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European Journal of Pharmaceutics and Biopharmaceutics 66 (2007) 244–252
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Tetronic micellization, gelation and drug solubilization: Influence of pH and ionic strength

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Received 28 July 2006; accepted in revised form 6 October 2006
Available online 20 October 2006

Abstract

The aim of this work was to gain an insight into the self-associative processes and drug solubilization ability of a Tetronic variety, T904 (4×15 EO units; 4×17 PO units; HLB 15), in aqueous media covering the physiological range of pH and ionic strength, applying isoperibol microcalorimetry, transmission electronic microscopy (TEM), dynamic light scattering (DLS), oscillatory rheometry, and drug diffusion experiments. T904 shows two pK_a (p$K_{a1} = 4.0$ and p$K_{a2} = 7.9$) and, at pH < 5.8, the diprotonated form predominates over the non-protonated one. Deprotonization of the central diamine group is a required condition for micellization, which is an endothermic entropy-driven process owing to hydrophobic interactions between the PPO chains. As the pH of the solutions decreases, the coulombic repulsions among the positively charged amine groups make the aggregation more difficult, raising the critical micellar concentration (CMC) and decreasing the size of the micelles. The changes in the conformation and hydrophilicity of the Tetronic were reflected in its gelation temperature (around 30 °C at neutral-alkaline pH; no gelation at pH < 2) and solubilization capacity for griseofulvin (2-fold greater at neutral-alkaline pH than at pH < 2) and rate of diffusion (slower at pH 7.4). Such alterations in self-assembly are relevant when using Tetronic in the design of drug delivery systems.

Keywords: Poloxamine surfactant; Polymeric micelles; Micellization enthalpy; Gel temperature; pH-sensitive micellization; Griseofulvin solubilization

1. Introduction

Amphiphilic copolymers that spontaneously aggregate in water forming nanosized colloidal particles, with a hydrophobic core and a hydrophilic shell, have shown a great potential as carriers of poorly water soluble and amphiphilic drugs [1–3]. These micelle-type polymer aggregates present important advantages compared to the micelles of low molecular weight common surfactants: lower critical micellar concentration (CMC), greater thermodynamic and kinetic stability against dilution, and an enhanced drug solubilizing and stabilizing capability in the biological fluids [4–6]. Additionally, an appropriate choice of the copolymer architecture enables control of important pharmacokinetic and therapeutic characteristics of the drug formulations, such as blood circulation time and control of drug release rate and site [7–10]. Drug-loaded micelles can passively accumulate in pathological sites with affected and leaky vasculature (tumours, inflammations, and infarcted areas) via the enhanced permeability and retention effect [11]. Furthermore, the shell can be provided with specific ligand molecules, such as antibodies, to transport the drug-loaded micelles to, and into, target cells (e.g., tumour cells) [1,3,12]. Additionally, micelles made of pH-responsive amphiphilic block copolymers can specifically release their contents in precise areas of the

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0939-6411/$ - see front matter © 2007 Published by Elsevier B.V.
doi:10.1016/j.ejpb.2006.10.010

gastrointestinal tract, in pathological tissues showing acidosis, or inside certain cell compartments. This may increase the bioavailability of poorly soluble or unstable drugs and, in general, the efficacy of the treatment [3,13–17].

Most research in the polymeric micelles field has been carried out with linear polyethylene oxide (PEO)/polypropylene oxide (PPO) triblock copolymers of the family of Pluronic®, and its derivatives, which proved to be highly effective drug delivery vehicles with temperature-sensitive behaviour [7,12,18]. By contrast, the related X-shaped copolymers formed by four poly(propylene oxide) (PPO) and poly(ethylene oxide) (PEO) block chains bonded to an ethylene diamine central group, known as Tetronic®, have been practically ignored until recently as pharmaceutical excipients. These commercially available surfactants, also known as poloxamines, are commonly used for industrial purposes at relatively high concentrations, as wetting, dispersant and thickener agents, and as emulsifiers particularly in the petroleum industry [19]. Although still only a few, the studies carried out in the pharmaceutical and biomedical fields have shown the potential of Tetronics as components of transdermal formulations [20], as tissue scaffolds [21], and in nanoparticle engineering [22]. Surface-adsorbed Tetronic can provide steric stabilization and modify the biodistribution of orally or parenterally administered drug-loaded nanoparticles [23,24]. For example, the presence of Tetronic® 908 contributes to preventing drug precipitation in the gastrointestinal tract, to facilitating the contact with the absorbing surface of the gut, and even the uptake of the nanoparticles across the gastrointestinal walls, and thus, to increased blood circulation time and reduced liver uptake [23,25].

