Pharmaceutical Research, Vol. 26, No. 4, April 2009 (© 2008)
DOI: 10.1007/s11095-008-9783-0

Research Paper

Influence of the Solid Form of Siramesine Hydrochloride on its Behavior in Aqueous Environments

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Received July 9, 2008; accepted November 4, 2008; published online November 13, 2008

Purpose. To study the influence of solid form on the behavior of the salt siramesine hydrochloride in aqueous environments.
Methods. The solubilities and dissolution rates of siramesine hydrochloride anhydrate and monohydrate were determined at pH 3.4 and 6.4, and precipitates were examined by X-ray powder diffraction. The mechanism of anhydrate–hydrate conversion was investigated by optical microscopy, and wet massing of the anhydrate was carried out using water and 60% (*v*/*v*) ethanol separately as granulation liquids. The wet masses were analyzed using Raman microscopy.
Results. At pH 3.4 the anhydrate and monohydrate salts exhibited similar dissolution profiles. At pH 6.4 both the anhydrate and monohydrate salts formed supersaturated solutions of high apparent solubility. From the anhydrate solution, precipitation of the free base occurred, while the solution of the monohydrate salt remained in the supersaturated state. This resulted in a superior dissolution profile of the monohydrate salt. Microscopy and wet massing experiments showed that the anhydrate–hydrate conversion of siramesine hydrochloride was solution-mediated and dissolution-controlled.
Conclusion. During development of a formulation based on the anhydrate salt, the risk of processing-induced transformation to the monohydrate form as well as precipitation of the free base should be considered.

KEY WORDS: dissolution rate; monohydrate; solution-mediated transformation; supersaturation; wet massing.

INTRODUCTION

A large number of pharmaceutical compounds are capable of forming hydrates (1). The incorporation of water molecules into the crystal lattice of an API changes the crystal structure and hence the physiochemical properties of the compound. This may affect the processing properties, such as hardness, flowability, and compactibility, as well as the performance of a formulation (2). Especially the solubility and dissolution properties of the hydrate form can be critical, since hydrates generally have lower solubilities than their corresponding anhydrate forms (3–5). This might affect the *in vivo* release and ultimately lead to lower compound bioavailability (2,4).

Hydrate formation can occur under various circumstances. It may be the result of a crystallization process, or it may be the result of moisture uptake from the surroundings (4). Hydrates may also form during processing of pharmaceuticals where aqueous solutions are involved. This is referred to as a processing induced transformation (6) and has been observed during wet granulation of for instance theophylline and carbamazepine (7–9). Furthermore, hydrate formation during *in vitro* dissolution, resulting in slower release, has been reported for theophylline, carbamazepine, and nitrofurantoin (10–12). Thus, if a compound is capable of hydrate formation, care must be taken to ensure that the most suitable form of the drug is chosen for development, and that phase transformations do not occur (or occur in a controlled manner) during processing and release (2,13). Therefore, an investigation of the behavior of the drug in aqueous environments is valuable.

The weakly basic drug siramesine hydrochloride (Lu 28–179, Fig. 1) can exist as an anhydrate and as a monohydrate. Furthermore, recent studies have shown that the anhydrate can exist in two considerably different morphologies, plates and needles. Since the drug is a hydrochloride salt, it has a free base, which represents a solid form different from the anhydrate and monohydrate salt forms. Thus, apart from anhydrate–hydrate conversion, transformation to the free base may also occur under certain aqueous conditions.

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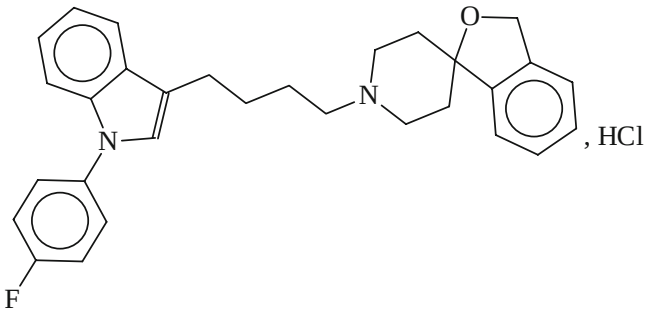

Fig. 1. Molecular structure of siramesine hydrochloride.

Williams *et al.* reported dissociation of a hydrochloride salt to the amorphous free base during wet granulation (14). Moreover, the enhanced dissolution rate of a salt as compared to the free form, which is the principal reason for development of salts (15–17), may be limited by precipitation of the free form or by the common ion effect for salts of basic compounds (15).

The aim of this study was to investigate the behavior of the anhydrate and the monohydrate forms of siramesine hydrochloride in aqueous environments. In order to do so, the study was divided into two parts; initially the solubilities and dissolution behaviors of the anhydrate and the monohydrate salts were investigated at two different physiologically relevant pH values, namely pH 3.4 and 6.4. A particular aim was to evaluate the effect of solid form on the pH dependent transformation of salt to its free base. In the second part of the study the conversion between anhydrate and monohydrate was studied using two different aqueous solvents to determine the conversion mechanism and the rate-limiting step of the conversion. To study the risk of anhydrate–hydrate conversion or transformation of the salt form to the free base during pharmaceutical processing, wet massing was carried out to mimic the wet granulation process. The two morphologically different anhydrate crystals were treated separately with two different solvents; pure water and an ethanol–water mixture.

