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Enhancing the solubility of hydrophobic drugs by solubilization in micellar surfactant solutions

SUMMARY

of the thesis, presented for the academic degree
“Doctor of philosophy” (PhD)

Professional area: 7.3 Pharmacy
Pharmaceutical technology and biopharmaceutics

Sofia, 2020

Thesis information

- The thesis consists of 77 pages, 33 figures and illustrations and 4 tables.
- Cited literature consists of 110 references.
- The thesis is based on 2 published papers that have 39 citations in total.
- The numbering of sections, figures and tables in the Summary of the thesis corresponds to the numbering in the complete text.

Contents

Chapter 1. Introduction

For a substance to be absorbed in the gastrointestinal tract (GIT), it must dissolve in the GIT fluids and cross the membrane of the enterocytes. The conceptual role of drug solubility and membrane permeability for the oral bioavailability has been established by the biopharmaceutics classification system (BCS), proposed by Amidon et al. [1]. BCS divides drugs in four main classes, depending on their solubility and membrane permeability. The membrane permeability is related to the ability of the drug molecules to cross the lipophilic cell membrane of the enterocytes, which is required for drug absorption [2]. Drugs from class I (high solubility, high permeability) are usually characterized with high oral bioavailability. In contrast, drugs belonging to class IV (low solubility, low permeability) have very low or highly variable oral bioavailability.

The importance of solubility and permeability for oral drug absorption is recognized also in physiologically-based pharmacokinetic (PBPK) modeling, where these parameters play a central role for the prediction of oral bioavailability [3–5]. To improve the predictive power of PBPK models, it is critical to establish the correct values of drug solubility in simple aqueous and biorelevant media, where solubilization can occur by excipients or endogenous surfactants (*e.g.* bile salts and phospholipids) [6–8].

The three drugs studied in the thesis (progesterone, danazol and fenofibrate) are BCS class II compounds, characterized by low solubility and high permeability [9,10]. Although the oral delivery of such poorly water-soluble drugs is challenging, pharmaceutical technology offers various solutions for drug solubility enhancement [11–13].

Solubility is constant for each solute-solvent pair (at constant temperature). Hence, drug solubilization in micellar surfactant solutions increases the *apparent* drug solubility [14–16]: drug molecules are incorporated in colloidal aggregates (micelles), which are homogenously dispersed in the solution. Micelles are self-assembled structures that are formed spontaneously in solutions of surfactants above the critical micelle concentration (CMC) [17].

Experimental evidence shows that the solubility of hydrophobic drugs increases at higher surfactant concentrations [18,19]. The driving force for solubilization is the difference of the

standard chemical potential of a molecule dissolved in aqueous medium, compared to a molecule incorporated in a surfactant micelle.

The solubilization of drugs by surfactant micelles has been extensively studied [61,71,73–84]. The effect of alkylsulfate, polysorbate, ethoxylated alcohol, ethoxylated alkyl ester and alkyltrimethylammonium bromide surfactants on drug solubilization has been evaluated. However, most of these studies report the measured drug solubilization without analyzing the link between the surfactant chemical structure and the drug solubilization capacity of the micelles. The lack of consensus on the main mechanisms that govern the strong effects of surfactant hydrophilic headgroup and hydrophobic chain on drug solubilization is also obvious. Therefore, despite the significant efforts to improve the understanding of drug solubilization by surfactants, the main molecular mechanisms and interactions that govern this process are still unclear. This also leads to the lack of rational surfactant selection criteria when drug solubility enhancement by solubilization is considered.

The main aims of the thesis are to clarify how the surfactant and drug molecular structures determine the micellar solubilization capacity and to provide mechanistic physicochemical interpretation of the observed effects. To achieve these goals, the following tasks were defined:

(A) To study systematically the solubilization of three hydrophobic drugs (progesterone, danazol and fenofibrate) by a wide range of surfactants.

(B) To establish the main trends from the obtained data and to define possible mechanisms and hypotheses to explain the results.

(C) To perform dedicated experiments to check the defined hypotheses by using model hydrophobic substances and/or appropriate complementary methods.

In Chapter 2 of the thesis are described all materials and methods used. To introduce the reader to the research approach used, the results for one of the drugs studied (progesterone) are presented first (Chapter 3) and then, the results for fenofibrate and danazol are presented in the same fashion (Chapter 4). The data for progesterone is compared to fenofibrate and danazol in Chapter 4. The conclusions and main contributions of the thesis are summarized at the end of the text, together with the published papers, presentations at scientific conferences and references.

Chapter 2. Materials and methods

2.1. Materials

The link between surfactant chemical structure and micellar drug solubilization was studied by using three drugs, one model hydrophobic compounds and 20 surfactants. The studied drugs included two compounds with steroidal structure, used in gynecology (progesterone and danazol), one drug with aromatic structure from the group of fibrates (fenofibrate) and one model hydrophobic compound with very low dipole moment, which is a steroidal sex hormone analogue (androstane).

Homologue series (from C_{10} to C_{18}) of surfactants with different charge were studied: nonionic (polysorbates; ethoxylated alcohols), anionic (alkylsulfates) and cationic (trimethylammonium bromides). The impact of the number of ethylene oxide units in the head group of ethoxylated dodecylsulfates and alcohols was also studied. Additional surfactant types that include specific molecular motifs like aromatic rings and double bonds were included as well (*e.g.* linear alkyl-benzen sulfonate, LAS). Although some of these surfactants are toxic and rarely used in drug delivery, they were used to clarify the general trends (*viz.* effect of surfactant charge).

2.2. Determination of drug solubilization in micellar surfactant solutions

The equilibrium drug or androstane solubility in micellar surfactant solutions was measured by high-performance liquid chromatography (HPLC) or gas chromatography (GC) by the following protocol: excess of solid drug or androstane was added to 10 mL freshly prepared surfactant solution, after which the suspension was stirred for 24 h at T = 37 °C. Afterwards, the suspension was filtered by a NYLON filter with 200 nm pore size to separate all undissolved particles. The obtained clear aqueous phase was analyzed by HPLC (for the drug molecules) or was extracted by chloroform and analyzed by GC (for androstane). Some of the experiments were performed in presence of 600 mM NaCl in the surfactant solution to study the effect of ionic strength on solubilization. The excess solid drug used in the solubilization experiments was at least three orders of magnitude higher than the equilibrium aqueous solubility of the studied substances (10 mg/mL progesterone, 1.0 mg/mL danazol, 1.5 mg/mL fenofibrate and 1 mg/mL androstane).

Preliminary experiments showed that drug solubilization depends linearly on surfactant concentration and, hence, the solubilization capacity can be calculated by using experiments at single surfactant concentrations. Therefore, most of the experiments with fenofibrate and danazol were performed at 0.5 wt % surfactant concentration, whereas the experiments with progesterone were performed at 40 mM surfactant concentration.

2.5. Calculation of the drug solubilization capacity of the micelles

The following equation was used to calculate the micellar solubilization capacity [19]:

$$\chi = \left(\frac{S_{tot} - S_W}{C_S - CMC} \right) \times 1000 \qquad (18)$$

where S_{tot} is the measured molar drug solubility in the presence of surfactants, S_W is the aqueous solubility of the drug, C_S is the molar surfactant concentration and CMC is the critical micelle concentration of the respective surfactant. Note that the subtraction of S_W from S_{tot}, and of CMC from C_S, allows one to consider only the drug and surfactant molecules that are incorporated in the micelles (surfactant monomers and drug molecules dissolved in water are disregarded).

2.6. Determination of the solubilization locus polarity

The polarity of the locus of fenofibrate solubilization was assessed by UV/Vis absorption spectrometry [22,32]. In this method, the shift of the absorption spectrum of the solubilized molecules is used to assess the polarity of their surroundings in the micelle. To determine the dependence of the spectral shift on solvent polarity, fenofibrate spectra were obtained in a series of solvents with increasing polarity (Figure 9): *n*-dodecane, *n*-octanol, methanol and several water:methanol mixtures (the most polar medium studied was 70:30 water:methanol, vol./vol.). The solvent shift was characterized by the shift in the shoulder between λ = 300 and 320 nm, determined at molar absorption coefficient of ε_{uv} = 5 $mM^{-1}.cm^{-1}$, which provided higher sensitivity and resolution, compared to the shifts of the absorption maxima, see Figure 10.

