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International Journal of Pharmaceutics 159 (1997) 215–222

international journal of pharmaceutics

Investigations on the micellisation behaviour of fenoprofen sodium

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Received 5 June 1997; received in revised form 8 August 1997; accepted 9 September 1997

Abstract

The aim of the present study was to investigate the micellisation behaviour of the non-steroidal anti-inflammatory drug fenoprofen sodium in aqueous solutions. The methods used were foam stability measurements, ^{1}H-NMR, solubilisation, photon correlation spectroscopy (PCS) and transmission electron microscopy (TEM). Results from surface tension measurements performed in a previous study were included. A great discrepancy between critical values from different experimental methods was found. Methods that detect changes in the surface properties of aqueous fenoprofen sodium solutions suggest that the critical concentration was $1.5*10^{-2}$ mol/l, while methods that detect changes in the bulk phase led to a critical concentration of $1.2*10^{-1}$ mol/l. In the concentration range between $1.5*10^{-2}$ and about $1.0*10^{-1}$ mol/l no formation of aggregates could be detected (concluded from the absence of an upfield shift of the phenyl proton signals in the NMR measurements and the finding that the oily, practically water insoluble fenoprofen acid could not be solubilised by fenoprofen sodium solutions below concentrations of $1.0*10^{-1}$ mol/l fenoprofen sodium). Association of fenoprofen sodium molecules to micelles starts at concentrations between 1.0 and $1.2*10^{-1}$ mol/l. In conclusion, the determination of the critical micelle concentration from surface tension measurements does not allow the determination of micellisation phenomena in the case of fenoprofen sodium. Further methods are required to assure micellisation. Photon correlation spectroscopy and TEM support the assumption that fenoprofen sodium forms disclike micelles, although with both methods the micelle size could not exactly be determined. From differences in the chemical shift of the two phenyl rings of fenoprofen sodium it was concluded that the micelles have a bilayer or partially overlapping bilayer structure. © 1997 Elsevier Science B.V.

Keywords: Fenoprofen sodium; Micelles; Photon correlation spectroscopy

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PII S0378-5173(97)00288-3

1. Introduction

Not only pharmaceutical excipients such as emulsifiers, solubilising agents and wetting agents (Aulton, 1988) but also a great number of drugs have an amphiphilic molecular structure. These drugs are surface active and are able to form micelles in aqueous solutions at concentrations higher than their critical micelle concentration (cmc) and temperatures higher than their Krafft temperature. Examples of surface active, micelle forming drugs can be found in the group of phenothiazines (Attwood et al., 1974), tricyclic and tetracyclic antidepressants (Albert, 1980), antihistamines, local anaesthetics, anticholinergics (Florence and Attwood, 1988) and nonsteroidal antiinflammatory drugs (Hamann, 1990; Kriwet and Müller-Goymann, 1993; Fini et al., 1995).

In the present study the non-steroidal anti-inflammatory drug fenoprofen sodium (FNa) was used as amphiphilic substance. The structural formula (Fig. 1) reveals that FNa consists of an anionic polar head group and a mainly aromatic lipophilic tail group (diphenyl ether). In previous studies it could be shown that FNa, besides its ability to form a thermotropic mesophase of the type smectic Ad (Rades, 1994), is a surface active drug, which is able to form an aqueous lamellar mesophase or an aqueous dispersion of a lamellar mesophase at high drug concentrations (Rades and Müller-Goymann, 1992). However, the micellisation behaviour of FNa remained unclear, as a great discrepancy between the cmc determined by surface tension measurements on the one hand (cmc: $1.5*10^{-2}$ mol/l) (Rades et al., 1993) and bulk methods, such as osmometry and solubilisation (cmc: $2.3–3.5*10^{-1}$ mol/l) (Hamann, 1990) on the other hand was found. The aim of the present study therefore was to investigate the micellisation of FNa using foam stability measurements, NMR, solubilisation, photon correlation spectroscopy (PCS) and transmission electron microscopy (TEM) as well as to elucidate physico chemical reasons for the great discrepancy mentioned above.

