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Diseño, preparación y caracterización de hidrogeles de agarosa para liberación controlada de fármacos

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

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DISEÑO, PREPARACIÓN Y CARACTERIZACIÓN DE HIDROGELES DE AGAROSA PARA LIBERACIÓN CONTROLADA DE FÁRMACOS

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Que la presente Tesis Doctoral titulada ***"DISEÑO, PREPARACIÓN Y CARACTERIZACIÓN DE HIDROGELES DE AGAROSA PARA LIBERACIÓN CONTROLADA DE FÁRMACOS"*** ha sido realizada por la Licenciada en Farmacia **Doña Tatiana Marras Marquez** bajo su dirección y, al estar concluida, autorizan su presentación a fin de que pueda ser juzgada por el tribunal correspondiente. Y, para que conste firman este informe en Madrid a 19 de octubre de 2015.

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Si no puedes volar entonces corre,
si no puedes correr entonces camina,
si no puedes caminar entonces arrástrate,
pero sea lo que hagas,
sigue moviéndote hacia adelante.

Martin Luther King

ÍNDICE

RESUMEN

En esta tesis doctoral se han abordado nuevas estrategias para la elaboración de sistemas farmacéuticos para la liberación de fármacos basados en hidrogeles de origen natural. La sustancia elegida para la fabricación de estos sistemas fue la agarosa para la que cada día se encuentran nuevas aplicaciones dentro de campos relacionados con la biomedicina, la biotecnología y la liberación controlada de fármacos. La agarosa presenta la gran ventaja, entre otras, de, gracias a su capacidad de gelificar en función de la temperatura, conformar diferentes tipos de materiales en piezas con notables prestaciones mecánicas que permiten su manipulación.

En una primera aproximación se procedió a probar la capacidad de los sistemas de agarosa de permitir la inclusión de tres tipos de surfactantes: pluronic® F68, tween® 80 y lauril sulfato de sodio, con objetivo de facilitar la liberación de los fármacos incluidos. Los tensoactivos incluidos no afectaron las propiedades de los sistemas obtenidos. Se pudo comprobar cómo, incluso a los mayores porcentajes de surfactante, se obtienen sistemas manejables que, en el caso del fármaco modelo hidrosoluble, teofilina, respondían al comportamiento esperado, una más rápida liberación del fármaco con pequeñas variaciones en función de la naturaleza y porcentaje del surfactante añadido. Sin embargo, en el caso del fármaco modelo de baja solubilidad en agua, la tolbutamida, el comportamiento es radicalmente diferente ya que la liberación es más sostenida independientemente del tipo de surfactante. Con el fin de aclarar este inesperado comportamiento se procedió a caracterizar estos sistemas desde el punto de vista microestructural, considerando las interacciones establecidas entre las micelas cargadas de fármaco y la agarosa y las modificaciones en la porosidad de los hidrogeles liofilizados. Se ha podido comprobar la marcada

influencia que tienen los surfactantes en la arquitectura de poro, lo que resulta crítico en la rehidratación a la que se ven expuestos los sistemas liofilizados durante su administración. El modo en que el medio acuoso restaura el status quo original, un hidrogel altamente hidratado, así como otros factores como el grado de cristalinidad del fármaco en esta situación, contribuyen a explicar este, aparentemente anómalo, comportamiento de liberación.

Teniendo en cuenta que la incorporación de tensoactivos de diversa naturaleza y concentración a hidrogeles de agarosa modifica la estructura final de este tipo de matrices se decidió afrontar el diseño y elaboración de sistemas de liberación de fármacos que permitiesen aportar alternativas a los sistemas de administración empleados en la actualidad. En primer lugar se planteó la posibilidad de mejorar las prestaciones de sistemas basados en pectina utilizados en la liberación en el tracto gastrointestinal, en especial para tratamientos de cáncer de colon. La incorporación de tensoactivos a hidrogeles de pectina origina sistemas de difícil manipulación. Por lo tanto, se establecieron dos alternativas para mejorar este comportamiento: realizar mezclas con agarosa o someter a los sistemas a un proceso de liofilización. La capacidad gelificante de la agarosa permite conformar sistemas que, a pesar de incluir los tensoactivos ya estudiados, tienen consistencia suficiente para ser manipulados incluso recién preparados. Asimismo, la liofilización, técnica de desecación empleada en la industria farmacéutica, es utilizada como herramienta para conformar piezas que presentan patrones de liberación diferentes a los observados en los sistemas no liofilizados. La presencia de los tensoactivos (pluronic, tween y lauril sulfato sódico) permite mejorar y controlar la liberación de tolbutamida en un

medio de pH progresivo que simula el encontrado a lo largo del tracto gastrointestinal. Los datos de liberación de tolbutamida desde los sistemas frescos se ajustan al modelo descrito por Higuchi y los datos de liberación de tolbutamida desde los sistemas liofilizados se ajustan al modelo Korsmeyer-Peppas; durante la rehidratación del sistema el proceso de reordenamiento de las cadenas del hidrogel es un paso primordial en la liberación del fármaco. El estudio de los cambios que estos diferentes factores causan en la microestructura de las matrices liofilizadas de pectina o de agarosa-pectina resulta crítico para entender estos diferentes perfiles de liberación.

La consistencia gelatinosa de los sistemas frescos permite su aplicación en la administración de medicamentos por vía oral en pacientes con problemas de disfagia.

La caracterización microestructural demuestra que tanto los tensoactivos como los polímeros que constituyen el sistema facilitan la disolución del fármaco, determinan el tamaño de los cristales de activo y luego del proceso de liofilización determinarán la estructura porosa del sistema obtenido. La caracterización realizada es de suma importancia debido a que gracias al proceso de liofilización es posible conservar muestras hidratadas. Los sistemas de pectina permiten una interacción con el fármaco y este fenómeno es demostrado a través de la obtención de perfiles de liberación de tolbutamida pH-independiente, comportamiento que va en función de la naturaleza y concentración de tensoactivo.

En segundo lugar, y una vez mejorada la liberación de fármacos poco solubles, se procedió a diseñar sistemas que permitiesen una liberación progresiva y controlada de un fármaco altamente soluble como la teofilina. Los perfiles de cesión de teofilina en pH progresivo muestran dos comportamientos que van en

función de la naturaleza del excipiente utilizado. Los sistemas con Eudragit® S-100 muestran perfiles similares a los obtenidos con los sistemas blanco. El derivado del ácido acrílico, Eudragit® RL-PO, permite obtener una liberación controlada y pH-independiente en un medio de pH progresivo. Este efecto puede ser atribuido a diversos fenómenos, la presencia de grupos de amonio cuaternario en la estructura de este derivado acrílico el cual afecta el proceso de rehidratación de las cadenas de agarosa debido al efecto filtrante, la arquitectura porosa de la matriz polimérica y a las interacciones de los nanocristales de fármaco con los componentes del sistema.

En esta tesis se demuestra cómo se pueden diseñar y optimizar sistemas multicomponentes basados en un vehículo común, hidrogeles de agarosa, que permite incluir diferentes aditivos en función del fármaco a liberar. Por otro lado la combinación de liofilización, que permite obtener estructuras porosas, junto con la modificación de la estructura cristalina de los principios activos por su incorporación a las micelas de los surfactantes incluidos en el sistema, resultan de interés en el desarrollo de formulaciones que requieran mejorar la biodisponibilidad del fármaco.

SUMMARY

This PhD. Thesis describes new strategies towards the elaboration of drug release pharmaceutical systems based on natural hydrogels. The substance chosen for the preparations of these systems is agarose, a thermoreversible hydrogel that allows to shape robust multicomponent formulations. Among the different polysaccharides employed in the biomaterials and biotechnological world, agarose is gaining several applications besides its traditional and extensive utilization in molecular biology separation techniques such as gel electrophoresis or gel filtration chromatography. Specifically, the gelling capacity of agarose has been applied to shape ceramic or metallic particles or fibres by gelcasting and similar techniques. Besides it has been employed in diverse templation and micropatterning techniques to create stamps, arrays and microfluidic devices for biotechnological devices. In addition agarose is being employed in the biomaterials field as a matrix to regenerate a damaged tissue or forming part of a drug controlled release device. In this sense agarose has been considered as a potential candidate to regenerate different types of tissues, especially hard (bone and cartilage), pancreas and nervous system.

In a first stage, the capacity of these agarose-based systems to accommodate three different types of anionic or non-ionic surfactants: Pluronic® F68, Tween® 80 and sodium laurylsulfate (SLS) was tested. These surfactants, included with the objective of enhancing the solubility and facilitating the release of different drugs, do not affect to the mechanical performance of the so obtained systems. In the case of the hydrophilic drug used as a model, theophylline, the presence of the surfactants enhances, as expected, the release of this drug. The presence of any surfactant produces a decrease of

the water surface tension that facilitates the rehydration process, the dissolution and diffusion of the surfactant molecules conditions, as well, the hydrophilic drug release. However, the much-sustained release of the water low-soluble tolbutamide, independently on the surfactant, cannot be so easily explained. In fact, this effect can only be clarified considering the interactions established between the drug loaded micelles and agarose but also to the alteration of the freeze-dried hydrogels microstructure. It has been observed that the modification of the porosity percentage as well as the pore size distribution during the lyophilization plays a critical role in the different phenomena that take place as soon as desiccated hydrogel is rehydrated: i) the dissolution of the drug, which depends on the crystallite size of the lyophilized drug, and ii) its diffusion through the hydrogel that it suffering at the same time a rehydration and reconstruction process.

It has been demonstrated that the inclusion of a surfactant within a polysaccharide-based hydrogel matrix deeply affects to the final arrangement of the different components in the resulting self-assembled structures that constitute this type of network. The consequential pore architecture of the freeze-dried matrices is deeply affected by the nature and percentage of the surfactant, the type of drug included and the created loaded or non-loaded micelles. The unexpected controlled release of a hydrophobic drug or the increased swelling degree values after the matrices rehydratation can be explained not only considering the interactions established between the drug loaded or non-loaded micelles and agarose but also to the alteration of the freeze-dried hydrogels microstructure. The possibility of tailoring the pore architecture as a function

of the surfactant nature and percentage can be applied from drug control release to the widespread and growing applications of materials based on hydrogel matrices.

Taking into consideration these results, it was faced the design and preparation of drug delivery systems that could be an alternative to those presently employed. The first objective was to improve the performance of pectine-based systems used in the gastrointestinal tract, especially for colon cancer treatments. These systems are thought to be versatile enough to allow the inclusion of substances (such as the surfactants tested: Pluronic, Tween, Na Lauryl sulphate) that may contribute to tailor the drug release patterns. The incorporation of different surfactants produces pectin-based hydrogels of difficult manipulation. In order to improve this drawback, two different strategies have been developed: blending with agarose or freeze-drying. The presence of agarose yields robust systems that can be handled and tested as prepared, in the fresh state. Freeze-drying not only allows shape pure pectin and blended systems, but also generates a porous structure whose microstructure, determined by the different components included, influences on the drug release behavior. The presence of the surfactants allows control the tolbutamide release within a pH progressive medium that resembles that found in the gastrointestinal tract. Tolbutamide release kinetics from freshly prepared matrices can be fitted to the Higuchi model while the freeze-dried ones adjust to the Korsmeyer-Peppas model; hence the hydrogel chains rearrangement processes rule the release during the rehydration process.

Despite the structural modifications induced by these surfactant agents, the hydrated systems, either directly

obtained or soaked after being freeze dried, can be handled and bear their manipulation throughout the release tests. In this sense, the jelly consistency of these materials, easy to swallow may be an adequate solution for oral administration in elderly patients with dysphagia problems. The microstructural characterization demonstrates that these substrates do not only influences by facilitating the drug solubilization and ulterior release, but also determines the drug crystal size and pore architecture after the freeze drying process. This characterization is of critical importance taking into consideration the widespread use of the lyophillization technique to ensure the preservation and off-the-shelf utilization of highly-hydrated formulations such as the hydrogel-based testes in this work. The pectin-based formulations do not behave merely as drug carriers but has been designed as a vehicle that interacts with the drug. This active role has been demonstrated through the progressively less pH-dependent release profiles of tolbutamide that can be tailored, as a function of the surfactants nature and percentage, for the same hydrophobic drug use as model.

Once reached the objective of improving the solubility and release of poorly soluble drugs, agarose-based systems are designed to accommodate additives that allow to tailor a controlled and pH-independent release of highly soluble drugs such as theophylline within a pH progressive medium. The theophylline release in a pH progressive medium show two different patterns that primarily depend on the additive nature: those systems containing Eudragit® S-100 show a similar behaviour to that observed in the absence of additives while the presence of Eudragit® RL-PO yields a progressive and pH-independent release. This effect can be mainly attributed

to the presence of quaternary ammonium groups that causes a sieving effect during the agarose network rehydration but also to the generated pore architecture and the interactions of the drug nanocrystals with the other components.

In this PhD. Thesis it is shown how can multicomponent systems based on a common vehicle: agarose hydrogels, be designed and optimized by including different additives as a function of the drug characteristics. In such a way the solubility or stability restraints imposed by the drug nature may be overcome. On the other hand, the combination of the freeze-drying preservation technique, that allows to obtain a porous structure, together with the modification of the drugs crystal structures as a consequence of its incorporation inside the included surfactants micellar aggregates may be of interest for the design and development of formulations with improved biodisponibility

INTRODUCCIÓN

La evolución en el desarrollo de sistemas de liberación controlada o modificada de fármacos se encuentra relacionada con las desventajas observadas en los sistemas convencionales de liberación de fármacos. Las desventajas de estos sistemas se relacionan principalmente con las fluctuaciones en la concentración plasmática del fármaco que disminuye el efecto terapéutico y conlleva la aparición de efectos secundarios en el paciente.

Los sistemas de liberación controlada de fármacos reducen los tratamientos multidosis, disminuyen los efectos secundarios y facilitan el cumplimiento de tratamientos a largo plazo.

Al realizar una búsqueda en los buscadores **Pubmed** y **Web of science** (WOS) utilizando las palabras clave: "control drug release" y "conventional drug delivery", se confirma la tendencia de diferentes líneas de investigación hacia el desarrollo de sistemas de liberación controlada.

Dentro de los requisitos para comenzar con el desarrollo de un sistema de liberación controlada existen tres elementos fundamentales que requieren un estudio exhaustivo: fármaco, formulación y vía de administración.

A la hora de elegir los excipientes que formarán parte del sistema de liberación controlada, es fundamental estudiar las propiedades fisicoquímicas de cada uno de ellos y una vez desarrollado el sistema, la caracterización estructural permitirá establecer las interacciones entre los componentes y predecir su comportamiento en contacto con medios acuosos del organismo.

Los polímeros naturales son excipientes de elección en la elaboración de sistemas de liberación controlada. Se encuentran disponibles en la naturaleza en recursos renovables como algas, plantas superiores y diversas especies animales,

son biocompatibles y biodegradables [1]. Como ejemplos de los polisacáridos más utilizados tenemos al quitosano, alginato, fibrina, colágeno, gelatina, ácido hialurónico, dextrano, agarosa, carragenina, goma guar, goma xantana, xiloglucano, inulina, pectina y los derivados de celulosa [2-5].

Los hidrogeles se encuentran dentro de la clasificación de los sistemas de liberación controlada como sistemas matriciales hidrofílicos. Estos sistemas son mezclas de fármaco y un polímero hidrófilo que puede hincharse en el agua. La liberación del fármaco desde estos sistemas dependerá de los materiales que conforman la matriz y de las características del medio de cesión [6, 7].

Los hidrogeles son redes poliméricas compuestas de homopolímeros o copolímeros hidrofílicos los cuales son capaces de retener una elevada cantidad de agua o fluidos biológicos en su matriz y a su vez mantener su estructura (Figura 1). Los hidrogeles exhiben una afinidad termodinámica con el agua que les permite humectarse en medios acuosos [8]. La red polimérica formada por los hidrogeles, posee un grado de flexibilidad similar a los tejidos naturales lo que permite su aplicación en ingeniería tisular y/o como transportadores de sustancias activas [8-11]. Las uniones entre las redes poliméricas se encuentran formadas por homopolímeros o copolímeros y son insolubles debido a la presencia de uniones químicas del tipo covalentes y no covalentes y a la formación de entramados y cristales [12, 13].

La clasificación de los hidrogeles se hace teniendo en cuenta los siguientes factores:

- **Naturaleza del polímero**: natural, sintético, híbrido.
- **Naturaleza de las uniones que lo conforman**: químicas o físicas

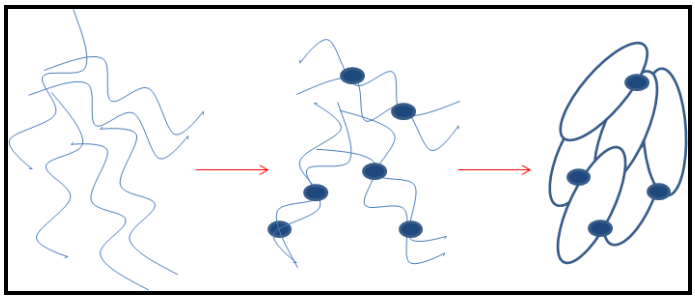

Figura 1. *Formación de hidrogeles en medio acuoso [14]*

- **Método de preparación:** homopolímeros, copolímeros, redes interpenetradas y semi-interpenetradas.
- **Grado de porosidad:** macroporosas, microporosas y nanoporosas.
- **Naturaleza de carga:** neutros, aniónicos y catiónicos.
- **Sensibles a estímulos externos:** Los denominados hidrogeles inteligentes o "smart" que presentan un hinchamiento que depende de los factores externos presentes en el medio. Según el factor que influye en el hinchamiento, estos pueden ser: sensibles a la temperatura, a señales eléctricas, a la luz, al pH, a la presencia de iones o biomoléculas y sensibles a la glucosa [10, 15-19].

Entre los factores que afectan el hinchamiento de los hidrogeles se encuentra el grado de unión de las cadenas poliméricas, un mayor grado de unión entre las cadenas poliméricas provoca una disminución en el hinchamiento, debido a la formación de una estructura compacta con un gran número de cadenas poliméricas que hacen que disminuya la velocidad a la cual ingresa medio al interior del hidrogel [8].

En función del tipo de polímeros que conforman las redes del hidrogel, la velocidad de expansión y humectación del hidrogel puede sufrir modificaciones. Los hidrogeles con mayor proporción en cadenas hidrofílicas presentan un aumento en la

velocidad de captación de solvente cuando se compara con hidrogeles compuestos en su mayoría por grupos hidrófobos [8, 20].

Los polímeros con elevado peso molecular presentan efectos en la viscosidad de la solución polimérica que formará el hidrogel y en las propiedades de hinchamiento de la matriz obtenida [21]. El estudio del proceso de hinchamiento en los hidrogeles se encuentra dentro de los métodos de caracterización más comunes para explicar su comportamiento en medios biológicos. La caracterización de este proceso permite conocer la cantidad de fluido que es embebido y/o retenido por el hidrogel. A partir de este estudio, es posible definir otros comportamientos asociados, como son difusión de moléculas, flujo de solventes, influencia de procesos de liofilización y secado, entre otros [22, 23].

La caracterización de las propiedades mecánicas de los hidrogeles permite definir el comportamiento que presentarán en el sitio de acción, así como su degradación e interacción con el medio [24, 25]. En función de la naturaleza y las propiedades intrínsecas de cada polímero, es posible obtener estructuras con propiedades físico-químicas adecuadas a una aplicación específica [26].

En distintas revisiones se incluyen los factores que influyen en las propiedades mecánicas de los hidrogeles:

1) Composición química y estructura del polímero [27]
2) Condiciones de polimerización [12]
3) Densidad de las uniones formadas [22]
4) Medio utilizado en la preparación del hidrogel [28]

En el campo de la liberación controlada de sustancias activas, la propiedad característica de los hidrogeles se encuentra en la capacidad de controlar la difusión de moléculas a través de

la red que conforman [29, 30]. La velocidad de difusión de la sustancia activa viene condicionada principalmente por las características estructurales del hidrogel y las interacciones del fármaco con las moléculas del polímero [31].

Los hidrogeles pueden ser utilizados como sistemas de liberación controlada de sustancias activas a nivel oral, rectal, ocular, subcutáneo y transdérmico [32-34].

La incorporación de principios activos en hidrogeles puede realizarse mediante:

a) **Incorporación durante la elaboración del hidrogel:** Este método se realiza mezclando el fármaco con la solución precursora del hidrogel, incluyendo o no agentes reticulantes. El fármaco quedará atrapado en la red polimérica formada una vez que se produzca la gelificación.
b) **Incorporación en el hidrogel formado:** Se sumerge el hidrogel dentro de una solución en la cual el fármaco se encuentra disuelto o disperso y se permite la humectación del sistema hasta alcanzar el estado de equilibrio [35, 36].

La mayor parte de los modelos matemáticos desarrollados para explicar los procesos de liberación que rigen las cinéticas de liberación en los hidrogeles, describen al mecanismo de difusión como el proceso principal por el que la liberación de sustancias activas tiene lugar. Debido a que el proceso de difusión se encuentra íntimamente relacionado con la estructura del material polimérico, la morfología del hidrogel es una característica que influye en el ajuste hacia un modelo matemático u otro.

A través del mecanismo de difusión es posible clasificar a los hidrogeles en tres sistemas de liberación controlada:

a) **Liberación controlada mediante difusión**

Como ya se ha mencionado se encuentra dentro de los principales mecanismos de liberación de fármacos. La difusión del fármaco se produce a través de los espacios existentes entre las cadenas poliméricas, denominados "poros".

Dentro de los factores que condicionan la difusión del fármaco podemos mencionar: la geometría y estructura interna del sistema y la concentración del fármaco.

La ecuación que mejor define este comportamiento es la propuesta por Higuchi en 1961 [22]. El proceso de difusión del fármaco viene descrito por la ley de Fick y depende de la raíz cuadrada del tiempo:

$$M_t/M_\infty = K_H \sqrt{t}$$

donde, M_t es la cantidad de fármaco liberado a tiempo t, M_∞ es la cantidad de fármaco liberado a tiempo infinito y K_H es la constante de disolución de Higuchi [37].

b) **Liberación controlada a través del hinchamiento**

En estos sistemas el fármaco se encuentra disperso/disuelto en la matriz polimérica sometida a un proceso de liofilización o secado. El polímero en contacto con medios acuosos comienza a humectarse y el solvente ingresa dentro de los espacios libres formados entre las cadenas macromoleculares, comienza a hincharse y se establecen dos fases: una vítrea y otra plástica; la liberación del fármaco se produce por el movimiento del activo desde la fase vítrea a la fase plástica del polímero.

En 1984, Korsmeyer y Peppas propusieron una ecuación que permitía establecer la influencia de las características geométricas del sistema en el proceso de difusión del fármaco. La ecuación viene representada de la siguiente forma:

$$M_t/M_\infty = Kt^n$$

Donde, M_t representa la cantidad de fármaco disuelto a tiempo t, M_∞ es la cantidad total de fármaco disuelto a tiempo infinito, K es la constante que incorpora las características estructurales y geométricas del sistema y n es el exponente de difusión. El valor de n viene representado para cada forma geométrica y un resumen del mecanismo de liberación en función de la geometría del sistema de liberación controlada viene representado en la tabla 1 [6, 38].

Exponente n			**Mecanismo de liberación**
Films	**Cilindros**	**Esferas**	
0,5	0,45	0,43	Difusión fickiana
0,5<n<1,0	0,45<n<0,89	0,43<n<0,85	Transporte anómalo
1,0	0,8	0,85	Transporte tipo II

Tabla 1. *Principales mecanismos de liberación de fármacos desde diferentes estructuras geométricas.*

c) **Liberación controlada químicamente**

Este mecanismo se utiliza para describir las reacciones que suceden dentro de la matriz polimérica gracias a las cuales se determina la liberación del fármaco. Dentro de las reacciones más comunes producidas entre la red polimérica y el fármaco podemos mencionar: la ruptura de las cadenas por vía hidrolítica y/o por degradación enzimática, siendo estas reacciones reversibles o irreversibles.

Este mecanismo de liberación controlada presenta dos subclases mediante las cuales es posible describir la liberación controlada del fármaco: sistemas erosionables y sistemas que incorporan grupos y/o cadenas a su estructura. En los sistemas erosionables, la liberación del fármaco se produce por la degradación o disolución del polímero y en los sistemas con grupos y/o cadenas incorporados en su estructura, la

liberación del fármaco se produce por la degradación de las uniones mediante las cuales el fármaco se encuentra unido al polímero [8, 35].

El agar es un polisacárido, coloidal e hidrofílico que puede ser extraído principalmente de 4 géneros de algas, *Rhodophyta* o alga roja, *Phaeophyta* o alga marrón, *Cyanophyta* o alga azul y *Chlorophyta* o alga verde [39-42]. Se encuentra compuesto de unidades alternas de D- y L- galactopiranosa; está constituido por 3 componentes: agarosa neutra, agarosa piruvato (ligeramente sulfatada) y galactosa sulfato (Figura 2).

