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Preferential adsorption of polysorbate 20 molecular species in aqueous paliperidone palmitate suspensions

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ABSTRACT

This work investigates the adsorption of polysorbate (Tween) 20 to paliperidone palmitate using the depletion technique, which analyzes the residual surfactant concentration in the aqueous continuous phase of the solid/liquid suspension. Total organic carbon determination was used to study the overall Tween concentration, whereas ^{1}H NMR was applied to differentiate between hydrophilic and amphiphilic ethoxylated sorbitan species. The investigation revealed that Tween 20 adsorption was proportional to the specific surface area of paliperidone palmitate particles, and can be approximated by the Langmuir model. The alkyl-chain-bound (amphiphilic) sorbitans were found to be more effectively adsorbed than the alkyl-chain-free (hydrophilic) sorbitan species, which indicates that the adsorption was driven by hydrophobic interactions. Temperature (room temperature versus 40 °C) was found to have an increasing effect on adsorption.

1. Introduction

Many pharmaceutical products are suspensions of a poorly water soluble solid active ingredient in an aqueous liquid phase. To enable dispersion of the hydrophobic solid particles, a surfactant is generally used. Besides, the addition of surfactant may give rise to either electrostatic or steric stabilization [1,2]. In the pharmaceutical industry, it is common to introduce an excess of surfactant in suspensions, which may be required to improve mechanical processing (e.g. during milling). However, an excessive amount of surfactant may result in micellar depletion flocculation and active ingredient solubilization. The latter may promote destabilization due to Ostwald ripening. Hence, quantification of surfactant adsorption is of significant importance to optimizing the formulation of pharmaceutical suspensions.

Adsorption of nonionic surfactants including Tween is largely governed by hydrophobic interactions [3]. Many nonionic surfactants have been found to form a monolayer on hydrophobic surfaces [4,5]. Many analytical methods have been used to quantify the adsorption of Tween. For example, Greff et al. [6] as well as Duro et al. [7] used a colorimetric method that took advantage of the blue coloration caused by the interaction between the polyethoxylated compound and ammonium cobaltothiocyanate, as described by Clesceri et al. [8]. Martin-Rodriguez et al. [5] utilized a nephelometric method based on the method developed by Attia and Rubio [9] whereas Hu et al. [10] quantified Tween 80 by determination of the fatty acid content upon hydrolysis using high performance liquid chromatography. However, Verbrugghe et al. [11] recently demonstrated that only part of the ethoxylated sorbitans are esterified to fatty acyl chains. Hence, both non-esterified and esterified PEO species exist, which will be characterized by a different EO to fatty acid ratio. Whereas the surface activity of mono-, and especially di- and tri-esters is high, unesterified ethoxylated sorbitan is expected to be hardly surface active. Therefore, the determination of the residual fatty acid or EO content in the supernatant is not appropriate to determine the residual amount of polysorbate upon adsorption equilibrium.

In this work, polysorbate (Tween) 20 (Fig. 1) adsorption to aqueous paliperidone palmitate (PP, Fig. 1) suspensions is studied. The active ingredient is a poorly water soluble and highly hydrophobic substance for the treatment of schizophrenia [12]. Tween 20 is widely used for both wetting and stabilizing purposes in pharmaceutics [13]; it comprises a hydrophobic C_{12} chain attached to an ethoxylated sorbitan ring. The sorbitan ring with its attached ethylene oxides (EO) will be referred to as polyethoxylate (PEO) in this work. To better understand the adsorption of Tween 20

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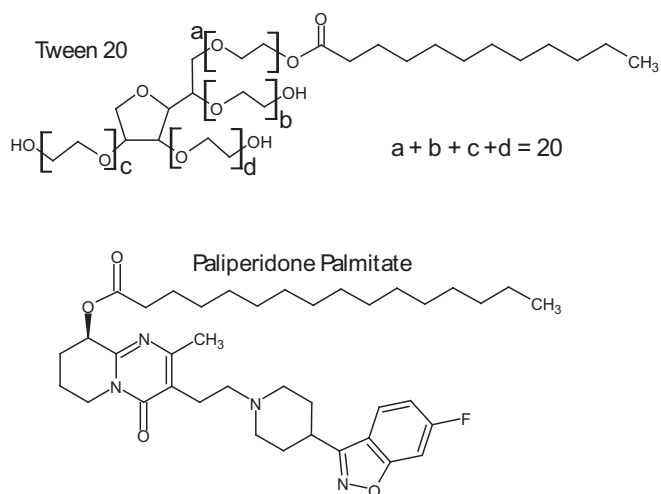E-mail address: Paul.VanderMeeren@UGent.be (P. Van der Meeren).

Fig. 1. Chemical structures of polysorbate (Tween) 20 and paliperidone palmitate (PP).

to paliperidone palmitate, this work investigates the influence of the adsorbate concentration, adsorbent particle size and temperature on the adsorption equilibrium. Hereby, both total carbon (TC) determination and high resolution NMR were used to evaluate the residual surfactant concentration in the continuous phase. Whereas the former approach allows detection of all present molecular species, the latter enables the quantification of the hydrophilic and amphiphilic ethoxylated sorbitans.

