modifications in the spectral features of the dye (compare curves 3 and 4 in Fig. 2A) which indirectly proved the occurrence of DDABr-phenamate interactions that competed with DDABr-CBBG ones. Direct confirmation of this interaction was obtained from DDABr - induced phenamate spectral changes (compare curve 1 with curves 2-4 in Fig. 2B). In the absence of DDABr, spectra from meclophenamic acid solutions at pH=5.9 (drug mainly in its basic form) showed two absorption peaks at 280 and 320 nm, which underwent

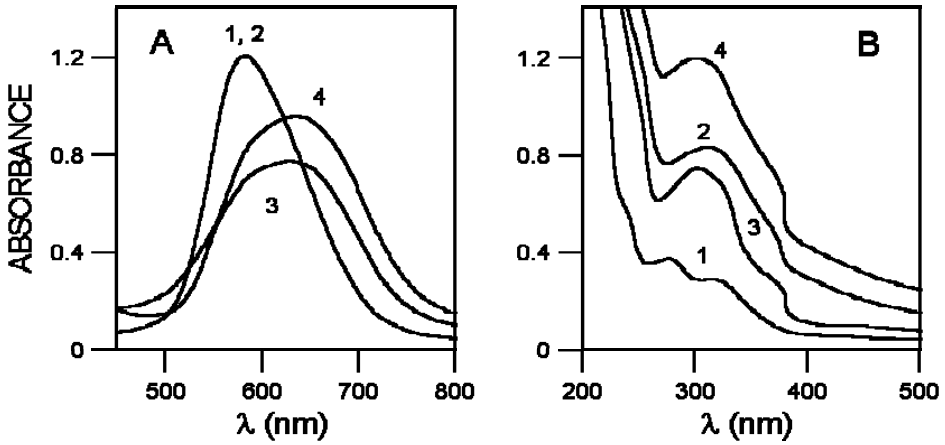


Fig.2. (A) Spectra for Coomassie Brilliant Blue G (2.4 x 10^{-5}) in: (1) the absence of amphiphile and the presence of (2) 1.2x10^{-5} meclophenamic acid, (3) 1.2 x 10^{-4} M DDABr and (4) 1.2 x 10^{-4} M DDABr and 1.2 x 10^{-5} M meclophenamic acid. (B) Spectra for meclophenamic acid (4.4 x 10^{-5} M) in (1) the absence and (2-4) the presence of DDABr (2) 4.4 x 10^{-5} M (3) 8.8 x 10^{-5} M and (4) 1.5 x 10^{-4} M. [phosphate buffer] = 0.05 M; pH = 5.9. Spectra 3 and 4 in (A) recorded against 1.2 x 10^{-4} M DDABr 0.05 M buffer phosphate blank solutions.

bathochromic and hyperchromic shifts in the presence of the surfactant.

The possibility of the formation of well-defined stoichiometry DDABr-phenamate aggregate was investigated by using the mole-ratio method. Experiments were carried out by mixing increasing DDABr concentrations within the range of analytical interest (0-1.5x10^{-4} M) and a constant concentration of meclophenamic acid (4.4x10^{-5}M) at pH 5.9, fixed with phosphate buffer. In order to obtain reproducible measurements in the presence of the light scattering produced by the formation of DDABr-meclophenamic acid aggregates, the absorbance of the mixture was measured at 295 nm at a fixed time (5 min). Fig. 3 shows the variation of the absorbance as a function of the DDABr/meclophenamic acid molar ratio. The broken line obtained suggested the formation of DDABr: drug aggregates of different stoichiometries (between 1:1 to 3:1) in proportion to the DDABr concentration increased.

Briefly, under our experimental conditions (pH=5.9), the cationic surfactant DDABr interacts with both the anionic dye CBBG and the anionic drug to form DDABr-CBBG and DDABr-drug aggregates. Therefore, the addition of phenamic acids into the DDABr-CBBG mixture strongly affects the extent of DDABr-CBBG aggregate formation.

Fig. 4 shows typical experimental titration curves obtained in the absence (curve 1) and presence of variable meclophenamic acid concentrations (curves 2-4) using 1.0x10^{-3} M DDABr as titrant. Under these experimental conditions, the formation of aggregates with DDABr:CBBG stoichiometries up to 3:1 and higher than 3:1 were successively recorded before and after the endpoint of the titration curves, respectively. Parameter m_S and the different m_S* values were easily calculated from the volume of titrant (V_S and V_S*, respectively) used at the endpoint.

3.2. Optimisation

The effect of different variables (pH, KH_2PO_4 buffer, organic additive and CBBG concentrations, and temperature) on m_S , m_S* and the measurement parameter $(m_S$*-$m_S)$

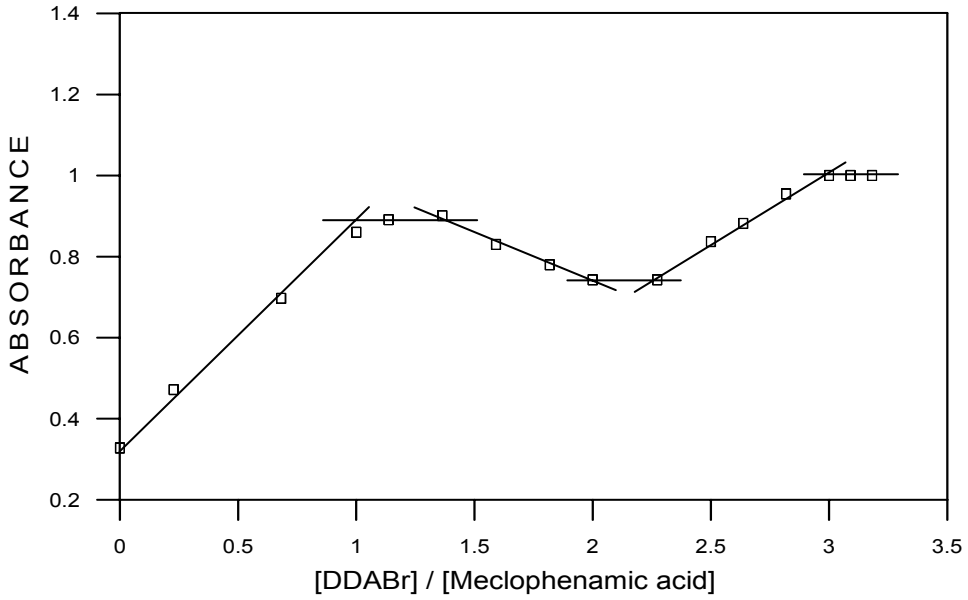


Fig.3. Variation of the absorbance of meclophenamic acid (4.4 x 10^{-5} M) at 295 nm as a function of the DDABr/meclophenamic acid molar ratio; [phosphate buffer] = 0.05 M; pH = 5.9.

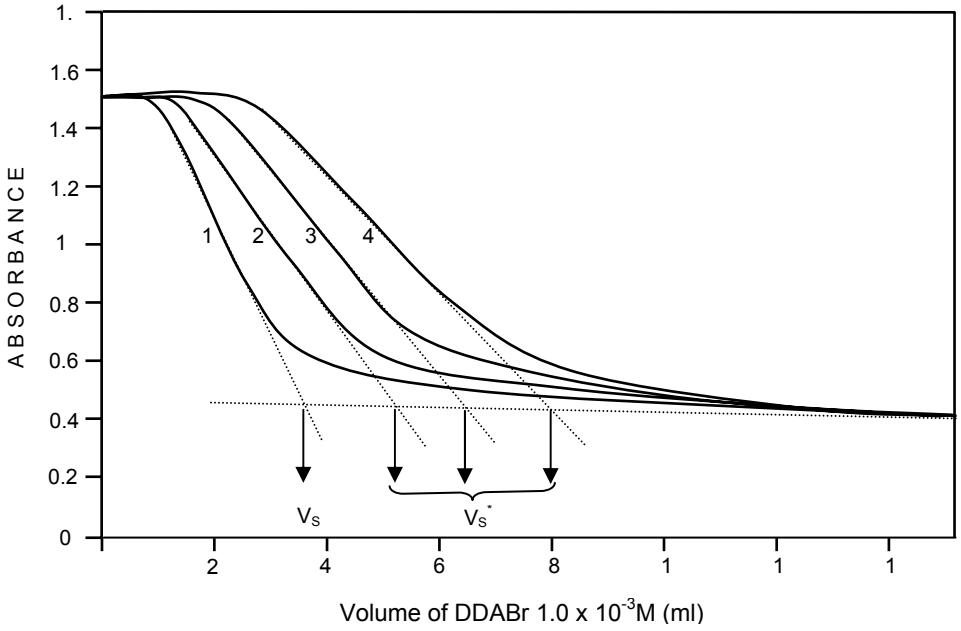


Fig.4. Variation of the absorbance of Coomassie Brilliant Blue G (2.4 x 10^{-5} M) at 610 nm as a function of the volume of titrant (1.0 x 10^{-3} M) DDABr added to a titration vessel containing (1) non phenamic acid or meclophenamic acid at a concentration of (2) 5 mg L^{-1}, (3) 9 mg L^{-1} and (4) 15 mg L^{-1}; [phosphate buffer] = 0.05 M; pH = 5.9.

was investigated in order to find the optimum experimental conditions for determining phenamic acids (e.g. the best possible sensitivity in their determination and the highest possible precision in the determination of the end-point of titration curves). Meclophenamic acid was the analyte selected to perform this optimisation study, which was carried out by changing each experimental variable in turn while keeping all others constants.

The influence of the CBBG concentration on m_S , m_S* and the measurement parameter was studied over the range (0.5–2.6)x10^{-5} M. Logically, the DDABr concentration required for DDABr-CBBG aggregates formation increased as a function of CBBG concentration in both the absence and the presence of meclophenamic acid (Fig. 5A1). This increase was no linear, which could be explained on the basis of a decrease in the DDABr concentration threshold value for the formation of DDABr-CBBG aggregates (cac) as the dye concentration in the titration medium was increased.

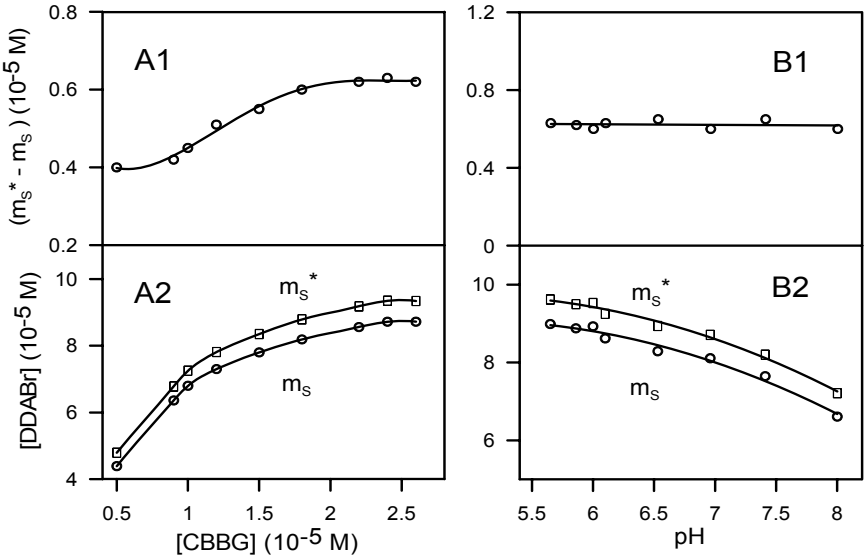


Fig.5. Influence of (A) CBBG concentration and (B) pH on (1) the measurement parameter and (2) m_S and m_S*; [meclophenamic acid] = 3 mg L^{-1}; (B) [CBBG] = 2.4 x 10^{-5} M; (A) pH = 5

Increase CBBG concentration has been previously reported to decrease the critical premicellar concentration of cationic surfactants such as dodecylpyridinium chloride and tetradecyltrimethylammonium bromide [6]. The measurement parameter, and hence, the degree of binding of analyte to cationic surfactant (β_A), was found to increase slightly for CBBG concentrations comprised between about $7x10^{-6}$ M and $1.8x10^{-5}$ M (Fig. 5A2). A $2.4x10^{-5}$ M CBBG concentration was selected as optimal in terms of sensitivity and precision.

The effect of altering the pH in the titration medium on dye-induced aggregates formation and on the measurement parameter was investigated using hydrochloric acid and sodium hydroxide for adjustment. The precision in the determination of the titration end-point was highly dependent on the pH of the titration medium because of changes provided in the shape of titration curves. Thus, pH values lower than 5.5 and higher than 8.0 proved inadvisable because the irreproducibility obtained. Fig. 5B shows the results obtained at pH values in the range 5.5-8.0. The formation of DDABr-CCBG aggregates was disfavoured as the pH value was increased (Fig. 5B2). This variable did not affect the formation of DDABr-analyte (Fig. 5B1), which is logical taking into account that the analyte was mainly as meclofenamate anion (pKa = 3,6) and therefore, negatively charged, over the whole pH range studied. Optimal precision in the end-point determination was obtained at pH values comprised between 5.5 and 6.5.

A phosphate buffer was used to adjust the pH of the titration medium to the required value. Parameters m_S and m_S^* were found to increase as a function of the buffer concentration. This behaviour is according to an electrolyte effect; electrolytes can decrease electrostatic interactions between oppositely charged groups in the anionic dye and the cationic surfactant. Although increased ionic strength also diminished electrostatic interactions between oppositely charged groups in meclofenamate anion and DDABr, this effect was less pronounced than that on the CBBG-DDABr system, which resulted in an increase in the measurement param-

eter (about 3 times) as the buffer concentration increased up to about 0.04 M. Buffer concentrations from 0.04 M to 0.055M did not affect to the measurement parameter.

The effect of the temperature, which was studied in the range 5-50 °C, caused a slight decrease in the DDABr concentration required to form CBBG-DDABr aggregates, in both the absence and the presence of analyte. Temperatures lower than 25 °C were found to favour the formation of meclofenamate-DDABr aggregates in a greater extent than that of CBBG-DDABr ones, which resulted in an increase in the measurement parameter (about 1.5 times from 20 to 5ºC). Nevertheless, measurements were made at room temperature in order to avoid the use of a cryostating unit and increase precision.

Organic solvents have been reported to disfavour the formation of surfactant aggregates [11, 21]. The influence of organic additives on CBBG-DDABr and drug-DDABr aggregates formation was studied by addition of ethanol at concentrations up to 10%. The alcohol was found to increase m_S and m_S^* in proportions higher than 0.7%. No effect of this solvent on the formation of meclophenamate-DDABr aggregates was observed at concentrations up to 6%. Higher alcohol contents was found to difficult the interaction between meclophenamate anion and DDABr, which resulted in a slight decrease in the measurement parameter (about 1.2 times for ethanol percentages from 6 to 10%). Ethanol disfavoured dye-surfactant and drug-surfactant aggregates formation because of its negative influence on hydrophobic interactions as a result of its ability to break down the structured water molecules around the hydrophobic parts of molecules [22]. Ethanol was the solvent used to prepare phenamic acid standard solutions and to treat pharmaceutical samples, since its effect on the measurement parameter could be easily controlled.

3.3. Analytical features

After all experimental variables were optimised, plots of the parameter m_S^*-m_S versus phenamic acid concentration (m_A) were constructed. The figures of merit of the calibration

graphs obtained are shown in Table 1. Linear calibrations with intercepts values no significantly different from zero were obtained in all cases [the standard error of the estimate and the correlation coefficient varied over the range (2.3-2.9)x10^{-6} and 0.998-0.9990, respectively]. Therefore, the results obtained fitted to Eq. (1) indicating that the degree of binding of the cationic surfactant DDABr to phenamate anions, β_A , remained constant over the linear concentration range and that binary mixtures of drug-surfactant behaved as those made of pure surfactants. The value of β_A can be easily calculated from the slopes obtained for each of the phenamic acids tested, the concentration expressed as molar.

Table 1

Analytical figures of merit of the proposed method for the determination of phenamic acids

Phenamic acid	Detection limit[a] (mg L^{-1})	Linear concentration range[b] (mg L^{-1})	Intercept ± s (10^{-5} M)	Slope ± s (10^{-5} M L mg^{-1})	r[c]	S_{yx}[d] (10^{-6})
Meclophenamic acid	0.25	0.8 – 15	0.1 ± 0.2	0.86 ± 0.02	0.998	2.8
Fluphenamic acid	0.23	0.8 – 15	0.1 ± 0.2	0.90 ± 0.02	0.9990	2.6
Mephenamic acid	0.24	0.8 – 15	0.2 ± 0.2	0.96 ± 0.02	0.998	2.9
Niflumic acid	0.27	0.9 – 15	0.1 ± 0.2	0.81 ± 0.01	0.9990	2.3

[a] Calculated as 3-fold the standard deviation of m_s

[b] Quantification limit calculated as 10-fold the standard deviation of m_s

[c] Correlation coefficient, n=11

[d] Standard error of the estimate

The precision of the proposed method, expressed as relative standard deviation, was 1.1% (n=11) for a concentration of meclophenamic acid of 7 mg L^{-1}.

Since the β_A value is a measure of the sensitivity of the SDBD method, we investigated the relationship between this parameter, that expresses the surfactant reagent (S^+) – drug (A^-) binding degree in the end-point of titration curves, and the intrinsic characteristics of phenamic acids. These analytes are expected to interact with DDABr through ionic (carboxylate - quaternary ammonium groups) and hydrophobic interactions. As the former ones should be similar for all phenamic acids, we studied how the hydrophobicity of the drug affected to the analytical sensitivity. Table 2 shows the β_A values obtained for each phenamic acid arranged according to decreasing hydrophobicity. The degree of binding of DDABr to drugs decreased as the octanol/water partition coefficient (P) of phenamic acids did, therefore, hydrophobic interactions, beside of attractive electrostatic interactions, played an important role in the formation of DDABr-drug aggregates. Since β_A is related with the successive $AS_n^{+(n-1)}$ association constants (e.g. K_1, K_2...K_n) through the expression given as:

$$\beta_A = \frac{K_1 m_{S,M} + 2K_1 K_2 m_{S,M}^{\ 2} + \ldots\ldots + nK_1 K_2 \ldots\ldots K_n m_{S,M}^{\ n}}{1 + K_1 m_{S,M} + K_1 K_2 m_{S,M}^{\ 2} + \ldots\ldots + K_1 K_2 \ldots\ldots K_n m_{S,M}^{\ n}}$$

It becomes clear that the surfactant reagent(S^+)-drug(A^-) bond strength, and all experimental factors that affect it, will determine the sensitivity of the SDBD approach. According to the β_A values exhibited in Table 2, DDABr: phenamate anion aggregates of stoichiometries 2:1 and 3:1 were present in the titration medium at the titration end-point for all the phenamic acids studied, although in different proportion.

