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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 $\mathbf{N_{agg}}$ = 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

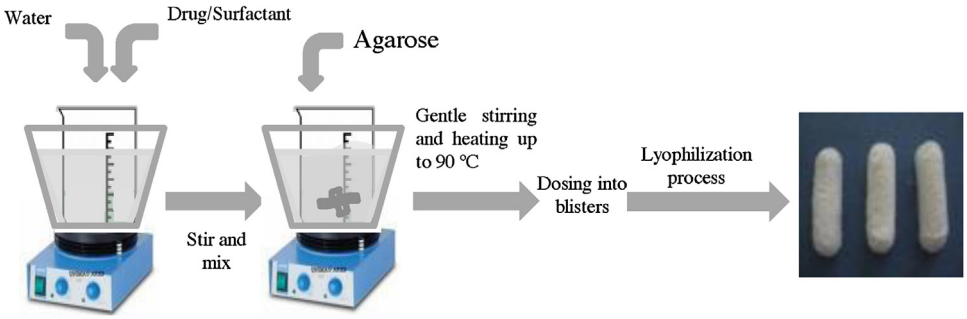

Fig. 1. Preparation and preservation route of drug containing systems.

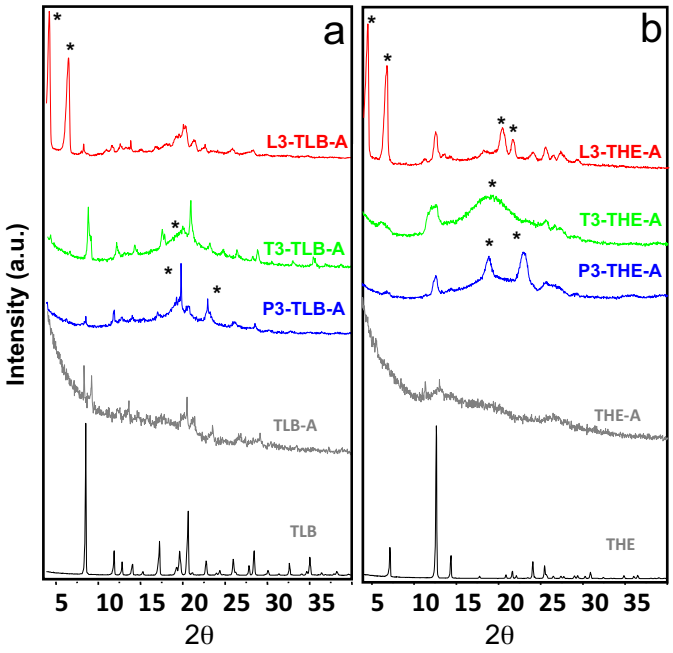

Fig. 2. X-ray diffraction of samples containing (a) Tolbutamide (TLB) or (b) Theophylline (THE). * Indicates the contribution of the surfactants included.

