Title

Solubilization of flurbiprofen with non-ionic Tween20 surfactant micelles: a diffusion ^{1}H-NMR study

Author names

M. Verbrugghe,*[a] P. Sabatino,[a,b] E. Cocquyt,[a] P. Saveyn,[a] D. Sinnaeve,[b] P. Van der Meeren[a] and J. C. Martins[b]

Affiliations

[a] Particle and Interfacial Technology Group, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Gent, Belgium. Fax: +32 (0)9 264 62 42; Tel: +32 (0)9 264 60 03

[b] NMR and Structure Analysis Unit, Department of Organic Chemistry, Faculty of Sciences, Ghent University, Krijgslaan 281 S4, B-9000 Gent, Belgium. Fax: +32 (0)9 264 49 72 ; Tel:+32 (0)9 264 44 69 ; E-mail: Jose.Martins@UGent.be

Corresponding author

Maarten Verbrugghe

Particle and Interfacial Technology Group, Faculty of Bioscience Engineering, Ghent University

Coupure Links 653, B-9000 Gent, Belgium.

Fax: +32 (0)9 264 62 42; Tel: +32 (0)9 264 99 11

E-mail: Maarten.Verbrugghe@UGent.be

Abstract

The diffusional behavior of solubilized flurbiprofen (a poorly water soluble anti-inflammatory drug) in the presence of non-ionic Tween20 micelles was studied in an acidic environment (pH 3) using ^{1}H-NMR. Two different concentrations of flurbiprofen were considered, each at temperatures ranging from 15 to 55 °C. The variability of the hydrodynamic micellar diameter, which was calculated from diffusion ^{1}H-NMR data, revealed a structural rearrangement of the Tween20 micelles, caused by the solubilization of flurbiprofen. It was found that the additional peaks (at about 0.1 ppm upfield to the original ones), which appeared upon clouding, corresponded to a slowly diffusing population of solubilized drug molecules, in slow exchange (on an NMR timescale) with the faster diffusing population. This phenomenon was only observed in saturated samples at a temperature of at least 45 °C. For saturated samples at 55 °C, it was found that the flurbiprofen existed in dissolved, solubilized, as well as precipitated form. Combined diffusion and peak integration data revealed that the solubilized fraction was linked to micellar flurbiprofen (rapidly diffusing), small aggregates (slowly diffusing) and larger aggregates that were no longer observable by ^{1}H-NMR.

Keywords

flurbiprofen, micelles, diffusion ^{1}H-NMR, solubilization

1. Introduction

Many new active pharmaceutical ingredients are poorly soluble in water [1], which reduces their applicability in aqueous systems. Micellar solutions, as well as nanosuspensions, are two possible formulation strategies to increase the solubility up to the millimolar range [2-4]. In both cases, a thorough knowledge of the drug solubilization within micelles is of utmost importance to ensure stability during storage. In micellar solutions, it is important to keep the active ingredient solubilized under different storage conditions. In nanosuspensions, part of the drug may become solubilized in the excess surfactant, which may badly affect their stability against Ostwald ripening [5,6].

Micellar solubilization can be defined as the spontaneous dissolution of a substance by reversible interaction with the micelles of a surfactant in water to form a thermodynamically stable isotropic solution [7]. The capacity of surfactants to solubilize drugs depends on numerous factors, such as the chemical structure of both drug and surfactant, temperature, pH and ionic strength amongst others [8]. In a previous paper, we studied the influence of temperature on the solubilization of the poorly water soluble anti-inflammatory drug flurbiprofen in non-ionic Tween20 surfactant micellar solutions in an acidic environment by both ^{19}F and ^{1}H-NMR spectroscopy. Using ^{19}F-NMR, an increased solubilization capacity was observed as temperature increased. This effect became more pronounced above the cloud point, which was found to be reduced by more than 30°C in the presence of an excess of flurbiprofen. Upon clouding, peak splitting was observed in the ^{19}F spectrum, which indicated the presence of two different pools of solubilized flurbiprofen in slow exchange on the ^{19}F frequency timescale. Peak splitting was also observed in the ^{1}H spectrum: additional small peaks occurred at about 0.1 ppm upfield to the original ones [9]. To investigate the nature of these different environments, we studied the diffusion behaviour of the different species present in both saturated and unsaturated mixtures

of flurbiprofen and Tween20 as a function of temperature by ^{1}H-NMR.

2. Materials and methods

2.1 Materials

Tween20 (Fig. 1) was obtained from Sigma-Aldrich. Flurbiprofen (Fig. 2) with purity ≥ 98 % was obtained from TCI Europe. Sodium acetate and acetic acid (p.a.) were from VWR prolabo. Deuterium oxide (D_2O) with ≥ 99.8 atom % D was used as purchased from Armar Chemicals.