MATERIALS AND METHODS

Materials

The anhydrous form (plate morphology) of the hydrochloride salt of siramesine (1′-[4-[1-(4-fluorophenyl)-1-*H*-indol-3-yl]-1-butyl]spiro[*iso*-benzofuran-1(3*H*), 4′ piperidine]) was supplied by H. Lundbeck A/S, Denmark (molecular weight 491.06 g/mol, solubility of the hydrochloride salt in water 150 μg/ml, solubility of the hydrochloride salt in 96% ethanol 24 mg/ml, solubility of the free base in water 0.003–0.03 μg/ml, pK_a~9, log *P*~8.5). The siramesine-HCl anhydrate needles were prepared from the anhydrate plates by dissolving them in 99% ethanol to a concentration of 5% at 50°C under stirring conditions, followed by cooling to room temperature. The supersaturated solution was left at room temperature for 24 h. During this time the salt crystallized as siramesine-HCl anhydrate needles, which were isolated by filtration and stored under dry conditions. For the dissolution and solubility experiments, only siramesine-HCl anhydrate plates were used, whereas both plates and needles were used for the investigations of the route of conversion between siramesine-HCl anhydrate and monohydrate and the stability of siramesine-HCl anhydrate during wet massing.

Siramesine-HCl monohydrate was obtained from siramesine-HCl anhydrate plates through antisolvent precipitation. The plates were dissolved in 96% ethanol (1% *w*/*v*, 50 ml) followed by rapid mixing with 200 ml of water under stirring conditions. Precipitation occurred instantly, and after 60 min the siramesine-HCl monohydrate particles were isolated by vacuum filtration and dried over anhydrous silica in a dessicator.

Methods

Particle Characterization of Drug Substance

The morphology of siramesine-HCl anhydrate (plates and needles), siramesine-HCl monohydrate, and the free base was studied by scanning electron microscopy (SEM). Micrographs were taken using a Philips XL30 scanning electron microscope (FEI Europe, Eindhoven, Netherlands). Samples were mounted on aluminum stubs with double adhesive carbon tape and coated with gold/palladium at 15 mA for 120 s in a nitrogen atmosphere (Polaron SC7640 sputter coater, Newhaven, UK).

The specific surface areas of siramesine-HCl anhydrate (plates and needles) and siramesine-HCl monohydrate were determined by the BET method on a Gemini III 2375 Surface Area Analyzer (Micromeritics Instrument Corporation, Mönchengladbach, Germany) using helium and nitrogen gases.

Kinetic Solubility at pH 6.4

Saturated solutions of siramesine-HCl anhydrate and siramesine-HCl monohydrate in phosphate buffer (0.05 M) containing 0.25% (*v*/*v*) Tween 80 were prepared by introducing excess amounts of drug (30 mg) into 3 ml of medium in vials. The sealed vials were placed on rotation at room temperature (21–23°C) and at specified time points (0.5, 4, 8, 16, and 24 h), three vials of each species were removed for analyses. The solid and liquid phases were separated by filtration. The liquid phase was analyzed by HPLC, while the solid phase was examined by XRPD.

Powder Dissolution

Dissolution studies were performed according to the USP paddle method in 900 ml dissolution medium, maintained at 37°C. The sample size was 20 mg and the stirring speed was 100 rpm. Each solid phase was evaluated in each medium in triplicate. The dissolution rate at pH 3.4 was measured in acetic acid (0.01 M; pH 3.4) using a VanKel 7000 apparatus (VanKel Industries Inc, Edison, NJ, USA) equipped with a fraction collector (VK 8000). At fixed time points, 3 ml aliquots were withdrawn, followed by quantification by HPLC. The dissolution rate at pH 6.4 was determined in phosphate buffer (0.05 M; pH 6.4) containing 0.25% (*v*/*v*) Tween 80 using a VanKel 7000 apparatus (VanKel Industries Inc, Edison, NJ, USA). Spectrophotometric detection at 258 nm (Varian Cary 50 BioUV–Visible) was used to determine the amount of drug dissolved. Data acquisition

was performed using Cary WinUV software. The results are reported as free base equivalents.

HPLC

HPLC analyses were performed as described by Christensen *et al.* (18). The HPLC system comprised an isocratic pump model L-6200, an autosampler (AS 2000A), a column thermostat (L-5025), a D-6000 Interface, and a L-4250 UV-VIS detector, all obtained from Merck Hitachi, Japan. Data acquisition and analysis were performed using D-7000 HSM software, also obtained from Merck Hitachi. Citric acid monohydrate, GR for analysis, was supplied by Merck, Darmstadt, Germany, and HPLC grade acetonitrile was obtained from LAB-SCAN analytical sciences, Dublin, Ireland.

X-ray Powder Diffraction (XRPD)

X-ray powder diffractograms were measured on a PANalytical X'Pert PRO X-Ray diffractometer (Almelo, Netherlands) using CuKα1 radiation (λ=1.5406 Å). The voltage and current were 45 kV and 40 mA respectively. Samples were measured in reflection mode in the 2θ-range 5–40° using an X'celerator detector; the resolution was 0.0334° 2θ. Data were collected using X'Pert Data Collector (PANalytical B.V., The Netherlands).

Light Microscopy

The conversion of siramesine-HCl anhydrate (needles) to siramesine-HCl monohydrate in aqueous dispersions was observed by a Zeiss Axiolab microscope (Carl Zeiss, Inc., Beograd, Österreich), and recorded every 20 s by a DeltaPix digital camera (Infinity X with 1.3 mega pixels CMOS, Maalov, Denmark). DeltaPix software 1.6 (Maalov, Denmark) was used for data acquisition.

Wet Massing

To mimic the wet granulation process, wet masses of siramesine-HCl anhydrate plates and needles were prepared with a mortar and pestle. One gram of drug was mixed with 500 µl of granulation liquid, and the wetted mass was grinded thoroughly for 3 min. As granulation liquids purified water and 60% ethanol (*v*/*v*) were used. The wet mass was sealed immediately to prevent moisture from escaping and samples were withdrawn over 3 h at specified time points. Each sample was dried on filter paper to prevent further conversion. Afterwards, the solid form of the samples was determined by Raman spectroscopy.