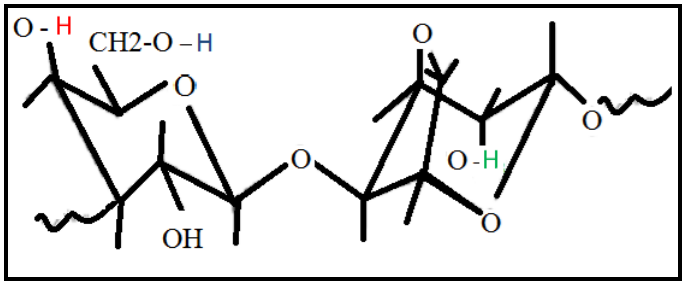

Figura 2.*Estructura química de la agarosa*

El agar puede ser dividido en dos fracciones: una que gelifica naturalmente, denominada agarosa y otra fracción que no gelifica, denominada agaro-pectina [43-45]. La agaro-pectina presenta la misma conformación que la agarosa y varios grupos aniónicos como son los grupos sulfato, piruvato y gluconato [46].

La fracción denominada agarosa está compuesta principalmente por unidades alternadas de β-D-galactopuranosil y 3,6-anhidro-α-L-galactopuranosil [47]. La agarosa presenta un peso molecular alrededor de 100.000 y 140.000 Daltons [42]. La temperatura de fusión se encuentra alrededor de 85°C y la de gelificación entre 30 – 40°C. La temperatura de gelificación se encuentra influenciada por el grado de metoxilación de la

agarobiosa en el C6 [41, 42, 48]. La agarosa presenta la propiedad de histéresis debido a la amplia diferencia entre las temperaturas de fusión (85°C) y gelificación (38°C)[42].
Realizando la caracterización de la agarosa mediante espectrofotometría de infrarrojo, se observa una amplia banda alrededor de 3420 cm^{-1} correspondiente a la vibración de grupos –OH. Estos grupos son los sitios de unión para otros grupos químicos. Otra banda característica se observa entre 2960-2850 cm^{-1}, que corresponde a los grupos –CH y por último la banda obtenida alrededor de 1065 cm^{-1} es atribuida a los grupos –CO del alcohol primario presente en la estructura de la agarosa [49-51].
La agarosa forma hidrogeles físicos mediante procesos exotérmicos a concentraciones por encima de 0,1% [42]. Factores tales como la concentración, tipo de agarosa, peso molecular, solvente y temperatura de trabajo presentan influencia en el proceso de formación de la red polimérica [52-54]. Se han registrado diversos tamaños de poro, desde 50 a 1200 nm, en sistemas con diferentes concentraciones de agarosa [55]. Los técnicas de liofilización y de secado permiten la elaboración de hidrogeles de agarosa con tamaños de poro que van de 2.1 a 125 µm [56, 57].
De acuerdo a diversos autores, el proceso de gelificación puede ser dividido en 3 estados: indución, gelificación y pseudo-equilibrio [58-60]. Durante el enfriamiento, la agarosa forma un gel a partir de una solución homogénea por debajo de la temperatura de transición espiral-hélice [61]. Este mecanismo fue propuesto previamente por diversos autores [62, 63], sugiriendo que la presencia de hélices dobles durante el enfriamiento es el fenómeno responsable de la agregación que determina la formación de la red tridimensional del hidrogel

gracias a interacciones hidrófobas y puentes de hidrógeno. Por otro lado, otros estudios sugieren que la formación de cadenas simples pueden ser capaces de formar un hidrogel [64, 65] o bien, la formación de complejos ternarios agarosa/agua/co-solvente pueden generar la misma estructura.

Con el fin de describir el proceso mediante el cual se produce la gelificación de la agarosa se han utilizado diversos métodos analíticos. La resonancia magnética de protón es una de las técnicas que permite explicar el proceso de gelificación. La solución de agarosa formada a altas temperaturas (50 a 55°C), presenta cadenas de polímero con gran movilidad que asumen una conformación aleatoria con un valor constante de difusión. Según disminuye la temperatura, se observa una disminución de la fricción entre los solutos, las cadenas de agarosa y las redes formadas; en este estado la constante de difusión aumenta (50 a 35°C). Cuando se produce una disminución de la temperatura por debajo de 35°C el valor de la constante incrementa debido al movimiento de las cadenas libres de agarosa remanentes [60].

La calorimetría diferencial de barrido permite estudiar la formación de hélices y de agregados en el proceso de gelificación de la agarosa. A una temperatura de 40°C se observa la presencia de un pico exotérmico que indica la formación de hélices. La obtención de picos endotérmicos alrededor de los 90°C indica la fusión progresiva de los agregados formados durante el proceso de gelificación [66, 67].

Con el objeto de incrementar los usos de la agarosa en diversos campos, se han realizado incorporaciones de diversos grupos funcionales a sitios específicos en la estructura de la agarosa.

Un ejemplo de estas modificaciones se encuentra en el uso de polímeros y biomoléculas unidos a los grupos hidroxilo de la agarosa con el objetivo de estudiar el crecimiento y adhesión celular [68]. La incorporación de Carbomer, propilenglicol, glicerina y colágeno a hidrogeles de agarosa ha permitido la aplicación de estos sistemas como soportes de diferentes cultivos celulares [69, 70]. La unión de glucosaminoglicanos a los grupos epoxi de la agarosa permite su utilización como transportadores de péptidos [71].

Se han obtenido estructuras tridimensionales de agarosa capaces de inmovilizar biomoléculas utilizadas en biología celular e ingeniería tisular mediante la activación de los grupos hidroxilo presentes en la estructura del polímero utilizando 1,1'-carbonildiimidazol y a través de radiación ultravioleta [72, 73].

En cromatografía, la agarosa tradicionalmente ha sido utilizada como medio de separación para moléculas con un peso molecular superior a 250.000 Daltons, sin embargo, la incorporación de grupos orgánicos del tipo azidas, alquinos y amino permite mejorar la inmovilización selectiva de este polímero [74, 75]. La unión covalente de agarosa y sulfuro de 6-bromo-7-hidroxicumarina permite la obtención de un sistema capaz de inmovilizar biomoléculas a través de la generación de grupos tiol al someter a los sistemas a procesos de excitación multifotónica [76].

La incorporación de citosina y adenina en la estructura de la agarosa permite la obtención de un material polimérico fluorescente utilizado como sensor [51, 77].

Con el fin de mejorar las propiedades fisicoquímicas de la agarosa, se han realizado mezclas con material cerámico y polímeros con diferentes propiedades mecánicas.

La obtención de un vehículo para ingeniería tisular fue posible gracias a la obtención de un estructura de agarosa y fosfato de calcio con propiedades mecánicas y químicas mejoradas [78]. Estructuras compuestas por quitosano-agarosa-gelatina con poros interconectados con suficiente longitud para promover el cultivo celular fueron desarrollados mediante un proceso de criogelación [79]. Mezclas poliméricas formadas por agarosa-alginato y agarosa-gelatina permitieron la obtención de estructuras con propiedades mecánicas estables y adecuadas para favorecer la proliferación y viabilidad celular [80-82].

Las diversas aplicaciones de la agarosa en ingeniería tisular tienen relación con las propiedades características de este polímero: biocompatibilidad, macroporosidad, ópticamente transparente, no inmunogénica y prácticamente inherte debido a la ausencia de ligandos capaces de interactuar con las células [13, 79, 83-87].

Las aplicaciones de la agarosa en este campo, pueden ser:

Como implantes de piel y tejido cardíaco, formando parte de mezclas con otros polisacáridos naturales: quitosano y gelatina [79] y en la reparación de daños cerebrales [88]; en cultivos celulares mezclada con alginato[47, 80]. Asimismo, en terapias de regeneración articular y regeneración ósea, mezclada con cerámicas del tipo hidroxiapatita, para desarrollar cartílagos calcificados [89-91], en el desarrollo de órganos artificiales, tales como un páncreas bioartificial a través de la incorporación de células de Langerhans [92, 93] y mediante la incorporación de un complemento del receptor tipo I, para incrementar la viabilidad del sistema [94, 95]. En la elaboración de material macroporoso, utilizado como soporte para el cultivo de células pancreáticas [96]. Como

soporte bioartificial unido a oligopéptidos, que le otorgan la capacidad de favorecer el crecimiento neuronal [97]. Formando parte de una matriz con laminina, utilizada como biomaterial multifuncional y en cultivos celulares [98].Unida a polimetacrilatos del tipo HEMA-MMA, que permite favorecer la proliferación, viabilidad y actividad metabólica celular [99]. Además, como soporte que permita estudiar las fuerzas mecánicas que controlan las funciones de los condrocitos [100, 101], para establecer el comportamiento celular en función de la ubicación en las articulaciones [102, 103], los procesos de difusión, las modificaciones estructurales y la producción de glucosaminoglucanos en el tiempo [104].

Como se ha descrito anteriormente, el uso de la agarosa se encuentra orientado principalmente a las técnicas de separación molecular, cromatografía, como medio de cultivo, como soporte en ingeniería tisular. En el campo de la industria farmacéutica se encuentran trabajos relacionados sobre el uso de tensoactivos y polisacáridos que facilitan la incorporación y modulan la liberación de fármacos anticancerígenos como Camptotecina [30, 105]. Asimismo, la elaboración de mezclas poliméricas se presentan como alternativa para la obtención de sistemas de liberación controlada de fármacos hidrosolubles [106].

OBJETIVO y PLANTEAMIENTO

Dado que la elaboración de sistemas de liberación controlada de fármacos presenta una gran aplicación en el campo farmacéutico y que dentro de los polisacáridos naturales la agarosa presenta propiedades físicas y mecánicas que le permiten su aplicación en diversas áreas de investigación, el objetivo de la tesis es:

Elaborar hidrogeles basados en agarosa como vehículos para modular la liberación de fármacos de diferente solubilidad.

Para cumplir con el objetivo expuesto el planteamiento del trabajo es:

Preparación de hidrogeles de agarosa, a los que se incorporan fármacos modelo con diferentes características de solubilidad, tolbutamida cómo modelo de fármaco con solubilidad pH-dependiente y teofilina como modelo de fármaco hidrosoluble. Posteriormente se procederá a la caracterización de los sistemas, en fresco y después de liofilizarlos, mediante técnicas de análisis térmico, microscopía, difracción de rayos X, porosidad e hinchamiento. El estudio de liberación de los principios activos en diferentes medios, agua desmineralizada y medio de pH progresivo, para emular las condiciones gastrointestinales, pondrá de manifiesto la influencia de las características físico-químicas del sistema en la liberación del fármaco que contiene en función de la naturaleza del mismo y de la naturaleza del medio de liberación.

De acuerdo con los resultados que se obtengan en la etapa anterior se prepararán nuevos sistemas incorporando aditivos de diferente naturaleza, otros polímeros como la pectina, tensoactivos de alto y bajo valor de HLB y derivados del ácido poliacrílico, con el fin de determinar si estos agentes son capaces de modificar la estructura de los hidrogeles y determinar su influencia en el perfil de liberación de cada

fármaco para conseguir una liberación sostenida y que además sea independiente de la variación del pH del medio en el que se encontrará la formulación a lo largo de su paso por el tracto gastrointestinal.

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Artículo I

Agarose drug delivery systems upgraded by surfactants inclusion: Critical role of the pore architecture

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Agarose drug delivery systems upgraded by surfactants inclusion: Critical role of the pore architecture

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ABSTRACT

Anionic or non-ionic surfactants have been introduced in agarose-based hydrogels aiming to tailor the release of drugs with different solubility. The release of a hydrophilic model drug, Theophylline, shows the predictable release enhancement that varies depending on the surfactant. However, when the hydrophobic Tolbutamide is considered, an unexpected retarded release is observed. This effect can be explained not only considering the interactions established between the drug loaded micelles and agarose but also to the alteration of the freeze-dried hydrogels microstructure. It has been observed that the modification of the porosity percentage as well as the pore size distribution during the lyophilization plays a critical role in the different phenomena that take place as soon as desiccated hydrogel is rehydrated. The possibility of tailoring the pore architecture as a function of the surfactant nature and percentage can be applied from drug control release to the widespread and growing applications of materials based on hydrogel matrices.

1. Introduction

Among the different polysaccharides in the biomaterials and biotechnological world (Borgogna, Bellich, & Cesaro, 2011; Coviello, Matricardi, Marianecci, & Alhaique, 2007; Laurienzo, 2010; Malafaya, Silva, & Reis, 2007; Murano, 1998; Oliveira & Reis, 2011), agarose is gaining several applications (Rinaudo, 2008) further than its traditional and extensive utilization in molecular biology separation techniques such as gel electrophoresis or gel filtration chromatography (Punna, Kaltgrad, & Finn, 2005; Stellwagen, 2009). In addition agarose is employed as an alternative to agar in particular situations as culture media, microorganism motility assays or food texture modifications. Besides its utilization in different aspects within the biotechnology field (Renn, 1984), agarose is being employed in the biomaterial field as a matrix to regenerate a damaged tissue or forming part of a drug controlled release device. Both functions are being combined, in what can be termed as functional scaffolds, aiming to achieve a better integration of a scaffold by the inclusion of biomolecules that avoids the immunological reactions, facilitate the cell colonization while avoiding a possible bacterial infection.... In this sense agarose has been considered as a potential candidate to regenerate different types of tissues, especially hard (bone and cartilage (Chung & Burdick, 2008; Ge, Li, Heng, Cao, & Yang, 2012)), pancreas (Bloch et al., 2005; Iwata et al., 1992; Teramura & Iwata, 2010) and nervous system (Bellamkonda, 2006; Cao, Gilbert, & He, 2009; Khaing & Schmidt, 2012; Stokols & Tuszynski, 2006). Many of these studies are based on the facility of this substance to form hydrogels that can be used to shape pieces in the required form for an actual case. Indeed, the relatively low gelling temperatures allow the direct inclusion of thermal labile biomolecules or even cells during the fabrication procedure (Cabanas, Pena, Roman, & Vallet-Regi, 2009; Pena, Roman, Cabanas, & Vallet-Regi, 2010). In such a way a system with a high content in water and a chemical composition that resembles that of extracellular matrix can be obtained and tailored by the inclusion, besides the already mentioned biomolecules, of structural components that contribute to improve the mechanical performance (Delair, 2012; Gupta, Vermani, & Garg, 2002; Jeong, Kim, & Bae, 2002; Lee & Mooney, 2001; Lin & Metters, 2006; Pena et al., 2010; Peppas, Bures, Leobandung, & Ichikawa, 2000; Slaughter, Khurshid, Fisher, Khademhosseini, & Peppas, 2009).

However it must be taken into consideration that this type of matrixes requires, due to their high water content and presence of labile components, the employment of a preservation technique that facilitates its off-the-shelf application. Freeze–drying

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has been extensively employed in different industries to eliminate water from solid, semisolid or liquid systems, thus enabling their conservation and ulterior application after a rehydration process (Claussen, Ustad, Strommen, & Waide, 2007; Sanchez, Hernandez, Auleda, & Raventos, 2011; Wang, 2000). At the same time this technique has been employed as a tool to generate a characteristic honeycomb pore architecture that consist on uniaxial parallel pores of around 100–200 μm that have been generated during the water extraction (Gutierrez, Ferrer, & del Monte, 2008; Lozinsky et al., 2003). This range of porosity is of critical importance for the fluid migration containing nutrients and metabolic waste throughout the whole matrix volume; in addition it facilitates the capillary vascularization.

Nevertheless, despite their several benefits the highly hydrophilic environment within hydrogels supposes a disadvantage to entrap homogeneously poorly soluble drugs and may not ensure an adequate protection even causing a premature degradation. In this sense several strategies are being developed in order to optimize the performance of these compounds as drug delivery devices or functional scaffolds (Alvarez-Lorenzo & Concheiro, 2003; Bai, Thomas, Rawat, & Ahsan, 2006; Coviello et al., 2007; Tadros, 2009). On one side, a better release control can be achieved by including the drug molecules in structures of greater size and lower diffusivity, while it can also be regulated through the incorporation of additives, especially surfactants (Alvarez-Lorenzo & Concheiro, 2008; Kawakami, Oda, Miyoshi, Funaki, & Ida, 2006; Paulsson and Edsman, 2001).

Surfactants are compounds very used in different industrial fields. In pharmaceutical technology may act as wetting agents, emulsifiers, foaming and dispersant agents in order to stabilize different systems (solutions, suspensions, emulsions, aerosols) (Eeckman, Moes, & Amighi, 2003; Moffat, Osselton, Widdop, & Clarke, 2004; Tadros, 2009). Moreover, their micelle-forming capability increases the solubility of poorly soluble drugs as well as hydrophobic organic compounds (Bromberg, Hatton, Barreiro-Iglesias, Alvarez-Lorenzo, & Concheiro, 2007; Kawakami et al., 2006; Laha, Tansel, & Ussawarujikulchai, 2009; Lin, Lin, & Yang, 2009). Sodium lauryl sulphate, an anionic surfactant, has been used to enhance water solubility of Camptothecin, Mefenamic acid, Nimesulide or Ibuprofen (Liu & Li, 2005, 2007; Park & Choi, 2006). Tween 80®, hydrophilic nonionic surfactant, has demonstrated its capability as stabilizing agent of solid lipid nanoparticles or in the water insoluble suspensions of Itraconazole and Budesonide (Olbrich & Muller, 1999; Owen, Graham, Werling, & Carter, 2009). Pluronic F-68®, non-ionic surfactant consist of polyethylene oxide–polypropylene oxide–polyethylene oxide (PEO–PPO–PEO) block copolymers has been incorporated to chemotherapeutic micelles formulation to enhance their effectiveness (Kabanov, Batrakova, & Alakhov, 2002a; Kabanov, Lemieux, Vinogradov, & Alakhov, 2002b; Tadros, 2009).

Besides their applications in the drug delivery field, the combination of polysaccharides and surfactants are being applied for different uses such as nanopore matrixes for separation(Sitaras, Naghavi, & Herrington, 2011; Stellwagen, 2009), generation of nanoparticles (Chatterjee, Chatterjee, & Woo, 2010), homogeneous dispersion of a compound within the gel matrix (Guo et al., 2013), unusual texture properties for food ingredients (Chhatbar, Godiya, & Siddhanta, 2012; Maurer, Junghans, & Vilgis, 2012), creation of superporous adsorbents (Shi, Wang, & Wang, 2013; Zohuriaan-Mehr, Omidian, Doroudiani, & Kabiri, 2010). . ..

Initially the aim of this research was to study the possible benefits of incorporating a surfactant within agarose matrixes with the objective of facilitating the release of poorly soluble drugs such as Tolbutamide in comparison with a model drug highly water soluble (Theophylline) (Alvarez-Mancenido, Landin, Lacik, & Martinez-Pacheco, 2008; Kondo, Niwa, Okamoto, & Danjo, 2009; Luo, Zhang, Wei, Liu, & Chen, 2009; Shaheen & Yamaura, 2002). However, the unexpected results obtained induced us to characterize the microstructural changes caused in the agarose matrixes by the surfactants presence and how do these alterations affect to the behavior of these materials when immersed in an aqueous medium. In this sense it should be remarked that part of this characterization must be carried out in a dry state, that is, freeze-dried. Considering this, the objective of this work is to characterize the microstructure, focusing specially on the porosity, of freeze-dried samples and try to correlate it with their behavior in a fluid, specifically with the release of a substance initially included in the formulation. Such characterization results of considerable interest taking into account the great number of hydrogels employed in applications related to the controlled release of active substances, and that these formulations are usually preserved by freeze–drying before administration or implantation.

2. Materials and methods

2.1. Materials

Theophylline (**THE**) (Lot: 093K0122), Tolbutamide (**TLB**) (Lot: 16H0898), Tween 80® (**T**) (Lot: 83H0550) and Pluronic F-68® (**P**) (Lot: 100K0199) were purchased from Sigma Chemical (St. Louis, MO, USA). Sodium lauryl sulphate (**L**) (Lot: 252368CS) was purchased from Panreac, (Barcelona, Spain). Agarose (**A**) for routine use (Lot: 085K0062) with a sulphate content < 0.15%, E.E.O. 0,09-0,13 a gel point at 36 °C and a gel strength >1200 g/cm^2 was also purchased from Sigma Chemical (St. Louis, MO, USA). Water was purified with the Milli-Q reagent system (Millipore). The physicochemical properties of the surfactants employed in this work are detailed in Table 1.

2.2. Preparation of freeze-dried hydrogels

As shown in Table 2, the different amounts of surfactants or drugs previously sieved to a particle size <100 μm were dissolved in demineralized water. Subsequently, the agarose was added into this solution/suspension and slowly heated at 90 °C (±0.1 °C) on a water bath under agitation until the agarose in completely hydrated. At that moment, each system was poured into PVC blisters (1 mL of capacity) and allowed to gel at room temperature for 24 h. Finally, as schematized in Fig. 1 all the systems were freeze dried (Lia-bor) reaching a freezing temperature, a sublimation temperature and a sublimation pressure into a chamber of −45 °C, from −45 to 25 °C and 4.54×10^{-4} atm, respectively.

2.3. Systems characterization

All samples were analyzed by X-ray diffraction (XRD) in a Philips X-Pert MPD diffractometer with Bragg–Brentano geometry, operating with Cu*K*α radiation (λ = 1.5406 Å) at 40 kV and 20 mA. The X-ray diffraction patterns were collected over the range between 4° and 40° 2θ with a step size of 0.02° 2θ and a contact time of 5 s per step. Thermogravimetric Analysis (TGA) and differential thermal analysis (DTA) were performed in a TA instruments TG/DTA analyser, with 10 °C/min heating ramps. In order to observe the behavior showed for every sample during its heating, thermomicroscopic examinations were carried out using a Thermogalen Hot Stage Microscope (HSM) fitted with a Kofler stage; every sample was heated at a rate of 2 °C/min between 30 and 350 °C. An Hg intrusion porosimetry study was carried out using a Micromeritics AutoPore III 9410 porosimeter between 0.1 and 30,000 psi.

Table 1
Physicochemical properties of SLS, Tween 80® and Pluronic F-68®.

Surfactant	Average molecular weight (g/mol)	Melting point (°C)	CMC (mg/L)	HLB	N_{agg}
SLS	288.38	204–207	230	40	62
Tween 80®	1310	–21	14	15	60
Pluronic F-68®	8400	52–57	4032	29	15

CMC – Critical micelle concentration **HLB** – hydrophilic–lipophilic balance N_{agg} – Aggregation number (Raymond, Rowe, Sheskey, Cook, Association & Fenton, 2012; Helgason et al., 2009).

Table 2
Composition of binary and ternary freeze-dried hydrogels.

	TLB (wt%)	THE (wt%)	L (wt%)	T (wt%)	P (wt%)	A (wt%)
A	–	–	–	–	–	100.0
THE-A	–	25.0	–	–	–	75.0
TLB-A	25.0	–	–	–	–	75.0
L1-A	–	–	25.0	–	–	75.0
L1-THE-A	–	20.0	20.0	–	–	60.0
L1-TLB-A	20.0	–	20.0	–	–	60.0
L3-A	–	–	50.0	–	–	50.0
L3-THE-A	–	14.4	42.8	–	–	42.8
L3-TLB-A	14.4.	–	42.8	–	–	42.8
T1-A	–	–	–	25.0	–	75.0
T1-THE-A	–	20.0	–	20.0	–	60.0
T1-TLB-A	20.0	–	–	20.0	–	60.0
T3-A	–	–	–	50.0	–	50.0
T3-THE-A	–	14.4	–	42.8	–	42.8
T3-TLB-A	14.4	–	–	42.8	–	42.8
P1-A	–	–	–	–	25.0	75.0
P1-THE-A	–	20.0	–	–	20.0	60.0
P1-TLB-A	20.0	–	–	–	20.0	60.0
P3-A	–	–	–	–	50.0	50.0
P3-THE-A	–	14.4	–	–	42.8	42.8
P3-TLB-A	14.4	–	–	–	42.8	42.8

TLB: Tolbutamide; **THE**: Theophylline; **L**: Sodium lauryl sulphate; **T**: Tween 80®, **P**: Pluronic F-68® and **A**: Agarose.

2.4. Swelling behavior

Swelling of all freeze-dried hydrogels prepared was evaluated in terms of weight gain after the samples were maintained in aqueous medium at 37 ± 0.1 °C. The samples, previously weighted, were introduced in a beaker that contained 500 mL of demineralized water and were left in a shaking water bath at 42 oscillations per minute. At specific time intervals, the hydrogels were removed from the test medium water blotted and carefully weighed. The maximum duration of assay was 4 h after which a constant equilibrium weight was reached.