The absorption spectra were measured in the range from 200 to 400 nm by an Unicam 8625 UV/Vis spectrophotometer. All solutions of fenofibrate (both in solvents and in surfactants) were diluted in the respective media to obtain an absorption of 1.0 ± 0.2 AU at λ_{max}, in order to maximize the sensitivity and accuracy of the measurement.

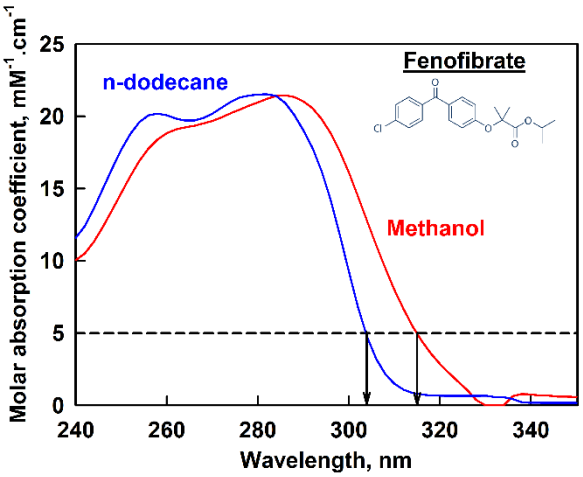


Figure 9. Absorption spectrum of fenofibrate in methanol (red line) and n-dodecane (blue line). Significant red shift of the spectrum is observed upon increasing the solvent polarity. The difference in the wavelengths at molar absorption coefficent of ε_{uv} = 5 $mM^{-1}.cm^{-1}$ (see the horizontal dashed line) is used to characterize the dependance of the shift on solvent polarity by using a series of solvents.

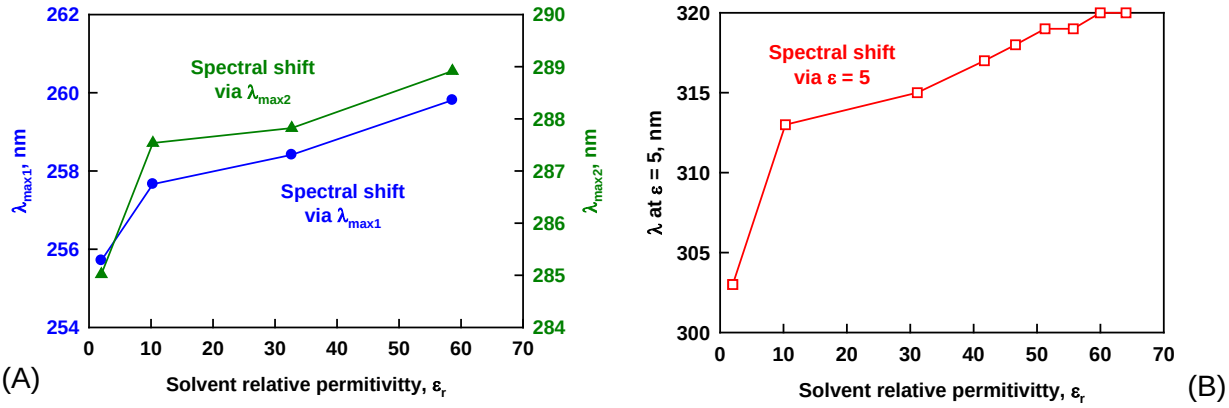


Figure 10. Shifts of (A) λ_{max1} and λ_{max2} or (B) λ at ε = 5 $mM^{-1}.cm^{-1}$, as a function of solvent relative permittivity for fenofibrate.

Chapter 3. Progesterone solubilization

3.1. Introduction and aim of the study

This Chapter presents the results from the study of progesterone solubilization in surfactant solutions. The results were interpreted in view of the molecular mechanisms of the observed effects. Additional experiments were performed with the model hydrophobic compound androstane to check the formulated hypotheses. The main conclusions from this part of the thesis are summarized at the end of the chapter.

3.2. Experimental results and discussion

3.2.1. Effect of surfactant type on progesterone solubility

The solubility of progesterone in micellar surfactant solutions is presented in Figure 12. One sees that drug solubility depends very strongly on surfactant type: solubilities in the range of 0.25 to 3.3 g/L (25 to 300-fold higher than the aqueous solubility of progesterone) were measured.

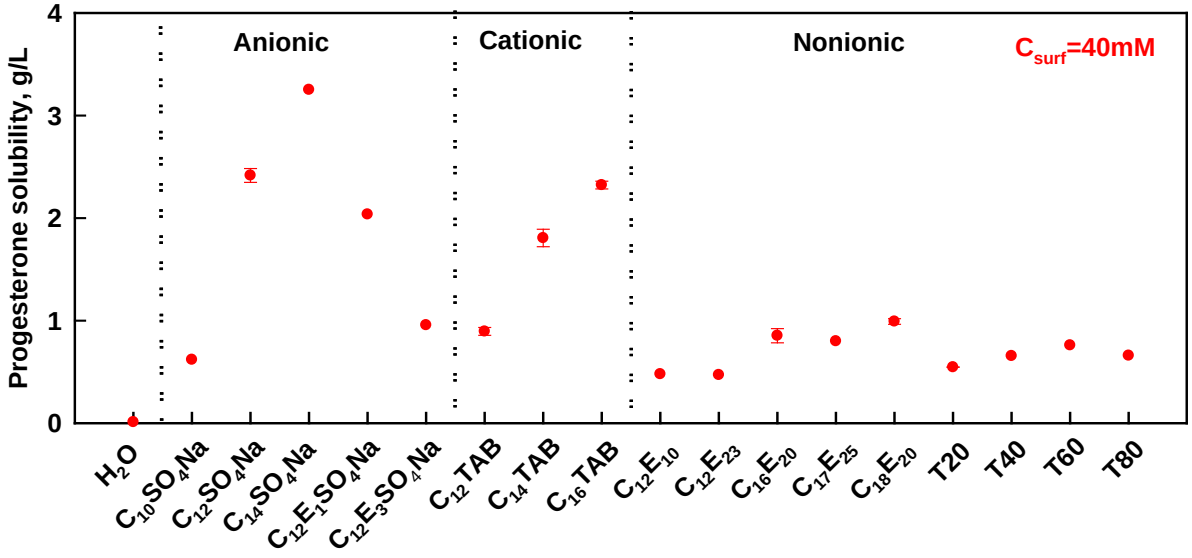


Figure 12. Progesterone solubility as a function of surfactant type. Experiments we performed at constant surfactant concentration of 40 mM and T = 37 °C. The error bars can be smaller than the symbols.

3.2.2. Link between surfactant structure and drug solubilization capacity

The effect of the two main structural properties of the surfactants (the hydrophobic chain length and head group type) was studied by comparing the solubilization capacity of the surfactants, calculated by equation (18), described in section 2.5.

The effect of the type of hydrophilic head group for a series of surfactants with the same hydrophobic chain length (C_{12}) is presented in Figure 13. The highest solubilization capacity of the sulfate group (≈ 250 mM/M) decreased dramatically (to ≈ 40 mM/M) when it was replaced with uncharged E_{10}, E_{23} or sorbitan-E_{20} group. The addition of 1 or 3 ethylene oxide units in between the sulfate head group and the hydrophobic alkyl chain also decreased strongly the solubilization capacity. The trimethylammonium bromide (TAB) group had intermediate properties: smaller solubilization capacity compared to the sulfate group, but much higher than all uncharged groups. The increase of ethylene oxide units from 10 to 23 had no significant effect on the solubilization capacity of the nonionic alcohol ethoxylates, which was rather low (≈ 40 mM/M) and similar to that of the polyoxyethylene-sorbitan group.

