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Towards the Determination of the Solubilities of the Two Enantiotropically Related Crystallographic Forms of Etiracetam in Methanol

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Supporting Information

ABSTRACT: This contribution focuses on the presentation and the comparison of five experimental methods for the determination of the solubilities of the two enantiotropically related crystallographic forms of etiracetam (racemic intermediate to the synthesis of leviteracetam, Keppra, UCB Pharma) in methanol. The five experimental methods can be divided into two sets of methods, depending on whether the method is isothermal or whether the temperature varies during the experiment. The results show that the most accurate and reliable determination of the solubility curves of the two crystallographic forms is obtained when combining the different methods. Using a single method limits the amount of information gained, does not allow for a verification of the obtained data, and can even limit the range for which solubility data can be experimentally determined. The results allow distinguishing the solubilities of the stable and the metastable forms and hence confirming the enantiotropic relationship of the two crystallographic forms. The transition temperature is determined to be equal to 30 °C, which confirms previous findings.

1. INTRODUCTION

Crystallization by cooling followed by the separation of the crystals from the resulting suspension is the most frequent method used to achieve the required purity of active pharmaceutical ingredients (API).[1,2] However, this unit operation is still poorly understood, leading to a lack of strategies for its design, optimization and control. Moreover, depending on the pharmaceutical compound and the crystallization process applied, different crystallographic forms may appear in the suspension.[3] Some of these forms may be undesirable from a pharmaceutical point of view. New crystallization methodologies and online control techniques are therefore developed to ensure that the solid form of interest is isolated.[4,5] The determination of the physicochemical characteristics of all the crystallographic forms possibly present in suspension is thus crucial for the crystallization process control and its optimization. One such property is the solubility in a given solvent, which forms the basis for the development of a robust crystallization process.

The solubility of the compound A in the solvent B at the temperature T is defined as the concentration of the dissolved compound A in the liquid phase, composed of the solvent B in which the compound A is dissolved, when this liquid phase is in equilibrium with macroscopic crystals of the compound A, at the temperature T. Hereafter, the compound A dissolved is called the solute, the crystals of A are called the solid phase and the liquid mixture of A and B is called the solution. In this paper, the solubility is expressed in grams of solute per gram of solution: g/g_{sol}. The solubility is also called the saturation concentration. The solubility can generally be determined by the measurement of the maximum mass of macroscopic crystals that can be dissolved in a given mass of solvent at the temperature T.[2] Over recent years, several experimental methods for the determination of the solubility have been described in the literature.[6−9] The choice of one method over another depends on many factors: the solute−solvent system studied, the available technical and analytical devices, the operator's expertise, the available time, or the accuracy and precision required.[10]

When a pure compound can crystallize in several crystallographic forms, only one of these is stable at a given temperature unless the solid forms are enantiotropically related, in which case a transition temperature, a temperature at which both forms are stable, exists. Thermodynamically, only the solubility of the stable crystallographic form, hereafter referred to as thermodynamic solubility, can be defined and determined. However, the solubility of metastable crystallographic forms, hereafter referred to as metastable solubility, can also be experimentally determined if the experiment duration is long enough for a metastable equilibrium state to be established between the solid and the liquid phases and short enough to avoid a polymorphic transition from the metastable form towards the more stable one.[7,11] Both the time needed for equilibrium to be reached and the time required for the polymorphic transition to occur are not only temperature dependent but also dependent on external

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parameters such as the stirring rate, the reactor design, the stirring time, etc. For enantiotropic systems, determining both the thermodynamic and the metastable solubilities can therefore be quite challenging, especially around the transition temperature where the solubility of both forms is comparable.