Despite this attractive potential, the paucity of Tetronic physical-chemical data, in particular regarding micellization in aqueous solutions, is noteworthy [26–28]. It is well-known that the performance of a surface-active agent for a particular use is intimately linked to its chemical structure as well as the physical–chemical solution properties. Despite Tetronics usually considered non-ionic surfactants, they have a central diamine group that can be protonated to add a positive charge to the polymer [19,27]. As for other pH-sensitive copolymers, the deprotonization would be a required previous step for micellization [17,27,29]. The properties of the core of these micelles can strongly differ from those of the Pluronic micelles since the hydrophilic diamine group connects the hydrophobic PPO chains [30]. The special architecture of Tetronics (Fig. 1) suggests that the pH and ionic strength of the medium may strongly influence the behaviour of both the unimers and micelles and, consequently, their performance as components of drug delivery systems. The pH-sensitiveness of Tetronic micelles could offer interesting features compared to the micelles of non-ionic block copolymers, such as Pluronics, for developing pharmaceutical nanocarriers with the ability to host/release drugs as a function of pH. Therefore, the aim of this work was to gain an insight into the self-associative processes of a Tetronic® variety, T904 [31], in aqueous media and to evaluate the sensitiveness of the micelles and the dispersions to changes in pH, ionic strength and temperature in the physiological range, in order to elucidate its potential as component of stimuli-sensitive drug delivery systems. The self-aggregation processes were characterized by isoperibol microcalorimetry, transmission electronic microscopy (TEM), dynamic light scattering (DLS), and oscillatory rheometry, and the ability of the micelles to load and control the release of griseofulvin was evaluated under different environmental conditions.

2. Materials and methods

2.1. Materials

Tetronic® 904 ($(OE_{15}OP_{17})_2NCH_2CH_2N(OP_{17}OE_{15})_2$) was from BASF Corporation (New Jersey, USA) and used as received. Griseofulvin and *N*,*N*,*N*′,*N*′-tetramethyl ethylene diamine (TEMED) were from Sigma-Aldrich (Spain). Purified water was obtained by reverse osmosis (resistivity > 18.2 MOhm cm; MilliQ®, Millipore Spain). All other reagents were of analytical grade.

2.2. Preparation of T904 solutions

Several T904 solutions (10–30% w/w) were prepared by adding the adequate amount of the surfactant to water, NaCl 0.9%, HCl 0.1 M, HCl 0.01 M, NaOH 0.02 M, HCl 0.01 M/NaCl 0.9% solutions, and pH 5.8 or 7.4 USP phosphate buffers. Magnetic stirring was applied until complete dissolution of the surfactant, and the solutions were left to settle for at least 24 h before characterization.

2.3. T904 potentiometric titration

Titration of T904 was performed using a pH-meter Crison, model GLP22 (Barcelona, Spain), equipped with

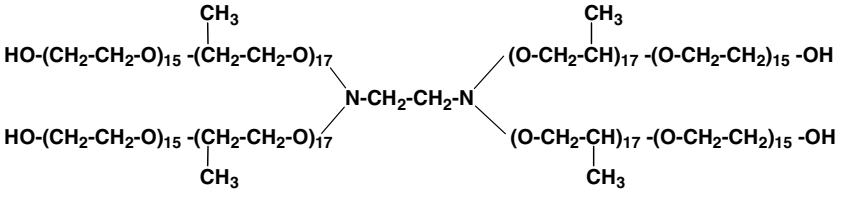

Fig. 1. Structure of Tetronic® 904.

a viscous sensor (Ag/AgCl) n°52-21. Hydrochloric acid (0.01 M, 25 ml) was added to 0.01 M T904 solutions (25 ml), which were then titrated with 0.01 M sodium hydroxide.

To calculate the concentration of un-ionized and protonated species at any point on the potentiometric curve, the following approach was made [28]:

(i) the total concentration of T904 chains in solution ($[T_{total}]$) is the sum of the non-protonated, mono-, and di-protonated forms:

$$[T_{total}] = [T] + [TH^+] + [TH_2^{2+}] \quad (1)$$

(ii) and the electroneutrality of the system is given by the expression:

$$[H^+] + [TH^+] + 2[TH_2^{2+}] + [Na^+] = [Cl^-] + [OH^-] \quad (2)$$

The concentrations of the monoprotonated and the diprotonated forms can be estimated from the expressions of the dissociation constants K_{a1} and K_{a2} as follows:

$$[TH^+] = \frac{[T][H^+]}{K_{a2}} \quad (3)$$

and

$$[TH_2^{2+}] = \frac{[T][H^+]^2}{K_{a2}K_{a1}} \quad (4)$$

The concentration of un-ionized T904 molecules is given by:

$$[T] = \frac{[T_{total}]}{1 + ([H^+]/K_{a2}) + ([H^+]^2/K_{a2}K_{a1})} \left(\frac{V_{initial}}{V_{initial} + V_{base}}\right) \quad (5)$$

where $V_{initial}$ is given by the volume of T904 solution and of HCl solution, and V_{base} is the volume of NaOH solution added.

