

Solubility analysis of venlafaxine hydrochloride polymorphs by shake-flask method and real time monitoring

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Abstract

Aims: The aqueous solubility of two polymorphic forms of venlafaxine hydrochloride was investigated.

Methods: The pH-dependent solubility (S_{pH}) over a wide pH range was measured by saturation shake-flask (SSF) method at 25 °C. The solubility of the free base form was depicted by the intrinsic solubility (S_0). To identify the solid form present at the solubility equilibrium, X-ray powder diffraction (XRPD) and Raman spectroscopy was carried out. The dissolution was studied using real time concentration monitoring applying fiber optic UV probes.

Results: No difference was found in the S_{pH} values of Form I and Form II, in the pH range 7.5-12. Solid phase isolated from pH 10-12 suspensions was identified as free base by XRPD and Raman spectroscopy. Precipitates separated from pH 7-8 samples were also identical product. The transition of polymorphs to the free base was supported by the real time dissolution analysis.

Conclusion: In this study we demonstrated a good agreement of equilibrium solubility measured by SSF method and in-situ UV fiber optic method. μ DISS ProfilerTM has the advantage to provide much more information about dissolution process; with this approach the dissolution kinetic, the supersaturation and the time needed to reach the equilibrium can be easily monitored.

Keywords: solubility-pH profile, polymorphs, shake-flask method, μ DISS Profiler, venlafaxine

1. Introduction

Solubility is one of the most important molecular properties of drugs. It plays a key role in determining their absorption potential and has fundamental impact on the bioavailability therefore. Solubility data refers to the given physical form of a solid compound thereby it is significantly affected by polymorphism. Polymorphism – the ability of a substance to exist as two or more crystalline forms that have different arrangements and/or conformations of the molecules in the crystal lattice – is common among the pharmaceutical solids [1]. More than one third of drugs show polymorphism and a further one third is capable of forming hydrates and solvates [2]. This subject has gained intensive attention in both original and generic drug research since in case of several drugs polymorphism caused serious safety problems [3]. The most known examples (from chloramphenicol palmitate, through carbamazepine, oxytetracycline, enalapril, etc. up to ritonavir) have been extensively reported in the literature [4-8]. These cases in-

duced severe regulatory considerations [9] resulting that polymorph screening and the investigation of physico-chemical properties and stability of different polymorphic forms has become inevitable during the process of drug development [10, 11].

Polymorphs have different lattice energy so they differ in those physical and chemical properties which associated with the internal free energy of the solid, such as: density, refractivity, conductivity, hygroscopicity (packing properties); melting temperature, vapor pressure, solubility (thermodynamic properties); dissolution rate, stability (kinetic properties); and several others (surface, mechanical, spectroscopic properties) [2]. From absorption and bioavailability point of view the solubility and the dissolution rate are the most relevant properties of a pharmaceutical compound, however from formulation and safety aspects stability is also essential. A general rule is that a polymorph having the lower lattice free energy is the more stable form and it has the lower solubility while that having higher energy is less stable but tends to dissolve faster and has higher solubility [1]. Based on litera-

ture solubility data *Pudipeddi* and *Serajuddin* [12] published a survey that the ratio of metastable/stable polymorph solubility is typically less than 2, though occasionally higher ratios can be observed as reported for sulindac (7x difference between Form I and II) [13] and for premafloxacin (23x) [14], etc. Similar trend was found for anhydrate/hydrate solubility ratio (~2) with many exceptions, while the amorphous/crystalline forms solubility differs with factor ~10, generally. Solubility difference of polymorphs will have effect on the bioavailability/bioequivalence (BA/BE) of a drug product if the solubility is the rate limiting factor upon absorption (for BCS II and IV drugs).

Solubility characterized by the equilibrium solubility data is the concentration of the compound in a saturated solution when solid and solute are at equilibrium. This parameter can be measured by several methods however the "gold standard" is still the saturation shake-flask technique (SSF). Recently *Avdeef et al.* published a consensus-based "white paper" commentary which summarized the recommendations for solubility measurement including SSF and other methods to improve the data quality [15]. The paper pointed out to the difficulties in the measurement of polymorphs due to the possible polymorph transitions upon equilibration and the importance of the analysis of both solution and solid phases. Well established methods for solid phase analysis (X-ray powder diffraction XRPD, differential scanning calorimetry DSC, FT-IR and Raman spectroscopy, etc.) should be used for characterization of the solid at the beginning and also at the end of the solubility experiment. Adhering and applying all these recommendations of the "white paper" one can measure only the thermodynamic solubility of that crystalline form which is in dynamic equilibrium with its solution form, at the temperature of the experiment. However, during the dissolution process polymorphs can show different kinetics, resulting in substantially various supersaturation solutions even so if later they convert to another form. The incubation time in the standard protocol of SSF is 24 h (6 h agitation and 18 h sedimentation) [16]. Since solvent induced changes in solid form structure might occur much faster or slower than this timeframe the *in situ* monitoring of the dissolution-time profile may provide more information about the behavior of polymorphs and may enable the optimization of incubation time. Moreover *in situ* monitoring with fiber optic UV probes under certain con-