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

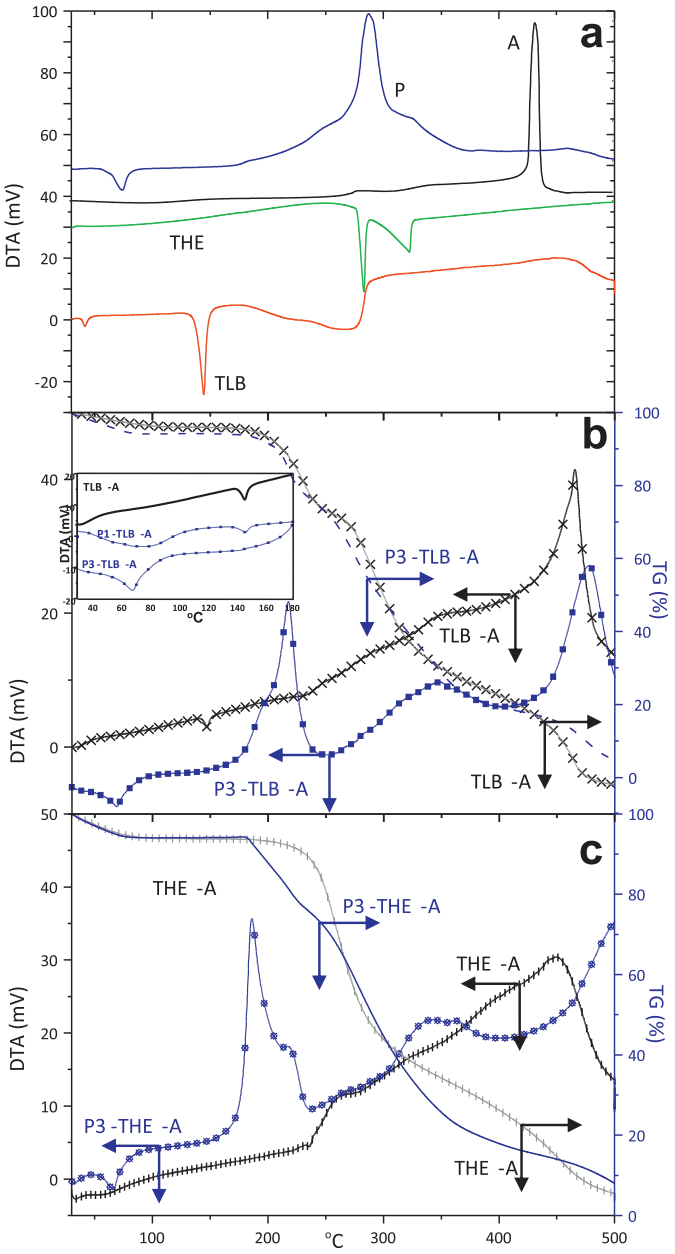

Fig. 3. (a) DTA patterns of the single components, (b) TG and DTA patterns of TLB-A and P3TLB-A, inset: augmentation of the low temperature region of TLB-A, P1-TLB and P3-TLB-A DTA curves, (c) TG and DTA patterns of THE-A and P3THE-A.

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 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.