2.2 Sample preparation

A buffer solution with a pH value of 3 was obtained by mixing acetic acid and sodium acetate with final concentrations of 19.68 mM and 0.35 mM, respectively. This buffer solution was used in all experiments, unless stated otherwise. In this condition, the majority (about 95 %) of the flurbiprofen molecules (pKa = 4.03 according to Avdeef [10]) is in the undissociated form and thus poorly soluble in aqueous medium. As it is well known that the pKa of amphiphilic carboxyl acids in nonionic micelles is typically 1 tot 2 units higher than in aqueous solution, it follows that nearly all solubilized flurbiprofen molecules will be in the undissociated form [11].

2.3 ^{1}H-NMR methods

All NMR experiments were performed on a Bruker DRX spectrometer operating at a ^{1}H frequency of 500.13 MHz. A 5 mm ^{1}H, ^{13}C, ^{15}N TXI-Z-gradient probe with a maximum gradient strength of 56.1 G·cm^{-1} was used throughout. Temperature was controlled to within ± 0.1 °C with a Eurotherm 2000 VT controller. Diffusion coefficients were measured by PFG-NMR with a convection compensated double-stimulated-echo experiment using monopolar smoothened square shaped gradient pulses and a phase cycle modified according to Connell et al. [12]. The echo-decay of the resonance intensity obtained with the double stimulated echo sequences obeys equation (1), from which the diffusion coefficient *D* is derived as a function of the parameter *k*. A detailed description of the PFG-NMR method and the sequences mentioned

above is given in a review written by Price [13].

$$I = I_0 \cdot \exp\left(-D \cdot (\gamma G \delta s)^2 \Delta'\right)$$

$$I = I_0 \cdot \exp(-D \cdot k) \qquad (1)$$

I = echo intensity with gradient

I_0 = echo intensity at zero gradient

γ = gyromagnetic ratio

G = maximum gradient amplitude

δ = duration of the gradient pulse

s = gradient shape factor (here 0.9)

Δ' = diffusion delay corrected for the finite gradient pulse duration ($\Delta' = \Delta - 0.6021 \cdot \delta$)

The determination of the diffusion coefficient with corresponding 67 % confidence interval was based on the fitting of a mono- or bi-exponential curve to the echo-decay of the peak integral of the selected resonances using the Monte Carlo procedure. This fitting procedure was repeated 100 times for each experiment; according to Alper and Gelb [14], 60 fits are sufficient to produce a constant confidence interval.

3. Results and Discussion

3.1 ^{1}H-NMR spectrum of flurbiprofen solubilized in 10 mM Tween20

Fig. 3 shows the spectrum at 25 °C of a 4 mM flurbiprofen suspension in a solution of 10 mM Tween20 in D_2O. To enable the characterisation of the peaks, this spectrum was compared to a spectrum of Tween20 in the absence of flurbiprofen and to a spectrum of dissolved flurbiprofen in a 10 mM NaOH solution in D_2O.

At 0.9 ppm, a peak is observed from the terminal methyl group of the surfactant lauryl chain, whereas at 1.3 ppm the aliphatic CH_2 groups of the hydrophobic moiety of Tween20 are visible.

The peak at 2.4 ppm belongs to the CH_2 group next to the carbonyl function (α-position) in Tween20 whereas the peak at 1.6 ppm belongs to the CH_2 group next to it (β-position). At 2.1 ppm, a signal originating from the buffer solution (HAc/Ac^-) is found. Whereas most ethylene oxide protons cause a large peak around 3.7 ppm, the CH_2 of the ethylene oxide group next to the ester function results in a contribution at 4.2 ppm. The peak at 4.8 ppm corresponds to residual HDO. The flurbiprofen contributions to the spectrum are mainly visible at 1.5 ppm (from the methyl group of flurbiprofen) on the one hand, and between 7 and 8 ppm (from the aromatic moiety), on the other hand. At pH 3, the aqueous solubility of flurbiprofen is only 30 µM at the given temperature [15]. Hence, the clear presence of flurbiprofen resonances in this spectrum must result from its enhanced solubilization in the presence of Tween20. As some undissolved flurbiprofen remained visible, these samples will be refered to as saturated.

Since most signals of flurbiprofen and Tween20 do not overlap with each other, it becomes possible to study both the diffusion coefficient and the chemical shift of each component separately. The insert in Fig. 3 shows the polyethylene oxide (PEO) peak of a 10 mM Tween20 solution in D_2O at 25 °C, both in the absence (dotted line) and presence of 4 mM flurbiprofen (full line). The broadening of the PEO peak upon the addition of flurbiprofen is due to a partial overlap of the signal from surfactant PEO with the CH group next to the methyl group of flurbiprofen. Hence, the peak at about 3.7 ppm cannot be used to obtain information that can be traced back to either flurbiprofen or the surfactant. As a further consequence, this peak will not be taken into consideration in all further analyses.