2.5. Drug release studies

A Sotax AT-7 dissolution apparatus with paddles was employed to carry out all the dissolution studies. Demineralized water (1000 mL) was employed as dissolution medium at a temperature of 37 ± 0.1 °C. The stirring speed was 100 r.p.m. in the case of TLB systems and 50 r.p.m. in the case of THE systems. An amount of 30 mg of TLB or THE, or their equivalent amount of lyophilized systems, were used for the dissolution studies. At determined time intervals samples of the dissolution medium were extracted and filtered with a Whatman® filter paper (type 42). The quantity of drug dissolved was determined using a Beckman spectrophotometer DU-7 at a wavelength of 228 nm (TLB) or 272 nm (THE). Three replicates of each dissolution assay were carried out.

3. Results and discussion

The characterization carried out focus, on one side, on determining the status of the drug crystallites in the different matrices prepared and to preview its dissolution behavior when rehydrated. As observed during the preparation stage, the utilization of surfactants contributes to a better solubilization of TLB, due to its incorporation into micelles. In the case of the hydrophilic THE its great solubility (12 mg/mL (Li et al., 2013)) is not affected by the presence of any surfactants. However, water elimination, as a consequence of the freeze–drying process, compromises the integrity

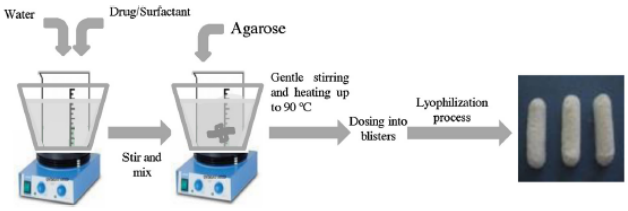

Fig. 1. Preparation and preservation route of drug containing systems.

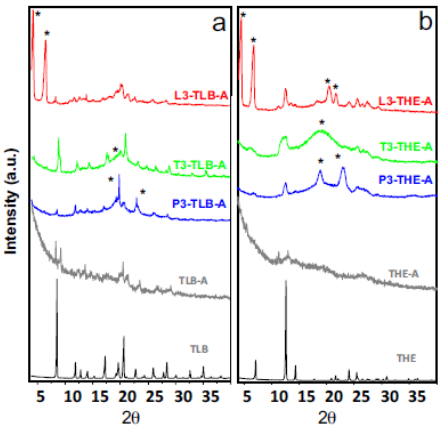

Fig. 2. X-ray diffraction of samples containing (a) Tolbutamide (TLB) or (b) Theophylline (THE). * Indicates the contribution of the surfactants included.

of the micelles, while the status of the drug crystallites inside (TLB) or outside the micelles (THE) surges as a question to solve.

On the other side, the study of the pore architecture should give critical information about the fluid migration once that the sample is introduced in an aqueous medium. The massive entrance of water is determined by the pore architecture and influences on the following series of successive or overlapping phenomena: reconstruction of the micelles, redissolution of the crystals and release of the drug.

The X-ray characterization of the samples shows (Fig. 2) a considerable decrease on the crystallinity of the drugs included in an agarose matrix. This lower crystallinity can be clearly corroborated by comparing single Theophylline or Tolbutamide X-ray diffraction patterns with those where the drugs are included within agarose matrices. Agarose, due to its non-crystalline nature, has a scarce presence of the X-ray patterns, but limits and addresses the growth of Tolbutamide or Theophylline crystals among the agarose chains. The decrease in the drug crystal size can, as well, be observed on surfactant containing samples despite the presence of additional diffraction peaks attributable to the different surfactants (marked in the figure with a *) that depend on their crystalline state, *i.e.* from well-defined and intense maxima for SLS to wider and weaker for Pluronic and Tween. Moreover, it must be pointed out that the surfactant influences on the X-ray diffractograms patterns as can be inferred from slight variations on the relative intensity of some significant reflections. The examination of the corresponding binary, not containing agarose, samples (not shown) evidences that the surfactant included has a critical role on this growth thus creating preferred orientations.

Additionally, when comparing Theophylline and Tolbutamide containing systems, the lower crystallinity observed for the THE samples can be explained considering that, due to its higher solubility, the growth of these drug crystals is considerable much slower when the water is frozen during the freeze–drying process (Fig. 2).

The use of a set of techniques (TG, DTA, HSM) based on the behavior of the materials with temperature variations allows to envisage possible interactions between the different components as well as to predict the crystalline state of the drugs included. Since

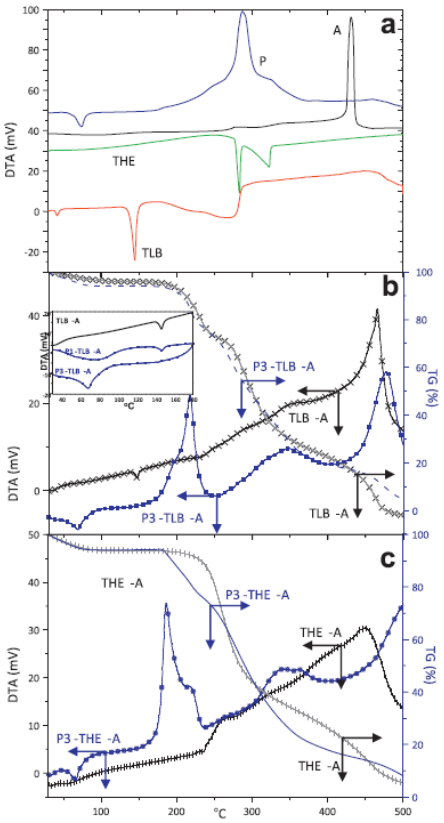

Fig. 3. (a) DTA patterns of the single components, (b) TG and DTA patterns of TLB-A and P3TLB-A, inset: augmentation of the low temperature region of TLB-A, P1-TLB and P3-TLB-A DTA curves, (c) TG and DTA patterns of THE-A and P3THE-A.

the representation of all the different samples prepared would be impossible to fit within this article, Fig. 3 shows only the characterization of the materials prepared with Pluronic. The characterization of the single components (Fig. 3a) facilitates the analysis of the multicomponent systems prepared and allows to confirm the melting process of Pluronic (52–57 °C), and its decomposition around 300 °C (Table 1). Tolbutamide suffers, as well, a melting around 140 °C while in the case of Theophylline the endothermic peak near to 300 °C can be attributed to the thermal decomposition of this drug. The decomposition patterns of the ternary systems including TLB (Fig. 3b) or THE (Fig. 3c) show a considerable complexity and do not result a mere addition of the individual components curves. An example of the interactions established that cause these different patterns is the exothermic peak that can be observed around 200 °C. This signal, which already appears in the binary P1-A and P3-A (not displayed) but not in the individual components patterns,

indicates some type of interaction between them. When a third component is included, THE or TLB, this peak shifts towards higher temperatures which gives idea of newly and not easy to determine bonds between these substances that, in addition, yield different decomposition patterns at much higher temperatures.

Moreover, a more detailed study of the peak attributable to the TLB-A melting point (Fig. 3b, inset) allows to deduce information about the state of these crystals. The decrease on this peak area can be related not only to the lower quantity of this drug when included in the agarose binary sample, but mainly to its lower crystallinity, as was previously observed by X-ray diffraction. Increasing percentages of the surfactant lead to the practical disappearance of this peak due to its dissolution action through the formation of micelles. This effect has been confirmed through Hot Stage Microscopy, a subjective technique that allows to monitor *in vivo* the progressive dissolution of the TLB crystals within the agarose/surfactant matrix, even in the absence of water.

Concerning the thermal behavior of the Tween or SLS containing samples, complex decompositions patterns that include the appearance of non-expected peaks are, as well, observed. At this point it should be stressed out the different thermal behavior of the three surfactants employed (Table 1) where pluronic can be considerate intermediate between SLS that remains solid over 200 °C, while Tween, with a much lower melting point: −21 °C (Helgason et al., 2009), is in the liquid form when manipulated to prepare the materials. This different behavior should not have a major influence when the materials are prepared since all the different components should be completely dissolved in the aqueous medium. However, the different thermal properties should have a critical role when the sample is freeze-dried; the temperature at which the samples are initially preserved (−20 °C) is not very distant from the melting point of Tween which means that remains in the liquid form till the very last moment. On the other side SLS (≈200 °C) should transform to the solid state immediately after the preparation procedure, while Pluronic (≈50 °C) is characterized an intermediate behavior. Furthermore, the presence of these compounds and the interactions between them may result in an alteration of the thermal behavior of the so obtained materials. In this sense, Pluronic containing materials, which should have an in-between situation, could remain in the liquid state much longer that expected. This could be applied, as well, to SLS samples but the high melting point value ensures its permanence as a solid throughout the preparation and preservation procedures.

The pore architecture results critical during the specimens rehydratation since determines the initial massive water entrance rate as well as the fluid interchange once the initial volume has almost been recovered and an dynamic equilibrium state is established. Fig. 4a shows the monomodal pore size distribution, centered between 100 and 200 μm, characteristic of samples containing only agarose (Table 3). This microstructure has been already observed in different freeze dried gels, and is formed as a consequence of the water extraction during its elimination in the lyophilization procedure. The addition of any of the two drugs causes no alteration on this distribution as can be tested in Fig. 4a. In the same way the inclusion of any of the surfactants within the agarose matrix does not considerably alter the microstructure already described. However, the simultaneous inclusion of a surfactant and a drug does induce modifications on the microstructure, especially when Tolbutamide is included and the surfactant employed is Tween or Pluronic. The inclusion of TLB within the SLS micelles does not vary the porosity percentage over a 90%, but causes a slight decrease in the pore size (80–130) that does not vary as a function of the surfactant percentage. However, the percentage increase does decisively affect to the pore distribution and porosity percentage for Tween or Pluronic containing samples; while

Table 3
Experimental density, porosity percentage and pore size of the prepared materials.

	Exp. density (mg/mL)	Porosity (%)	Pore size (μm)
A	28	>90	100–200
THEA	37	>90	100–200
TLBA	39	>90	100–200
L1A	38	>90	100–200
L1THEA	48	>90	100–200
L1TLBA	50	>90	80–135
L3A	57	>90	100–200
L3THEA	68	>90	100–200
L3TLBA	67	>90	80–125
T1A	38	85	80–120
T1THEA	52	85	100–200
T1TLBA	53	70	70–120
T3A	56	80	70–200
T3THEA	69	80	100–200
T3TLBA	66	55	2–5, 8–15
P1A	49	90	70–120
P1THEA	46	90	65–135
P1TLBA	49	90	1–5, 40–110
P3A	68	85	70–200
P3THEA	66	90	70–135
P3TLBA	65	85	1–5, 15–50, 60–140

a 1% of surfactant causes a pore size distribution similar to that observed in SLS samples when TLB is included (Fig. 4c and d), a higher quantity of surfactant (3%) provokes the complete (Tween) or partial (Pluronic) disappearance of the characteristic pore distribution around 100–200 μm. The resulting microstructures are characterized by a bimodal (Pluronic) or trimodal (Tween) pore distribution, where smaller pores around 1–5 μm and 5–50 μm can be detected (Table 3). As a consequence of this modification the porosity percentages decreases specially in the case of Tween containing samples. Concerning the influence of the hydrophilic drug, Fig. 4b–d show scarce differences between non-loaded and Theophylline loaded samples showing pores within the range observed in agarose samples.

The cumulative intrusion curves of Hg within these systems contributes to show the great differences between Theophylline (Fig. 5a) and Tolbutamide (Fig. 5b) loaded samples, especially in the case of those prepared with Tween or Pluronic at a 3%. As can be deduced from its denomination these curves represent the quantity of mercury that enters within the sample when the pressure is progressively increased and gives an idea of how a fluid penetrates the matrix starting from the bigger pores and occupying progressively those smaller. A lower degree of mercury intruded can be observed for T3-TLB-A samples, followed by P3-TLB-A, while that prepared with SLS show levels similar to those observed for samples loaded with Theophylline. This can be related to the pore size distribution consisting on smaller pores for those samples with lower intrusion values, that hinders the fluid penetration. However, it must be taken into consideration that these intrusion values are calculated for the intrusion of mercury, a bigger and heavier substance that water, that requires elevated pressures to penetrate within the solids, while in the case of the solids being characterized water enters freely reaching the original dimensions of the pieces in a few minutes. In any case this minor degree of intrusion agrees with the lower porosity percentages observed for these samples (Table 3).

The microscopy characterization confirms (Fig. 6) the progressive transformation of the characteristic pore architecture consisting in parallel uniaxial 100–200 μm pores into a denser surface for samples containing Tolbutamide and Pluronic or Tween. This network is characteristic of freeze-dried materials and has been described by several authors as a honeycomb or spongy like structure (Dal Pozzo et al., 2000; Muzzarelli, 2009; Roman, Cabanas, Pena, & Vallet-Regi, 2011). This spongy aspect can be explained by

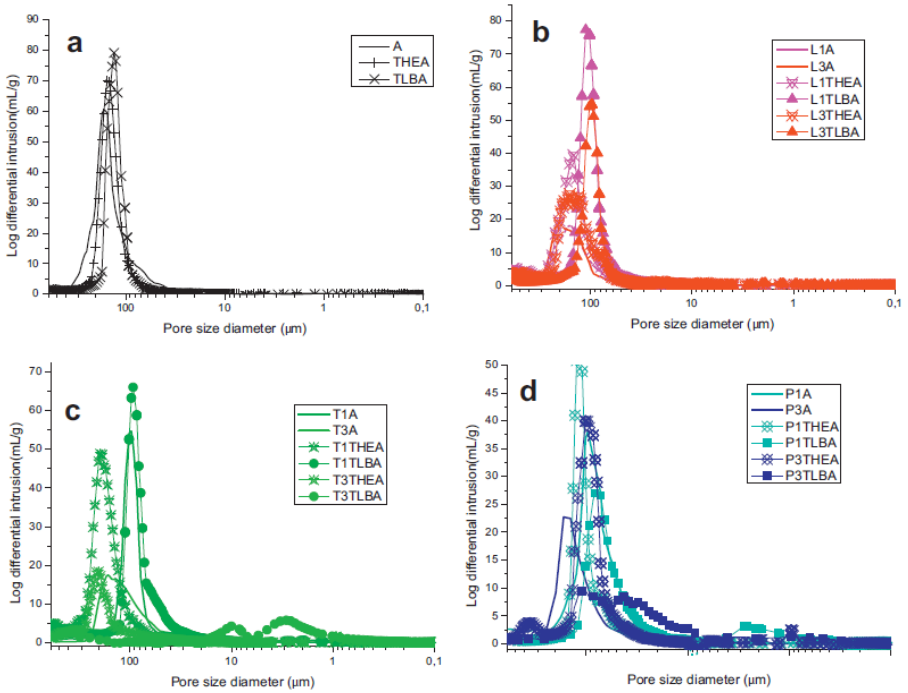

Fig. 4. Pore size distribution of (a) agarose, tolbutamide-agarose and Theophylline agarose samples, (b) theophylline and tolbutamide loaded and non-loaded samples prepared with (b) SLS, (c) Tween and (d) pluronic.

considering the elimination of the large amount of water contained in the freshly prepared materials. During the freezing process, ice crystals nucleate from the solution and grow along the lines of thermal gradients; the subsequent lyophilization generates a porous material. Throughout the desiccation process of the hydrogel the agarose chains approximate to a point that induces a collapsed system where the ulterior rehydration is considerable reduced, when compared to the original water contents of the as

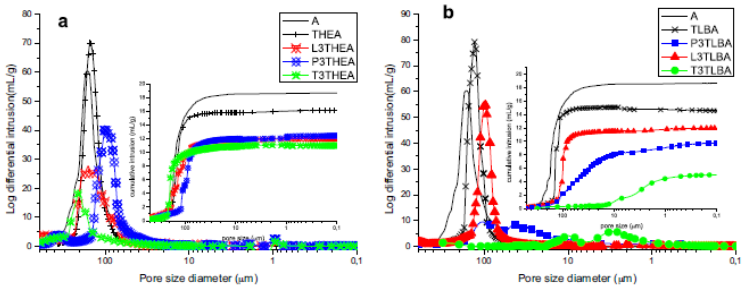

Fig. 5. Pore size distribution and cumulative intrusion of (a) theophylline and (b) tolbutamide containing systems prepared with different surfactants at a 3%. Agarose and agarose-drug curves are also included.

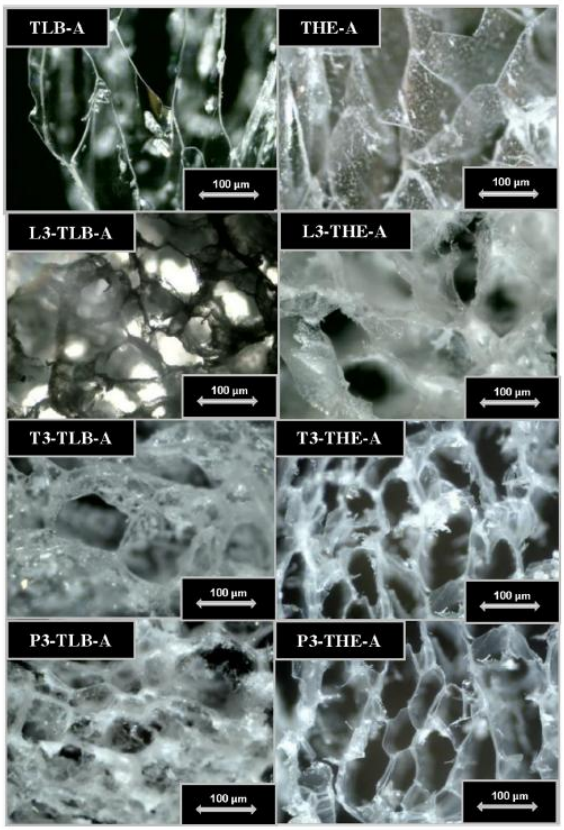

Fig. 6. Microscope micrographs of the binary (TLB-A and THE-A) and ternary systems with TLB or THE and the three surfactants employed.

prepared (fresh) systems. However, this collapse would be influenced, and even tailored, by the presence of different additives within the hydrogel matrix as has been extensively demonstrated by the use of the well-known cryoprotectants (Julca, Alaminos, Gonzalez-Lopez, & Manzanera, 2012; Wang, 2000).

In order to compare swelling data obtained, which reflects the collapse degree, Fig. 7 shows the swelling expressed as water uptake units (W.U.U.), which represents the milligrams of water taken by the same quantity of agarose in each system.

The water uptake units have been calculated using the following expression:

$W.U.U = \frac{W_S - W_F}{W_{Ag}}$ where W_S is the weight of swollen hydrogel at a predetermined time, W_F is the initial weight of the freeze-dried hydrogel and W_{Ag} is the amount of agarose in each analysed system.

The W.U.U. value observed for pure agarose system, which will be used in this discussion as a reference threshold, is considerably affected by the presence of Tolbutamide whose hydrophobic nature reduces the water uptake. On the contrary the THE-A system shows a swelling value very similar to the pure agarose reference. The key factor of the water recovery depends clearly on the hydrophobic or hydrophilic nature of the drug since, as observed on the microstructure characterization, the presence of any of the drugs does not alter the pore size distribution or porosity percentage.

The introduction of a surfactant into the hydrogel increases, in almost all cases, the water uptake due to the diminution of the surface tension value of the water that causes the rehydration. The more significant W.U.U. values for Tween (T1A and T3A) can be explained considering the much lower critical micelle concentration (CMC) of Tween (14 mg/L, Table 1) when compared to Pluronic (4032 mg/L) or SLS (230 mg/L). This parameter indicates the extreme facility of Tween to form micelles even for very low concentrations of this surfactant. Consequently, it can be assumed that Tween micelles are formed ubiquitously all over the matrix thus reducing the freedom degree of the agarose chains that induces

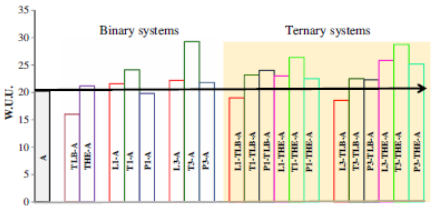

Fig. 7. Swelling behavior as a function of the water uptake units (W.U.U.).

a minor collapse when water is eliminated. The presence of the micelles, which actuates as a framework, reduces the hysteresis observed after rehydrating and allows higher swelling values.

In the ternary systems the W.U.U. value is determined by both the hydrophilic or hydrophobic nature of the drug, as well as by the hydrogel microstructure determined by the interactions between the surfactant molecules or the loaded micelles with the agarose chains. In this sense, those values below the considered reference value can be explained adding the hydrophobic nature of TLB to high porosity percentages, *i.e.* samples that are prepared with Sodium lauryl sulfate.

In the case of TLB loaded Pluronic or Tween agarose matrices the decreasing effect of the drug on the swelling behavior is compensated by a completely modified less porous microstructure. In addition, the presence of molecules or particles between the agarose chains avoids the collapse of the structure thus enabling the reversibility of this process, although a complete recovery of the initial weight and dimensions cannot be reached and a certain hysteresis that depend on the nature and quantity of the "spacers" is observed (Pena et al., 2010).

The simultaneous presence of Theophylline and any of the surfactants induces, in all cases, superior values of water absorption to that considered as threshold. This effect can be attributed to the additive combination of the hydrophilic nature of drug, the surfactant decreasing action over the water surface tension and the role of the surfactants molecules on the agarose chains condensation during the desiccation process.

Before considering the drug release of Tolbutamide or Theophylline, it must be taken into consideration the status of these drugs within the materials. When a drug is formulated in a hydrogel that incorporates surfactants, the drug may be in different states depending on its hydrophilic or hydrophobic nature. In the case of a hydrophilic drug, as THE, drug molecules can be found in two different forms: free or adsorbed on the hydrogel chains. Furthermore, when drug is hydrophobic, like TLB, its molecules are in three different states: free form, inside micellar aggregates and adsorbed on the agarose. (Kapoor, Thomas, Tan, John, & Chauhan, 2009; Kapoor, Bengani, Tan, John, & Chauhan, 2013) Besides, it must be taken into account the different phenomena that take place when the initially lyophilized materials are immersed in an aqueous media and rehydrates: the agarose chains expands and redistribute from a collapsed state that has been minimized by the presence of the micelles which at the same time must recover their original "status quo". Additionally, it must be taken into consideration the immediate and progressive destruction of the micelles due to the surfactant diffusion through the hydrogel. According to the studies carried

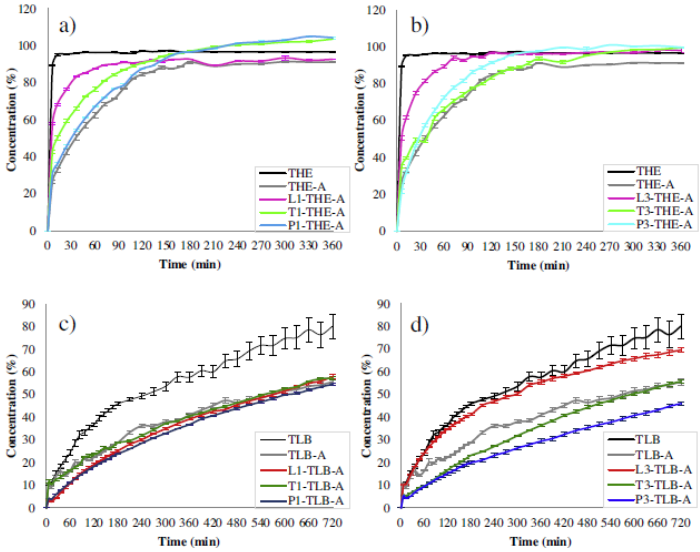

Fig. 8. Drug release profiles of freeze-dried hydrogels. Ternary THE containing systems with 1% (a) or 3% (b) of surfactant and equivalent TLB ternary formulations with 1% (c) and 3% (d).

out by Kapoor et al. (2009) the diffusion rate through the hydrogel depends on its molecular weight: higher rates for decreasing weights. According to this the diffusion rate of the surfactants utilized in this work would be L > T > P. This phenomenon decisively affects to the drug release since the voids left by the surfactants molecules are occupied by the solution medium thus facilitating the dissolution and diffusion of the drug.

The drug release of Tolbutamide and Theophylline from the different formulations prepared is depicted in Fig. 8. The influence of the surfactant is discussed taking into consideration the situation of the release pattern within the window created between the dissolution profile of pure drug and that of the same drug from the agarose matrix where no surfactants have been included.