3.4. Determination of phenamic acids in pharmaceutical preparations

The proposed method (see section 2.3) was applied to the determination of phenamic acids in seven commercially available pharmaceutical formulations. Table 3 compares the results obtained using the

Table 2
Influence of the hydrophobicity of phenamic acids on the degree of binding of the cationic surfactant DDABr to anionic drugs (β_A)

Phenamic acid	Log P[a] ± s	$\beta_A \pm$ s
Meclophenamic acid	5.9 ± 0.4	2.55 ± 0.06
Fluphenamic acid	5.6 ± 0.5	2.53 ± 0.06
Mephenamic acid	5.3 ± 0.4	2.31 ± 0.05
Niflumic acid	4.7 ± 0.5	2.28 ± 0.03

[a] Octanol/water partition coefficient

Table 3
Determination of phenamic acids in pharmaceutical preparations

Phenamic acid	Commercial formulation[a]	Phenamic acid nominal value	Phenamic acid found (s)[b]
Meclofenamic acid	Meclomen (capsules)	276 mg g^{-1}	274 (7) mg g^{-1}
Flufenamic acid	Movilisin (Gel)	30 mg g^{-1}	30 (1) mg g^{-1}
	Movilisin (Solution)	30 g L^{-1}	31 (1) g L^{-1}
Mefenamic acid	Coslan 500mg (Suppositories)	221 mg g^{-1}	220 (4) mg g^{-1}
	Coslan 250mg (Suppositories)	209 mg g^{-1}	204 (6) mg g^{-1}
	Coslan 125mg (Suppositories)	103 mg g^{-1}	103 (2) mg g^{-1}
Niflumic acid	Niflactol (capsules)	820 mg g^{-1}	823 (27) mg g^{-1}

[a]Composition of commercial formulations: Meclomen (Parke-Davis, Spain): excipients, 72.4% (w/w). Movilisin (gel) (Sankyo Pharma, Spain): salicylic acid, 2.0% (w/w); polyglycosaminoglycan sulphate, 0.2% (w/w); excipients, 94.8% (w/w). Movilisin (solution) (Sankyo Pharma, Spain): salicylic acid, 2.0% (w/v); polyglycosaminoglycan sulphate, 0.2% (w/v); excipients, 94.8% (w/v). Coslan 500 mg (Parke-Davis, Spain): excipients, 77.9% (w/w). Coslan 250 mg (Parke-Davis, Spain): excipients, 79.1% (w/v).

surfactant-dye binding degree method with nominal contents. As can be seen, both nominal and found values were quite consistent, which proves the applicability of the proposed method for the quality control of drugs in pharmaceutical formulations.

4. Conclusions

The surfactant-dye binding degree (SDBD) method was extended to the determination of amphiphilic drugs with structure very different to that of typical surfactants, e.g. phenamic acids. Binary mixtures of drug-surfactant were demonstrated to behave as those made up of pure surfactants, and therefore, the expressions of analytical interest previously derived for determining surfactants (Eq. 1) could be used for determining amphiphilic drugs. The proposed method permitted the determination of phenamic acids at the mg/L level (sensitivities obtained by using the SDBD method were similar to those afforded by photometric [23, 24] and fluorimetric [25, 26] monomer-based methods) with the precision required for quality control of both pure drugs and the drugs in pharmaceutical preparations. Other features of the SDBD method that make it useful for the routine work needed in quality control included: 1) high experimental simplicity, only the mixing of a dye, a surfactant and the analyte to form dye-surfactant and analyte-surfactant aggregates was required; 2) rapidity, the formation equilibrium for aggregates was reached very rapidly, so that, titration curves could be recorded in a few minutes; 3) low cost in both instrumentation and reagents; and 4) minimum sample treatment, only dissolution of the samples in ethanol was required for analysing most of the pharmaceutical formulation. The method is not recommended for the determination of phenamic acids in blood or urine since other drugs, also amphiphiles, can be present in these samples at higher concentration than phenamic acids (e.g. only about 0.05-1% of the phenamic acid ingested doses are usually found in urine) and besides the composition of the sample and the surfactant-interfering drugs association constants are not known.

Further work is required in order to determine the real scope of the SDBD method. Current research in this respect is being aimed in different directions: 1) use of the CBBG-DDABr system to the determination of other drug families containing anionic groups in their molecular structure; 2) selection of cationic dye-anionic surfactant systems to be used in the determination of drugs bearing positive charge; and 3) investigations on the applicability of the SDBD method to the determination of hydrotropes, organic compounds of low molecular weight and relatively low hydrophobicity, such as salicylic acid, which are known to influence the self-association behaviour of surfactants in solution.

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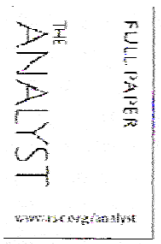
Pharmaceutical quality control of acid and neutral drugs based on competitive self-assembly in amphiphilic systems



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An aggregation parameter-based methodology for determining acid and neutral drugs in pharmaceutical dosage forms is presented. The method is based on competitive self-assembly in ternary dye-surfactant-drug aqueous mixtures. Dyes bearing charge of opposite sign to that of surfactants bind to surfactant to form mixed dye-surfactant aggregates, which are monitored from changes in the spectra features of the dye. The drug competes with the dye to interact with the surfactant to form drug-surfactant aggregates, which results in a decrease in the surfactant to dye binding degree proportional to the drug concentration in the aqueous solution. Coomassie Brilliant Blue G (CBBG) and didodecyldimethy-lammonium bromide (DDABr) were the dye and surfactant reactant used, respectively. The suitability of the surfactant to dye binding degree (SDBD) method to determine drugs with very different molecular structure: propionic (flurbiprofen, ibuprofen, naproxen and ketoprofen) and acetic (diclofenac, felbinac and zomepirac) acids, indolines (indomethacin and sulindac), glycyrrhetinic acid derivatives (carbenoxolone and enoxolone), salicylates (diflunisal and phenyl salicylate), oxicams (meloxicam, piroxicam and tenoxicam), pyrazolones (phenylbutazone and sulphinpyrazone) and hydrocortisones (dexamethasone and prednisolone) has been proved. The proposed method was successfully applied to the determination of drugs in commercial formulates (effervescent granulates, tablets, suppositories, gels and blisters) with a minimum sample treatment (dilution of liquid samples and dissolution of solid samples).

INTRODUCTION

Amphiphiles, compounds that have both hydrophobic and hydrophilic groups, play an essential role in our world. Natural amphiphiles (e.g. phospholipids, glycolipids, lipopeptides, lipolysaccharides, etc.) are the principal component of biological membranes and guarantee the transport and exchange of materials.[1] Both synthetic and natural amphiphilic substances are widely used in the industry, agriculture, medicine, pharmacology, etc. They are key ingredients of household products in every day use[2] (e.g. laundry detergents, shampoos, textile, softeners, cosmetics, etc.) and are widely used as foodstuffs additives[3,4] (antimicrobial preservatives, antioxidants, emulsifier, dough conditioners, etc.). Finally, amphiphiles acquire a special relevance in the pharmaceutical industry, since most of therapeutic drugs are selected or designed to be amphiphilic in order to penetrate cells and tissues and to favour interaction of drug molecules with receptor sites[5]. Thus, antibiotics, anti-cancer drugs, antihistamines, tricyclic antidepressant, phenothiazines and anti-inflammatory drugs, among others, are amphiphiles.

The characteristic molecular structure of amphiphiles, known as amphipatic structure, is the base of two phenomena which differentiate these compounds from other chemical substances: a) adsorption at interfaces (gas-liquid, liquid-liquid and solid-liquid), which results in the formation of monolayers and multilayers, and b) self-assembly association in bulk solution, which results in the formation of ordinary and reverse micelles, vesicles, bilayers, microemulsions, etc.

Based on the capability of amphiphiles to form self-assemblies in aqueous bulk solutions, our research group has developed two new analytical approaches,[6,7] which have been demonstrated to be a powerful tool for the determination of amphiphilic compounds. The mixed aggregate (MA) method[6,8], based on measurements of the critical aggregate concentration of amphiphile mixtures, has been successfully used for the determination of global indexes (total concentration of non-ionic,[6,9] anionic[10]

and cationic surfactants[11] in aqueous environmental samples) and for quality control of household products,[11,12] foodstuffs[13] and pharmaceuticals.[14-16] The surfactant to dye binding degree (SDBD) method[7], based on the effect of amphiphilic compounds on the degree of binding of a surfactant to dye molecules, has been also demonstrated to be suitable for environmental analysis[7] and pharmaceutical quality control.[17-19] The quantification of the total concentration of anionic surfactants in sewage samples[7] and the determination of phenamic acids[17] and fusidane antibiotics[18] in pharmaceutical preparations have been reported. The amphiphilic character of the analyte is not an essential requirement for MA and SDBD methods; the determination of aromatic hydrotropic drugs using the SDBD method has been recently described[19]. However, the occurrence of both ionic/polar and hydrophobic moieties in the analyte molecules results in increased drug-surfactant bond strength, which permits to develop more sensitive methods.

This work deals with the evaluation of the SDBD method to be used as a general methodological approach for the quality control of drugs in pharmaceutical preparations. For this study, a wide number of acid, neutral and basic amphiphilic drugs with very different molecular structure: propionic and acetic acids, indolines, glycyrrhetinic acid derivatives, salicylates, oxicams, pyrazolones, hydrocortisones, phenothiazines, ethanolamines and dibenzazepines were selected. The anionic dye Coomassie Brilliant Blue G (CBBG) was used as inductor of aggregates of the cationic surfactant didodecyldimethylammonium bromide (DDABr), which were monitored from changes in the spectral features of the dye. In ternary CBBG-DDABr-drug mixtures, the drug competed with the dye to interact with the surfactant, which resulted in a decrease in the degree of binding of surfactant to dye molecules. Ibuprofen was selected as a model for the optimization of experimental conditions and the analytical features of the proposed method for the determination of the different drugs studied were evaluated. The feasibility of the SDBD

method for the direct quantitation of drugs in pharmaceutical preparations was investigated by analysing effervescent granulates, tablets, suppositories, gels and blisters.

EXPERIMENTAL

Apparatus

A Metrohm 794 Basic Titrino titrator (Herisau, Switzerland) equipped with a 20-mL autoburet, a fan stirrer and a titration vessel was used for titrations. The detection unit was a Metrohm 662 spectrophotometer furnished with an immersion probe (1-cm path length). The instrument control and data processing were performed using a computer made up of a Pentium 4 processor, a Microsoft Windows XP operating system and a Metrohm TiNet 2.5 Light software. Light scattering measurements for drug-surfactant interactions studies were performed using a Hitachi U-2000 spectrophotometer (Tokyo, Japan).

Reagents and solutions

Commercially available highest-grade reagents were used throughout, without further purification. Aqueous solutions (1.0 mM) of the surfactants: didodecyldimethylammonium bromide (DDABr), didecyldimethylammonium bromide (DDeABr), ditetradecyldimethylammonium bromide (DTeABr) (Fluka Chemie GmgH, Buchs, Germany) and cetylpyridinium chloride (Serva Feinbiochemica GmbH, Heidelberg, Germany) were prepared in distilled water. A 100 mM dodecyltrimethylammonium bromide (DTABr, Sigma Aldrich Chemie GmbH, Steinheim, Germany) was also prepared in distilled water. Aqueous solutions of the dyes: Coomassie Brilliant Blue G (CBBG, 0.14 mM), Acid Red 97 (AR97, 0.4 mM), Acid Red 88 (AR88, 1.2 mM) and Acid Orange 6 (AO6, 0.4 mM), were made by dissolving the reagent in 1 L of distilled water with sonication for 15 min. These solutions were prepared at least 24 h prior to use. CBBG was purchased from Sigma Chemical CO. (St. Louis, MO, USA) and AR97, AR88 and AO6 were supplied by Sigma Aldrich Chemie GmbH (Steinheim, Germany). The buffer solution used consisted of 0.1M KH_2PO_4 with the pH

adjusted to 5.9 with 2 M NaOH. Stock solutions (2 g L^{-1}) of therapeutic drugs, ibuprofen, enoxolone (Fluka Chemie GmbH), flurbiprofen, ketoprofen, diclofenac, zomepirac, indomethacin, sulindac, phenyl salicylate, diflunisal, phenylbutazone, suphinpyrazone, meloxicam, carbenoxolone, dexamethasone, prednisolone (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), naproxen and felbinac (Aldrich Chemie GmbH, Steinheim, Germany), were prepared in ethanol. Piroxicam (0.5 g L^{-1}) and tenoxicam (0.1 g L^{-1}) stock solutions were also made by dissolving each drug (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) in ethanol. Promethazine (2 g L^{-1}), diphenhydramine (1 g L^{-1}) and nortiptyline (1 g L^{-1}) stock solutions were prepared by dissolving the corresponding chlorhydrate (Sigma Chemical CO., St. Louis, MO, USA) in distilled water.

Recommended procedure for the determination of acid and neutral drugs

Volumes of 4.0 mL of 0.14 mM CBBG solution (stable for one month), 12.5 mL of 0.1M phosphate buffer (pH=5.9) and an aliquot of standard or treated sample solution to give a final drug concentration between about 0.2 and 50 mg L^{-1} were placed in a 25-ml volumetric flask. Then, distilled water was added to the mark. This solution was placed in a 50-ml titration vessel and titrated with 1.0 mM DDABr delivered from the buret at a rate of 10 mL min^{-1}. The stirring rate was set at 700 rpm. Titration curves were obtained by recording the absorbance at 590 nm as a function of the titrant volume.

The concentration of therapeutic drug was determined from the following equation[7]:

$$m_S^* - m_S = \beta_A \, m_A \qquad (1)$$

where m_S^* and m_S denote the amount of DDABr, expressed as a molar concentration, required to reach a given degree of binding of DDABr to CBBG, in the presence and the absence of drug, respectively, m_A the concentration of analyte and β_A the DDABr-analyte binding degree. Calibration graphs were constructed by plotting the measurement parameter ($m_S^* - m_S$) as a function of the con-

centration of analyte (m_A). The m_S* and m_S values were calculated from the volumes of DDABr consumed in titrations performed in the absence (V_S) and presence (V_S*) of drug, respectively (Fig. 1B).

Light scattering studies on the formation of drug-surfactant aggregates

In a 25-mL volumetric flask were placed, in sequence, 12.5 mL of 0.1 M phosphate buffer (pH=5.9), 75 µL of 2 g L^{-1} ibuprofen solution, appropriate volumes of 1.0 mM DDABr to give a final surfactant concentration between 0 and 75 µM, and distilled water to the mark. These ingredients were mixed by shaking and, then, the stopclock was started. An aliquot of the mixture was transferred to a 1-cm cell and its absorbance spectra between 200 and 500 nm was recorded exactly 5 min after the stopclock was started.

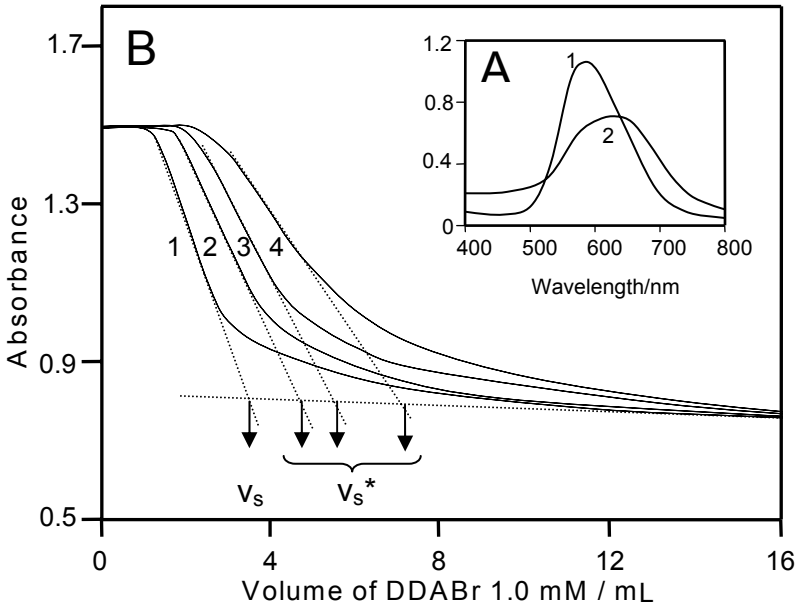


Fig. 1 (A) Spectra for CBBG (22 µM) in (1) the absence and (2) the presence of 120 µM DDABr. (B) Variation of the absorbance of CBBG (22 µM) at 590 nm as a function of the volume of titrant (1 mM DDABr) added to a titration vessel containing (1) non-drug or ibuprofen at a concentration of (2) 14.5 µM, (3) 24.0 µM and (4) 44.0 µM. [phosphate buffer] = 50 mM; pH = 5.9. Spectra 2 in (A) recorded against 120 µM DDABr 50 mM buffer phosphate blank solutions.

Determination of anti-inflammatory drugs in pharmaceutical preparations

Several pharmaceutical samples including blisters, tablets, gels, suppositories and effervescent granulates were analysed. Their compositions are shown in Table 1. Blisters were diluted with ethanol without further treatment; these diluted solutions contained about 0.5 g L^{-1} of drug. For the analysis of solid formulations (tablets, gels, suppositories and effervescent granulates), an amount of sample containing about 200 mg of drug was accurately weighed and dissolved in about 80 mL of ethanol with sonication for 15 min (tablets and effervescent granulates) or with stirring for 30 min (gels and suppositories). Extraction of the drug from suppositories was performed under slight heating (about 30ºC) to aid dissolution of samples. If any insoluble material was present in the solution after this treatment, it was removed by filtration and washed several times with ethanol. Dissolved samples were diluted to 100 mL with ethanol and aliquots of them were analysed as described above.