Increasing the temperature of the 10 mM Tween20 solution containing 4 mM flurbiprofen, a completely transparent liquid (without any precipitate) was obtained at temperatures above 35°C. This indicates that all the flurbiprofen becomes either dissolved in the aqueous phase or solubilized within the micelles and hence the micellar solution becomes unsaturated in these conditions. From the integration of the peak area of the flurbiprofen signals (using the acetic acid signal as internal standard), it was calculated that 2.5 mM was solubilized at 15 °C, 2.8 mM

at 25 °C, 3.6 mM at 35 °C and 4.0 mM at 45 °C and 55 °C. Hence, the NMR peak integration data corroborated the visual observation that the system underwent a transition from saturated to unsaturated when the temperature increased from 35 °C to 45 °C. From these data, it follows that the molar Tween20 to flurbiprofen ratio ranges from 2.5 to 3.6 within the temperature range considered. Considering an aggregation number of about 30 molecules of Tween20, each micelle contains about 8 to 12 flurbiprofen molecules. Based on ^{1}H-NMR peak shift data, as well as 2D NOESY spectroscopy, it has been shown before that the flurbiprofen molecules are hereby preferably incorporated in the palissade layer of the Tween20 micelles, with the carboxyl groups in contact with the polyethylene oxide groups and the aromatic part directed towards the hydrophobic core of the micelle [9]. The increased micellar solubilization of the flurbiprofen molecules at higher temperatures is in line with similar observations on carbamezapine solubilization in Pluronic micelles [16]. These authors attributed the enhanced solubilization to the increase in thermal vibrations of the monomers in the micelles which results in an increase in the space available for solubilization of the drug in the micelle.

Increasing the flurbiprofen content up to 20 mM, an exces of solid flurbiprofen remained visually observable at the bottom of the recipient, up to the highest temperature considered (i.e. 55 °C). In this case, a weak turbidity was observed in the supernatant at 45 °C, whereas a much more pronounced turbidity was obtained at 55 °C. The fact that the cloud point was already reached at a temperature of 45 °C can be assigned to the presence of flurbiprofen molecules in the micelles. It is indeed well known that solubilization may affect properties of surfactant solutions, such as the cloud point [17] or CMC [18].

The transition from a transparent to a cloudy aspect coincided with the emergence of an additional upfield peak for every Tween20 or flurbiprofen related peak in the NMR spectrum, as becomes obvious from the comparison of Fig. 3 and Fig. 4. The emergence of these additional peaks indicates that a distinct environment (which allows for sufficient molecular flexibility to enable detection by NMR) has become available for flurbiprofen molecules and that the

molecular exchange with this environment is slow with respect to the NMR frequency time-scale. Based on the inequality $k_{ex} < 2\pi\Delta\nu$, and noting that the frequency difference $\Delta\nu$ between Tween20 or flurbiprofen protons in both environments is of the order of 50 Hz, an upper limit of about 300 s^{-1} can be estimated for the overall exchange rate constant k_{ex}. In order to acquire a better understanding of this complex behaviour, translational diffusion experiments were performed.

3.2 Diffusion NMR data on Tween20 in the absence and presence of flurbiprofen

Since the critical micelle concentration (CMC) of Tween20 is about 58 µM at 25°C in H_2O [19], it can be stated that almost all of the Tween20 molecules in a 10 mM solution were associated into micelles and that the relative amount of free molecules can be neglected. As a consequence, the diffusion coefficients related to the signals of Tween20 can all be dedicated to Tween20 micelles. In Fig. 5, the diffusion coefficients of Tween20 micelles are depicted as a function of temperature, at 3 different concentrations of flurbiprofen: 0, 4 and 20 mM. The corresponding hydrodynamic diameter d_h of the Tween20 micelles was calculated via the Stokes-Einstein equation: $d_h = k_B \cdot T/(3 \cdot \pi \cdot \eta \cdot D)$. Hereby, k_B is the Boltzmann constant. The viscosity η of D_2O was calculated as a function of the absolute temperature T, as prescribed by Kestin et al. [20]. According to Table 1, the hydrodynamic diameter of Tween20 micelles in the absence of flurbiprofen changed from 7.22 ± 0.04 nm to 5.23 ± 0.03 nm upon changing the temperature from 15 to 55 °C. These values compare well to the reported hydrodynamic diameter of 6 to 7 nm for Tween20 in water at 21 °C obtained by both FCS and PCS [21]. According to Rosen [7], a (limited) increase of the aggregation number of the micelles of ethoxylated non-ionic surfactants with increasing temperature is possible. Hence, the decreasing micellar diameter at higher temperature points to the fact that, within the temperature range considered, the hydrodynamic behaviour is governed mainly by the dehydration of the PEO-groups with increasing temperature rather than by the increased aggregation number [22,23].