2. Materials and methods

2.1. Materials

Three paliperidone palmitate powders (referred to as A, B and C) were provided by Janssen Pharmaceutica (Belgium) in dry state. Tween 20 (Sigma Ultra) was purchased from Sigma–Aldrich (USA), deuterium oxide (D_2O, >99.8% atom D) from Armar Chemicals (Switzerland) and sodium acetate (analytical grade) from VWR Prolabo (Belgium). The water used was de-ionized with a maximum conductivity of 0.2 μS/cm.

2.2. Adsorbent characterization

A 10% (w/w) PP suspension containing 1% (w/w) Tween 20 was first prepared. To enable optical microscopy, this suspension was then diluted 100 times with water. The photos of the samples were taken using an Olympus CX40 microscope (Japan) of 1000× total optical magnification.

Laser diffraction measurements were performed using a Mastersizer S (Malvern Instruments, UK) equipped with a 300 mm RF (Reverse Fourier) lens and a MS17 auto sample dispersion unit. Prior to measurement, an aliquot of the 10% (w/w) PP suspension in 1% (w/w) Tween 20 suspension was added dropwise to the sample dispersion unit to obtain an obscuration between 10 and 15%. Both the pumping and stirring speeds during measurement were fixed at 30% of their maximum values. The selected real, imaginary and dispersant refractive indices were 1.55, 0.01 and 1.33. The data were analyzed using the polydisperse option.

Surface area measurements were performed at 77 K with a TriStar 3000 gas adsorption analyzer (Micromeritics, USA) using nitrogen gas as adsorbate. The adsorbed amount of adsorbate was measured as a function of the ratio of equilibrium pressure (P) to saturation pressure (P_0), from which the specific surface area (A_s) was calculated using the Brunauer–Emmet–Teller (BET) model.

2.3. TC measurement

Tween 20 solutions of different concentrations (up to 2 mg/ml) were first prepared. PP suspensions were prepared by mixing 780 mg dry powder with 10 ml of each Tween 20 solution. The suspensions were then fixed to undergo constant vertical end-over-end rotation at 200 RPM for 48 h. Afterwards, each suspension was incubated for another 48 h at either room temperature (RT, $22 \pm 2\,°C$) or 40 °C for the particles to settle. After incubation, the supernatant of each sample (4.5 ml) was centrifuged at $725 \times g$ at the desired temperature to ensure the absence of particles in the supernatant. Blank samples were prepared by excluding PP in the aforementioned preparation so that adsorption can be deduced by subtraction. TC contents were measured using a TOC-5000 combustion oxidation analyzer (Shimadzu, Japan) equipped with an infrared gas analyzer operating at 680 °C. As the inorganic carbon content was negligibly small (<1 ppm, which was the detection limit) in all samples, the total carbon content was considered in all further analyses as organic carbon content. The data fitting utilized the Langmuir adsorption model described by Eq. (1) [14].

$$\Gamma = \Gamma_{max} \frac{c}{c + (1/a)} \tag{1}$$

In (1), Γ is the concentration of the adsorbed surfactant; Γ_{max} is the maximum concentration of adsorbed surfactant to form a monolayer; c is the equilibrium surfactant concentration; and a is the adsorption activity. The data fitting was based on linear regression of c/Γ versus c, whereby Γ_{max} (with its 95% confidence interval) was calculated as the reciprocal of the slope.

2.4. ^1H NMR measurement

A 700.13 MHz Bruker AVANCE II spectrometer equipped with a 5 mm ^1H, ^{13}C, ^{15}N TXI-Z-gradient probe capable of a maximum gradient strength of 56.1 G/cm was used for both ^1H spectroscopy and diffusion ordered spectroscopy (DOSY). The temperature during all measurements was controlled at $25 \pm 0.01\,°C$ with a Eurotherm 3000 VT digital controller. Full relaxation for quantitative one dimensional measurements was ensured by allowing a 22 s interscan delay. Diffusion measurements utilized the convection compensated double-stimulated-echo sequences [15] with smoothened square shaped gradient pulses and a modified phase cycle [16]. The diffusion coefficient was derived from the echo decay as a function of the parameter k (Eqs. (2) and (3)).

$$I = I_0 \exp[-D(\gamma G \delta s)^2 (\Delta - 0.6021\delta)] \tag{2}$$

$$I = I_0 \exp(-Dk) \tag{3}$$

where I is the echo intensity with gradient; I_0 is the echo intensity without gradient; D is the diffusion coefficient; γ is the gyromagnetic ratio; G is the maximum gradient amplitude; δ is the duration of the gradient pulse; s is the gradient shape factor (0.9); and Δ is the diffusion delay.