RESULTS AND DISCUSSION

Competitive self-assembly in ternary aqueous drug-CBBG-DDABr mixtures

In aqueous solutions containing the surfactant didodecyldimethylammonium bromide (DDABr), the dye Coomassie Brilliant Blue G (CBBG) and an amphiphilic drug, two types of mixed aggregates, dye-surfactant and drug-surfactant aggregates, are simultaneously formed. The formation of CBBG-DDABr aggregates can be monitored from changes in the features of the dye spectrum (compare curves 1 and 2 in Figure 1A). Thus, at DDABr concentrations higher than a threshold value (37±4 μM under the experimental conditions given in the Experimental Section), the absorbance measured for CBBG at 590 nm decreases as a function of the surfactant concentration (Fig. 1B). These absorbance changes have been previously demonstrated to be related to the formation of CBBG-DDABr aggregates with well-defined stoi-

Table 1 Determination of drugs in pharmaceutical preparations

Drugs	Commercial formulation[a]	Drugs nominal value	Drugs found (s)[b]
Ibuprofen	Neobrufen (Effervescent granulates)	92.7 mg g^{-1}	93.1 (0.4) mg g^{-1}
Diclofenac	Voltarén Retard (Tablets)	314 mg g^{-1}	314 (1) mg g^{-1}
Indomethacin	Inacid (Suppositories)	60 mg g^{-1}	60 (1) mg g^{-1}
Carbenoxolone	Sanodin (Gel)	20 mg g^{-1}	20.1 (0.4) mg g^{-1}
Diflunisal	Dolobid (Tablets)	590 mg g^{-1}	590 (7) mg g^{-1}
Piroxicam	Feldene (Blisters)	20 g L^{-1}	20 (0.2) g L^{-1}
Phenylbutazone	Butazolidina (Suppositories)	125.4 mg g^{-1}	125.3 (0.7) mg g^{-1}
Dexamethasone	Fortecortín (Tablets)	10.05 mg g^{-1}	10.1 (0.1) mg g^{-1}

[a]Composition of commercial formulations: Neobrufen (Abbott Laboratories, Spain): saccharose, 51.45% (w/w) and others excipients (sodium carbonate, cellulose, sodium croscarmellose, malic acid, povidone, sodium hydrogen carbonate, orange essence, sodium dodecyl sulphate and sodium saccharin), 39.28% (w/w). Voltarén Retard (Novartis Pharmacéutica, Spain): saccharose, 40.16% (w/w) and others excipients (colloidal anhydrous silica, cetylic alcohol, magnesium stearate, povidone, hypromellose, red iron oxide, polysorbate 80, talc, titanium IV oxide and polyethylene glycol 8000), 26.08% (w/w). Inacid (Merck Sharp & Dohme, Spain): excipients (butylhidroxyanisol and butylhidroxytoluene), 93.9% (w/w). Sanodin (Altana Pharma, Spain): excipients (karaya gum, vaseline oil and polyethylene), 98% (w/w). Dolobid(Frosst Laboratories, Spain): excipients (sodium croscarmellose, hydroxypropyl methylcellulose, starch, propylene glycol, sodium stearyl fumarate, and titanium dioxide), 41% (w/w). Feldene (Nefox Pharma, Spain): ethanol, 12.8% (v/v) and others excipients (benzilic alcohol, sodium phosphate, nicotinamide, 1,2 propanediol, sodium hydroxyde, hydrochloric acid and water), 85.2% (w/v). Butazolidina (Padró Laboratory, Spain): excipients (triglycerides), 87.45% (w/w). Fortecortín (Merck Pharma and Chemistry, Spain): excipients (lactose, starch, talc and magnesium stearate), 98.99% (w/w). [b]s denotes standard deviation (n=6).

chiometries.[7]

The amount of DDABr required to form dye-surfactant aggregates of a given stoichiometry increases as a function of the drug concentration in the aqueous mixture (compare curves 1 with curves 2-4 in Fig. 1B) as a result of the competition established between drug and dye molecules to interact with the cationic surfactant to form mixed aggregates. The capability of amphiphilic drugs to form mixed aggregates with DDABr have been previously demonstrated.[17,18] The Fig. 2 shows the results obtained from light scattering studies for the forma-

tion of ibuprofen-DDABr aggregates. The addition of DDABr to ibuprofen aqueous solutions (pH = 5.9) results in an increase in the absorbance measured for the drug in the UV region (compare spectra 1 with spectra 2-3 in Fig. 2A), which is related to the formation of drug-surfactant aggregates. The broken line obtained by plotting the absorbance measured for ibuprofen at 220 nm as a function of the [DDABr]/[ibuprofen] molar ratio indicates the formation of DDABr: ibuprofen aggregates of different stoichiometries (between 1:2 and 5:2) as the cationic surfactant concentration increases.

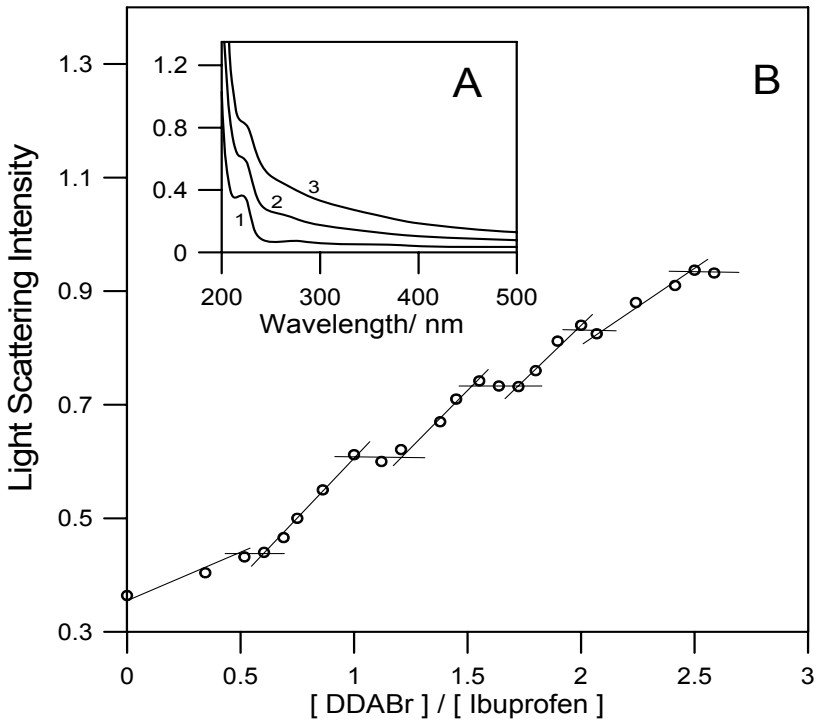


Fig. 2 (A) Spectra for ibuprofen (29 μM) in (1) the absence and (2,3) the presence of DDABr: (2) 29 μM and (3) 58 μM. (B) Variation of the absorbance of ibuprofen (29 μM) at 220 nm as a function of the [DDABr]/[ibuprofen] molar ratio. [phosphate buffer] = 0.05 M; pH = 5.9.

Experimental variables affecting the formation of mixed aggregates and sensitivity

Analytical response obtained for drugs using the SDBD method depends on both the molecular structure of the analyte and the experimental conditions used for its determination. The effect of the nature of the analyte on the analytical signal obtained will be discussed later. In this section, a systematic study of the effect of different variables (molecular structure of the dye and the surfactant reactant, pH, dye and buffer concentration, organic additives and temperature) on m_S , m_S^* and the measurement parameter $(m_S - m_S^*)$ is presented. Ibuprofen was the drug selected as a model to perform this study.

The surfactant reactants tested were dodecyltrimethylammonium bromide (DTABr), cetylpyridinium chloride (CPC) and dialkyldimethylammonium bromides (DDeABr, DDABr and DTeABr). No response was obtained for ibuprofen at concentrations up to 30 mg L^{-1} by using cationic surfactants containing a unique long alkyl chain in their molecular structure (i.e. DTABr or CPC). However, all double long alkyl chain cationic surfactants assayed (alkyl chain length between 10 and 14 carbon atoms) provided m_S^* values significantly higher than that of m_S at ibuprofen concentrations at the low mg L^{-1} level. Increased drug-surfactant hydrophobic interactions due to the occurrence of a second hydrocarbon chain in the molecular structure of the cationic surfactant could explain the different behaviour observed for both types of ammonium salts. The highest reproducibility in the determination of the titration end-point was obtained using DDABr as titrant, so it was the surfactant reactant selected for further studies.

Figure 3A shows the molecular structure of the dyes investigated in this study. Addition of DDABr to dye aqueous solutions caused spectral modifications for all dyes evaluated. Figure 3B shows these modifications and the wavelength at which the titration curves were recorded. The results obtained for m_s and m_s^* as a function of the dye concentration are depicted in Figure 4. The lowest dye concentration tested was that

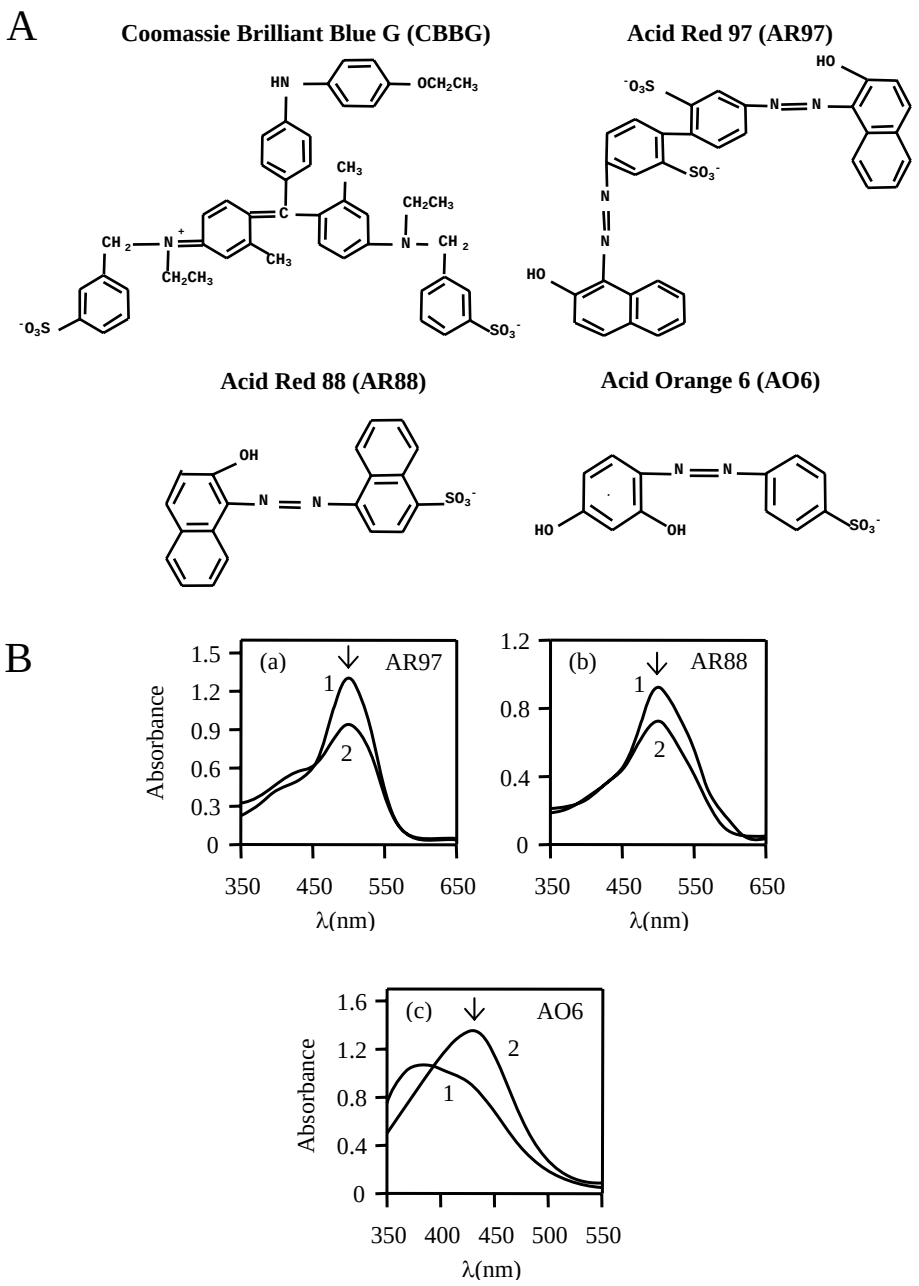


Fig. 3 (A) Molecular structures of the tested dyes. (B) Spectra for Acid Red 97 (AR97, 25 μM), Acid Red 88 (AR88, 50 μM) and Acid Orange 6 (AO6, 65 μM) in (1) the absence and (2) the presence of DDABr at a concentration of: (a) 30 μM, (b) 20 μM and (c) 120 μM. pH = 5.9 adjusted with 1M NaOH. Wavelengths selected to record titration curves for each dye are marked on their corresponding spectra with the symbol: ↓.

providing a reproducible measurement of the titration end-point, whereas the highest dye concentration assayed was that no causing saturation of the detector response. Since both m_S and m_S^* values linearly increased as a function of the dye concentration in a similar way, the measurement parameter was kept constant over the whole concentration range studied for each dye. The molecular structure of the dye greatly affected the sensitivity achieved for the determination of drugs using the SDBD method. The sensitivity decreased as the dye-surfactant bond strength increased and followed the order AR97<AR88 <AO6<CBBG. The low sensitivity obtained using AR97 (Fig. 4A) is a consequence of the two anionic groups and the great hydrophobic moiety in its molecular structure, which results in strong electrostatic and hydrophobic interacttions with DDABr. For dyes bearing a unique anionic group (i.e. AR88 and AO6), the sensitivity is higher when the hydrophobic moiety is lower (see Fig. 4B and C). Repulsive electrostatic interactions between positively charged groups in CBBG and DDABr probably disfavour the formation of CBBG-DDABr aggregates, which permitted the drug very effectively compete with the dye to interact with the surfactant (Fig. 4D).

The pH of the titration medium should be adjusted between 5.5 and 7.0 in order to ensure reproducibility in the determination of the titration end-point. Within this interval, decreased m_s and m_S^* values were obtained as the pH value increased. The measurement parameter, however, was kept constant. For adjustment of the pH, a phosphate buffer (pH = 5.9) was used. Electrolytes are known to decrease electrostatic interactions between oppositely charged organic molecules, so, phosphate buffer disfavoured the formation of both dye-surfactant and drug-surfactant aggregates. The effect of phosphate buffer on the interaction between dye and surfactant was more pronounced than that on the drug-surfactant interaction, which resulted in an increase in the measurement parameter (about two times) as the buffer concentration increased up to about 0.04 M. No influence of the buffer concentration in the interval

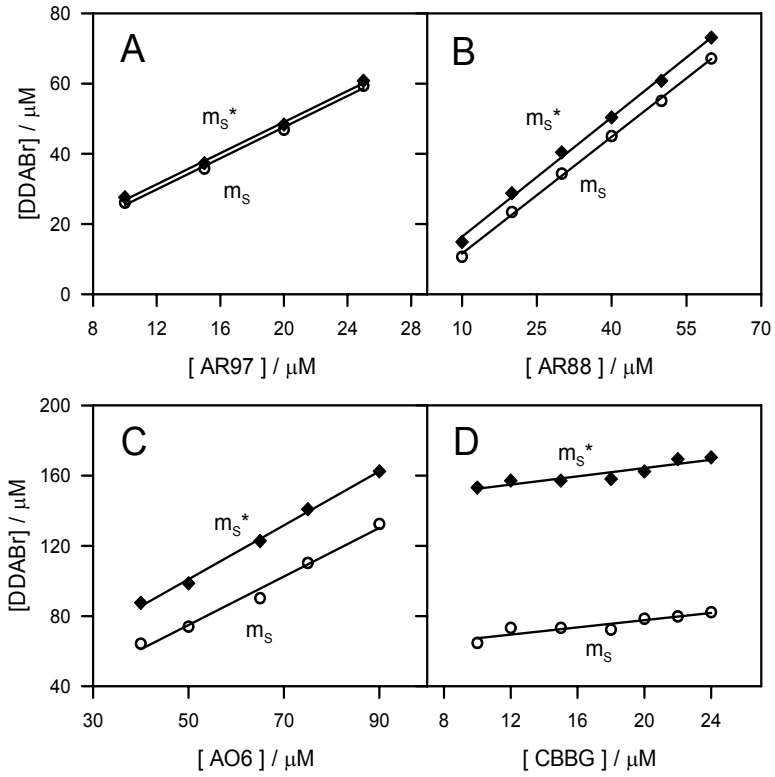


Fig. 4 . Influence of the concentration of the dyes: (A) AR97, (B) AR88, (C) AO6 and (D) CBBG on (O) m_S and (♦)m_S*. pH = 5.9 adjusted with 1M NaOH; [ibuprofen] = (A) 50 mg L^{-1}, (B) 30 mg L^{-1}, (C) 12 mg L^{-1} and (D) 12 mg L^{-1}.

0.04-0.06 M on the sensitivity achieved for the determination of ibuprofen was observed.

The influence of the temperature on the formation of mixed dye-surfactant and drug-surfactant aggregates and on the measurement parameter was studied in the range 5-60 °C. The DDABr concentration required to form dye-surfactant aggregates slightly decreased as a function of the temperature. At temperatures higher than about 40 °C, this effect was more pronounced in the presence than in the absence of drug, which resulted in a decrease of the measurement parameter by a factor of about 2 times when the temperature increased from 40 to 60 °C. The measurement parameter was kept constant at temperatures comprised between 5 and 40 °C,

therefore, measurements were performed at room temperature.

