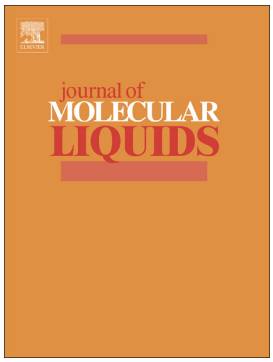


Poloxamine/D-α-Tocopheryl polyethylene glycol succinate (TPGS) mixed micelles and gels: Morphology, loading capacity and skin drug permeability

Joan Puig-Rigall, María J. Blanco-Prieto, Carlos Aydillo, Aurel Radulescu, Dolores Molero-Vilchez, Cécile A. Dreiss, Gustavo González-Gaitano

PII: S0167-7322(20)37172-5

DOI: https://doi.org/10.1016/j.molliq.2020.114930

Reference: MOLLIQ 114930

To appear in: *Journal of Molecular Liquids*

Received date: 11 October 2020

Revised date: 24 November 2020

Accepted date: 30 November 2020

Please cite this article as: J. Puig-Rigall, M.J. Blanco-Prieto, C. Aydillo, et al., Poloxamine/D-α-Tocopheryl polyethylene glycol succinate (TPGS) mixed micelles and gels: Morphology, loading capacity and skin drug permeability, *Journal of Molecular Liquids* (2020), https://doi.org/10.1016/j.molliq.2020.114930

Poloxamine/D-α-Tocopheryl polyethylene glycol succinate (TPGS) mixed micelles and gels: morphology, loading capacity and skin drug permeability

Joan Puig-Rigall[a], María J. Blanco-Prieto[b,f], Carlos Aydillo[b,f], Aurel Radulescu[c], Dolores Molero-Vilchez[e], Cécile A. Dreiss[*d], Gustavo González-Gaitano*[a]*

[a] Departamento de Química, Universidad de Navarra, 31080 Pamplona, Spain
[b] Departamento de Tecnología y Química Farmacéuticas, Universidad de Navarra, 31080 Pamplona, Spain
[c] Jülich Center for Neutron Science, JCNS at Heinz Maier-Leibnitz Zentrum MLZ, Forschungszentrum Jülich GmbH, Lichtenbergstraße 1, 85747 Garching, Germany
[d] Institute of Pharmaceutical Science, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, UK
[e] CAI-RMN, Universidad Complutense de Madrid, Facultad de Químicas, Avda. Complutense s/n, Madrid, 28040, Madrid, Spain
[f] Instituto de Investigación Sanitaria de Navarra, IdiSNA, Irunlarrea 3, 31008, Pamplona, Spain

ABSTRACT

The combination of polymeric surfactants with different features into mixed micelles give access to properties that may be superior to the single-component micelles. In this work, we investigated synergistic effects in mixtures of D-α-Tocopheryl polyethylene glycol succinate (TPGS) with poloxamines (also known as Tetronic), pH-responsive and thermogelling polyethylene oxide (PEO)-polypropylene oxide (PPO) 4-arm block copolymers. We examined the morphology of the self-assembled micelles of TPGS with Tetronic 1107 (T1107) and 908 (T908) in the presence of naproxen (NA), used as a model drug, and assessed the capacity of the single and mixed micelles to trap the guest, using a combination of small-angle neutron scattering (SANS) and NMR spectroscopy (1D, 2D-NOESY and diffusion NMR), over a range of compositions and temperatures, in the dilute regime and gel state. NA did not interact with T1107 or T908 in their unimer form, but it was incorporated into the hydrophobic core of the micelles above the critical micellar temperature (CMT). In contrast, TPGS dissolved NA at any temperature, mainly in the tocopherol core, with some partitioning in the PEG-shell. The micellar structure was not altered by the presence of NA, except for an expansion of the core size, a result of the preferential accumulation of NA in that compartment. The solubility of the drug in single component micelles increased markedly with temperature, while mixed micelles produced an intermediate enhancement of the solubility between that of TPGS and the poloxamines, which increased at higher TPGS/poloxamine ratios. Micellar hydrogels formed by the packing of the polymeric mixed micelles in a BCC macrolattice, whose structure was not altered by the presence of the drug (at least at 0.2 wt %). The applicability of the drug-loaded

gels for topical formulations was explored by transdermal diffusion testing using a synthetic model of skin, showing that the diffusion of NA across the membrane was enhanced by incorporating small amounts of TPGS to the hydrogel, especially with the more hydrophilic T908.

Keywords: polymeric surfactants; micelles; gels; SANS; DLS; diffusion NMR; Tetronic; TPGS; naproxen; transdermal

Abbreviations. Critical micellar concentration (CMC); critical micellar temperature (CMT); core-shell sphere (CSS); diffusion coefficient (*D*); dynamic light scattering (DLS); Diffusion Ordered Spectroscopy (DOSY); Food and Drug Administration (FDA); hard-sphere structure factor (HS); aggregation number (N_{agg}); small-angle neutron scattering (SANS); scattering length density (*sld*); Tetronic 908 (T908); Tetronic 1107 (T1107); temperature of gelation (T_{gel}); D-α-Tocopheryl polyethylene glycol succinate (TPGS); naproxen (NA)

1. INTRODUCTION

Amphiphilic block copolymers spontaneously-self-assemble into nanosized colloidal structures with narrow size distributions,[1] and constitute the simplest type of polymer-based nanocarriers. Specifically, polymeric micelles form above the critical micellar concentration (CMC) and critical micelle temperature (CMT),[2] where the micelle core, formed by the hydrophobic segments of the copolymer, provides the entrapment locus for hydrophobic molecules and controls their release profile, while the hydrophilic shell stabilizes the core and ensures the solubilization stability of the aggregate.[3–7] Raising the concentration and/or temperature can lead to further organization of the polymeric micelles into physical gels, which provides opportunities for sustained delivery from a depot and other biomedical applications.[8–10] A wide variety of copolymers can be used to produce polymeric micelles and hydrogels, whose physicochemical and biological properties can be tuned by changing the configuration, architecture or ratio of the constituting blocks.[10]

Among the amphiphilic block copolymers based on polyethylene oxide (PEO) and polypropylene oxide (PPO), the family of Tetronic® (BASF) surfactants (also known by the generic name of poloxamines), is attracting increasing attention.[11–13] They present a four-arm star structure, in which a central ethylene diamine spacer connects the arms, each formed by a PPO and a PEO block. They are characterized by pH- responsiveness, due to the protonation of the central diamine group,[14,15] as well as a rich phase behaviour, modulated by varying the length of the blocks. Different molecular weights, hydrophilic-lipophilic balance (HLB), CMC and CMT, or gel point can thus be obtained.[16,17] Response to temperature and pH changes make Tetronic micelles and gels interesting, especially as nanocarriers for controlled drug and protein release,[18–21] thus expanding the range of applications of their linear counterparts, Pluronic®[22–24].

D-α-Tocopheryl polyethylene glycol succinate (TPGS) is a water-soluble derivative of vitamin E with a polyethylene glycol (PEG) chain which is attracting great interest.[25,26] TPGS shares with poloxamines the ability to form polymeric micelles and the type of hydrophilic part, while inhibiting more efficiently the activity of P-glycoprotein transporters (P-gp), responsible for the poor permeability of many anticancer drugs through physiological barriers.[27,28] TPGS-1000, which comprises a 23 ethylene oxide (EO)-unit hydrophilic block, has been the most studied to date. It self-aggregates at 0.02%, forming spherical core-shell micelles of aggregation number around 100, which are very stable with temperature.[29]

Considering the interesting features of both Tetronic and TPGS, it is expected that their mixtures may combine pH- and temperature responsiveness and gelation capacity (Tetronic), with the higher biocompatibility and potential antioxidant properties of TPGS, expanding the range of drugs that can be loaded, their release profiles, and modifying their permeability across

barriers.[30] There are very few studies in the literature on the combinations of different Tetronic for the solubilisation of drugs,[31,32] as well as on the combined use of TPGS and poloxamines.[33,34] Recently, we have reported a detailed structural characterisation by scattering and spectroscopic methods of TPGS-Tetronic mixtures, using Tetronic 1107 (60 EO and 20 PO units per arm), and Tetronic 908 (114 EO and 21 EO units per arm).[35] Spherical core-shell micelles comprising both surfactants in their structure (mixed micelles) were found to form, in which Tetronic unimers incorporate into TPGS aggregates below the CMT of the poloxamine, while mixed micelles only form under limited conditions with T908. At high concentration and body temperature, small proportions of TPGS extend the gel phase and significantly improve the cell viability of NIH/3T3 fibroblasts, making poloxamine gels doped with TPGS promising platforms for drug delivery as ointments, to promote the topical administration of drugs.

In this work, we investigate the capacity of the mixed micelles and gels formed from TPGS, Tetronic 1107 and Tetronic 908 to encapsulate the anti-inflammatory naproxen. This molecule was selected because of its well-established quantification methods[36–38] and its hydrophobic character, which makes it a suitable model to test the solubility and permeability of poorly water-soluble drugs. Specifically, we examined the morphology of the self-assembled structures of TPGS, Tetronic and their mixtures in the presence of the drug over a range of compositions and temperatures, using a combination of small-angle neutron scattering (SANS), NMR methods (1D, 2D-NOESY and diffusion NMR). The solubilisation capacity of the micelles was quantified by diffusion NMR and fluorescence spectroscopies, and the applicability of the gels for topical formulations explored by transdermal diffusion testing using a synthetic model of skin (Strat-M® membranes).

2. MATERIALS AND METHODS

Materials. Tetronic® 1107 (T1107) and Tetronic® 908 (T908) were a gift from BASF (Ludwigshafen, Germany). The reported composition per arm is 60 ethylene oxide (EO) and 20 propylene oxide PO for T1107, with an average molecular weight 15,000 $gmol^{-1}$. T908 comprises 114 EO and 21 PO, with an average molecular weight of 25,000 $gmol^{-1}$. D-α-Tocopheryl polyethylene glycol succinate (TPGS), with PEG molecular weight of 1000 (23 EO), and naproxen (NA, purity ≥ 98.5%) were obtained from Sigma-Aldrich (Merck KGaA). All the solutions were prepared by weight, unless stated otherwise, and the concentrations expressed in wt%.