The Monte Carlo procedure [17] was used for data fitting, which enables a 67% confidence interval for diffusion coefficient determination. To produce a constant interval, 100 repetitions were used for fitting; this number exceeds the required number of 60. In order to prevent interference of H_2O, 5 ml of each clear supernatant sample (as described in Section 2.3) was first mixed with 1 ml of 2.5 mM sodium acetate solution (as an internal standard) and then lyophilized using a Heto FD 3 freeze-dryer (Denmark). The dry material was subsequently dissolved in 0.5 ml D_2O for measurement. This procedure not only enabled the replacement of H_2O by D_2O, but also achieved 10 times higher Tween concentrations.
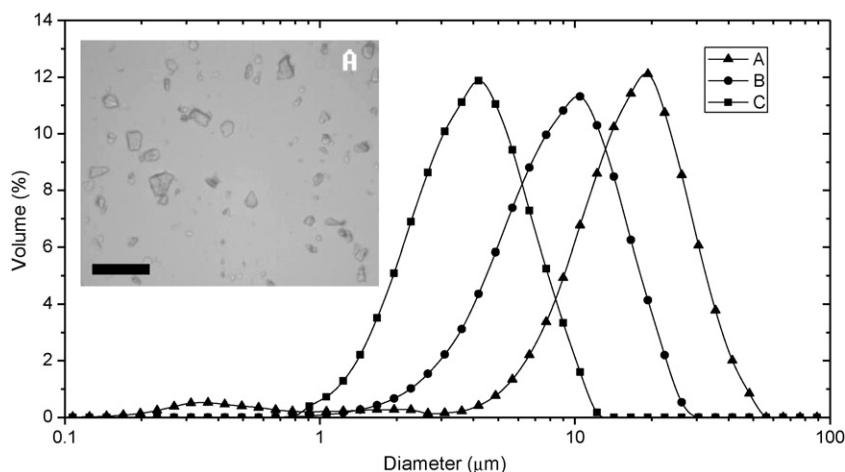
Fig. 2. Volume weighted size distributions of the PP powders by laser diffraction; the insert shows a $1000\times$ optical microscopic photo of a 1 mg/ml suspension of PP powder A. The black mark corresponds to 50 µm.

3. Results and discussion

3.1. PP powder characterization

The three PP powders were prepared by dry milling and differed merely in size. As indicated by optical microscopy in Fig. 2, the dry-milled particles were irregular in shape. Laser diffraction measurement results revealed that the particles were characterized by a log-normal distribution.

The Sauter mean diameters measured by laser diffraction roughly followed a logarithmic order with values of 12.4, 6.5 and 3.2 µm for powder A, B and C, respectively, from which a volume-specific surface area of 0.5, 0.9 and 1.9 m²/ml is calculated assuming spherical geometry. Since the density of paliperidone palmitate is 1.17 g/ml [18], the mass-specific surface area is estimated as 0.4, 0.8 and 1.6 m²/g for powder A, B and C, respectively. However, when using laser diffraction for size characterization, due to the Mie theory that the measurement depends upon, particles are assumed spherical [19,20], which hinders the method's abilities to reveal the true characteristics of non-spherical particles. Since the PP powders are highly irregular in shape, the specific surface areas of the three PP powders were evaluated by gas adsorption measurement to obtain more accurate results. From the nitrogen gas adsorption isotherms, the specific surface areas of powder A, B and C determined by the BET model were 2.1, 2.4 and 3.7 m²/g, respectively. The difference between the specific surface areas of powder A and B is much smaller than predicted from the Sauter mean diameters calculated from the laser diffraction data, which must be ascribed to differences in sphericity of the various samples.

3.2. Adsorption isotherms of Tween 20 to PP from TC measurements

Tween 20 adsorption measurements were performed with the depletion method, i.e. from the difference in surfactant concentration in the aqueous phase in the absence and presence of the adsorbent. Since the aqueous solubility of PP is negligibly small (<1 ppm at room temperature), TC content analysis can be used to determine the surfactant concentration because Tween is the only (organic) carbon containing compound present in the continuous phase. All three PP powders were used to investigate the influence of particle size. The adsorption isotherms at room temperature and at 40 °C were calculated from the results of the TC measurements.

The isotherms in Fig. 3 show a resemblance to the L type isotherm according to the classification by Giles [21], which sug-

gests monolayer adsorption of Tween 20 to PP. The amount of adsorbed Tween 20 per unit mass of PP increased as the particle size of PP powder decreased. According to the Langmuir model used to describe the L type isotherms, the adsorption is proportional to the number of binding sites on the adsorbate surface [22]. In this case, the adsorption is proportional to the surface area of the adsorbate. For the sake of completeness, it has to be mentioned that the Langmuir model is based on some assumptions, such as lack of interaction between adsorbate molecules. Whereas these assumptions are almost never valid in nonionic surfactant adsorption, still Rosen mentions that many surfactants show Langmuir-type adsorption from solution because of the mutual compensation of several factors that affect the shape of the isotherm [23]. Whereas Zhu and Gu [24] as well as Gonzalez-Garcia et al. [25] proposed some further refinements of the Langmuir model by introducing two additional parameters, Jodar-Reyes et al. mention that in case more detailed properties, such as the structure of the adsorbed layer, have to be described, more detailed models are required, which may be based on statistical mechanical techniques [26].