This study focuses on a reference compound, etiracetam (Figure 1), encountered as a racemic intermediate in the synthesis of leviteracetam, the active ingredient of Keppra, an antiepileptic drug commercialized by UCB Pharma. The etiracetam compound can crystallize in two distinct crystallographic forms, form I (Figure 2a) and form II (Figure 2b), which are enantiotropically related. As shown in previous contributions, analytical techniques such as differential scanning calorimetry (DSC),[12] Raman spectroscopy (Figure 3a),[13] X-ray powder diffraction (XRPD) (Figure 3b), or granulometry[14] can be used to distinguish between the two crystallographic forms. Moreover, form I has been shown to be the most stable form below 30.5 °C, whereas form II is the most stable one above 30.5 °C.[13] Earlier results have shown the characteristic polymorphic transition time required for the metastable form II to transform into the stable form I at temperatures below 30.5 °C to be approximately 1 h. However, above 30.5 °C, the characteristic polymorphic transition time for the metastable form I to transform into the stable form II is less than 5 min. In this paper, the solubility of the two enantiotropically related crystallographic forms of the etiracetam compound in methanol is determined experimentally for temperatures between −10 and 60 °C using five different methods. The goal of this paper is to show how an accurate phase diagram can be obtained, even for an enantiotropic system, by combining several experimental methods, hereby circumventing the limitations of each single method.[10]

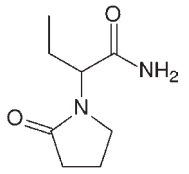

Figure 1. Molecular structure of the etiracetam compound.

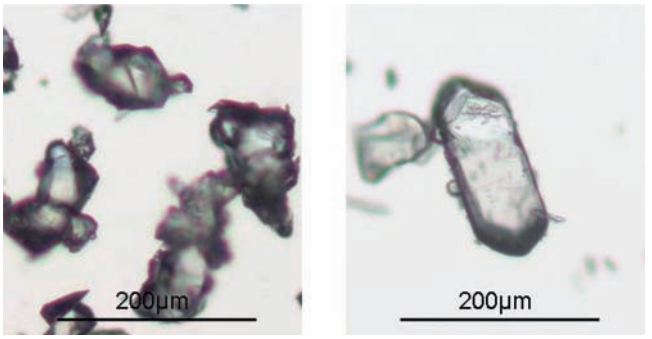

Figure 2. Optical microscopy images of (a) form I crystals and (b) form II crystals.

2. MATERIALS AND METHODS

Five different experimental methods were used throughout this work, which could be divided into two distinct groups, depending on whether the solubility was determined at a fixed temperature (isothermal) or by variation of the temperature (dynamic).

2.1. Isothermal Methods. The isothermal methods are based on the following principle. A suspension (solution + suspended crystals) of macroscopic crystals is brought to a given temperature T and ***kept at this temperature***. The determination of the solubility at this temperature T consists in determining the concentration of the solute in the solution when the equilibrium is reached between the solid and the liquid phases.[2] This saturation concentration is the *equilibrium solubility* at the temperature T. Three isothermal methods were investigated in this work for the determination of the saturation concentration (Figure 4). Gravimetry was the first of the isothermal methods used. The second method consisted in determining the refractive index of the solution in equilibrium with crystals. The third isothermal method was based on the measurement of the infrared spectrum of the saturated solution, using an online attenuated total reflectance-Fourier transformed infrared (ATR-FTIR) probe. For the latter two methods, the measured values