NMR spectroscopy. A Bruker Avance Neo 400 MHz spectrometer was used for DOSY and NOESY experiments. For the diffusion experiments, bipolar pulse longitudinal eddy current

delay (BPLED) pulse sequences were used (ledbpgp2s1d and ledbgpg2s). 1D proton spectra were recorded first for each gradient applied, and the attenuation of selected resonances of each spectrum analysed by integration. The separation of the gradients and their duration, defined as Δ and δ, respectively were optimized to ensure a complete exponential decay of the signal as the gradient increases. 2D Diffusion Ordered Spectroscopy representation (DOSY), with the NMR spectra on the x-axis and the diffusion coefficient (*D*) on the y-axis, was obtained with Mnova (Mestrelab research), by the Bayesian transformation of the collected spectra, and TopSpin (Bruker) software. The transformation of diffusion coefficients to hydrodynamic radii (R_h) was done by introducing the measured viscosities (obtained with a Cannon-Fenske viscosimeter) into the Stokes-Einstein equation. All samples were prepared by dissolving the required amounts of NA, TPGS and the poloxamines in D_2O (Aldrich, deuterium content > 99.96%).

Small-angle neutron scattering (SANS). SANS experiments were carried out on the KWS-2 diffractometer at the Jülich Centre for Neutron Science (JCNS), Heinz Maier-Leibnitz Zentrum (MLZ), Garching, Germany.[39] An incidental wavelength of 5 Å was used with detector distances of 1.7 and 7.6 m, with a collimation length of 8 m, to cover the *q* range from 0.008 to 0.5 $Å^{-1}$. A wavelength spread $\Delta\lambda/\lambda$ = 15% was used in the standard mode, while in the concentrated regime a high-resolution mode was achieved by using a collimation length of 20 m in combination with the double-disc chopper and time-of-flight data acquisition for an improved wavelength spread of $\Delta\lambda/\lambda$ = 5%. All samples were measured in quartz cells (Hellma) with a path length of 2 mm using D_2O as the solvent. The cells were placed in an aluminium rack where water was recirculated from an external cryostat, with temperatures ranging from 20 ºC to 50 ºC (0.1 ºC precision). Absolute scattered intensities (obtained after correction for detector pixel efficiency, empty cell scattering and background) were reduced with the QtiKWS software provided by JCNS in Garching.[39] For the micellar systems, the scattering data were described by core-shell spheres (CSS), combined to a hard-sphere (HS) structure factor. For the micellar gels, SANS data were analysed with a BCC (body centred cubic) paracrystal model.[17,40] SasView 4.2.0 was used to fit the SANS data.[41] The equations, parameters definition and fitting procedures are detailed in the SI.

Sol-gel diagrams. Solutions of T1107, T908, TPGS-T1107 and TPGS-T908 with concentrations up to 30% were prepared in water, in the absence or presence of NA (0.2% or 1%). Repeated cycles of stirring and cooling (4 ºC) were performed until a homogeneous and transparent solution was obtained. About 1.5 mL of each sample was placed in glass tubes and introduced in a Thermo Scientific digital cooling dry bath, where they were gradually heated up from 20 to 70 ºC, and the physical appearance (sol, viscous liquid or gel) noted after 5 min of thermal homogenization and rapid tube inversion at each temperature.

Solubility studies. Solubility of NA up to 24 hours was determined in solutions of TPGS, T1107, T908, TPGS-T1107 and TPGS-T908 in water for a total concentration of surfactants of 1% at 37 ºC. Three independent experiments were performed. For each experiment, 20 mL of the solutions placed in glass tubes were introduced in a Unitronic reciprocating shaking bath (JP Selecta) with an excess of the drug at a speed of 30 oscillations per minute. At selected times, 1 mL of the solution was removed and filtered with 0.45 μm pore size nylon filters. Dissolved NA was quantified by measuring the intrinsic fluorescence of the drug. The emission spectra were recorded with a FLS920 spectrofluorimeter (Edindburgh Instruments), in 1 cm path-length quartz cuvettes. A 450 W Xe lamp was used as the source, by exciting at 330 nm and collecting the emission at 351 nm, with 2.5 nm excitation and emission slits. The concentration of the drug was calculated by linear calibration of the normalized fluorescence in the $6 \cdot 10^{-6}$ – $7 \cdot 10^{-5}$ M range. Under the instrumental conditions used, an average value of the slope of 15600 ± 300 M^{-1} was obtained (three replicates, $R^2 > 0.99$ each).

Permeability studies. Strat-M® synthetic membranes were used as test models for transdermal diffusion (Merck Millipore Ltd). The membranes (25 mm diameter), were mounted on the Franz type diffusion cells, with an available diffusion area of 1.77 cm^2 (Hanson Research Corporation). 2 g of formulations with 20 mg of the drug (NA) or the drug suspension in water were placed in the donor chamber, in contact with the membrane. The receptor chamber contained 7 mL of phosphate buffer solution (pH= 7.4) and subjected to continuous stirring (350 rpm). Temperature was set at 32.5 ± 0.1 ºC to simulate the 32 ºC of the skin, in accordance with manufacturer recommendation. At given intervals of time, 1 mL aliquots were collected and replaced with the same volume of fresh buffer. The quantification of NA in the receptor compartment was performed by measuring the intrinsic fluorescence of the drug, as in the solubility studies (see above). The methodology provides a linear response of the detector in the concentration range 0.020-12 μg/mL (three replicates, $R^2 > 0.999$ each), with a limit of quantification of 0.015 μg/mL.

3. RESULTS AND DISCUSSION

3.1. Dilute regime. Interaction of naproxen with T1107, TPGS and TPGS-T1107 micelles

3.1.1 Solubilisation of NA in T1107 and TPGS micelles

Tetronic 1107 self-aggregates into core-shell micelles above 35ºC, at 1% concentration.[40] The interactions between a saturated solution of NA and 1% T1107 was studied first by ^{1}H NMR at 25 ºC, below the CMT of the poloxamine. No changes in the resonances of the aromatic protons of NA (7-8 ppm, SI, Figure S1) are detected (Figure 1A), suggesting a lack of interaction between the drug and the poloxamine in unimer form. To further confirm this, NMR diffusion

experiments were performed on NA + T1107 mixtures at this temperature. Two different profiles are obtained for NA and T1107, with diffusion coefficients, *D*, of $4.44 \cdot 10^{-10}$ and $4.76 \cdot 10^{-11}$ $m^2 \cdot s^{-1}$, respectively, in accordance with the diffusion of NA molecules and T1107 unimers alone (SI, Figure S2). In contrast, above the CMT (1% T1107 and 40 ºC), the signals of the aromatic protons of NA undergo slight downfield shifts (Figure 1B), revealing a different magnetic environment of the drug. ^{1}H NMR spectra of the drug in the presence of PEG 4000 were recorded for comparison (0.66%, the same effective concentration of EOs as in a 1% T1107 solution), but no significant shifts in the aromatic region of the spectrum were detected at either 25 or 40 ºC (Figure 1C), constituting evidence that the solubilisation of NA takes place mainly in the core of the poloxamine micelles.

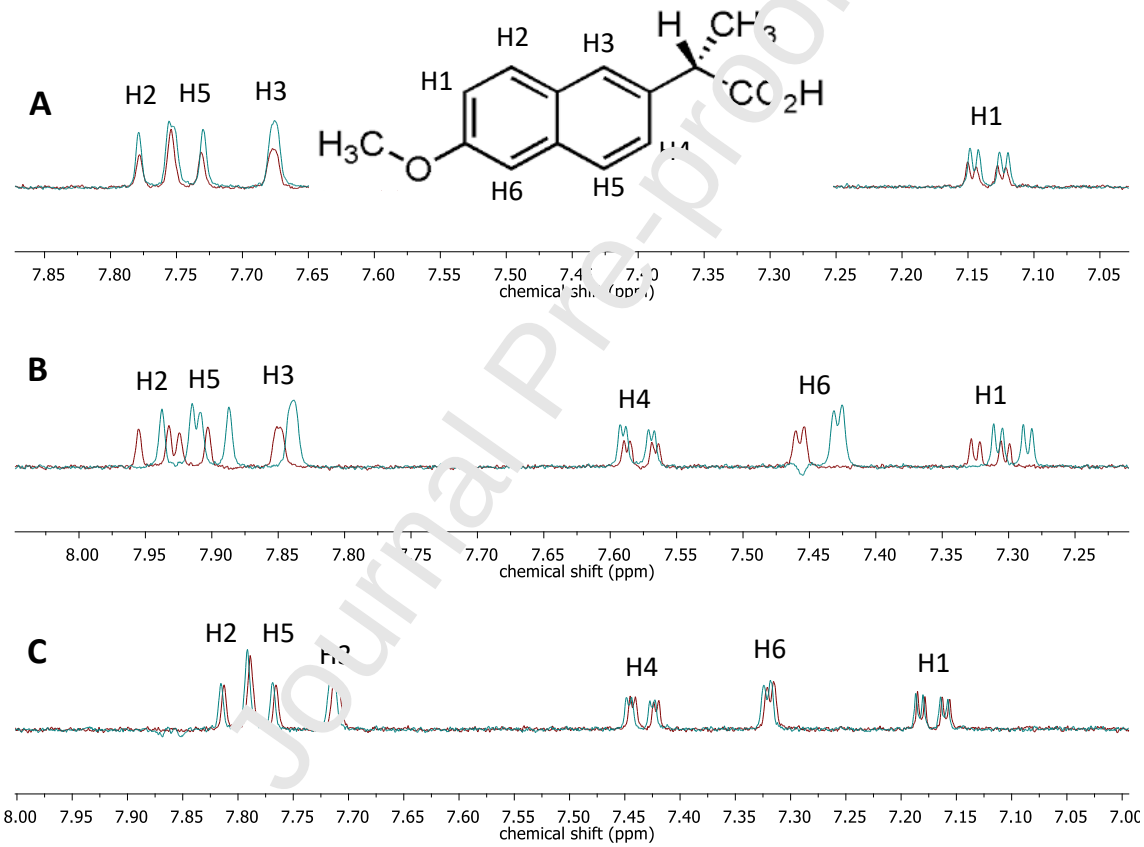


Figure 1. ^{1}H NMR spectra of the aromatic protons of NA alone (red trace) with the addition of either (A) 1% T1107 at 25 ºC, (B) 1% T1107 at 40 ºC, and (C) 0.66% PEG 4000 at 25 ºC (blue traces).