2. Materials and methods

2.1. Fenoprofen

FNa was prepared from fenoprofen calcium (Eli Lilly, Gießen, Germany). The calcium salt was dispersed in an aqueous HCl solution (10% w/v). To extract the free acid, the dispersion was shaken several times with dichloromethane. After evaporation of the dichloromethane, fenoprofen acid was obtained as a clear, yellow liquid of which the refractive index at 20°C varied between 1.569 and 1.572. According to the Merck Index the refractive index is 1.574 (Windholz, 1983). To prepare the sodium salt, the acid was dissolved in an equimolar amount of 1 N NaOH. The solvent was evaporated until fenoprofen sodium crystallised as dihydrate with a water content of 12%, determined by thermogravimetry (TGA 2/TADS 3600, Perkin Elmer, Überlingen, Germany) and by KARL-FISCHER titration (701 KF Titrino/703 Ti, Metrohm, Herisau, Switzerland). The melting point of the sodium salt in a closed system, determined by DSC, was 79°C (DSC2-C/TADS 3600, Perkin Elmer, Überlingen, Germany).

2.2. Methods

2.2.1. Foam stability

Five ml of aqueous FNa solutions were filled in test-tubes of equal diameter. The solutions were shaken ten times with the hand. In preliminary experiments the reproducibility of the shaking procedure was assured, measuring the foam

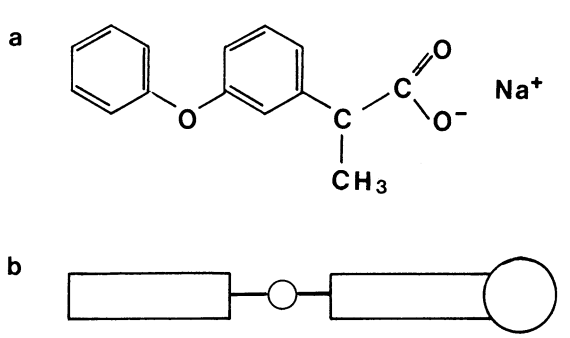

Fig. 1. (a) Structural formula of FNa, (b) schematic representation of polar and non polar areas of FNa.

height of samples, containing the same FNa concentration ten times. One min after shaking the foam height (F1) and the height of the liquid phase in the test tube (L) were determined. After 5 min the foam height was determined again (F2). F1/L gives the foam number FN1. F2/L gives the foam number FN2. FN1/FN2 is the foam stability (FST). Each experiment was performed in triplicate at 20°C. Errors in the FST determination are ± 5%.

2.2.2. ^{1}H-NMR experiments

These experiments were carried out with a Bruker AM 400 Mhz ($B_0 = 9.4$ T), Bruker analytische Meßtechnik GmbH, Rheinstetten, Germany) of varying concentrations of FNa dissolved in D_2O (Deuterium Oxide for NMR, Merck, Darmstadt, Germany) at a temperature of 20°C. Errors in the chemical shift determinations ($\delta =$ 0.1–8.9 ppm) were ± 0.002 ppm.

2.2.3. Photon correlation spectroscopy

PCS was performed on a Zetasizer 3 (Malvern, Herrsching, Germany) equipped with a 35 mW He/Ne-laser (Spectra Physics, Göttingen, Germany) to determine micelle sizes in aqueous FNa solutions. For micelle size determination all samples were filtered through a 0.22 μm filter.

2.2.4. Turbidity measurements

The count rate given by the PCS apparatus was used to investigate the solubilisation of fenoprofen acid in aqueous, buffered solutions of FNa, (pH 6.6, phosphate buffer). For this purpose FNa was dissolved at room temperature in an aqueous 0.05 mol phosphate buffer of pH 6.6 to assure a certain amount of the drug in its acidic form.