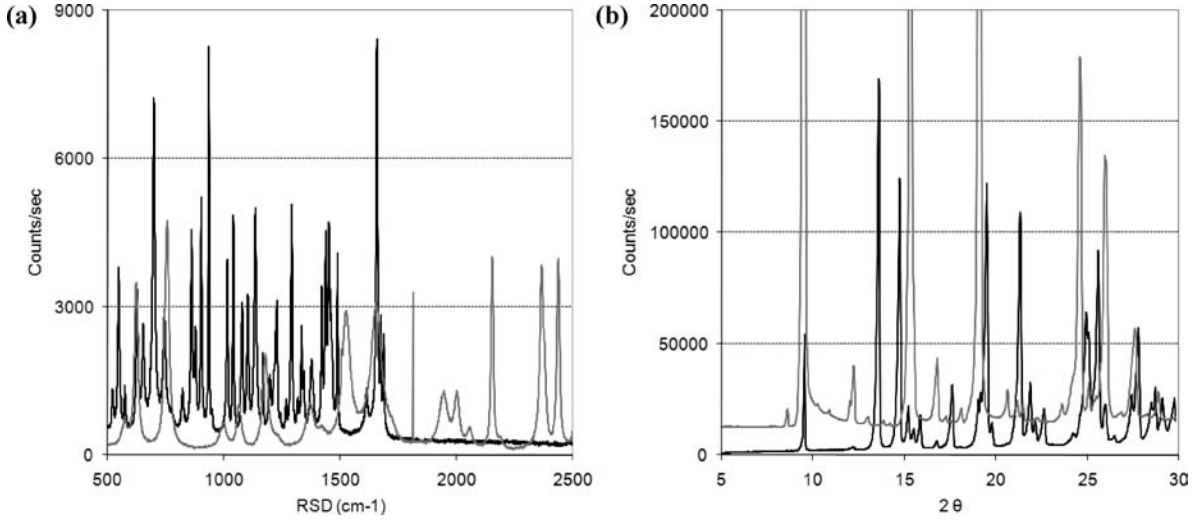

Figure 3. (a) Raman spectra (Horiba Jobin Yvon, LabRam Aramis, 120 s exposure time at 785 nm[13]) and (b) XRPD patterns (Bruker B8 Advance diffractometer, monochromatic Cu radiation[14]) of the form I crystals (black line) and the form II crystals (grey line) (RSD: Raman shift displacement).

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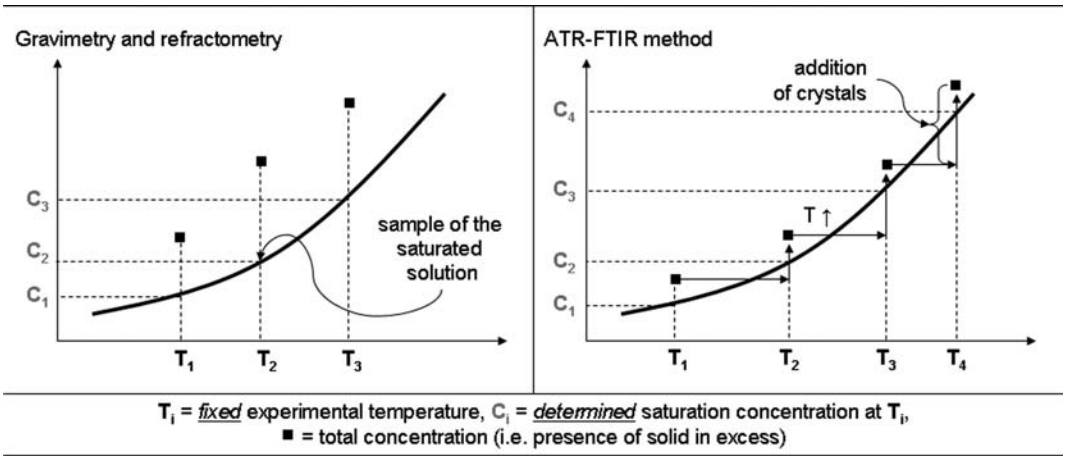

Figure 4. Principle of the three isothermal methods.

were related to the saturation concentration using an appropriate model obtained through calibration prior to the experimental measurements.