The effect of the incorporation of NA in T1107 aggregates was then assessed by SANS. The experiments were performed at 37 and 50 ºC with 5% poloxamine with a saturated solution of the drug (Figure 2). A core-shell form factor combined to a hard-sphere structure factor (CSS-HS) was used to fit the data for T1107 and NA+T1107 (cf. Materials and Methods section and SI). In the calculations, the scattering length density (*sld*) of the core was fixed at $3.44 \cdot 10^{-7}$ $Å^{-2}$

for T1107 (reflecting a completely dehydrated core, as found in previous studies),[40] while it was left free for NA+T1107 mixtures. The scattering curves and corresponding fits are plotted in Figure 2A and the relevant parameters obtained for both systems gathered in Table 1. The analysis of the fits reveals that the overall micellar size is not affected by the addition of the drug, with a total radius of ca. 7.5 and 8 nm at 37 and 50 ºC, respectively. However, the dimension of the core increases in the presence of NA and the *sld* slightly increases from $3.44 \cdot 10^{-7}$ Å^{-2} (pure PO) up to $9 \cdot 10^{-7}$ Å^{-2} (the *sld* of NA is $2 \cdot 10^{-6}$ Å^{-2}), which could be explained by the incorporation of the drug in the hydrophobic core of the micelle, as also suggested by NMR (Figure 1B). The volume fraction of the loaded micelles is similar to that of T1107, and so is the *sld* of the shell, whose values are close to that of D_2O, reflecting a hydration of ca. 93% and 91% at 37 and 50 ºC, respectively. The large extent of water contained in the corona justifies the large micellar volume fractions observed. Polydispersity was left as a floating parameter in the fits, and values between 0.15-0.30 were obtained, in agreement with previous studies.[35]

Table 1. Structural parameters obtained from SANS data analysis of 5% surfactant micelles in D_2O in the absence and presence of naproxen (NA): R_c (core radius, Å), t (shell thickness, Å), ϕ (volume fraction of micelles), ρ_{shell} (*sld* of the shell, Å^{-2}).

	T (ºC)	R_c	t	ϕ	$\rho_{shell} \cdot 10^6$
T1107	37	27	47	0.20	6.02
	50	32	47	0.19	5.84
NA+T1107	37	30	43	0.19	5.98
	50	37	43	0.20	5.86
TPGS	20	36	29	0.13	5.68
	37	36	27	0.12	5.60
NA+TPGS	20	35	26	0.14	5.80
	37	35	24	0.12	5.71

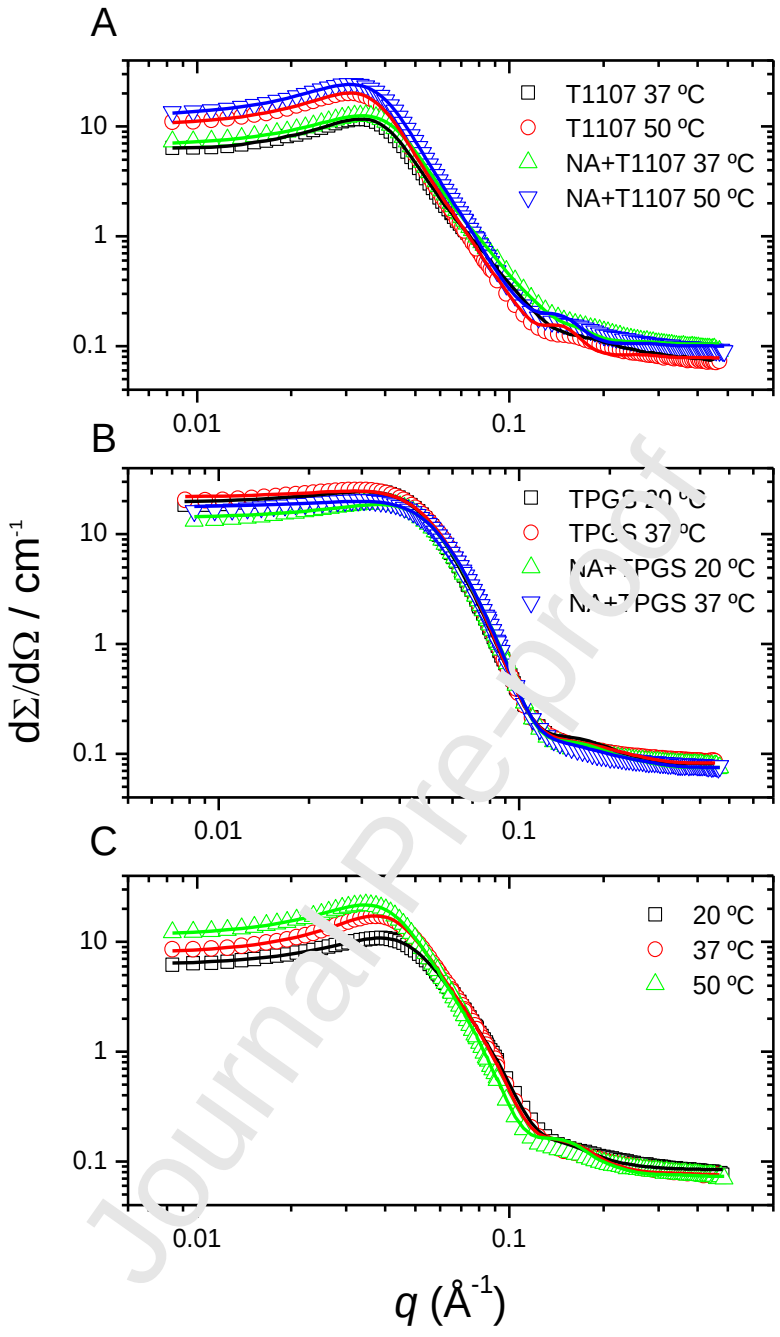


Figure 2. SANS curves from (A) 5% T1107 with and without NA, (B) 5% TPGS with and without NA, and (C) 2.5% TPGS + 2.5% T1107 with NA as a function of temperature in D_2O. Solid lines correspond to fits to a CSS-HS model (see text for details).

We next turn to solubilisation of the drug in TPGS micelles. Unlike T1107, TPGS forms micelles over a wide interval of temperatures and concentrations.[29] The core of TPGS micelles is formed by the packing of hydrophobic tocopheryl moieties, which makes this surfactant capable of dissolving non-polar drugs like NA, as confirmed by the significant shifts of the

signals from the aromatic protons of NA in the ^{1}H NMR spectrum (SI, Figure S3). This is corroborated by the 2D NOESY spectrum by the distinct cross-peaks between the aromatic protons of the drug and the tocopheryl moiety (aliphatic region, 0.5-2 ppm) (Figure 3). It is worth noting that some interactions also occur between NA and the PEG block (3.5 ppm) of TPGS, indicating some presence of the drug in the shell.