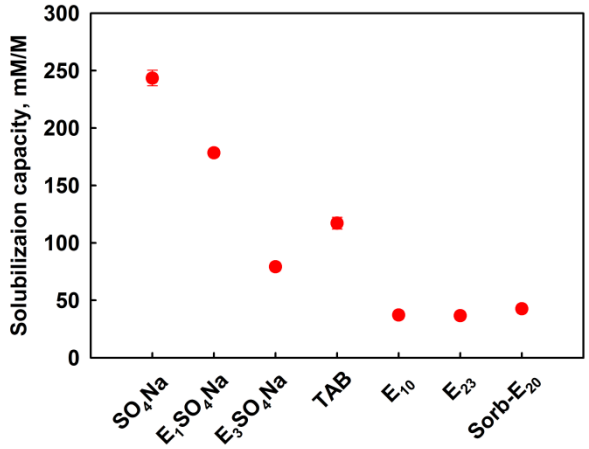


Figure 13. Progesterone solubilization capacity as a function of hydrophilic head group type for surfactants with the same hydrophobic chain length (C-12). The error bars can be smaller than the symbols ($n \geq 2$).

The strong impact of the hydrophilic head group type on Progesterone solubilization capacity clearly indicates that the drug molecules are solubilized in the palisade layer of the surfactant micelles. Effects of such magnitude are not expected for molecules that are solubilized

in the anhydrous hydrophobic core of the micelles, where hydrophobic and dispersion interactions govern the solubilization capacity [33]. Furthermore, only molecules with very simple aliphatic structure have been shown to be located in the hydrophobic core of the micelles [33–36], whereas polar molecules such as Progesterone are usually solubilized in the palisade layer [37–42].

To gain further insight about the micellar microenvironment of solubilized Progesterone and the main intermolecular interactions that determine the solubilization capacity, we can examine additional details of the experimental data. Best solubilization is observed in charged surfactants micelles, which suggests that electrostatic interactions play a key role in solubilization. As Progesterone molecules are not charged, the interactions are most likely of the ion-dipole type. The latter explanation suggests that the lower solubilization capacity of the positively charged TAB group is due to lower binding energy (*viz.* weaker ion-dipole interactions) of the TAB group to Progesterone. In support of the latter suggestion, the sulfate group of alkylsulfate surfactants was shown experimentally to bind water molecules via ion-dipole interactions much more strongly than the TAB group [43].

The validity of the ion-dipole interaction hypothesis was checked by solubilization experiments with Androstane: a hydrophobic molecule with simple steroid structure, which in contrast to Progesterone does not contain any polar atoms (O, N, S) or unsaturated groups (C=C, C≡C). If the proposed hypothesis is correct, one would expect low solubilization capacity of Androstane in ionic surfactant micelles, due to the very low dipole moment of Androstane, which results in very weak ion-dipole interactions. The results for Androstane solubilization in $C_{12}SO_4Na$, $C_{12}TAB$ and Tween 20 surfactants are presented in Figure 15. One sees that indeed, the solubilization capacity of the ionic surfactants for Androstane is much lower than for Progesterone. Therefore, ion-dipole interactions between Progesterone and surfactant head groups are key for the micellar solubilization capacity.

To further clarify the role of surfactant charge and electrostatic interactions we performed additional experiments at high ionic strength of 600 mM NaCl with one cationic, anionic and nonionic C-12 chain-length surfactant, see Figure 15B. The solubilization capacity of the charged surfactants decreased very strongly, whereas no effect was observed for the nonionic surfactant. The lack of effect of ionic strength for the nonionic surfactants is not surprising, as their head

groups are uncharged. However, screening the charged surfactant head groups (sulfate and TAB) resulted in drastic decrease of the solubilization capacity, due to the decreased ion-dipole interactions strength. Note that the change in the number of micelles (*viz*. the CMC) is accounted for in the calculation of the solubilization capacity and thus cannot explain the observed decrease.

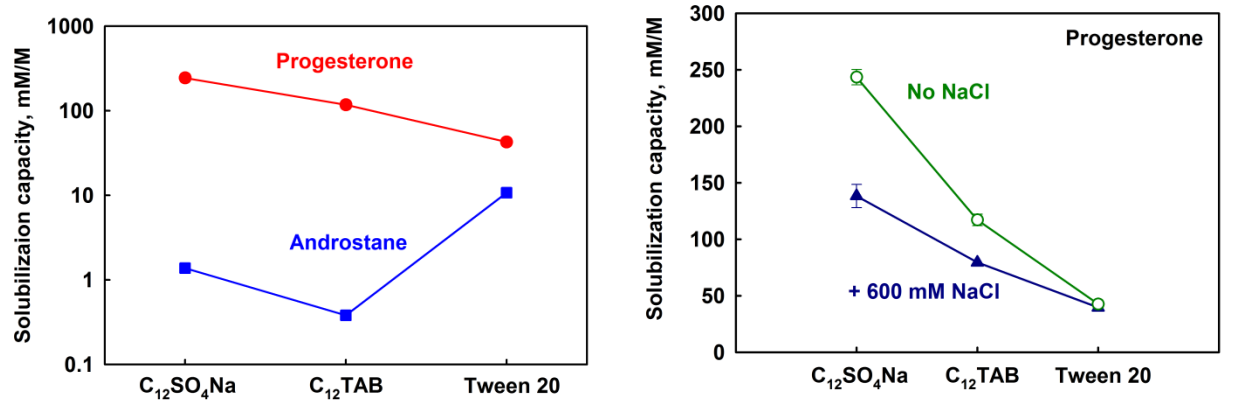


Figure 15. Solubilization capacity of sodium dodecyl sulfate, dodecyl trimethylammoinum bromide and Tween 20 for (A) Progesterone (red circles) and Androstane (blue squares) and (B) Progesterone in absence of NaCl (empty green circles) and at high ionic strength of 600 mM NaCl (full dark blue triangles). The error bars can be smaller than the symbols ($n \geq 2$).

3.2.3 Effect of the hydrophobic chain length

The effect of hydrophobic chain length on the solubilization capacity of alkylsulfate, alkyltrimethylammonium bromide (TAB), alcoholethoxylate and polyoxyethylene sorbitan ester surfactants is presented in Figure 16. The increase of hydrophobic chain length increases linearly the solubilization capacity of all studied types of surfactants. The effect is most pronounced for surfactants with charged head group (alkylsulfates and alkyl-TABs), whereas it is much smaller for the nonionics. The different intercept of the curves illustrates the effect of the hydrophilic head group type on solubilization capacity, which was already discussed in the previous subsection.

The effect of hydrophobic chain length on drug solubilization is well documented in literature and is usually explained with the decrease of CMC or the increase of the micellar hydrophobic core volume [33]. However, the differences in CMC have been accounted for in the

calculation of the solubilization capacity and thus cannot explain the obtained results. On the other hand, the large effect of surfactant hydrophilic head on solubilization showed that the locus of Progesterone solubilization is most likely in the palisade layer, hence, the increase of hydrophobic core volume cannot explain the observed increase in the solubilization capacity. Most likely, the improved solubilization is due to increased palisade layer volume.

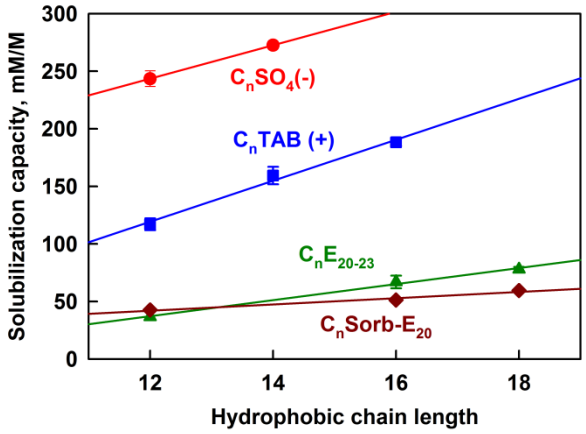


Figure 16. Molar progesterone solubilization capacity as a function of surfactant hydrophobic chain length for alkylsulfate (red circles), alkyltrimethylammonium bromide (blue squares), alcoholethoxylate (green triangles) and polyoxyethylene sorbitan ester (brown diamonds) surfactants. The error bars can be smaller than the symbols ($n \geq 2$).