Dividing the maximum adsorbed amount (derived from the best fitting Langmuir isotherm, expressed in mg/g) by the mass-specific surface area, the maximum adsorbed amount per unit adsorbent surface area corresponded to about 1.7 ± 0.3, 1.5 ± 0.3 and 1.7 ± 0.2 mg/m² PP for powder A, B and C at room temperature,

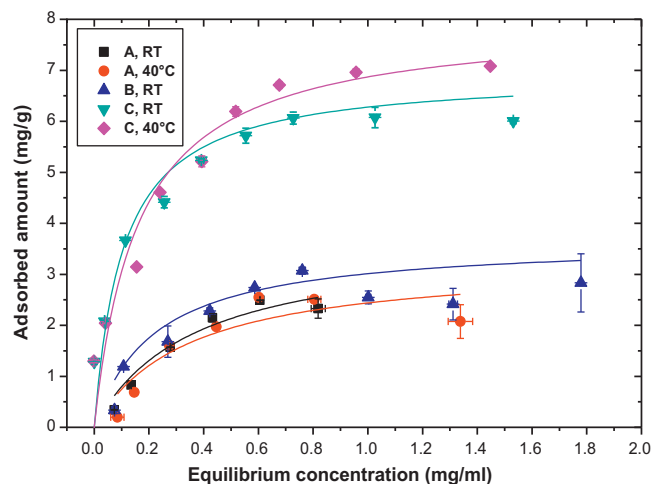**Fig. 3.** Adsorption isotherms of Tween 20 to PP powder A, B and C at room temperature (RT) and 40 °C and the best fitting curves using a Langmuir model.

Table 1
The Γ_{max} values of Tween 20 adsorption to PP powder A, B and C as a function of the specific surface area of the particles (A_s) and temperature (T). The values with the same superscript are not significantly different from each other based on a 95% confidence level.

Powder	A_s (m^2/g)	T	Γ_{max} (mg/g)	Γ_{max} (mg/m^2)
A	2.1	RT	3.67 ± 0.66[a]	1.74 ± 0.31[a,b]
		40 °C	3.27 ± 0.60[a]	1.55 ± 0.28[a]
B	2.4	RT	3.67 ± 0.65[a]	1.54 ± 0.27[a]
C	3.7	RT	6.93 ± 0.29[b]	1.71 ± 0.15[a]
		40 °C	7.98 ± 0.51[c]	2.15 ± 0.14[b]

respectively. As can be seen in Table 1, these values are not significantly different. Assuming a monomolecular layer, the adsorption maximum values correspond to a projected area of 109 to 133 Å^2 per Tween 20 molecule. These values correspond quite well to the projected area of 86 Å^2 of a dodecyl chain with is adsorbed parallel to the surface. The latter value was estimated assuming a cylindrical geometry of the saturated hydrocarbon hydrophobic tail. Using the equations proposed by Tanford [27], the length and diameter of this idealized cylindrical dodecyl chain correspond to 16.72 and 5.16 Å, respectively.

The Γ_{max} values revealed that the temperature difference studied did not result in a significantly different maximum adsorption to powder A, which had the smallest specific surface area. However, for powder C that had the biggest specific surface area, the higher temperature resulted in a significantly higher maximum adsorption. The latter observation is in line with the findings of Steinby et al. [28] and Qing-Feng et al. [29], who also observed that a temperature increase gave rise to a nonionic surfactant adsorption enhancing effect onto a hydrophobic surface. In fact, the more pronounced adsorption at higher temperature is a logical consequence of the decreased hydrophilicity and hence increased surface activity of ethoxylated surfactants at higher temperature.

3.3. Adsorption of Tween 20 studied by high resolution NMR

In previous studies, it has been shown that commercial surfactants, such as Tween 20, consist of a variety of molecular species. Commercial Tween 20 appears to be a complex mixture of unesterified, mono-, di- and tri-ester of ethoxylated sorbitans and fatty acids [11]. Hereby, TC measurements provide information on all molecular species simultaneously. More detailed information may be obtained by high resolution NMR.

In the first experiment, the influence of adsorbent concentration was studied. Considering the relatively lower sensitivity of NMR measurements (compared to TC measurements), suspensions containing a high concentration of Tween 20 (15 mg/ml), different concentrations (100, 200 or 312 mg/ml) of PP powder A and 10 mM sodium acetate (as internal standard) in D$_2$O were prepared. The suspensions were incubated at room temperature for 2 days for adsorption equilibration. An additional sample containing only 3 mg/ml Tween 20 and 312 mg/ml PP powder A was also prepared to further increase the percentage of Tween adsorption.