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

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

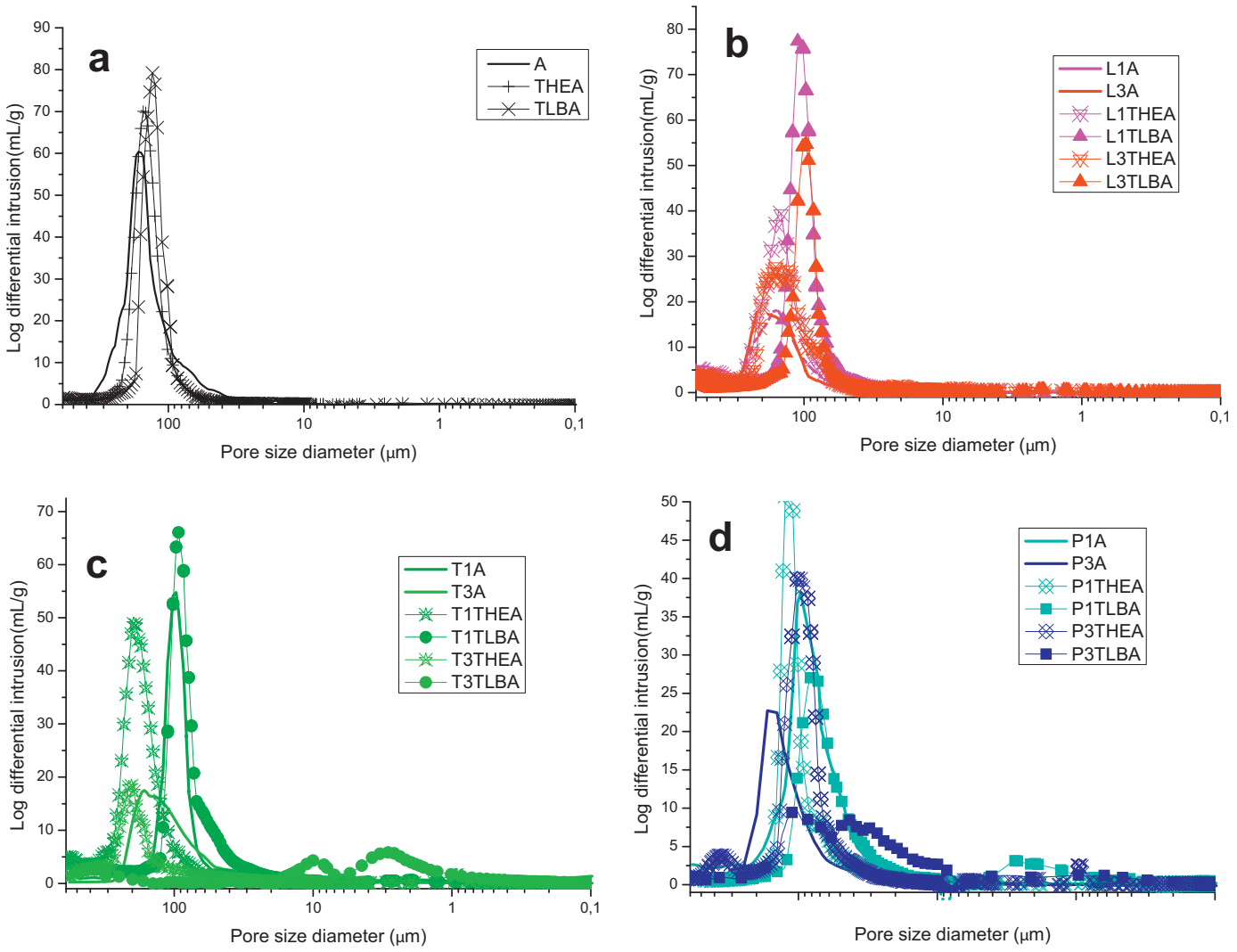

Fig. 4. Pore size distribution of (a) agarose, tolbutamide-agarose and Theophylline agarose samples, (b) theophylline and tolbutamide loaded and non-loaded samples prepared with (b) SLS, (c) Tween and (d) pluronic.

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

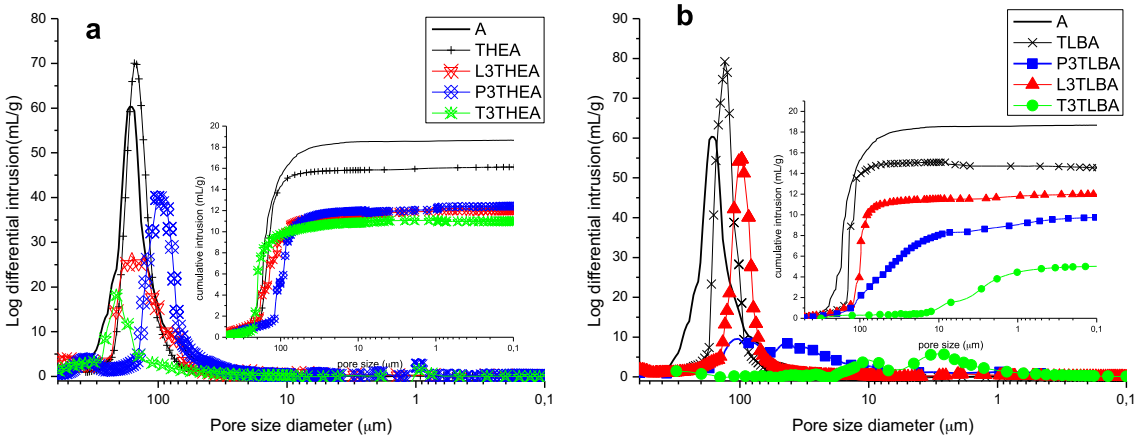

Fig. 5. Pore size distribution and cumulative intrusion of (a) theophylline and (b) tolbutamide containing systems prepared with different surfactants at a 3%. Agarose and agarose-drug curves are also included.