2.1.1. Gravimetry. The gravimetric method is based on weighing the mass of a saturated solution and the mass of crystals obtained after evaporation of the total amount of solvent.[11] Suspensions of form I or form II crystals in methanol were prepared in thermostatted and mechanically stirred (Mixel TT, 1000 rpm) 50-mL glass reactors of an automated Mettler-Toledo Multimax reactor system. For form I, the temperatures 0, 10, 20, and 30 °C were investigated, whereas for form II, 30, 40, and 50 °C were considered. The amount of solid added to the methanol was such that total dissolution of the crystals did not occur at the given temperature. Both the jacket and the suspension temperatures were continuously monitored. An approximately 4 h isothermal hold was introduced prior to sampling to ensure that the polymorphic transition from the metastable form towards the stable form had occurred (~1 h for II → I at T < 30.5 °C and ~5 min for I → II at T > 30.5 °C) and the equilibrium between the solid and the liquid phases was reached. After stopping the mechanical agitation, some crystals were sampled for further verification of their crystallographic form by DSC analysis. Moreover, about 20 mL of the solution was sampled and poured into an Erlenmeyer flask. No filtering was needed as sedimentation occurred rapidly. The saturation concentration of the solution at the fixed temperature was determined by measuring the initial mass of the sampled saturated solution and the final mass of the crystallized solid after total evaporation of the solvent. Complete solvent evaporation was assumed after one week as the sample weight no longer varied. Moreover, no solvate form of the etiracetam compound in methanol exists.

2.1.2. Refractometry. The basic idea behind this method is to relate the concentration of a solute in solution to a physical property of the solution. The physical property measured here is the refractive index of the solution.[6] Suspensions of form I or form II crystals in methanol were prepared in thermostatted and magnetically stirred (300 rpm) 50-mL glass flasks. The lowest temperatures investigated were −4 and −3 °C for the form I and the form II, respectively. The six highest temperatures (−2.5, 0.5, 4, 10.5, 14, and 20 °C) were the same for both crystallographic forms. An excess amount of solid was added to avoid total dissolution. The temperature within the suspension was continuously controlled. A maximum 1 h isothermal hold was introduced prior to sampling to avoid the polymorphic transition from the metastable form II towards the stable form I (~1 h for II → I at T < 30.5 °C). After stopping the magnetic agitation, some crystals were sampled for further confirmation of their crystallographic form by XRPD analysis. Moreover, solution was sampled for the determination of its refractive index (DR-A1, Atago refractometer (η^{Na}, between 1.3 and 1.7 nD)). No filtering was needed as sedimentation occurred rapidly. Regular sampling (30 min, 45 min, and 1 h) showed that no variation in refractive index occurred after 30 min. The thermodynamic equilibrium between the stable form I crystals and the solution as well as the metastable equilibrium between the metastable form II crystals and the solution were therefore assumed at this stage. On the basis of the calibration curve (Supporting Information, Figure SI.1), the saturation concentration of the solution was then determined.

2.1.3. ATR-FTIR Method. The basic idea behind this method is to relate the concentration of a solute in solution to a given physical property of the solution. In this case, one is relating the concentration of a solute to the absorbance observed in the IR region of the electromagnetic spectrum.[6,8,11] An online in situ ATR-FTIR probe (Mettler-Toledo, ReactIR4000, diamond dicomp 16-mm tip, K6 conduct) was used, thereby avoiding issues that might occur during sampling or offline analysis (Supporting Information, Figure SI.2).[15−19] The solubility curves were constructed using a continuous one-pot methodology. For this, initial suspensions of form I or form II crystals in methanol were prepared at an initial temperature of −10 °C in a magnetically stirred (45° pitched-blade-turbine, 400 rpm) 1-L Mettler-Toledo automated Labmax reactor. An excess amount of crystalline material was added to avoid total dissolution. The temperature and the concentration of the solute in solution were continuously monitored. Approximately 30 min were required for the IR signal to stabilize, a time-period similar to the one observed for the refractometric method. At this moment, several infrared spectra of the saturated solution were recorded with 5-min time intervals. A maximum of five spectra were taken for the saturated solutions of metastable form II to avoid the polymorphic transition towards the stable form I (~1 h for II → I at T < 30.5 °C). An in situ verification of the crystallographic form of the suspended crystals was performed by online Raman analysis. The saturation concentration of the solution was determined using the calibration model (Supporting Information, Figure SI.3).[20,21] The