No visual turbidity was observed in the 10 mM Tween20 solutions containing 4 mM flurbiprofen

within the temperature range considered, which is an indication that the cloud point was not reached. Diffusion NMR measurements revealed a mono-exponential decay of the echo-intensity as a function of the parameter *k* at all temperatures up to 55 °C. Comparing the diffusion characteristics of the Tween20 micelles in the absence and presence of 4 mM flurbiprofen (Fig. 5), it is clear that the diffusion coefficient of the Tween20 micelles was lowered upon flurbiprofen solubilization, which pinpoints a size increase. This effect was especially pronounced at higher temperature, in agreement with our previous work, in which it was found that the amount of solubilized flurbiprofen increases with temperature [9]. However, incorporation of 4 mM flurbiprofen (i.e. 977 mg/L) into 10 mM Tween20 (i.e. 12275 mg/L) increases the mass of the micelles by less than 9% (assuming no significant change in aggregation number), which would yield a size increase of less than 3%. Hence, the diffusion data indicate that the micelles do not only become swollen by the incorporated drug, but will also have a higher surfactant aggregation number.

As long as the solution is saturated with flurbiprofen, i.e. at 15 °C, 25 °C and 35 °C, highly similar diffusion coefficients of the micelles are obtained after addition of 4 mM and 20 mM flurbiprofen (Fig. 5). For 20 mM flurbiprofen, the system remains saturated up to the maximum temperature of 55 °C used in the current study. In this case, an additional peak emerged for every Tween20 or flurbiprofen related signal in the NMR spectrum at a temperature of at least 45 °C. As these additional peaks are slightly shifted by about 0.1 ppm, it follows that they originate from species with a slightly different molecular environment. Considering the first (zero gradient strength) and last spectrum (highest gradient strength) acquired in a diffusion experiment under these circumstances, only the additional peaks remain visible at high gradient strength (Fig. 6). Hence, it is obvious that these additional peaks at higher temperatures originate from species that have a much smaller diffusional mobility, and hence must be ascribed to larger structures as compared to the micelles. In fact, this diffusion filtered spectrum is simplified by the complete disappearance of the water, acetate, as well as free ethoxylate

contributions, which are all fast decaying [24]. The peak area ratio of the flurbiprofen resonances (at 7-8 ppm) to the Tween20 lauryl chain resonance (aliphatic CH_2 groups and terminal methyl group) increased by 14% at the end of the diffusion experiment. The increased importance of the flurbiprofen signal compared to the lauryl signal may indicate that the additional peaks of the hydrophobic part of Tween20 are more rapidly decaying than those of flurbiprofen. However, this increased relative peak area may also indicate a relatively higher flurbiprofen to Tween20 ratio in the surfactant aggregates that give rise to the additional peaks in the spectrum at 55 °C. It is indeed quite probable that the larger aggregates are enriched in more hydrophobic components and hence have a higher solubilizing capacity. In fact, it has been shown that larger rodlike micelles have a higher solubilization capacity as compared to smaller spherical aggregates [25].

When the main and additional peak of each functional group were considered together, a bi-exponential decay of the echo intensity was seen. Hereby, the diffusion coefficient of the main peak was similar to the one obtained in the presence of 4 mM flurbiprofen, whereas the diffusion coefficient of the additional upfield peak had a much lower diffusion coefficient. The diffusion data obtained from bi-exponential analysis were corroborated by mono-exponential analysis of the separate major and minor peak, as illustrated by Fig. 7 for the terminal methyl group of the surfactant hydrophobic tail. Thus, the diffusion coefficient of the terminal methyl group of Tween20 for the additional (minor) peak was $(6.2 \pm 0.3).10^{-12}$ m^2s^{-1}, and $(13.8 \pm 0.3).10^{-12}$ m^2s^{-1} at 45°C and 55°C, respectively, which is an order of magnitude smaller than the diffusion coefficient found for the main peak, which was equal to $(6.68 \pm 0.05).10^{-11}$ m^2s^{-1} and $(10.76 \pm 0.05).10^{-11}$ m^2s^{-1}, respectively. Table 2 indicates that especially the fastest diffusion coefficient was reproduced to within roughly 10%, whereas the slowest diffusion coefficient showed more variability. Based on the relative peak area of the main and the additional peak, the former corresponds to 90 and 81% at 45 and 55 °C, respectively. The last column in table 2 reveals that similar results for the contribution of the fastest diffusion coefficient were obtained from the

relative contributions of the bi-exponential fitting procedure. Hence, the results obtained by a simple bi-exponential fit may be considered as accurate. Comparing the bi-exponential fit results for the Tween20 aliphatic CH_2 groups to these for the terminal methyl group, no significant differences were observed in both the diffusion coefficients and their relative contributions, both at 45 and 55 °C (Table 2).