ditions (low amount of excess solid, long analysis time, etc) enables the determination of dissolution kinetics and also equilibrium solubility from a single experiment.

The goals of the present study were: (1) investigation of equilibrium solubility of different polymorphs of venlafaxine hydrochloride (VENL) as model compound, in wide pH range by SSF method coupled with solid phase analysis; (2) real time monitoring of the dissolution profile using *in situ* UV fiber optic; (3) to compare the equilibrium solubility results obtained by SSF and μ DISS methods and (4) to reveal the advantages and limits of the latter technique for solubility measurement.

2. Material and methods

2.1. Materials

The structure of model compound is presented in [Figure 1](#). Venlafaxine hydrochloride (313.86 g/mol) was purchased from Sigma-Aldrich Co. LLC. (St. Louis, MO, USA). From the commercially available polymorph the other polymorphic form was prepared in house based on patent (US6924393B2) and verified by XRPD. Distilled water of Ph. Eur. grade was used. All other reagents were of analytical grade.

A Britton-Robinson (BR) buffer stock solution (a mixture of acetic acid, phosphoric acid and boric acid, each at 0.04 M) was prepared, and the required amount of 0.2 M NaOH or 1M NaOH was added to give the pH specified for the solubility experiments. BR buffer solutions were used in pH range 7.5-11, while at pH 11 and above NaOH was applied.

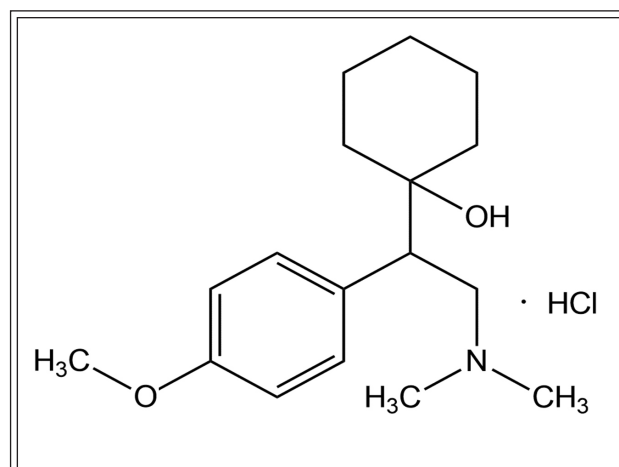


Figure 1 Structure of venlafaxine hydrochloride

2.2. Determination of the thermodynamic solubility by saturation shake-flask method (SSF)

2.2.1. Preparation of saturated solution

The equilibrium solubility of VENL in the examined pH region was determined by the SSF method [16, 17]. The sample was added in excess to the aqueous buffer solutions to produce a suspension. The amount of solid added was accurately measured: 10-300 mg/4 mL. At controlled temperature of 25.0 ± 0.1 °C the solution containing solid excess of the sample was vigorously stirred for a period of 6 h (agitation time), what followed a further 18 h of sedimentation period (stirrer turned off).

2.2.2. Concentration determination of the saturated solution

2.2.2.1. Off-line: sampling, dilution and concentration measurement with UV spectrophotometer

The concentration of the saturated solution was measured by UV spectroscopy using JASCO V 550 UV/VIS spectrophotometer. Three aliquots were carefully withdrawn from the liquid, using a fine glass pipette, and were diluted with the solvent if necessary. Three replicate solubility measurements were carried out at each of the tested conditions.

The specific absorbance ($A^{1\%}_{1\text{cm}}$, the absorbance of 1 g/100 mL solution over a 1 cm optical pathlength at a given wavelength) of VENL was determined separately at pH 8.0 using 12 points of two parallel dilution series, from the linear regression equation (Lambert-Beer law) where the regression coefficient (r) was higher than 0.9998. The specific absorbance data obtained is 38.4 at λ_{max} 273.5 nm. This value was used for concentration calculation at all pHs since the UV spectrum of VENL is not pH-dependent due to the distance between the protonation site (aliphatic N atom) and the chromophore in the structure.