2.2.5. Transmission electron microscopy

Samples were cryo-fixed with a jet-freeze device JFD 030 with liquid propane (Baltec, Walluf, Germany) The frozen samples were freeze fractured at − 100°C at a pressure of $5*10^{-6}$ bar (BAF 400, Balzers, Wiesbaden, Germany). Shadowing of the samples was performed with platinum/carbon (layer thickness 2 nm) at 45° and with carbon (layer thickness 20 nm) at 90°. The replica were cleaned with chloroform/methanol (1:1 (v/v)) and water. Replica on uncoated grits were viewed with a transmission electron microscope at 80 kV (EM 300, Philips, Kassel, Germany) at various magnifications.

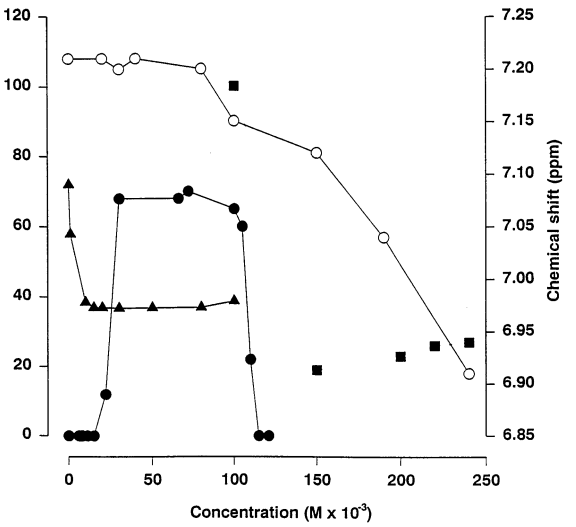

Fig. 2. Surface tension (in mN/m, ▲), foam stability (in %, ●), and scattering intensity (in arbitrary units, ■) of aqueous FNa solutions and chemical shift of the mean of the phenyl proton signals of FNa solutions in D_2O (in ppm, ○).

3. Results

3.1. Surface tension measurement

In a previous study, using the Wilhelmy plate method (Prozessor Tensiometer K12, Krüss, Hamburg, Germany), the surface tension reduction isotherm of aqueous FNa solutions was determined. The critical concentration at which further addition of FNa did not lead to a further reduction of the surface tension was found at 1.5×10^{-2} mol/l FNa (Rades et al., 1993). For a micelle forming substance this concentration is usually regarded as the critical micelle concentration (cmc).It must however be pointed out that an effect is measured in the interface solution/air. Surface tension measurements therefore do not allow direct determination of association phenomena in the bulk phase. The surface tension at this concentration was 37 mN/m (Fig. 2). The calcula-

tion of the surface area per molecule from the slope of the surface tension versus log FNa concentration in the air–solution interface, using Gibbs equation, led to a value between 80 and 100 $Å^2$.

3.2. Foam stability

The physico-chemical changes which are determined using tensiometry clearly are changes in the surface properties of the surfactant solutions and changes in the properties of the bulk phase can only be concluded from effects measured in the surface. Foaming is a process which depends on properties of the interface and the bulk phase. The formation of a foam is accompanied by an increase in the total surface area. Due to the Gibbs–Marangoni effect (Myrs, 1988), stable foams cannot be formed if the concentration of the surfactant in the bulk phase is low. The formation of a stable foam was not observed until the FNa concentrations exceeded $1.5*10^{-2}$ mol/l (0.4% (w/v)). At $2.6*10^{-2}$ mol/l the foam formed had reached its maximum height and stability. For many anionic surfactants the concentration required to attain maximum foam height is found at concentrations 1.2–3 times higher than the cmc (Myrs, 1988). At concentrations higher than $1.0*10^{-1}$ mol/l (2.7% (w/v)) foam stability decreased and was found to be zero at $1.1*10^{-1}$ mol/l FNa. Compared with foams formed by ionic surfactants such as sodium lauryl sulphate, cetrimide or benzalkonium chloride using the same technique, the foam had a low stability at all FNa concentrations.