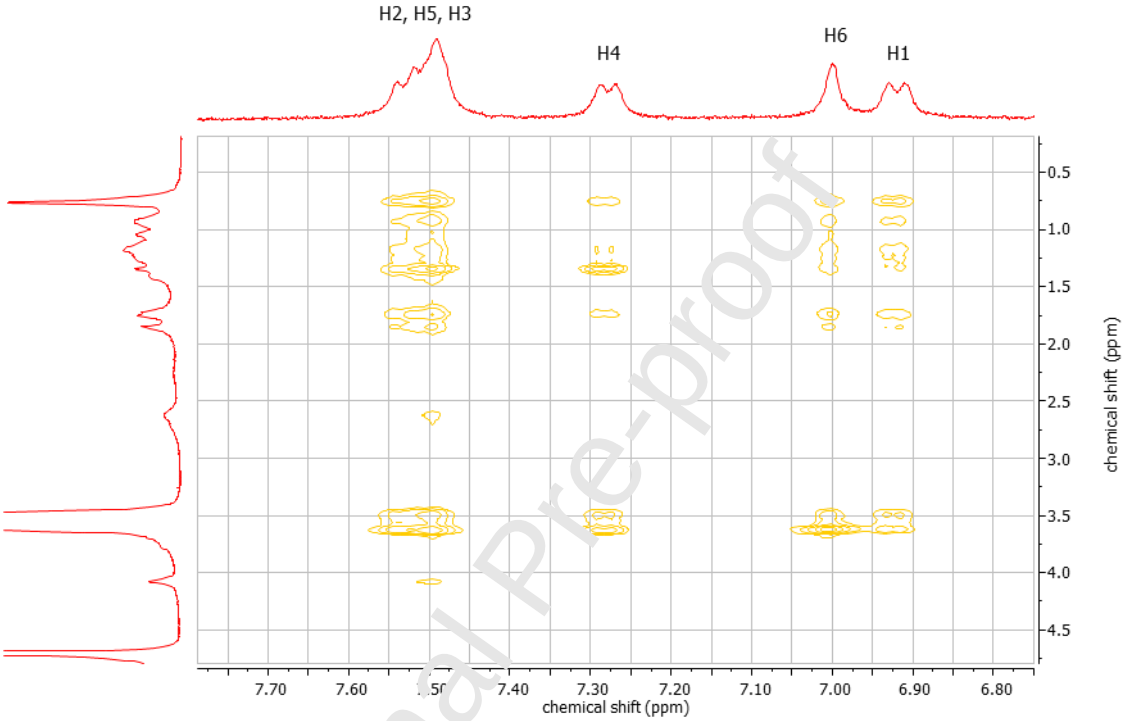


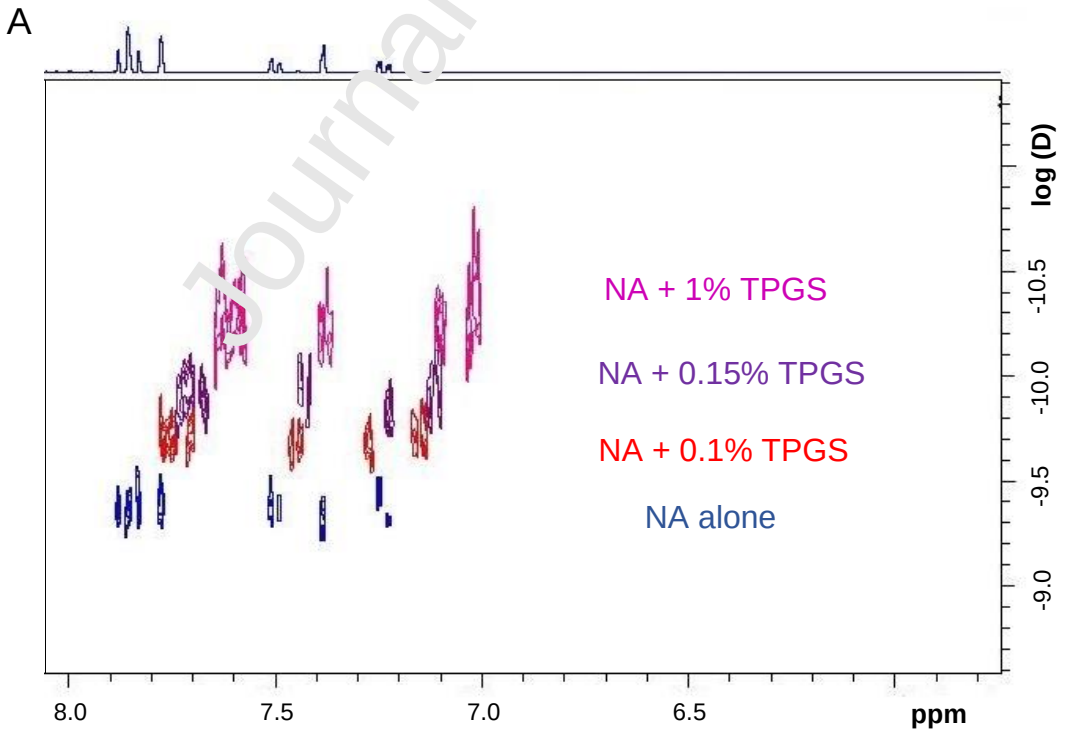
Figure 3. Partial view of the 2D NOESY spectrum of NA + 1% TPGS (D_2O, at 25ºC).

NMR diffusion experiments were performed to quantify the maximum amount of NA encapsulated. When the drug becomes incorporated into the micelle, its diffusion coefficient is reduced considerably with respect to its value in water, since its moves at the same speed as the aggregate. From the analysis of the variations in the diffusion coefficient as a function of the surfactant concentration (up to 1%, with an excess of NA, 6 mg/g in our experiments), the partitioning of the drug between the solvent and the micelles can be obtained. Figure 4A shows the DOSY representation of the diffusion coefficients obtained with the aromatic protons of the drug. The NA signals shift along the x-axis (upfield) while the diffusion coefficient decreases with increasing surfactant concentration. The fact that a single set of signals is observed in the plot at each surfactant concentration indicates a fast exchange of the drug between the solution and the micelles. The fraction of bound NA ($X_{NA,m}$) can be quantified from the measured diffusion coefficient, D_{NA}, according to:

$$D_{NA} = D_{NA,s}X_{NA,s} + D_{NA,m}X_{NA,m} \qquad (1)$$

where $D_{NA,s}$ corresponds to the diffusion coefficient of free naproxen ($4.44 \cdot 10^{-10}$ $m^2 \cdot s^{-1}$), $D_{NA,m}$ to the diffusion coefficient of the drug in the TPGS micelle, which is equated to that of a TPGS micelle ($2.77 \cdot 10^{-11}$ $m^2 \cdot s^{-1}$), and *X* represents the corresponding fraction of NA (free or bound to the micelle). This equation is conceptually similar to the one applied to describe inclusion complexes between different types of surfactants and cyclodextrins by gradient NMR.[42,43] Figure 4B shows the values of *D* and calculated fractions of NA from equation 1. As TPGS concentration increases, so does the fraction of bound drug, up to 95% for 1% TPGS, while nearly 80% of the drug is encapsulated at significantly lower concentrations of surfactant (0.15%), concentration below which a drastic drop of the NA fraction is observed.

Once the fraction of encapsulated and free NA is known, the concentration of drug solubilised can be estimated by ^{1}H NMR, taking into account the relative areas of the signals of TPGS (terminal methyls, 12 protons) and NA (aromatic region, 6 protons) in the spectrum. The results, gathered in Table 2, show the increasing solubilisation of the drug by TPGS, up to 20-fold its water solubility with 1% TPGS.[44]



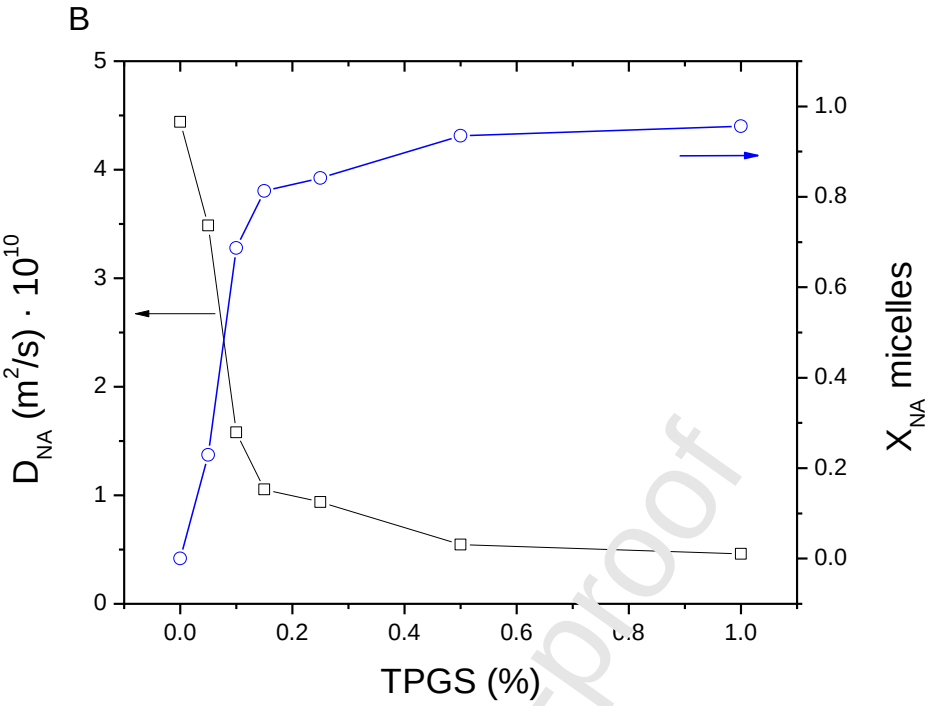


Figure 4. (A) Diffusion coefficient of NA as a function of TPGS concentration at 25 ºC. (B) Diffusion coefficient and fraction of drug encapsulated in TPGS micelles as a function of surfactant concentration at 25 ºC.

The interactions between NA and TPGS were also studied at higher temperatures. Similarly to 25 ºC, changes in the chemical shifts of the aromatic protons of the drug confirm its solubilisation in the micelles at 40 ºC (SI, Figure S3). The solubilisation of the drug is favoured by increasing the concentration of TPGS, as revealed by the comparison of the measured chemical shifts in the presence of surfactant with those from NA alone, δ_0 (SI, Figure S4). The total amount of NA solubilised, quantified by the integration of the ^{1}H NMR spectra as described above (Table 2A) and yields results that are in agreement with the results from fluorescence at 1% TPGS (540 mg·L^{-1}, shown further down).

Table 2A. Solubility of NA as a function of TPGS concentration at 25 and 40 ºC, estimated by NMR ($c_{NA,m}$ is the concentration of NA solubilised in the micelles; *Total* represents $c_{NA,m}$ plus the concentration of free NA (in the solvent)

		c_{NA} (mg·L^{-1})	
Temperature	**TPGS (%)**	$c_{NA,m}$	***Total***
25 ºC	0	-	16*
	0.1	71	104

	0.15	114	141
	0.25	192	228
	0.5	308	330
	1	461	482
40 ºC	0	-	28**
	0.1	-	137
	0.15	-	156
	1	-	533

* Extracted from Yalkowsky et al.[45]

** Obtained from solubility studies (shown further down)

The effect of NA on TPGS micelles was studied by SANS at 20 and 37 ºC, conditions under which micelles are fully formed. The previous CSS-HS model was also used to fit the data. Scattering curves and fits are plotted in Figure 2B, and the fitted parameters gathered in Table 1. The analysis reveals that the volume fraction, hydration of the shell and polydispersity of the loaded micelles are unaffected by either temperature or NA solubilisation. As observed for T1107 micelles, the incorporation of the drug induces a slight shrinking of the shell of the micelles, while, in this case, the core size is barely affected, producing slightly smaller micelles. This agrees with the slightly faster diffusion of NA-TPGS micelles (D = 2.95·10^{-11} m^2·s^{-1}) compared to TPGS alone (D = 2.77·10^{-11} m^2·s^{-1}).

3.1.2 Solubilisation of NA in mixed T1107-TPGS micelles

The simultaneous presence of TPGS and T1107 induces the formation of mixed micelles.[35] Below the CMT of the poloxamine, the region of micellar existence is expanded due to the presence of TPGS, while the fraction of T1107 in the mixed micelles increased above the CMT (40 ºC).[40] The fraction of poloxamine in the micelles, X_{T1107}, can be assessed by gradient NMR, using the CH_3 signal of the PPO block at 1 ppm. We assume that this mode is the weighted average of free T1107 unimers (D = 4.76·10^{-11} m^2·s^{-1}) and the poloxamine that takes part into the mixed micelles (ranging from 2.4 to 3.2·10^{-11} m^2·s^{-1}, as obtained from the DOSY map of each system). Since T1107 does not micellise at 25 ºC,[40] the slower diffusion mode must correspond to mixed TPGS-T1107 micelles. Up to 30% of the poloxamine added forms micelles in a 0.75% TPGS + 0.25% T1107 mixture (Table 2B), close to the value obtained for 1% TPGS + 1% T1107 system (ca. 33%).[35]

Table 2B. NA solubility in TPGS-T1107 mixtures (total surfactant concentrations of 1%) at 25 ºC as obtained from diffusion NMR.