For the sake of completeness, it can be mentioned that the hydrodynamic diameter of the surfactant aggregates at 45 °C and 55 °C as determined by the Stokes-Einstein equation was 9.56 ± 0.06 nm and 6.60 ± 0.05 nm for the largest diffusion coefficient and 198.21 ± 1.05 nm and 44.17 ± 0.65 nm for the smallest diffusion coefficient, respectively.

3.3 Diffusion NMR data on dissolved and solubilized flurbiprofen

In Fig. 5, the diffusion coefficients of flurbiprofen (i.e. the mean diffusion coefficient of the different aromatic signals in the 7 to 8 ppm region) in a solution of 10 mM Tween20 and either 4 mM or 20 mM flurbiprofen at a pH value of 3 are summarised. In order to enable the comparison of these data with molecular diffusion, a flurbiprofen solution in 10 mM NaOH was considered.

The temperature dependence of the diffusion coefficient of dissolved flurbiprofen (in 10 mM NaOH) is adequately described by the Arrhenius equation: linear regression of the logarithm of the diffusion coefficient as a function of the reciprocal temperature yielded a determination coefficient R^2 of 0.99 with diffusion coefficient values ranging from $(3.6 \pm 0.2)\cdot10^{-10}$ m^2s^{-1} at 15 °C to $(11.8 \pm 0.6)\cdot10^{-10}$ m^2s^{-1} at 55 °C.

The diffusion coefficient of flurbiprofen in a micellar solution (Fig. 5) was always one order of magnitude smaller than the diffusion coefficient of truely dissolved flurbiprofen, which is caused by the solubilization of flurbiprofen in the micelles. In the micellar solutions containing 4 mM flurbiprofen, all decay curves were mono-exponential, which means that there is a quick exchange between the dissolved form of flurbiprofen and the solubilized form. At 15 °C and 25 °C, the diffusion coefficient of flurbiprofen was equal to the diffusion coefficient of the micelles.

At 35°C, 45°C and 55°C, however, the diffusion coefficient of flurbiprofen was higher than the diffusion coefficient of the micelles. This phenomenon is caused by the fact that the observed diffusion coefficient D_{obs} is a weighted average of the diffusion coefficient of the dissolved fraction $D_{dissolved}$ and the diffusion coefficient of the solubilized fraction $D_{solubilized}$.

$$D_{obs} = p*D_{solubilized} + (1 - p)*D_{dissolved} \quad (2)$$

In equation (2), *p* represents the solubilized fraction. Assuming that the diffusion coefficient of the solubilized flurbiprofen is equal to the diffusion coefficient of the micelles, whereas the diffusion coefficient of the free flurbiprofen may be approximated by the value of the dissolved species in 10 mM NaOH, equation (2) allows estimating the dissolved fraction (*1-p*) and the solubilized fraction (*p*) of flurbiprofen. At 15 and 25 °C, the dissolved fraction of flurbiprofen could not be discerned from 0, whereas it increased from 0.9% at 35 °C over 1.2% at 45°C to 1.4% at 55°C. In fact, the dissolved fraction at 45 °C, i.e. 1.2% of 4 mM, which equals 48 µM, corresponds well to the reported aqueous solubility at pH 3.0, which is 30 µM at 25 °C according to Li & Zhao [15]. In a solution of 10 mM Tween20 and 20 mM flurbiprofen, mono-exponentially decaying functions for the aromatic protons of flurbiprofen were only observed at 15, 25 and 35 °C. Hereby, no significant difference between the diffusion coefficient of flurbiprofen and the diffusion coefficient of the micelles was observed at 15 and 25 °C (Fig. 5). At 45 °C and 55 °C, the integrated flurbiprofen signal in the 7 to 8 ppm region yielded a bi-exponentially decaying curve, with similar properties as the Tween20 terminal methyl signal and the aliphatic methylene signals (Table 2). However, as was also observed in the presence of only 4 mM of flurbiprofen, the fastest diffusion coefficient of flurbiprofen was significantly larger than the fastest diffusion coefficient of the hydrophobic part of the surfactant, which was earlier ascribed to the enhanced flurbiprofen solubility at higher temperatures. The slow diffusion coefficient, on the other hand, was highly similar for both flurbiprofen and Tween20. In addition, the relative contribution of the fastest diffusing flurbiprofen species was significantly smaller as compared to the Tween

molecules, which once more points towards a higher flurbiprofen to Tween20 ratio in the slowest diffusing structures.