2.2.2.2. In situ: with fiber optic UV probe

μ DISS Profiler™ (Pion Inc. Billerica MA, US, [Figure 2](#)) has been applied as UV fiber optic instrumentation. The equilibrium concentration was concomitantly determined *in situ* in the equilibrated solubility suspension (without dilution) immersing the UV dip probe. Calibration was performed with the same (2-5-10-20 mm) tip adjusted to the fiber optic UV dip probe which was used in the given



Figure 2 μ DISS Profiler™

solubility experiment. Solubility values obtained from the same experiments analysed with both the *off-line* and the *in situ* UV method were compared (see section 3.3.).

2.3. In situ real time monitoring of dissolution, supersaturation, precipitation and the equilibrium solubility

The drug solution concentration *versus* time (0-24 h) was investigated with the μ DISS Profiler™. The instrument measures the real time concentration with fiber optic UV dip probe inserted in 6 temperature controlled 20 mL vessels, stirred with magnetic stirrer. Dissolution of VENL was registered in BR buffer pH ~ 11.5, in three parallel channels, where 10 mg sample was added to 4 mL dissolution media. Using the appropriate 2 mm tip adjusted to the UV probe, the UV spectra were registered in the following timing protocol: per 1 min in 0-2 h, per 10 min in 2-6 h and per 30 min in 6-24 h periods. The stirrer was turned off at 6 h. For the evaluation of the concentration previously determined calibration data and second derivative spectra were used.

2.4. Methods for the analysis of the solid phase

2.4.1. XRPD

Small amount of the solid phase at the end of solubility measurement was isolated and dried to a glass plate. X-ray powder diffraction patterns of these samples were recorded by means of a PANalytical (Amelo, The Netherlands) X'pert ProMDP X-ray diffractometer using Cu-K α radiation (1.524 Å) and a Ni filter. The applied voltage

was 40 kV, while the current was 30 mA. The samples were analysed between 4° and 42° 2 θ .

2.4.2. Raman spectroscopy

Raman spectra were collected using a Horiba Jobin-Yvon LabRAM system coupled with an Olympus 97 BX-40 optical microscope (Olympus Corporation, Tokyo, Japan). The sample was illuminated by a 785-nm diode laser (TEC 510 type, Sacher Lasertechnik, Marburg, Germany) and an objective of $\times 10$ (laser spot size, approximately 4 μm) was used for optical imaging and spectrum acquisition. The laser beam is focused through the objective, and the backscattered radiation is collected with the same objective, a common configuration found in most confocal spectroscopic systems. The collected radiation is directed through an edge filter that removes the Rayleigh photons and then through a confocal hole (500 μm) and the entrance slit (100 μm). Finally, a 950 groove/mm grating monochromator disperses the Raman photons before reaching the CCD detector. Each spectrum of the image was collected in the spectral range of 300 to 1600 cm^{-1} .

2.4.3. Polarized light microscopy

Polarized light microscopic images were taken using Amplitval (Carl Zeiss, Jena, Germany) type microscope coupled with Olympus C4040 Z type camera (Olympus, Japan).

2.5. Statistical analyses

Concentrations were expressed as means \pm SD, and were compared using "two-sample" Student's *t* test. Differences were considered statistically significant when $p < 0.05$.

3. Results and Discussion

In this study we measured the equilibrium solubility of different polymorphic forms of venlafaxine hydrochloride modelling the possible behaviour of a pharmaceutical solid. The pH-dependent equilibrium solubility (S_{pH}) over a wide pH range was investigated by SSF method at 25 °C temperature. The solubility of the free base form was depicted by the intrinsic solubility (S_0) measured at high pH value. To identify the solid form that is present at the solubility equilibrium, the approach was completed with the solid phase analysis at the end of the solubility experiment. We also studied

the dissolution kinetics and the extent of supersaturation and the incubation time needed to reach the equilibrium, using real time monitoring by μDISS Profiler™ to reveal the differences between the stable and less stable/metastable forms.