	X_{T1107}	X_{NA}	$c_{NA,m}$ (mg·L^{-1})
0.25% TPGS + 0.75% T1107	0.182	0.804	248
0.5% TPGS + 0.5% T1107	0.196	0.916	264
0.75% TPGS + 0.25% T1107	0.308	0.952	312

The solubilisation of NA in TPGS-T1107 micelles was studied at 1% total polymer concentration, at 25 and 40 ºC, varying the ratio of the two surfactants. As in the single-surfactant systems, the aromatic protons of NA shift upfield in the ^{1}H NMR spectra. The relative changes, plotted in Figure 5, reveal the same behaviour at both temperatures: as the TPGS fraction in the mixed micelle increases, larger differences in δ are observed, indicating a gradual incorporation of the drug into the micelles, in accordance with the fraction of NA, X_{NA} (Table 2B). The concentration of loaded drug, $c_{NA,m}$, was estimated by diffusion NMR at 25 ºC, following the procedure previously described. The results reveal that $c_{NA,m}$ increases with TPGS concentration (Table 2B), following the trend observed with TPGS alone.

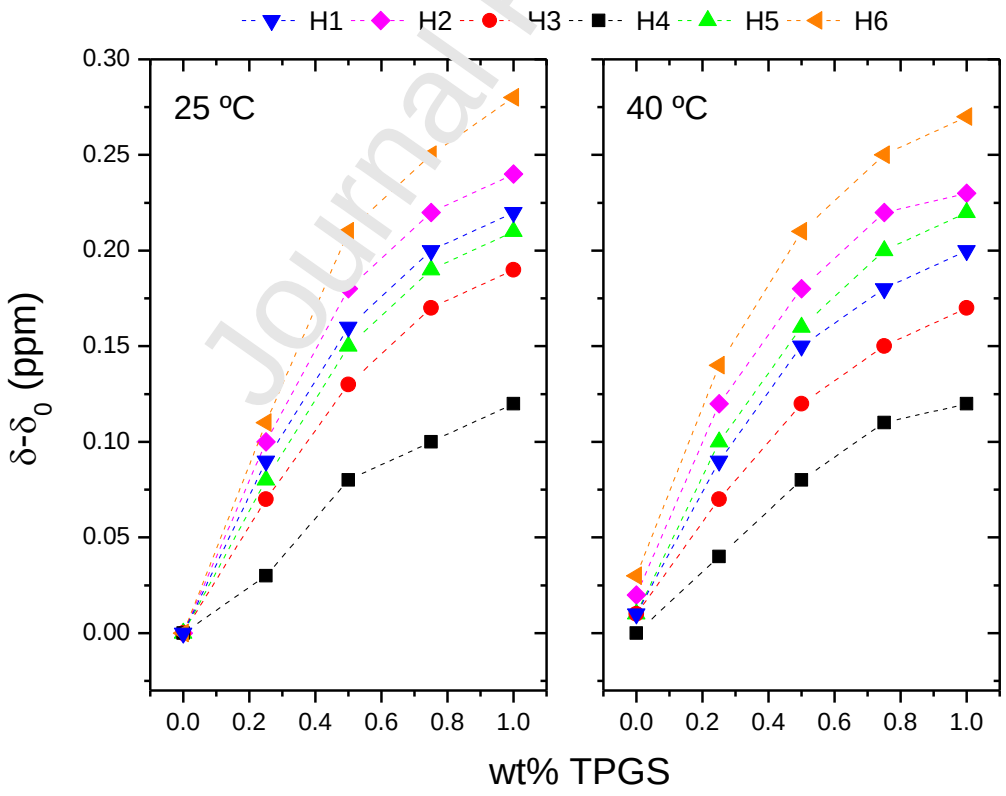


Figure 5. Relative chemical shifts of the aromatic protons of NA in TPGS-T1107 mixtures as a function of TPGS fraction at 25 and 40 ºC (1% total surfactants concentration).

The solubility of NA in the TPGS-T1107 mixture was also studied at 37 ºC and the results compared with the single-component solutions, by using the intrinsic fluorescence of the drug, as described in Material and Methods and represented in SI, Figure S5. Profiles of the solubilised drug in the different systems reveal that the maximum solubility is reached after 5 hours (Figure 6A). At 24 hours and 1% total polymer concentration, the TPGS-T1107 mixture solubilise an amount of drug (385 mg·L^{-1}) intermediate between that of TPGS (the highest, 540 mg·L^{-1}), and T1107 (the lowest, 190 mg·L^{-1}), about an order of magnitude higher than the solubility of NA in water (28 mg·L^{-1}) (Figure 6B). The results for 0.5% TPGS and TPGS + T1107 (0.5% each) at 37 ºC contrast with those obtained at lower temperatures. While at 25 ºC, less naproxen is solubilised in the mixture compared to TPGS alone (Tables 2A and 2B), at 37 ºC the drug solubilised is 380 mg·L^{-1} for the mixture (Figure 6B), close to the value for 0.5% TPGS (365 mg·L^{-1}), as interpolated from the solubilities at 25 ºC and at 40 ºC (Table 2A). The higher fraction of Tetronic unimers forming the mixed micelles, which, in turn, produces larger aggregates, and the increase in the volume fraction of the micelles as temperature increases[35] may explain this observation.

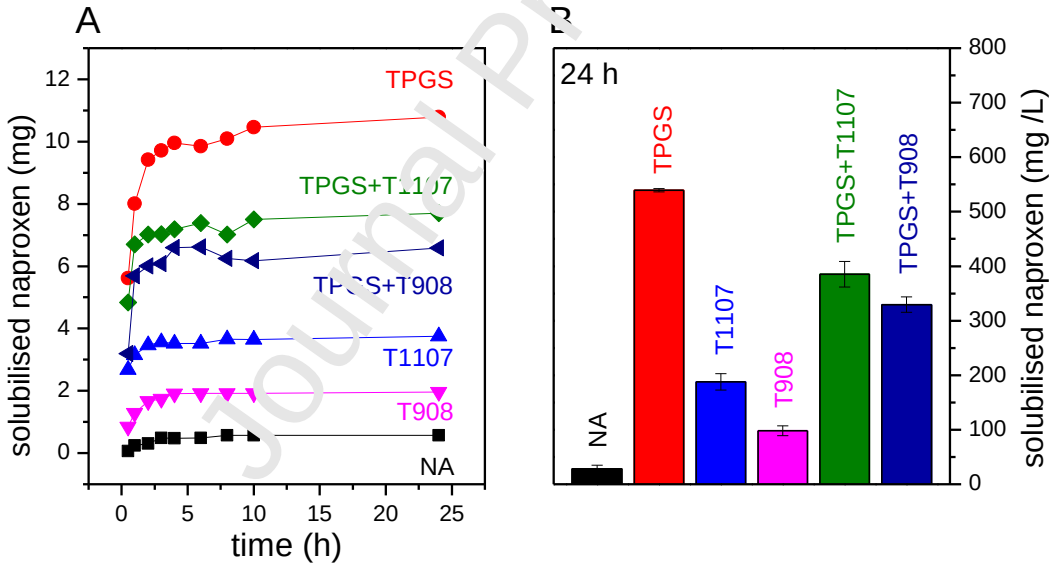


Figure 6. (A) Solubility profiles of NA, 1% TPGS, 1% Tetronic and 0.5% TPGS + 0.5% Tetronic systems in water at 37 ºC; (B) total amount of NA solubilised after 24 h (mg/L).

As done for micelles of T1107 and TPGS alone, the effect of NA on the structure of the mixed micelles was studied by SANS, using the same CSS-HS model to fit the experimental data. In this case, the *sld* of the core was left free, obtaining fitted values lower than 1·10^{-6} Å^{-2}, in accordance with previous studies,[35] confirming the little hydration of the core compared to that of the shell (ca. 6·10^{-6} Å^{-2}). The corresponding scattering curves and fits are plotted in Figure

2C and the parameters returned by the fits are shown in Table 3. The presence of the drug does not seem to modify the volume fraction and the structure of the micelles, except for a slight shrinking of the shell, as observed with the pure TPGS and T1107 micelles (Table 1). The formation of micelles is favoured by temperature, resulting in larger sizes due to the increased fraction of T1107 in the aggregates.[35] The polydispersity of the micelles does not vary in the presence of NA, ranging from 0.15 to 0.20.

Table 3. Structural parameters of TPGS + T1107 micelles with and without NA from SANS data analysis: R_c (core radius, Å), t (shell thickness, Å), ϕ (volume fraction), ρ_{shell} (*sld* of the shell, Å^{-2}).

	T (ºC)	R_c	t	ϕ	$\rho_{shell} \cdot 10^6$
1% TPGS + 1% T1107*	20	33	37	0.054	6.03
	40	37	42	0.066	5.95
5% TPGS + 5% T1107	20	32	31	0.23	6.12
	40	37	35	0.31	6.10
2.5% TPGS + 2.5% T1107 + NA	20	34	29	0.15	6.26
	37	35	34	0.19	6.09
	50	37	34	0.18	6.00

* Data from Puig-Rigall et al.[35]

3.2. Concentrated regime

3.2.1 Effect of NA on Tetronic and TPGS-Tetronic gels

T1107 forms micellar thermogels at high concentration, induced at intermediate-to-high temperatures.[40] T908, which has larger PEO blocks and therefore is more hydrophilic, also forms gels[17] and mixed micelles with TPGS.[35]. The solubility of NA was also studied for T908-containing systems in the dilute regime (Figure 6 and SI, Figure S5). A similar behaviour to the one reported for T1107 was found, but with lower solubilities; the amount of solubilised NA in TPGS-T908 mixtures (330 mg·L^{-1}), is also intermediate between the one obtained with TPGS (540 mg·L^{-1}) and T908 (100 mg·L^{-1}).