From the integrated peak area values in the 7-8 ppm region (corresponding to the flurbiprofen aromatic signals) and using acetic acid as internal standard, it was calculated that 2.4 mM of flurbiprofen was solubilized at 15 °C, 2.9 mM at 25 °C, 4.8 mM at 35 °C, 8.8 mM at 45 °C and 6.7 mM at 55 °C by 10 mM Tween20 in the presence of 20 mM flurbiprofen. According to these data, the flurbiprofen solubilization seems to reach a maximum around the temperature of 45 °C, i.e. when the cloud point occurs. However, it is important to notice that the relative peak area of (the terminal methyl group of) Tween20 in the 20 mM flurbiprofen containing sample at 55°C was about 20% lower than in the similar sample without flurbiprofen, whereas it was not affected by the presence of 4 mM flurbiprofen (Table 3). Hence, the apparently lower flurbiprofen solubilization at 55 °C must be ascribed to excessive aggregation of Tween20 surfactant molecules, whereby the molecular mobility in (at least part of) these large aggregates becomes too low to enable its detection by high resolution NMR.

4. Conclusions

In the present work, flurbiprofen solubilization by micellar Tween20 solutions (pH 3) was studied by diffusion NMR. Peak integration revealed that flurbiprofen solubilization increased as the temperature was increased, whereas diffusion data showed that solubilization induced an increased hydrodynamic diameter of Tween20 micelles at low flurbiprofen loads, which is partly due to an increased aggregation number upon sorption. At higher flurbiprofen loads and temperatures of at least 45 °C, visible clouding occured, which gave rise to the emergence of two distinct flurbiprofen populations which are in very slow (or no) exchange within the NMR frequency timescale.

Combining all diffusional data, it follows that the flurbiprofen-saturated Tween20 samples at

elevated temperature do not only consist of micelles, but also incorporate nanosized aggregates (which are still observable by NMR), as well as still larger aggregates in which the molecular flexibility is reduced to such an extent that they can no longer be observed by NMR techniques. As Ostwald ripening is promoted by solubilization in nonionic surfactants [6], the stability of nanosuspensions may be troublesome upon storage at elevated temperature. In addition, the formation of larger surfactant aggregates may affect the organ distribution upon administration of micellar solutions and may even cause blockage of small blood vessels [26].

Acknowledgements

The authors thank the Fund for Scientific Research – Flanders (FWO-Vlaanderen) for a Ph.D. fellowship to Paolo Sabatino, Pieter Saveyn and Davy Sinnaeve, as well as for various research and equipment grants (G.0365.03; G.0064.07; G.0678.08; G.0102.08).

References

[1] C.A. Lipinski, Drug-like properties and the causes of poor solubility and poor permeability, J. Pharmacol. Toxicol. Methods. 44 (2000) 235-249.

[2] E. Merisko-Liversidge, G.G. Liversidge, E.R. Cooper, Nanosizing: A formulation approach for poorly-water-soluble compounds, Eur. J. Pharm. Sci. 18 (2003) 113-120.

[3] V.B. Patravale, A.A. Date, R.M. Kulkarni, Nanosuspensions: A promising drug delivery strategy, J. Pharm. Pharmacol. 56 (2004) 827-840.

[4] B.C. Stephenson, C.O. Rangel-Yagui, A. Pessoa, L.C. Tavares, K. Beers, D. Blankschtein, Experimental and theoretical investigation of the micellar-assisted solubilization of ibuprofen in aqueous media, Langmuir. 22 (2006) 1514-1525.

[5] J. Weiss, C. Canceliere, D.J. McClements, Mass transport phenomena in oil-in-water emulsions containing surfactant micelles: Ostwald ripening, Langmuir. 16 (2000) 6833-6838.

[6] S. Ariyaprakai, S.R. Dungan, Influence of surfactant structure on the contribution of micelles to Ostwald ripening in oil-in-water emulsions, J. Colloid Interface Sci. 343 (2010) 102-108.

[7] M. Rosen, Surfactants and Interfacial Phenomena, John Wiley and Sons, Inc., New York.

[8] V.P. Torchilin, Structure and design of polymeric surfactant-based drug delivery systems, J. Controlled Release. 73 (2001) 137–172.