3.3. ^{1}H-NMR measurements

The determination of chemical shifts of ^{1}H-NMR signals of the surfactant is a sensitive bulk method to determine the cmc of a surfactant in aqueous solution assuming that the association behaviour of the surfactant is the same in deuterium oxide and in water. If the surfactant contains an aromatic lipophilic group, micelle formation can be observed, determining the signal shift of the phenyl protons, which is stronger than the shift of the alkyl protons, due to an intermolecular aromatic ring current effect (Gao et al., 1990). Additionally, in the case of FNa, there is the possibility to allocate the proton signals of the phenyl protons to either ring A or ring B (see Fig. 3) of the diphenyl ether structure, allowing further structural interpretation.

Plotting the chemical shift of the mean of the diphenyl ether proton signals versus the molar concentration of FNa, no upfield shift of the signals could be detected until the FNa concentration was higher than $1.0*10^{-1}$ mol/l. At concentrations higher than $1.0*10^{-1}$ mol/l large shifts to lower frequencies (by 0.06–0.3 ppm) were found. The upfield shift increased with increasing FNa concentrations, indicating that the concentration of FNa molecules forming associates increased with respect to the concentration of free FNa molecules (Fig. 2). To allow for comparison of ^{1}H-NMR measurements with the other techniques used in this study to determine the cmc, the chemical shifts are plotted versus concentration and not in the usual way as chemical shift versus reciprocal concentration. The critical concentration, however, was determined from the intercept of two straight lines drawn through the values of the observed shifts at high and low FNa concentrations versus the reciprocal FNa concentration (Gao et al., 1990). This critical concentration was found to be $1.2*10^{-1}$ mol/l (3.2% (w/v)) and can be regarded as the cmc.

At concentrations higher than the cmc, it was found that the proton signals of ring B showed a comparatively stronger upfield shift than the signals of the ring A protons (Fig. 3).

3.4. Solubilisation of fenoprofen acid, turbidity measurements

Fenoprofen acid is practically insoluble in water ($7.1*10^{-4}$ mol/l, ca. 0.019% (w/v) (Hamann, 1990). Aqueous FNa solutions were adjusted to pH 6.6 (aqueous solutions react alkaline, the sodium salt of fenoprofen is fully ionised in aqueous solutions) so that the percentage of fenoprofen existing as acid is about 0.79% (pK_a of fenoprofen = 4.5 (Florence and Attwood, 1988)). While solutions containing $1.5*10^{-1}$ mol/l or more did not show any macroscopical turbidity