Organic solvents are frequently used to extract active ingredients from solid pharmaceutical samples and to dilute liquid formulates. So, the effect of organic additives on m_S , m_S* and the measurement parameter was studied by adding ethanol to the titration medium at concentrations up to 10%. These parameters were found to remain constant at alcohol concentrations up to 3%. Higher ethanol concentrations disfavoured the formation of both dye-surfactant and drug-surfactant aggregates, which resulted in a decrease in the measurement parameter by a factor of 1.7 when the alcohol content was increased from 3 to 10%. Ethanol has been reported to break down the structured water around the hydrophobic parts of organic molecules hindering hydrophobic interactions between them.[2]

Analytical performance

Calibration curves were run for therapeutic drugs belonging different structural groups, namely, propionic (flurbiprofen, ibuprofen, naproxen and ketoprofen) and acetic (diclofenac, felbinac and zomepirac) acids, indolines (indomethacin and sulindac), glycyrrhetinic acid derivatives (carbenoxolone and enoxolone), salicylates (diflunisal and phenyl salicylate), oxicams (meloxicam, piroxicam and tenoxicam), pyrazolones (phenylbutazone and sulphinpyrazone), hydrocortisones (dexamethasone and prednisolone), phenothiazines (promethazine), ethanolamines (diphehydramine) and dibenzazepines (nortriptyline). Linear calibration curves were obtained for all drugs tested bearing no charge or opposite charge to that of the cationic surfactant DDABr at the working pH 5.9 (molecular structures are depicted in Table 2). The sensitivity, defined as the slope of the calibration graph, the detection limit and the linear concentration range of the proposed method for the determination of these pharmaceuticals are shown in Table 3. Standard errors of the estimate and correlation coefficients varied over the ranges 0.3 - 2.3 and 0.995 -0.9998, respectively and the intercept values of the calibration graph were not

Table 2 Structures of anionic and neutral drugs tested

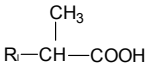
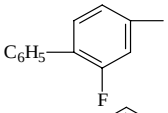
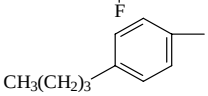
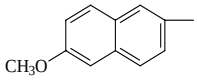
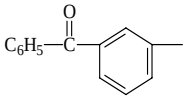
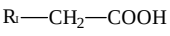
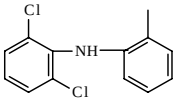
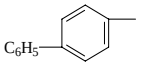
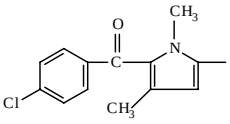
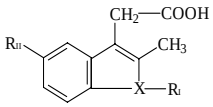
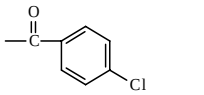
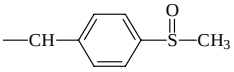
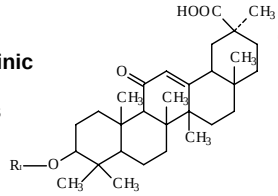
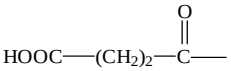
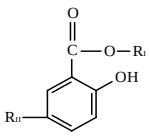
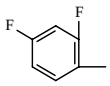
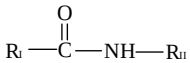
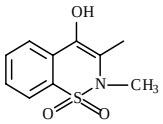
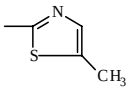
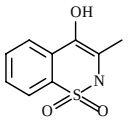
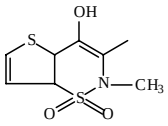
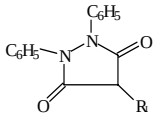
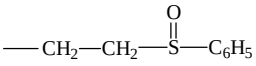
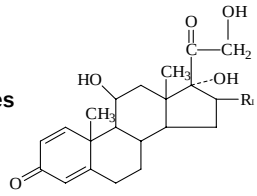
Structural group	General formula	Drug	X	R_I	R_{II}
Propionic acids		Flurbiprofen			
		Ibuprofen			
		Naproxen			
		Ketoprofen			
Acetic acids		Diclophenac			
		Felbinac			
		Zomepirac			
Indolines		Indomethacin	N		—OCH_3
		Sulindac	CH		—F
Glycyrrhetinic acid derivatives		Carbenoxolone			
		Enoxolone		—H	

Table 2 Structures of anionic and neutral drugs tested *(Continued)*

Structural group	General formula	Drug	R_I	R_{II}
Salicylates		Diflunisal	—H	
		Phenyl salicylate	—C_6H_5	H
Oxicams		Meloxicam		
		Piroxicam		
		Tenoxicam		
Pyrazolones		Phenylbutazone	—CH_2—$(CH_2)_2$—CH_3	
		Sulphinpyrazone		
Hydro-cortisones		Dexamethasone	—CH_3	
		Prednisolone	—H	

significantly different from zero. Therefore, results obtained for neutral and acid drugs, fitted to eqn (1) and the drug-surfactant binding degree at the titration end-point, β_A, remained constant over the linear concentration range. The precision, expressed as relative standard deviation, was estimated to be 0.1% (n=11) for a concentration of ibuprofen of 6.0 mg L^{-1}.

Basic pharmaceuticals (e.g. promethazine, diphenhydramine and nortriptyline) provided no analytical response at least up to drug concentrations of 200 mg L^{-1}, using DDABr as titrant. Repulsive electrostatic forces between drug and surfactant molecules bearing the same charge disfavoured the formation of drug-DDABr aggregates, which could not effectively compete with the formation of CBBG-DDABr aggregates in ternary drug-surfactant-dye aqueous mixtures.

The parameter β_A , which can be easily calculated from the slopes obtained for each of the drug tested taking into account their molecular weight, is a measure of the sensitivity of the SDB method. Table 4 shows the β_A values obtained for the neutral and acid drugs tested. The value of this parameter, and, therefore, the sensitivity of the proposed method, was highly dependent on the molecular structure of the drug. The drug-DDABr binding degree reached at the titration end-point was found to depend on the occurrence and number of anionic groups in the drug; it was higher for anionic than for neutral drugs and it increased with the number of anionic groups (e.g. compare the β_A values for carbenoxolone and enoxolone). Generally, the β_A value was higher for drug molecules with large hydrophobic moieties (e.g. compare the β_A values for diclofenac and zomepirac), although some exceptions were found (e.g. drugs belonging the oxicam structural group).

Determination of drugs in pharmaceutical preparations

The proposed method (see Procedure) was applied to the determination of neutral (dexamethasone)

Table 3 Analytical figures of merit of the proposed method for the determination of acid and neutral drugs

Drug	Detection limit[a]/ mg L^{-1}	Linear concentration range[b] /mg L^{-1}	Intercept ± s/µM	Slope ± s/ µM L mg^{-1}	r[c]	S_{yx}[d]
Flurbiprofen	0.1	0.3 – 3.5	0.3 ± 0.3	14.2 ± 0.1	0.9998	0.4
Ibuprofen	0.1	0.4 – 10	1 ± 1	11.4 ± 0.2	0.9994	1.7
Naproxen	0.2	0.7 – 12	1 ± 1	6.0 ± 0.1	0.9990	1.2
Ketoprofen	0.3	1 – 20	-2 ± 2	4.4 ± 0.1	0.997	2.3
Diclofenac	0.1	0.4 – 10	1 ± 1	9.7 ± 0.2	0.9990	1.8
Felbinac	0.1	0.5 – 7	1 ± 1	9.0 ± 0.1	0.9996	0.8
Zomepirac	0.3	1 – 15	-0.3 ± 0.5	3.90 ± 0.05	0.9997	0.6
Indomethacin	0.1	0.4 – 3	1 ± 1	12.0 ± 0.4	0.997	1.1
Sulindac	0.15	0.5 – 7	1 ± 1	8.2 ± 0.2	0.998	1.5
Carbenoxolone	0.1	0.4 – 7	1 ± 1	12.1 ± 0.3	0.9990	1.7
Enoxolone	0.2	0.6 – 9	-1 ± 1	7.0 ± 0.1	0.9994	0.9
Diflunisal	0.1	0.4 – 12	1 ± 1	9.9 ± 0.2	0.9991	1.9
Phenyl salicylate	0.8	3 – 30	0.7 ± 0.9	2.50 ± 0.05	0.9997	1.2
Meloxicam	0.1	0.4 – 12	-1 ± 1	11.6 ± 0.2	0.9994	2.1
Piroxicam	0.06	0.2 – 2	1 ± 1	21 ± 1	0.995	1.8
Tenoxicam	0.06	0.2 – 2	0.4 ± 0.4	22.1 ± 0.4	0.9997	0.5
Phenylbutazone	0.1	0.5 – 4	-0.1 ± 0.6	9.0 ± 0.2	0.9996	0.9
Sulphinpyrazone	0.1	0.3 – 3	2 ± 2	8.4 ± 0.8	0.995	2.2
Dexamethasone	2.5	8 – 50	0.2 ± 0.2	0.52 ± 0.01	0.9992	0.4
Prednisolone	2.3	8 - 25	-0.2 ± 0.2	0.55 ± 0.01	0.998	0.3

[a]Calculated as 3-fold the standard deviation of m_s

[b]Quantification limit calculated as 10-fold the standard deviation of m_s

[c]Correlation coefficient

[d]Standard error of the estimate

Table 4 Binding degree (β_A) of the cationic surfactant DDABr to acid and neutral drugs

Drug	$\beta_A \pm s^a$	Log P	Drug	$\beta_A \pm s^a$	Log P
Propionic acids			***Salicylates***		
Flurbiprofen	2.4 ± 0.2	4.2[b]	Diflunisal	2.47 ± 0.05	4.3[c]
Ibuprofen	2.35 ± 0.04	4.0[b]	Phenyl salicylate	0.53 ± 0.01	3.6[c]
Naproxen	1.38 ± 0.02	3.2[b]	***Oxicams***		
Ketoprofen	1.12 ± 0.02	3.1[b]	Meloxicam	4.32 ± 0.07	3.4[b]
Acetic acids			Piroxicam	6.9 ± 0.3	3.1[b]
Diclofenac	3.08 ± 0.06	4.5[b]	Tenoxicam	7.4 ± 0.1	1.4[c]
Felbinac	1.91 ± 0.02	3.3[c]	***Pyrazolones***		
Zomepirac	1.13 ± 0.01	2.3[c]	Phenylbutazone	2.77 ± 0.03	3.2[b]
Indolines			Sulphinpyrazone	3.4 ± 0.3	2.3[b]
Indomethacin	4.3 ± 0.1	4.3[b]	***Hydrocortisones***		
Sulindac	2.92 ± 0.07	3.4[b]	Dexamethasone	0.204 ± 0.004	1.8[b]
Glycyrrhetinic acid derivatives			Prednisolone	0.198 ± 0.004	1.6[b]
Carbenoxolone	6.9 ± 0.2	7.3[c]			
Enoxolone	3.29 ± 0.05	6.6[c]			

[a]s denotes standard deviation (n=5). Octanol/water partition coefficient values were obtained from [b]Virtual Computational Chemistry Laboratory (www.vcclab.org) and [c]Scifinder Scholar

and acid (ibuprofen, diclofenac, indomethacin, carbenoxolone, piroxicam and phenylbutazone) drugs in commercial formulations. Results obtained for different pharmaceutical dosage forms: effervescent granulates, tablets, suppositories, gels and blisters, are shown in Table 1. As it can be seen, all of them were consistent with the nominal contents, which prove the suitability of the SDBD method to analyse a high number of drugs in pharmaceutical preparations with a minimum sample treatment (only dilution of liquid samples and dissolution of solid samples).

CONCLUSIONS

The use of the surfactant DDABr and the dye CBBG has permitted the accurate, precise and selective determination of a variety of acid and neutral drugs, based on surfactant to dye binding degree measurements. Because of the advantageous features of the SDBD method (versatility, rapidity, simplicity and low cost) and the great number of drugs that can be determined, this methodology can be considered a useful tool for pharmaceutical quality control. Nowadays, our investigations on the use of the SDBD method to determine drugs in formulates continue with the aim of extending this new aggregation parameter-based method to the determination of basic drugs. Promising results have been already obtained using the anionic surfactant sodium dodecylsulphate (SDS) and the dye Cresyl Violet.

ACKNOWLEDGEMENTS

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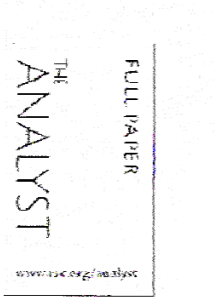
PARTE II

DETERMINACIÓN DE COMPUESTOS HIDROTRÓPICOS

CAPÍTULO 3

DETERMINACIÓN DE DROGAS HIDROTRÓPICAS

Analyst, 2005, 130, 1102-1107



Determination of aromatic hydrotropic drugs in pharmaceutical preparations by the surfactant-binding degree method

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An aggregation parameter-based analytical approach, the surfactant-dye binding degree (SDBD) method, was used, for the first time, to determine aromatic hydrotropic compounds. The anionic dye Coomassie Brilliant Blue G (CBBG) was used as inductor of didodecyldimethylammonium bromide (DDABr) aggregates, whose formation was monitored from changes in the spectral features of the dye. Interactions between hydrotrope and DDABr molecules resulted in a decrease of the degree of binding of the cationic surfactant to CBBG, which was proportional to the concentration of hydrotrope in the aqueous solution. The CBBG-DDABr-hydrotrope chemical system was found to fit to the mathematical expression previously derived for the determination of amphiphilic compounds. The hydrotrope-surfactant bond strength determined the sensitivity achieved for the determination of hydrotropic compounds, which was highly dependent on the molecular structure of the analyte. The high precision (the relative standard deviation for 7 mg L^{-1} of salicylic acid was 0.8%), rapidity (measurements were performed in a few minutes) and low cost (in both instrumentation and reactants) of the proposed method, made it especially suitable for quality control. The practical analytical applicability of the SDBD method for the control of hydrotropic drugs in pharmaceutical preparations was demonstrated by quantifying salicylic acid and acetyl salicylic acid in liquid (solutions) and solid (tablets, granulates, unguents, gels and creams) samples, which were directly analyzed after dissolution of the samples.

INTRODUCTION

Hydrotropes are a special class of compounds that exhibit distinct solution properties. They increase the aqueous solubility of sparingly-soluble solutes[1] and may self-assemble in solution[2] similarly to amphiphiles, although the minimum hydrotrope concentration required for aggregates formation (MHC) is very high (molar level) compared with that required for amphiphile aggregation. This behaviour can be explained on the basis of their molecular structure; they are structurally similar to amphiphiles in that they have hydrophilic and hydrophobic regions, but possess shorter hydrophobic moieties, which disfavours aggregation. Hydrotropes can also interact with amphiphilic compounds (i.e. surfactants) influencing their solution behaviour. Thus, they have been reported to modify phase-forming behaviour of micelle, microemulsion and liquid crystal-forming systems, clouding of non-ionic surfactants and polymers, conductance percolation behaviour of microemulsions and surfactant aggregation.[3-5]

Many drugs used in pharmaceutical formulations are hydrotropic. Some representative ones include aromatic carboxylic acids (salicylic, acetyl-salicylic, benzoic and p-amino-benzoic acid), aromatic sulphonic acids (p-hydroxybenzenesulphonic acid), poly-hydroxy benzenes (resorcinol), alkaloids (nicotine), aromatic amines (procaine) and non-aromatic compounds (ascorbic acid and urea). Since their structure and physico-chemical properties widely range, their quality control in formulations requires the use of very different analytical methods. Because their roughly amphiphilic character, it is interesting to explore the potential of aggregation parameter-based methods[6,7] for the determination of hydrotropic drugs, in order to have a common methodological approach for their quality control.

Aggregation parameter-based methods have emerged in the last few years as an advantageous alternative to monomer-based approaches used for determining amphiphilic compounds. Two new methodologies have been developed to the date: the mixed aggregate[6,8] (MA) and the sur-

factant-dye binding degree (SDBD)[7] methods. The critical micelle concentration (cmc) is the aggregation parameter measured in the MA method; the cmc value of mixtures of amphiphilic substances (a surfactant reactant and one/several analytes) decreases by a factor proportional to the mole fraction of the analyte in the aqueous solution. The cmc-based method has been proved to offer important advantages over the traditional analytical methods used for determining surfactants in environmental[6,9-12] and quality control,[11,13] and to be a useful tool for the sensitive and rapid determination of drugs in pharmaceutical preparations.[14-16]

The SDBD method is based on the measurement of the degree of binding of a surfactant to dye molecules, which induce the formation of surfactant aggregates at concentrations below its critical aggregate concentration (cac). An amphiphilic compound added to the dye-surfactant mixture causes a decrease in the dye-surfactant binding degree as a result of the interaction between amphiphile (analyte) and surfactant (reactant) molecules, being this decrease the basis of the measurement. The SDBD method has been demonstrated to permit the accurate and selective determination of total ionic surfactants in environmental samples[7] and amphiphilic drugs in pharmaceutical preparations.[17]

This research deals with the study of the potential of the binding-degree method for the determination of hydrotropic drugs. The MA method, based on cmc measurements, was considered less suitable for this purpose because the high concentration values required for hydrotrope aggregation. The surfactant used as reactant was didodecyldimethylammonium bromide (DDABr), which formed aggregates induced by the dye Coomassie Brilliant Blue G (CBBG) at concentrations far below its critical aggregation concentration (cac). The formation of these dye-induced aggregates was monitored from changes in the spectral features of the dye. The molecular interactions involved in the determination of hydrotropic compounds were studied, the variables affecting the hydrotrope-DDABr binding degree were evalu-

ated, the fit of the hydrotrope-DDABr-CBBG chemical system to the mathematical expression previously derived for calibration of amphiphiles was investigated and the suitability of the SDBD method for determining aromatic hydrotropic drugs in pharmaceutical preparations was demonstrated.

EXPERIMENTAL

Apparatus

A Metrohm 794 Basic Titrino titrator (Herisau, Switzerland) furnished with a 20-mL autoburet, a fan stirrer and a titration vessel was used for titrations. A Deward titration vessel thermostated by means of circulating water (temperature uncertainty = ±0.1 °C) was used to control the temperature for optimization studies. The detection unit was a Metrohm 662 spectrophotometer equipped with an immersion probe (1-cm path length). The instrument control and data processing was performed using a computer made up of a Pentium 4 processor, a Microsoft Windows XP operating system and a Metrohm TiNet 2.5 Light software. A Hitachi U-2000 spectrophotometer (Tokyo, Japan) was used to study molecular interactions in mixed aggregates.

Reagents and solutions

Highest-grade commercially available reagents were used throughout, without further purification. A $1.4x10^{-4}$ M aqueous solution of Coomassie Brilliant Blue G (CBBG, Sigma Chemical CO., St. Louis, MO, USA) was prepared by dissolving 0.125 g of the reagent in 1 L of distilled water with sonication for 15 min. This solution was prepared at least 24 h prior to use and was stable for one month. The buffer solution used consisted of 0.1 M KH_2PO_4 with the pH adjusted to 5.9 with 2 M NaOH. A $1x10^{-3}$ M aqueous solution of didodecyldimethylammonium bromide (DDABr, Fluka Chemie GmgH, Buchs, Switzerland) was also prepared. Stock solutions (1 g L^{-1}) of the hydrotropic compounds, salicylic acid, benzoic acid, p-aminobenzoic acid, p-hydroxybenzenesulphonic acid, resorcinol and procaine (Aldrich Chemie GmbH & Co KG, Steinheim, Germany), acetyl-

salicylic acid and ascorbic acid (Fluka Chemie GmgH, Buchs, Switzerland), nicotine (Riedel-deHaën, Seelze, Germany) and urea (G. Merck, Darmstadt, Germany) were prepared in distilled water.

Procedure

Volumes of 4.0 mL of $1.4x10^{-4}$ M CBBG solution, 11.2 mL of 0.1 M phosphate buffer (pH=5.9) and an aliquot of standard or treated sample solution of hydrotropic drug to give a final concentration between about 0.6 and 200 mg L^{-1} were placed in a 25-mL volumetric flask. Then, distilled water was added to the mark. This solution was placed in a 50-mL titration vessel and titrated with $1.0x10^{-3}$ M DDABr delivered from the buret at a rate of 10 mL min^{-1}. The stirring rate was set at 700 rpm. Titration curves were obtained by recording the absorbance at 610 nm as a function of the titrant volume.