Raman Microscopy

A Renishaw Ramascope System 1000 with a NIR diode laser (λ=785 nm) was employed in this study. For the wet massing samples, each wet mass was placed separately on a microscopy slide, and viewed under an optical Raman microscope through a ×20 objective. The Raman laser beam, with a spot area of approximately 12×89 µm, was then focused on each sample using the same objective. A Rencam Charge Coupled Device (CCD) silicone detector was used to acquire Raman shifts with an exposure time of 10 s and two accumulations at a laser power of 50 mW. The physical state of plate crystals converted from needle anhydrate dispersed in ethanol–water mixture (60% *v*/*v* ethanol) was also assessed using the same Raman instrument. The crystals were viewed and focused with a ×20 objective, and the Raman spectra were then recorded at an exposure time of 30 s and two accumulations with a laser power of 100 mW. Wire V.2.0 software was used for instrument control and data acquisition.

RESULTS AND DISCUSSION

Solubilities of Siramesine-HCl Anhydrate and Monohydrate

To investigate the influence of solid form on the pH-dependent solubility and dissolution rate of siramesine-HCl, solubility and dissolution rate determinations were carried out at two different physiologically relevant pH values; namely pH 3.4 and 6.4. The pH-solubility profile for a weakly basic drug, such as siramesine-HCl, resembles the one shown in Fig. 2.

The pH-solubility relationship of ionizable compounds is based on the Henderson–Hasselbalch relationship, which relates the solubility of the completely 'unionized' compound ($[B]_s$, intrinsic solubility) to the total solubility measured at a given pH (S_T) and the pK_a of the compound:

$$S_T, base(pH > pH_{max}) = [B]_s\left[1 + 10^{(pK_a - pH)}\right] \quad (1)$$

$$S_T, salt(pH < pH_{max}) = [BH^+]_s\left[1 + 10^{(pH - pK_a)}\right] \quad (2)$$

where $[BH^+]_s$ is the salt solubility. pH_{max} (Fig. 2) is defined as the point of maximum solubility, and below this pH the solid phase in equilibrium with the solution is the salt. Above pH_{max} the solid phase in equilibrium with the solution is the free base. Thus, if the pH_{max} is lower than the pH of the intestine, precipitation of the free base may occur (15,19).

Of the chosen pH values, pH 3.4 is almost six pH-units below the pK_a of the drug. At this pH the drug is entirely in its ionized form, and therefore the determined values correspond to the solubilities of the anhydrate and monohydrate salts. The solubilities at this pH have been determined previously using acetic acid (0.01 M) and were found to be 200 µg/ml for siramesine-HCl anhydrate and 90 µg/ml

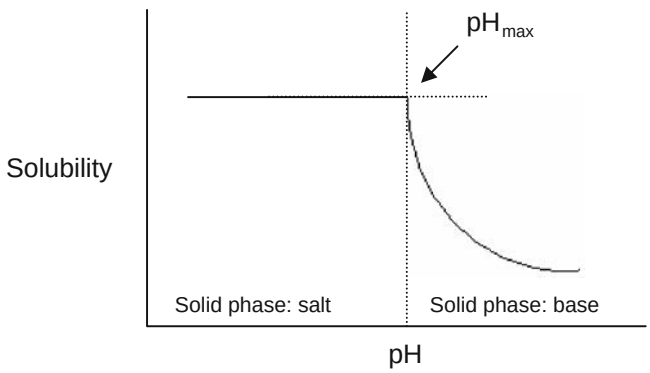

Fig. 2. pH-Solubility profile for weakly basic drugs.

for siramesine-HCl monohydrate. X-ray analysis of the precipitates showed that no conversion between the forms or conversion to the free base had occurred during the experiments. The solubility at pH 7.4 has also been determined previously (0.5 μg/ml), and from this information the pH-solubility profile for siramesine can be drawn using the Henderson–Hasselbalch relationship (Fig. 3). From Fig. 3 it is evident that pH_{max} for the anhydrate salt is 4.8 and pH_{max} for the monohydrate salt is 5.1.

The solubility at pH 6.4 was investigated in this study using phosphate buffer (0.05 M) containing 0.25% (*v*/*v*) Tween 80 as medium. This pH is 2.6 pH-units below the pK_a of the compound and hence the drug is almost entirely in its ionized form. However, this pH is higher than pH_{max} of both the anhydrate and the monohydrate salts, and therefore precipitation of the free base would be expected to occur. In order to follow the salt-base conversion and its effect on the solubility, the apparent solubility of the anhydrate and monohydrate salts as a function of time was determined over 24 h. The solid form of the precipitates was determined by XRPD (Fig. 4).

As evident from Fig. 4, very high apparent solubility values (500 μg/ml) were achieved for both solid forms within the first 8 h of the experiment. This value is much higher than the solubilities determined at pH 3.4, which theoretically should represent the maximum solubilities of the systems. Tween is a surfactant which is known to enhance the aqueous solubility of poorly water-soluble drugs, and thus the high solubility may be attributed to solubility enhancement by Tween 80.