3.3. Conclusions from Chapter 3

The solubilization of Progesterone by 17 surfactants with a variety of hydrophilic head groups and hydrophobic chain lengths was studied. Charged surfactants showed highest solubilization capacity, increasing Progesterone solubility above 3 mg/mL, whereas all nonionic surfactants had much smaller effect (0.5 to 1 mg/mL Progesterone solubility). The high solubilization of Progesterone in charged surfactant micelles was explained by ion-dipole interactions. The increase of hydrophobic chain length improved drug solubilization for all studied surfactants, regardless of the type and charge of the hydrophilic head. In respect to the effect of hydrophilic head group, the solubilization capacity of C-12 surfactants decreased in the order SO_4^- > $E_1SO_4^-$ > $^+N(CH_3)_3$ > $E_3SO_4^-$ > $SorbEO_{20}$ = E_{10} = E_{23}. All obtained results indicate that the locus of Progesterone solubilization is in the micelle palisade layer, where electrostatic ion-dipole interactions with charged surfactant head groups, combined with hydrophobic

interactions between the hydrophobic moiety of the Progesterone molecule and the alkane chain of the surfactant, lead to high solubilization capacity. Therefore, the best candidates to improve oral Progesterone absorption through solubility enhancement are surfactants with long hydrophobic chain and charged hydrophilic head group (*e.g*. alkylsulfates).

Chapter 4. Solubilization of fenofibrate and danazol

4.1. Introduction and aim of the study

Drug solubilization in micellar surfactant solutions was further explored in this Chapter by studying two drugs with different structure: fenofibrate, which contains two aromatic rings, and danazol, which has a steroidal structure (similar to progesterone). The same approach as in Chapter 3 was used to study drug solubilization. First, drug solubility in solutions of surfactants with different charge and hydrophobic chain length was determined and the main trends were established. Then, the suggested hypotheses and mechanisms were checked by dedicated experiments: comparison of danazol solubilization with a less polar compound with similar structure (androstane) and determination of the fenofibrate solubilization locus by UV-spectrometry. The data for fenofibrate and danazol was compared to that obtained for progesterone. The main conclusions from this Chapter are summarized at the end of this part of the thesis.

4.2. Experimental results

4.2.1. Solubilization of fenofibrate and danazol in surfactant solutions

The drug solubilization capacity of the studied surfactant micelles is compared in Figure 19. Two general trends are observed: (a) ionic surfactants solubilize danazol much more efficiently than fenofibrate and (b) the nonionic surfactants solubilize fenofibrate better than danazol. Thus, maximal solubilization capacity for fenofibrate ($\chi_{max} \approx 50$ mM/M) is attained by several nonionic ($C_{18}E_{20}$, T60 and T80) and one anionic surfactant ($C_{14}SO_4Na$). In contrast, danazol is solubilized best by the ionic surfactants $C_{14}SO_4Na$ and $C_{14}TAB$ and its maximal solubilization (χ_{max} = 90-100 mM/M) is much higher than that of fenofibrate. The obtained results clearly demonstrate that the solubilization capacity is particularly sensitive to both drug and surfactant type, in agreement with the results presented in Chapter 3.

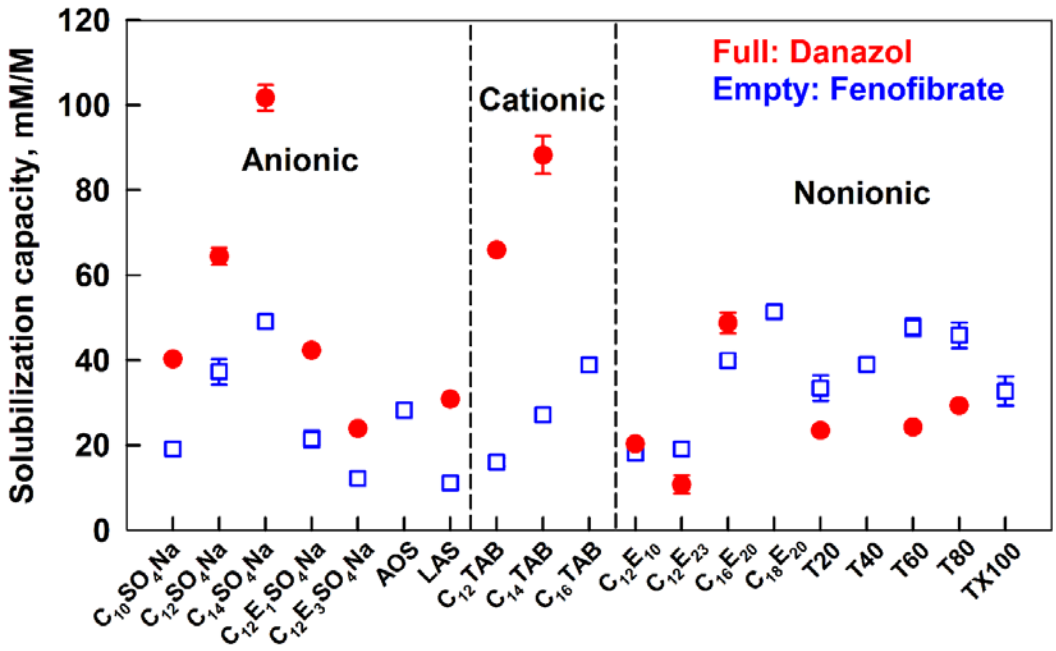


Figure 19. Solubilization capacity of fenofibrate (empty blue squares) and danazol (full red circles) as a function of the surfactant type. The error bars can be smaller than the symbols.

To analyze the effect of surfactant structure on solubilization, the solubilization capacity is plotted as a function of the hydrophobic chain length for the different surfactant head groups, see Figure 21. For each of the plots in Figure 21, the chain length is varied while the type of hydrophilic head is the same: trimethylammonium bromide for the cationics, sulfate for the anionics, and ethylene oxide (20-23) for the nonionics. The increase of the chain length increases linearly the solubilization of both drugs for all surfactant types studied (nonionic, cationic and anionic). Comparing the magnitude of solubilization capacity increase per CH_2-group (*viz.* the slopes of the lines in Figure 21), one sees that the effect is greater for danazol than for fenofibrate, for all surfactants studied. In respect to the type of surfactant, the magnitude of the chain length effect decreases in the order $C_nSO_4Na > C_nTAB > C_nE_{20\text{-}23}$ for both drugs studied.