Pure theophylline, due to its hydrophilic nature, shows a typical burst release where almost the 100% of the drug is released within the first 15 min (Fig. 8a and b). However, the inclusion of the drug within the agarose matrix results into a much-sustained release that takes up to 180 min to completely liberate the Theophylline. This difference can be attributed to the phenomena that take place when water enters within these agarose matrices: (i) the dissolution of the drug, which depends on the crystallite size of the lyophilized drug, and (ii) its diffusion through the hydrogel that it suffering at the same time a rehydration and reconstruction process. When surfactants are introduced in Theophylline containing systems, drug release is conditioned by the nature of the surfactant. Although the presence of any surfactant produces a decrease of the water surface tension that facilitates the rehydration process, the dissolution and diffusion of the surfactant molecules conditions, as well, the hydrophilic drug release. This can be attributed to the gaps left when the surfactants molecules migrate out of the hydrogel matrix. Considering the molecular weight influence, systems containing L show higher release rate when compared to P or T. Indeed, the higher THE released values at shorter times found for lauryl sulfate containing systems can be attributed to their higher hydrophilic character as can be deduced from the HLB values of the surfactants (Table 1).

A hydrophobic drug as Tolbutamide has a completely different behavior when immersed in an aqueous solution (Fig. 8c and d). On one side, the release from a hydrogel matrix is, as well, retarded for similar reasons to those mentioned above. The presence of surfactants should "a priori" minimize this hindered decrease. Though, the inclusion of a 1% of any surfactant has a scarce influence on the drug release showing release profiles very similar to that observed from the agarose matrix (TLB-A). However, when higher surfactant percentages are included (3%) completely different release patterns can be observed (Fig. 8d). On one side, the SLS surfactant behaves as expected, *i.e.* facilitating the drug solubilization and its release due to the gaps created as a consequence of the L surfactant molecules diffusion.

On the other hand, when Pluronic or Tween is included, a more controlled release, below that observed for the matrix without surfactants (TLB-A), can be described. This behavior can be explained taking into consideration the three factors that condition and differentiate TLB release from systems with Tween or Pluronic:

(i) The microstructure modification observed in these materials and its critical role on the water entrance. The lower porosity percentages provoke and alteration on the rehydration process due to the different pore architecture.
(ii) TLB molecules can be found, besides free or adsorbed on the hydrogel as in the case of THE, bounded to the hydrophobic core of the surfactants aggregates. Only free and adsorbed TLB molecules can be directly dissolved, while those bounded to the surfactant aggregates core generate a depot that prolongs the total drug release duration. Moreover, the surfactant nature determines the drug diffusion rate through the surfactant head region into the agarose matrix.
(iii) In a similar way to what has been observed for THE release, the higher molecular weights of Tween and Pluronic cause a more progressive migration of the surfactant molecules and, consequently, lower TLB dissolution/diffusion rates.

4. Conclusions

It has been demonstrated that the inclusion of a surfactant within a polysaccharide-based hydrogel matrix deeply affects to the final arrangement of the different components in the resulting self-assembled structures that constitute this type of network. The consequential pore architecture of the freeze-dried matrices is deeply affected by the nature and percentage of the surfactant, the type of drug included and the created loaded or non-loaded micelles. In such a way it becomes possible to tailor and tune the final performance of this type of networks as a function of a particular application: superporous adsorbents, food additives, matrixes for separation. . .besides the controlled release of a drug considered in this work.

The unexpected controlled release of a hydrophobic drug or the increased swelling degree values after the matrices rehydratation can be explained not only considering the interactions established between the drug loaded or non-loaded micelles and agarose but also to the alteration of the freeze-dried hydrogels microstructure.

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Artículo II

Robust and versatile pectin-based drug delivery systems

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Robust and versatile pectin-based drug delivery systems

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ABSTRACT

Pectin-based resistant, interactive and versatile hydrogel vehicles for oral administration have been prepared. These systems are thought to be versatile enough to allow the inclusion of substances (such as the surfactants tested: Pluronic, Tween, Na Lauryl sulphate) that may contribute to tailor the drug release patterns. Tolbutamide, that shows a discrete and pH-dependent solubility in water, has been employed as a model drug to test the capability of these matrices to overcome such drug-imposed restraints. The incorporation of different surfactants produced pectin-based hydrogels of difficult manipulation. In order to improve this drawback, two different strategies have been developed: blending with agarose or freeze-drying. The presence of agarose yields robust systems that can be handled and tested as prepared, in the fresh state. Freeze-drying not only allows to shape pure pectin and blend systems, but also generates a porous structure whose microstructure, determined by the different components included, influences on the drug release behavior. Tolbutamide release kinetics from freshly prepared matrices can be fitted to the Higuchi model while the freeze-dried ones adjust to the Korsmeyer–Peppas model; hence the hydrogel chains rearrangement processes rule the release during the rehydration process.

1. Introduction

Pectin is a natural polysaccharide structurally composed by homogalacturonan (HG), also termed as the smooth region, and rhamnogalacturonan-I (RG-I), known as the hairy region. According to its degree of methyl esterification, pectin can be classified as high methoxyl pectin (HM) or low methoxyl pectin (LM), that yield some differences in their properties (Mohnen, 2008; Moreira et al., 2014; Rowe et al., 2009).

This polysaccharide is being employed within the pharmaceutical field to control local bleeding and to reduce cholesterol blood levels (Liu et al., 2007) as well as in wound-care products, implying bactericidal and wound-healing effects (Munarin et al., 2011, 2012). In the food industry it has been extensively used as jellifying agent, thickener, texturizer, emulsifier . . . (Brejnholt, 2009; Maxwell et al., 2012; Thakur et al., 1997) as well as in the preparation of edible films intended as active food packaging (Perez Espitia et al., 2014). Pectin has also been considered for tissue engineering, especially in hard tissue reparation (Girod Fullana et al., 2010; Munarin et al., 2010, 2011).

The increasing use of pectin in drug delivery is facilitated by the high availability from renewable sources and non-toxic nature of this natu
ral polysaccharide (Liu et al., 2007; Sriamornsak, 2011; Willats et al., 2006). Moreover some of the physic-chemical properties of pectin – muco-adhesiveness, ease of dissolution in basic environments, resistance to degradation by proteases and amylases of the upper gastrointestinal tract and the ability to form gels in acid environments – allow to target different drug delivery formulations (microspheres, beads, pellets and microparticles) into completely different environments such as nasal, vaginal, ocular, gastric and, specially, large intestine (Bosio et al., 2012; Liu et al., 2003; Morris et al., 2010; Wong et al., 2011). The use of pectin based formulations in colon cancer treatments has been extensively considered due to the high degradation rate of this vehicle at the basic pH that characterizes this area and to the presence of specific colonic bacteria (Alvarez-Lorenzo et al., 2013; Semdé et al., 2000; Wong et al., 2011). Finally, pectin has been proposed for cancer treatment (Maxwell et al., 2012; Munarin et al., 2012).

However, pectin has not fulfilled its potential for drug delivery systems due to variability on its formulation depending on its

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source and processing that affects to its stability over time and behavior in a hydrated media. In order to minimize these effects and to improve their properties, several strategies, implying its combination with other polymers: ethylcellulose, hydroxypropyl methylcellulose, gelatin, zein, chitosan, xyloglucan . . . (Das et al., 2011; He et al., 2008; Itoh et al., 2008; Miyazaki et al., 2000; Morris et al., 2010; Ribeiro et al., 2014) or with divalent or trivalent cations (Das et al., 2011; Dhalleine et al., 2011), have been adopted. In this sense, the improved gelation verified in the presence of calcium cation allows to tailor the drug release rate and even create in situ gelling vehicles for oral administration (Itoh et al., 2006, 2007).

In this regard, agarose is proposed to be blended to pectin in order to ensure tougher gels able to include different drugs or additives. Agarose is a biocompatible polysaccharide constituted by (1→3)-β-D-galactopyranose-(1→4)-3,6-anhydro-α-L-galactopyranose units that forms a thermoreversible hydrogel. (Phillips and Williams, 2009; Renn, 1984). Among the different polysaccharides employed in the biomaterials and biotechnological world (Borgogna et al., 2011; Coviello et al., 2006; Laurienzo, 2010; Malafaya et al., 2007; Matricardi et al., 2013; Murano, 1998; Oliveira and Reis, 2011), agarose is gaining several applications (Rinaudo, 2008) besides its traditional and extensive utilization in molecular biology separation techniques such as gel electrophoresis or gel filtration chromatography (Punna et al., 2005; Stellwagen, 2009). Specifically, the gelling capacity of agarose has been applied to shape ceramic or metallic particles or fibers by gelcating and similar techniques (Drisko et al., 2011; Ma et al., 2012; Pena et al., 2010; Roman et al., 2011; Zhou et al., 2006). Besides it has been employed in diverse templation and micropatterning techniques to create stamps, arrays and microfluidic devices for biotechnological devices (Dahlmann et al., 2013; Mercey et al., 2010). In addition agarose is being employed in the biomaterials field as a matrix to regenerate a damaged tissue or forming part of a drug controlled release device. In this sense agarose has been considered as a potential candidate to regenerate different types of tissues, especially hard (bone and cartilage (Chung and Burdick, 2008; Ge et al., 2012)), pancreas (Bloch et al., 2005; Teramura and Iwata, 2010) and nervous system (Bellamkonda, 2006; Cao et al., 2009; Stokols and Tuszynski, 2006).

On the other side freeze-drying is proposed as an alternative strategy to shape pectin based systems. Freeze-drying is one of the most commonly used technologies that ensure a long-term conservation of raw materials for different uses (Nakagawa et al., 2013; Zhang et al., 2014). In pharmaceutical and biomedical fields, freeze-drying can be used in order to preserve unstable molecules by water removal, thus enabling their conservation and ulterior application after a rehydration process (Sanchez et al., 2011; Wang, 2000). At the same time the characteristic honeycomb pore architecture formed by uniaxial parallel pores of around 100–200 μm, generated during the water extraction (Gutierrez et al., 2008; Lozinsky et al., 2003), favors the colonization and vascularization of agarose based scaffolds (Alcaide et al., 2010; Roman et al., 2011). This porosity growth also induces an increase on the specific surface that can be employed to design drug delivery devices for nasal or gastric (Luppi et al., 2010; Risbud et al., 2000) tissues.

In order to test the drug release capabilities of these matrices, a poorly and pH-dependent water soluble drug, Tolbutamide, has been included in the formulations as a model drug. At the same time three different surfactants have been introduced in order to enhance the dissolution of drugs with dissimilar behavior in an aqueous medium. In this sense the introduction of surfactants to improve the drug release performance of hydrogel based systems is becoming an emergent approach (Alvarez-Lorenzo and Concheiro, 2008; Kapoor et al., 2009, 2013). Besides their applications in the drug delivery field, the combination of polysaccharides and surfactants is being put to different uses such as nanopore matrices for separation, generation of nanoparticles (Chatterjee et al., 2010), homogeneous dispersion of a compound within the gel matrix (Guo et al., 2013), unusual texture properties for food ingredients (Chhatbar et al., 2012; Maurer et al., 2012), creation of superporous adsorbents (Zohuriaan-Mehr et al., 2010) . . . In order to succeed in these different aims it is necessary to have a deep knowledge of the polysaccharide/surfactant microstructure as well as of the interaction of these components with additional substances included.

The objective of this work is to design an "open" multicomponent formulation which allows to tailor the release of orally-administered drugs with a wide range of solubility restraints. In order to proceed, these vehicles should be resistant enough not only to overcome the different phenomena to which they may be exposed before and after their administration, but also in terms of structural robustness. This point has been tested through the three surfactants added to facilitate the inclusion and ulterior control release of tolbutamide, i.e., to transform a mere carrier into a vehicle that interacts with the substance loaded and influences on its delivery.

2. Materials and methods

2.1. Materials

Tolbutamide (TLB) (Lot: 16H0898), Tween 80® (T) (Lot: 83H0550), Pluronic F-68® (P) (Lot: 100K0199) and agarose (A) for routine use (Lot: 030M0150) with a sulfphate content <15%, E.E. O. 0.09–0.13 a gel point at 36 °C and a gel strength >1200 g/cm^3 were purchased from Sigma Chemical (St. Louis, MO, USA). Sodium lauryl sulphate, SLS (L) (Lot: 252368CS) was purchased from Panreac, (Barcelona, Spain). Pectin (Pec) (Lot: 9373200010) with 60–70% of galacturonic acid was purchased from Guinama (Valencia, Spain). All other reagents were of analytical grade. Water was purified with the Milli-Q reagent system (Millipore).

2.2. Solubility studies

An excess amount of TLB was weighed into tubes to which 20 mL of five aqueous solutions with different pH values (1.5, 4, 5.4, 6.8 and 7.4) was added. The buffers were prepared according to USP 34: hydrochloric acid (1.5), acid phthalate (4) and phosphate (5.4, 6.8 and 7.4). After closing the tubes, they were shaken at room temperature (25 ± 0.5 °C) in a shaking water bath at 40 oscillations per minute. To make sure that equilibrium was established, samples were withdrawn after 3 days. Every sample was filtered by Millipore filter (0.45 μm) and after suitable dilution with the respective medium; the quantity of dissolved tolbutamide was assayed at a wavelength of 228 nm in a Beckman spectrophotometer DU-7. Three replicates were made in every case.

2.3. Preparation of pectin and agarose/pectin blends hydrogels

2.3.1. Pectin hydrogels

TLB, previously sieved to a particle size <100 μm and different amount of surfactants were dispersed in demineralised water (Table 1). This solution/suspension was added into a beaker containing pure pectin. In order to allow the complete wetting of pectin, the system was gently mixed with a magnetic stirrer overnight. After 24 h, each system was poured into PVC blisters (1 mL of capacity) and allowed to equilibrate at room temperature for 24 h, so fresh hydrogels were obtained. To prepare freeze-dried systems, the fresh systems were freeze-dried (Lia-bor®; Telstar, Barcelona, Spain) reaching a freezing temperature, a sublimation

Table 1
Composition (mg) of each hydrogel systems.

Pectin systems (mg)	Pec	Pec-L1	Pec-L3	Pec-T1	Pec-T3	Pec-P1	Pec-P3
Pec (Pectine)	30	30	30	30	30	30	30
L (SLS)	–	10	30	–	–	–	–
T (Tween)	–	–	–	10	30	–	–
P (Pluronic)	–	–	–	–	–	10	30
TLB (Tolbutamide)	10	10	10	10	10	10	10
Blend systems (mg)	**APec**	**APec-L1**	**APec-L3**	**APec-T1**	**APec-T3**	**APec-P1**	**APec-P3**
A (Agarose)	15	15	15	15	15	15	15
Pec (Pectine)	15	15	15	15	15	15	15
L (SLS)	–	10	30	–	–	–	–
T (Tween)	–	–	–	10	30	–	–
P (Pluronic)	–	–	–	–	–	10	30
TLB (Tolbutamide)	10	10	10	10	10	10	10

temperature and a sublimation pressure into the chamber of −45 °C, from −45 to 25 °C and 4.54×10^{-4} atm, respectively.

2.3.2. Agarose/pectin blend hydrogels

Demineralized water was added into a beaker containing pure pectin. The system was gently mixed overnight in order to allow the complete wetting of pectin. On the other hand, different amounts of surfactants and drug previously sieved to a particle size <100 μm were dissolved in demineralised water. Agarose was added into this solution/suspension and slowly heated at 90 °C (±0.5 °C) on a water bath under agitation until the agarose was completely hydrated.

Afterwards, warm agarose solution was added to the beaker with the moisturized pectin previously prepared. A gentle mixing was necessary to obtain a homogeneous system. The fresh and freeze-dried blends hydrogels were prepared using the same procedure for the freeze-dried pectin hydrogels preparation. Table 1 shows the composition of all systems prepared.

2.4. Systems characterization

The freeze-dried samples were analyzed by X-ray diffraction (XRD) in a Philips X-Pert MPD diffractometer with Bragg–Brentano geometry, operating with CuKα radiation (λ = 1.5406 Å) at 45 kV and 40 mA. The X-ray diffraction patterns were collected over the range between 4° and 40° 2θ with a step size of 0.02° 2θ and a counting time of 5 s per step. Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA) and Differential Thermal Analysis (DTA) were performed in a TA instruments TG/DTA analyser, with 10 °C/min heating ramps. An Hg intrusion porosimetry study was carried out using a Micromeritics AutoPore III 9410 porosimeter between 0.1 and 30,000 psi. Surface morphology was analyzed by scanning electron microscopy (SEM) in a JEOL 6400 Electron microscope.

2.5. Swelling behavior

The swelling degree was determined in demineralized water. The samples, previously weighted, where placed in beakers that contained 500 mL of demineralized water. These beakers were then placed in a thermostatic water shaking bath with an experimental temperature of 37 ± 0.1 °C and a shaking rate of 42 U/min. At specific time intervals, the hydrogels were removed from the test medium water, blotted and carefully weighed. The maximum duration of assay was 24 h after which a constant equilibrium weight was reached. Taking into consideration that all the systems essayed contain the same amounts of components that can swell, this behavior was expressed in terms of Water Gain (WG) directly by subtracting the weight of the swollen hydrogel from the dried one. All quantitative results are expressed as means ± standard deviation (SD) for n = number of samples analyzed. Statistical analysis was carried out using the test of the difference between two means. Results were taken as significantly different at p values less than 0.05.

2.6. Release studies in water and kinetic fits

A Sotax AT-7 dissolution apparatus with paddles was employed to carry out all the dissolution studies in demineralized water (vol. = 1000 mL) at a temperature of 37 ± 0.5 °C. The stirring speed was 100 r.p.m. An amount of 30 mg of TLB, or their equivalent amount of fresh/lyophilized system, was used for these studies. At given time intervals, samples of the dissolution medium were extracted and filtered with a Whatman® filter paper (type 42). The quantity of drug dissolved was determined using a Beckman spectrophotometer DU-7 at a wavelength of 228 nm. Three replicates of each dissolution assay were made.

In order to predict and define the mechanism of TLB release from the systems, the release data were evaluated following the mathematical models proposed by Higuchi and Korsmeyer and Peppas.

Higuchi model:

$$Q_t = K_H \sqrt{t}$$

where Q_t is the amount of drug released in time t and K_H is the Higuchi dissolution constant. This equation was first proposed by Higuchi to predict drug release from a non-degradable monolithic system whereby drug particles are dispersed uniformly throughout the matrix (Lao et al., 2011).

Korsmeyer–Peppas model:

$$\frac{M_t}{M_\infty} = Kt^n$$

where M_t represents the amount of drug dissolved in time t, M_∞ is the total amount of drug dissolved when the pharmaceutical dosage form is consumed, K is a constant incorporating structural and geometric characteristics of the device and n is the diffusional exponent. The n value is used in order to characterize different drug release mechanisms. In the case of cylindrical samples, such as the samples studied, n = 0.45 indicates a Fickian release; values between 0.45 and 0.89 reveal an anomalous non-Fickian (diffusion/relaxation) drug release. Finally, when n is 0.89, a case-II (erosion/relaxation) drug release mechanism can be attributed (Costa et al., 2001; Siepmann and Peppas, 2001).

2.7. Release studies in progressive pH medium

A similar methodology to that used in demineralized water was employed but a progressive pH medium (1.5→4→6.8) was essayed as dissolution medium. The progressive medium was composed of an aqueous mixture of 0.05 M hydrochloric acid 37%, 0.05 M ortho-phosphoric acid 85% and 0.05 M glacial acetic acid with a final pH value of 1.5, which was maintained during the first one hour and half of assay. After this period, the required quantity of a 10 M NaOH solution was added to reach pH 4 which was maintained for one hour more. Finally, in order to reach a pH value of 6.8, the necessary amount of the alkaline solution above mentioned was added. These conditions were maintained until the test was finished. The quantity of drug dissolved was determined using a Beckman spectrophotometer DU-7 at a wavelength of 229 nm. Three replicates of each dissolution assay were carried out.

3. Results and discussion

3.1. Systems preparation

As it has been schematized in Fig. 1, two different strategies have been carried out to succeed preparing pectin containing hydrogels. On one side, the gelling capability of agarose allows to obtain stable and easy to handle hydrogels even immediately after being prepared, shortened in this work as "fresh" or "freshly-prepared". Alternatively, the lyophillization process allows to shape these types of hydrogels in the absence of agarose. As will be described below, the pectin-based samples suffer a progressive and complete dissolution when immersed in a hydrated medium while the presence of agarose ensures their stability.

3.2. Microstructure characterization

The microstructural characterization of the so obtained hydrogels is hindered by the massive presence of water that hides and camouflages many revealing evidences or, directly, prevents the use of some characterization techniques. The analysis of the freeze-dried materials is an inevitable indirect way of determining the microstructure of a hydrogel in the swollen state. The characterization of the crystalline state of the different components by X-ray diffraction and Thermogravimetric techniques is a footprint of the degree of dissolution of the hydrophobic drug used as a model in this work. The degree of solubility, which depends on the presence and nature of the surfactants, determine, together with the interaction of the drug with the rest of the components, the crystal growth within the hydrogel matrix. It must be taken into consideration the influence of the possible states of the drug (Kapoor et al., 2013; Kapoor et al., 2009), i.e., free form, inside micellar aggregates and adsorbed on the hydrogel, on its growth that is triggered when the water is eliminated during the freeze drying process. Additionally, the detection by different thermogravimetric techniques of new exo and endothermic transitions that are not detected in the individual components, indicates the generation of interactions between the different components: hydrogel matrix (agarose and pectin), surfactants and drug.

In the case of the freeze-dried materials, the possibility of directly characterizing the drug crystal size and the components "status quo" is of critical importance in order to explain the different phenomena that take place when water rehydrates these materials. In this sense, the use of Mercury Intrusion Porosimetry (MIP) allows to characterize the pore architecture that determines the fluid entrance within the desiccated hydrogels and their swelling behavior (Marras-Marquez et al., 2014). At this point, the partial rearrangement, due to hysteresis, of the components original "fresh" state determines the immediate drug dissolution and release from the matrix.

3.2.1. Drug crystallinity characterization

The examinations of the drug loaded materials by X-ray diffraction confirms the lower crystallinity of the tolbutamide included in any of the agarose or agarose–pectin systems. Fig. 2 shows how the most intense diffraction maxima of TLB are hard to

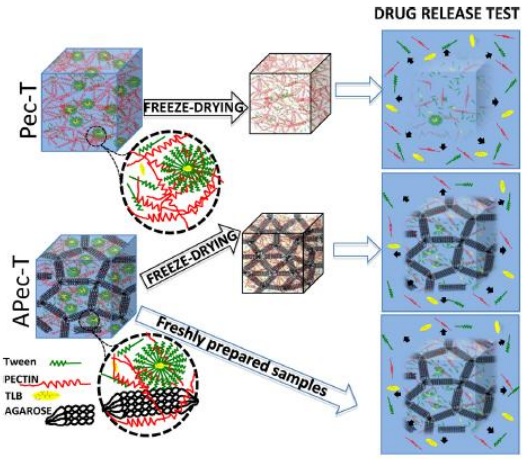

Fig. 1. Schematic representation of the (fresh) pectin and (fresh or freeze-dried) agarose–pectin based systems prepared before and after being subjected to the drug release test. Only Tween containing samples are represented to illustrate the surfactant effect.

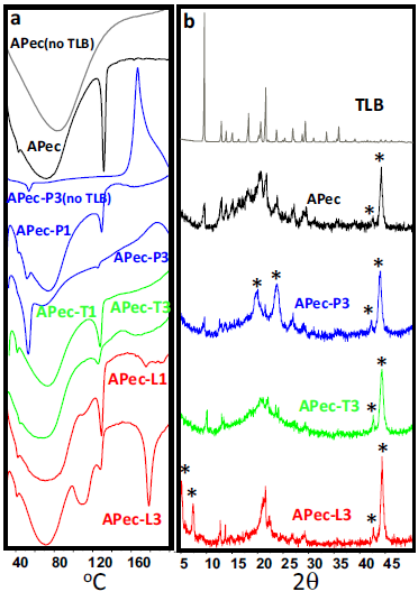

Fig. 2. (a) Differential scanning calorimetry and (b) X-ray diffraction of agarose–pectin based systems. *stands for the maxima attributable to the surfactant or the agarose–pectin matrix.

distinguish from the background attributable to the amorphous nature of agarose–pectin; consequently, no differences can be discerned between the crystallinity of the TLB introduced within the different samples. The different surfactants included further induce lower crystallinity as can be deduced from the almost complete disappearance of the most characteristic diffraction maxima of tolbutamide. In fact the most intense maxima that now appear in these diffraction patterns can be attributed to the surfactants or the matrix. The decrease on the drug crystal size has been corroborated by means of the DSC curves that show a decrease on the area of the endothermic peak at 120 °C attributable to tolbutamide melting (Fig. 2a). This decrease can be associated to a lower crystal size that requires less heat for this transition. A progressive diminution of this peak area can be observed for all the surfactants when their percentage is increased. The reduction of the crystal size when the drug is included in a hydrogel matrix can be explained considering that, during the freeze-drying process, the agarose or pectin chains limit and address the growth of the tolbutamide crystals thus rendering smaller ones. Furthermore, the presence of the surfactants exacerbates this decrease as a consequence of the enhanced solubility of this hydrophobic drug.