Calculations

Quantification of hydrotropic drugs was based on the mathematical expression previously derived for the determination of amphiphilic compounds.[3] The anionic dye CBBG (D^-) interacts with the cationic surfactant DDABr (S^+) to form successive aggregates ($DS_n^{+(n-1)}$). The CBBG-DDABr binding degree (β_D) parameter, which expresses the extent of the aggregates formed, is calculated from: $\beta_D = (m_S - m_{S,M})/m_D$ where m_S denotes the surfactant concentration required to reach a determined β_D value, m_D is the total concentration of dye in the aqueous surfactant-dye mixture and $m_{S,M}$ is the concentration of surfactant in monomeric form. When a hydrotropic compound is added to the dye-surfactant mixture, the interaction between hydrotrope and surfactant molecules results in a decrease of the β_D value, which is now calculated from: $\beta_D = (m_S^* - m_{S,M} - \beta_H m_H)/m_D$, where m_S^* is the surfactant reagent concentration necessary to reach the same β_D value in the presence of hydrotrope than that obtained in its absence, β_H is the hydrotrope-surfactant binding degree and m_H the hydrotrope concentration. Combination of both equations gives:

$$m_S^* - m_S = \beta_H m_H \qquad (1)$$

from which calibrations for hydrotropic drugs are obtained by plotting the parameter $m_S^*-m_S$ as a function of m_H. The values of m_S^* and m_S are calculated from the volumes of DDABr consumed in titrations performed in the absence (V_S) and presence (V_S^*) of hydrotrope, respectively (see Fig.1).

The parameter β_H express the extent of the hydrotrope-surfactant aggregates formed in the presence of dye at the titration end-point. The values of β_H for the different hydrotropes tested were calculated from the slope of the linear calibration graphs (eqn.1), the hydrotrope concentration expressed as molar.

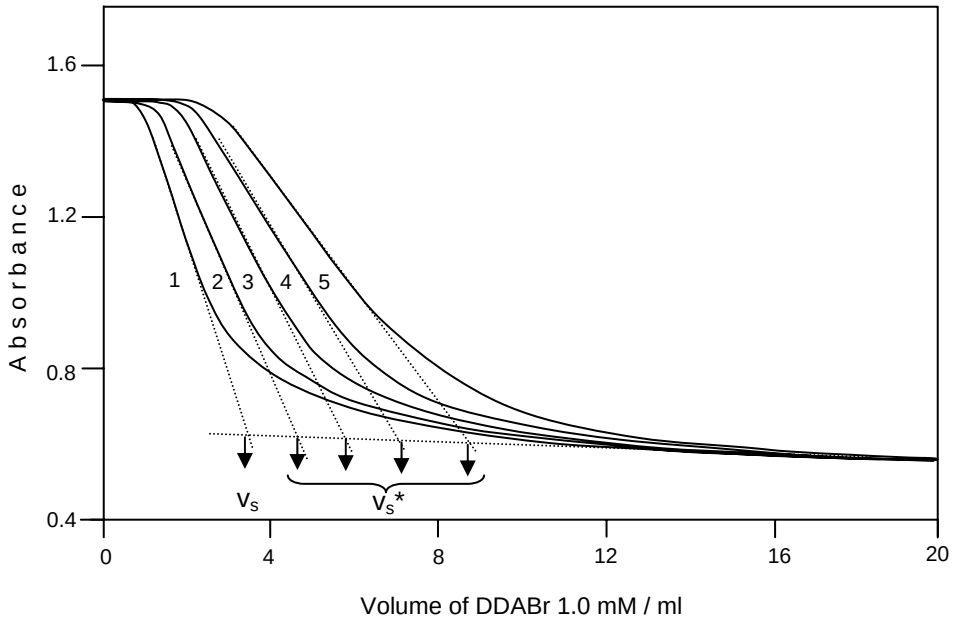

Fig. 1 Variation of the absorbance of Coomassie Brilliant Blue G (22 µM) at 610 nm as a function of the volume of titrant (1.0 mM) DDABr added to a titration vessel containing (1) no salicylic acid or salicylic acid at a concentration of (2) 22 µM, (3) 44 µM, (4) 66 µM and (5) 88 µM. [phosphate buffer] = 40 mM; pH = 5.9.

Determination of hydrotropic drugs in pharmaceutical preparations

Both liquid (solutions) and solid (tablets, granulates, unguents, gels and creams) pharmaceutical samples were analysed. Liquid formulations were diluted with ethanol to obtain clear solutions, which contained about 1g L^{-1} of hydrotropic drug. For the analysis of solid formulations, an amount of sample containing about 100 mg of hydrotropic drug was accurately weighed and dissolved in about 80 mL of ethanol (unguents and gels), or in about 200 mL of distilled water (tablets, granulates and creams) with magnetic stirring for 30 min or 2 hours, respectively, to aid dissolution of samples. If any insoluble material was present in the solution after this treatment, it was removed by filtration and washed several times with ethanol (unguents and gels) or distilled water (tablets, granulates and creams). Ethanol and aqueous solutions were made up to 100 and 250 mL, respectively. Aliquots of these sample solutions were analysed as described above.

RESULTS AND DISCUSSION

Molecular interactions in the dye-surfactant-hydrotrope system

The dye CBBG and the surfactant DDABr form aggregates of well-defined stoichiometries at DDABr concentrations higher than a thresh-old value, which is highly dependent on the experimental conditions.[7,17] The formation of dye-surfactant aggregates can be monitored from DDABr-induced changes in the spectral characteristics of CBBG (compare curves 1 and 2 in Fig. 2A). Under the experimental conditions recommended for the determination of hydrotropic drugs (see the Proce-dure in the Experimental Section), the threshold value for the formation of CBBG-DDABr aggregates was found to be 34±2 μM.

The addition of a hydrotropic drug (e.g. salicylic acid) to dye-surfactant mixtures causes further modifications in the spectral features of CBBG (compare curves 2 and 3 in Fig. 2A). The interaction occurring between the hydrotrope and the surfactant was confirmed from the hydrotrope

spectral changes induced by DDABr. As an example, Figure 2B shows the spectra of salicylate in DDABr solutions. They showed a slight bathochromic shift in the maximum absorption peak from 294 to 296 nm and an important hyperchromic shift over all the wavelength range studied (compare curves 1 with curves 2-3 in Fig. 2B). These changes are usually associated to minor electronic changes in the structure of drug molecules.[18]

Investigations on the formation of well-defined stoichiometry salicylate-DDABr aggregates were carried out by measuring the absorbance of salicylate (50 µM, pH=5.9) at 295 nm as a function of the cationic surfactant concentration within the interval of analytical interest (0-100 µM). In order to avoid irreproducibility due to

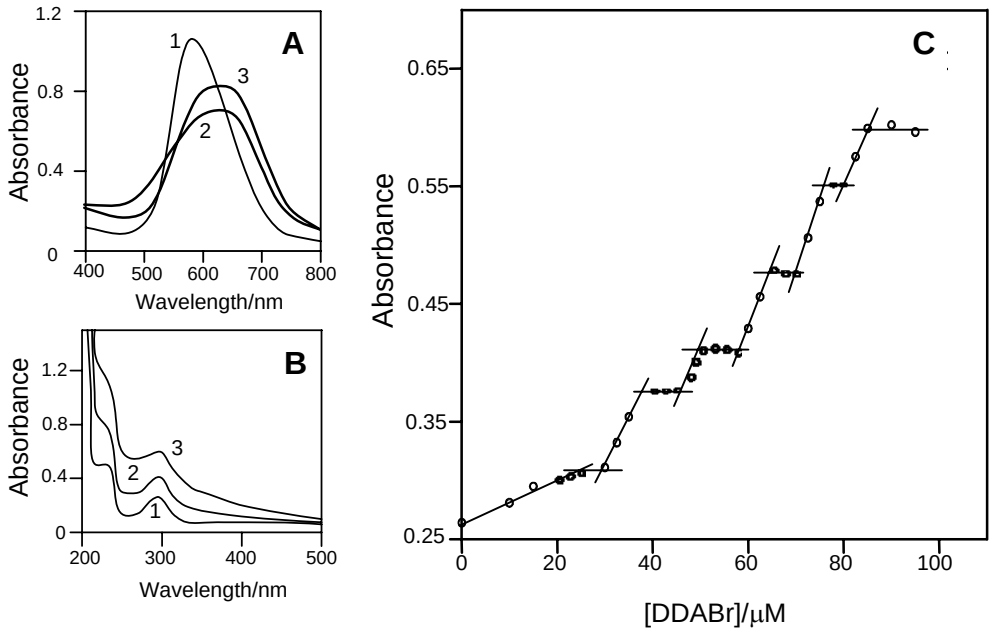

Fig. 2 (A) Spectra for 22 µM Coomassie Brilliant Blue G (1) alone and in the presence of (2) 120 µM DDABr and (3) 120 µM DDABr and 30 µM salicylic acid. (B) Spectra for salicylic acid (50 µM) in: (1) the absence and (2,3) the presence of DDABr: (2) 50 µM and (3) 85 µM. (C) Variation of the absorbance of salicylic acid (50 µM) at 295 nm as a function of the DDABr concentration. [phosphate buffer] = 40 mM; pH = 5.9. Spectra 2 and 3 in (A) recorded against 120 µM DDABr 40 µM phosphate buffer blank solutions.

light scattering produced by DDABr-salicylic acid aggregates, absorbance measurements were performed at a fixed time (5 min). The broken line obtained (Fig. 2C) indicated the formation of DDABr:salicylate aggregates of different stoichiometry (between 1:2 to 5:3) in proportion to the DDABr concentration increased. Therefore, dye-surfactant and hydrotrope-surfactant aggregates are simultaneously formed, with stoichiometries depending on the surfactant concentration and the relative interaction energies. More cationic surfactant will be necessary in the presence of hydrotropic drug in order to reach the same dye-surfactant stoichiometry (compare curve 1 with curves 2-5 in Fig 1).

Optimisation

The influence of different variables (pH, phosphate buffer and CBBG concentrations and temperature) on m_s , m_s* and the measurement parameter $(m_s*–m_s)$ was studied. Both, the sensitivity obtained for the quantification of hydrotropic drugs and the precision in the determination of the titration end-point were taking into account to select the optimal experimental conditions. Salicylic acid was the hydrotropic drug selected as model to perform this study.

The influence of the pH was determined using hydrochloric acid and sodium hydroxide for adjustment. The spectral changes of CBBG caused by DDABr and the hydrotropic drugs only produced appropriate titration curves, i.e. those permitting a reproducible determination of the titration end-point, in the range 5.5-6.5. The value of the parameters m_s* and m_s were found to decrease as a function of the pH value in this interval (Fig. 3A), however the difference between them, i.e. the measurement parameter, was kept constant. In order to obtain reproducible measurements, the selected pH (i.e. 5.9) was adjusted using a phosphate buffer. The DDABr concentration required to form dye-surfactant aggregates slightly increased as the buffer concentration did in both the presence and absence of analyte (Fig. 3B). This was the consequence of the decreasing that electrolytes cause in the electrostatic interactions between

oppositely charged organic molecules. The measurement parameter $(m_s^* - m_s)$ was not affected by the phosphate buffer concentration over all the range studied (0-50 mM). A 45 mM phosphate buffer concentration was selected.

The effect of the CBBG concentration on m_s , m_s^* and the measurement parameter was studied over the 10-25 µM range. Dye concentrations lower than 10 µM were inadvisable since the absorbance decrease as a function of the DDABr concentration was very small and this fact detracted from precision in the determination of the titration end-point. At CBBG concentrations higher than 25 µM, the initial absorbance at 610 nm was out of the detection unit measurement range. Both m_s and m_s^* values were found to linearly increase as a function of the CBBG concentration in a similar way (Fig. 3C) so, the measurement parameter remained constant over the whole dye concentration range studied.

The temperature, the effect of which was studied between 10 and 60°C, did not affect m_s and m_s^* values up to 30°C. Increased temperatures resulted in a decrease of the DDABr concentration required to form dye-surfactant aggregates in both the absence and presence of the analyte. The measurement parameter was found to decrease about three-fold from 30 to 60°C. The range from 10 to 30°C was optimal, and measurements could be performed at room temperature without thermostating.

The influence of organic solvents on the formation of CBBG-DDABr and salicylate-DDABr aggregates was investigated by addition of ethanol concentrations up to 10%. Ethanol was found not to affect the formation of dye-surfactant aggregates in proportions below about 2%. Higher alcohol contents disfavoured the formation of CBBG-DDABr aggregates as a result of its ability to disrupt the structured water surrounding hydrophobic moieties in organic molecules, which hindered hydrophobic interactions between them. The alcohol did not influence the salicylate-DDABr aggregates formation at concentrations up to 4%. Above this ethanol content, a slight increase in the analyte-surfactant binding

degree was observed (the measurement parameter increased in a factor of about 1.5 times in the presence of 10% ethanol), probably due to the fact that ethanol disfavour hydrotrope-surfactant aggregates formation to a lesser extent than dye-surfactant aggregates formation, since hydrophobic interactions play a more important role in the formation of mixed amphiphile aggregates than in the formation of hydrotrope-amphiphile aggregates.

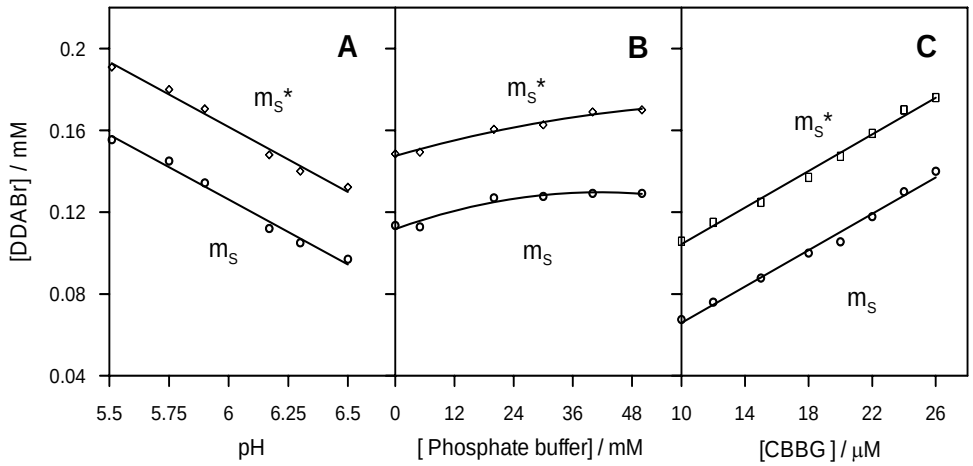

Fig. 3 Influence of (A) pH, (B) phosphate buffer and (C) CBBG concentration on m_s^* and m_s. [salicylic acid] = 22 μM; (A) and (B) [CBBG] = 24 μM; (A) and (C) [phosphate buffer] =45 mM; (B) and (C) pH = 5.9.

Calibration

Calibration graphs were run for different hydrotropic drugs, namely, aromatic (negatively charged: benzoic, salicylic, acetylsalicylic, p-aminobenzoic and p-hydroxybenzenesulphonic acids; neutral: resorcinol; and positively charged: procaine and nicotine) and aliphatic (ascorbic acid and urea) hydrotropes. Linear calibration curves were obtained for all aromatic hydrotropes tested bearing no charge or opposite charge to that of the cationic surfactant DDABr (Table 1). Standard errors of the estimate and correlation coefficients varied over the ranges $(0.6\text{-}2.1)\times10^{-6}$ and 0.994-0.9998, respectively. The linearity obtained indicated that the results for

these hydrotropic drugs fitted to eqn. (1) and the degree of binding of the cationic surfactant DDABr to aromatic hydrotropes at the titration end-point, β_H, remained constant over the linear concentration range. Therefore, surfactant-aromatic hydrotrope mixtures behaved as those made of amphiphilic compounds (surfactant-surfactant[7] and surfactant-drug[17] mixtures). The detection limits reached were highly dependent on the molecular structure of the hydrotropic drug. The precision, expressed as relative standard deviation, was estimated to be 0.8% for a concentration of salicylic acid of 7.0 mg L^{-1}.

No response was obtained for hydrotropic drugs bearing the same charge to that of the surfactant (e.g. procaine and nicotine) at least up to drug concentrations of 200 mg L^{-1}. Repulsive electrostatic forces prevented the formation of drug-DDABr aggregates and therefore, their quantitation by the SDBD method. Non-aromatic hydrotropes (e.g. ascorbic acid and urea) could not be

Table 1 Analytical figures of merit of the proposed method for the determination of aromatic hydrotropic drugs

Hydrotropic drug	Detection Limit[a] / mg L^{-1}	Linear Concentration range[b] / mg L^{-1}	Intercept ± s (µM)	Slope ± s (µM l mg^{-1})	r[c]	S_{yx}[d] (10^{-6})
Salicylic acid	0.2	0.6 – 15	0.1 ± 0.1	11.7 ± 0.1	0.9993	2.1
Benzoic acid	2.8	9 – 100	-0.04 ± 0.04	0.776 ± 0.007	0.9998	0.6
Acetylsalicylic acid	5	16 – 200	0.1 ± 0.1	0.46 ± 0.01	0.9994	1.3
p-Aminobenzoic acid	23	75 – 200	-0.08 ± 0.09	0.096 ± 0.008	0.996	1.2
p-Hydroxybenzene-sulphonic acid	3.5	12 – 30	0.1 ± 0.1	0.61 ± 0.06	0.994	1.4
Resorcinol	4	14 – 200	-0.1 ± 0.1	0.507 ± 0.007	0.9997	1.2

[a] Calculated as 3-fold the standard deviation of m_s

[b] Quantification limit calculated as 10-fold the standard deviation of m_s

[c] Correlation coefficient

[d] Standard error of the estimate

quantified either; the very short hydrophobic moiety in these molecules resulted in weak hydrotrope-surfactant interactions, which could not effectively compete with CBBG-DDABr interactions.