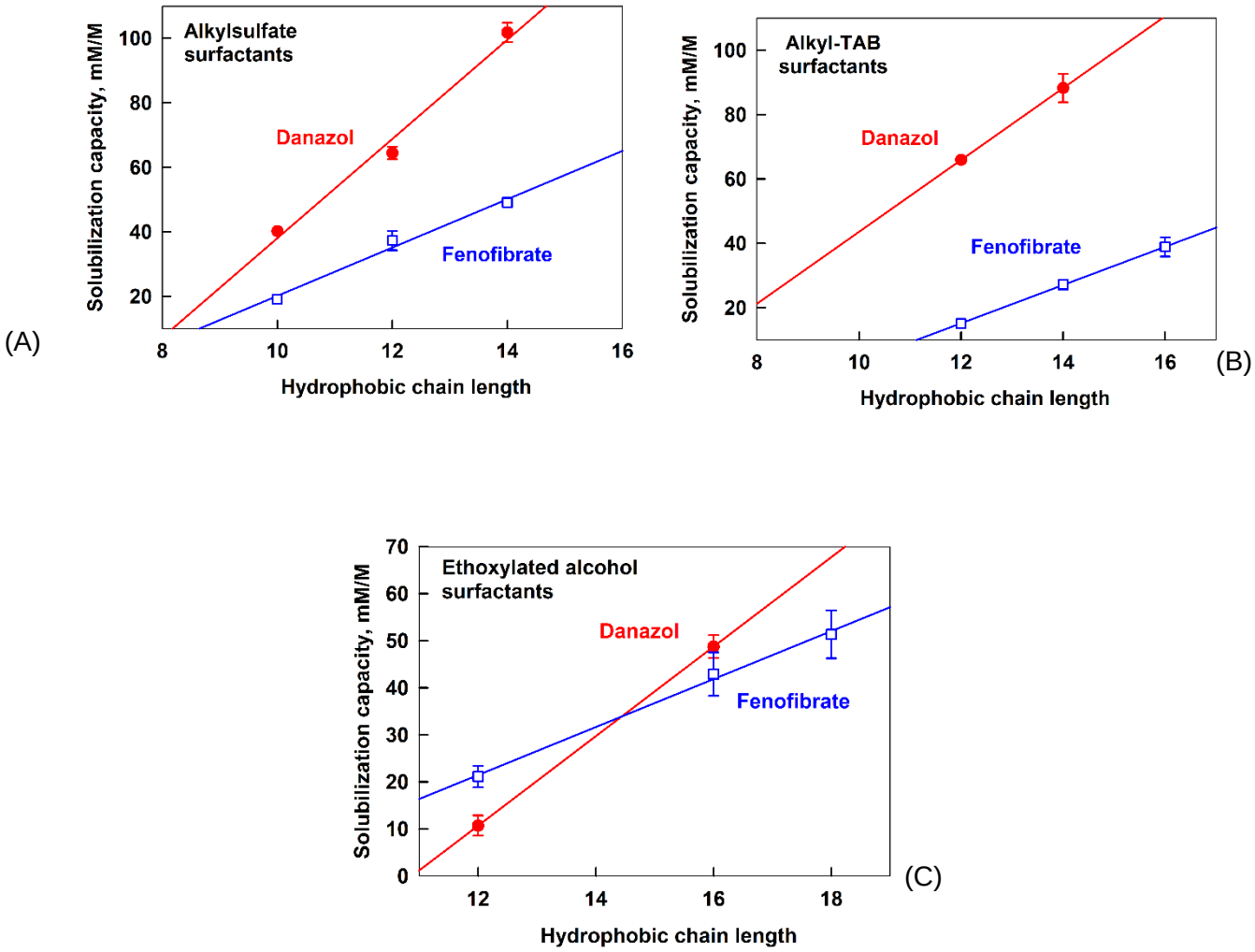


Figure 21. Solubilization capacity of fenofibrate (empty blue squares) and danazol (full red circles) as a function of the hydrophobic chain length of (A) alkylsulfate, (B) trimethylammonium bromide, and (C) ethoxylated alcohol (≈ 20 ethylene oxide units) surfactants. The results are averaged over at least two independent measurements. The error bars can be smaller than the symbols.

The effect of the hydrophilic head group on the solubilization capacity of surfactants with C_{12} hydrophobic chain is compared in Figure 23. As already explained, one sees that danazol is solubilized much more efficiently by ionic surfactants, compared to fenofibrate.

For fenofibrate, the solubilization capacity decreases in the order SO_4Na > E_1SO_4Na > E_{10} ≈ E_{23} ≈ TAB > E_3SO_4Na ≈ benz-SO_3Na. Thus, best solubilization is achieved for the surfactant with sulfate head group. The addition of ethylene oxide groups in between the sulfate

group and the alkyl chain decreases very strongly the solubilization capacity: χ = 37 and 12 mM/M for $C_{12}SO_4Na$ and $C_{12}E_3SO_4Na$, respectively. In contrast, the increase of ethylene oxide units from 10 to 23 has no significant effect on the solubilization capacity of the nonionic alcohol ethoxylates (χ = 18-19 mM/M).

For danazol, the solubilization capacity decreases in the order $SO_4Na \approx TAB > E_1SO_4Na > benz\text{-}SO_3Na > E_3SO_4Na \approx E_{10} > E_{23}$. Best solubilization is obtained by surfactants with sulfate or TAB head group. On the other hand, all surfactants with nonionic hydrophilic head have low solubilization capacity. The solubilization effectiveness of the nonionics decreases with the increased number of EO units in the head group: from χ = 20 mM/M for E_{10}, to χ = 11 mM/M for E_{23}.

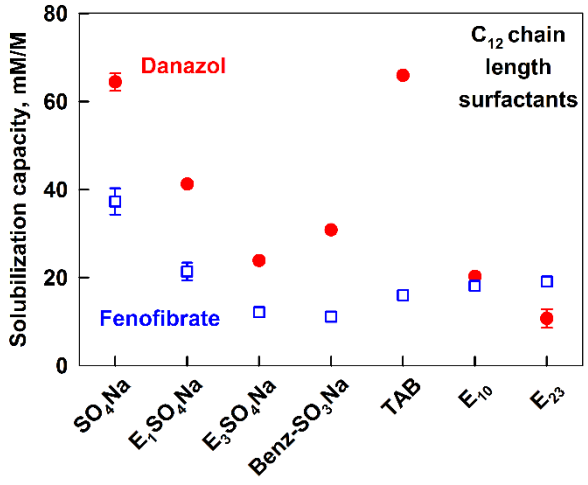


Figure 23. Solubilization capacity of fenofibrate (blue squares) and danazol (red circles), as a function of the type of hydrophilic head for surfactants with hydrophobic chain length of C_{12}. The error bars can be smaller than the symbols.

4.2.2. Relative polarity of fenofibrate solubilization locus in the micelles and correlation with solubilization capacity

The relative polarity of the fenofibrate microenvironment, measured in micelles of several surfactants, is presented in Figure 26. The relative polarity of the nonionic surfactant micelles solubilization locus ($\varepsilon_r \approx 5.5$) is comparable to that of *n*-dodecane (ε_r = 2.0). In contrast, the relative polarity of charged surfactant micelles in the absence of electrolyte is much higher ($\varepsilon_r \geq$ 19) and reaches values characteristic for polar solvents, such as methanol and methanol-water

mixtures. The increase of the chain length of C_nTAB surfactants from C = 14 to 16 decreases ε_r from ≈ 35 to 23, whereas the increase from C-12 to C-14 has no effect on ε_r.

Addition of ethylene oxide units to $C_{12}SO_4Na$ also decreases strongly the polarity of the solubilization locus: from ε_r = 41 (no EO units) to ε_r = 19 for 3 ethylene oxide units.

High electrolyte concentration (600 mM NaCl) decreases significantly the solubilization locus polarity for C_nTAB surfactant micelles, whereas no such effect is observed for $C_{12}SO_4Na$ micelles.

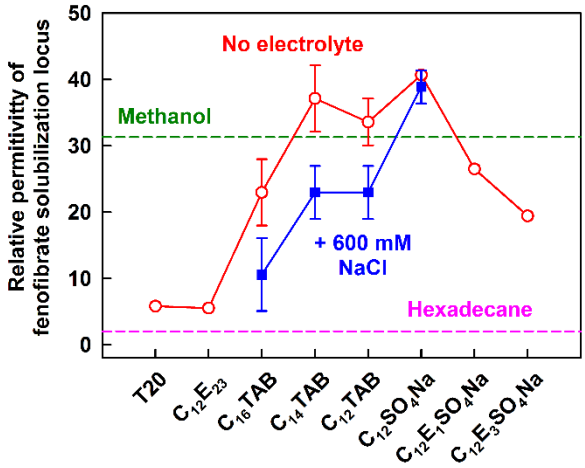


Figure 26. Relative dielectric permittivity of the locus of fenofibrate solubilization in different surfactant micelles, as determined by UV absorption spectroscopy and solvent calibration. The experiments were performed in absence of electrolyte (empty red circles) and in presence of 600 mM NaCl (full blue squares).

4.3. Discussion of the obtained results

4.3.1. Locus of fenofibrate solubilization in surfactant micelles

The location of the solubilized molecule inside the surfactant micelles is one of the factors that is expected to have a major influence on the micellar solubilization capacity. The experimental results show that, for the nonionic surfactant micelles (Tween 20 and $C_{12}E_{23}$), the aromatic part of fenofibrate is located in a medium with relative polarity similar to that of normal hydrocarbons (Figure 26). This result evidences that the locus of fenofibrate solubilization in these micelles is in the anhydrous hydrophobic core, Figure 28A. In contrast, the much higher relative polarity measured for ionic surfactant micelles in the absence of electrolyte shows that

the drug is located in the transition region between the anhydrous hydrophobic core and the micelle surface, *viz*. in the palisade layer, Figure 28B. Note that danazol molecules are more polar than those of fenofibrate which means that danazol is solubilized predominantly in the palisade layer, at least for the ionic surfactant micelles.