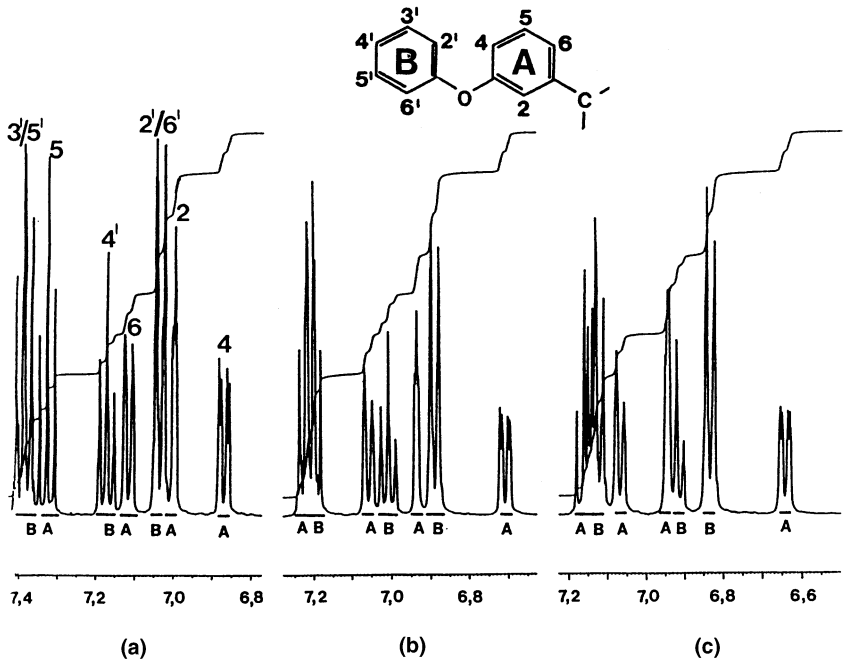

Fig. 3. ^{1}H-NMR spectra of the phenyl proton signals of FNa in D_2O at different FNa concentrations: (a) $1.1*10^{-1}$ mol/l, (b) $1.9*10^{-1}$ mol/l, (c) $3*10^{-1}$ mol/l.

and had a low count rate in the PCS measurements, a concentration of $1.0*10^{-1}$ mol/l was a macroscopically turbid dispersion, showing a high count rate in the PCS instrument. Using light and polarising light microscopy (Photomikroskop III, Zeiss, Oberkochen, Germany) it was found that the dispersion was an emulsion of a non birefringent oil phase in an aqueous continuous phase, i.e. at this concentration the oily, amorphous fenoprofen acid separated from the aqueous solution. It was concluded that fenoprofen acid was kept in solution at higher FNa concentrations although it was present in the solution at concentrations higher than its saturation concentration in water, because it was solubilised by FNa associates. The strong increase in the count rate (turbidity) by diluting the solution from $1.5*10^{-1}$ to $1.0*10^{-1}$ mol/l FNa shows that the solubilisate (fenoprofen acid) is coming out of solution between these two concentrations, due to the fact that FNa micelles no longer exist to solubilise the oily water insoluble fenoprofen acid. With this method the cmc was found to be between $1.0*10^{-1}$ mol/l and $1.5*10^{-1}$ mol/l FNa. It must be pointed out however, that due to the addition of phosphate buffer to adjust the pH, the ionic strength of the fenoprofen solutions was altered. This alteration may affect the cmc of fenoprofen sodium. Cmc values determined by methods using surfactant solutions with different ionic strengths cannot be directly compared. It can however be stated that the cmc using this method was in the same concentration range as the cmc determined by ^{1}H-NMR measurements and about 10 times higher than the critical concentration, determined with surface tension measurements.

3.5. PCS and TEM

At concentrations higher than $1.5*10^{-1}$ mol/l, i.e. at FNa concentrations which are above the cmc, PCS measurements showed a high polydispersity index (0.3–0.4). High polydispersity is more likely for rodlike or disclike micelles, because rods or discs can grow in one or two dimensions, respectively, without a change of the

packing parameter of the surfactant molecule. The size distribution of spherical micelles however, should be narrow as the packing parameter of the surfactant molecule already determines the micelle shape and size. The mean size for a solution containing a FNa concentration of $1.3*10^{-1}$ mol/l was 200 nm and mean sizes up to 400 nm were measured at increasing FNa concentrations ($5*10^{-1}$ mol/l FNa). These values have to be regarded as very high, being about 10–100 times larger than the usual micelle size. The high polydispersity, the low scattering intensity of the particles as well as the possibility of the formation of anisodiametric associates, make a PCS measurement very difficult. It is questionable whether these results can be regarded as true values for a mean micelle size, especially as all samples have been filtered through a 0.22 μm filter. The measured values however, seem to indicate that the micelle size may be larger than that of common surfactants and that it may increase with increasing FNa concentrations.