Hereby, ^{1}H spectroscopy was used to differentiate the fatty acyl chain (expressed as (CH$_2$)$_n$ and CH$_3$) and PEO as illustrated in Fig. 4, whereas DOSY measurement was used to quantify the fast and slow diffusing PEO species as illustrated by Fig. 5, which shows a mono-exponential signal decay of the fatty acyl chain and a bi-exponential decay of PEO. This reveals the existence of two PEO species in this sample: a slowly diffusing species of fatty-acyl-bound amphiphilic PEO molecules that associate into micelles and a fast diffusing species of unesterified (free) hydrophilic PEO molecules that are present in the monomeric state.

Table 2
^{1}H NMR relative integral peak areas of (CH$_2$)$_n$, CH$_3$ and PEO as well as the fast diffusing PEO fraction of PP suspensions containing 15 mg/ml Tween 20 as a function of PP concentration at room temperature.

PP (mg/ml)	(CH$_2$)$_n$ (%)	CH$_3$ (%)	PEO (%)	Fast fraction (%)
0	100	100	100	38.5 ± 0.3
100	92.7	95.5	95.3	38.8 ± 0.5
200	91.2	91.9	95.9	39.3 ± 0.4
312	86.1	75.6	91.0	40.9 ± 0.6

The data in the 2nd, 3rd and 4th columns of Table 2 are the integral area values of the Tween 20 components relative to those of the blank sample in the absence of PP. These data show that in the presence of 15 mg/ml Tween 20, there was only a slight reduction in PEO, (CH$_2$)$_n$ and CH$_3$ concentrations upon increasing the PP concentration, indicating little decrease in Tween 20 concentration by adsorption. These results reveal that at the higher surfactant concentration (15 mg/ml), even when an amount of PP as high as 312 mg/ml was present, the suspension still contained a
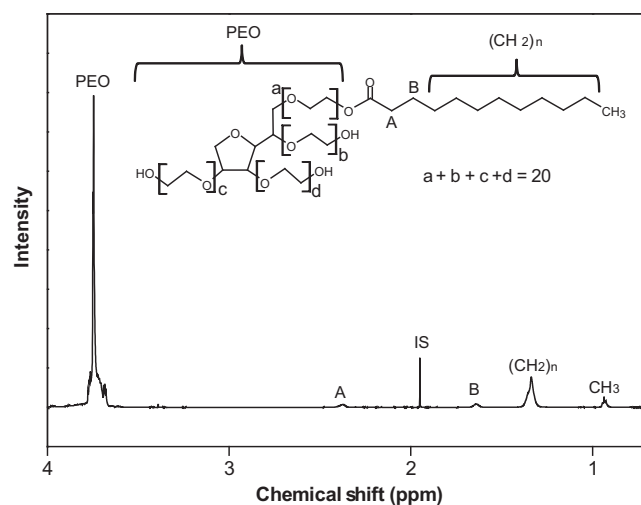

Fig. 4. The ^{1}H NMR spectrum of the supernatant of a sample containing 10 mM sodium acetate as an internal standard (IS), 200 mg/ml PP powder A and 15 mg/ml Tween 20. Tween 20 components can be discriminated by their chemical shifts and their concentrations are proportional to the integral areas of the peaks. The (CH$_2$)$_n$ group does not include the alpha and beta positions indicated as A and B.
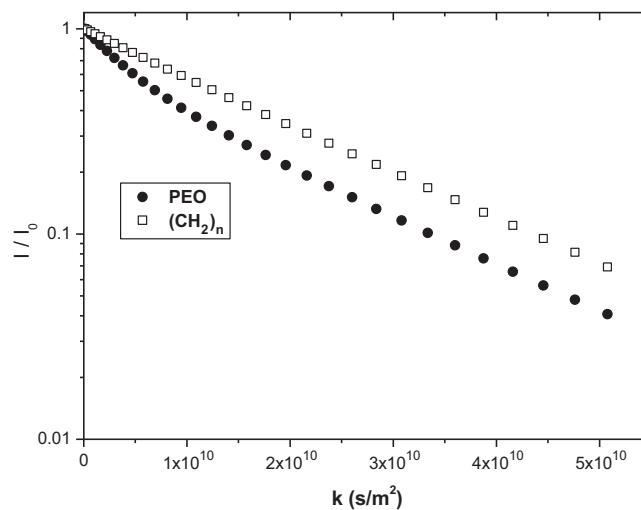

Fig. 5. Relative signal intensity (I/I_0) decays of the PEO group (filled symbols) and the fatty acyl chain (open symbols) of Tween 20 as a function of k of the sample containing 200 mg/ml PP (powder A) and 15 mg/ml Tween 20.

large excess of unadsorbed Tween 20. A close inspection of these data reveals a more pronounced reduction in the fatty acyl related peaks as compared to the PEO peaks.

On the other hand, at the same PP concentration of 312 mg/ml, when the initial Tween 20 concentration (15 mg/ml) was reduced by a factor of 5 (to 3 mg/ml), the relative peak areas of $(CH_2)_n$ and CH_3 (corresponding to the fatty acyl chain) were decreased from 86.1% and 75.6% to 5.4% and 6.7%, respectively. In this case, only 44.5% of the PEO groups were still present after the addition of 312 mg/ml of PP. Hence, at the smaller concentration of Tween 20, most of the surfactant became adsorbed.