2.4. Isoperibol microcalorimetry

Calorimetric experiments were performed in triplicate using a Tronac-450 isoperibol microcalorimeter and Tronac FS101 calorimetry software (Tronac Inc., Orem, Utah). In each experiment, a T904 10% (0.0149 M) solution was loaded into a 2 ml calibrated buret, and 47.5 ml of water, or of the medium in which T904 was dissolved, was placed in a Dewar reaction vessel. The entire assembly was then immersed into a constant temperature (310.0 K) water bath. After thermal equilibration, the T904 solution was delivered at 0.3332 ml/min constant rate into the reaction vessel, in which a stirrer mixed the two solutions rapidly. The evolution of the temperature in the system was monitored using a thermistor, and later reproduced using a heating coil in the reaction vessel. The apparent enthalpy was calculated from the applied current and voltage and the heating time. Calibration of the system was assured by titration of tris(hydroxymethyl)aminomethane with a HCl standard solution. In order to obtain the heat associated to Tetronic and/or buffer ionization, Tetronic was replaced in the buret by TEMED at the same concentration (0.0149 M) to produce the same change in pH as the addition of the copolymer solution. The integral demicellization enthalpy was estimated by subtracting from the measured heat produced by addition of Tetronic to a given medium (ΔH_{obs}), the heat effects due to Tetronic/buffer ionization (ΔH_{ion}) [32,33].

$$\Delta H_{demic} = \Delta H_{obs} - \Delta H_{ion} \quad (6)$$

The final concentration of T904 in the Dewar was well below that required for gel formation at the temperature of the experiment. Therefore, no interference due to phase transition should be expected in the estimation of the enthalpy [32]. The standard Gibbs free energy of micellization (ΔG_{mic}) was calculated using the following expression:

$$\Delta G_{mic} = (1 + \beta)RT \ln X_{cmc} \quad (7)$$

where R is the gas constant, T is the absolute temperature, X_{cmc} is the CMC in mole fraction units, and β is the counterion binding evaluated from the potentiometric titration experiments. The standard entropy change (ΔS_{mic}) was derived from the following relation:

$$\Delta S_{mic} = (\Delta H_{mic} - \Delta G_{mic})/T \quad (8)$$

2.5. Surface tension measurements

Surface tension of Tetronic solutions was determined by the platinum ring method using a Lauda Tensiometer TD1 (Lauda-Königshofen, Germany) [6].

2.6. Transmission electron microscopy (TEM)

A 5 μl drop of the Tetronic solution was placed on grids covered with Fomvar film. After 30 s, the excess was carefully removed with a tip of filter paper, and a drop of water (5 μl) was added. After another 30 s, the excess was removed; a drop of 2% w/v phosphotungstic acid (5 μl) was added and left for 30 s before removing. The samples were then dried in a closed container with silicagel, and observed using a Philips CM-12 TEM apparatus (FEI Company, The Netherlands). Diameter of the micelles/aggregates was measured using a calibrated scale.

2.7. Dynamic light scattering (DLS)

The DLS measurements were performed using an ALV-5000 F optical system equipped with CW diode-pump Nd:YAG solid-state laser (400 mW) operated at 532 nm (Coherent Inc., Santa Clara, CA, USA). The intensity scale was calibrated against scattering from toluene. The T904 solutions were filtered (Millipore® 0.45 μm, Ireland) into the quartz cell (previously washed with condensing acetone vapour) and maintained at 20–37 °C. The diffusion

coefficient was deduced from the standard second-order cumulant analysis of the autocorrelation functions measured at 90° angle. The experiments were carried out in triplicate and the apparent hydrodynamic radius ($r_{h,app}$) of the micelles was calculated from the apparent diffusion coefficients.

2.8. Oscillatory rheology

The storage or elastic (G′) and the loss or viscous (G″) moduli of T904 30% solutions were evaluated in triplicate at 20 °C, applying 0.5% strain and angular frequencies of 0.05–50 rad/s in a Rheolyst AR-1000N rheometer (TA Instruments, Newcastle, UK) equipped with an AR2500 data analyzer, a Peltier plate and a cone geometry (6 cm diameter, 2.1°). To determine the influence of temperature on both moduli, the tests were carried out in triplicate, at 1 rad/s from 20 to 60°C with a heating rate of 1.5 °C/min. Evaporation was prevented using an adequate solvent trap.

2.9. Griseofulvin solubility studies

The T904 solutions (10%, 4 ml) were placed in glass ampoules containing an excess of drug (40 mg). The ampoules were flame-sealed and rocked at 25 °C and 50 rpm for 5 days. Then, the suspensions were filtered through 0.45 μm cellulose acetate membrane filters (Millipore®, Ireland). Control experiments were carried out using as solvent the media in which the T904 solutions were prepared. The concentration of the dissolved drug was determined by UV spectrophotometry (Agilent 8453, Germany) at 294 nm. The solubilization capacity per gram or per mol of T904 was calculated as the amount (gram or mol) of griseofulvin dissolved in the T904 solution in excess of that dissolved in an equivalent volume of each solvent medium. The solubilization capacity per gram of hydrophobic block was calculated similarly, using the mass fraction (60%) of the hydrophobic blocks in T904.