The parameter β_H , which can be easily calculated from the calibration graph slopes (see Calculations in the Experimental Section), is a measure of the sensitivity of the SDBD method for the determination of hydrotropes. Variations in the β_H values observed for aromatic hydrotropic drugs (Table 2) could be explained on the basis of the strength of hydrophobic and ionic hydrotrope - surfactant interactions. Thus, β_H values increased as the octanol/ water partition coefficient for the drug did (see the β_H values in Table 2 for carboxylic aromatic acids). For similar hydrophobicity, the ammonium quaternary-sulphonate interacttion was higher than that of the ammonium quaternary - carboxylate one. A sulphonate group gives less resonance interaction with the aromatic ring than a carboxylate group, as a result, negative charge is localized on the sulphonate group, while it is delocalized and distributed over the carboxylate group and the aromatic ring. Finally, the presence of a hydroxyl group in aromatic acids permitted the electrostatic interactions of one hydrotropic molecule with two surfactant molecules, which resulted in higher hydrotrope-surfactant binding degree. An example is the β_H value obtained for salicylic acid, which was 17 times higher than that obtained for benzoic acid, although both organic molecules only differ in a hydroxyl group. This effect was not observed for para-isomers of hydroxy substituted aromatic acids, probably due to steric accessibility problems.

Determination of hydrotropic drugs in pharmaceutical samples

The analytical applicability of the SDBD method for pharmaceutical quality control was evaluated by determining salicylic acid and acetyl salicylic acid in liquid (solutions) and solid (tablets, granulates, unguents, gels and creams) samples. Ten commercially available pharmaceutical formulations were analysed using the recommended procedure (see the

Table 2 Binding degree (β_H) of the cationic surfactant DDABr to negatively charged and neutral aromatic hydrotropic drugs

Aromatic hydrotropic drug	$\beta_H \pm$ s	Log P[a] ± s
Carboxylic aromatic acids		
Salicylic acid	1.62±0.01	2.1±0.2
Benzoic acid	0.095±0.001	1.9±0.2
Acetylsalicylic acid	0.083±0.01	1.2±0.2
p-Aminobenzoic acid	0.013±0.001	0.0±0.2
Sulphonic aromatic acids		
p-Hydroxibenzenesulphonic acid	0.14±0.01	0.2±0.2
Polyhydroxybenzenes		
Resorcinol	0.056±0.001	0.8±0.2

[a] Octanol/water partition coefficient from SciFinder Scholar

Experimental Section). Liquid formulations were diluted with ethanol in order to avoid precipitation of pharmaceutical excipients, which permitted the determination of hydrotropic drugs in unfiltered samples. Hydrotropic drugs were quantitatively extracted from tablets, granulates and creams using water and from unguents and gels using ethanol. For all the pharmaceutical preparations tested, both nominal and found values (see Table 3) were quite consistent, which proves the suitability of the proposed method for the intended purpose.

CONCLUSIONS

The ability of the surfactant to dye binding degree-based method for determining aromatic hydrotropic drugs in pharmaceutical preparations has been demonstrated. The strength of the interaction between the hydrotrope and the surfactant used as

Table 3 Determination of aromatic hydrotropic drugs in pharmaceutical preparations

Hydrotropic drug	Commercial formulation[a]	Nominal concentration	Found (s)[b] concentration
Salicylic acid	Morry (Unguent)	500 mg g^{-1}	500 (10) mg g^{-1}
	Movilat (Gel)	20 mg g^{-1}	20.2 (0.4) mg g^{-1}
	Bazalin (Cream)	30 mg g^{-1}	29.7 (0.7) mg g^{-1}
	Diprosalic (Solution)	20 mg g^{-1}	20.0 (0.2) mg g^{-1}
	Quocin (Solution)	120 g L^{-1}	120 (1) g L^{-1}
Acetyl salicylic acid	Aspirina (Tablets)	830 mg g^{-1}	831 (8) mg g^{-1}
	A.A.S. (Tablets)	437 mg g^{-1}	435 (7) mg g^{-1}
	Couldina Instant (Effervescent granulates)	144 mg g^{-1}	145 (2) mg g^{-1}
	Aspirina Complex(Effervescent granulates)	141 mg g^{-1}	142 (2) mg g^{-1}
	Gripal (Effervescent tablets)	99 mg g^{-1}	99 (1) mg g^{-1}

[a] Morry: Teofarma S.R.L., Italy. Movilat: Sankyo Pharma, Spain. Bazalin: Yamanouchi Pharma, Spain. Diprosalic: Schering-Plough, Spain. Quocin: Isdin Laboratories, Spain. Aspirina, Aspirina Complex and Gripal: Pharmaceutical Chemical Bayer, Spain. A.A.S.: Sanofi-Synthelabo, Spain. Couldina Instant: Alter Laboratories, Spain. [b]s denotes standard deviation (n=6).

reactant determines the sensitivity of the method. Maximum sensitivity was achieved for hydrotropic compounds able to bond to the surfactant through hydrophobic and electrostatic interactions. The analytical features of the proposed method in terms of sensitivity (the detection limit achieved was similar to that afforded by monomer-based methods[19-22]), selectivity (most of active ingredients or excipients did not interfere), precision (relative standard deviation for the whole analytical process including sample treatment < 2%) and rapidity (not only because of the speed of the determination step, titration curves could be recorded in a few

minutes, but also because a minimum sample treatment was required) supported its usefulness for the quality control of aromatic hydrotropic drugs. The proposed method is not adequate to analyze pharmaceuticals containing mixtures of hydrotropes with similar analyte-surfactant binding degree values; however, it could be a useful tool to determine aromatic hydrotropic compounds able to strongly interact with the surfactant used as reactant, in the presence of hydrotropes with low β_H values.

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CAPÍTULO 4

DETERMINACIÓN DE ADITIVOS ALIMENTARIOS

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ANALYTICAL & BIOANALYTICAL CHEMISTRY

Surfactant to dye binding degree based approach for the selective determination of L-glutamate in foodstuffs

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A selective method for the determination of L-glutamate in foodstuffs has been developed. It was based on the competition established between the analyte and the dye Coomassie Brilliant Blue G (CBBG) to interact with the surfactant didodecyldimethylammonium bromide (DDABr). The measurement parameter was the amount of DDABr required to reach a given dye-to-surfactant binding degree. It was obtained by photometric titration on the basis of the changes observed in the spectral characteristics of the dye when CBBG-DDABr aggregates were formed. The calibration graph obtained was linear in the L-glutamate concentration interval 0.2–5 mM (detection limit 0.05 mM). The high selectivity of the proposed method (other amino acids and food additives did not interfere at the concentrations present in foodstuffs) permitted the direct analysis of food samples after dissolution of the analyte in hot water. The accuracy of the surfactant to dye binding degree method was demonstrated by determining L-glutamate in different kinds of foodstuffs (liquid and dried soups, seasonings, pasta sauces and dried mushroom creams) and comparing the results obtained with those provided by the commercial Boehringer Mannheim essay.

Keywords Surfactant to dye binding degree method, L-Glutamate, Food analysis, Routine quality control

Introduction

L-Glutamate is one of the most widely used flavor enhancers in the food industry (world production 500,000 t $year^{-1}$). It is added to foodstuffs either as purified monosodium L-glutamate or as L-glutamic acid, the latter being obtained from the hydrolysis of proteins. L-Glutamate is generally recognized as safe at the typical ingestion levels, but high doses may result in certain adverse physiological effects. Its consumption has been linked to neurodegenerative diseases such as Alzheimer's and Parkinson's [1, 2] and has been alleged to cause and/or exacerbate obesity problems (because of its appetite-enhancing effect), asthma, atopic dermatitis, ventricular arrhythmia, neuropathy, etc. [3] and the so-called Chinese restaurant syndrome [4]. The World Health Organization has set an acceptable L-glutamate daily intake of 120 mg kg^{-1} body weight [5, 6]. The maximum concentration permitted by the European Union [7] is 10 g kg^{-1} foodstuff (sum of the total content of both natural and added glutamic acid and its salts). However, no limits have been established for condiments and seasonings.

The routine quality control of L-glutamate in foodstuffs requires the use of selective analytical methods, able to accurately determine this amino acid without the interference from other amino acids or additives. Rapidity, simplicity and low cost are also desirable features. Commercially available [8, 9] and widely used methods in the food industry are those based on enzymatic reactions and photometric detection [8-13]. Enzyme-based fluorimetric [10, 14, 15], potentiometric [16, 17] and amperometric [18-21] approaches have also been reported. High cost and instability of enzymes are the main disadvantages of these methods. The use of amino acid analyzers and liquid chromatography is also common, but these methods require sample cleanup by ion exchange [22] or solid-phase extraction [23] and/or the postcolumn or precolumn derivatization of the analyte [23-25], which greatly increases analysis time.

This paper reports a new approach for the routine monitoring of L-glutamate in foodstuffs based on the surfactant

to dye binding degree (SDBD) method. This method, recently developed by our research group [26], is founded on the measurement of the amount of surfactant needed to reach a given degree of binding to a dye. This amount increases as a function of the concentration of analyte, owing to the competition established between it and the dye to interact with the surfactant. It is determined by photometric titration, monitoring the formation of dye-surfactant aggregates. The energy of binding between the analyte and the surfactant determines the sensitivity of the SDBD method and this energy compared with that afforded by the interferent species determines its selectivity. To date, selective approaches based on the SDBD method have been developed. They include the determination of the total concentration of anionic surfactants in tap, natural and residual waters without the interference of non-ionic surfactants [26, 27] and the quantification of different drugs in pharmaceutical preparations without the interference of other active ingredients or excipients [28-32].

The dye and the surfactant selected for determining L-glutamate in foodstuffs were Coomassie Brilliant Blue G (CBBG) and didodecyldimethylammonium bromide (DDABr), respectively. The molecular interactions in the CBBG-DDABr-glutamic acid system were studied, the experimental variables affecting the sensitivity and precision of the determination of L-glutamate were optimized, the analytical features of the proposed method were determined and its applicability to the analysis of real samples was evaluated by analyzing different kinds of foodstuffs.

Experimental

Apparatus

A Metrohm 794 Basic Titrino titrator (Herisau, Switzerland) equipped with a 20-mL autoburet, a fan stirrer and a titration vessel was used for titrations. The detection unit was a Metrohm 662 spectrophotometer furnished with an immersion probe (1-cm path length). The instrument control and data processing were performed using a computer made up of a Pentium 4

processor, Microsoft Windows XP operating system and Metrohm TiNet 2.5 Light software. For optimization studies, a Deward titration vessel thermostated by means of circulating water (temperature uncertainty ±0.1 °C) was used to control the temperature of the titration medium. Absorption measurements were performed with a Hitachi U-2800A spectrophotometer.

Reagents and solutions

Highest-grade commercially available reagents were used throughout, without further purification. A 0.14 mM CBBG (Sigma Chemical Co., St. Louis, MO, USA) solution was prepared by dissolving 0.125 g of the dye in 1 L of distilled water with sonication for 15 min. This solution was prepared at least 24 h prior to use and was stable for 1 month. The buffer solution used consisted of 0.5 M NaH_2PO_4 with the pH adjusted to 5.5 with 2 M NaOH. DDABr (Fluka Chemie, Buchs, Switzerland) solutions (1 mM) were made by dissolving 0.4626 g of the cationic surfactant in 1 L of distilled water with magnetic stirring for 5 min at room temperature. Fresh aqueous DDABr solutions were prepared monthly. A 2.5 M NaCl solution was also prepared. Aqueous stock solutions of 14 mM L-glutamic acid (Sigma-Aldrich Chemie, Steinheim, Germany) were made weekly in distilled water. L-Amino acids used in the selectivity studies were obtained from Fluka Chemie (Buchs, Switzerland) – arginine, serine, threonine, alanine, valine, methione, histidine and cystine – and Sigma-Aldrich Chemie (Steinheim, Germany)–lysine, aspartic acid, asparagine, glutamine, glycine, proline, isoleucine, leucine, phenylalanine, tryptophan and tyrosine. Other substances tested as possible interferents were: sodium benzoate (Sigma-Aldrich Chemie, Steinheim, Germany), sodium dihydrogen citrate, sodium sorbate, ascorbic acid, sodium succinate (Fluka Chemie, Buchs, Switzerland) and sodium acetate (Merck, Darmstadt, Germany).

Sample preparation

Both the liquid and the solid foodstuffs analyzed (Table 1) were

treated as follows. An amount of sample containing about 50 mg of L-glutamate was accurately weighed and mixed with about 80 mL of water by magnetic stirring for 10 min at about 70 °C. After it had cooled, the whole aqueous solution was filtered through paper filters (Filter-lab, Barcelona, Spain, porosity 7-9 µm, diameter 125 mm) and made up to 100 mL with distilled water. Aliquots of these sample solutions were analyzed as described below.

Table 1 Determination of L-glutamate in foodstuffs

Foodstuff	Label ingredients	L-Glutamate found[a] (s)[b]	
		SDBD method	Boehringer Mannheim kit
Seasoning	Salt, spices, aroma, yeast extract, milk serum, modified starch, flour, soya, onions, potatoes, carrots, celery, vegetable fat and maltodextrin	128 (2)	128 (1)
Liquid soup	Water, salt, aroma, spices, yeast extract, hydrolyzed vegetable protein, onions, potatoes, carrots, celery and chicken fat	6.75 (0.09)	6.8 (0.1)
Dried soup	Salt, spices, yeast extract, hydrolyzed vegetable protein, onions, potatoes, carrots, celery, parsley, chicken, chicken fat, vegetable oil and corn starch	8.6[c] (0.1)	8.7[c] (0.1)
Dried mushroom cream	Salt, aroma, yeast extract, milk serum, hydrolyzed vegetable protein, modified starch, onions, carrots, celery, potatoes puree, corn flour, vegetable oil, maltodextrin, mushroom and sweetener	5.7[c] (0.1)	5.8[c] (0.1)
Pasta sauce	Water, sugar, spices, aromatic plants, tomatoes, onions, carrots, celery, vegetable oil and modified starch	4.8 (0.1)	4.86 (0.04)

SDBD: surfactant-to-dye binding degree

[a]Expressed as grams of L-glutamic acid per kilogram of food sample
[b]Standard deviation (n=6)
[c]L-Glutamate concentrations in the reconstituted food

Recommended procedure for the quantification of L-glutamate

Volumes of 4.3 mL of 0.14 mM CBBG solution, 2 mL of 0.5 M phosphate buffer (pH 5.5), 0.35 mL of 2.5 M NaCl and an aliquot of standard or treated sample solution to give a final concentration of L-glutamate between about 0.2 and 5 mM were placed in a 25-mL volumetric flask. Then, distilled water was added to the mark. This solution was placed in a 50-mL titration vessel and titrated with 1.0 mM DDABr delivered from the buret at a rate of 10 mL min^{-1}. The stirring rate was set at 700 rpm. Titration curves were obtained by recording the absorbance at 610 nm as a function of the titrant volume (Fig. 1).

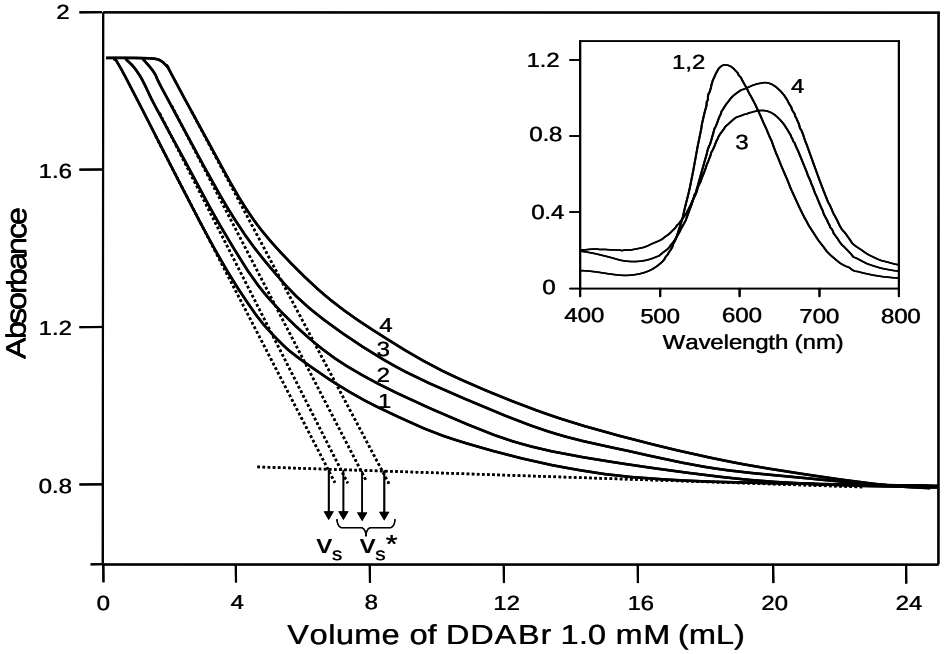

Fig. 1 Variation of the absorbance of Coomassie brilliant blue G (CBBG; 24 μM) at 610 nm as a function of the volume of titrant (1 mM didodecyldimethylammonium bromide; DDABr) added to a titration vessel containing (1) non-analyte or L-glutamate at a concentration of (2) 1 mM, (3) 3 mM and (4) 5 mM. Inset: Spectra for 24 μM CBBG in (1) the absence of analyte and the presence of (2) 4 mM L-glutamate, (3) 0.2 mM DDABr and (4) 0.2 mM and 4 mM L-glutamate. Other conditions were 40 mM phosphate buffer, 35 mM NaCl and pH 5.6.

The concentration of L-glutamate was determined from the following equation [26]:

$$m_S^* - m_S = \beta_G \, m_G \qquad (1)$$

where m_S^* and m_S denote the amount of DDABr, expressed as a molar concentration, required to reach a given degree of binding of DDABr to CBBG, in the presence and the absence of L-glutamate, respectively, m_G is the concentration of L-glutamate and β_G is the glutamic acid-DDABr binding degree. Calibration graphs were constructed by plotting the measurement parameter ($m_S^* - m_S$) as a function of the concentration of L-glutamate (m_G). The m_S^* and m_S values were calculated from the volumes of DDABr consumed in titrations performed in the absence (V_S) and presence (V_S^*) of L-glutamate, respectively.

Determination of L-glutamate using the Boehringer Mannheim essay

Measurements were performed according to the instructions [8] of the manufacturer (R-Biopharm, Darmstadt, Germany).

Results and discussion

Molecular interactions in the CBBG-DDABr-glutamic acid system

The anionic dye CBBG and the cationic surfactant DDABr form mixed dye-surfactant aggregates of well-defined stoichiometry [26] at surfactant concentrations higher than a threshold value (11.5±0.5 μM under the experimental conditions recommended for the determination of L-glutamate; "Experimental"). The formation of CBBG-DDABr aggregates can be monitored photometrically from changes in the spectral features of the dye (compare spectra 1 and 3 in Fig. 1, inset).