Venlafaxine is a 2nd generation, SNRI (serotonin-norepinephrine reuptake inhibitor) antidepressant drug nowadays widely used in depression, in generalized anxiety disorder, post-traumatic stress disorder, etc. (US Patent, 2006, US7030164B2). VENL is used as racemate of its hydrochloride salt which is known to exist in two polymorphic forms, Form I and Form II. Though Form I is more stable (melting temperature: 210-212 °C, ΔH : 125.8 J/g) than Form II (melting temperature: 208-210 °C, ΔH : 130.3 J/g) the latter is preferred in the formulation because it has larger particle size with better filtration and drying characteristics [18]. VENL is a monovalent base ($\text{p}K_a$: 9.63), its solubility is pH-dependent. Since the compound belongs to BCS I class, the solubility is not an absorption limiting factor from GI tract. Investigation of salt solubility in biorelevant medium was not the goal of this study. However VENL is a good model for studying the pH-dependent solubility of polymorphs and for comparison the SSF and μDISS methods in the measurement of intrinsic solubility.

Table I Equilibrium solubility of venlafaxine hydrochloride polymorphs in BR buffer measured by SSF method

VENL Form I			VENL Form II		
pH	$S_{\text{pH}} \pm \text{SD}$ (mg/mL)	n	pH	$S_{\text{pH}} \pm \text{SD}$ (mg/mL)	n
7.50	59.35 \pm 8.23	9	7.54	54.32 \pm 4.43	3
7.70	32.08 \pm 2.60	9	7.60	45.67 \pm 2.34	9
7.83	23.34 \pm 0.45	3	7.67	38.35 \pm 2.99	9
8.30	8.655 \pm 0.267	9	7.78	26.79 \pm 0.27	3
8.34	7.194 \pm 0.043	3	8.20	8.175 \pm 1.061	9
8.37	7.495 \pm 0.601	3	8.40	7.689 \pm 0.345	9
8.43	6.983 \pm 0.103	3	8.52	5.506 \pm 0.104	3
8.47	6.364 \pm 0.071	3	8.58	4.985 \pm 0.053	3
9.30	0.948 \pm 0.031	9	8.64	4.587 \pm 0.013	3
9.50	0.796 \pm 0.094	9	8.70	3.809 \pm 0.104	3
9.72	0.574 \pm 0.011	3	9.45	0.831 \pm 0.027	9
9.76	0.507 \pm 0.004	3	9.76	0.567 \pm 0.014	4
9.81	0.559 \pm 0.062	3	9.96	0.544 \pm 0.011	3
9.96	0.570 \pm 0.013	3	10.06	0.495 \pm 0.052	4
10.34	0.521 \pm 0.186	3	10.26	0.523 \pm 0.037	4
10.41	0.454 \pm 0.011	3	10.41	0.498 \pm 0.034	4
11.51	0.275 \pm 0.004	4	11.62	0.296 \pm 0.025	4
12.11	0.292 \pm 0.007	4	12.01	0.285 \pm 0.004	4

Concentration is expressed as free base equivalents.

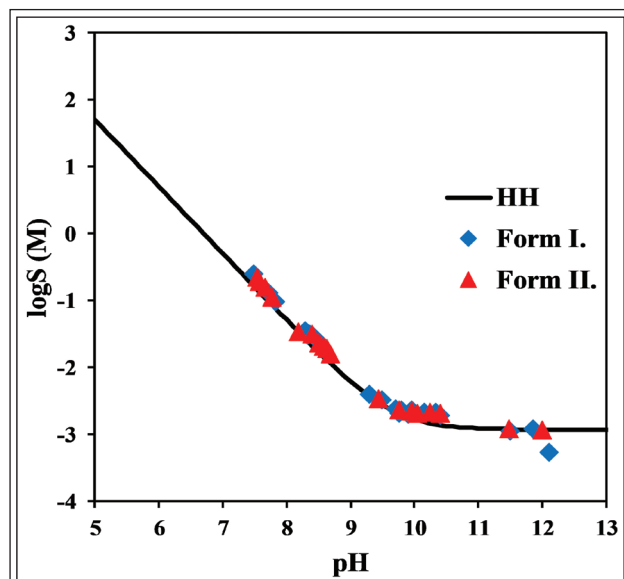


Figure 3 Solubility-pH profile of VENL Form I and Form II (solid line represents the theoretical HH curve calculated from pK_a : 9.63 and $\log S_0$: -2.93, points represent the experimental data)

3.1. Solubility-pH profile of venlafaxine polymorphs

The equilibrium solubility of VENL Form I and II were measured by the standard protocol of SSF method at 18 different points in pH range 7.5-12, at 25 °C. The $S_{pH} \pm SD$ results are expressed in mg/mL unit as average value of parallel measurements and are presented in [Table I](#). The standard deviation was in the range of 1-13 %, average SD: 6 %. No significant difference was found between the S_{pH} values of polymorphs at the same pH in the whole exam-

ined region. This can be interpreted with the transition of polymorphs to a common product as supported by the solid phase analysis (see below 3.2).