To obtain further information on FNa solutions, TEM investigations of freeze fractured and replicated samples were performed. Fig. 4 shows TEM micrographs of a $8*10^{-2}$ mol/l FNa solution (Fig. 4(b), FNa concentration was between the critical concentrations found with surface and bulk methods) and a 1.5 mol/l-FNa solution (Fig. 4(a), FNa concentration ten times above the critical concentration found with bulk methods). While at low FNa concentrations only very fine structures were found, at high FNa concentrations the appearance of the samples changed into a more coarse structure. Although these structures may be interpreted as disclike associates with a size around 100 nm, an accurate determination of the size or size distribution of the associates from the TEM investigations was not possible.

4. Discussion

At low FNa concentrations the amphiphilic drug adsorbs at the air–solution interface, lowering the surface tension of water from 72 to 37 mN/m. The area per molecule in the interface is about twice as high as that of sodium lauryl sulphate (Hamann, 1990). The reason for this high value may be the high solubility of FNa molecules in the bulk phase (causing a dynamic exchange of FNa molecules between surface and bulk phase) and/or a possible flat arrangement of the surfactant molecules in the interface (facilitated by the ability of the ether oxygen to act as a hydrogen bond acceptor). The high solubility of the surfactant molecules in the bulk phase can also be regarded as the reason for the lack of a good foam stabilisation.

While surface methods (surface tension measurements and foam stability) suggest a critical concentration of $1.5*10^{-2}$ mol/l FNa, bulk methods reveal a critical concentration of $1.2*10^{-1}$ mol/l. With NMR measurements no association of FNa molecules to micelles could be detected at

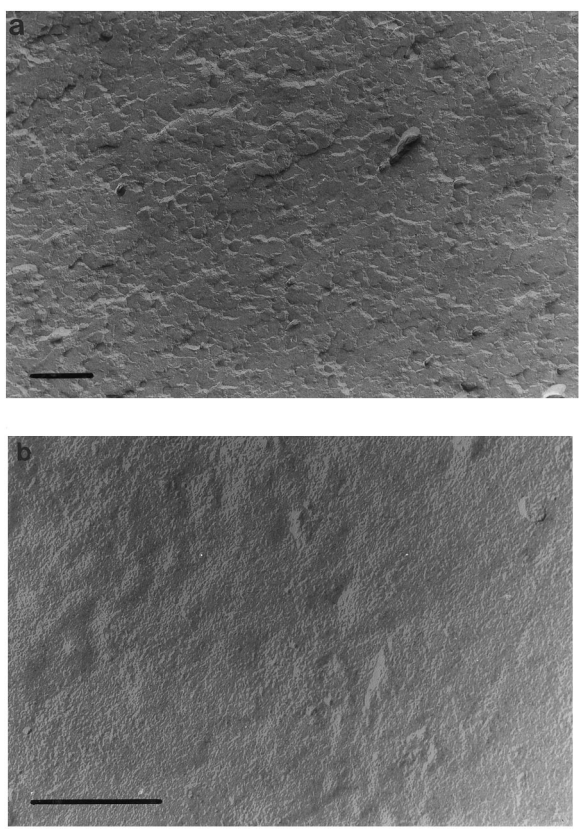

Fig. 4. micrographs of aqueous FNa solutions at different FNa concentrations: (a) $15*10^{-1}$ mol/l, (b) $8*10^{-2}$ mol/l, bar represents 300 nm.

concentrations lower than $8*10^{-2}$ mol/l. Therefore, it can be concluded that the FNa molecules do not undergo a continuous stepwise association process in the concentration range, between $1.5*10^{-2}$ and $1.2*10^{-1}$ mol/l FNa as would class II solutes (according to the classification of Mukerjee (1974)). The critical concentration found in the surface tension versus FNa concentration isotherm and the first critical concentration in the foam stability isotherm therefore cannot be regarded as a critical micelle forming concentration. They only reflect on the fact that the surface concentration of the amphiphile is saturated at this concentration. At concentrations higher than $1*10^{-1}$ mol/l micellisation to larger associates seems to occur. The NMR results are confirmed by the solubilisation experiments. In the experiment the amount of fenoprofen acid at $1*10^{-1}$ mol/l FNa was just above the saturation concentration. Even this small amount of fenoprofen acid however, could not be solubilised, indicating that at concentrations smaller than $1*10^{-1}$ mol/l FNa molecules are still molecular dispersed or only form very small associates (e.g. dimers, as it is reported for molecules with a relatively rigid hydrophobic ring system (Attwood et al., 1974).