In order to show just an example of some of the new transitions that have been detected in binary or ternary samples, the curves of the non TLB containing agarose–pectin (APec) and the 3% Pluronic agarose–pectin (APec-P3) samples have been included in Fig 2a. It can be observed how the inclusion of Pluronic in the agarose–pectin matrix causes the generation of a strong exothermic peak around 165 °C. This transition shifts toward higher temperatures when tolbutamide is included. The thermo-analysis based characterization by TG-DTA reveals complex decomposition patterns at higher temperatures (not shown) that are not a mere addition of the individual component curves.

3.2.2. Pore architecture characterization

The examination of the pore size architecture generated as a consequence of water elimination during the freeze-drying process shows a honeycomb-like structure formed by pores whose width slightly varies as a function of the matrix-forming polysaccharide (Fig. 3a). The agarose–pectin lyophilized samples are characterized by a bimodal pore distribution between that of single agarose (130 μm) and single pectin (75 μm). The combined introduction of surfactants and tolbutamide induce changes on this microstructure, already described for agarose gels (Marras-Marquez et al., 2014), that depends on the nature and percentage of surfactant included. This different degree of alteration can be confirmed in Fig. 3b, where the presence of SLS induces a slight decrease on the pore size: from 50 (Pec-L1) to 32 μm (Pec-L3). The introduction of Pluronic induces the disappearance of this pore distribution and a less porous structure formed by smaller pores is created. In the case of Tween, its presence does not induce a decrease on the pore size, on the contrary, not only contributes to maintain the honeycomb pore architecture but also generates slightly bigger pores for the sample with the higher percentage (Pec-T3). A similar tendency was described for agarose-based matrices (Marras-Marquez et al., 2014) and is confirmed in agarose–pectin ones (Fig. 3c and d). SLS inclusion (Fig. 3c) does not alter the monomodal pore size distribution that slightly decreases with the surfactant percentage when compared to the respective matrices in the absence of the surfactant. However, when Pluronic is introduced at a 3% percentage the characteristic honeycomb pore architecture collapses yielding a denser microstructure with pores around 32 and 2 μm (Fig. 3d). The third surfactant used, Tween, causes (Fig. 3e), on blend systems, a similar effect yielding a less porous matrix with smaller pores (23 μm). The porosity decrease could be quantified to evolve from porosities over a 90% for samples without surfactants or in the presence of SLS to values around a 70% for those systems with the smallest pore sizes: APec-T3 and APec-P3.

These results agree with those obtained by Scanning Electron Microscopy, Fig. 4, that confirms the smaller pores of the honeycomb structure observed for pectin containing samples when compared to the equivalent agarose–pectin. Simultaneously, these materials show more defined channels as a consequence of the superior gelling capacity of agarose over pectin. As a consequence of this, the pore size decrease can be more clearly observed for agarose–pectin samples that show a clear evolution from APec-L3, in the same order of magnitude of agarose–pectin without surfactant (APec) toward the smaller channels observed for APec-T3 and APec-P3.

3.3. Swelling behavior

The progressive dissolution of the pectin hydrogel hampers a feasible measurement of weight variation; hence only systems containing agarose and pectin are discussed. As can be observed in Fig. 5, the introduction of the hydrophobic drug produces a noticeable decrease of the weight gain that is recovered in the presence of any of the surfactants tested, in fact, no remarkable differences have been observed in weight gain measured for these different systems. Despite this apparent similarity, different tendencies can be observed when comparing samples containing the same percentage of different surfactants. The test of the difference between two means contribute to analyze the scarce differences between the samples studied. The more significant

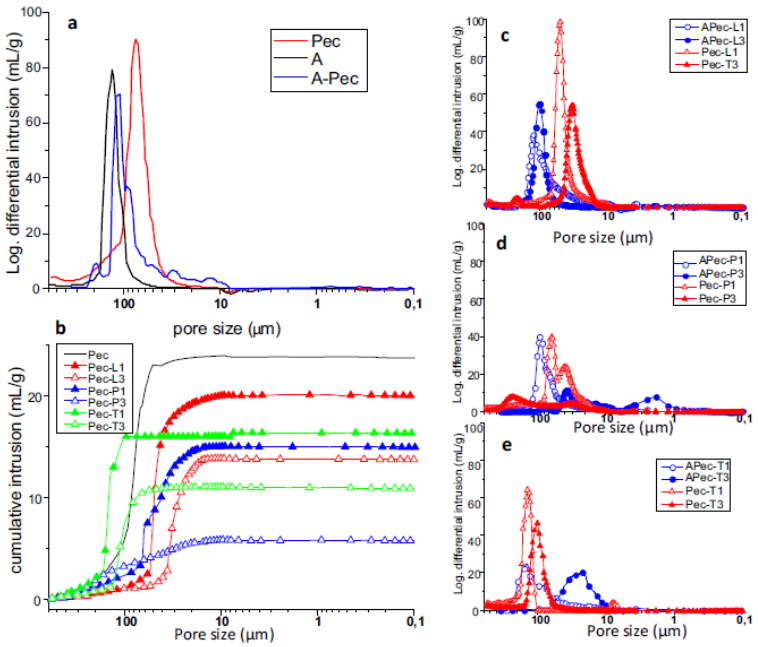

Fig. 3. (a) Pore size distribution of agarose (A), pectin (Pec) and agarose–pectin (APec) matrixes (b) Cumulative intrusion of pectin based systems, Pore size distribution of (c) L, (d) P and (e) T containing systems.

differences ($p < 0.01$) are found between the system without surfactant (APec) and the rest of the samples. At a minor extent ($p < 0.05$), some differences can be appreciated in systems containing a 3% of Pluronic or Tween. The surfactant effect on the water surface tension decrease seems to have a more critical role at low (1%) percentages as can be deduced from the slightly higher swelling degree observed for the material containing 1% of SLS (APec-L1); this anionic surfactant shows the highest hydrophilic–lipophilic balance (HLB) value among the surfactants essayed. Additionally, the structural modifications induced by the surfactants have a significant contribution to the swelling behavior as can be evidenced by the different tendency observed at higher percentages (3%). The higher weight gains experienced for APec-P3 and APec-T3 can be related to the above mentioned less porous architecture but also to a minor collapse of the hydrogel matrix after the freeze-drying, due to the presence of the micelles that act as spacers between the agarose chains that facilitates a better hydration and a minor swelling hysteresis.

Besides these phenomena, already described for single agarose systems (Marras-Marquez et al., 2014), it must be taken into consideration the disruptive effect of both Pluronic and Tween, and to a minor extent SLS, on the formation of consistent pectin gel. Taking all this into account it can be deduced that the swelling behavior of these systems are governed by sometimes complementary and others contradictory factors which explain the scarce differences in water gains among the studied systems.

3.4. Release characterization and kinetic fits in water

The drug release behavior from the different systems prepared has been essayed in two different media: water and pH progressive medium. The more standardized release in distilled water has been completed by fitting the so obtained data to determine the release kinetics in all the systems considered. On the other hand, considering the potential use of this type of materials in the treatment of different pathologies in the intestinal tract, a pH progressive medium has been essayed to test the tolbutamide release. Prior to these essays it was necessary to determine the solubility of this drug in solutions with these different pH values. Fig. 6 exhibits a pH-dependent solubility, i.e., higher solubility values are obtained for a pH over 5.4. This increase can be explained considering that the $pK_a = 5.3$ favors the ionized form of tolbutamide (Moffat et al., 2011).

The drug release characterization could only be carried out in freeze-dried pectin samples due to the lower gelling capacity of pectin (Liu et al., 2003; Thakur et al., 1997) that difficult the preparation of consistent pieces. In fact Pec-T3 and Pec-P3 systems show a liquid aspect and not the semisolid consistency observed in the rest of the samples; this can be attributed to the excessive amount of surfactant molecules and micellar aggregates that hinder the pectin chains arrangement. The drug release of TLB from freeze dried gels requires a previous rehydration process that implies several processes, retarding the drug release as can be

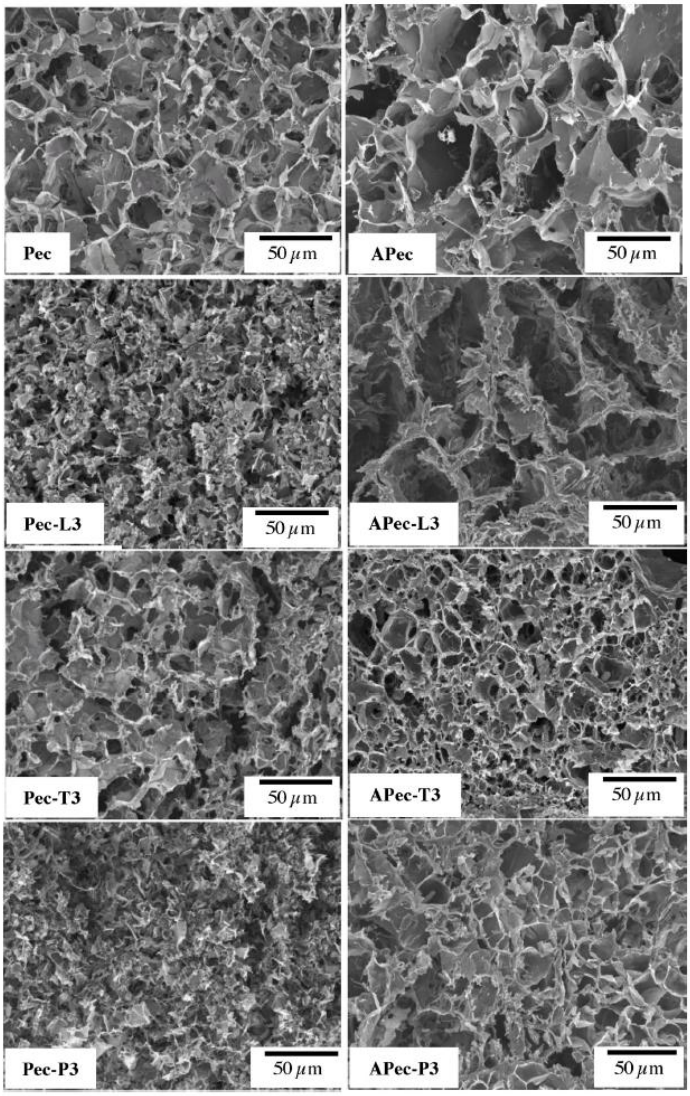

Fig. 4. SEM micrographs of freeze-dried pectin and blend systems.

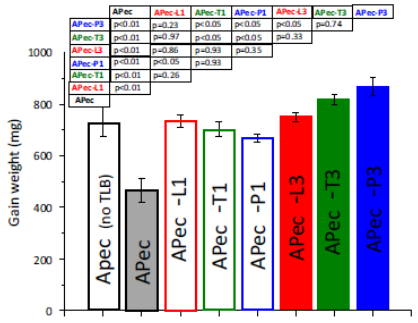

Fig. 5. Swelling behavior of the agarose blend systems expressed in terms of gain weights. Inset: Test of the difference between two means ($p < 0.05$ evidence significant differences).

observed in the Pec binary sample which shows an initial latency period of 48 min (Fig. 7a). The presence of surfactants minimizes this delay due, partially, to the diminution of the surface tension value of the water that causes the rehydration. At the same time surfactant parameters such as the Critical Micellar Concentration (CMC) or the molecular weight, the physical properties of the generated micelles and their interaction with the hydrogel backbone allows to explain the different release patterns observed. Additionally, Fig. 3b helps to understand the contribution of the pore architecture generated by freeze-drying to the drug release patterns. Despite the two compositions including a 3% of Pluronic (Pec-P3) or Tween (Pec-T3) that show an immediate and chaotic release due to a deficient three dimensional structure, a clear correlation can be established between the pore size and the percentage of drug released. The curves shown in Fig. 3b represent the direct determination of the pore sizes at which significant amounts of mercury enter within the lyophilized matrix and allow to obtain a pore size distribution. Even considering that mercury is completely different to water and that increasingly high pressures are necessary to intrude mercury within solids, this representation give us idea of how a fluid can penetrate in the studied matrices. However, although this factor may help to understand the initial steps of the rehydration process, the following phenomena must

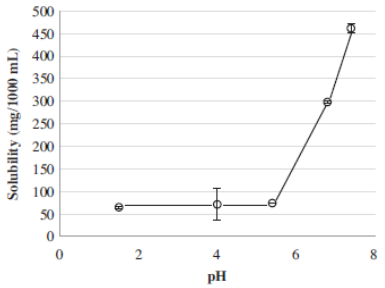

Fig. 6. Solubility of TLB in different aqueous buffers at 25 °C ± 0.5.

be considered in order to understand the rearrangement of the original "status quo" from the collapsed state and the simultaneous release of the drug:

i) The size and structure of the micelles originally formed, which actuates as a framework during the freeze drying and minimize, at a different extent, the hysteresis observed after the rehydratation.
ii) Surfactant molecules dissolution and diffusion through the hydrogel that depends on the surfactants molecular weight (Pluronic > Tween > SLS). The voids left by the surfactants molecules can be occupied by the dissolution medium thus facilitating the dissolution and diffusion of the drug.
iii) Besides these more "physical" phenomena, the chemical interaction of the drug in the different possible states: free, inside micellar aggregates and adsorbed on the hydrogel, with the rest of the components must be considered.

In the case of the blend matrices the introduction of agarose contributes to generate a more robust structure for all the different surfactants even at the highest percentages. In this sense, freeze-dried (Fig. 7b) or freshly (Fig. 7c) prepared agarose–pectin matrices can be subjected to the drug release test yielding jelly-consistent pieces that maintain their integrity. This stability in an aqueous medium is accompanied by a controlled drug release from both types of samples, slightly over the curve obtained in the absence of any surfactant. Agarose generates a more stable network capable of bearing the microstructural and chemical changes induced by the presence of the surfactants. In fact gels based only in agarose containing any of the surfactants considered in this work show tolbutamide release rates below those obtained in their absence (Marras-Marquez et al., 2014). As can be deduced from Fig. 7b and c the presence of pectin minimizes these effects while, at the same time, surfactants induce higher release rates. The systems containing 3% of surfactant are not only viable and able to withstand the different essays but at the same time show the highest release rates. The modification of the microstructure has a critical role on the rehydration process of freeze-dried samples. The higher porosity of APec-L3 facilitates the water entrance while samples characterized by a less porous architecture composed by smaller pores (APec-P3 and APec-T3) show much lower drug release rates. At the same time, the enhanced release rates observed with increasing surfactant percentages included reveal the importance of the surfactant properties and the so generated micelles such as the hydrophilic–lipophilic balance (HLB), molecular weight, micellar size . . . .

As has been observed the release from a freeze dried matrix depends on an initial process of rehydration, governed by the microstructure, but also on the subsequent drug dissolution and diffusion processes. In the case of freshly prepared samples the release is only directed by these processes which, in addition, take place unidirectionally under a more static environment, i.e., the drug in any of the possible states diffuses from the already highly hydrated hydrogel to the surrounding medium. The characterization of the TLB release kinetics confirms the fitting of the freeze-dried and freshly prepared materials to two different models: Korsmeyer–Peppas and Higuchi (Table 2, Fig. 7d–f). These models have been developed to explain drug release from solid (Korsmeyer–Peppas) or semisolid (Higuchi) polymeric systems (Costa et al., 2001; Siepmann and Peppas, 2011; Siepmann and Siepmann, 2008). The fitting of the freeze dried samples (Fig. 7d and e) to the Kosmeyer–Peppas model showing the highest regression coefficient value (R^2) and n values between 0.45 and 0.89 (Table 2), confirms that the release from these matrices is ruled by the relaxing and rearrangement processes that suffer the hydrogel chains during rehydration. This anomalous transport is

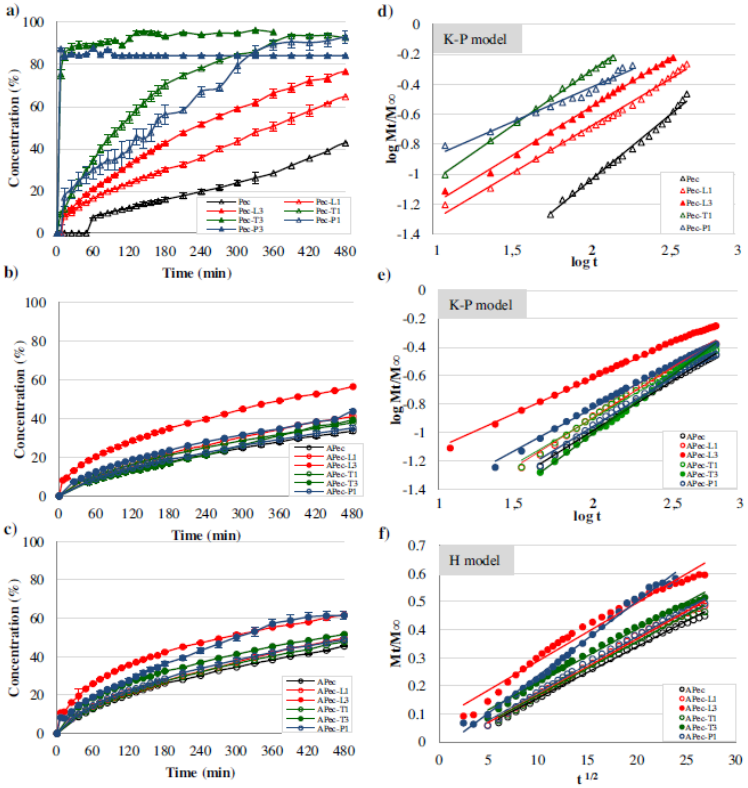

Fig. 7. Dissolution profiles of TLB from a) freeze-dried pectin (b) freeze-dried blend and (c) fresh blend systems in demineralized water. Corresponding Korsmeyer–Peppas and Higuchi models fitting (d–f).

commonly employed to describe the release from lyophilized formulations (Boateng et al., 2009; Labovitiadi et al., 2012); the initial superposition of different phenomena such as the massive fluid entrance and the progressive and partial restoration of the hydrated "status quo" difficult the explanation of the early drug delivery stages. After this initial step, dominated by a substantial swelling in which microstructure plays a critical role, a more static equilibrium is reached due to synchronization of swelling, drug diffusion through the hydrogel and dissolution. At this point erosion adds to the different factors that determine the drug delivery as has been observed for those systems containing pectin that progressively dissolves during the release tests. In fact the highest diffusional exponent (n) value observed for the material containing only pectin (PEC), 0.80, indicates certain resemblance to the case-II (erosion/relaxation) drug release mechanism described for values over 0.89. The presence of surfactants minimizes this effect as a consequence of the superior swelling rates derived from a more porous loose structure that facilitates the fluid entrance. Diffusion/relaxation drug release mechanism describe the kinetics of agarose–pectin blend systems as can be deduced from the n values, between 0.45 and 0.89, depicted in table 2. However the erosion contribution cannot be neglected due to presence of pectin that progressively dissolves together with the surfactant molecules; in fact, it contributes to explain the superior drug release values compared to systems containing only agarose (Marras-Marquez et al., 2014).

On other side, curves of fresh systems (Fig. 7f) can be fitted to the Higuchi model ($R^2 > 0.99$, Table 2), hence diffusion is the main release mechanism. As already mentioned, fresh systems are constituted by a highly hydrated network of agarose and pectin chains in the relaxed state. The drug diffuses more or less easily depending on its situation: free from, inside the micelles or entrapped within the polysaccharide chains. Consequently, the physic-chemical properties of the surfactants and those of the

Table 2
Release kinetics analysis in demineralized water of freeze-dried and fresh systems using Korsmeyer–Peppas and Higuchi models.

Freeze-dried systems	Korsmeyer–Peppas		
	R^2	K^a	n^b
Pec	0.9914	0.0021	0.8072
Pec-L1	0.9933	0.0128	0.5944
Pec-L3	0.9973	0.0156	0.6147
Pec-T1	0.9981	0.0180	0.7003
Pec-P1	0.9750	0.0475	0.4401
APec	0.9986	0.0047	0.6590
APec-L1	0.9954	0.0057	0.6632
APec-L3	0.9967	0.0266	0.4712
APec-T1	0.9939	0.0071	0.6144
APec-T3	0.9984	0.0026	0.7731
APec-P1	0.9952	0.0054	0.6447
APec-P3	0.9961	0.0103	0.5707

Fresh systems	Higuchi	
	R^2	K_H^c
APec	0.9961	0.0183
APec-L1	0.9962	0.0198
APec-L3	0.9749	0.0207
APec-T1	0.9950	0.0191
APec-T3	0.9924	0.0231
APec-P1	0.9960	0.0199
APec-P3	0.9949	0.0265

[a] Constant which takes into account the structural and geometric characteristics of the device.
[b] Diffusional exponent for drug release.
[c] Higuchi dissolution constant.

micelles have a predominant role on the drug release as can deduced from the higher release rates and Higuchi dissolution constant (k_H) values obtained for the systems containing the higher surfactants percentages (3%).

3.5. Release characterization in a pH progressive medium

Besides this characterization of the release behavior and kinetics in deionized water, the tolbutamide release from the different materials prepared was also essayed in a progressive pH medium. The pH condition of this medium has been designed to simulate the conditions that a drug formulation suffers when orally administered: 1.5 h in the gastric medium (pH 1.5), 1 h in the small intestine (pH 4) and under pH 6.8 until the essay finalization simulating the large intestine environment.

The study of the lyophilized pectin-based systems show (Fig. 8) that, in the presence of the different surfactants, a progressive increase of the tolbutamide release rates and diminution of the latency period as well as a minimization of the pH dependence imposed by the drug solubility is observed. In fact, when 1% of Tween is included (Pec-T1) an almost pH-independent behavior can be observed. Intermediate release curves with a different degree of pH-dependence can be designed by including different surfactants (Pluronic, SLS) at different percentages (Pec-L1, Pec-L3). In a similar way to what happens when Pec-P3 and Pec-T3 are essayed in water, these inconsistent systems release the included tolbutamide in the first 10 min.

The tougher agarose–pectin systems show less dependence on the surrounding pH. In the case of the freeze-dried system the inclusion of surfactants allows to reach higher TLB release rates and to minimize the pH dependence. In fact the APec-L3 system shows an almost linear release thus demonstrating how an appropriate formulation can minimize particular drug restraints. The essay of the freshly prepared systems show more pH dependent release curves, in any case not as extreme as those

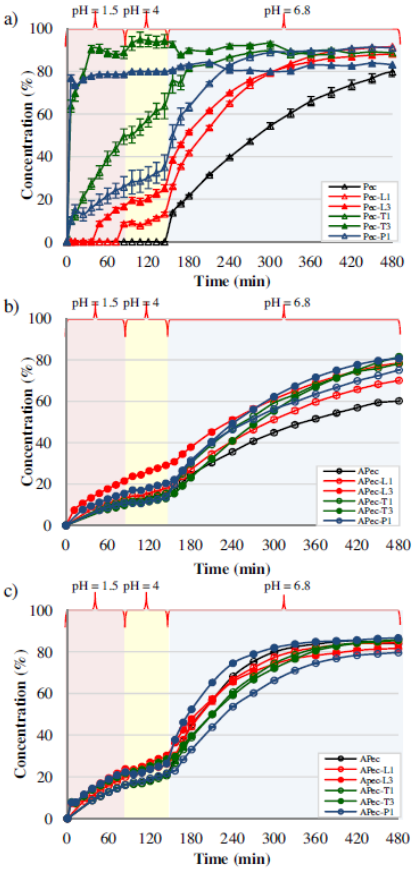

Fig. 8. Dissolution profiles of TLB from freeze-dried pectin (a), freeze-dried blend (c) and fresh blends systems (e) in pH progressive medium. Data are shown as means ± standard deviations ($n = 3$).

obtained from pectin, where the presence of the surfactants control the tolbutamide cession. The differences between freshly prepared and freeze-dried samples can be explained considering the different release kinetics that depend on diffusion or more complex mechanisms governed by the fluid penetration and the hydrogel chains relaxing and rearrangement processes, respectively.

4. Conclusions

- The versatility, i.e., the possibility of designing multicomponent, modular systems, has been demonstrated through the inclusion of surfactants with different nature at percentages up to a 3%

that form drug-loaded micelles within a hydrogel matrix composed by pectin or pectin–agarose.