[Figure 3](#) shows the solubility-pH profile of VENL Form I and Form II as plotted $\log S$ [M] values *vs* pH. As it can be seen in [Figure 3](#) the curve is typical for a monoprotic base as described by the theoretical Henderson-Hasselbalch (HH) relationship ($\log S = \log S_0 + \log(10^{pK_a - pH} + 1)$ [17, 19]).

The intrinsic solubility of VENL base (calculated as the average of values measured at $pH \geq 11.5$) is $\log S_0$ [M]: -2.93 ± 0.014 ($n=4$). This value was used to generate the HH curve of VENL (solid line on [Figure 3](#)). The experimental data points closely follow the theoretical HH curve. Below pH 10 the VENL free base starts to convert to cationic form and the solubility is increasing. Below pH 7 the solubility was not studied because the salt solubility is very high (> 500 mg/mL) and its saturated solution can be considered non-ideal solution.

3.2. Characterization of the solid phase isolated from solubility experiment

First the starting materials, Form I and Form II were identified by powder X-ray diffraction (see in [Figure 4](#)). Next the samples isolated and dried from the solubility suspension after incubation at different pHs were studied. Diffractograms shown representatively in increasing pH order in [Figure 4](#) demonstrate that the samples are not identical with the starting polymorphic forms in neither case. Results indicate that both polymorphs re-

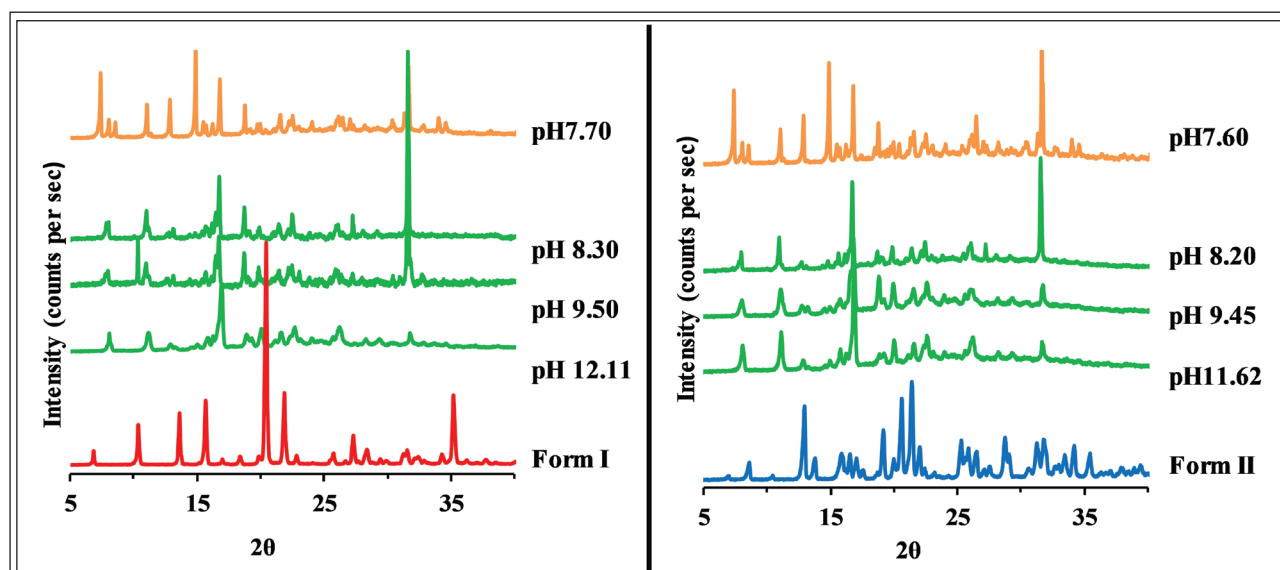


Figure 4 XRPD patterns of VENL Form I and Form II and the solids isolated from solubility suspensions at different pH values