2.10. Griseofulvin release studies

Griseofulvin release profiles from 30% T904 solutions in HCl 0.1 M or pH 7.4 phosphate buffer, previously loaded with griseofulvin following the procedure explained above, were obtained in triplicate at 37 °C in Franz-Chien diffusion cells (VidraFoc, España) fitted with cellulose acetate filters (0.45 μm pore size, Teknokroma, Spain). Samples of 0.5 ml were put into the donor cell. The receptor compartment of 5.5 ml capacity was filled with the corresponding medium (which ensured sink conditions), and magnetic stirring was applied. The area available for diffusion was 0.7654 cm^2. Samples of the receptor solution (0.5 ml) were withdrawn at predetermined times and drug concentration was determined by UV spectrophotometry (Agilent 8453, Germany) at 294 nm. The samples were immediately returned to the receptor cell. Diffusion coefficients, D, were estimated using the equation [34]:

$$Q/A = 2C_0(Dt/\pi)^{1/2} \quad (9)$$

where Q/A is the amount of griseofulvin released per unit area at the time t, and C_0 is the initial concentration of griseofulvin in the donor solution (0.18 and 0.27 mg/ml in the systems prepared in HCl 0.1 M and pH 7.4 buffer, respectively).

3. Results and discussion

Among the different varieties of Tetronic commercially available, T904 was chosen because it has been previously shown to be biocompatible and useful in nanoparticle and tissue scaffold engineering [22] and has an intermediate molecular weight and HLB (6700 Da; HLB 12–18; Fig. 1) [31].

3.1. T904 protonization behaviour

The titration profile of T904 (Fig. 2A) showed two inflection points that correspond to proton dissociation from the nitrogen atoms on the central ethylene diamine

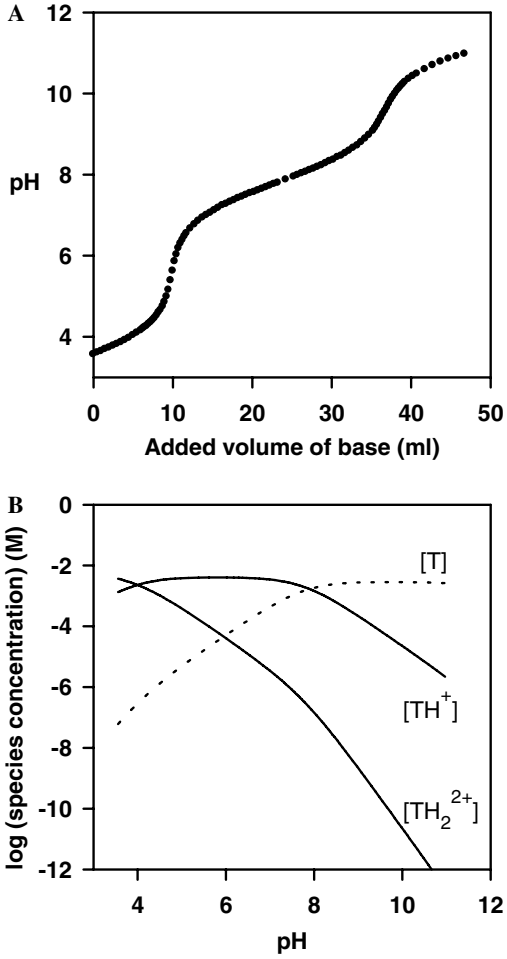

Fig. 2. Potentiometric titration curve obtained for T904 (A) and dependence of the concentrations of the non-protonated and protonated forms of T904 as a function of pH (B).

group. The pK_a values obtained were $pK_{a1} = 4.0$ and $pK_{a2} = 7.9$. These values are similar to those previously found for T701 [28]. At a constant temperature of 25 °C, the diprotonated form is the predominant one at pH values below 4 and its concentration is greater than that of the non-protonated form up to pH 5.8. The monoprotonated form is the predominant form in the pH range between 4.0 and 7.9 (Fig. 2B). Since the coulombic repulsions among the positively charged amine groups at the centre of the PPO chains can prevent aggregation, at pH values below pK_a a necessary condition for micellization is the proton dissociation [27]. The balance between the free energy of micellization and the free energy of protonization will determine the possibility of micelle formation, which can be represented as follows for the pH range in which the monoprotonated form predominates:

$$n\ \mathrm{TH}^+ \leftrightarrow \mathrm{T}n + n\mathrm{H}^+ \tag{10}$$

$$K_{\text{micellization}} = \frac{[\mathrm{T}n][\mathrm{H}^+]^n}{[\mathrm{TH}^+]^n} \tag{11}$$

As the pH decreases, the deprotonation-micellization would become more difficult. To evaluate the practical repercussions of these events, we first determined the CMC and the size of the micelles in media covering a wide range of pH and ionic strength conditions.