The DOSY data in the 5th column of Table 2 indicate a slight increase in the fast diffusing fraction of PEO upon increasing the PP concentration at a surfactant concentration of 15 mg/ml, suggesting some limited adsorption of the slowly diffusing amphiphilic PEO molecules. This can be explained by effective adsorption of the fatty-acyl-bound PEO and poor adsorption of the hydrophilic PEO to the hydrophobic surface of PP. At the lower surfactant concentration (3 mg/ml), the typical bi-exponential decay of PEO in the absence of PP transformed into a mono-exponential decay when PP was added. In this case, the slowly diffusing PEO molecules (in micellar form) were effectively removed by adsorption to the PP particles and hence only the fast diffusing hydrophilic PEO (free of fatty acyl) remained in the supernatant after incubation.

3.4. Correlation of TC and NMR measurements

The above-mentioned experiments indicate that large amounts of adsorbent at low surfactant concentrations are needed to enable direct measurement of the reduction in adsorbate concentration. However, concentrated suspensions are more viscous and hence more difficult to ensure proper mixing whereas lower concentrations require a longer NMR acquisition time. To overcome these limitations, a concentration increasing step was included consisting of lyophilization followed by dissolution in a 10 times smaller volume.

The influence of surface area and surfactant concentration was further investigated by NMR measurements. PP powder B and C that had markedly different total particle surface areas were used. The samples for NMR measurements were prepared in the same way as the samples for the TC experiment. Prior to measurement, 5 ml of supernatant was first mixed with 1 ml 2.5 mM sodium acetate (used as internal standard) and then freeze-dried. The dried material was afterwards dissolved in 0.5 ml D_2O for NMR measurement.

NMR peak area results in Fig. 6a show that at the highest Tween 20 concentration (2 mg/ml), the residual concentration of PEO in either sample was found to be slightly lower than that in the blank sample (without adsorbent), indicating limited PEO adsorption at this Tween 20 concentration. In addition, the effect of particle size was less obvious considering the relative PEO peak areas. This behavior is partly due to the fact the PEO peak is rather broad, which makes accurate peak integration more troublesome. On the other hand, the specific surface area significantly affected the remaining amount of fatty acyl methylene and methyl groups (Fig. 6b and c). The much smaller amount of residual fatty acyl methylene and methyl groups in sample C (square symbols) shows more adsorption due to the increased specific surface area of these smaller PP particles.

Table 3 presents the comparison between the overall TC adsorption determined by combustion oxidation and the adsorption of different Tween 20 components determined by ^{1}H NMR spectroscopy. In the ideal case, due to the weak adsorption of the hydrophilic PEO, adsorption derived from $(CH_2)_n$ resonance intensities should be similar to that derived from the CH_3 resonance (due to amphiphilic PEO adsorption), and greater than PEO adsorption, while adsorption derived from TC values is expected to lie
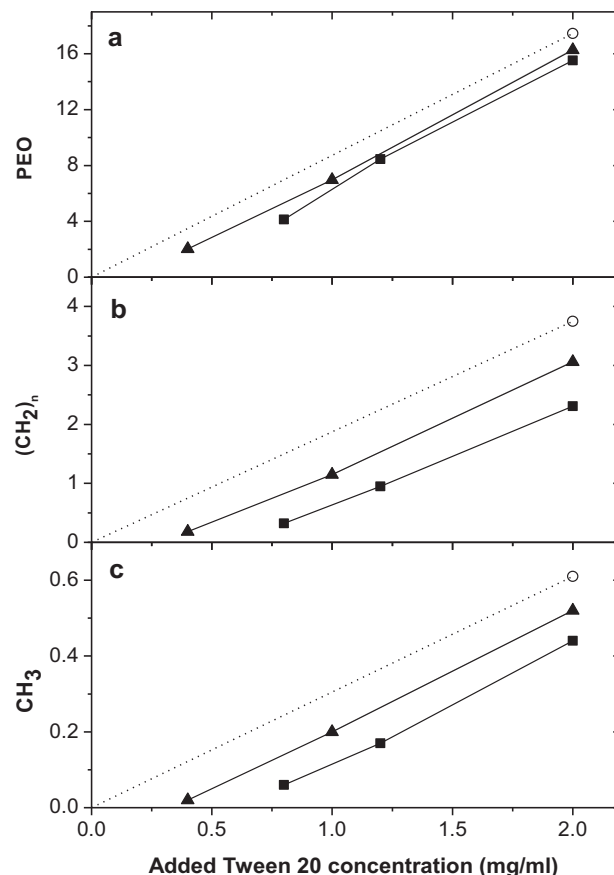

Fig. 6. ^{1}H NMR relative peak areas of Tween 20 components of the supernatants of PP suspensions as a function of the added surfactant concentration and particle specific surface area; the circle ($\bigcirc$), triangle ($\blacktriangle$), and square ($\blacksquare$) symbols represent the results obtained in the absence of PP, in the presence of powder B (A_s = 2.4 m^2/g), and in the presence of powder C (A_s = 3.7 m^2/g), respectively.

in between if all data are expressed as a percentage relative to the original concentration. Table 3 shows that the experimental results in percentage confirm this hypothesis. It should also be noted that, due to the lower proton number of the CH_3 group, its integration was not as accurate as that of the $(CH_2)_n$ resonance.