During the first 8 h, the XPRD analysis showed no conversion of the salts to the free base, even though the solution phase was highly supersaturated with respect to the base (from Fig. 3 the solubility of the base is found to be 5 μg/ml at pH 6.4). Several investigations of pH-solubility profiles of drug-salt systems have shown that, specifically in the region around pH_{max}, supersaturation may occur (15,20). For the theophylline Na salt system, the level of supersaturation obtained exceeded four times the solubility of the salt (21). Ledwidge and Corrigan studied several drug-salt systems and found that the greatest degrees of supersaturation were achieved with systems in which self-association of dissolved drug molecules lowered the activity of the drug in solution. In this way the solutions were kept in the metastable zone, rather than exceeding the threshold for spontaneous nucleation (20). During the remainder of the solubility experiment at pH 6.4, siramesine-HCl monohydrate remained in a supersaturated state, reaching a solubility value of 800 μg/ml, while after 16 h, precipitation of the free base had occurred in the vials containing siramesine-HCl anhydrate. The drug concentration in these vials had dropped to around 50 μg/ml, which is higher than the theoretical base solubility of 5 μg/ml. This could be attributed to the fact that there was still some anhydrate left, as evident from Fig. 4, where small anhydrate reflections were still present after 16 and 24 h, and/or to the solubility enhancing effect of Tween 80.

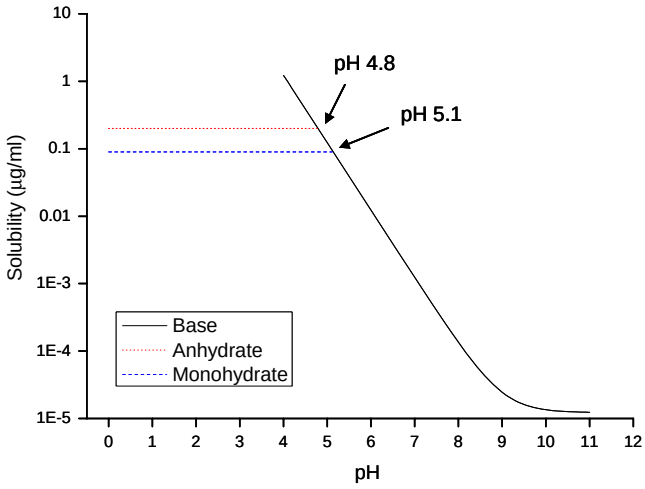

Fig. 3. pH-Solubility profile for siramesine-HCl.

The results indicate that the free base of siramesine-HCl is more susceptible to nucleate and grow in a suspension containing solid anhydrate salt than in a suspension containing solid monohydrate salt. For drug-salt systems it has been shown that adding seed crystals (nucleation substrates) of either the drug or the salt can induce precipitation from a supersaturated solution (20,22). Thus, the anhydrate crystals may act as seeds causing nucleation of the free base. The nucleation step of crystallization is an area that is still poorly understood. However, there is evidence that when crystals of a particular solid phase form in a suspension containing a different solid phase of the same compound, nucleation of the new crystalline phase takes place on the surface of the original crystals, or in the highly supersaturated phase surrounding the original crystals. Rodrigues-Hornedo *et al.* studied the theophylline anhydrate–monohydrate system and found that anhydrous theophylline crystals acted as nucleation substrates for the monohydrate phase, followed by epitaxial growth of monohydrate crystals (23). Similar observations were reported by Davey *et al.* who found that nucleation and epitaxial growth of polymorph 2 of dihydroxybenzoic acid took place on the surface of polymorph 1 crystals (24). If these findings are applied to the results presented here, the anhydrate crystals are able to act as nucleation substrates for the free base, while nucleation of the free base does not occur as readily on the monohydrate crystals. Due to the differences in crystal packing between the anhydrate and monohydrate salts, their surface structures differ, which may explain why nucleation can occur on the anhydrate crystals and not on the monohydrate crystals.

Influence of Solid Form on *In Vitro* Dissolution

The described findings would be expected to affect the dissolution rate of the two solid forms of the salt at pH 6.4. Powder dissolution using the USP paddle method was performed at pH 3.4 and 6.4 to asses the influence of solubility enhancement and base precipitation on the release of drug from the anhydrate (plate morphology) and monohydrate salts (Fig. 5).

At pH 3.4, the dissolution rates of siramesine-HCl anhydrate and siramesine-HCl monohydrate are similar with the anhydrate dissolving slightly faster than the monohydrate. The solubility of siramesine-HCl anhydrate is twice that of siramesine-HCl monohydrate, and therefore faster dissolution of the anhydrate would be expected, but due to the larger surface area of the monohydrate (1.00 and 0.33 m^2/g for the monohydrate and the anhydrate plates respectively), the dissolution rates in