- Despite the structural modifications induced by these surfactant agents, the hydrated systems, either directly obtained or soaked after being freeze dried, can be handled and manipulated throughout the release tests. In this sense, the jelly consistency of these materials, easier to swallow, may be an adequate solution for oral administration in elderly patients with dysphagia problems.
- The microstructural characterization shows that these substrates do not only facilitate the drug solubilization and ulterior release, but also determine the drug crystal size and pore architecture after the freeze drying process. This characterization is of critical importance taking into consideration the widespread use of the lyophillization technique to ensure the preservation and off-the-shelf utilization of highly-hydrated formulations such as the hydrogel-based tested in this work.
- The pectin-based formulations do not behave merely as drug carriers; they have been designed as a vehicle that interacts with the drug. This active role has been demonstrated through the progressively less pH-dependent release profiles of tolbutamide that can be tailored, as a function of the surfactants nature and percentage, for the same hydrophobic drug use as model.

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Artículo III

Agarose-based systems tailored for the controlled release of highly soluble drugs within a pH progressive medium

En fase de revisión en Pharmaceutical Research

Agarose-based systems tailored for the controlled release of highly soluble drugs within a pH progressive medium.

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RUNNING HEAD: Agarose systems for the controlled release of soluble drugs

ABBREVIATURES

DSC	Differential Scanning Calorimetry
DTA	Differential Thermal Analysis
GW	Gain of Weight
K_H	Higuchi dissolution constant
K_{KP}	Constant incorporating structural and geometric characteristics of the device
L	Labrafac
M_t	Amount of drug dissolved in time t
M_∞	Total amount of drug dissolved when the pharmaceutical dosage form is consumed
MIP	Mercury Intrusion Porosimetry
n	the diffusional exponent
S	Eudragit® S-100
SEM	Scanning Electron Microscopy
R	Eudragit® RL-PO
THE	Theophylline
Ws	weight of swollen system
W_f	weight of freeze-dried system
XRD	X-ray diffraction

INTRODUCTION

Different solid-state techniques are being used to improve solubility and optimize delivery of drugs with special bioavailability requirements, especially poorly soluble ones(1). Particle size reduction and, to a further extent, crystal engineering, even amorphization, are pursued by means of different techniques (milling, spray and freeze-drying, melt extrusion, wet granulation, roller compaction…), preparation routes (coprecipitation within polymers, desolvation of solvent and hydrates, aqueous film coating…), inclusion of different additives such as surfactants…(2-6).

In previous works (7, 8) these strategies were employed to enhance the solubility and control release of poorly aqueous soluble drugs in freeze-dried agarose based matrixes. The synergic effect of the use of surfactants, the overall integration together with the drug within the agarose network and the changes induced in the pore and molecular architecture allowed to create robust systems capable of solubilize, preserve and control release tolbutamide, a poorly soluble drug used a model.

However, a similar approach with highly soluble drugs did not yield a sustained release system(8). The lyophillization of such type of drugs within the polysaccharide matrix ensure their homogeneous distribution throughout the system and an intimate contact with the agarose chains that determine the crystal growth of the drug when the formulation is rehydrated. Unfortunately, despite the dispersion of the nano-sized drug crystals and their physical/chemical entrapment, its release in such a hydrated medium cannot be controlled. In order to design new agarose-based formulations capable of release highly soluble drugs in a sustained way, new type of additives are included in these systems: Labrafac, and two different Eudragit® compounds (S 100, RL PO).

The drug chosen as a model, theophylline, is a highly soluble methylxanthine alkaloid used in the treatment of asthma as a bronchodilator (9, 10). Due to its narrow

therapeutic index, it has received a considerable attention in sustained release formulations (11).

Labrafac, a mixture of caprylic and capric triglyceride, is a surfactant employed to enhance both oral bioability(12) as well as a cream penetration in topical applications(13). The low hydrophilic-lipophilic balance (HLB) value, 1, makes this substance an attractive candidate to retard the release of a highly soluble product.

Eudragit is the brand name for diverse polymethacrylate-based copolymers. Eudragit® S-100 is an anionic powder based on methacrylic acid and methyl methacrylate. It is insoluble in acid but soluble in intestinal medium from pH 7, providing gastroresistance to formulations (14, 15). For controlled release, it is effective and it forms stable enteric coating with a fast dissolution in the upper Bowl. Eudragit® RL PO is a polymethacrylate with a quaternary ammonium group branching out of their polymer backbone. The presence of this cationic group as chlorides salts and their dissociation in aqueous media is responsible for the swellability and permeability of these polymers. (14, 16). The drug release mechanism in based on the fluid penetration into the core and subsequent dissolution and outward diffusion of the active substance. It is indicated as a coating material for tablets, pellets and microspheres in pH independent dosage forms.

Agarose, the thermoreversible hydrogel that allows to shape robust multicomponent formulations, is a biocompatible polysaccharide constituted by (1→3)-β-D-galactopyranose-(1→4)-3,6-anhydro-α-L-galactopyranose units. (17). Among the different polysaccharides employed in the biomaterials and biotechnological world (18-20), agarose is gaining several applications (21) besides its traditional and extensive utilization in molecular biology separation techniques such as gel electrophoresis or gel filtration chromatography. Specifically, the gelling capacity of agarose has been applied to shape ceramic or metallic particles or fibres by gelcasting and similar techniques (22-

25). Besides it has been employed in diverse templation and micropatterning techniques to create stamps, arrays and microfluidic devices for biotechnological devices (26). In addition agarose is being employed in the biomaterials field as a matrix to regenerate a damaged tissue or forming part of a drug controlled release device. In this sense agarose has been considered as a potential candidate to regenerate different types of tissues, especially hard (bone and cartilage (27)), pancreas (28) and nervous system (29).

Freeze-drying is one of the most commonly used technologies that ensure a long-term conservation of raw materials for different uses (30). In pharmaceutical and biomedical fields, freeze-drying can be used in order to preserve unstable molecules by water removal, thus enabling their conservation and ulterior application after a rehydration process (31, 32). At the same time the characteristic honeycomb pore architecture formed by uniaxial parallel pores of around 100-200 μm, generated during the water extraction (33), facilitates the rehydration process. When this technique has been employed to shape scaffolds, this open porosity favors the colonization and vascularization. This porosity growth also induces an increase on the specific surface that can be employed to design drug delivery devices for nasal or gastric (34) tissues as well as for rapidly dissolving producing oral dosage forms or floating systems(35).

MATERIALS AND METHODS

Materials

Theophylline (Lot: 093K0122) and Agarose for routine use (Lot: 030M0150) with a sulphate content <15%, E.E.O. 0.09-0.13 a gel point at 36ºC and a gel strength >1200 (g/cm^3) were purchased from Sigma Chemical (St. Louis, MO, USA). Eudragit® S-100 (**S**) (Lot: B071005090) and Eudragit® RL-PO (**R**) (Lot: G120536083) were a gift from Evonik (Darmstand, Germany). Labrafac WL1349 (Lot: 450529011) was purchased

from Gattefossé (SAINT-PRIEST Cedex, Francia). All other reagents were of analytical grade. Water was purified with the Milli-Q reagent system (Millipore).

Preparation of freeze-dried hydrogels

In order to prepare the freeze-dried acrylic hydrogels, Theophylline (**THE**), previously sieved to a particle size < 100 μm as well as the different amounts of Labrafac (**L**) or Eudragit® (**R** or **S**) depicted in table I were dissolved/dispersed in demineralised water. Subsequently, agarose (**A**) was added into this solution/suspension and slowly heated at 90°C (± 0.1 °C) in a water bath under agitation until complete hydration of the agarose component. At this point, the resulting suspension was poured into PVC blisters (1 mL of capacity) and allowed to gel at room temperature for 24 hours. Finally, all the systems were freeze dried (Lia-bor) reaching a freezing temperature, a sublimation temperature and a sublimation pressure into a chamber of -45 °C, from -45 to 25°C and 4.54 x 10^{-4} atm, respectively.

In addition, a binary system (THE-A) was prepared using the same methodology. Table I summarizes the composition (mg/unit) and nomenclature of all systems prepared.

Systems characterization

The freeze-dried samples were analyzed by X-ray diffraction (XRD) in a Philips X-Pert MPD diffractometer with Bragg-Brentano geometry, operating with CuKα radiation (λ = 1.5406 Å) at 45 kV and 40 mA. The X-ray diffraction patterns were collected over the range between 4º and 40º 2θ with a step size of 0.02º 2θ and a counting time of 5 s per step. Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA) and Differential Thermal Analysis (DTA) were performed in a TA instruments TG/DTA

analyser, with 10°C/min heating ramps. An Hg intrusion porosimetry study was carried out using a Micromeritics AutoPore III 9410 porosimeter between 0.1 and 30,000 psi. Surface morphology was analyzed by scanning electron microscopy (SEM) in a JEOL 6400 Electron microscope.

Dissolution studies and mathematical fits

A Sotax AT-7 dissolution apparatus with paddles was employed to carry out all the dissolution studies. Demineralized water (1000 mL) was employed as dissolution medium at a temperature of 37± 0.5 °C. The stirring speed was 50 r.p.m. Samples containing 30 mg of THE were used for the dissolution studies. At determined time intervals, samples of the dissolution medium were extracted and filtered with a Whatman® filter paper (type 42). The quantity of drug dissolved was determined using a Beckman spectrophotometer DU-7 at a wavelength of 272 nm. Three replicates of each dissolution assay were carried out.

A progressive pH medium (1000 mL) (1.5→4→6.8) was employed as dissolution medium in order to simulate different parts of gastrointestinal tract. The progressive pH medium was composed of an aqueous mixture of 0.05 M hydrochloric acid 37%, 0.05 M ortho-phosphoric acid 85% and 0.05 M acetic acid glacial with a final pH value of 1.5, which was maintained during the first one hour and half of assay. After this time, a sufficient quantity of 10 M NaOH was added until pH reached a value of 4.0, which was maintained one hour more. Finally, in order to reach a value of pH = 6.8 enough quantity of 10 M of the NaOH solution was added. This condition was maintained until the test was finished. The treatment of the samples was the same that in demineralized water.

Dissolution data obtained were evaluated following the mathematical models proposed by Higuchi and Korsmeyer and Peppas. The Higuchi model was the first mathematical model to describe drug release from matrix systems postulated in 1961. In this model the amount of drug released is proportional to the square root of time and the drug release rate varies as the reciprocal of the square root of time (36). Higuchi describe drug release as a diffusion process based in the Fick's first law (37). On the other hand, Korsmeyer-Peppas model use release exponent calls "*n*" in order to characterize different release mechanisms. For cylinders, Fickian diffusion is represented for the exponent n=0.45, values of "*n*" between 0.45 and 0.89 can be regarded as an indicator for the superposition of diffusion and swelling-controlled drug release (anomalous transport or non-Fickian diffusion). Release of active substance depends simultaneously on the matrix swelling and diffusion phenomena. Finally, a release exponent n=0.89 can serve as an indication for case-II transport.

Swelling behaviour

Swelling of freeze-dried acrylic hydrogels was evaluated in terms of weight gain after the samples were maintained in pH progressive medium at 37 ± 0.5ºC. The samples, previously weighted, were introduced in a beaker that contained 500 mL of aqueous medium and were left in a shaking water bath at 42 oscillations per minute. At specific time intervals, the hydrogels were removed from the test aqueous medium blotted and carefully weighed. Similarly, to the dissolution assay this test was extended to a maxiumum of 8 hours.

The swelling behaviour results have been represented by means of the gain of weight of each system (GW). The GW has been calculated using the expression:

$$GW = W_s - W_f$$

where *Ws* is the weight of swollen system and *Wf* is the weight of freeze-dried system.

RESULTS AND DISCUSSION

Release characterization in water

The dissolution data obtained in demineralized water shown in figure 1 has been employed as a preliminary screening to check the capacity of the essayed formulations to modify the release pattern of the highly soluble model drug tested. The inclusion of theophylline within a polysaccharide matrix retards the release of such a water-soluble molecule; this can be attributed to the required hydration process of the freeze dried samples that allows the drug release from the reconstituted hydrogel to the surrounding medium. However, the different additives included to achieve a more sustained release do not alter significantly this release pattern. In fact some of them, A-L3, A-L12 and A-S3, show higher and faster release rates when compared with the biphasic sample (A). Bearing in mind these results, and taking into consideration the manipulation and fabrication difficulties derived from the use of the Labrafac additive, only the samples prepared with the Eudragit compounds were further characterized.

Release characterization and kinetic fits in a pH progressive medium

The Eudragit containing formulations were subjected to a release test in a progressive pH medium, a critical characterization considering their potential usage for oral administration. Figure 2 show two completely different release patterns that mainly depend on the type of additive included and to a minor extent on its percentage. Eudragit® S100 containing samples, despite its pH-dependent solubility (pH>7), show

at the high percentage (A-S12) a more sustained but very similar pattern to that obtained for the binary sample (A). The only hint of an enhanced solubility at pH>7 is given by a slight jump on the release curve in the transition between the pH 4,0 and 6,8 media. On the other side, those formulations containing Eudragit® RL-PO show, almost irrespectively on the percentage included, a much sustained drug release completely pH-independent. It has been estimated that the total drug release is prolonged in more than a 50%, that is, its takes around 12-14 hours, depending on the percentage, to fulfil the release.

In order to deepen in the characterization of the drug release mechanism from Eudragit® RL-PO systems, dissolution data were fitted to the mathematical models proposed by Higuchi and Korsmeyer and Peppas (fig. 3). In the Higuchi model, the amount of drug release is proportional to the square root of time and diffusion is the dominating process (36). Korsmeyer-Peppas model allows describing the drug release by means of two phenomena: the diffusion of water into the device and drug out of the system and the polymer swelling (38).

It can be deduced that the A-R3 and A-R12 systems release adjust both to the Higuchi and the Korsmeyer-Peppas models. The R^2 values: 0.9840 and 0.9986, respectively, indicate, that according to Higuchi, the release behavior is ruled by a diffusion process. At the same time, the n values observed (table II) for both systems: 0.4460 and 0.4201, respectively, points, as well, to a Fickian diffusion. Considering that this diffusion process takes place within media with different pH, it can be stated that the drug release from both systems can be described as sustained and pH-independent.

The explanation for these different release behaviors is not simple since it can not only be attributed to the particular properties of the additive included. The main features of these additives, pH-dependent solubility (S) and insolubility and high permeability (R)

have been extensively tested for its use in enteric formulations or tablets or particles coating, respectively, in which a continuous layer with a controlled and homogeneous thickness is assumed. However, it should be considered that the studied samples show a much complex structure in which the interaction of the acrylic component with the agarose matrix and the loaded drug must be clarified. The first question to be solved is related to the way that these Eudragit additives affect to the rehydration of the freeze-dried formulations. As it has been previously discussed (7, 8), the inclusion of additives may modify the honeycomb-like pore architecture generated during the lyophillization process thus affecting the initial massive water entrance. In no time, only a few minutes are required to recover the original rubbery texture and dimensions, the drug is released to the surrounding medium. At this stage, characterized by a more stable status quo within the highly hydrated hydrogel, the molecular interaction between the different components takes a predominant role and needs, as well, to be characterized.

Swelling behavior

All these agarose-based freeze-dried systems experience, when immersed in an aqueous medium, an immediate, within minutes, hydration process that allows to recover almost completely the original weight and dimensions. As can be observed in figure 4 the system without any type of additives shows a considerable weight uptake that slightly increases as a consequence of the test duration but not to the pH changes; the swelling can be described as a dynamic process that progressively takes place independently on the pH medium. The inclusion of the pH solubility dependent additive (S) at low percentages does only cause a slight decrease on the gain weights changes. However, higher quantities of this additive (A-S12) enhance the pH discriminatory effect as can be deduced by the decreasing gain weights. The photographs included in this figure

contribute to explain this swelling behavior: sample A-S12 suffers a considerable dissolution, almost erosion, due to the higher solubility at pH over 7. This evident, even with the naked eye, degradation cannot be detected at lower percentages (A-S3) or when the R additive is included (A-R3 or A-R12). Noticeably, these last formulations show much lower gain weights when compared with the rest of the formulations. The explanation for this limited swelling and the sustained release of theophylline is not evident and is faced considering the effect of the additive on both the pore distribution as well as on the molecular interaction of the drug with the agarose and Eudragit components.

Microstructure characterization

Previous works have demonstrated the necessity to characterize the pore architecture generated on agarose-based matrixes as a consequence of the freeze-drying process (7, 8). It has been established that the dimensions of these pores can be tailored as a function of the additive included. The resulting microstructure determines the rate and degree of rehydration that precedes the drug release from a progressively reconstituted highly hydrated matrix. Figure 5 combines the results obtained from two of the techniques used to determine the microstructure of these formulations. Scanning Electron Microscopy allows to confirm the honeycomb-like structure that characterize freeze-dried samples and that this microstructure partially degrades with increasing doses of the additive introduced. Both types of additives show a very similar morphology more dependent on the Eudragit percentage than on its nature. Taking this into account micrographs of only one of the two series are displayed in this figure. The second technique used, Mercury Intrusion porosimetry, not only confirms the evolution of the microstructure but also allows to quantify the pore size distribution. This type of

agarose-based hydrogels generate, when lyophilized, uniaxial pores of around 100 microns as can be observed in figure 5 for the biphasic material (A). The introduction of any of the additives at low percentages causes a slight evolution, more pronounced for A-S3, towards smaller pore sizes. Higher additive inclusion induces a much considerable change on the pore architecture now characterized by pores around 80μm and a much denser morphology as can be observed in figure 5.

Once considered the most relevant influence of the amount of additive when compared to its nature on the microstructure of the freeze-dried formulations, the role of these additives at a molecular level is analyzed. The interactions between the different components are characterized by FTIR and DSC; this last technique, together with XRD, allows to determine the cystallinity of the drug included. Figure 6 shows how the diffraction maxima of theophylline almost completely disappears, in fact only the most intense maxima (020, 120, 101) remain as signs of crystallinity within the amorphous background of the agarose matrix. This alteration on the drug crystallinity is confirmed through the DSC technique by the total disappearance of the fusion peak of theophylline around 275ºC ((10)) when this drug is introduced in the agarose matrix in any of the formulations prepared (figure 7). The loss of this endothermic peak indicates that no energy is required to melt the theophylline thus indicating a minimum crystal size. The reduction of the crystal size when the drug is included in a hydrogel matrix can be explained considering that, during the freeze-drying process, the agarose chains limit and address the growth of the theophylline crystals thus rendering smaller ones.

Besides the particular situation of the THE crystals, the analysis of the behavior of these samples with temperature variations allows to envisage possible interactions between the different components. Since the representation of all the different samples prepared would be impossible to fit within this article, figures 7 and 8 only depicts the results

obtained with the highest Eudragit percentage (A-S12, A-R12). Additionally these figures include the spectra of some samples subjected to the similar conditions to those set in the release test but, in this case, a sample was extracted, dried and studied after each pH variation. In order to facilitate the analysis of these multicomponent formulations inclusion, the thermographs of the single components have been included in figure 7 (DSC) as well as in figure 8 (FTIR). This last technique allows to test the progressive disappearance of THE during the release essay as can be deduced from the elimination of the most characteristic bands of theophylline (marked with arrows).

The thermograph of the A sample shows, as previously mentioned, no evidence of the endothermic peak of the THE fusion; on the contrary, an exothermic signal assignable to agarose contributes to completely hinder the minimum energy necessary to melt the nanometric drug crystals. The combination with any of the Eudragit additives modifies the curves at high temperatures as can be deduced by the generation of exothermic peaks that are related to their thermal decomposition. In addition, it must be remarked that while samples containing the R additive maintains the curve shape throughout the release experiment, those containing Eudragit S show some variations that can be related to the preferred solubility at pH>7 that this component imparts.

The absence of significant alterations on the FTIR spectra depicted in figure 8 in the form of the appearance of new bands or their shifting indicates that no evidences of chemical links could be established. These predominant physical interactions can be explained considering the neutral nature of the gelling component, agarose that limits ionic interactions with the quaternary ammonium groups of the Eudragit R such as that observed with the carboxyl groups of alginate, a similar polysaccharide hydrogel(39). At the same time the hydroxyl substituents of agarose restricts the formation of

hydrogen bonds with theophylline that could retard the release of the drug as has been observed when chitosan is employed as matrix(11).

Another point to be considered is the role of the quaternary ammonium groups, characteristic of the Eudragit® RL PO, in the much-sustained release of formulations containing this additive. Previous studies have demonstrated that the degree of swelling and related drug release of this component is a function of the chloride counterion interaction with the quaternary ammonium groups, i.e. the ion exchange has proved to be the responsible mechanism of controlling polymer permeability as a function of anionic species and concentration in the surrounding medium (16, 39, 40). The chloride concentration, essential in oral dosage forms, is critical for the design of controlled drug delivery systems based on the combination of Eudragit additives in coating or its mixture with polysaccharide hydrogels in a similar system to that considered in this work.

Despite the scarce chemical interactions between the different components, the swelling dependence of the Eudragit® RL PO on the varying release medium influences on its permeability to the aqueous solution that enters the matrix and allows the agarose chains to expand and partially recover the original status quo. Hence, the acrylic component acts as a filter that limits the liquid interaction with the hydrogel thus limiting its expansion and the substance migration between the highly hydrated matrix and the surrounding medium. This filtering effect does not affect to the pore architecture (figure 5) but critically determines, even at the lowest additive percentages, the swelling behavior and the drug release. The pore size reduction from 100 to 80 μm at high percentages is not a hindrance for the fluid migration during the rehydration process and the immediate drug release.

On the other side, the absence of these quaternary ammonium groups, whose percentage determines the permeability, in the Eudragit S 100 confirms this sieve effect. In this case the swelling and drug release is governed not only by the pH-dependent dissolution of the Eudragit additive, the drug release do not markedly increases at pH>7, but is mainly determined by the agarose behavior. In this particular case the interaction of this acrylic component with the hydrogel chains practically do not limit their rearrangement and expansion having a scarce influence on the drug release.

CONCLUSIONS

It has been demonstrated that among the additives essayed the inclusion of Eudragit RL-PO, even at the lowest amounts essayed, within an agarose-based hydrogel system ensures the sustained release of theophylline, the highly soluble drug used as a model. A pH non-dependent control release was verified in a pH progressive essay that simulates an oral dosage administration. The presence of this additive does not significantly affect to the pore architecture but does has a marked influence on the swelling behaviour due to a sieving effect during the agarose network rehydration.

The potential applications of these agarose-based systems capable of accommodating different type of additives that ensures the sustained release of a particular drug with special restraints has been extended to highly soluble drugs. At the same time the combination of the freeze-drying preservation technique with the crystallization within these polysaccharide hydrogel matrix may be of interest for the crystal engineering and preservation required by the nature of certain drugs thus expanding their potential application and bioavailability.

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Figure captions

Figure 1. Theophylline release from freeze-dried ternary systems in demineralized water. Samples including 3% (a) and 12 % (b) of additives.

Figure 2. Theophylline release from freeze-dried ternary systems in pH progressive medium. Samples including Eudragit®S100 (a) and RL PO (b).

Figure 3. Mathematical models used to fit the dissolution profile of THE in pH progressive medium.

Figure 4. Swelling behaviour of binary (A) and Eudragit-containing ternary systems in a pH progressive medium.

Figure 5. Pore size distribution of binary (A) and Eudragit-containing ternary systems. Inset: Micrographs of A, A-S3 and A-S12 samples

Figure 6. X-ray diffract patterns of binary (A) and Eudragit-containing ternary systems.

Figure 7. FTIR spectra of single components (Agarose, Theophylline), binary (A), A-R12 (left) and A-S12 (right) ternary systems. Below are included the spectra of the corresponding samples subjected to the release test within the pH progressive medium.

Figure 8. DSC curves of single components (Agarose, Theophylline), binary (A), A-R12 (left) and A-S12 (right) ternary systems. Below are included the curves of the corresponding samples subjected to the release test within the pH progressive medium.

Table I. Composition (mg/unit) of the binary and ternary freeze-dried hydrogels

	Labrafac WL 1349 (mg)	Eudragit® S100 (mg)	Eudragit® RL PO (mg)	Theophylline (mg)	Agarose (mg)
A	-	-	-	10	30
A-L3	30	-	-	10	30
A-L12	120	-	-	10	30
A-S3	-	30	-	10	30
A-S12	-	120	-	10	30
A-R3	-	-	30	10	30
A-R12	-	-	120	10	30

Table II. Parameters obtained using Higuchi and Korsmeyer-Peppas model

Mathemathical model	Formula	A-R3	A-R12
Higuchi	$M_t/M_\infty = K_H \sqrt{t}$	$K_H = 0.0293$ $R^2 = 0.9840$	$K_H = 0.0283$ $R^2 = 0.9986$
Korsmeyer-Peppas	$M_t/M_\infty = K_{KP} t^n$	$n = 0.4460$ $K_{KP} = 0.0407$ $R^2 = 0.9811$	$n = 0.4201$ $K_{KP} = 0.0508$ $R^2 = 0.9991$

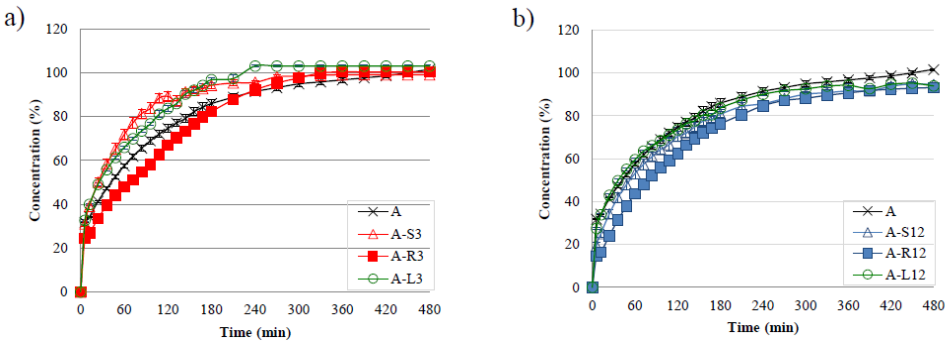

Figure 1.