The phase diagrams of both poloxamines are compared in Table 4 and SI, Table S1, with 1% and 0.2% of NA, respectively. For T1107 and TPGS-T1107 gels, the addition of NA affects gelation by shifting the transition to higher temperatures (by about 5 ºC) for a total concentration of 25% polymer, attributed to the acidification of the medium when the drug is added, which produces the protonation of the amino groups of the poloxamine (from pH of 7.8 ± 0.2 to 5.6 ± 0.2). This shift in gelation is not observed for T908 and TPGS-T908 gels.

Table 4. Phase diagram of poloxamine gels in the absence and presence (1%) of NA in water at natural pH. ○ Solution; □ viscous solution; ● gel.

	System	*T* (ºC)								
		20	**25**	**30**	**35**	**37**	**40**	**50**	**60**	**70**
no NA	24.5% T1107 + 0.5% TPGS	□	□	□	●	●	●	●	●	●
	29.5% T908 + 0.5% TPGS	□	□	□	●	●	●	●	●	●
	29.9% T908 + 0.1% TPGS	□	□	□	●	●	●	●	●	●
	30% T908	□	□	□	●	●	●	●	●	●
with 1% NA	24.5% T1107 + 0.5% TPGS	□	□	□	●	●	●	●	●	●
	29.5% T908 + 0.5% TPGS	□	□	□	●	●	●	●	●	●
	29.9% T908 + 0.1% TPGS	□	□	□	●	●	●	●	●	●
	30% T908	□	□	□	●	●	●	●	●	●

The structure of the gels was determined by SANS. The best fits to the scattering patterns were obtained with a BCC paracrystal model, a model previously used for the poloxamine alone and TPGS-Tetronic gels.[17,35,40] In this model, the radius of the sphere corresponds to the core of the micelles. In the absence of NA, the *sld* of the spheres was set to that of PPO ($3.44 \cdot 10^{-7}$ Å^{-2}) and polydispersity fixed to 0.2, while the volume fraction of the spheres (ϕ) and lattice spacing (*dnn*) were set as floating parameters, in accordance with previous studies.[35] In the presence of 0.2% NA, the *sld* of the core may change because of the presence of the drug. In order to limit the number of floating parameters, either the *sld* of the core or the volume fraction were fixed to the value obtained in the absence of the drug, but no significant changes on *dnn*, core radius or distortion factor, *d*, were observed. The total radius of the micelles ($R_{micelle}$) is given by $R_{micelle} = \frac{\sqrt{3}}{4} dnn$, assuming an ideal packing, where spheres are in contact along the diagonal of the BCC cube. The analysis of the fits reveal that for T1107 (SI, Figure S6 and Table 5) and TPGS-T1107 (SI, Figure S7 and Table 5), the structural parameters of the gels are not affected by the addition of 0.2% NA, maintaining their BCC structure and micellar size. Compared to the diluted systems, the radius of the micelles forming the paracrystal cell is smaller in all cases, either with the addition of NA or not, which indicates a certain overlap of the PEO shells, already reported for the single poloxamines gels[35].

Table 5. Structural parameters of 25% T1107 and 1% TPGS + 24%T1107 gels in D_2O in the absence and presence of naproxen (0.2%) from SANS data analysis. *d factor* (paracrystal distortion factor), *dnn* (lattice spacing), ϕ (volume fraction), R_c (sphere radius, Å), $R_{micelle}$ (total micellar radius, Å).

	***T* (ºC)**	***d factor***	***dnn***	R_c	$R_{micelle}$
T1107*	37	0.078	156	33	68
	50	0.079	161	37	70
T1107 + NA	37	0.077	160	33	69
	50	0.080	165	37	71
TPGS + T1107*	37	0.079	160	34	69
	50	0.081	162	38	70
TPGS + T1107 + NA	37	0.078	160	34	69
	50	0.080	164	37	71

* Data from Puig-Rigall et al.[35]

3.2.2 Drug permeability studies

The applicability of the gels for topical formulations was next assessed by measuring the permeability of NA using Strat-M® membranes, which share structural and chemical characteristics with the human epidermis and are considered appropriate test models for transdermal diffusion.[46–48] Permeability of NA was measured for up to 24 hours with different Tetronic and TPGS-Tetronic systems, whose concentrations were chosen based on the results of cytotoxicity studies of TPGS-Tetronic gels,[35] and their sol-gel transition (Table 4). The amount of permeated drug for different formulations with 1% NA is represented in Figure 7. A control suspension of the drug (NA in water) was included for comparison purposes, showing higher concentration of NA in the receptor medium compared to all formulations tested (significant differences, $p < 0.05$, were found at 24 hours), in accordance with the fact that Strat-M® membranes inhibit more efficiently the penetration of the drug in semisolid forms, such as gels.[49]

Regarding the effect of the composition of the mixture on the permeated drug, by comparing TPGS-T908 formulations first, a significantly higher amount of NA was found in the receptor medium with 29.5% T908 + 0.5% TPGS with respect to 30% T908 (5-times higher at 24 hours). Interestingly, despite the relatively low proportion of added TPGS, its presence in the mixture increases drug penetration through the membranes, which is in agreement with the reported capacity of TPGS to promote drug permeation across physiological barriers.[26] On the other hand, the type of poloxamine also has an effect on permeation. At a fixed TPGS concentration, while similar amounts of NA are present in the receptor medium for 29.5% T908 + 0.5% TPGS and 24.5% T1107 + 0.5% TPGS formulations up to 12 hours, the concentration of drug permeated for 29.5% T908 + 0.5% TPGS is twice the amount for 24.5% T1107 + 0.5% TPGS at 24 hours. Structural and rheological factors may account for these results. The facility of T1107 to micellize[40] and the stability of TPGS-T1107 micelles compared to T908[35] makes T1107 more efficient to encapsulate naproxen (Figure 6B), which in turn may hamper the release of the drug. In addition, the fact that T1107 hydrogels are generally stiffer than T908 ones[35] would hinder the diffusion of the drug from the gel. Although further studies would be necessary to determine to what extent each mechanism is contributing to the membrane permeability, as well as other factors, such as the effect of the hydrophobicity of the drug in the diffusion process, or the effect of the skin pH on the structure and drug release of the gels, these preliminary studies point out that the permeation of the drug can be easily controlled by the adequate selection of the poloxamine (HLB, molar mass) and by changing the proportion TPGS/poloxamine in the formulation.

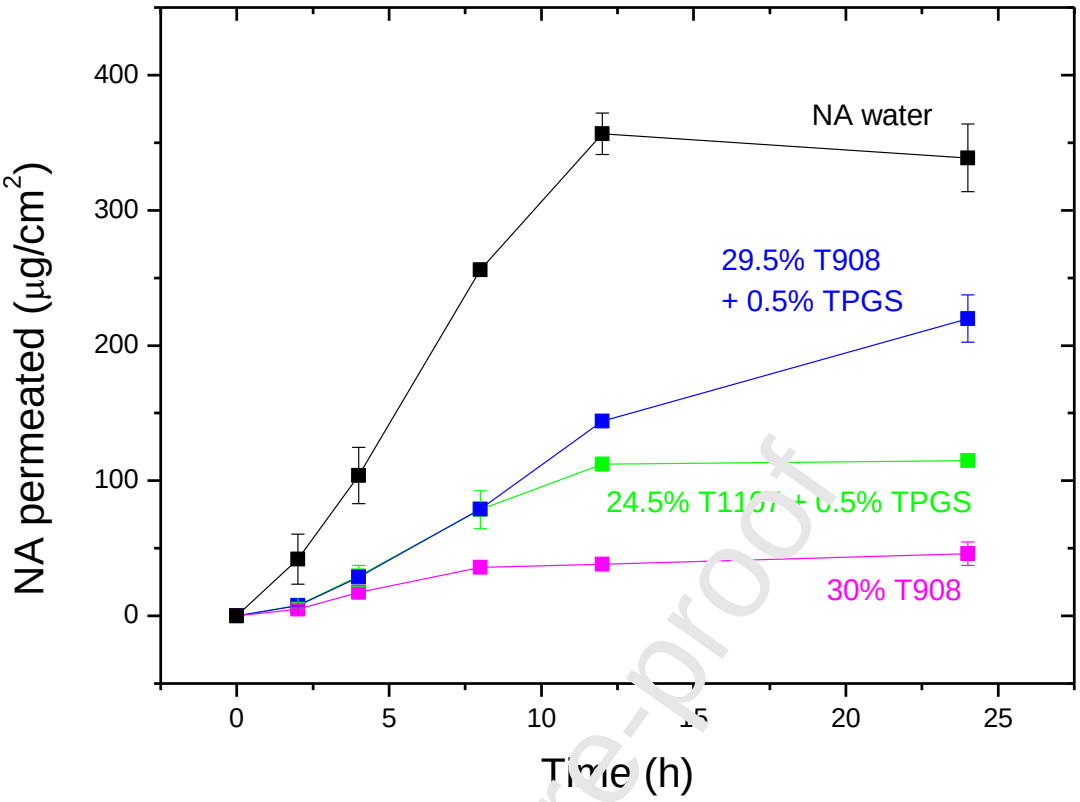


Figure 7. Amount of NA permeated through synthetic membranes Strat-M® as a function of time for different gel formulations containing 1% NA and the control (suspension of NA in water).

4. CONCLUSIONS

Mixtures of TPGS, a water soluble derivative of vitamin E, with two Tetronic surfactants (T1107 and T908) were investigated in the sol and gel state in the presence of naproxen (NA). Both types of surfactants are PEO-based copolymers capable of forming mixed micelles that combine the biocompatibility and antioxidant properties of TPGS with the pH- and temperature responsiveness, and gelation capacity of the poloxamines.