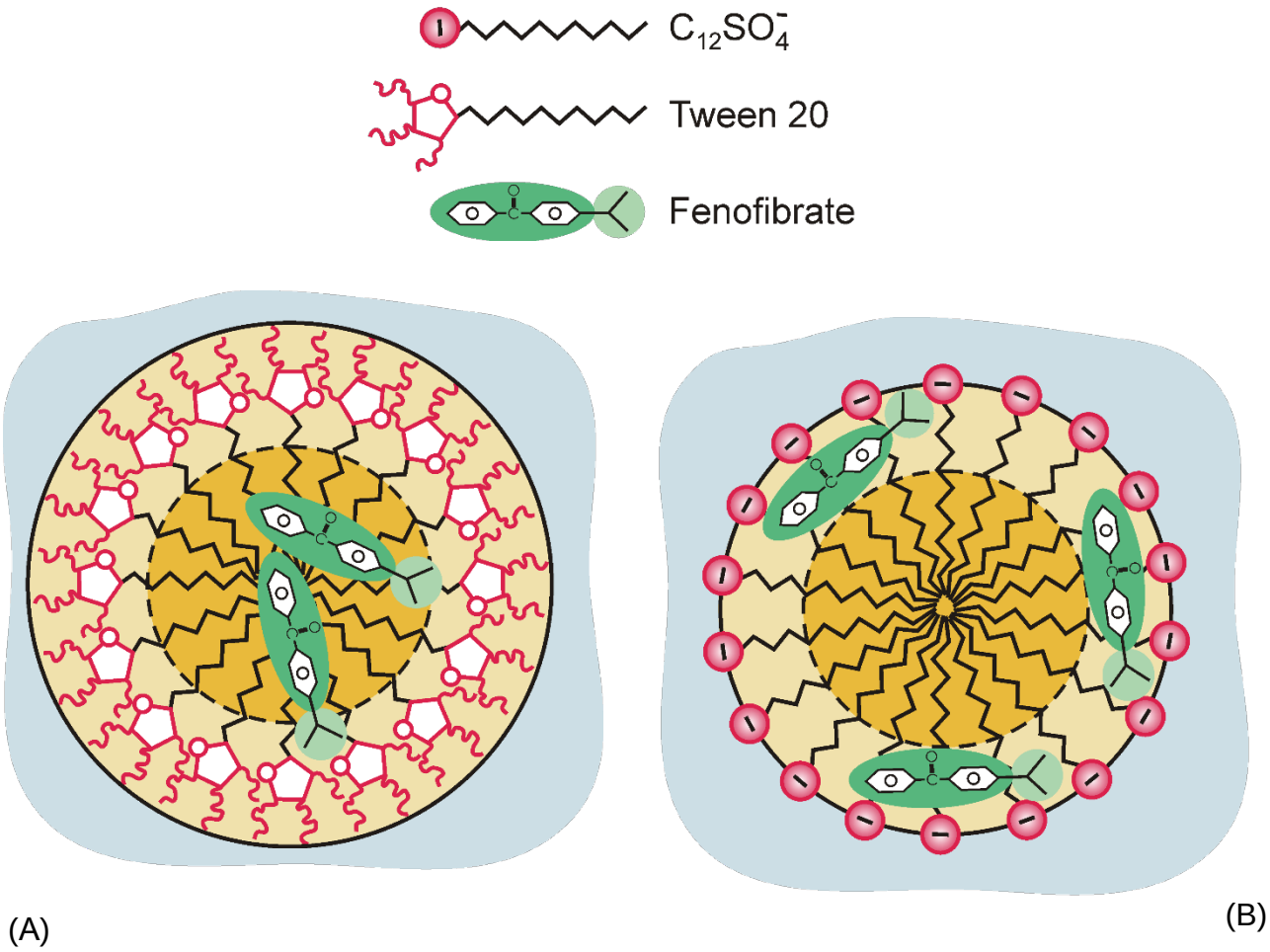


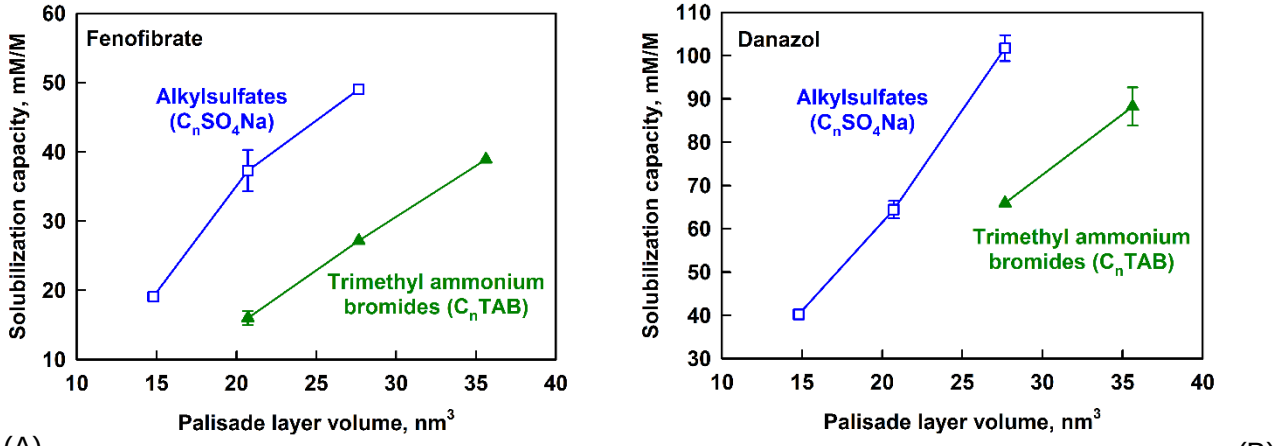
Figure 28. Schematic illustration of the fenofibrate solubilization: (A) inside the core of the micelles of nonionic surfactants, and (B) in the palisade layer of the micelles of ionic surfactants.

4.3.2. Effect of the hydrophobic chain length

Linear increase of surfactant solubilization capacity with the increase of hydrophobic chain length was observed for all studied drugs (danazol, fenofibrate and progesterone). The effect is present for all studied surfactants: nonionic (ethoxylated alcohols, polysorbates), anionic (alkylsulfates) and cationic (TABs).

The presence of double bond in the hydrophobic chain of polysorbates has no significant effect on the solubilization of both danazol and fenofibrate, as demonstrated by the similar solubilization capacity of T60 and T80.

The mechanism of improved solubilization at longer chain length for non-polar or slightly polar molecules is the increased volume of the hydrophobic core, where the solubilizate is located [33,44]. As discussed in Chapter 3, similar mechanism can be pictured for polar molecules like fenofibrate and danazol, which are solubilized in the palisade layer of ionic surfactants: increase of the hydrophobic chain length leads to bigger volume of the palisade layer and thus increases the space available for solubilization (Figure 28B). In agreement with the latter explanation, very good correlation is observed between the palisade layer volume and the solubilization capacity, see Figure 29.



(A) (B)

Figure 29. Solubilization capacity of (A) fenofibrate and (B) danazol, as a function of the palisade layer volume for alkylsulfate (empty blue squares) and trimethylammonium bromide (full green triangles) surfactants. The error bars can be smaller than the symbols.

To calculate the volume of the palisade layer constant depth of penetration of water molecules in the micelle (the first 3 methylene groups of micellized surfactant [45,46]) was assumed, whereas the approximation of Tanford (see the discussion in [17]) was used to calculate the total length of the surfactant hydrophobic chain. Therefore, the increased solubilization capacity with increasing surfactant chain length can be explained by the bigger volume of the palisade layer.