The addition of L-glutamate to CBBG-DDABr aqueous mixtures resulted in further changes in the spectral features of the dye (compare spectra 3 and 4 in Fig. 1, inset) compatible with a decrease in the CBBG-DDABr binding degree. Consequently, the amount of DDABr necessary to reach a given surfactant to dye binding degree increased as the L-glutamate concentration did (compare curves 1-4 in Fig. 1). This effect could be

explained on the basis of the L-glutamate-DDABr interactions that effectively competed with the CBBG-DDABr ones, decreasing the amount of surfactant available to form CBBG-DDABr aggregates. Analyte-surfactant binding occurred through both attractive electrostatic interactions between the γ-carboxylate group in the analyte, negatively charged at the working pH of 5.5 (pKa = 4.1), and the ammonium quaternary group in the cationic surfactant, and hydrophobic interactions between the hydrophobic moieties in both L-glutamate and DDABr molecules. No evidence of interaction between L-glutamate and CBBG was obtained (compare curves 1 and 2 in Fig. 1, inset).

Optimization

The variables optimized were pH, KH_2PO_4 buffer, CBBG and sodium chloride concentrations, and temperature. The optimization was performed by studying the effect of these variables on the measurement parameter $(m_S^* - m_S)$, changing each of them in turn while keeping all other parameters constant, and selecting the experimental conditions that provided maximum sensitivity and precision.

The influence of the pH was studied in the interval 4.5-8.0. Titration curves providing reproducible determination of the titration end point were obtained at pH values in the interval 5.3–6.3. Beyond this range, the titration curves showed very short straight lines before and/or after the end point, which resulted in lack of precision in the determination of V_S and V_S^*. A 40 mM phosphate buffer was used to adjust the pH inside this interval. Both m_S^* and m_S decreased as the pH increased, this effect being more pronounced in the presence of L-glutamate (m_S^*) than in its absence (m_S). As a result, the measurement parameter decreased at pH values higher than about 5.6 (Fig. 2, panel a). A pH of 5.5 was selected.

Increased phosphate buffer concentrations resulted in decreased electrostatic interaction between oppositely charged groups in the dye and the surfactant, and, hence, in an increase in the DDABr concentration required for mixed dye-surfactant aggregate

formation. This effect was observed in both the presence and the absence of L-glutamate. However, the phosphate buffer did not affect the degree of binding of the analyte to DDABr (β_G) and the measurement parameter remained constant over all the concentration range studied (0–50 mM). A 40 mM-phosphate buffer was used to adjust the pH of the titration medium to 5.5.

The CBBG concentration greatly affected the sensitivity and precision obtained for the determination of L-glutamate. Logically, the amount of DDABr needed to form CBBG-DDABr aggregates increased as a function of the dye concentration. Because the increase was more pronounced in the presence of analyte than in its absence, an increase in the measurement parameter was observed (Fig. 2, panel b). Improved precision in the determination of the titration end point was also observed as the dye concentration increased because of the greater absorbance decrease obtained as a function of the DDABr concentration. A CBBG concentration of 24 µM was considered optimal for the quantification of L-glutamate.

The parameters m_S and m_S^* slightly decreased as the temperature increased from 20 to 40 °C. However, the measurement parameter was kept constant at temperatures between 20 and 30°C, decreasing by a factor of about 2 when the temperature increased from 30 to 40 °C. Measurements were performed at room temperature.

Because sodium chloride is a common ingredient in most foodstuffs, its effect on the measurement parameter was investigated. Increases observed in the m_S and m_S^* values when the salt was present in the titration medium could be explained on the basis of an electrolyte effect, similar to that described above for phosphate buffer. Sodium chloride also decreased L-glutamate-DDABr interactions, which resulted in a decrease in the analyte-surfactant binding degree and, hence, in diminished measurement parameter values (Fig. 2, panel c). Addition of 35 mM NaCl to the titration medium was recommended to avoid the interference of this salt in the determination of L-glutamate in foodstuffs by the SDBD method.

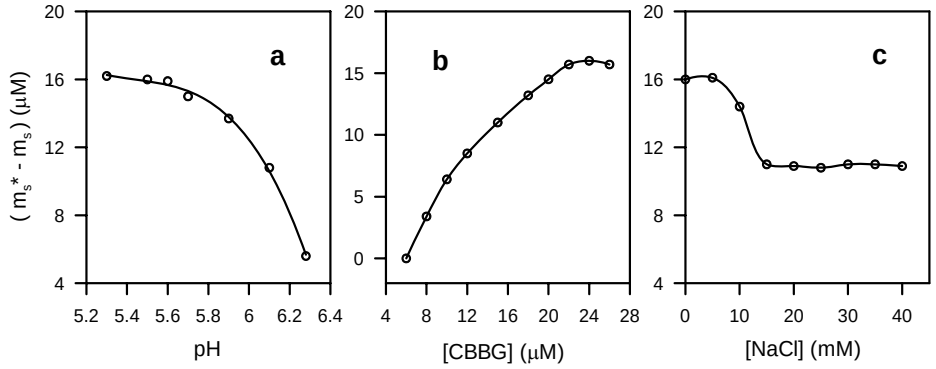

Fig. 2 Influence of *a* pH, and *b* CBBG and *c* sodium chloride concentrations on the measurement parameter. The conditions were as follows: 1.4 mM L-glutamate; *a* and *c* 24 μM CBBG; *b* and *c* pH 5.6; 40 mM phosphate buffer

Calibration

The calibration graph was constructed by plotting the measurement parameter (m_S*– m_S) versus the concentration of L-glutamate (m_G). It was linear in the L-glutamate concentration range 0.2–5 mM and its intercept value was not significantly different from zero (0.2±0.4 μM). Therefore, the measurements obtained fitted to Eq. 1, and the degree of binding of L-glutamate to DDABr (β_G , sensitivity, defined as the slope of the calibration graph) remained constant [(7.1±0.1) x10^{-3}]. The standard error of estimate and the correlation coefficient obtained were 5.1x10^{-4} and 0.9994 (n=9), respectively. The detection limit, calculated as 3 times the standard deviation of m_S , was 0.05 mM.

The precision of the proposed SDBD method, expressed as relative standard deviation, was 1.2 % (n=11) for 1.5 mM L-glutamate.

Selectivity

The main interferences commonly found in the enzymatic determination of L-glutamate arise from other amino acids and additives (i.e., preservatives, acidity regulators, emulsifiers, etc.) [10, 11, 14, 15, 17, 21], so specific procedures have been proposed for their removal in the commercially available methods [8,

9]. Usually, these methods work adequately for interferent to L-glutamate molar ratios around 1, but they fail at higher proportions, with cysteine and ascorbic acid being the most common interferences.

The selectivity of the SDBD method in the presence of common amino acids and additives was investigated. A given compound was considered not to interfere with the determination of L-glutamate if the mixture of interference plus analyte yielded a signal in the range $S_x \pm s$, where S_x is the signal provided by the analyte in the absence of interferent and s is the standard deviation of the method. The maximum molar ratio of foreign species to L-glutamate tested was 100, except for low water soluble amino acids (i.e., isoleucine, methionine, phenylalanine, leucine, tryptophan, cysteine and tyrosine), which were assayed at their corresponding water solubility values. The results obtained are shown in Table 2.

Amino acids were tolerated at high amounts by the non-enzymatic SDBD method. Arginine, histidine and lysine, the more interfering ones, did not give any analytical response at the same molar ratio as L-glutamate. Higher concentrations produced a decrease in the analytical signal probably owing to the interaction between the positive charge in their side chain at the working pH of 5.5 (i.e., pK_a = 12.5, 6.0, 10.5, for arginine, histidine and lysine, respectively) and the anionic dye CBBG, which resulted in a decrease in the amount of the CBBG-DDABr aggregates formed.

The selectivity of the SDBD method against some food additives was very high (Table 2). However, the tolerated ratio for other additives (e.g., ascorbate, sorbate, citrate and benzoate) was similar to that afforded by the enzymatic methods. These additives caused a positive interference in the determination of L-glutamate as a result of their interaction with DDABr.

In conclusion, noninterferences are expected from other amino acids, at the concentrations usually present in foodstuffs, for the determination of L-glutamate. Interferences could occur from foods containing ascorbate, sorbate, citrate or benzoate as

Table 2 Effect of foreign species on the determination of 1 mM L-glutamate, expressed as tolerated molar ratio of foreign species to L-glutamate

Foreign species	Tolerated molar ratio	Foreign species	Tolerated molar ratio	Foreign species	Tolerated molar ratio
Amino acids				*Food additives*	
Aspartic acid	>100	Methionine	>60	Oxalate	>100
Serine	>100	Phenylalanine	>30	Succinate	>100
Threonine	>100	Leucine	>20	Acetate	>100
Asparagine	>100	Tryptophan	>3	Ascorbate	1
Glutamine	>100	Cysteine	>2	Sorbate	0.5
Glycine	>100	Tyrosine	>1	Citrate	0.5
Proline	>100	Arginine	2	Benzoate	0.5
Valine	>100	Histidine	2		
Alanine	>100	Lysine	1		
Isoleucine	>60				

additives, if their amounts are higher than that of L-glutamate.

Determination of L-glutamate in foodstuffs

The applicability of the proposed method to the analysis of real samples was investigated by analyzing different kinds of foodstuffs (seasonings, liquid and dried soups, dried mushroom creams and pasta sauces) and comparing the results obtained with those provided by a commercial enzymatic – spectrophotometric approach supplied by Boehringer Mannheim [8]. The results obtained are summarized in Table 1. As can be seen, the results provided by the two methods were quite consistent proving the suitability of the SDBD method for the determination of L-glutamate in foods. Concentrations of this amino acid below the threshold value permitted by the European Union (10 g kg^{-1}, except for condiments and seasonings, for which no limit has been established [7]) were obtained for all the foodstuffs analyzed.

Conclusions

The SDBD method has proven to be a valuable tool for the determination of L-glutamate in foodstuffs. Among the analytical features, it is worth pointing out its selectivity; tolerated interferent to L-glutamate molar ratios higher than those reported for enzymatic methods [10, 11, 14, 15, 17, 21] are obtained for most amino acids and food additives tested. The sensitivity and selectivity afforded by the proposed method permit the direct determination of the analyte in food samples with a minimum sample treatment. The simplicity of the sample treatment and that of the analytical signal measurement steps contribute greatly to the precision of the method (relative standard deviations for the whole analytical process were 1.4-2.4%). Finally, the SDBD method greatly surpasses commercially available enzyme-based methods using photometric detection [8, 9], the most widely used in the food industry, in terms of rapidity, cost and convenience. The total time of the assay is reduced to a half of that required using enzymatic methods, the cost is about 30 times lower and analytical measurements are based on an automatic titration in contrast with enzymatic methods, where repeated absorbance readings should be performed.

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CONCLUSIONES

Los trabajos de investigación realizados en esta Tesis Doctoral han permitido consolidar la metodología de grado de enlace tensioactivo-colorante como una ventajosa alternativa a los métodos basados en propiedades monoméricas para la determinación de diferentes tipos de analitos en una amplia variedad de matrices, de acuerdo con los objetivos inicialmente establecidos.

Las principales conclusiones obtenidas a partir de los estudios realizados se resumen a continuación:

1) La expresión matemática previamente desarrollada para la determinación de tensioactivos, es de aplicación para la cuantificación de compuestos con estructura molecular muy diferente a la de éstos, concretamente:
    a) Drogas terapéuticas anfifílicas
    b) Drogas terapéuticas aromáticas con carácter hidrotrópico
    c) Aditivos alimentarios hidrotrópicos.

2) El sistema tensioactivo reactivo-colorante utilizado para implementar la metodología basada en agregación competitiva debe cumplir los siguientes requisitos:
    a) El tensioactivo reactivo y el colorante deben formar agregados mixtos que modifiquen las características espectrales del colorante con respecto a las obtenidas para éste en forma monomérica.
    b) El tensioactivo reactivo debe tener carga opuesta al o a los analitos iónicos a determinar o poseer grupos que le permitan interaccionar fuertemente con analitos neutros, por ejemplo grupos de amonio cuaternario que den lugar a interacciones π-catión con analitos aromáticos.
    c) El colorante no debe interaccionar con el o los analitos a las concentraciones de ambos presentes en el medio de valoración.

3) El mecanismo a través del cual el analito ejerce su efecto sobre el grado de enlace tensioactivo reactivo-colorante es la formación de agregados mixtos tensioactivo reactivo-analito independientemente del tipo de sustancia

química determinada (compuesto anfifílico o hidrotrópico). Estos agregados se forman mediante interacciones electrostáticas, hidrofóbicas y/o específicas π-catión. Los agregados tensioactivo reactivo-analito y tensioactivo reactivo-colorante se forman simultáneamente y ambos tipos de agregados tienen estequiometrías bien definidas que varían en función de la concentración de tensioactivo reactivo adicionada al medio de valoración. El valor del grado de enlace tensioactivo reactivo-colorante, β_C, y tensioactivo reactivo-analito, β_A, obtenido en unas condiciones experimentales dadas depende de la energía de las interacciones entre moléculas en cada tipo de agregado mixto. La diferencia entre la cantidad de tensioactivo reactivo necesaria para alcanzar un determinado valor de β_C en presencia y en ausencia de una determinada concentración de analito será tanto mayor cuanto mayor sea la energía de interacción entre las moléculas de tensioactivo reactivo y analito en el agregado mixto.

4) La sensibilidad obtenida utilizando el método de grado de enlace tensioactivo-colorante depende de:

   a) *La estructura molecular del tensioactivo reactivo y del colorante*. La sensibilidad aumenta a medida que disminuye la fuerza de las interacciones electrostáticas e hidrofóbicas que se producen entre las moléculas de tensioactivo reactivo y colorante. El número de grupos iónicos en el colorante y su naturaleza determinan la fuerza de las interacciones electrostáticas y el tamaño de las regiones hidrófobas de las moléculas de tensioactivo reactivo y colorante las interacciones hidrofóbicas.

   b) *La estructura molecular del analito*. Los valores de grado de enlace tensioactivo reactivo-analito más elevados y por lo tanto, los límites de detección más bajos, se obtienen para la determinación de analitos iónicos, debido a las fuertes interacciones electrostáticas atractivas que se producen entre las moléculas de tensioactivo reactivo y analito en el

agregado mixto formado. Dentro de un mismo grupo estructural, la sensibilidad aumenta a medida que lo hace el número de grupos iónicos con carga opuesta a la del tensioactivo reactivo en la molécula de analito. El tamaño de la región hidrófoba del analito también afecta de forma decisiva a la sensibilidad obtenida, la sensibilidad generalmente aumenta al aumentar la hidrofobicidad de las sustancias químicas determinadas.

5) La metodología basada en agregación competitiva proporciona excelentes prestaciones analíticas en diferentes ámbitos de aplicación:

   a) *Determinación no específica de tensioactivos iónicos*. El método de grado de enlace tensioactivo-colorante en combinación con una etapa previa de extracción en fase sólida, aventaja al método estándar del azul de metileno (AM) para la determinación de la concentración total de tensioactivos aniónicos en muestras acuosas medioambientales (aguas naturales y residuales) en términos de:

      a1) Exactitud. Al contrario que el método AM, el método de grado de enlace tensioactivo-colorante proporciona respuestas independientes de la estructura molecular del tensioactivo aniónico. Se han ensayado tensioactivos con diferentes grupos hidrófilos (alquilbenceno sulfatos, alquilsulfatos, alquiletoxi-sulfatos y alquilsulfonatos) y con grupos hidrófobos constituidos por cadenas hidrocarbonadas con un número de átomos de carbono entre 10 y 18.

      a2) Selectividad. Importantes interferencias del método AM entre las que cabe destacar el anión sulfuro, ácidos húmicos y tensioactivos no iónicos, especies que con frecuencia se encuentran presentes en muestras acuosas medioambientales, no interfieren en la determinación no específica de los tensioactivos aniónicos usando el método propuesto, fundamentalmente debido a la efectividad del proceso de

extracción en fase sólida para eliminar determinadas interferencias y al hecho de que el método de grado de enlace tensioactivo-colorante responde preferentemente a compuestos anfifílicos iónicos con carga opuesta a la del tensioactivo reactivo.

a3) Sensibilidad. El límite de detección obtenido usando el grado de enlace tensioactivo-colorante es entre 3 y 9 veces inferior a los límites de detección obtenidos para los diferentes tensioactivos aniónicos utilizando el método AM.

a4) Precisión. La desviación estándar relativa obtenida para el método propuesto incluida la etapa de tratamiento de muestra es de 2,5% para una cantidad total de tensioactivos aniónicos de 25 μg frente a 5,8% obtenida con el método AM para 100 μg de tensioactivos. Esto es debido a la simplicidad del método propuesto en contraste con el método AM en el que se realizan seis extracciones líquido-líquido.

a5) Robustez. Usando el método de grado de enlace tensioactivo-colorante apenas se observa diferencia entre las desviaciones estándar relativas obtenidas dentro de ensayos y entre ensayos (2,5% y 2,8%, respectivamente), al contrario que para el método AM para el que la desviación estándar relativa entre ensayos aumenta hasta 8,7%.

a6) Otras propiedades analíticas. El volumen de muestra usado en el método propuesto es inferior al requerido en el método MA debido a que la sensibilidad obtenida es mayor. También se reduce considerablemente el volumen de disolventes orgánicos usados, de 900 a 60 mL, y el tiempo total del análisis, de 280 a 120 minutos, incluyendo calibración y análisis.

b) *Control de calidad de drogas en fármacos.* Se han propuesto métodos para la determinación de drogas terapéuticas anfifílicas e hidrotrópicas

pertenecientes a diferentes grupos estructurales: ácidos fenámicos, propiónicos y acéticos, indolinas, derivados del ácido glicirretínico, salicilatos, oxicams, pirazolonas e hidrocortisonas (anfifilos), ácidos aromáticos carboxílicos y sulfónicos y polihidroxibencenos (hidrótropos) en una gran variedad de preparados farmacéuticos: disoluciones, tabletas, capsulas, granulados esfervescentes, supositorios, cremas, geles y ungüentos. Las características analíticas de los métodos propuestos los hacen especialmente adecuados para análisis de rutina. Son métodos versátiles, precisos, rápidos, simples y de bajo coste. La sensibilidad obtenida es similar a la proporcionada por los métodos basados en propiedades monoméricas de las drogas, pero aventajan a estos en selectividad. Esto ha permitido llevar a cabo la determinación directa de los principios activos en los fármacos con un mínimo tratamiento de la muestra que consiste en la puesta en disolución de la droga. En el caso de fármacos que contengan varias drogas que den lugar a similares grados de enlace tensioactivo reactivo-droga es necesario llevar a cabo un proceso previo de separación de las mismas.

c) *Control de calidad de alimentos*. El método desarrollado para la determinación de L-glutamato en alimentos elaborados (condimentos, salsas, cremas y sopas) aventaja a los métodos enzimáticos, los más ampliamente usados en la industria alimentaria, en selectividad, rapidez y coste, el que se reduce aproximadamente 30 veces, y evita los problemas derivados de la inestabilidad de las enzimas. El tratamiento de la muestra previo a la determinación basada en agregación competitiva es muy simple, consiste en la disolución del analito en agua y filtrado de la muestra. Finalmente, el método propuesto es muy preciso, las desviaciones estándar relativas para el proceso analítico completo se encuentran en el intervalo 1,4-2,4 %.