Regarding the dilute regime, NA does not interact with T1107 or T908 in their unimer form, but it incorporates into the micelles above the CMT of the poloxamines (ca. 35ºC). SANS analysis shows that the drug does not produce any change in the shape, polydispersity or overall size of the micelles, but the core size and *sld* increases as a result of the preferential accumulation of NA in the hydrophobic core of the aggregates, as corroborated by NMR. In contrast, TPGS dissolves the drug in the 20-50 ºC range, with no measurable changes in the dimensions of the aggregates apart from the core, which increases when loading the drug, and incorporates some NA in the PEG shell. The exchange of the drug from the solution to the micelles is fast on the

NMR-timescale. With 1% TPGS, up to 95% of the solubilised drug is encapsulated, as determined from the concentration dependence of the self-diffusion coefficient of the drug by gradient NMR, and its solubility in water augmented in a 20-fold ratio at 25ºC. At 37ºC, the solubilizing capacity of 1% TPGS micelles is higher than that of T1107 and T908 at the same mass percentage (3 and 5 times higher, respectively).

The combination of TPGS with either poloxamine produces mixed micelles; the proportion of Tetronic in the micelles increases above its CMT, as assessed by gradient NMR. NA can be solubilised in the mixed micelles, its incorporation being favoured by increasing the TPGS/poloxamine ratio. The solubility of the drug in the mixed micelles at 37ºC and 1% total concentration of surfactants is intermediate between that of TPGS and the poloxamines. The presence of the drug does not change the morphology of the mixed micelles, except a slight shrinking and expansion of the shell and core, respectively, as observed for the single component systems, compatible with the location of the drug in the core.

High concentrations of T1107 and T908 doped with TPGS (up to 1%) induce the formation of physical gels. In the T1107-TPGS mixtures, the analysis of the SANS data reveal that the micelles pack in a BCC macrolattice, and that the spacing and size of the micelles is not affected by the drug or by temperature. The presence of 1% NA shifts gelation by 5ºC in T1107-containing gels, a change that must be ascribed to the drop in pH, which partially ionizes the amino groups of the poloxamine, but not with T908 and TPGS-T908 gels.

Finally, permeability studies using synthetic polymeric membranes show that the diffusion of NA from the hydrogels across the membrane can be modified by incorporating TPGS into the formulation in small proportions, being T908 the poloxamine that permits the passage of higher amounts of drug. Hydrogels formed from mixtures TPGS-Tetronic could thus be used to deliver drugs across the skin, the characteristics of the hydrogels easily tuned by varying the type of poloxamine and the ratio TPGS/poloxamine.

ACKNOWLEDGEMENTS

The authors thank JCNS for the provision of beam time to the KWS-2 diffractometer at the Heinz Maier-Leibnitz Zentrum (MLZ, Garching, Germany) and to Prof. G. Tardajos and Prof. A. Guerrero (UCM), for their help with the NMR measurements. H. Lana (UN) is acknowledged for his assistance with the permeability studies. The authors also thank undergraduate students J. Burriel and J. Carriles for their help with the diffusion NMR experiments. Financial support from project SAF2017-83734-R of the Spanish MINECO and the Asociación de Amigos de la Universidad de Navarra for the doctoral grant of J.P.-R are

acknowledged. This work benefited from the use of the SasView application, originally developed under NSF Award DMR-0520547, which contains code developed with funding from the EU Horizon 2020 programme under the SINE2020 project Grant No 654000.

REFERENCES

(1) Cagel, M.; Tesan, F. C.; Bernabeu, E.; Salgueiro, M. J.; Zubillaga, M. B.; Moretton, M. A.; Chiappetta, D. A. Polymeric Mixed Micelles as Nanomedicines: Achievements and Perspectives. *Eur. J. Pharm. Biopharm.* **2017**, *113*, 211–228.

(2) Biswas, S.; Kumari, P.; Lakhani, P. M.; Ghosh, B. Recent Advances in Polymeric Micelles for Anti-Cancer Drug Delivery. *Eur. J. Pharm. Sci.* **2016**, *83*, 184–202.

(3) Kreuter, J. Nanoparticles. In *Colloidal drug delivery systems*; Kreuter, J., Ed.; Marcel Dekker: New York, 1994; pp 219–342.

(4) Kwon, G. S.; Kataoka, K. Block Copolymer Micelles as Long-Circulating Drug Vehicles. *Adv. Drug Deliv. Rev.* **1995**, *16* (2–3), 295–309.

(5) Kwon, G. S.; Okano, T. Polymeric Micelles as New Drug Carriers. *Advanced Drug Delivery Reviews*. 1996, pp 107–116.

(6) Scholes, P. D.; Coombes, A. G. A.; Davis, M. C.; Illum, L.; Davis, S. S. Particle Engineering of Biodegradable Colloids for Site-Specific Drug Delivery. In *Controlled drug delivery. Challenges and strategies*; Park, K., Ed.; American Chemical Society: Washington, 1997; pp 73–106.

(7) Gothwal, A.; Khan, I.; Gupta, U. Polymeric Micelles: Recent Advancements in the Delivery of Anticancer Drugs. *Pharm. Res.* **2016**, *33* (1), 18–39.

(8) Milcovich, G.; Lettieri, S.; Antunes, F. E.; Medronho, B.; Fonseca, A. C.; Coelho, J. F. J.; Marizza, P.; Perrone, F.; Farra, R.; Dapas, B.; et al. Recent Advances in Smart Biotechnology: Hydrogels and Nanocarriers for Tailored Bioactive Molecules Depot. *Adv. Colloid Interface Sci.* **2017**, *249*, 163–180.

(9) Liow, S. S.; Dou, Q.; Kai, D.; Karim, A. A.; Zhang, K.; Xu, F.; Loh, X. J. Thermogels: In Situ Gelling Biomaterial. *ACS Biomater. Sci. Eng.* **2016**, *2* (3), 295–316.

(10) Dreiss, C. A. Hydrogel Design Strategies for Drug Delivery. *Curr. Opin. Colloid Interface Sci.* **2020**, *48*, 1–17.

(11) Puig-Rigall, J.; Fernández-Rubio, C.; González-Benito, J.; Houston, J. E.; Radulescu, A.; Nguewa, P.; González-Gaitano, G. Structural Characterization by Scattering and Spectroscopic Methods and Biological Evaluation of Polymeric Micelles of Poloxamines and TPGS as Nanocarriers for Miltefosine Delivery. *Int. J. Pharm.* **2020**, *578*, 119057.

(12) Pillai, S. A.; Sharma, A. K.; Desai, S. M.; Sheth, U.; Bahadur, A.; Ray, D.; Aswal, V. K.; Kumar, S. Characterization and Application of Mixed Micellar Assemblies of PEO-PPO Star Block Copolymers for Solubilization of Hydrophobic Anticancer Drug and in Vitro Release. *J. Mol. Liq.* **2020**, *313*, 113543.

(13) Guan, S.; Munder, A.; Hedtfeld, S.; Brachbach, P.; Glage, S.; Zhang, L.; Lienenklaus, S.; Schultze, A.; Hasenpusch, G.; Garrels, W.; et al. Self-Assembled Peptide–Poloxamine Nanoparticles Enable in Vitro and in Vivo Genome Restoration for Cystic Fibrosis. *Nat. Nanotechnol.* **2019**, *14* (3), 287–297.

(14) Gonzalez-Lopez, J.; Alvarez-Lorenzo, C.; Taboada, P.; Sosnik, A.; Sandez-Macho, I.; Concheiro, A. Self-Associative Behavior and Drug-Solubilizing Ability of Poloxamine (Tetronic) Block Copolymers. *Langmuir* **2008**, *24* (19), 10688–10697.

(15) Larrañeta, E.; Isasi, J. R. Phase Behavior of Reverse Poloxamers and Poloxamines in Water. *Langmuir* **2013**, *29* (4), 1045–1053.

(16) Egan, R. W.; Jones, M. A.; Lehninger, A. L. Hydrophile-Lipophile Balance and Critical Micelle Concentration as Key Factors Influencing Surfactant Disruption of Mitochondrial-Membranes. *J. Biol. Chem.* **1976**, *251* (14), 4442–4447.

(17) Puig-Rigall, J.; Obregon-Gomez, I.; Monreal-Pérez, P.; Radulescu, A.; Blanco-Prieto, M. J.; Dreiss, C. A.; González-Gaitano, G. Phase Behaviour, Micellar Structure and Linear Rheology of Tetrablock Copolymer Tetronic 908. *J. Colloid Interface Sci.* **2018**, *524*, 42–51.

(18) Simoes, S. M. N.; Veiga, F.; Torres-Labandeira, J. J.; Ribeiro, A. C. F.; Concheiro, A.; Alvarez-Lorenzo, C. Poloxamine-Cyclodextrin-Simvastatin

Supramolecular Systems Promote Osteoblast Differentiation of Mesenchymal Stem Cells. *Macromol. Biosci.* **2013**, *13* (6), 723–734.

(19) Puga, A. M.; Rey-Rico, A.; Magariños, B.; Alvarez-Lorenzo, C.; Concheiro, A. Hot Melt Poly-ε-Caprolactone/Poloxamine Implantable Matrices for Sustained Delivery of Ciprofloxacin. *Acta Biomater.* **2012**, *8* (4), 1507–1518.

(20) Larrañeta, E.; Isasi, J. R. Non-Covalent Hydrogels of Cyclodextrins and Poloxamines for the Controlled Release of Proteins. *Carbohydr. Polym.* **2014**, *102*, 674–681.

(21) Larrañeta, E.; Martínez-Ohárriz, C.; Vélaz, I.; Zornoza, A.; Machín, R.; Isasi, J. R. In Vitro Release from Reverse Poloxamine/α-Cyclodextrin Matrices: Modelling and Comparison of Dissolution Profiles. *J. Pharm. Sci.* **2014**, *103* (1), 197–206.

(22) Gao, L.; Wang, X.; Ma, J.; Hao, D.; Wei, P.; Zhou, L.; Liu, G. Evaluation of TPGS-Modified Thermo-Sensitive Pluronic PF127 Hydrogel as a Potential Carrier to Reverse the Resistance of P-Gp-Overexpressing SMMC-7721 Cell Lines. *Colloids Surfaces B Biointerfaces* **2016**, *140*, 307–316.