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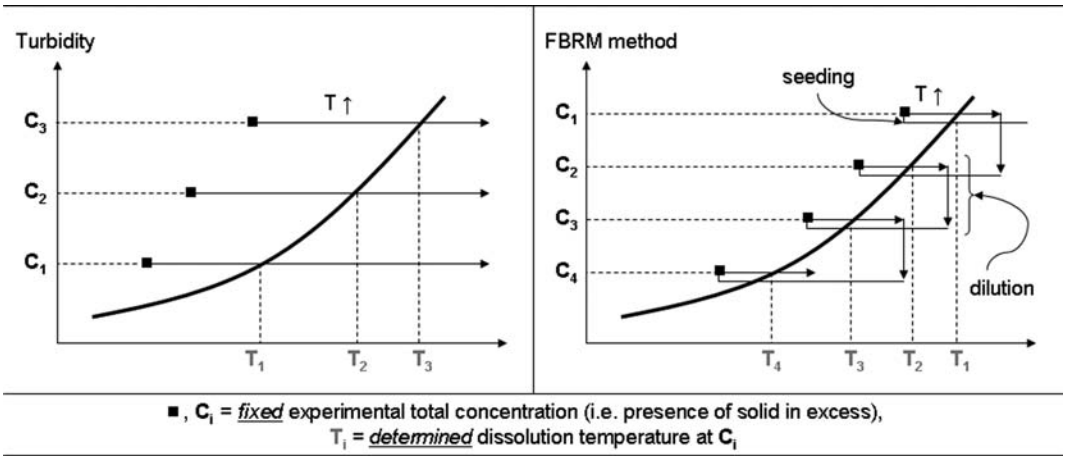

Figure 5. Principle of the two dynamic methods.

temperature was then increased by 10 °C increments up to 60 °C, adding solid compound at each stage of the experiment. For temperatures beyond 30.5 °C, the solubility of form I could not be determined using this method due to the small characteristic time for the polymorphic transition from the metastable form I towards the stable form II (∼5 min for I → II at T > 30.5 °C).

2.2. Dynamic Methods. The dynamic methods are based on the following principle. A suspension of macroscopic crystals at a known total concentration is prepared. This suspension ***is then heated*** and the temperature at which all the crystals of the sample are dissolved is determined.[2] This temperature is called the dissolution temperature. The known total concentration is the solubility at the determined dissolution temperature. Due to the heating rates applied and the non instantaneous dissolution of the crystals, the solubility determined by dynamic methods is generally referred as *kinetic solubility*, which may differ from the *equilibrium solubility* determined by isothermal methods. Two experimental methods were investigated in this work for the determination of the dissolution temperature (Figure 5). They were both based on the detection of the presence or the absence of crystals in suspension over time, while applying a heating rate. The first method was based on the analysis of the time evolution of the transmittance of a light signal through the sample, and the second method consisted in analysing the time evolution of the laser reflectance signal provided by a focused beam reflectance measure (FBRM) probe.

2.2.1. Turbidity. The basic idea behind the turbidity method is to send a monochromatic beam through the suspension and analyze the transmitted signal. Suspensions of form I or form II crystals in methanol were prepared in magnetically stirred 12 mL glass tubes of an automated React-Array RS-10 reactor box. Two different stirring rates, 350 and 1000 rpm, were used in order to estimate their influence on the dissolution rate of the crystals. The total concentrations under study extended from 0.1 g/g_{sol} to 0.3 g/g_{sol} with 0.05 g/g_{sol} intervals. The initial temperatures were set approximately 10 °C below the respective estimated dissolution temperatures to ensure the presence of crystals in suspension. The temperature and the turbidity (calibrated Thermo Fisher Scientific probe) were simultaneously monitored online over time. At the initial temperature, crystals present in suspension diffracted the beam of light. At this stage, the transmittance signal value was calibrated at 0%. The temperature was then slowly increased (0.15 °C/min) and the transmittance signal