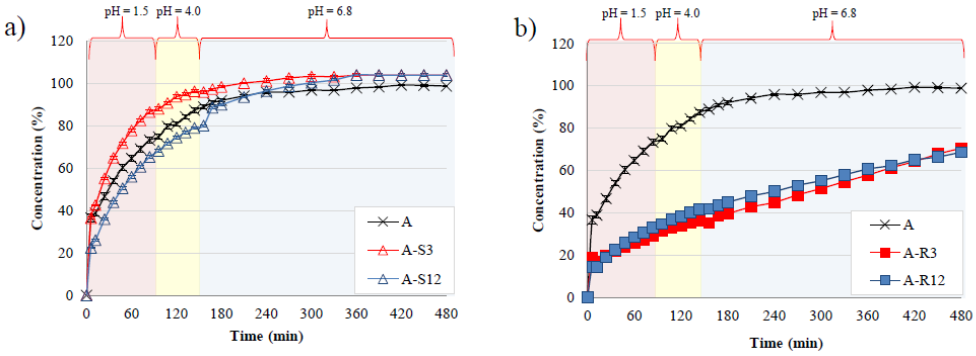

Figure 2.

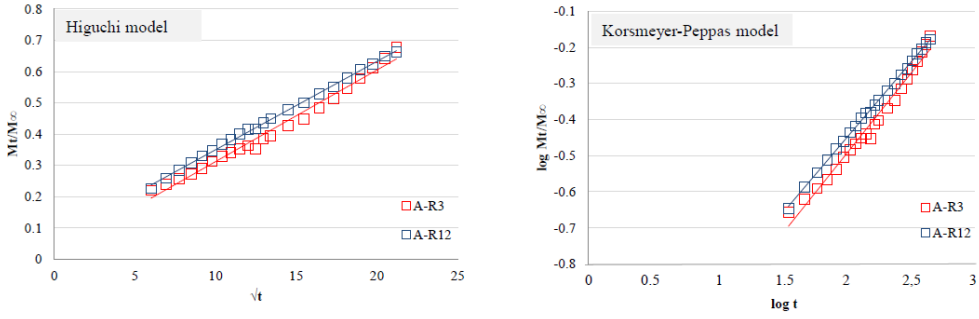

Figure 3..

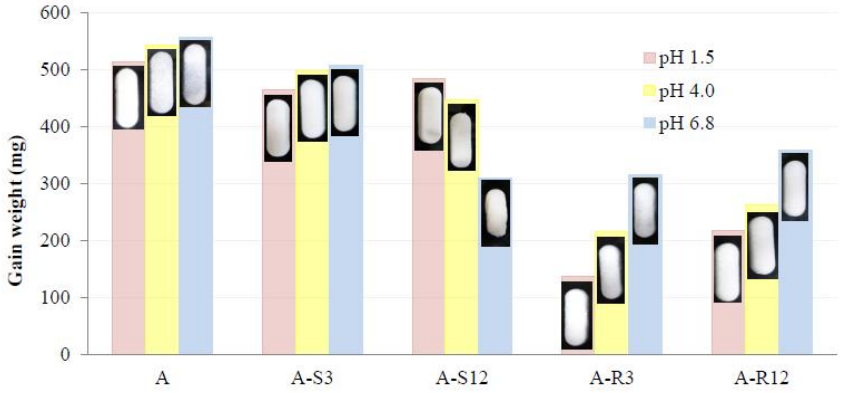

Figure 4.

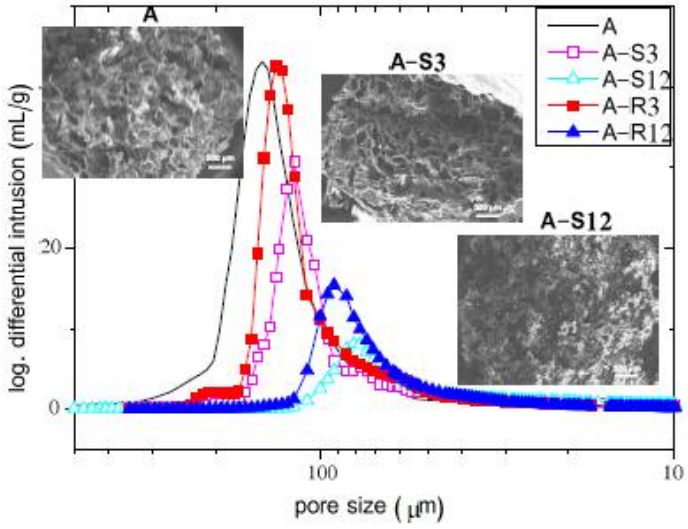

Figure 5.

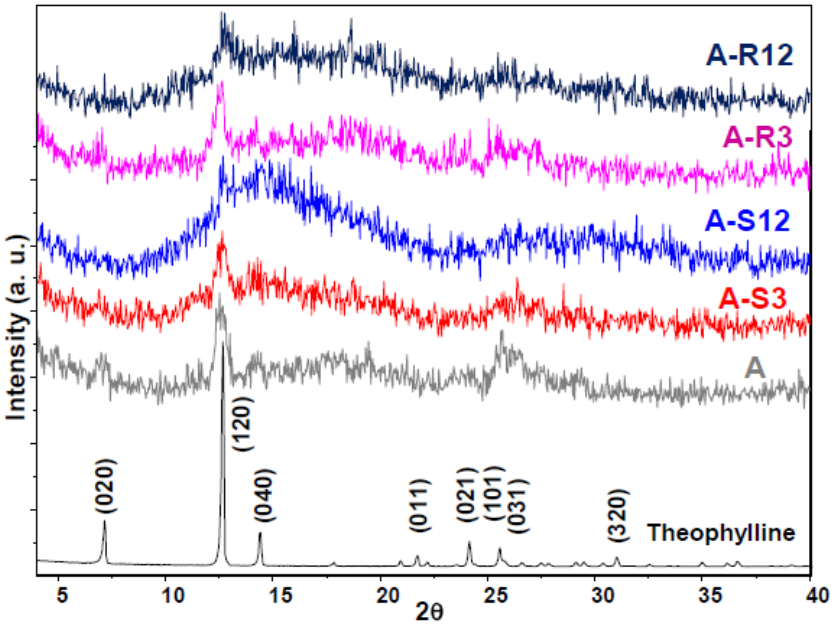

Figure 6.

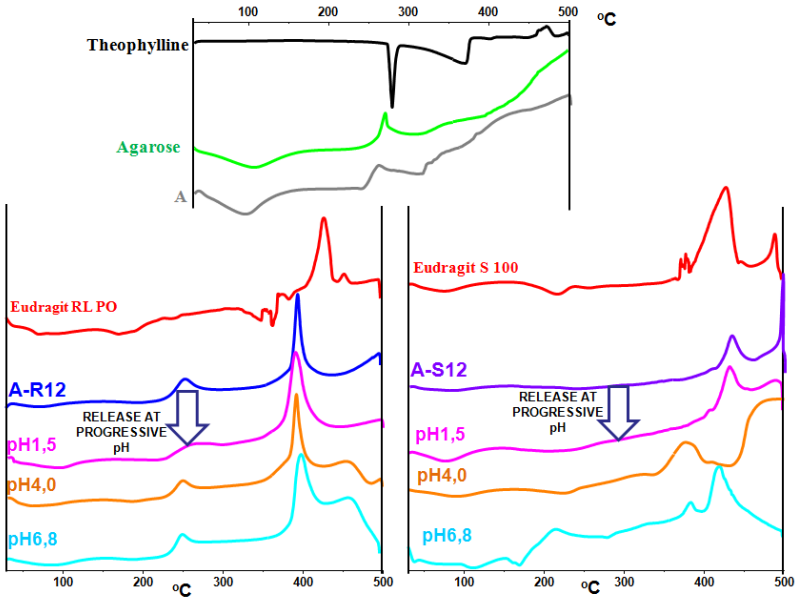

Figure 7.

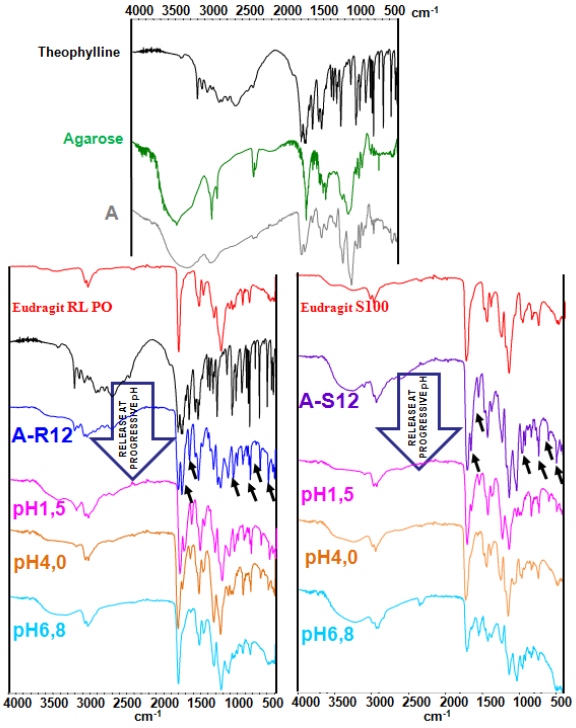
4000 3500 3000 2500 2000 1500 1000 500 cm-1
Theophylline
Agarose
A
Eudragit RL PO
A-R12
RELEASE AT PROGRESSIVE pH
pH1,5
pH4,0
pH6,8
Eudragit S100
A-S12
RELEASE AT PROGRESSIVE pH
pH1,5
pH4,0
pH6,8
4000 3500 3000 2500 2000 1500 1000 500 cm-1
4000 3500 3000 2500 2000 1500 1000 500 cm-1

Figure 8.

DISCUSIÓN INTEGRADORA

La Real Farmacopea Española describe las formas farmacéuticas de liberación modificada como aquellas preparaciones en las que la velocidad y el lugar de liberación de la sustancia o sustancias activas, es diferente del de la forma farmacéutica de liberación convencional administrada por la misma vía. Esta modificación deliberada se consigue por una formulación particular o por un método de fabricación especial.

Las formas farmacéuticas de liberación modificada presentan numerosas ventajas con respecto a las formas farmacéuticas convencionales, entre las que destacan:

- Mejor cumplimiento del tratamiento por parte del paciente gracias a una administración más fácil, más adecuada, menos frecuente.
- Reducción de los efectos secundarios relacionados con dosis elevadas, es decir, incremento en la seguridad del fármaco al emplearse, en muchos casos, dosis menores y menor distribución del fármaco hacia órganos o tejidos no involucrados en la respuesta farmacológica y reducción de la formación de metabolitos tóxicos.
- Disminución de la fluctuación de niveles plasmáticos al tener la posibilidad de conseguir la velocidad de liberación más adecuada al lugar y al mecanismo de acción del fármaco.
- Efecto terapéutico más uniforme.
- Disminución de trastornos asociados con la intolerancia gástrica cuando se recurre a la administración oral.

Dentro de las diferentes vías de administración de medicamentos, la vía oral sigue siendo la más utilizada, por ello goza de la mayor concentración de esfuerzos investigadores para hallar nuevas formas farmacéuticas de liberación modificada en el tracto gastrointestinal.

Los polímeros de origen natural biocompatibles son objeto de investigación para el desarrollo de formulaciones de liberación modificada, como se puede comprobar al hacer una búsqueda bibliográfica en las bases de datos de ciencias biomédicas. La agarosa ha demostrado ser, tal y como se recoge en la introducción de esta tesis, un vehículo muy versátil utilizándose con éxito en diferentes campos, como soporte para cultivos celulares, como biomaterial formando matrices que favorezcan la regeneración de tejidos dañados y cómo soporte para sistemas de liberación controlada de fármacos, por su capacidad de captar medio acuoso formando un hidrogel. Dicho hidrogel es capaz de modular la liberación del fármaco que alberga en función de la estructura del hidrogel, naturaleza del fármaco, naturaleza del medio en el que se encuentre, etc.
Con estos antecedentes y de acuerdo con el objetivo de esta tesis se han desarrollado hidrogeles basados en agarosa como vehículos para modular la liberación de fármacos. Los fármacos modelos incluidos en los sistemas de la tesis han sido tolbutamida y teofilina. La tolbutamida se ha seleccionado por su solubilidad pH-dependiente y la teofilina como fármaco modelo hidrosoluble.
Los sistemas basados en agarosa se han desarrollado cronológicamente incorporando diferentes aditivos para estudiar la influencia de los mismos en los perfiles de liberación de los fármacos incluidos.
El objetivo del primer artículo que constituye esta tesis "*Agarose drug delivery systems upgraded by surfactants inclusión: Critical role of the pore architecture*" fue la elaboración de sistemas basados en agarosa para conseguir formas de liberación controlada de fármacos. Con este objetivo se elaboraron hidrogeles de agarosa a los cuales se

incorporaron tensoactivos con diferentes características fisicoquímicas: lauril sulfato sódico, tween® 80 y pluronic® F-68 para determinar su influencia en los perfiles de cesión de fármacos de distinta solubilidad: teofilina y tolbutamida. La caracterización microestructural realizada a los sistemas binarios y ternarios liofilizados utilizando diversas técnicas analíticas permitió conocer cómo la arquitectura de los sistemas actúa en el comportamiento de cesión de los fármacos incluidos.

Así, mediante difracción de rayos X se observa una disminución de la cristalinidad de ambos fármacos tanto en los sistemas binarios (fármaco-agarosa) como en los sistemas ternarios (fármaco-agarosa-tensoactivo). En los sistemas binarios (teofilina-agarosa y tolbutamida-agarosa), se observa la influencia de la agarosa sobre el crecimiento y dirección de los cristales de teofilina y tolbutamida dentro de las cadenas de agarosa. Comparando los difractogramas del sistema THE-A (teofilina/agarosa) con el sistema TLB-A (tolbutamida/agarosa) se observa una baja cristalinidad del fármaco teofilina debido a su alta solubilidad en medios acuosos. Asimismo, en los sistemas sin agarosa teofilina-tensoactivo y tolbutamida-tensoactivo se observa que la orientación y crecimiento de los cristales de fármaco se encuentran influenciados por el tipo de tensoactivo presente en cada sistema.

La interacción entre los componentes que constituyen los sistemas binarios y ternarios, así como el estado cristalino de los fármacos incluidos en los sistemas ternarios fueron estudiados mediante análisis termogravimetrico, análisis térmico diferencial (TG/DTA) y microscopía de platina caliente (HSM). El análisis térmico de los sistemas ternarios de tolbutamida permite definir la acción del tensoactivo en estos

sistemas. La desaparición del pico correspondiente al activo en los termogramas demuestra la acción solubilizante de las micelas formadas en el hidrogel fresco. Asimismo, mediante microscopía de platina caliente se observa la disolución de los cristales de tolbutamida lo que confirma la acción solubilizante de los tensoactivos.

Las técnicas de análisis térmico, termogravimetría, análisis térmico diferencial y microscopía de platina caliente permiten conocer el comportamiento de los materiales al someterlos a un programa preestablecido de calentamiento, detectando las interacciones que se producen entre sus componentes, así como el estado cristalino de los fármacos incluidos. Los característicos picos endotérmicos de fusión de los fármacos tolbutamida y teofilina desaparecen al incorporar cada uno de ellos a los sistemas con agarosa. Similar comportamiento se detecta en sistemas con tensoactivo. Por microscopía de platina caliente se confirma la desaparición de los picos de fusión de los fármacos, debido a la disolución de los microcristales de los mismos en la agarosa fundida. Estos estudios revelan la interacción que se produce entre los componentes.

Los estudios de porosimetría de los sistemas de agarosa muestran una distribución porosa monomodal compuesta por poros con tamaños que van de 100 a 200 µm. La adición de un segundo componente: fármaco o tensoactivo, no altera la microestructura formada durante el proceso de liofilización. Sin embargo, la inclusión simultánea de fármaco y tensoactivo conlleva modificaciones de la microestructura. Los sistemas formados por tolbutamida y lauril sulfato sódico, presentan una disminución en el tamaño de poro que es independiente de la concentración de tensoactivo presente en el sistema. Un

comportamiento diferente se observa cuando el tensoactivo es tween® 80 o pluronic® F-68, donde una concentración del 3% produce la desaparición completa o parcial, de la estructura característica de la agarosa. Los sistemas con tween® 80 presentan una distribución trimodal con poros de tamaño 2-5, 70-120 y 100-200 µm. Los sistemas con pluronic® F-68, presentan una distribución bimodal, con poros de tamaño 2-5 y 70-200 µm. Las curvas de intrusión de mercurio contribuyeron a demostrar las diferencias de porosidad entre los sistemas T3-TLB-A y P3-TLB-A (tween® 80 3%/tolbutamida/agarosa y pluronic® F-68 3%/tolbutamida/agarosa), los cuales presentan un bajo grado de intrusión de mercurio relacionado con la distribución de tamaños de poro muy pequeños que influyen en el porcentaje de porosidad.

Por otro lado, la caracterización por microscopía óptica permite confirmar la estructura de la agarosa y la transformación de los poros paralelos uniaxiales que van de 100 a 200 µm a una superficie densa observada en los sistemas ternarios.

A través de los ensayos de hinchamiento, se demuestra la influencia que tiene la naturaleza del fármaco presente en el sistema. Así, los sistemas binarios con tolbutamida, a diferencia de los sistemas con teofilina, no absorben la misma cantidad de agua que los sistemas de agarosa sola. Del mismo modo, la presencia de un tensoactivo aumenta la captación de agua gracias a la disminución de la tensión superficial del agua.

Los sistemas binarios con tween® 80 presentan valores de hinchamiento por encima de los obtenidos con los otros tensoactivos, lo cual indica la facilidad de este agente para generar micelas a bajas concentraciones. La presencia de

micelas en los hidrogeles frescos de tween® 80 evita el colapso del sistema en el proceso de liofilización.

En el caso de los sistemas ternarios con tolbutamida, se observa que la presencia de moléculas de fármaco y tensoactivo entre las cadenas de agarosa permite un aumento en el grado de hinchamiento respecto a los sistemas binarios agarosa-tolbutamida, a pesar de no alcanzar una recuperación del peso inicial. Por otro lado, la presencia simultánea de teofilina y tensoactivos permite una mejor captación de agua gracias a la acción sinérgica entre la hidrosolubilidad del fármaco y la disminución de la tensión superficial del agua producida por la presencia del tensoactivo. En estos sistemas los valores de hinchamiento superan los valores obtenidos por los sistemas de agarosa sola.

Los resultados obtenidos en los estudios de cesión de los sistemas binarios y ternarios aparecen condicionados por la naturaleza del fármaco y del tensoactivo. La liberación de teofilina en medio acuoso a partir del hidrogel formulado sólo con agarosa (teofilina/agarosa) es sostenida en el tiempo. Este comportamiento es atribuido a dos procesos diferenciados que suceden cuando la matriz de agarosa entra en contacto con medios acuosos: disolución de los cristales de fármaco y difusión de fármaco disuelto a través del hidrogel sometido a procesos de rehidratación y reconstrucción.

En los sistemas ternarios con teofilina, la cesión de este fármaco hidrosoluble se encuentra condicionada por la naturaleza del tensoactivo, peso molecular y HLB. Así la presencia de lauril sulfato sódico en estos sistemas facilita la liberación más que cuando los tensoactivos son tween® 80 o pluronic® F-68 (ambos con menor valor de HLB).

Los perfiles de cesión de los sistemas ternarios con tolbutamida presentan un comportamiento completamente diferente al obtenido con los sistemas ternarios con teofilina. La concentración de 1% de tensoactivo en los sistemas ternarios (lauril sulfato sódico 1%/ tolbutamida/agarosa, tween® 80 1%/tolbutamida-agarosa, pluronic® F-68 1%/tolbutamida/agarosa) no es suficiente para modificar el perfil de liberación de tolbutamida, siendo similar al observado con el sistema binario (tolbutamida/agarosa). En los sistemas ternarios con un 3% de lauril sulfato sódico (lauril sulfato sódico 3%/tolbutamida/agarosa), se observa que la presencia del tensoactivo incrementa la disolución de tolbutamida. Sin embargo, cuando el tensoactivo es tween® 80 o pluronic® F-68 (tween® 80 3%/tolbutamida/agarosa y pluronic® F-68 3%/tolbutamida/agarosa) se obtienen perfiles de liberación inesperados que se encuentran por debajo de los obtenidos con el sistema binario (tolbutamida/agarosa).

Este comportamiento, se debe a la modificación estructural observada en estos sistemas, pues la baja porosidad hace que se alteren los procesos de rehidratación de la matriz de agarosa. Por otro lado, sólo las moléculas del fármaco que se encuentran libres y/o absorbidas en la matriz de agarosa se disolverán más fácilmente que aquellas unidas al centro hidrófobo del tensoactivo, las cuales generan un depósito que prolonga la cesión. Asimismo, la disolución de estos depósitos fármaco-tensoactivo depende de la naturaleza y peso molecular del tensoactivo que condicionará la migración de moléculas de tensoactivo hacia el exterior del hidrogel y disminuirá la velocidad de disolución/difusión de tolbutamida.

Los resultados obtenidos permiten concluir que la incorporación de un tensoactivo en la matriz del hidrogel de agarosa modifica profundamente la disposición final de los componentes del sistema. La arquitectura de poros de los hidrogeles liofilizados está afectada por la naturaleza y cantidad de tensoactivo presente en cada sistema, así como por la presencia de sus micelas. Esta profunda modificación estructural es la responsable de los perfiles de liberación de los fármacos, siendo más acusado en el caso de tolbutamida por su menor solubilidad en medio acuoso.

A partir del conocimiento sobre las modificaciones estructurales que se presentan en las matrices de hidrogeles de agarosa cuando se incorporan tensoactivos, se decidieron formular geles mixtos compuestos por agarosa y pectina por su diferente capacidad de gelificación, ya que los geles de agarosa son rígidos, pero los de pectina son muy flexibles y fácilmente solubles en medio acuoso.

El objetivo de este trabajo es desarrollar formulaciones para administración oral con diversos componentes capaces de modular la liberación de fármacos con solubilidad pH-dependiente. Estos sistemas deben ser lo suficientemente resistentes para no verse afectados por las peculiaridades del entorno a lo largo del tracto digestivo. Los resultados de esta investigación se encuentran descritos en el artículo "*Robust and versatile pectin-based drug delivery systems*".