3.2. Thermodynamics of micellization

Isoperibol microcalorimetry experiments provided information about both the CMC of T904 and the energy associated to the demicellization/micellization process. Before being added to the Dewar, T904 (10% w/w) was in the buret above its CMC in all media tested, as confirmed by the low values of surface tension recorded (<38 mN/m). Therefore, when the T904 solution was slowly added into the Dewar solution, the micelles broke up until the concentration in the Dewar reached the CMC. Afterwards, the micelles were only diluted in a solution of micelles. In all media evaluated, the demicellization process was exothermic (enthalpy change negative), although remarkable differences in enthalpy and CMC were observed depending on the composition of the medium (Fig. 3). To correctly determine the enthalpy associated to the demicellization, the contribution of the ionization of the Tetronic and/or the buffer was taken into account [33]. T904 10% significantly raised the pH of the medium of un-buffered solutions (2–3 units) and, to a much lower extent (0.5 units), of the buffer solutions. Therefore, when the concentrated Tetronic solution was delivered into the Dewar, some protons could be taken up/exchanged with the components of the medium. To quantify the heat associated to this latter process, TEMED was used to carry out some blank experiments instead of T904. TEMED consists of exactly the same central ethylene diamine groups as T904 but has only two methyl groups at the end of each nitrogen, instead of two PPO-PEO blocks. Therefore, no associative phenomena at the concentration

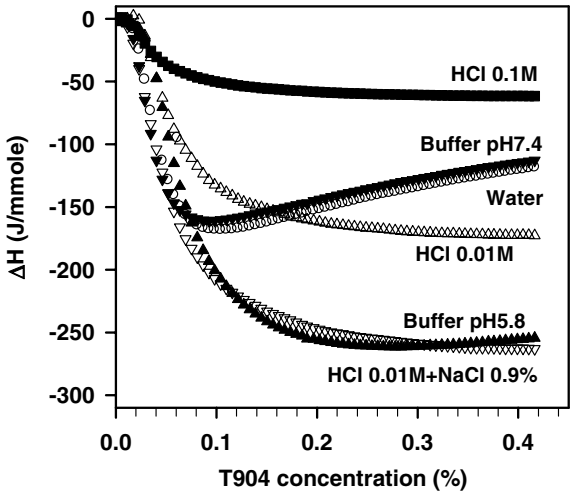

Fig. 3. Calorimetric profiles of the demicellization process, at 310 K, of 10% T904 solutions prepared in different media: water (open circles), pH 7.4 buffer (full down triangles), pH 5.8 buffer (full up triangles), HCl 0.1 M (full squares), HCl 0.01 M (open up triangles), and HCl 0.01 M with 0.9% NaCl (open down triangle).

used for the experiments are expected, and the calorimetric titrations just provide the enthalpy associated to changes in the protonization degree of the diamine group. For the media evaluated, the ionization enthalpy ranged from −5 to −10 J/mmol. Therefore, it was remarkably lower than the contribution of demicellization to the heat evolved in the Dewar when T904 was used. Fig. 3 shows the already corrected enthalpies.

The calorimetric profiles indicate that micellization is an endothermic entropy-driven process owing to hydrophobic interactions between the PPO chains. The exothermic dilution process is attributed to hydrogen-bonding formation between the PEO blocks and water after breakage of water-water and surfactant-surfactant hydrogen-bonds [32]. This behaviour is also typical of other structurally related surfactants such as Pluronics [35,36]. Table 1 summarizes the values of the thermodynamic parameters of T904 micellization in each medium. In un-buffered water (pH 8-9) and pH 7.4 buffer phosphate (Fig. 3), as well as in NaOH 0.02 M or NaCl 0.9% solutions (superimposable profiles), a clear CMC is observed at 0.09–0.10%. As the pH of the T904 solutions decreases, with the consequent increase in the degree of protonization of the diamine moiety in the middle of PPO blocks, the aggregation becomes more difficult. In pH 5.8 buffer phosphate, the inflexion point located at 0.25%. In a strongly acidic medium (HCl 0.1 M, pH 1.2), the dilution profile showed a progressively less exothermic process, but no inflexion point was observed below 0.4%. This means that at pH 1.2, T904 requires a remarkably greater concentration to induce the self-associative process (CMC ~ 0.4%) and, once the micelles are formed, they are easily broken by dilution. The coulombic repulsions among the positively charged amine groups prevent micellization in diluted T904 solutions. The decrease in ΔH may be motivated by an apparent reduction

Table 1
Thermodynamic parameters of micellization of T904 in aqueous solutions of different pH and ionic strength

Medium	pH of the T904 solution	X_{cmc} (mole fraction)	ΔH_{mic} (KJ/mol)	ΔG_{mic} (KJ/mol)	ΔS_{mic} (J/molK)
Water	8.5	$2.69\ 10^{-6}$	168	−49.56	701
pH 7.4 buffer	7.4	$2.42\ 10^{-6}$	161	−49.97	680
pH 5.8 buffer	5.8	$7.00\ 10^{-6}$	261	−61.15	1039
HCl 0.01 M	6.0	$7.00\ 10^{-6}$	167	−61.15	735
HCl 0.01 M + NaCl 0.9%	6.0	$7.00\ 10^{-6}$	255	−61.15	1019
HCl 0.1 M	1.2	$1.08\ 10^{-5}$	60	−88.40	478

Standard deviations were in all cases lower than 2%.

in the entropic driven force for micellization (Table 1), as found for Pluronics modified with terminal amine chains [37]. The protonization disturbs the microenvironment around the PPO blocks and hence the hydrophobic associations.