The $(CH_2)_n$, PEO and CH_3 adsorption results (expressed in mg/m^2) in Table 3 were calculated according to Eqs. (4) and (5). In these equations, F_{A,CH_n} is the adsorbed fraction based on NMR spectroscopy area, which corresponds to the complement of the relative peak area of the supernatant to the relative peak area of the corresponding blank. The fast diffusing fraction of PEO in the absence of PP was 38.5% (Table 2), from which the adsorbable (amphiphilic) molar fraction X_{amphi} was calculated to be 62.4% according to Verbrugghe et al. [11]. Given that the molecular weight of the hydrophilic PEO ($C_{46}H_{92}O_{25}$) is 1044 g/mol and the molecular weight of Tween 20 ($C_{58}H_{114}O_{26}$) is 1227 g/mol, the average molecular weight of the adsorbable amphiphilic PEO is hence 1335 g/mol, which accounts for 68.8% in mass fraction (x_{amphi}). Consequently, the adsorbable Tween concentration is obtained by multiplying the added Tween concentration C_{Tween} (in mass per unit volume) by the adsorbable mass fraction x_{amphi}. The adsorbed amount per unit surface area Γ_{amphi,CH_n} (mg/m^2) based on the $(CH_2)_n$ or CH_3 contribution in the NMR spectrum is then obtained from the adsorbent concentration C_{PP} (0.078 g PP/ml), as well as the specific surface area A_s of the adsorbent:

$$\Gamma_{amphi,CH_n} = \frac{C_{Tween} \cdot x_{amphi} \cdot F_{A,CH_n}}{C_{PP} \cdot A_s} \qquad (4)$$

Table 3
The adsorbed amount of TC, PEO, $(CH_2)_n$, and CH_3 at room temperature (both expressed as percentage of the added amount and in adsorbed amount per unit surface area) by 2 different PP powders (B and C) of different surface areas (A_s) as a function of added Tween 20 concentration.

A_s (m²/g)	Tween 20 (mg/ml)	TC		PEO		$(CH_2)_n$		CH_3	
		%	mg/m²	%	mg/m²	%	mg/m²	%	mg/m²
2.4	0.4	35.3	0.76	34.8	0.82	76.0	1.12	83.6	1.23
	1.0	24.5	1.31	20.2	1.20	38.7	1.43	34.4	1.27
	2.0	11.1	1.19	6.8	0.80	18.4	1.36	14.8	1.09
3.7	0.8	56.9	1.56	40.9	1.24	78.7	1.49	75.4	1.42
	1.2	38.5	1.58	19.2	0.87	57.8	1.64	53.6	1.52
	2.0	30.1	2.06	10.5	0.79	31.2	1.47	23.0	1.09

Table 4
The fast and slow diffusion coefficients (D) of PEO (characterized by a bi-exponential model), the diffusion coefficient of the fatty acyl chain (characterized by a mono-exponential model) of Tween 20 and the fast diffusing fraction of PEO as a function of the specific surface area (A_s) of the PP powders and the added Tween 20 concentration.

A_s (m²/g)	Tween 20 (mg/ml)	D ($\times 10^{-11}$ m²/s)			Fast fraction (%)
		$(CH_2)_n$	Fast PEO	Slow PEO	
2.4	0.4	26.8 ± 4.2	20.3 ± 0.1	–	100
	1.0	8.3 ± 0.4	21.1 ± 0.2	5.8 ± 0.1	65.9 ± 0.9
	2.0	6.5 ± 0.1	21.1 ± 0.1	5.6 ± 0.0	52.2 ± 0.4
3.7	0.8	17.4 ± 2.2	20.3 ± 0.1	–	100
	1.2	11.3 ± 0.8	21.5 ± 0.3	7.6 ± 0.6	83.7 ± 2.2
	2.0	7.6 ± 0.4	20.8 ± 0.3	5.9 ± 0.1	63.0 ± 1.1

Considering the PEO NMR data, due account of the fact that part of PEO is not adsorbable must be taken. Hereby, the percentage of PEO that is effectively adsorbed is derived from the complement of the ratio of the relative peak area in the supernatant to the relative peak area of the blank $F_{A,PEO}$, divided by the molar fraction of amphiphilic ethoxylate X_{amphi}. Further multiplication by the adsorbable amount (i.e. $C_{Tween} \cdot x_{amphi}$) and dividing by the PP concentration and by the specific surface area of the PP yields the adsorbed amount per unit surface area $\Gamma_{amphi,PEO}$ (mg/m²):

$$\Gamma_{amphi,PEO} = \frac{(F_{A,PEO}/X_{amphi}) \cdot C_{Tween} \cdot x_{amphi}}{C_{PP} \cdot A_s} \qquad (5)$$