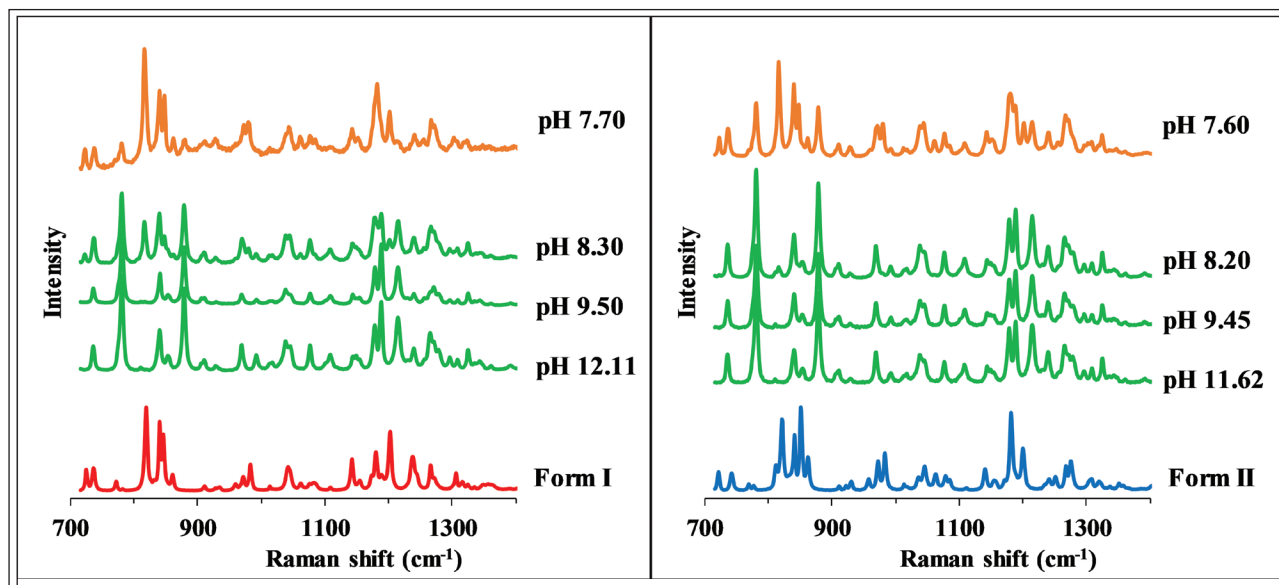


Figure 5 Raman spectra of solid phase of VENL polymorphs isolated from solubility suspensions

crystallized to a common form. Product obtained at lower pHs (7.5-9) could not be identified using Cambridge Structural Database. Based on patent US20030114536A1 we identified the product as venlafaxine hydrochloride monohydrate. Patterns registered at higher pH (11-12) values were found identical with one of the free base VENL polymorphs, specified in the database as free base Form I. A peak (if any) appeared with various intensity in the patterns of free base at $9 (2 \Theta)$ is due to the presence of sodium acetate remained in some samples. XRPD finding was supported by Raman spectroscopy as well (Figure 5). The polarized light microscopic images in Figure 6 (A and B panels) indicate that crystal shape of Form I and II is column but they substantially differ in their

size. Panel C shows the shape of the free base form isolated at pH 12.

All above results of solid phase analysis confirm and can interpret the equilibrium solubility data measured experimentally (Table 1) in pH range 7.5-12.

3.3. Comparison of concentration measurement with off-line UV method and in situ UV probe

μ DISS Profiler™ measures the concentration of the solute *in situ* in the saturated solution in the presence of solid. In order to prove that it has no impact on the results (e.g. distorting the absorption measurement), in a part of SSF experiment at the end of incubation time the concentration of the saturated solution was measured by two ways: (1) after the necessary dilution with traditional UV spectrophotometer (UV-JASCO), and (2) *in situ* in the solubility vessel (without dilution) by UV probe (UV- μ DISS). Solubility values shown in Table II are in good agreement. Difference between the results