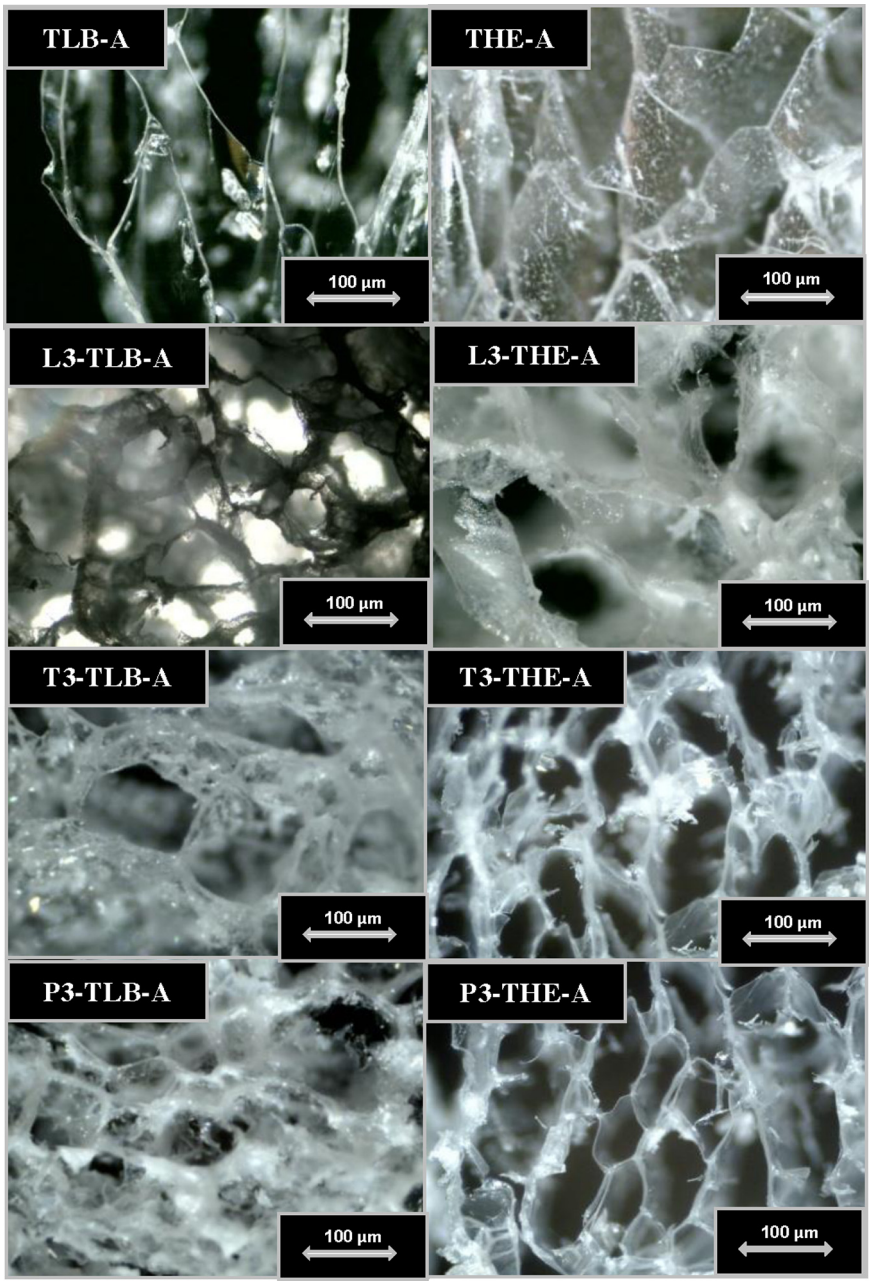

Fig. 6. Microscope micrographs of the binary (TLB-A and THE-A) and ternary systems with TLB or THE and the three surfactants employed.