It is interesting to note the important effect of the salt concentration on the demicellization enthalpy in the less acidic solution (pH 6.0) of T904 in HCl 0.01 M medium. The CMC values (0.25%) obtained with HCl 0.01 M/NaCl 0.9% solutions were similar to those recorded in HCl 0.01 M or pH 5.8 phosphate buffer media. However, T904 showed a remarkably greater demicellization enthalpy in the HCl 0.01 M/NaCl 0.9% medium than in the HCl 0.01 M solution; the former being similar to the one recorded in pH 5.8 phosphate buffer (Fig. 3). As mentioned above, the partial protonization of T904 increases the hydrophilicity of the macromolecule and makes the micellization more difficult. In contrast, the increase in ionic strength shields the ionic repulsions and can cause a small salting-out effect that decreases the hydrophilicity of the poly(ethylene oxide) chains and promotes hydrogen bonding interactions [36,38]. Consequently, the entropic driven force for aggregation is enhanced and also the enthalpic compensation (Table 1). Greater demicellization enthalpies in high ionic strength medium, compared to water, have also been reported for Pluronic F127 [36].

3.3. Micellar size

Not only the CMC, but the aggregation number (i.e., the number of T904 molecules that constitute each micelle) should be altered by a change in pH or ionic strength. TEM micrographs of T904 30% systems, prepared and observed at 20 °C, clearly showed the influence of this variable on the micellar size. As can be observed in Fig. 4, a great number of significantly small micelles (2 nm) were formed in HCl 0.1 M. By contrast, in water and NaOH 0.02 M the micelles presented a greater and quite homogeneous size (10–20 nm). In the phosphate buffers, the micelles presented a wider size distribution, in which micelles of different size can be observed. This same tendency was observed by DLS at 20 °C; only samples prepared in pH 7.4 phosphate buffer showed a bimodal distribution, in which micelles and clusters coexist (Fig. 5). DLS experiments were also carried out at 37 °C to elucidate if the influence of pH on the size of the micelles also occurs at

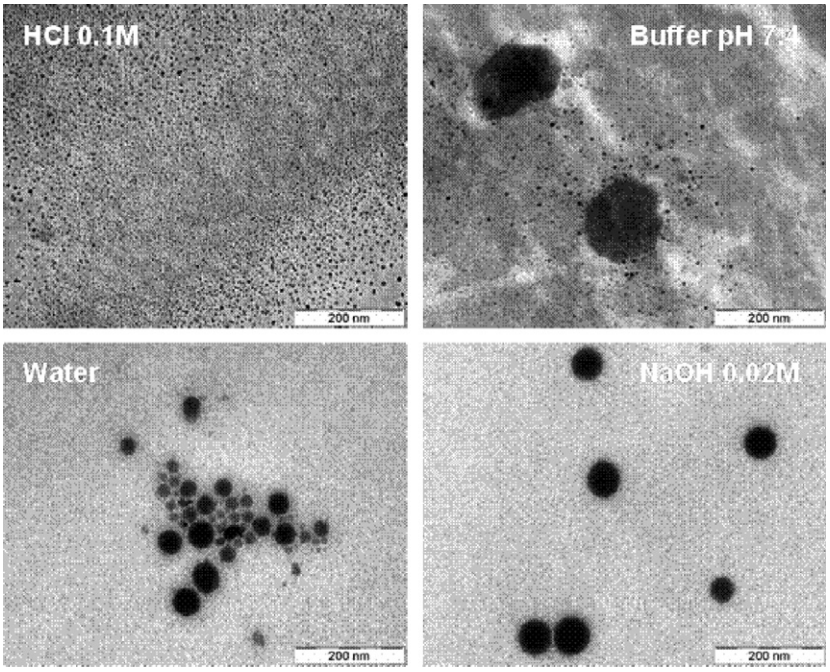

Fig. 4. TEM micrographs of 30% T904 solutions prepared in different media and negatively stained with phosphotungstic acid (110,000×).