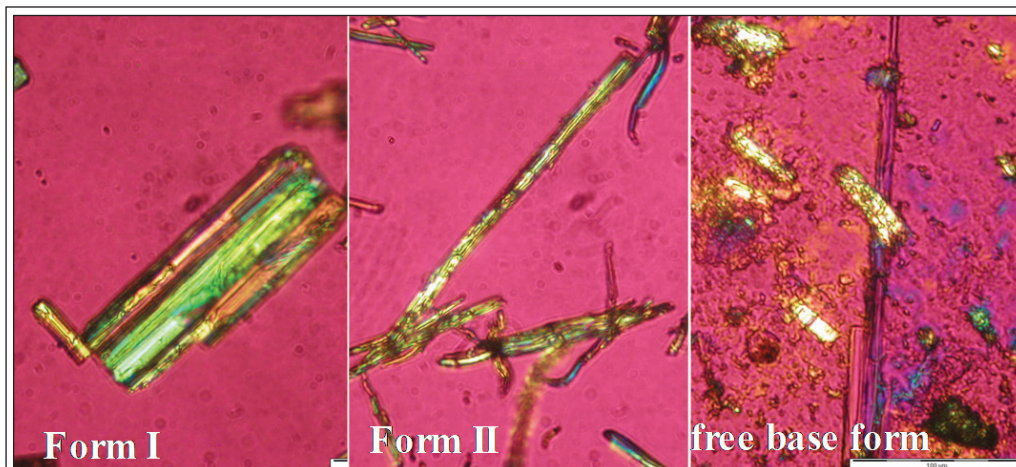


Figure 6 Polar microscopic images of VENL samples (A: Form I, B: Form II, C: free base isolated at pH 12)

between the results

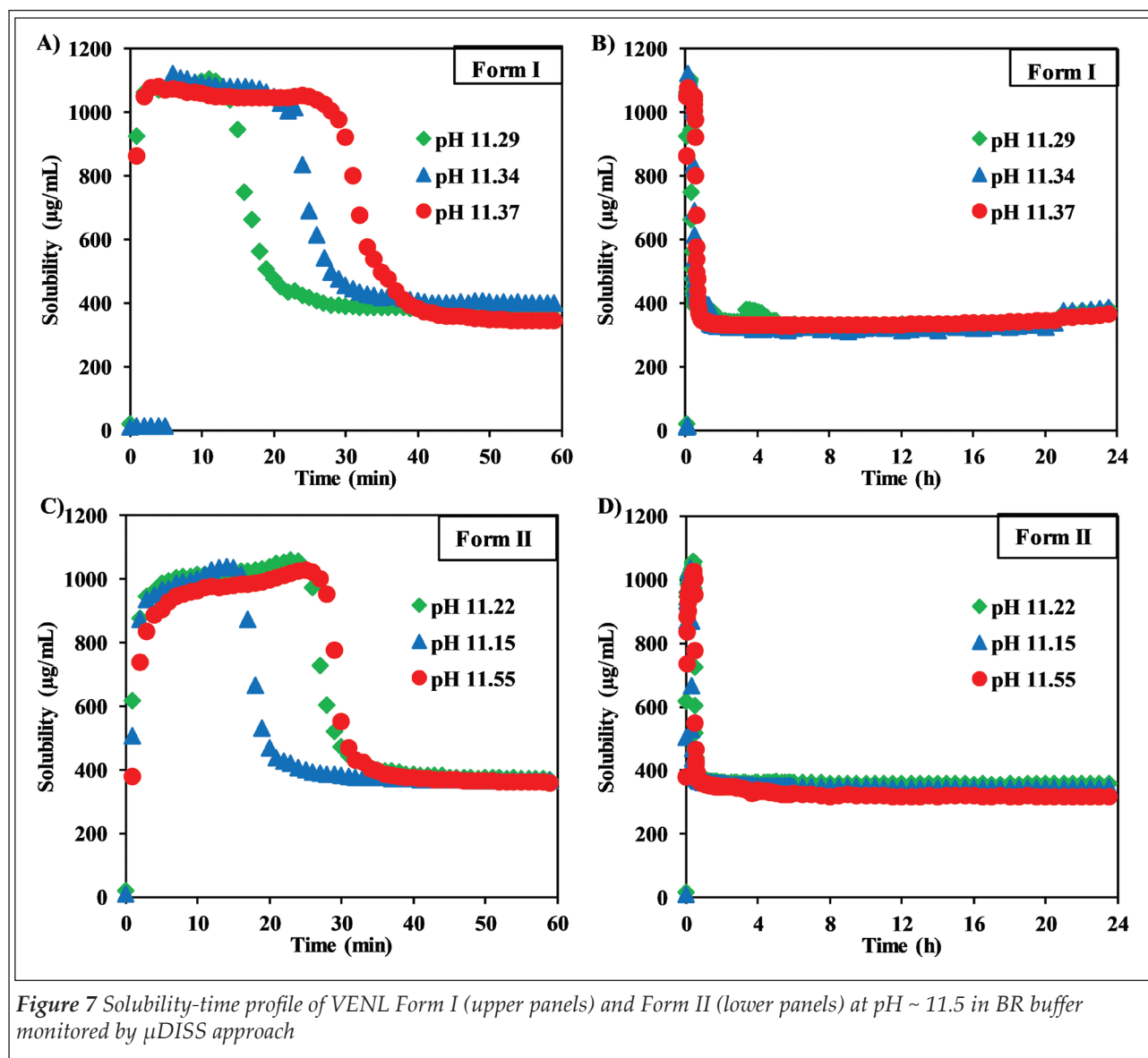


Table II Equilibrium solubility of venlafaxine hydrochloride polymorphs measured by SSF method using different concentration detection