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

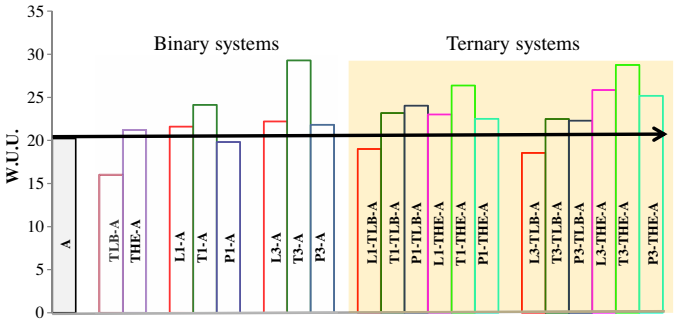

Fig. 7. Swelling behavior as a function of the water uptake units (W.U.U.).

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

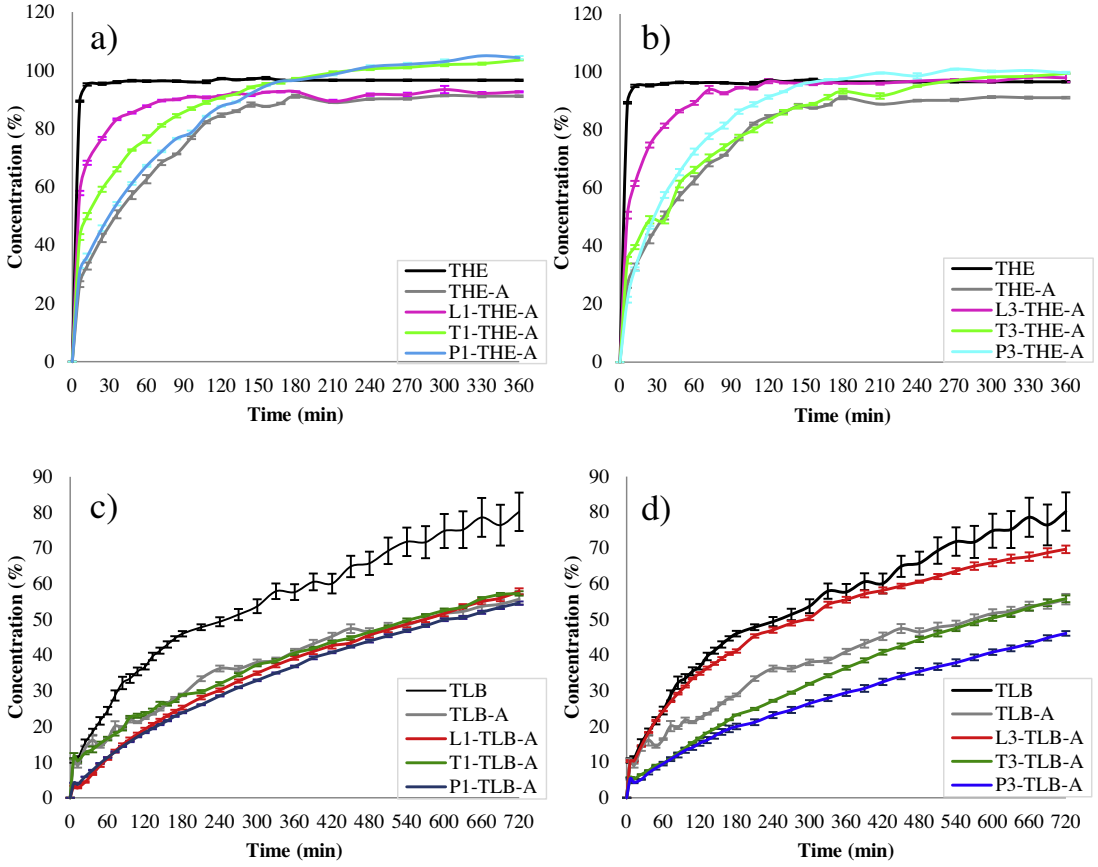

Fig. 8. Drug release profiles of freeze-dried hydrogels. Ternary THE containing systems with 1% (a) or 3% (b) of surfactant and equivalent TLB ternary formulations with 1% (c) and 3% (d).

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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