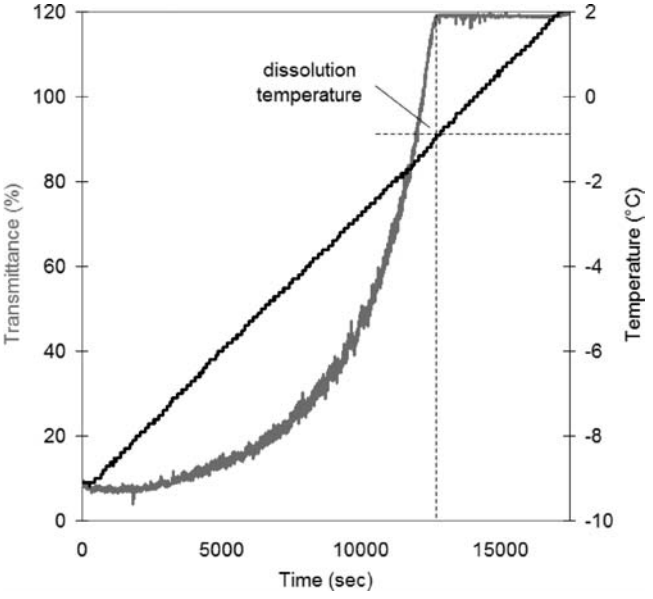

Figure 6. Transmittance and temperature signals recorded over time in a solution of etiracetam in methanol (total concentration: 0.15 g/g_{sol}).

recorded over time (Figure 6). When all crystals had dissolved, diffraction no longer occurred, and the signal no longer increased. The dissolution temperature of the sample was determined when the transmittance reached this stable value, theoretically set at 100%. Values could, however, deviate from 100% if prior calibration was not performed under the same conditions as those for the experimental ones. By taking the temperature at which the signal leveled off as dissolution temperature, the calibration became obsolete. Due to the small heating rate used, average experimental times of 10 h were observed, thus always leading to polymorphic transitions from the metastable form II towards the stable form I (∼1 h for II → I at T < 30.5 °C), as confirmed by Raman analysis.

2.2.2. FBRM Method. The basic idea behind the FBRM method is to send a laser radiation through the suspension and analyze the backscattered signal.[22] An online in situ FBRM probe (Mettler-Toledo, Lasentec, D600L) was used.[23−25] To determine the solubility curves, two one-pot continuous experiments

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were realized: one in a high concentration range (approximately from 0.55 g/g_{sol} to 0.31 g/g_{sol} with 0.03 g/g_{sol} intervals) and the other in a low concentration range (from approximately 0.44 g/g_{sol} to 0.21 g/g_{sol} with 0.05 g/g_{sol} intervals). For this, initial suspensions of form I or form II crystals in methanol were prepared at an initial total concentration in a mechanically stirred (45° pitched blade turbine, 400 rpm) 600-mL Mettler-Toledo automated Labmax reactor. The initial temperature was set approximately 10 °C above the estimated dissolution temperature, so that no crystals remained in solution. The temperature and the presence of suspended crystals were continuously monitored online over time. The temperature of the solution was then slowly decreased until the hypothetical solubility curve (obtained by previous methods) was crossed. Before spontaneous primary nucleation occurred, cooling was stopped: the solution was then seeded with 10% of form I or form II crystals with respect to the initial mass of crystals added and was reheated at a rate of 1 °C/min (Figure 7). Although the variations in chord length distribution (Supporting Information, Figure SI.4) over time, as given by the FBRM probe, contain information on phenomena such as crystal breaking, nucleation, and crystal growth,[26,27] for the purpose of this work only the presence or absence of crystals was of importance.[22] It was therefore sufficient to simply follow the time evolution of the total number of chord lengths recorded. This signal was converted into its derivative. As shown in Figure 7, the dissolution temperature was determined as the temperature at which the time derivative of this reflectance signal stabilized at 0. The average time between the incorporation of seeds in the solution and their disappearance was ~10 min. While this short time period allowed avoiding the polymorphic transition from the metastable form II towards the stable form I below 30.5 °C (~1 h for II → I at T < 30.5 °C), the transformation of the metastable form I to the stable form II above 30.5 °C (~5 min for I → II at T > 30.5 °C) could not be avoided. The polymorphic transition could be detected visually and was confirmed by offline DSC analysis. After the first dissolution temperature was determined, the solution was once more cooled and methanol was added to obtain an under-saturated solution at the next desired experimental concentration. By successive cooling - diluting and reheating - seeding cycles, the dissolution temperature at different concentrations could be determined (Figure 7), and a solubility curve constructed.