4.3.3. Effect of the hydrophilic head group

To check if the higher solubilization of danazol in ionic surfactant solutions is due to ion-dipole interactions, the same approach as in Chapter 3 was used. The solubilization of a hydrophobic molecule (androstane) with simple steroid structure which, in contrast to danazol, does not contain polar atoms (O, N, S) or unsaturated groups (C=C, C≡C), was studied. If the ion-dipole interactions are important for solubilization, one would expect much lower solubilization of androstane in ionic surfactant micelles, due to the apolar structure of androstane molecules, which results in very weak ion-dipole interactions. The results for androstane solubilization in $C_{12}SO_4Na$, $C_{12}TAB$ and Tween 20 surfactants are compared in Figure 31 with those for danazol. As predicted, the solubilization capacity of the ionic surfactants for androstane is much lower than that for danazol. Therefore, the ion-dipole interactions between danazol and surfactant head groups are key for the observed high solubilization capacity of the ionic micelles. The latter conclusion is supported further by the decreased solubilization of danazol in anionic surfactant micelles at high ionic strength.

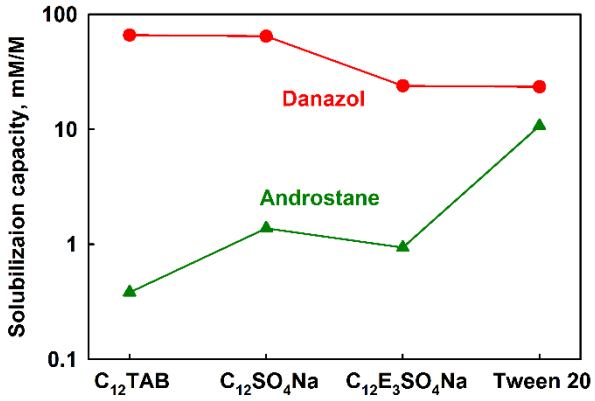


Figure 31. Solubilization capacity of danazol (red circles) and androstane (green triangles), as function of surfactant type.

4.3.4. Comparison between progesterone, danazol and fenofibrate

In Chapter 3, the effect of surfactant structure on the solubilization of the steroidal drug progesterone were clarified. In the current Chapter, the same approach was used to study the solubilization of danazol and fenofibrate. It would thus be interesting to compare the results for the three studied drugs, especially since danazol and progesterone share a similar (steroidal) chemical structure. To facilitate the interpretation of the observed trends, the impact of the hydrophobic chain length and the hydrophilic head group (which encompass most of the studied surfactant types) on drug solubilization is compared.

The solubilization capacity of surfactants with a hydrophobic chain length of C12 for the three studied drugs is presented in Figure 32.

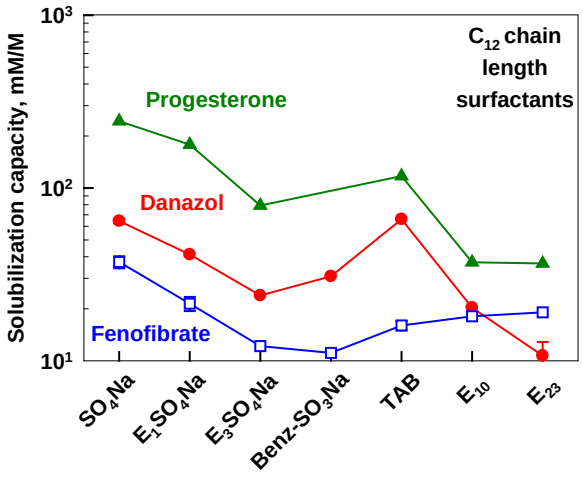


Figure 32. Solubilization capacity for fenofibrate (blue squares), danazol (red circles) and progesterone (green triangles), as function of the surfactant hydrophilic head group type for surfactant with the same hydrophobic chain length of C12. The error bars can be smaller than the symbols.

For the same hydrophilic head groups, the solubilization capacity is highest for progesterone, followed by danazol, while it is lowest for fenofibrate. The only exception from this trend is $C_{12}E_{23}$, for which the fenofibrate solubilization capacity is higher than for danazol.

The only trend shared by all three studied drugs is the decrease of the solubilization capacity with increase of ethylene oxide units in the head group of dodecyl sulfate surfactants, which is most likely due to the more difficult packing of the bulkier ethylene oxide group.

Ionic surfactants have higher solubilization capacity for the steroidal drugs studied (progesterone and danazol), compared to the nonionic surfactants, due to the additional ion-dipole interactions. This was not observed for fenofibrate, where some of the ionic and nonionic surfactants have the same effect.

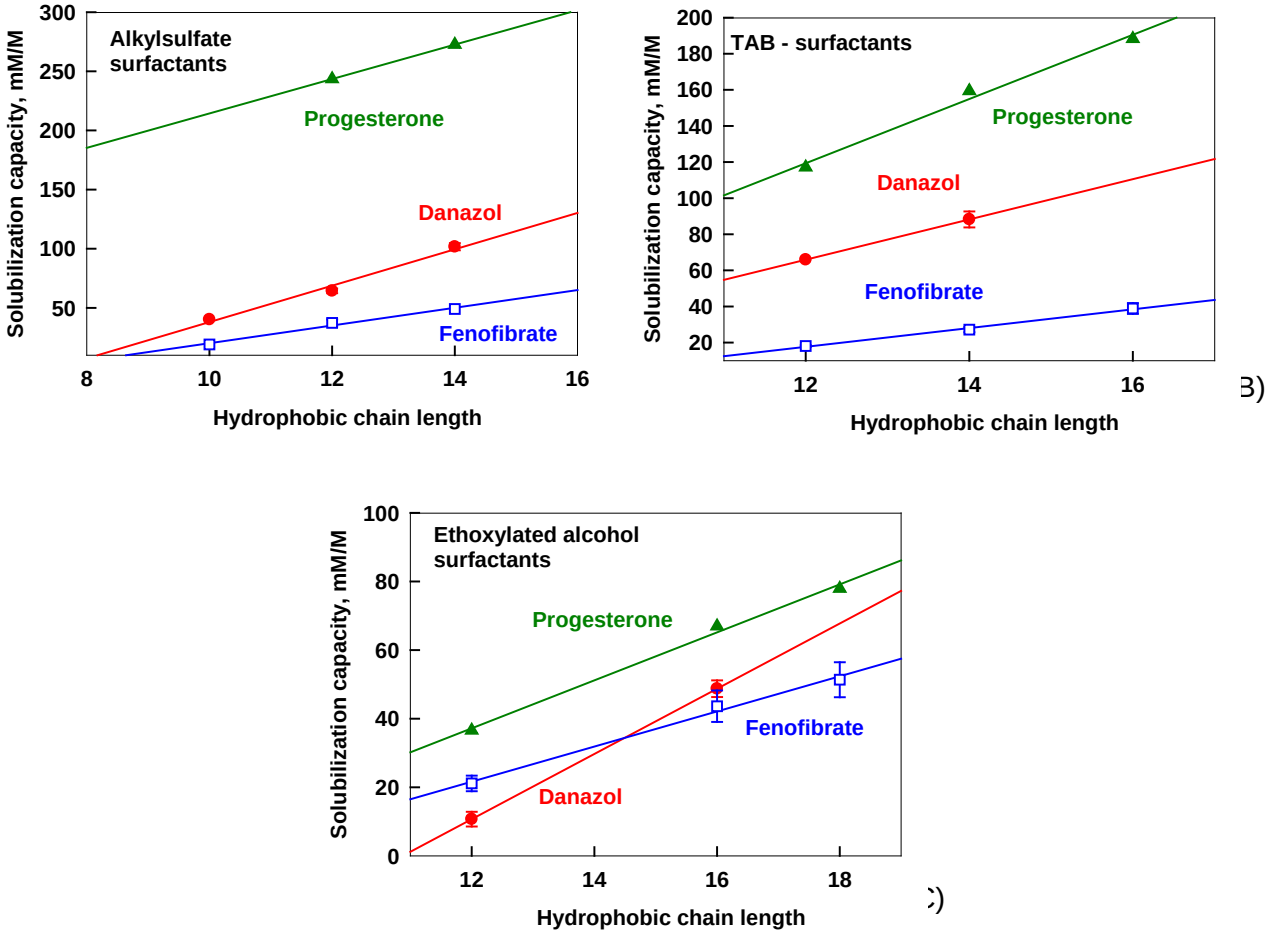


Figure 33. Solubilization capacity of fenofibrate (empty blue squares), danazol (full red circles) and progesterone (full green triangles), as a function of the hydrophobic chain length of (A) alkylsulfate, (B) trimethylammonium bromide and (C) ethoxylated alcohol (with ca. 20 EO units) surfactants. The results are averaged from at least 2 independent experiments. The error bars can be smaller than the symbols.