APÉNDICES

APÉNDICE A

PUBLICACIONES CIENTÍFICAS DERIVADAS DE LA TESIS DOCTORAL

Publicaciones científicas derivadas de la Tesis Doctoral

1.- "Surfactant-dye binding degree method for the determination of amphiphilic drugs".

Ana Pedraza, María Dolores Sicilia, Soledad Rubio, Dolores Pérez-Bendito.

Analytica Chimica Acta, 2004, 522, 89-97.

2.- "Determination of aromatic hydrotropic drugs in pharmaceutical preparations by the surfactant-binding degree method".

Ana Pedraza, María Dolores Sicilia, Soledad Rubio, Dolores Pérez-Bendito.

Analyst, 2005, 130, 1102-1107.

3.- "Pharmaceutical quality control of acid and neutral drugs based on competitive self- assembly in amphiphilic systems".

Ana Pedraza, María Dolores Sicilia, Soledad Rubio, Dolores Pérez-Bendito.

Analyst, 2006, 131, 81-89.

4.- "Assessment of the surfactant-dye binding degree method as an alternative to the methylene blue method for the determination of anionic surfactants in aqueous environmental samples**"**.

Ana Pedraza, María Dolores Sicilia, Soledad Rubio, Dolores Pérez-Bendito.

Analytica Chimica Acta, 2007, 588, 252-260.

5.- "Surfactant to dye binding degree based approach for the selective determination of L-glutamate in foodstuffs".

Ana Pedraza, María Dolores Sicilia, Soledad Rubio, Dolores Pérez-Bendito.

Analytical Bioanalytical Chemistry, 2007, 389, 2297-2302.

APÉNDICE B

PRESENTACIÓN DE COMUNICACIONES A CONGRESOS

PRESENTACIÓN DE COMUNICACIONES A CONGRESOS

1.- "Surfactant-Dye Binding Degree Method: A Useful Approach for the Determination of Drugs". **Póster.**

Ana Pedraza, María Dolores Sicilia, Soledad Rubio, Dolores Pérez-Bendito.

Euroanalysis XIII; Septiembre de 2004, Salamanca (España).

2.- "An Aggregation Parameter-Based Approach for determination of hydrotropes". **Póster.**

Ana Pedraza, María Dolores Sicilia, Soledad Rubio, Dolores Pérez-Bendito.

Euroanalysis XIII; Septiembre de 2004, Salamanca (España).

3.- "Aggregation Parameter Measurements as the Basis of Analytical Determinations for Pharmaceutical Quality Control". **Póster.**

Ana Pedraza, María Dolores Sicilia, Soledad Rubio, Dolores Pérez-Bendito.

19th Conference of the European Colloid and Interface Society; Septiembre de 2005, Geilo (Noruega).

4.- "Selective determination of L-glutamate in foodstuffs based on aggregation parameter measurements**"**. **Póster.**

Ana Pedraza, María Dolores Sicilia, Soledad Rubio, Dolores Pérez-Bendito.

3rd International Symposium on Recent Advances in Food Analysis; Noviembre de 2007, Praga (República Checa)

euroanalysisXIII

European Conference on Analytical Chemistry

5-10 September 2004. Salamanca, Spain

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Universidad Complutense de Madrid. Madrid (Spain)

Surfactant-Dye Binding Degree Method: A Useful Approach for the Determination of Drugs

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Most of therapeutic drugs are selected or designed to be amphiphilic in order to penetrate cells and tissues and to favour interaction of drug molecules with receptor sites. As a result of their amphiphilic character, drugs show surface activity and self-assembly ability in a similar manner to those exhibited by surfactants. Thus, amphiphilic drugs self-associate to form premicellar, micellar aggregates and, from the interaction between drug and surfactant, mixed aggregates, which can be studied in the framework of theories originally developed for surfactant-surfactant mixtures.

The surfactant-dye binding degree (SDBD) method [1] is a new approach recently developed in our laboratory for the determination of amphiphilic substances. This method is based on the effect of amphiphiles on the degree of binding of a surfactant to dye molecules. The dye carries out a 2-fold role in the analytical process: 1) to induce the formation of surfactant premicellar aggregates and 2) to allow this process to be monitored from changes in their spectral features. The addition of an amphiphilic compound to the dye-surfactant mixture causes a decrease in the degree of binding of surfactant to dye molecules as a result of the interaction between amphiphile (analyte) and surfactant (reactant) molecules. Based on the theoretical formulation provided by Simončič et al. [2] for dye-surfactant intermolecular interactions in mixed surfactant systems, and expression that provides linear calibrations for the determination of amphiphiles have been derived. The analytical use of this methodology has been demonstrated by quantifying classical anionic surfactants [1].

In this work, the SDBD method was extended to the determination of drugs. Phenamic acids, non-steroidal anti-inflammatory drugs (NSAIDs), were selected as a model. The interaction between the anionic dye Coomassie Brilliant Blue G (CBBG) and the cationic surfactant didodecyldimethylammonium bromide (DDABr) was used for the sensitive determination of phenamic acids. The addition of NSAIDs resulted in a strong interaction with DDABr and, hence, in decreased interactions CBBG-DDABr aggregates. Binary mixtures of phenamic acid-surfactant were demonstrated to behave as those made up of pure surfactant, and therefore, the expression previously derived for determining surfactant could be used for determining amphiphilic drugs.

The proposed method permitted the determination of phenamic acids at the mg/l level with the precision required for quality control (the relative standard deviation for 7 mg/l of phenamic acid was 1.1%). Pharmaceutical preparations could be analysed directly after dissolution of the samples in ethanol. Other features of the SDBD method that make it useful for the routine work needed in quality control included high experimental simplicity, rapidity and low cost.

[1]R. Fabios, D. Sicilia, S. Rubio, D. Pérez-Bendito, Anal. Chem.,75(2003) 6011
[2] B. Simončič, M. Kert, Dyes Pigments, 54 (2002) 221

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Universidad de Salamanca. Salamanca (Spain)

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An Aggregation Parameter-Based Approach for the Determination of Hydrotopes

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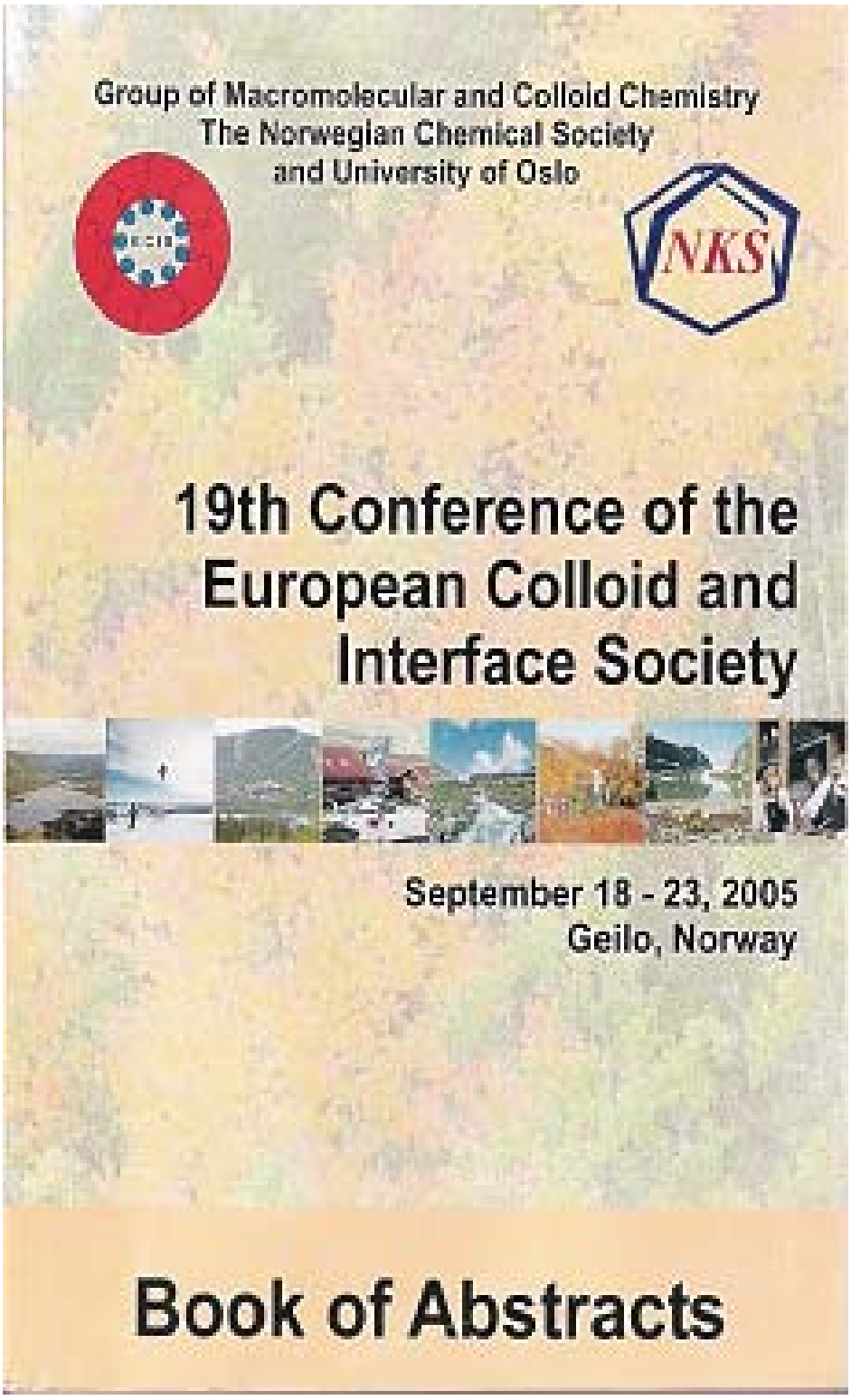
In the last years, our research group has performed intense investigations on the use of the self-assembly capability of amphiphiles with a nalytical purposes. As a result of these studies, two new aggregation parameter-based methodologies have been developed to date: the mixed aggregate (MA) method [1] and the surfactant-dye binding degree (SDBD) method [2]. The MA method is based on measuring the critical micelle concentration (cmc) value of mixtures of amphiphilic substances, one of which is the analyte. The SDBD method is founded on the effect of amphiphiles on the degree of binding of a surfactant to dye molecules, which induce the formation of surfactant premicellar aggregates. Both aggregation parameter-based approaches have been successfully applied to the determination of classical surfactants and drugs in different real samples.

Continuing with our investigations to explore the analytical potential of supramolecular aggregation-based methods, we study here the possibility of using the SDBD method for the determination of hydrotropes, organic compounds of low molecular weight, which are known to interact with amphiphiles and, hence, to influence their aggregate behaviour.

Hydrotropic compounds added to dye-surfactant mixtures caused a decrease in the degree of binding of surfactant to dye molecules as a result of the interaction between hydrotrope and surfactant molecules. Linear calibrations for the determination of hydrotropes were obtained on the basis of the expression previously derived to quantify amphiphiles [2]. Sensitivity was related to the degree of binding of the analyte (hydrotrope) to surfactant molecules. Therefore, in order to establish the optimal experimental conditions for the determination of hydrotropes, variables affecting the analyte-surfactant binding degree (pH, electrolyte and organic additive concentration, temperature and molecular structure of the surfactant) were studied. The dependence of the sensitivity achieved with the molecular structure of the analyte was also studied.

The analytical applicability of the SDBD method for the determination of hydrotropic compounds was demonstrated by quantifying salicylic acid in pharmaceutical formulations. The analytical features of the proposed method in terms of sensitivity (the detection limit was 0,6 mg/L), precision (the relative standard deviation for 7 mg/l of salicylic acid was 0,8%) and selectivity (organic compounds such as aliphatic acids present along with salicylic acid in pharmaceutical preparations were found not to interfere) supported the usefulness of the SDBD method to solve real analytical problems.

[1]D.Sicilia, S. Rubio, D. Pérez-Bendito, D. *Anal. Chem.*, 67 (1995)1872.
[2]R. Fabios, D. Sicilia, S. Rubio, D. Pérez-Bendito, Anal. Chem..75 (2003) 6011.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P7.4

Aggregation parameter measurements as the basis of analytical determinations for pharmaceutical quality control

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The effects of solutes on the aggregation behaviour of surfactants have been exploited by our research group to develop analytical methods based on aggregation parameter measurements. Up to now, two new aggregation parameter-based methods have been proposed: the mixed aggregate (MA) [1] and the surfactant-dye binding degree (SDBD) method [2]. The MA method is based on the measurement of the critical aggregate concentration (cac) of mixtures of amphiphilic substances one of which is the analyte.The aggregation parameter measured in the SDBD method is the degree of binding of a surfactant to dye molecules. Dyes bearing charge of opposite sign to that of surfactants induce the formation of surfactant aggregates at concentrations far below its cac, which is monitored from changes in the spectral features of the dye. In the presence of amphiphilic substances (analytes), a decrease in the dye-surfactant binding degree is observed as a result of the interaction between the amphiphile added to the aqueous mixture and the surfactant (reactant). The SDBD has been proved to be an advantageous analytical tool for determining total ionic surfactants in environmental samples [2]. The feasibility of this aggregation parameter-based method to be used to quantify amphiphilic drugs has been demonstrated by determining phenamic acids in pharmaceutical samples [3].In this work, the real scope of the SDBD method in pharmaceutical quality control was investigated by measuring the analytical response provided by a wide number of therapeutic drugs (non-steroidal and steroidal anti-inflammatory drugs, phenothiazines, antihistamines and tricyclic antidepressant drugs) using the anionic dye Coomassie Brilliant Blue G (CBBG) as inductor of aggregates of the cationic surfactant didodecyldimethylammonium bromide (DDABr). Linear calibration curves were obtained for all drugs tested bearing no charge or opposite charge to that of the DDABr, the sensitivity achieved depending on the drug-surfactant bond strength. Thus, maximum sensitivity was obtained for amphiphilic drugs able to provide electrostatic and hydrophobic interactions with the surfactant. Within a structural group, the sensitivity increased as the octanol/water partition coefficient did. For similar hydrophobicity, the sensitivity obtained for drugs containing two ionized groups was higher than that achieved for amphiphiles with one ionized group in their molecular structure. Finally, no response was obtained for drugs bearing the same charge to that of the surfactant because repulsive electrostatic forces prevented the formation of drug-DDABr aggregates. The analytical applicability of the SDBD method for pharmaceutical quality control was demonstrated by determining different drugs (ibuprofen, diclofenac, indomethacin, diflunisal, phenylbutazone, piroxican, carbenoxolone and dexamethasone) in commercial formulates.From the above results, and taking into account that most of therapeutic drugs are amphiphilic compounds, it could be asserted that the SDBD method could be used as a common methodological approach for drug quality control, with the only requirement being the use of surfactant reactants bearing opposite charge to that of ionic drugs.

1. D. Sicilia, S. Rubio, D. Pérez-Bendito, Anal. Chem., 67 (1995) 1872
2. R. Fabios, D. Sicilia, S. Rubio, D. Pérez-Bendito, Anal. Chem.,75 (2003) 6011
3. A. Pedraza, M.D. Sicilia, S. Rubio, D. Pérez-Bendito, Anal. Chim. Acta., 522 (2004) 89

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A-3
SELECTIVE DETERMINATION OF L-GLUTAMATE IN FOODSTUFFS BASED ON AGGREGATION PARAMETER MEASUREMENTS

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L-Glutamate is used worldwide to enhance the flavour of many foodstuffs. This additive is generally recognized as safe at typical ingestion levels, but high doses may result in certain adverse physiological effects. The maximum content of this substance in food products is regulated in most countries (e.g. the limit established by the European Union is 10 g/kg). Because of the complexity of samples, laborious and expensive analytical procedures involving ion-exchange extraction, chromatographic separations or enzymatic reactions are used by food industry laboratories to achieve the required selectivity to determine L-glutamate in foodstuffs.

In this work, a simple, rapid and low-cost analytical method, suitable for the direct routine monitoring of L-glutamate in food products, is presented. It is founded on an aggregation parameter-based methodology recently developed by our research group, namely the surfactant to dye binding degree (SDBD) method [1]. This method is based on the competition established between an ionic dye and the analyte to interact with an ionic surfactant bearing opposite charge to that of the dye. The dye and surfactant used for the determination of L-glutamate were Coomassie Brilliant Blue G (CBBG) and didodecyldimethylammonium bromide (DDABr), respectively. CBBG and DDABr form mixed dye-surfactant aggregates, which are monitored photometrically. In the presence of L-glutamate, the amount of DDABr required to reach a given CBBG-DDABr binding degree (m_s') increases compared with that needed in its absence (m_s), as a result of the interaction between analyte and DDABr molecules. This interaction occurs through both attractive electrostatic interactions between the γ-carboxylate group in the analyte and the ammonium quaternary group in the cationic surfactant, and hydrophobic interactions between the hydrophobic moieties in both L-glutamate and DDABr molecules. A linear calibration is obtained by plotting m_s'-m_s as a function of the analyte concentration.

The proposed method provides the sensibility (quantitation limit= 0.2 mM) and selectivity required for the direct determination of L-glutamate in foodstuffs. Amino acids, the main interferent compounds in L-glutamate quantitation, do not interfere at the concentrations present in food products. The applicability of this new approach to the determination of L-glutamate in food samples was demonstrated by analyzing liquid and dried soups, paste sauces, mushroom creams and seasonings. Measurements were performed after a minimum sample treatment; dilution of liquid samples and dissolution in water of solid samples, and filtration to remove non-soluble components. Results obtained were consistent with those provided by a commercial enzymatic colorimetric test, the Boehringer Mannheim kit. The precision, expressed as relative standard deviation for the whole analytical process ranged between 1.3 and 1.9 % (n=6).

[1] R. Fabos, D. Sicilia, S. Rubio, D. Pérez-Bendito, Anal. Chem.,75 (2003) 6011.