3. RESULTS

Solubilities of the two enantiotropically related crystallographic form of etiracetam are given in Table 1 and Table 2 for the isothermal and the dynamic methods, respectively. Moreover, the results are graphically presented in Figure 8a and 8b for the form I and the form II, respectively. As shown by these data, none of the five methods allowed determining the solubility of the metastable form I above 30.5 °C while most of the methods allowed determining the solubility of the metastable form II below 30.5 °C.

3.1. Isothermal Methods. The isothermal methods usually comprise a waiting period, introduced to ensure that the equilibrium is reached and that the *equilibrium solubility* is determined. At this stage, when studying a metastable form, a polymorphic transition from the metastable form towards the most stable form can occur. The major set-back of most of the isothermal methods is therefore that they are usually limited to finding the thermodynamic solubility of the most stable form at a given temperature. The solubility of a metastable form cannot always be obtained using these methods unless the experiment duration is long enough for a metastable equilibrium state to be established between the solid and the liquid phases and short enough to avoid a polymorphic transition from the metastable form towards the more stable one. In this work, for temperatures below 30.5 °C, the solubility of the metastable form II could be determined using the refractometric and ATR-FTIR methods as the time required to ensure a metastable equilibrium (30 min) was shorter than the experimental duration (between 30 min and 1 h) and the time required for the polymorphic transition to occur (~1 h for II → I at T < 30.5 °C). Due to the 4 h isothermal hold used for the gravimetric method before sampling, the metastable form II transformed into the stable form I as indicated by the symbol PT (polymorphic transition) in Table 1. None of the three isothermal methods allowed determining the solubility of the metastable form I beyond 30.5 °C, as the characteristic time for the polymorphic transition from the metastable form I towards the stable form II was estimated a mere 5 min, which was shorter than all of the waiting periods considered (from 30 min to 4 h).

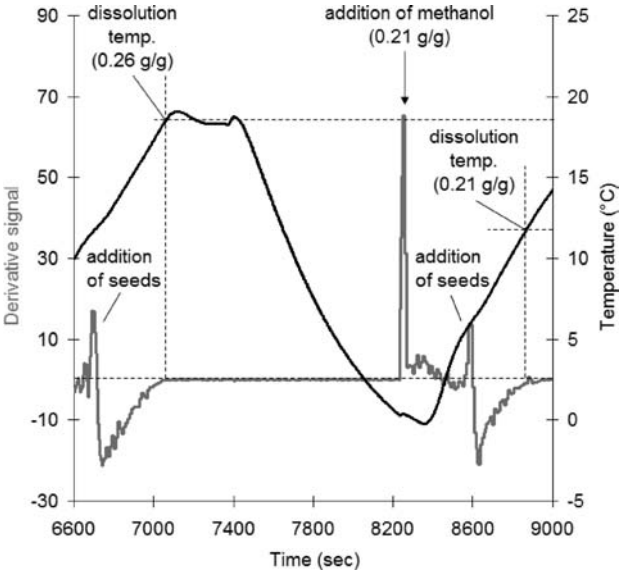

Figure 7. Derivative signal of the total number of counts/s and temperature signal recorded over time in a solution of etiracetam in methanol (Seeds = form I crystals).