Para ello se prepararon sistemas que contenían sólo pectina, como agente gelificante, o una mezcla agarosa-pectina, además de fármaco (tolbutamida). De forma paralela se desarrollaron sistemas que contenían además un agente tensoactivo, tween® 80, Pluronic® F-68 o laurilsulfato sódico en dos proporciones, 1 y 3%. Se prepararon hidrogeles frescos y liofilizados. El

proceso de preparación de los hidrogeles sirvió como un primer paso para el conocimiento de las interacciones que se producen entre los componentes de algunos sistemas. Así los hidrogeles que contenían pectina cómo único agente gelificante y tween o pluronic al 3 % no presentaban una consistencia adecuada, lo que impedía su manejabilidad. El proceso de liofilización permitió su caracterización igual que el resto de sistemas con pectina y todos los sistemas desarrollados con mezcla agarosa-pectina. Los estudios de difracción de rayos X realizados sobre los sistemas muestran que la tolbutamida incluida en hidrogeles de pectina o mezcla agarosa-pectina ha disminuido su grado de cristalinidad, observándose espectros de difracción de rayos X en los que es difícil detectar los picos característicos de tolbutamida. Esta disminución en la cristalinidad de la tolbutamida cuando el fármaco se incluye en la matriz del hidrogel se atribuye a que durante el proceso de liofilización las cadenas de agarosa y/o pectina limitan y dirigen el crecimiento de los cristales de fármaco, originando un menor tamaño de cristal. Cuando en el sistema existen además tensoactivos, éstos exacerban la amorfización de la tolbutamida, debido al poder solubilizante de sus micelas. Las curvas de DSC permiten apreciar el pico de fusión de tolbutamida en los sistemas que solo contienen pectina o mezcla agarosa/pectina, mientras que al incluir un agente tensoactivo desaparece el fenómeno endotérmico del fármaco debido al poder solubilizante del tensoactivo a temperaturas inferiores a la de fusión de tolbutamida. La evaluación de la porosidad originada en los hidrogeles durante el proceso de liofilización muestra que el tamaño de los poros es función del polisacárido que constituye cada muestra. Así en la muestras de la mezcla agarosa-pectina se diferencian los poros

de agarosa, que presentan un tamaño del orden de 130 μm, de los de pectina de 75 μm aproximadamente. Pero la incorporación a estas matrices de tensoactivo produce una modificación estructural que se cuantifica por la modificación en el tamaño de los poros. Esta modificación estructural coincide con la observada en los sistemas desarrollados en el artículo anteriormente discutido "*Agarose drug delivery systems upgraded by surfactants inclusión: Critical role of the pore architecture"*. Es decir que no sólo los tensoactivos tienen capacidad para modificar la porosidad de los hidrogeles de agarosa, sino que también lo hacen en hidrogeles mixtos como los de agarosa-pectina. La capacidad para modificar la porosidad depende tanto de la naturaleza como de la proporción de tensoactivo en el sistema. Así el lauril sulfato sódico no modifica sensiblemente el tamaño de poros ni en los sistemas de pectina ni en los de la mezcla agarosa/pectina si está al 1%, pero si en los sistemas la proporción del tensoactivo es del 3% se disminuye el tamaño de los poros, atribuible a que el elevado número de micelas disminuye el colapso del sistema durante la liofilización. La influencia de la incorporación de pluronic® F-68 o tween® 80 en la porosidad de los sistemas es diferente según la naturaleza del polisacárido gelificante. Por ello, en los sistemas agarosa/pectina la presencia de pluronic al 3% disminuye drásticamente el tamaño de los poros, lo mismo que en el caso de tween® 80. Pero en los sistemas de pectina, la incorporación de pluronic® F-68 o tween® 80 al 3% origina sistemas con poca porosidad, y los poros son de pequeño tamaño. Estos datos corresponderían a sistemas poco estructurados, característica que se observó en los sistemas antes de su liofilización, es decir presentaban dificultad para manejarlos. La presencia de ambos tensoactivos en una

proporción del 3% (elevado número de micelas) impide la conformación espacial de las cadenas de pectina durante su hidratación, lo que se traduce en ausencia de gelificación. La observación de las muestras por microscopía electrónica de barrido corrobora los datos obtenidos por porosimetría. Los poros de los sistemas con pectina se ven de menor tamaño que los de agarosa/pectina. En estos últimos se observan canales más definidos, debido a la mayor capacidad gelificante de agarosa respecto de la pectina. Las fotografías muestran una clara disminución en el tamaño de los poros al incorporar lauril sulfato sódico, pluronic® F-68 o tween® 80 tanto en los sistemas de agarosa/pectina como en los de pectina sola. Así mismo, se aprecia la alteración en la estructura de los poros detectada por porosimetría.

En los ensayos de hinchamiento, si se compara el sistema blanco (agarosa/pectina) con el sistema que incorpora tolbutamida (agarosa/pectina/tolbutamida) se observa que la incorporación de un fármaco hidrófobo produce la disminución del valor de ganancia de peso. Además, comparando los sistemas con tensoactivos, a pesar de obtener valores de ganancia de peso similares entre los sistemas compuestos por fármaco y tensoactivo, es posible definir diversas tendencias entre los sistemas utilizando un tratamiento estadístico. Mediante el test de diferencia entre dos medias se determinó la escasa diferencia entre las muestras estudiadas. Las diferencias más significativas se encuentran entre aquellas muestras con agarosa-pectina-fármaco respecto a las muestras con las diferentes concentraciones de tensoactivos. Entre los sistemas con un 1% de tensoactivo se observa que los sistemas con 1% de lauril sulfato sódico producen un ligero aumento en los valores de ganancia de peso, lo cual se atribuye al tipo de

tensoactivo y a su alto valor de HLB respecto al resto de tensoactivos. También se observa que las modificaciones estructurales producidas por los diferentes tensoactivos en la matriz agarosa/pectina influyen en los valores de ganancia de peso obtenidos (agarosa/pectina-/tween® 80 3%/tolbutamida y agarosa/pectina/pluronic® F-68 3%/tolbutamida). Este efecto se encuentra relacionado con la obtención de una estructura menos porosa y minoritariamente colapsada debido a la formación de micelas en el hidrogel fresco que actúan como espaciadores facilitando la hidratación del sistema liofilizado.

Se realizaron ensayos de cesión en agua desmineralizada como medio estandarizado para realizar estudios de cesión de fármacos y en medio pH progresivo debido a la aplicación potencial de estos sistemas en patologías del tracto gastrointestinal.

Los resultados obtenidos en el sistema liofilizado pectina/tolbutamida muestran un tiempo de latencia (48 minutos) lo cual demuestra que la liberación del fármaco desde el sistema liofilizado requiere el proceso de rehidratación previo. La presencia del tensoactivo permite disminuir el periodo de rehidratación gracias a la disminución de la tensión superficial del medio acuoso. Asimismo, la concentración crítica micelar, las propiedades físicas de las micelas formadas y las interacciones con la matriz agarosa/pectina son parámetros que permiten explicar los diferentes perfiles de cesión obtenidos.

Los perfiles de cesión instantáneos obtenidos en los sistemas liofilizados pectina/tween® 80 3%/tolbutamida y pectina/pluronic® F-68 3%/tolbutamida pueden explicarse a través de los datos obtenidos en los ensayos de intrusión de mercurio donde se determina la distribución de tamaño de

poros, lo cual permite predecir como el fluido acuoso ingresa dentro de la matriz polimérica. A su vez, los siguientes fenómenos permiten explicar los fenómenos que suceden desde la rehidratación del sistema hasta la posterior liberación del fármaco:

- El tamaño y estructura de las micelas formadas en el sistema fresco, que disminuirán el colapso del sistema una vez liofilizado.
- La disolución y difusión de las moléculas de tensoactivo.
- Las interacciones químicas del fármaco en los diferentes estados: libre, disuelto dentro de las micelas y absorbido en el hidrogel.

En el caso de los sistemas agarosa-pectina preparados con diferentes concentraciones de tensoactivo, la incorporación de agarosa permite obtener sistemas robustos que mantienen su estructura al estado fresco y liofilizado. La agarosa genera una red estable capaz de soportar los cambios micro-estructurales y químicos producidos por la presencia de tensoactivos. La modificación estructural observada en los sistemas con un 3% de tensoactivo (agarosa/pectina/lauril sulfato sódico/tolbutamida, agarosa/pectina/tween® 80/tolbutamida y agarosa/pectina/pluronic® F-68/tolbutamida) presenta un rol crítico en el proceso de rehidratación.

En los sistemas agarosa/pectina/lauril sulfato sódico 3%/tolbutamida los valores de porosidad facilitan la entrada de medio acuoso lo cual incrementa los valores de cesión del fármaco. En los sistemas agarosa/pectina/tween® 80/tolbutamida y agarosa/pectina/pluronic® F-68/tolbutamida la arquitectura menos porosa es uno de los factores que determina la obtención

de valores de cesión de tolbutamida por debajo de los obtenidos con lauril sulfato sódico.

En los sistemas agarosa-pectina frescos, a diferencia de los sistemas agarosa-pectina liofilizados, la liberación del fármaco se realiza a través de los procesos de disolución y difusión. El fármaco disuelto difunde desde la matriz hidratada hacia el medio acuoso.

El ajuste matemático de los resultados obtenidos en los perfiles de liberación de tolbutamida confirma que la cesión del fármaco desde los sistemas pectina liofilizados, agarosa-pectina liofilizados y agarosa-pectina frescos se ajustan a dos modelos ampliamente estudiados: Higuchi y Korsmeyer y Peppas.

En los sistemas pectina y agarosa-pectina liofilizados se confirma que los procesos que rigen la liberación del fármaco se producen por el relajamiento y reajuste que sufren las cadenas de hidrogel durante el proceso de rehidratación. Los valores n obtenidos para este ajuste son 0,45 y 0,89, lo cual se ajusta a un transporte anómalo. El transporte, denominado anómalo, se describe como una superposición de fenómenos relacionados con la entrada masiva de medio, reestructuración progresiva y parcial del sistema, sincronización entre el hinchamiento del sistema y la difusión del fármaco a través del hidrogel (Korsmeyer-Peppas). En los sistemas pectina-fármaco liofilizados, se observa además un proceso de erosión y el ajuste de la cinética indica un comportamiento de liberación del fármaco semejante al denominado caso II, gobernado por procesos de relajación y erosión de la matriz polimérica.

El mecanismo de liberación del fármaco desde los sistemas agarosa/pectina liofilizados se realiza mediante un proceso de

difusión y relajación, como se demuestra por los valores calculados de n entre 0,45 y 0,89. Al mismo tiempo, en estos sistemas se produce un fenómeno de erosión debido a la disolución de pectina y de tensoactivo. Este fenómeno permite explicar la obtención de valores de liberación de fármaco superiores a los obtenidos con sistemas compuestos por agarosa sola.

Por otro lado, los datos de cesión obtenidos en los sistemas agarosa/pectina frescos exhiben como principal mecanismo de liberación un proceso de difusión. Como se ha mencionado anteriormente, el fármaco puede encontrarse en estado libre, dentro de las micelas o atrapado en las cadenas de polímeros. Los valores obtenidos de la constante de Higuchi para los sistemas elaborados con un 3% de tensoactivo demuestra la acción coadyuvante de los tensoactivos en el proceso de liberación del fármaco (K_H = 0,0207, 0,0231 y 0,0265).

Los valores de cesión en medio pH progresivo de los sistemas pectina-tensoactivo-fármaco liofilizados demuestran que en presencia de un tensoactivo se produce un aumento en la velocidad de liberación de tolbutamida, la disminución del periodo de latencia y la disminución de la solubilidad pH-dependiente del fármaco. Este comportamiento es más evidente en los sistemas con 1% de tween® 80. En los sistemas pectina liofilizados con 3% de tween® 80 y pluronic® F-68 se observa la liberación total de fármaco a los 10 minutos de ensayo debido a la inconsistencia de los sistemas, fenómeno observado durante los ensayos realizados en medio acuoso.

En el caso de los sistemas agarosa-pectina liofilizados, la incorporación de un tensoactivo permite obtener una mayor velocidad de cesión de tolbutamida y a su vez, minimizar la dependencia de solubilidad del fármaco en función del pH.

Comparando estos resultados con los perfiles obtenidos en los sistemas agarosa-pectina frescos se observa el aumento de la dependencia al pH del medio acuoso, que es inferior a los valores obtenidos con los sistemas compuestos por pectina sola liofilizados.

El análisis de los resultados permite concluir que la formación de geles mixtos agarosa-pectina a los cuales se incorpora tensoactivos capaces de modificar la estructura polimérica, permite obtener un sistema que interactúa con las moléculas de la sustancia activa y es capaz de modular la liberación de un fármaco hidrófobo.

Con el fin de estudiar si los sistemas basados en agarosa, desarrollados en las etapas anteriores eran aptos para modular la liberación de fármacos hidrosolubles se prepararon otros sistemas de agarosa incorporando aditivos más hidrófobos como un tensoactivo de bajo valor de HLB o derivados del ácido poliacrílico. Los resultados se recogen en el tercer trabajo de esta tesis doctoral titulado "*Agarose-based systems tailored for the controlled release of highly soluble drugs within a pH progressive medium*". A los hidrogeles de agarosa se incorporan un tensoactivo de bajo HLB (labrafac) y polimetacrilatos (Eudragit® S100 y Eudragit® RL PO) con diferentes características de solubilidad y permeabilidad.

Los sistemas fueron elaborados con 3 y 12% de cada excipiente manteniendo constantes las proporciones de fármaco y agarosa (1 y 3%).

La obtención de valores de cesión similares entre los sistemas A-L3 (agarosa/labrafac al 3%) y A-L12 (agarosa/labrafac al 12%) con los sistemas A-S3 (agarosa/Eudragit® S100 al 3%) en medio acuoso permitieron definir los sistemas A-R3 (agarosa/Eudragit® RL PO al 3%) y A-R12 (agarosa/Eudragit® RL

PO al 12%) como los más idóneos para llevar a cabo su caracterización.

Comparando los perfiles de cesión en medio pH progresivo de los sistemas A-S3 (agarosa/Eudragit® S 100 al 3%) y A-S12 (agarosa/Eudragit® S 100 al 12%) con los sistemas A-R3 (agarosa/Eudragit® RL PO al 3%) y A-R12 (agarosa/Eudragit® RL PO al 12%) se observa la influencia de la naturaleza del derivado del ácido poliacrílico presente en cada sistema. En los sistemas compuestos por Eudragit® S100 al 12%, a pesar de su solubilidad pH dependiente se observa perfiles de cesión similares a los obtenidos por el sistema binario (teofilina-agarosa). Por otro lado, los sistemas A-R3 (agarosa/Eudragit® RL PO al 3%) y A-R12 (agarosa/Eudragit® RL PO al 12%) presentan una cesión sostenida pH independiente.

Realizando los ajustes cinéticos de los perfiles de cesión de los sistemas A-R3 (agarosa/Eudragit® RL PO al 3%) y A-R12 (agarosa/Eudragit® RL PO al 12%) a los modelos de Higuchi y Korsmeyer-Peppas, es posible predecir que el mecanismo principal por el cual se produce la disolución del fármaco es a través de un proceso de difusión donde, para que la difusión del fármaco tenga lugar, es necesario el ingreso de medio en el sistema, la difusión del fármaco hacia el exterior y que a su vez se produzca el hinchamiento del sistema.

Como se ha explicado anteriormente, el comportamiento observado en los sistemas A-R3 (agarosa/Eudragit® RL PO al 3%) y A-R12 (agarosa/Eudragit® RL PO al 12%) en medio pH progresivo se encuentra influenciado por diversos factores, por lo cual el siguiente paso fue realizar la caracterización de estos sistemas con el fin de conocer la influencia de los polimetacrilatos en el proceso de hinchamiento de hidrogeles

de agarosa y las interacciones moleculares entre los componentes del sistema.

El estudio del proceso de hinchamiento de los sistemas Eudragit® S100 muestra la solubilidad pH dependiente de este polimetacrilato, donde el efecto del cambio de pH contribuye a la disolución de los sistemas con concentraciones del 12%. Asimismo, en los sistemas A-R3 (agarosa/Eudragit® RL PO al 3%) y A-R12 (agarosa/Eudragit® RL PO al 12%) se observa una ganancia de peso inferior si se comparan con el resto de formulaciones. Los resultados de hinchamiento sugirieron la presencia de interacciones entre el fármaco, agarosa y eudragit, así como en la distribución de tamaño de poro del sistema, por lo cual se siguieron diversas técnicas de caracterización microestructural.

A través de la microscopia electrónica de barrido se observó la estructura característica de panal de abeja de estos sistemas y a su vez, la degradación parcial de esta microestructura cuando mayor es la concentración de polimetacrilato en el sistema.

A través de la porosimetría de intrusión de mercurio, se observó la evolución en el tamaño de poro de los sistemas en los cuales se ha incorporado polimetacrilatos. Los sistemas binarios (teofilina-agarosa) presentan tamaños de poros característicos de alrededor 100 µm, sin embargo la incorporación de 1% de polimetacrilatos determina la evolución hacia tamaños de poro más pequeños. La presencia de 3% de polímero en los sistemas de agarosa produce un cambio en la estructura porosa del sistema así como la obtención de sistemas con estructuras internas más densas.

De la misma forma a la caracterización de los sistemas de agarosa realizada en el primer trabajo, se observa la

influencia de la matriz de agarosa en el crecimiento y orientación de los cristales de teofilina durante el proceso de liofilización. La difracción máxima de teofilina observada mediante difracción de Rayos X en la materia prima desaparece cuando se realiza el análisis de los sistemas binarios y ternarios. La alteración de la cristalinidad del fármaco se confirma mediante Calorimetría diferencial de barrido donde se observa la desaparición del pico endotérmico a la temperatura de fusión de teofilina (275°C).

Con el fin de determinar las interacciones entre los componentes de cada sistema se utilizó la espectrofotometría de infrarrojos por transformada de Fourier (FTIR) y la calorimetría diferencial de barrido (DSC). Las muestras analizadas fueron obtenidas en los ensayos de cesión a cada variación de pH (1,5, 4,0 y 6,8).

Nuevamente se observa la ausencia del pico endotérmico característico de teofilina en el sistema binario. Los sistemas ternarios desarrollan un pico exotérmico a elevadas temperaturas que está relacionado a los procesos de descomposición del polimetacrilato.

La ausencia de alteraciones significativas en los espectros obtenidos mediante FTIR de los sistemas ternarios confirma la ausencia de interacciones químicas entre los componentes del sistema.

Por lo tanto, es posible relacionar la influencia de los sistemas A-R3 (agarosa/Eudragit® RL PO al 3%) y A-R12 (agarosa/Eudragit® RL PO al 12%) en los perfiles de cesión de teofilina en medio pH progresivo con la formación de interacciones físicas. La naturaleza neutra de la agarosa y la presencia de grupos hidroxilo en su estructura son factores que limitan la interacción con los grupos de amonio

cuaternario presentes en Eudragit® RL PO y la formación de enlaces de hidrógeno que condiciona la cesión de teofilina.

Otro factor que explica este comportamiento, es el grupo de amonio cuaternario presente en Eudragit® RL PO. Este grupo condiciona el grado de permeabilidad del sistema a través del mecanismo de intercambio iónico con el medio, por lo que el proceso de rehidratación del sistema y la posterior difusión del fármaco se encuentra comprometido. Asimismo, el comportamiento observado en el hinchamiento de los sistemas A-R3 (agarosa/Eudragit® RL PO al 3%) y A-R12 (agarosa/Eudragit® RL PO al 12%) permiten explicar el rol de Eudragit® RL PO en los sistemas. Este polímero actúa como un filtro que limita la interacción del hidrogel con el medio acuoso, limita su expansión y la migración del fármaco disuelto a través de la matriz hidratada hacia el exterior.

El efecto de filtro observado en Eudragit® RL PO no afecta la estructura porosa de los sistemas, pero presenta gran influencia en el hinchamiento y en la liberación del fármaco. Asimismo, la obtención de tamaños de poro más pequeños en los sistemas con Eudragit® RL PO a bajas concentraciones no condiciona la migración de medio hacia el interior del sistema.

Por último, en el caso de los sistemas con Eudragit® S100, el hinchamiento y liberación del fármaco desde los sistemas ternarios se encuentra influenciado principalmente por la presencia de la agarosa, la ausencia del grupo de amonio cuaternario permite confirmar el efecto filtrante de los sistemas compuestos por Eudragit® RL PO. Las interacciones producidas entre los componentes de los sistemas Eudragit®

S100 no limitan los procesos de hinchamiento, re-estructuración y liberación del fármaco.

Como conclusión de este trabajo podemos decir que la incorporación de un polimetacrilato de baja permeabilidad a hidrogeles de agarosa permite obtener un sistema de liberación controlada que modula la liberación de un fármaco de alta solubilidad. A pesar de la ausencia de modificaciones significativas en la estructura porosa de agarosa, se observa la influencia del efecto filtrante de este agente en el proceso de hinchamiento de los sistemas, lo cual condiciona la rehidratación y posterior cesión de fármaco.

Los resultados obtenidos en los tres trabajos que conforman la tesis permiten concluir que la estructura de los geles de agarosa puede ser modificada mediante la incorporación de aditivos de distinta naturaleza para de esta forma obtener sistemas útiles para controlar la liberación de fármacos solubles en agua o con solubilidad pH-dependiente, con lo que se han cumplido los objetivos propuestos al inicio de esta tesis.

CONCLUSIONES

En esta Tesis doctoral se han demostrado las posibilidades que presentan los sistemas basados en hidrogeles de agarosa como sistemas de liberación de fármacos. El origen natural de este polisacárido, su renovabilidad y gran disponibilidad facilitan su aplicación industrial con un menor impacto ecológico. Su capacidad gelificante ha permitido diseñar vehículos en los que se pueden incluir muy diversos aditivos que faciliten la integración y posterior disolución controlada de un determinado fármaco en un medio con condiciones específicas.

En este sentido, en el trabajo "*Agarose based systems upgraded by surfactants inclusión: Critical role of the pore architecture on their use as drug delivery systems*" se han diseñado sistemas que permiten la inclusión de tres surfactantes (Lauril sulfato de sodio, Tween® 80 y Pluronic® F-68) con el objetivo de mejorar la integración de ciertos fármacos de diferente solubilidad en medio acuoso y su posterior liberación. La presencia de estas moléculas así como de las micelas formadas influye de manera decisiva en el ordenamiento de las cadenas de agarosa durante el proceso de gelificación, lo que determina la arquitectura de poro resultante de someter estos hidrogeles a un proceso de liofilización. La modificación del tamaño de poro y su influencia sobre el proceso de rehidratación resulta clave para poder explicar los perfiles de liberación de los fármacos estudiados, en especial en el caso de la tolbutamida, de muy baja solubilidad en medio acuoso.

En el trabajo "*Robust and versatile pectin-based drug delivery systems*" se demuestra como la capacidad gelificante de la agarosa es capaz de soportar la inclusión de otro polisacárido, la pectina, además de los surfactantes ya estudiados en el trabajo anterior. De hecho, la consistencia

de estos sistemas mixtos agarosa-pectina permite trabajar con ellos inmediatamente después de su obtención, sin necesitar un proceso de liofilización. Estos sistemas resultan de gran interés para la administración de fármacos por vía oral en aquellos pacientes con problemas de disfagia. Por esta razón estos sistemas han sido ensayados a un pH que va incrementándose de manera análoga a lo que le ocurriría a un sistema que fuese administrado por vía oral. En cualquier caso, muestras "en fresco" o liofilizadas, se consigue una mejora en el proceso liberación del fármaco de baja solubilidad utilizado como modelo (tolbutamida). Estos sistemas agarosa-pectina no se comportan meramente como transportadores sino que interactúan con el fármaco. Este hecho se ha podido constatar porque los patrones de liberación, en los que se logra una menor dependencia del pH variable en el que han sido ensayados, son función de la naturaleza y porcentaje del surfactante incluido.

En el trabajo "*Agarose based systems tailored for the controlled release of highly soluble drugs within a pH progressive médium*" se demuestra cómo se ha logrado extender la aplicabilidad de los sistemas basados en agarosa a la liberación de fármacos con alta solubilidad en agua. Esta liberación controlada pH-independiente se ha logrado mediante la inclusión de un compuesto de la familia de los Eudragit, el RL-PO. En este caso, la influencia de este aditivo está relacionada en gran medida con el efecto filtrante que ejerce en el proceso de hinchamiento de los sistemas, lo que determina el proceso de liberación Asimismo, hay que tener en cuenta el efecto que causan los aditivos en la microestructura, en especial la arquitectura de poro y la cristalinidad del fármaco.

Además de mostrar la versatilidad y flexibilidad que presentan estos sistemas para alojar diferentes aditivos, en estos trabajos se ha manifestado la necesidad de caracterizar la porosidad de este tipo de sistemas. La arquitectura de poro, es decir el tamaño y distribución de los poros, su grado de conexión y el porcentaje de porosidad, son parámetros que determinan de manera crítica los procesos de rehidratación de sistemas liofilizados. Hay que tener en cuenta la relevancia y frecuente uso de la liofilización en la industria farmacéutica y otras como técnica de conservación o preparativa y que en todos ellos hay someter al producto liofilizado a un proceso de hidratación. En este sentido es relevante el uso que se ha hecho de la Porosimetría de Intrusión de Mercurio, no habitualmente empleada en este campo, así como de otras técnicas más extendidas como la Microscopía Electrónica de Barrido.

En el caso de la liberación de fármacos resulta crucial, el estudio del grado de cristalinidad del fármaco introducido en el hidrogel de agarosa. Hay que considerar que el fármaco introducido en una red de cadenas de agarosa tiene limitado, y en ocasiones orientado, su crecimiento lo que causa un proceso de amorfización durante el proceso de liofilización. Este hecho puede ser utilizado como una herramienta que permita controlar el crecimiento cristalino de una determinada molécula activa y con ello su comportamiento durante y tras su liberación. Por todo ello resulta de alto interés caracterizar el estado cristalino de los fármacos incluidos en un sistema liofilizado mediante técnicas como la difracción de rayos X o técnicas calorimétricas.

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