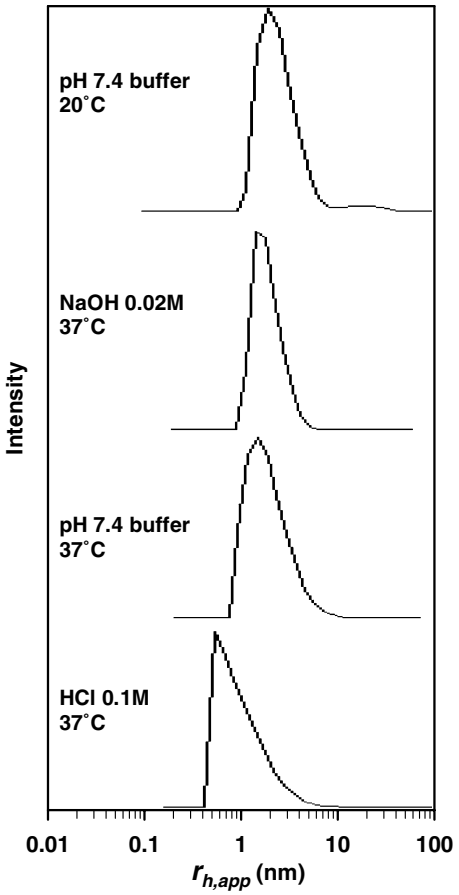

Fig. 5. Intensity fraction distribution of the apparent hydrodynamic radius ($r_{h,app}$) for 30% T904 solutions prepared in different media.

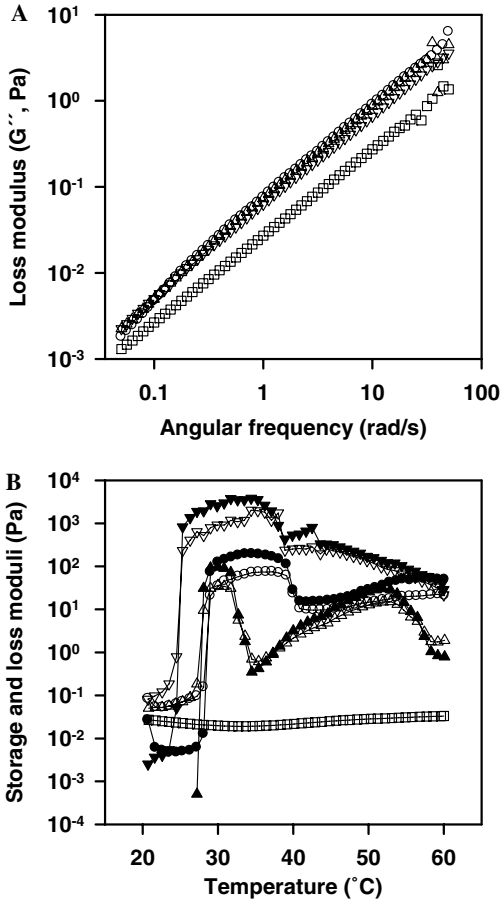

Fig. 6. Dependence of loss modulus on angular frequency at 20 °C (A) and of loss (open symbols) and storage (full symbols) moduli on temperature at 1 rad/s (B) for 30% T904 solutions prepared in water (circles), HCl 0.1 M (squares), pH 7.4 phosphate buffer (up triangles), and NaOH 0.02 M (down triangles).

the physiological temperature. Once again, the lower the pH, the smaller the micelles, ranging from 0.9 to 4 nm (Fig. 5). However, bimodal distributions were not observed at any pH and no important differences in polydispersity were detected. A similar influence of the temperature on micellization has been previously described for Pluronic P94: at 20 °C, micelles, and clusters were observed; whilst at higher temperature (40 °C), only the micelle peak with decreased polydispersity was recorded [39]. The increase in temperature enhances the hydrophobic interactions, which promotes the micelle formation with a lower content in water. Thus, the clusters are dissolved and the hydrodynamic size of the micelles decreases [39,40].

3.4. Rheological behaviour

To evaluate the repercussions of the pH-sensitive aggregation process on the bulk properties of the copolymer solutions, the viscoelastic behaviour of the Tetronic solutions was analysed. At 20 °C, the values of the storage modulus (G′) of T904 30% solutions were negligible, and the log-log plot of the loss modulus (G″) vs. angular frequency showed a slope close to 1 (Fig. 6A). These findings evidence that the T904 solutions behave as Newtonian viscous fluids. The lower loss modulus of the solution prepared in HCl 0.1 M can be attributed to the more hydrophilic character of the Tetronic chains, which is responsible for the formation of small micelles that

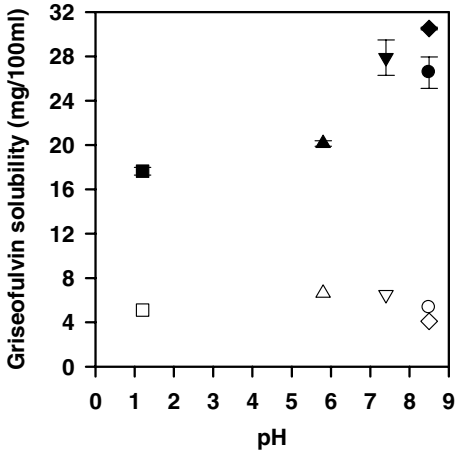

Fig. 7. Griseofulvin solubility in media of different pH and ionic strength without (open symbols) or with (full symbols) 10% T904. Legend: HCl 0.1 M (squares), pH 5.8 phosphate buffer (up triangles), pH 7.4 phosphate buffer (down triangles), water (circles) and 0.9% NaCl solution (diamond).