The average adsorbed amount of Tween 20 of both powders was 1.41 ± 0.44 mg/m² based on the TC data, 1.34 ± 0.18 mg/m² based on the $(CH_2)_n$ and CH_3 data and 0.95 ± 0.21 mg/m² based on the PEO data. These values are quite in line which each other, even though the percentages of adsorption are quite different when considering the data by TC, PEO, $(CH_2)_n$ or CH_3 NMR spectroscopy. Statistical analysis indicates that the adsorption data (in mg/m²) obtained by TC and from the $(CH_2)_n$ and CH_3 intensities in the NMR spectra are not significantly different based on a 95% confidence level. This confirms that only the amphiphilic PEO was effectively adsorbed. For the sake of completeness, it has to be mentioned that this adsorption behavior does not apply to all hydrophobic materials: thus, previous studies indicated that not only amphiphilic but also hydrophilic PEOs were adsorbed to activated carbon [30]. On the other hand, the adsorbed amount derived from the PEO NMR signal data was significantly lower which indicates that molecular species with a lower degree of ethoxylation (such as ethoxylates esterified to 2 or 3 fatty acyl groups) are preferentially adsorbed.

Diffusion measurement results in Table 4 show that the particle surface area significantly affected the remaining amount of the hydrophobic components of Tween 20 in the samples containing powder B ($A_s = 2.4$ m²/g) and C ($A_s = 3.7$ m²/g). The much smaller intensities of $(CH_2)_n$ and CH_3 signals in the samples of the smaller particles, as deduced from the larger contribution of the fast fraction, indicate more adsorption due to the increased specific surface area of the PP particles in the sample with the larger specific surface area. In addition, the increase in the fast decaying fraction of the PEO peak provides further evidence for the hypoth-

esis that the adsorption affinity of the fatty-acyl-linked PEO groups is much larger than the affinity of the free PEO groups. As the overall fatty acyl to sorbitan ratio is 1, the presence of unesterified sorbitans indicates that species of different fatty acyl to sorbitan ratio exist within Tween 20, whereby the species with the largest ratio (e.g. surfactant species containing 2 or 3 fatty acyl chains per sorbitan ring) are preferentially adsorbed whereas the species with the lowest ratio (i.e. free PEO) mainly remain dissolved in the continuous phase. Hillgren et al. [31] also reported that increasing the Tween 80 concentration in a lactate dehydrogenase solution caused a decrease in the apparent diffusion coefficient of Tween. Their data seem to be in agreement with our observations because an increase in Tween concentration will lead to a smaller fraction of adsorbed surfactant and hence a decreased portion of the fast diffusing PEO.

The diffusion coefficient of the residual fatty acyl ethylene groups increased as the equilibrium concentration decreased, and approached the value of the monomeric ethoxylates at the lowest surfactant concentrations. This behavior indicates that the contribution of micelles is steadily decreasing as the surfactant concentration is reduced down to the critical micelle concentration (CMC). According to Farhadie [32] and Wan and Lee [33], the CMC of Tween 20 is approximately 0.07 mg/ml at 25 °C, whereas according to Deechongkit et al. [34], the initiation of micellization begins already at 0.02 mg/ml. Below the CMC, only monomeric surfactant molecules remain, which are characterized by a much larger diffusion coefficient as compared to micelles. This behavior also induces the transformation from a bi-exponential to a mono-exponential decay for the PEO peak due to the greatly decreased difference in diffusion speed between the amphiphilic and the hydrophilic PEO molecules, in combination with the increased contribution of the hydrophilic component of PEO, as a result of its lower sorption affinity.

4. Conclusions

This study shows that the combination of TC measurements and high resolution NMR techniques allowed correlating the overall Tween 20 adsorption based on the residual total carbon content with the preferential adsorption of specific ethoxylated sorbitan species. This combination enabled a more detailed investigation

on the adsorption of Tween 20 to hydrophobic particles, such as paliperidone palmitate.

The Tween 20 adsorption could be approximated by a Langmuir model, whereby the maximum adsorption was proportional to the specific surface area of the particles. Temperature (ranging from room temperature to 40 °C) exhibited a significant enhancing effect on the adsorption of Tween 20 for the PP powder of the smallest particle size distribution.

The correlation between adsorption quantities derived from TC and NMR measurement indicated that the free PEO in Tween 20 was poorly adsorbed due to its high hydrophilicity. On the other hand, the amphiphilic PEO was much more effectively adsorbed. The latter's higher affinity to the hydrophobic surface of PP may be ascribed to the hydrophobicity of the linked fatty acyl chain. In this regard, PEO molecules with more than one fatty acyl chain attached were even more effectively adsorbed to PP.

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