[9] P. Saveyn, E. Cocquyt, W.X. Zhu, D. Sinnaeve, K. Haustraete, J.C. Martins, P. Van der Meeren, Solubilization of flurbiprofen within non-ionic Tween20 surfactant micelles: a ^{19}F and ^{1}H NMR study, Phys. Chem. Chem. Phys. 11 (2009) 5462-5468.

[10] A. Avdeef, Solubility of sparingly-soluble ionizable drugs, Adv. Drug Delivery Rev. 59 (2007) 568-590.

[11] L. Dupont-Leclercq, S. Giroux, B. Henry, P. Rubini, Solubilization of amphiphilic carboxylic acids in nonionic micelles: determination of partition coefficients from pKa measurements and NMR experiments, Langmuir. 23 (2007) 10463-10470.

[12] M.A. Connell, P.J. Bowyer, P.A. Bone, A.L. Davis, A.G. Swanson, M. Nilsson, G.A. Morris, Improving the accuracy of pulsed field gradient NMR diffusion experiments: Correction for gradient non-uniformity, J. Magn. Reson. 198 (2009) 121-131.

[13] W.S. Price, Pulsed-field gradient nuclear magnetic resonance as a tool for studying translational diffusion: Part II. Experimental aspects, Concepts Magn. Reson. 10 (1998) 197-237.

[14] J.S. Alper, R.I. Gelb, Standard errors and confidence-intervals in nonlinear regression – comparison of Monte-Carlo and parametric statistics, J. Phys. Chem. 94 (1990) 4747-4751.

[15] P. Li, L. Zhao, Solubilization of flurbiprofen in pH-surfactant solutions, J. Pharm. Sci. 92 (2003) 951-956.

[16] Y. Kadam, U. Yerramilli, A. Bahadur, Solubilization of poorly water-soluble drug carbamezapine in Pluronic (R) micelles: Effect of molecular characteristics, temperature and added salt on the solubilizing capacity, Colloids Surf., B. 72 (2009) 141-147.

[17] W.N. Maclay, Factors affecting the solubility of nonionic emulsifiers, J. Colloid Sci. 11 (1956) 272-285.

[18] S.K. Mehta, N. Bala, S. Sharma, Thermodynamics of aggregation of Tweens in the presence of diclofenac sodium, Colloids Surf., A. 268 (2005) 90-98.

[19] C.C. Ruiz, J.A. Molina-Bolivar, J. Aguiar, G. MacIsaac, S. Moroze, R. Palepu, Effect of ethylene glycol on the thermodynamic and micellar properties of Tween20, Colloid Polym. Sci. 281 (2003) 531-541.

[20] J. Kestin, N. Imaishi, S.H. Nott, J.C. Nieuwoudt, J.V. Sengers, Viscosity of light and heavy water and their mixtures, Phys. A. 134 (1985) 38-58.

[21] F. Luschtinetz, C. Dosche, Determination of micelle diffusion coefficients with fluorescence correlation spectroscopy (FCS), J. Colloid Interface Sci. 338 (2009) 312-315.

[22] V.M. Garamus, A new method for the determination of the dehydration coefficient of ethylene oxide groups of non-ionic surfactants in mixed micelles, Chem. Phys. Lett. 290 (1998) 251-254.

[23] F. Sterpone, C. Pierleoni, G. Briganti, M. Marchi, Molecular dynamics study of temperature dehydration of a $C_{12}E_6$ spherical micelle, Langmuir. 20 (2004) 4311-4314.

[24] M. Verbrugghe, E. Cocquyt, P. Saveyn, P. Sabatino, D. Sinnaeve, J.C. Martins, P. Van der Meeren, Quantification of hydrophilic ethoxylates in polysorbate surfactants using diffusion ^{1}H-NMR spectroscopy, J. Pharm. Biomed. Anal. 51 (2010) 583-589.

[25] B. Herzog, Micelle shape and capacity of solubilization, Prog. Colloid Polym. Sci. 84 (1991) 325-326.

[26] W.H. De Jong, W.I. Hagens, P. Krystek, M.C. Burger, A.J.A.M. Sips, R.E. Geertsma, Particle size-dependent organ distribution of gold nanoparticles after intravenous administration, Biomaterials. 29 (2008) 1912-1919.

Figure legends

Fig. 1 Chemical structure of polysorbate 20 (commercially available as Tween20). *n = a + b + c + d = 20*.

Fig. 2 Chemical structure of flurbiprofen.

Fig. 3 ^{1}H-spectrum at 25 °C of 4 mM flurbiprofen in a 10 mM Tween20 solution in D_2O (pH 3). The insert shows a detailed view of the ethylene oxide related peak both in the absence (dotted line) and presence (solid line) of 4 mM flurbiprofen.