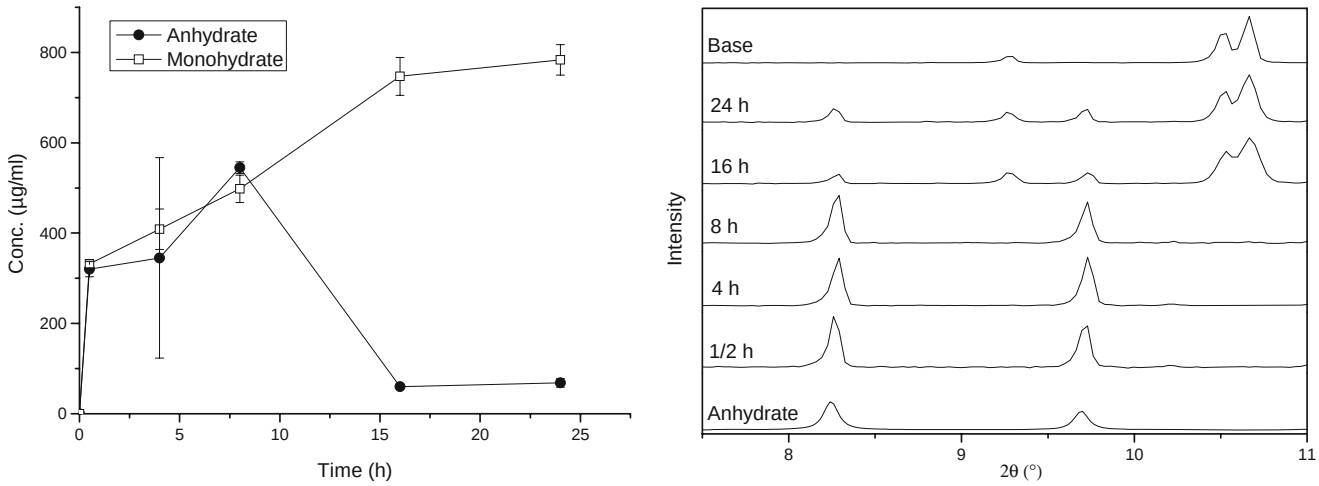

Fig. 4. *Left* Kinetic solubility of siramesine-HCl anhydrate and monohydrate in phosphate buffer + Tween 80, pH 6.4 (n=3). *Right* XRPD of precipitates formed during dissolution of siramesine-HCl anhydrate; XRPD of anhydrate and free base are shown for reference.

acetic acid did not differ greatly. Complete dissolution was achieved very quickly since at pH 3.4, dissolution is occurring under sink conditions (solubility data of the salt forms show the final concentrations in the pH 3.4 medium to be less than 20% of the solubilities).

Dissolution at pH 6.4 was performed in phosphate buffer (0.05 M). Tween 80 was added in a concentration of 0.25% (v/v) to overcome the poor wetting of the drug in the phosphate buffer. In this medium siramesine-HCl monohydrate exhibited a dissolution profile superior to that of siramesine-HCl anhydrate. Again, the higher surface area of the hydrate can explain the higher initial dissolution rate. However, the plateau was also higher for the monohydrate than for the anhydrate, indicating that the profiles were not achieved under sink conditions (as illustrated in Fig. 4, no single solubility value could be determined and therefore sink conditions could not be calculated). At the end of the experiment it was possible to recover some material from the anhydrate dissolution vessels, and XRPD showed complete conversion to the free base. In the monohydrate vessels, all material had dissolved. Using the information obtained from the solubility experiments, it seems likely that solutions supersaturated with respect to the free base were formed, and that the monohydrate remained in the supersaturated state, not forming nuclei of the base, while at some point, conversion of the anhydrate to the free base occurred. In the anhydrate vessels approximately 15 mg had dissolved resulting in a drug concentration of 17 µg/ml, which is higher than the theoretical 5 µg/ml. This higher value may be explained by the solubility enhancing effect of Tween 80. On the other hand, the found concentration is lower than the value of 50 µg/ml obtained in the solubility experiment, which may be due to the fact that not all anhydrate salt had converted into free base during the solubility experiment. Furthermore, it was not possible to recover all of the 20 mg of drug added to each dissolution vessel. This may be due to drug adsorption to equipment walls and tubing, caused by the high log P of the drug. Further investigation on this issue is ongoing.

Overall, the results suggest that under certain conditions, the monohydrate exhibits a more favorable dissolution behavior compared to the anhydrate. This is of interest, because hydrates generally show poorer dissolution characteristics than their corresponding anhydrous forms (4).

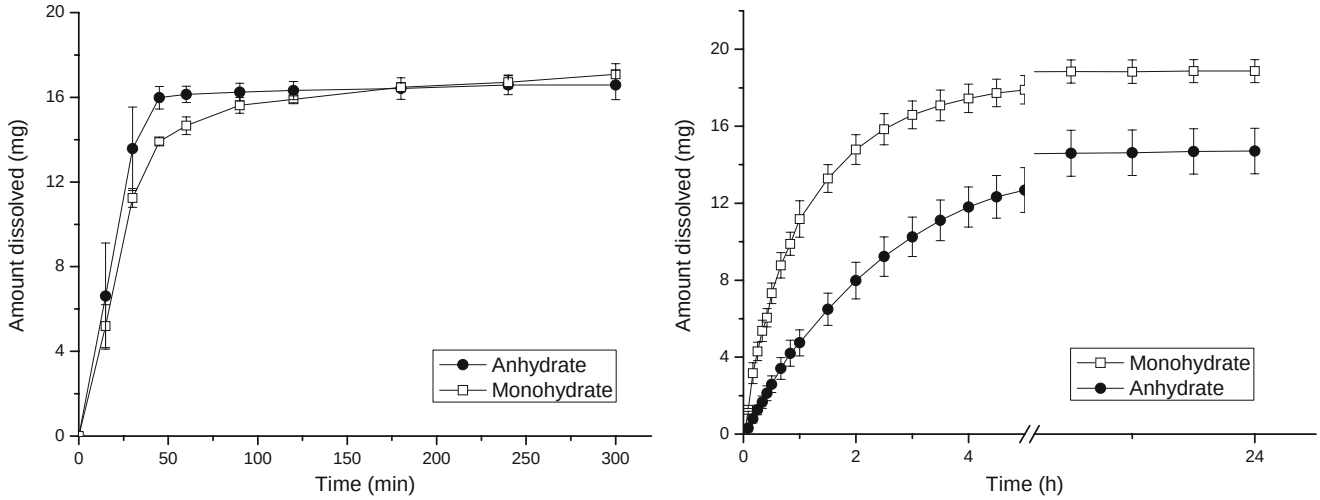

Fig. 5. *Left* Dissolution of siramesine-HCl anhydrate and monohydrate in acetic acid, pH 3.4 (n=3). *Right* Dissolution of siramesine-HCl anhydrate and monohydrate in phosphate buffer + Tween 80, pH 6.4 (n=3).