The effect of the hydrophobic chain length on the solubilization of the three studied drugs is compared in Figure 33 above. For all studied drugs, the solubilization capacity increases linearly with the hydrophobic chain length.

4.4. Conclusions from Chapter 4

1. Danazol is solubilized much better than fenofibrate and androstane by the ionic surfactants. The effect is due to ion-dipole interactions between the polar danazol molecules and the charged surfactant head-groups.

2. Ethoxylation of sodium dodecyl sulfate decreases significantly the solubilization capacity of both studied drugs, which is explained by the hindered packing of the surfactant and drug molecules in the palisade layer of the micelles.

3. The solubilization locus polarity of sodium dodecyl sulfate surfactant micelles decreases with the addition of ethoxy groups to the surfactant head group, due to their partially hydrophobic character, and is in excellent correlation with the decreased solubilization capacity.

4. Drug solubilization increases linearly with the increase of hydrophobic chain length for all types of surfactants (nonionic, cationic and anionic). The effect is due to the increased volume for solubilization in the micelles. The locus of fenofibrate solubilization is in the palisade layer of ionic surfactant micelles and in the hydrophobic core of the nonionic surfactant micelles.

Main conclusions from the thesis

1. The solubilization of hydrophobic drugs increases linearly with the increase of the surfactant hydrophobic chain length, regardless of the charge and type of the surfactant hydrophilic head group.
2. Ion-dipole interactions contribute significantly to the solubilization of steroidal drugs in ionic surfactant micelles.
3. The ethoxylation of sodium dodecyl sulfate decreases drug solubilization, most likely due to more difficult packing of the molecules in the micelles.
4. Fenofibrate is solubilized in the hydrophobic core of nonionic surfactant micelles and in the palisade layer of ionic surfactant micelles, as determined via UV-spectroscopy.

Contributions of the thesis

1. The influence of surfactant structure on the solubilization of hydrophobic drugs was clarified. The obtained information can be used for rational solubilizer selection during the formulation development of poorly water-soluble drugs.
2. The role of specific intermolecular interactions on the micellar solubilization of hydrophobic drugs was clarified. The identified interactions and mechanisms can be used to develop theoretical models that predict solubilization.
3. The generated database of drug solubilization by surfactants can be used to define drug solubilization in physiologically-based pharmacokinetic models.

Literature

Published research papers

D1. Z. Vinarov[ǂ], P. Dobreva, S. Tcholakova. Effect of surfactant molecular structure on progesterone solubilization. *Journal of Drug Delivery Science and Technology* **43** (2018), 44-49. **(IF = 2.734; 11 citations)**

D2. Z. Vinarov[ǂ], V. Katev, D. Radeva, S. Tcholakova, N. Denkov. Micellar solubilization of poorly water-soluble drugs: effect of surfactant and solubilizate molecular structure *Drug Development and Industrial Pharmacy* **44** (2018), 677–686. **(IF = 2.365; 28 citations)**

[ǂ]***Corresponding author***

Total: 39 citations (SCOPUS, self-citations removed)

The thesis has been presented on the following venues:

1. Z. Vinarov, S. Tcholakova, N. D. Denkov, Cost-effective delivery of hydrophobic drugs by surfactant solubilization: fundamental insights for practical applications, 6th FIP Pharmaceutical Sciences World Congress 2017, Sweden/Stockholm **2017** – **poster**
2. Z. Vinarov, Micellar solubilization of hydrophobic drugs, VI Congress of Pharmacy with international participation, Bulgaria/Sandanski **2016 – oral presentation**
3. Z. Vinarov, Micellar solubilization of hydrophobic drugs, Ninth National Conference on Chemistry (9NCC), Bulgaria/Sofia **2016 – oral presentation**
4. Z. Vinarov, D. Radeva, S. Tcholakova, N. Denkov, Micellar solubilization of fenofibrate by saponins and classical surfactants, 42nd Annual meeting and exposition of the Controlled Release Society (CRS), UK/Edinburgh **2015** – poster
5. Z. Vinarov, V. Katev, N. Burdzhiev, S. Tcholakova, N. Denkov, Impact of Surfactant−Bile Interactions on the Solubility of Hydrophobic Drugs in Biorelevant Dissolution Media, 8th World Conference on Physico Chemical Methods in Drug Discovery and Development, Croatia/Split **2019 – oral presentation**
6. Z. Vinarov, S. Tcholakova, N. Denkov, The impact of excipient-bile interactions on the solubility of poorly-water soluble drugs, Meeting of the European Network on Understanding Gastrointestinal Absorption-related Processes, Bulgaria/Sofia **2019 – oral presentation**
7. Z. Vinarov, V. Katev, D. Radeva, S. Tcholakova, N. Denkov, Micellar Solubilization of Poorly Water-soluble Drugs: Effect of Surfactant and Solubilizate Molecular Structure, Meeting of the European Network on Understanding Gastrointestinal Absorption-related Processes, Belgium/Leuven **2018 – poster**

8. S. Tcholakova, Z. Vinarov, N. D. Denkov, Effects of drug and surfactant molecular structure on drug solubilization in aqueous and biorelevant dissolution media, 9th International Colloids Conference, Spain/Barcelona **2019 - poster**
9. S. Tcholakova, Z. Vinarov, N. D. Denkov, Effects of drug and surfactant molecular structure on drug solubilization in aqueous and biorelevant dissolution media, 1st International Balkan Chemistry Congress, Turkey/Edirne **2018 – oral presentation**

List of other publications linked to the topic of the thesis:

1. Z. Vinarov‡, G. Gancheva, N. Burdzhiev, S. Tcholakova. Solubilization of Itraconazole by Surfactants and Phospholipid-Surfactant Mixtures: Interplay of Amphiphile Structure, pH and Electrostatic Interactions. ***Journal of Drug Delivery Science and Technology (IF = 2.7)*** 57 (2020) 101688
2. B. J. Boyd, C. A.S. Bergström, Z. Vinarov, M. Kuentz, J. Brouwers, P. Augustijns, M. Brandl, A. Bernkop-Schnürch, N. Shrestha, V. Préat, A. Müllertz, A. Bauer-Brandl, V. Jannin‡. Successful Oral Delivery of Poorly Water-soluble Drugs Both Depends on The Intraluminal Behavior of Drugs and of Appropriate Advanced Drug Delivery Systems. ***European Journal of Pharmaceutical Sciences (IF = 3.6)*** 137 (2019) 104967
3. Z. Vinarov‡, G. Gancheva, V. Katev, S. Tcholakova. Albendazole Solution Formulation via Vesicle-To-Micelle Transition of Phospholipid-Surfactant Aggregates. ***Drug Development and Industrial Pharmacy (IF = 2.4)*** 44 (2018) 1130-1138
4. Z. Vinarov‡, V. Katev, N. Burdzhiev, S. Tcholakova, N. Denkov. Effect of Surfactant–Bile Interactions on the Solubility of Hydrophobic Drugs in Biorelevant Dissolution Media. ***Molecular Pharmaceutics (IF = 4.3)*** 15 (2018) 5741−5753
5. K. Stoyanova, Z. Vinarov‡, S. Tcholakova. Improving Ibuprofen Solubility by Surfactant-Facilitated Self-Assembly into Mixed Micelles. ***Journal of Drug Delivery Science and Technology (IF = 2.7)*** 36 (2016) 208–215.
6. Z. Vinarov‡, D. Radeva, V. Katev, S. Tcholakova, N. Denkov. Solubilisation of Hydrophobic Drugs by Saponins. ***Ind. J. Pharm. Sci***. ***(IF = 0.72)*** 80 (2018) 709−718

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Total: 74 citations (SCOPUS, self-citations removed)

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