Fig. 4 Stacked diffusion ^{1}H-spectrum of 20 mM flurbiprofen in a 10 mM Tween20 solution in D_2O (pH 3) at 55 °C. Each spectrum represents a specific gradient strength, increasing from the first spectrum in front to the last recorded spectrum at the back. Additional upfield peaks for Tween20 as well as for flurbiprofen occur and remain visible up to the highest gradient strength applied, whereas the original peaks at that point are severely reduced and thus no longer visible.

Fig. 5 Diffusion coefficient (and standard deviation) of 10 mM Tween20 micelles in D_2O (pH 3) in the absence (non-shaded) and presence of 4 mM (single shaded) or 20 mM (double shaded) flurbiprofen as a function of the temperature. The data were obtained from a mono-exponential fit to the pfg-NMR decay curves from either the terminal methyl (grey), or the aliphatic methylene protons (white) of the lauryl moiety of 10 mM Tween20, or the aromatic moiety (black) of 4 mM or 20 mM flurbiprofen at 15 °C, 25 °C, 35 °C, 45 °C, and 55 °C.

Fig. 6 Diffusion filtered ^{1}H-spectrum of 20 mM flurbiprofen in a 10 mM Tween20 solution in D_2O (pH 3) taken at 55 °C. The spectrum was recorded at the highest experimental gradient strength (0.449 $G \cdot cm^{-1}$).

Fig. 7 Echo intensity of the surfactant terminal methyl group as a function of the parameter *k*, in the presence of 20 mM flurbiprofen at 55 °C. The decay of the main peak is represented by the black dots, whereas the decay of the additional (small) peak is represented by the white dots, both fitting mono-exponential. The bi-exponential decay of the total methyl resonance (including both the main and additional peak) is given by the semi-filled dots.

Tables

Table 1. Hydrodynamic diameter of Tween20 micelles as a function of temperature in a 10 mM solution in D_2O (pH 3) in the absence and presence of 4 mM and 20 mM flurbiprofen.

		Hydrodynamic diameter d_h (nm)		
T (°C)	η (mPa.s)	0 mM flur	4 mM flur	20 mM flur
15	1.44 ± 0.07	7.22 ± 0.04	7.51 ± 0.04	7.50 ± 0.05
25	1.09 ± 0.05	7.32 ± 0.04	7.49 ± 0.05	7.56 ± 0.04
35	0.87 ± 0.04	6.69 ± 0.04	8.10 ± 0.05	8.76 ± 0.05
45	0.71 ± 0.04	6.05 ± 0.03	8.97 ± 0.05	12.01 ± 0.10
55	0.60 ± 0.03	5.23 ± 0.03	9.77 ± 0.07	10.71 ± 0.14

Table 2. Comparison of the diffusion coefficients (in 10^{-11} m²/s) obtained by either a mono-exponential or a bi-exponential fit to the experimentally determined pfg-NMR decay curves for the terminal methyl and aliphatic methylene protons of the lauryl chain of Tween20, as well as for the aromatic protons of flurbiprofen in a 10 mM Tween20 solution (pH 3) containing 20 mM flurbiprofen at both 45 °C and 55 °C. The rapidly diffusing fraction is given as a percentage ($fraction_{fast}$).

T (°C)	Group	Mono-exponential data analysis	Bi-exponential data-analysis		
			D_{fast}	D_{slow}	$fraction_{fast}$ (%)
45	$-CH_3$	5.47 ± 0.18	6.84 ± 0.04	0.33 ± 0.00	91.2 ± 0.2
	$-CH_2-$	5.42 ± 0.17	6.82 ± 0.03	0.33 ± 0.00	91.0 ± 0.2
	flurbiprofen	5.26 ± 0.29	7.73 ± 0.05	0.31 ± 0.00	86.4 ± 0.2
55	$-CH_3$	7.50 ± 0.59	12.18 ± 0.27	1.82 ± 0.16	79.3 ± 1.5
	$-CH_2-$	7.52 ± 0.56	11.67 ± 0.20	1.61 ± 0.15	81.3 ± 1.3
	flurbiprofen	7.29 ± 0.73	14.13 ± 0.23	1.72 ± 0.09	75.2 ± 0.9

Table 3. Ratio of the relative peak area (to the peak area of acetic acid) of the terminal methyl group of Tween20 in the presence of 4 mM and 20 mM flurbiprofen to the relative peak area in the absence of flurbiprofen at 15, 25, 35, 45, and 55 °C.

	methyl peak ratio in presence of	
T (°C)	4 mM flurbiprofen	20 mM flurbiprofen
15	0.97	1.00
25	0.99	0.97
35	1.00	0.99
45	0.97	0.99
55	1.00	0.80