Siramesine-HCl is a poorly water-soluble drug with a high log *P*, and therefore dissolution in the gastro-intestinal tract is likely to be the rate-limiting step controlling the bioavailability of the drug (25). The ability to retain itself in a supersaturated state at physiologically relevant pH may have implications to the *in vivo* dissolution and absorption of siramesine-HCl since, in other studies, it has been demonstrated that supersaturation can offer advantages to drug delivery by enhancing the flux across biological membranes (26–28).

Mechanism of Anhydrate–Hydrate Conversion

The mechanism of anhydrate–hydrate conversion was elucidated by optical microscopy. This was possible due to the morphological differences between siramesine-HCl anhydrate and siramesine-HCl monohydrate. Figure 6 shows SEM micrographs of siramesine-HCl anhydrate, siramesine-HCl monohydrate, and siramesine base. As shown in Fig. 6a and b, the anhydrate can exist in two different crystal morphologies—plates and needles—sharing the same crystal structure. The morphology of the monohydrate (Fig. 6c) is similar to that of the anhydrate plates, but different from the anhydrate needles. This morphological difference between needle siramesine-HCl anhydrate and plate siramesine-HCl monohydrate allows a direct visual detection of the anhydrate–hydrate conversion mechanism. Although the particles of siramesine base (Fig. 6d) were larger than the anhydrate and monohydrate plates, their morphologies were similar, and therefore the formation of free base as observed in the solubility and dissolution experiments could not be studied visually using microscopy.

Initially the anhydrate needles were dispersed in pure water and placed on a microscope slide. The dispersion was followed over 48 h, but no conversion was observed. It was anticipated that conversion would happen eventually and that the very low rate of conversion in water was due to the low water solubility of siramesine-HCl anhydrate (150 μg/ml). To increase the solubility, a mixture of ethanol and water (60% *v*/*v* ethanol) was applied, since the solubility in ethanol is higher than in water (24 mg/ml). In this medium the needles dissolved readily, and the morphology change during dissolution was observed. As seen in Fig. 7, the surrounding liquid was clear in the beginning, but became noticeably turbid at

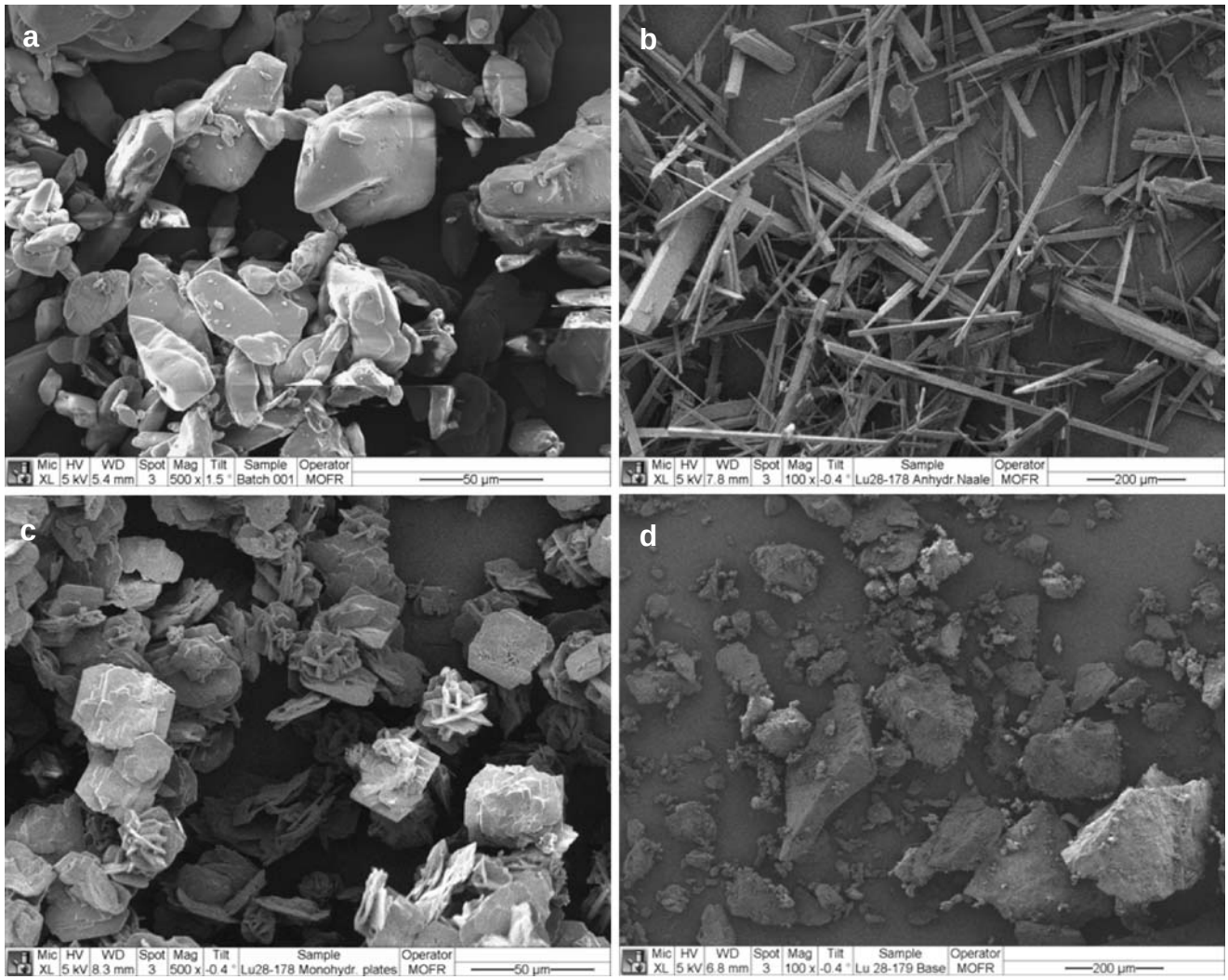

Fig. 6. SEM micrographs of siramesine-HCl **a** anhydrate plates (×500), **b** anhydrate needles (×100), **c** monohydrate (×500), and **d** siramesine base (×100).