Sample VENL	pH	$S_{pH} \pm SD$ (mg/mL)	
		UV-JASCO	UV- μ DISS
Form I	7.50	59.35 \pm 8.23	56.98 \pm 6.89
	7.70	32.08 \pm 2.60	31.34 \pm 2.40
	7.83	23.34 \pm 0.45	22.44 \pm 0.31
	9.30	0.948 \pm 0.031	1.121 \pm 0.038
	9.50	0.796 \pm 0.094	0.764 \pm 0.080
Form II	7.54	54.32 \pm 4.43	48.51 \pm 6.32
	7.60	45.67 \pm 2.34	45.54 \pm 1.25
	7.67	38.35 \pm 2.99	36.49 \pm 0.45
	7.78	26.79 \pm 0.27	26.96 \pm 0.40
	9.45	0.831 \pm 0.027	0.853 \pm 0.030

Concentration is expressed as free base equivalents.

varies in 0.2-15%, average is about 5%. It can be observed that in 7/10 cases μ DISS measures the lower value.

3.4. Real time monitoring of dissolution by μ DISS ProfilerTM

The real time dissolution profile was monitored by μ DISS ProfilerTM. It was performed in BR buffer pH ~11-11.5 (exact pH was measured at the end of the experiments) in 0-24 h period, where in the first 6 h stirrer was turned on. The UV spectra were registered according to a protocol (see in section 2.3.) in three parallel vessels and the concentration was calculated using calibration. Figure 7 shows that dissolution process of VENL can be followed well and the equilibrium solubility can

also be measured by this approach. In case of Form I (Figure 7 upper panels) the supersaturation is very similar in the three parallel experiments and solute reaches 3.5 times concentration relative to the equilibrium solubility. Precipitation starts at different time in the parallels (12-25-30 min, respectively) and the samples are reaching the equilibrium in rather short time (20-40 min). The concentration is not changing further when at 6 h stirrer is turned off up to 20 h. A small (~13%) increase in concentration can be observed in 20-24 h period for one of the channels. It may be due to the adsorption of solid crystals to the UV probe without stirring of the suspension. The dissolution-time profile of Form II as starting material (Figure 7 lower panels) is very similar. It reaches a bit slower the same supersaturation as Form I but the precipitation and time needed to reach the equilibrium is the same. We can conclude from this profiling that VENL polymorphs are converting to the common free base form immediately after dissolution. Intrinsic solubility measured by μ DISS Profiler™ at 24 h is 0.290 ± 0.004 mg/mL ($\log S_0$ [M]: -2.98) (n=4), it perfectly agrees with S_0 obtained by SSF method.

4. Conclusions

In this study we have demonstrated the importance of solid phase analysis in the solubility investigation of drug polymorphs giving reliable information about which form is present in solubility equilibrium. We presented an example for the possible behavior of pharmaceutical compounds.

Venlafaxine hydrochloride Form I and Form II transformed to a common product in aqueous buffer solution in pH 7.5-12 region and thus the measured solubility values at a given pH were identical. Solid isolated from pH 10-12 was identified as the free base, while between pH 7-8 the isolated common product was identified as VENL hydrochloride monohydrate based on XRPD results. Since VENL dissolves freely in acidic medium not the solubility but the better manufacturability can explain why the less stable Form II is preferably used in tablets.

We have also presented the good agreement of equilibrium solubility measured by SSF coupled with *off-line* analytics and *in situ* fiber optic concentration monitoring. μ DISS Profiler™ has the advantage to provide much more information about dissolution process, with this approach the dissolution kinetic, the supersaturation and the

time needed to reach the equilibrium can be easily monitored. It is simpler and occasionally can be faster than traditional methods however there are limits of its application. Neither too high nor too low solubility can be measured with this instrument, the optimal solubility window is about 1-500 μ g/mL. Since the new APIs in drug research are mainly poorly soluble compounds, for their solubility study μ DISS Profiler seems to be advantageous.

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