It is interesting that the foam stability of FNa solutions only starts to drop at concentrations higher than $1*10^{-1}$ mol/l FNa, to reach zero at the critical concentration determined using NMR measurements ($1.2*10^{-1}$ mol/l). It is however not clear if the formation of associates is the reason for the decrease in foam stability or if this effect is just due to the high surfactant concentration which starts to counteract the Gibbs–Marangoni effect (Myrs, 1988).

The cmc of an anionic surfactant is mainly determined by its lipophilic tail group (Brezesinski and Mögel, 1993). The tail group of FNa consists of 14 carbon atoms, 12 of which form the two phenyl groups. The influence of a phenyl group on the cmc can be compared to 3.5 methylene groups (Brezesinski and Mögel, 1993), resulting in an equivalent hydrocarbon chain length of nine carbon atoms for FNa. This value has to be reduced further, because one carbon atom is present as a branched methyl group and because of the presence of a heteroatom (oxygen) in the lipophilic tail group. The estimated equivalent hydrocarbon chain length therefore is about eight carbon atoms. The cmc of sodium octyl sulphate is $1.36*10^{-1}$ mol/l, the cmc of sodium octyl sulphonate is $1.6*10^{-1}$ mol/l, both being in the same concentration region as the critical micelle concentration of aqueous FNa solutions determined by NMR experiments (Myrs, 1988). FNa however, has a much larger volume and a much lower flexibility of the lipophilic tail group and therefore different sterical properties from a surfactant with a straight hydrocarbon chain.

From small angle X-ray scattering measurements (SAXS) and geometrical considerations, Hamann (1990) concluded that FNa forms disc-like micelles. The high polydispersity index in the PCS investigation (rather than the size distribution measured with this technique) as well as the structures found in the TEM investigations point in the same direction. It must also be pointed out that fenoprofen forms lyotropic lamellar mesophases at higher fenoprofen concentration (Rades and Müller-Goymann, 1992) which is the mesophase that corresponds to a disclike micellar shape.

Although the thickness of the lamella could not be determined exactly using SAXS, it was found to be clearly larger than the length of a single, fully stretched FNa molecule (ca. 1.2 nm, note that this is not the size of the micelle!), indicating that the associates are not formed by an FNa monolayer of interdigitated bilayer. In the NMR investigations it was found that the phenyl protons of ring B showed a higher upfield shift than the phenyl protons of ring A. The NMR measurements therefore confirm that an association of the FNa molecules to an interdigitated bilayer structure is unlikely, because in this model, the chemical environment of both phenyl rings would be the same. The association to a bilayer or a partially overlapping bilayer however, is in agreement with the NMR measurements.

5. Conclusion

The present study indicates that FNa is a surface active substance. The formation to larger

associates (micelles) however, does not occur immediately at concentrations higher than the critical concentration found with surface methods. The fact that a critical concentration of $1.2*10^{-1}$ mol/l can be detected using bulk methods, suggests that FNa does not undergo a continuous stacking process, starting once the surface concentration of the surfactant is saturated ($1.5*10^{-2}$ mol/l), but forms micelles in a comparatively narrow concentration region. Further investigations are necessary to determine the micelle size of fenoprofen micelles at different concentrations above the cmc.

Acknowledgements

We want to thank the Eli Lilly Company for supporting us with fenoprofen calcium. We also acknowledge the availability of the TEM and NMR at the Departments of Botanics and Organic Chemistry at the Technical University of Braunschweig.

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