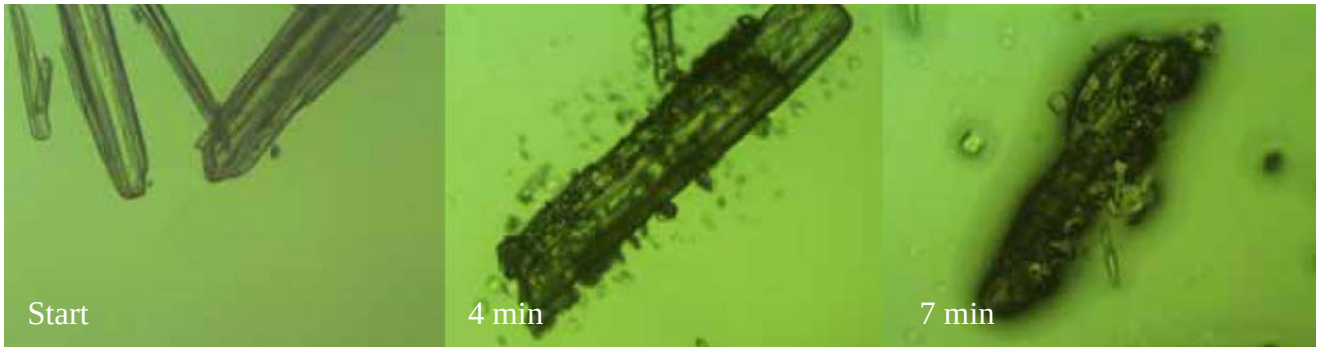

Fig. 7. Light microscopy pictures taken during the conversion of siramesine-HCl needle anhydrate to the monohydrate in 60% (*v*/*v*) ethanol.

4 min, indicating dissolution of the anhydrate needles. Simultaneously with anhydrate dissolution, monohydrate plates started to grow, primarily on the surface of the anhydrate needles. Some monohydrate crystals could also be observed in the surroundings of the dissolving needle crystals. Raman microscopy confirmed that the observed plate crystals were in fact monohydrate crystals.

These observations suggest that the conversion of siramesine-HCl anhydrate to the monohydrate is solution-mediated. This requires two essential steps (a) dissolution of metastable solid, and (b) self-recognition of the molecular units to nucleate a more stable solid phase, followed by growth of the stable phase. Depending on the solubility of the drug in question, the rate can be either dissolution-controlled or nucleation/growth-controlled (29). In the present investigation, the results suggest that the rate-controlling step of conversion from anhydrate to monohydrate is dissolution, since the rate of conversion was greatly decelerated in water. By replacing water with an ethanol–water mixture, the solubility of siramesine-HCl anhydrate increased greatly, and dissolution was no longer the rate-limiting step. Instead, the fast dissolution facilitated nucleation and growth of the monohydrate.

Monitoring Phase Transformations During Wet Massing

Wet granulation is a process where processing induced transformations are known to occur. Transformation between solid forms, as well as dissociation of salts to the free form, has been observed (14). The first step towards solid form control during processing is an assessment of the potential transformations the drug may undergo during processing. Therefore, a wet massing experiment was conducted to investigate the risks of conversion during granulation of siramesine-HCl anhydrate. As granulation liquids pure water and 60% *v*/*v* ethanol were chosen to allow comparison with the observations obtained with the microscope. Both anhydrate plate and needle crystals were applied, to see if the morphology differences would influence the dissolution-controlled conversion to monohydrate. Raman microscopy was used off-line to monitor the wet massing process, since it enables non-destructive and fast probing of the molecular structure of small pharmaceutical compounds.

The wet masses were followed over 180 min. For the siramesine-HCl anhydrate plates, no solid state transformations were detected during the time of the experiment. Also, no conversion was seen for the wet masses of needle anhydrate when water was used as granulation liquid. However, conversion to the monohydrate was found for the needle anhydrate when granulated with the ethanol-water mixture (60% ethanol *v*/*v*). Raman spectra demonstrating this conversion process are shown in Fig. 8.

The Raman spectra of siramesine-HCl anhydrate and siramesine-HCl monohydrate are different, and the gradual spectral changes with wet massing can be observed especially in the highlighted areas. Tentative assignments of these bands were carried out. The bands at around 1,460 and 1,488 cm^{-1} (shaded areas in Fig. 8) could be associated with (C–H) aromatic bending and stretching respectively. The doublet bands located at around