(23) Alakhova, D. Y.; Kabanov, A. V. Pluronics and MDR Reversal: An Update. *Mol. Pharm.* **2014**, *11* (8), 2566–2578.

(24) Batrakova, E. V.; Kabanov, A. V. Pluronic Block Copolymers: Evolution of Drug Delivery Concept from Inert Nanocarriers to Biological Response Modifiers. *J. Control. Release* **2008**, *130* (2), 98–106.

(25) Yu, L.; Bridgers, A.; Polli, J.; Vickers, A.; Long, S.; Roy, A.; Winnike, R.; Coffin, M. Vitamin E-TPGS Increases Absorption Flux of an HIV Protease Inhibitor by Enhancing Its Solubility and Permeability. *Pharm. Res.* **1999**, *16* (12), 1812–1817.

(26) Yang, C.; Wu, T.; Qi, Y.; Zhang, Z. Recent Advances in the Application of Vitamin E TPGS for Drug Delivery. *Theranostics* **2018**, *8* (2), 464–485.

(27) Duhem, N.; Danhier, F.; Preat, V. Vitamin E-Based Nanomedicines for Anti-Cancer Drug Delivery. *J. Control. Release* **2014**, *182*, 33–44.

(28) Mei, L.; Zhang, Z.; Zhao, L.; Huang, L.; Yang, X.-L.; Tang, J.; Feng, S.-S.

Pharmaceutical Nanotechnology for Oral Delivery of Anticancer Drugs. *Adv. Drug Deliv. Rev.* **2013**, *65* (6), 880–890.

(29) Puig-Rigall, J.; Grillo, I.; Dreiss, C. A.; González-Gaitano, G. Structural and Spectroscopic Characterization of TPGS Micelles: Disruptive Role of Cyclodextrins and Kinetic Pathways. *Langmuir* **2017**, *33* (19), 4737–4747.

(30) Grimaudo, M. A.; Pescina, S.; Padula, C.; Santi, P.; Concheiro, A.; Alvarez-Lorenzo, C.; Nicoli, S. Poloxamer 407/TPGS Mixed Micelles as Promising Carriers for Cyclosporine Ocular Delivery. *Mol. Pharm.* **2018**, *15* (2), 571–584.

(31) Ribeiro, A.; Sosnik, A.; Chiappetta, D. A.; Veiga, F.; Concheiro, A.; Alvarez-Lorenzo, C. Single and Mixed Poloxamine Micelles as Nanocarriers for Solubilization and Sustained Release of Ethoxzolamide for Topical Glaucoma Therapy. *J. R. Soc. Interface* **2012**, *9* (74), 2059–2069.

(32) Úriz, A.; Sanmartín, C.; Plano, D.; de Melo Barbosa, R.; Dreiss, C. A.; González-Gaitano, G. Activity Enhancement of Selective Antitumoral Selenodiazoles Formulated with Poloxamine Micelles. *Colloids Surfaces B Biointerfaces* **2018**, *170*, 463–469.

(33) Cagel, M.; Bernabeu, E.; Gonzalez, L.; Lagomarsino, E.; Zubillaga, M.; Moretton, M. A.; Chiappetta, D. A. Mixed Micelles for Encapsulation of Doxorubicin with Enhanced in Vitro Cytotoxicity on Breast and Ovarian Cancer Cell Lines versus Doxil®. *Biomed. Pharmacother.* **2017**, *95*, 894–903.

(34) Calienni, M. N.; Cagel, M.; Montanari, J.; Moretton, M. A.; Prieto, M. J.; Chiappetta, D. A.; del Valle Alonso, S. Zebrafish (Danio Rerio) Model as an Early Stage Screening Tool to Study the Biodistribution and Toxicity Profile of Doxorubicin-Loaded Mixed Micelles. *Toxicol. Appl. Pharmacol.* **2018**, *357*, 106–114.

(35) Puig-Rigall, J.; Blanco-Prieto, M. J.; Radulescu, A.; Dreiss, C. A.; González-Gaitano, G. Morphology, Gelation and Cytotoxicity Evaluation of D-α-Tocopheryl Polyethylene Glycol Succinate (TPGS) - Tetronic Mixed Micelles. *J. Colloid Interface Sci.* **2020**, *582*, 353–363.

(36) Arancibia, J. A.; Escandar, G. M. Determination of Naproxen in Pharmaceutical Preparations by Room-Temperature Phosphorescence. A Comparative Study of

Several Organized Media. *Analyst* **2001**, *126* (6), 917–922.

(37) Sebok, Á.; Vasanits-Zsigrai, A.; Palkó, G.; Záray, G.; Molnár-Perl, I. Identification and Quantification of Ibuprofen, Naproxen, Ketoprofen and Diclofenac Present in Waste-Waters, as Their Trimethylsilyl Derivatives, by Gas Chromatography Mass Spectrometry. *Talanta* **2008**, *76* (3), 642–650.

(38) García-Herrero, V.; Torrado-Salmerón, C.; García-Rodríguez, J. J.; Torrado, G.; Torrado-Santiago, S. Submicellar Liquid Chromatography with Fluorescence Detection Improves the Analysis of Naproxen in Plasma and Brain Tissue. *J. Sep. Sci.* **2019**, *42* (9), 1702–1709.

(39) Radulescu, A.; Szekely, N. K.; Appavou, M. S.; Pipich, V.; Kohnke, T.; Ossovyi, V.; Staringer, S.; Schneider, G. J.; Amann, M.; Zhang-Haagen, B.; et al. Studying Soft-Matter and Biological Systems over a Wide Length-Scale from Nanometer and Micrometer Sizes at the Small-Angle Neutron Diffractometer KWS-2. *J. Vis. Exp.* **2016**, No. 118, e54639.

(40) Serra-Gómez, R.; Dreiss, C. A.; González-Benito, J.; González-Gaitano, G. Structure and Rheology of Poloxamine T1107 and Its Nanocomposite Hydrogels with Cyclodextrin-Modified Barium Titanate Nanoparticles. *Langmuir* **2016**, *32* (25), 6398–6408.

(41) Doucet, M. SasView Version 4.1.2. August , p http://doi.org/10.5281/zenodo.825675.

(42) Guerrero-Martínez, A.; González-Gaitano, G.; Viñas, M. H.; Tardajos, G. Inclusion Complexes between β-Cyclodextrin and a Gemini Surfactant in Aqueous Solution: An NMR Study. *J. Phys. Chem. B* **2006**, *110* (28), 13819–13828.

(43) Guerrero-Martínez, A.; Montoro, T.; Viñas, M. H.; González-Gaitano, G.; Tardajos, G. Study of the Interaction between a Nonyl Phenyl Ether and β-Cyclodextrin: Declouding Nonionic Surfactant Solutions by Complexation. *J. Phys. Chem. B* **2007**, *111* (6), 1368–1376.

(44) National Center for Biotechnology Information (2020). PubChem Compound Summary for CID 156391, Naproxen. https://pubchem.ncbi.nlm.nih.gov/compound/Naproxen (accessed Aug 18, 2020).

(45) Yalkowsky, S. H.; Yan, H.; Parijat, J. *Handbook of Aqueous Solubility Data*; CRC Press, Boca Raton, FL, 2010.

(46) Santoyo, S.; de Jalón, E. G.; Ygartua, P.; Renedo, M. J.; Blanco-Prieto, M. J. Optimization of Topical Cidofovir Penetration Using Microparticles. *Int. J. Pharm.* **2002**, *242* (1–2), 107–113.

(47) Haq, A.; Dorrani, M.; Goodyear, B.; Joshi, V.; Michniak-Kohn, B. Membrane Properties for Permeability Testing: Skin versus Synthetic Membranes. *Int. J. Pharm.* **2018**, *539* (1–2), 58–64.

(48) Uchida, T.; Kadhum, W. R.; Kanai, S.; Todo, H.; Oshizaka, T.; Sugibayashi, K. Prediction of Skin Permeation by Chemical Compounds Using the Artificial Membrane, Strat-M^{TM}. *Eur. J. Pharm. Sci.* **2015**, *67*, 113–118.

(49) Ng, S.-F.; Rouse, J.; Sanderson, D.; Eccleston, G. A Comparative Study of Transmembrane Diffusion and Permeation of Ibuprofen across Synthetic Membranes Using Franz Diffusion Cells. *Pharmaceutics* **2010**, *2* (2), 209–223.

Joan Puig-Rigall: Investigation, Conceptualization, Methodology, Formal analysis, Writing - Original Draft, Writing - Review & Editing, Visualization

María J. Blanco-Prieto: Conceptualization, Methodology, Formal analysis, Writing - Original Draft, Writing - Review & Editing, Project administration, Funding acquisition, Supervision

Carlos Aydillo: Investigation, Conceptualization, Methodology, Formal analysis, Writing - Review & Editing

Aurel Radulescu: Investigation, Conceptualization, Methodology, Formal analysis, Writing - Review & Editing

Dolores Molero-Vilchez: Investigation, Conceptualization, Methodology, Formal analysis, Writing - Review & Editing

Cécile A. Dreiss: Conceptualization, Methodology, Formal analysis, Writing - Review & Editing, Project administration, Funding acquisition, Supervision

Gustavo González-Gaitano: Investigation, Conceptualization, Methodology, Formal analysis, Writing - Original Draft, Writing - Review & Editing, Project administration, Funding acquisition, Supervision

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

HIGHLIGHTS for: Poloxamine/D-α-Tocopheryl polyethylene glycol succinate (TPGS) mixed micelles and gels: morphology, loading capacity and skin drug permeability

- NA is extensively included into the hydrophobic core of TPGS-poloxamine mixed micelles
- Micelle core expands because of the preferential accumulation of the drug in that compartment
- Naproxen load in the micelles increases markedly with the temperature
- Micellar thermogels of TPGS-poloxamine maintain their 3D structure upon drug loading
- Diffusion of NA from the thermogels increases by incorporating TPGS into the micelles