Table 2
Fractions of drug that are free in the medium (f_f) and micelle-incorporated (f_m), and solubilization capacities of T904 in solutions prepared with the copolymer at 10% in different media

Medium	Drug distribution		Solubilization capacity		
	f_f	f_m	mg/g T904	mol/mol T904	mg/g Hydrophobic block
NaCl 0.9%	0.134	0.866	2.64 (0.01)	0.050 (0.001)	4.48 (0.02)
Water	0.201	0.799	2.12 (0.14)	0.040 (0.002)	3.60 (0.24)
pH 7.4 buffer	0.250	0.750	2.09 (0.16)	0.040 (0.003)	3.54 (0.27)
pH 5.8 buffer	0.379	0.621	1.25 (0.02)	0.024 (0.001)	2.12 (0.04)
HCl 0.1 M	0.288	0.712	1.25 (0.03)	0.024 (0.001)	2.13 (0.06)

remain individualized. Fig. 6B shows the evolution of the loss and the storage moduli as the temperature raises. All systems, except the one prepared in HCl 0.1 M, exhibit a sol-gel transition around 30 °C. As the temperature increases, the PPO and PEO blocks become less soluble in water and, thus, more hydrophobic. Consequently more micelles are formed at a given concentration as the temperature increases. This causes gelation due to the close packing of the micelles into body centred cubic phase gels [41]. The increase of the volume fraction of micelles is shown as a maximum in the values of both moduli for a certain temperature. At greater temperatures, the dehydration of PPO chains, and even of PEO blocks, causes the polymer to phase separate, and G′ and G″ values to decrease. The absence of storage modulus for T904 solutions prepared in HCl 0.1 M clearly indicates that the copolymer chains cannot entangle when they are strongly protonated. Therefore, in an high acidic physiological environment it is not expected that T904 systems become gel-like.

3.5. Solubilization ability

From the point of view of the pharmaceutical use of Tetronics, its ability to solubilize hydrophobic drugs is a critical property. Since this solubilizing effect is a consequence of the incorporation of the solute to the micelles, the changes in the CMC and micelle conformation could notably condition their performance. Fig. 7 shows the effect of the pH of the medium on the solubility of griseofulvin in 10% T904 solutions; griseofulvin was chosen as a model of pH-independent poorly soluble drug (4 mg/100 ml). As can be observed, although T904 micelles are able to increase drug solubility even in HCl 0.1 M (3-fold), the solubilizing capability is twice as big at alkaline pH (6-fold). The fraction of drug that is hosted by the micelles as well as the amount of drug that is incorporated per gram of PPO blocks clearly raised as the pH increased (Table 2). These findings are related to the influence of pH on the protonization and hydrophobicity of the unimers and, consequently, on the concentration and properties of the micelles. The lower the pH, the lower the number of micelles in the medium. The griseofulvin solubilization capacity values obtained for T904 are in range of those previously reported for Tween 80 (3.4 mg/g) and Cremophor EL (2.6 mg/g) [42].

3.6. Drug release

Griseofulvin diffusion from the 30% T904 solutions to the receptor medium proceeded slowly and the profiles of amount released per unit area vs. the square root of time were linear ($r^2 > 0.98$). The values of the diffusion coefficients obtained for 30% T904 systems prepared in HCl 0.1 M ($1.46\ 10^{-4}$; s.d. $8.5\ 10^{-6}$ cm^2/min) were almost twice those recorded for the same systems prepared in pH 7.4 phosphate buffer ($0.89\ 10^{-4}$; s.d. $8.7\ 10^{-6}$ cm^2/min). The higher affinity of the drug for the micelles and the greater viscosity of the systems prepared at pH 7.4 explain the pH-dependence of the diffusion.

4. Conclusions

T904 cannot be considered as a non-ionic surfactant since its self-aggregation properties are strongly dependent on pH and ionic strength of the medium in the range of physiological values. This dependence of the conformation and hydrophilicity of the Tetronic unimers and micelles has a great repercussion on the critical micellar concentration, on the sensitiveness of its solutions to the temperature, and on the drug solubilizing capability of the micelles. At pH < pK_{a1}, the central diamine group is diprotonated and causes the unimers to repel each other. This makes the self-aggregation more difficult and the size of the micelles significantly lower in a higher acidic pH medium than in neutral-alkaline solutions, which is reflected in changes in the aggregation number. At acid pH not only micellization is hindered but also the *in situ* gelling behaviour is lost; consequently drug diffusion is facilitated. Such alterations in self-assembly have to be taken into account when using Tetronic in the design of drug carriers and, if conveniently modulated, this pH-responsive behaviour could be useful for obtaining site-specific drug delivery systems.

Acknowledgments

This work was financed by the Ministerio de Educación y Ciencia (SAF2005-01930; RYC2001-8) FEDER, and Xunta de Galicia (PGIDT01PX1203014IF; PGIDIT05BTF20301PR), Spain. The authors express their gratitude to BASF Corporation for providing samples of Tetronic® varieties and to Dr. P. Taboada for his help with the DLS experiments.

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