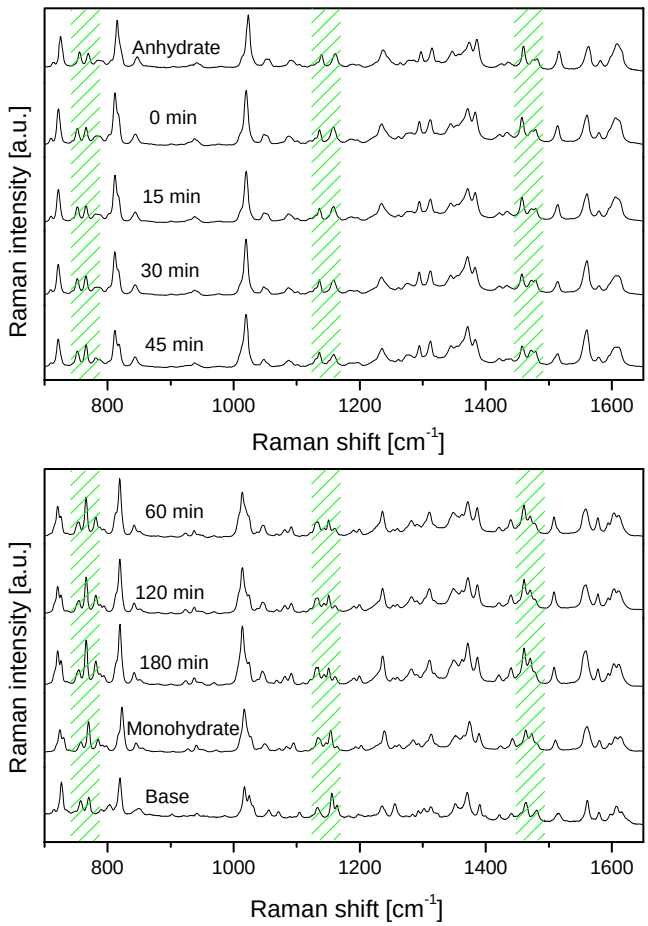

Fig. 8. Raman spectra of siramesine-HCl anhydrate and monohydrate, siramesine base and wet masses (off-line analysis).

1,135 and 1,160 cm^{-1} (shaded in Fig. 8) are associated with ρ(N–H) and ν(C–C)ring/(C–N–C) asymmetric respectively, and those at around 755 and 771 cm^{-1} (shaded in Fig. 8) might be caused by the (C–O–H) bending. The obvious changes in these bands with the progress of wet massing indicate a structural change of anhydrate when forming its monohydrate. Further information about the structures of siramesine-HCl anhydrate and siramesine-HCl monohydrate can be found in Cambridge Crystallographic Data Centre (anhydrate 122 K CCDC 689613; anhydrate 293 K CCDC 689614; monohydrate CCDC 689612). The conversion from anhydrate to hydrate started at approximately 45 min as illustrated in Fig. 8, and it was not completed at 180 min, since some differences are still discernable between the spectra of the monohydrate reference and the wet mass obtained at 180 min.

The fact that the anhydrate did not convert to the monohydrate when water was used as granulation liquid reflects the poor wetting and dissolution of the anhydrate crystals. The presence of water was not sufficient to dissolve enough of the drug to initiate nucleation and growth of the monohydrate. This agrees with the observations from the light microscope where the solution-mediated conversion between anhydrate and monohydrate was found to be dissolution rate controlled. The fact that the anhydrate needles started to convert during the experiment while the anhydrate plates did not further supports this hypothesis. The larger surface area of the needles as compared to the plates (0.39 *vs.* 0.33 m^2/g) facilitated faster dissolution of the anhydrate needle crystals. Furthermore, the dissolution rates of crystals with different habits may vary because the exposure of hydrophilic functional groups at the surface may be different. The fact that conversion was only observed for the needles may also be due to breakage of the needle crystals during grinding. This increases the surface area and creates an activated surface where phase transitions are facilitated. Wikström *et al.* studied the rate of transformation of theophylline anhydrate to theophylline monohydrate during wet granulation and observed an increased rate of transformation with increasing impeller speed, due to the higher shear forces imposed on the drug (9).

No transformation of the salt to free base was detected in the wet masses. Thus, the results indicate that there is a risk of hydrate formation when siramesine-HCl anhydrate is processed in the presence of organic aqueous solvents due to enhanced solubility, which facilitates solution-mediated transformation to the monohydrate. This should be taken into consideration when designing granulation liquids for this compound. The wet granulation process was chosen in this study, but anhydrate–hydrate conversion may also occur during other processes, for instance during film coating.

CONCLUSION

The behavior of siramesine-HCl in aqueous environments was investigated. Solubility and dissolution studies showed that in phosphate buffer pH 6.4 containing Tween 80, siramesine-HCl anhydrate and siramesine-HCl monohydrate formed supersaturated solutions of high apparent solubility. In the case of siramesine-HCl anhydrate this was followed by precipitation of the free base with almost complete transformation to base after 16 h. In contrast, the drug was able to remain in the supersaturated state in a solution containing solid monohydrate salt for 24 h, i.e. the free base did not nucleate from this suspension. The different susceptibilities of the two salt forms to transform to the free base may be due to the structural differences between them. In the case of the anhydrate, the anhydrate crystals were able to act as seed crystals for the base, causing faster precipitation of the base, which has very low aqueous solubility. This resulted in a more favorable dissolution profile of the monohydrate compared to that of the anhydrate.

The conversion from siramesine-HCl anhydrate to siramesine-HCl monohydrate in aqueous dispersion was monitored by optical microscopy and the mechanism was found to be solution-mediated. The conversion was dissolution rate controlled, since conversion was not observed in pure water, where siramesine-HCl is poorly soluble, but occurred readily in an ethanol–water mixture (60% *v/v* ethanol), due to the higher solubility in this medium. Assessment of the risk of processing-induced transformations occurring during wet granulation, as exemplified by wet massing of siramesine-HCl anhydrate plate and needle crystals, revealed that neither of the two morphologically different anhydrate batches transformed to a different solid phase when pure water was used as granulation liquid. However, when 60% (*v/v*) ethanol was applied, the siramesine-HCl anhydrate needles converted to the monohydrate. Thus, if siramesine-HCl anhydrate is processed with solvents in which its solubility is good, there is a potential risk of transformation to the monohydrate form.

ACKNOWLEDGEMENTS

H. Lundbeck A/S (Copenhagen, Denmark) and the Drug Research Academy (Copenhagen, Denmark) are thanked for financial support. Mogens Frost, H. Lundbeck A/S, Preformulation, is thanked for assistance with SEM micrographs and BET measurements. Furthermore, Erling Bonne Jørgensen, H. Lundbeck A/S, Preformulation, is thanked for commenting on the manuscript.

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