3.2. Dynamic Methods. The dynamic methods involve a continuous increase of the temperature. The solution is therefore always slightly under-saturated with respect to the form under study. When studying a metastable form, the under-saturated solution may still be super-saturated with respect to the stable form, but even so, this supersaturation will be less so compared to that in the isothermal methods. Theoretically, a decreased supersaturation level induces a decrease of the risk of nucleation of the more stable form, thus implying an increase of the characteristic transition time from the metastable form towards the stable form. Nevertheless, depending on operating conditions (when the time required for the polymorphic transition to occur is shorter than the time needed for the experimental measure to be taken), a polymorphic transition from the metastable form towards the most stable form can occur. In this work, such polymorphic transitions were observed and indicated by the symbol PT (polymorphic transition) in Table 2. For temperatures below 30.5 °C, due to the small heating rate used

Table 1. Solubilities of form I and form II for different temperatures obtained using the three isothermal methods[a]

	form I			form II		
temp.* °C	gravimetry g/g_{sol}	refractometry g/g_{sol}	ATR-FTIR g/g_{sol}	gravimetry g/g_{sol}	refractometry g/g_{sol}	ATR-FTIR g/g_{sol}
−10	–	–	0.11 ± 0.04 (8)	–	–	0.15 ± 0.039 (4)
−4	–	0.15 ± 0.03	–	–	–	
−3	–	–	–	–	0.17 ± 0.03	–
−2.5	–	0.16 ± 0.03	–	–	0.18 ± 0.03	–
0	0.1679 ± 0.0001	–	0.16 ± 0.02 (13)	–	–	0.18 ± 0.03 (4)
0.5	–	0.17 ± 0.03	–	–	0.19 ± 0.03	–
4	–	0.19 ± 0.03	–	–	0.21 ± 0.03	–
10	0.2375 ± 0.0001	–	0.22 ± 0.02 (9)	–	–	0.24 ± 0.03 (5)
10.5	–	0.23 ± 0.04	–	–	0.24 ± 0.04	–
14	–	0.25 ± 0.03	–	–	0.27 ± 0.03	–
20	0.3073 ± 0.0001	0.30 ± 0.05	0.29 ± 0.02 (13)	–	0.32 ± 0.05	0.30 ± 0.02 (5)
30	0.3947 ± 0.0001	–	0.36 ± 0.01 (30)	PT	–	0.37 ± 0.02 (3)
30.5			transition temperature			
40	–	–	PT	0.5063 ± 0.0002	–	R
50	–	–	PT	0.6012 ± 0.0001	–	R
60	–	–	PT	–	–	R

[a] Symbol "–": the operational condition was not investigated; symbol "PT" (polymorphic transition): the metastable form transformed into the stable one; symbol "R" (rejected): the results were rejected due to model inadequacy in this temperature range; symbol "*": the error on the temperature depends on the precision of the thermometer used for the experiment (0.1°C for the gravimetry, 0.5°C for the refractometry, and 0.1°C for the ATR-FTIR method). The numbers given in parentheses for the ATR-FTIR method are the numbers of spectra considered. Error estimation on the concentration is clarified in the Supporting Information.

Table 2. Dissolution temperatures of form I and form II for different total concentrations using the two dynamics methods[a]

	form I		form II	
concentration g/g_{sol}	turbidity °C	FBRM* °C	turbidity °C	FBRM* °C
0.0999 ± 0.0003	−11.4 ± 0.8	–	PT	–
0.1498 ± 0.0007	−0.1 ± 0.3	–	PT	–
0.2005 ± 0.0063	8.2 ± 3.2	–	PT	–
0.214 ± 0.0023	–	11.0−12.0	–	6.7−9.4
0.2500 ± 0.0013	14.7 ± 0.6	–	PT	–
0.260 ± 0.0017	–	17.3−18.9	–	12.2−13.9
0.2991 ± 0.0003	20.2 ± 1.9	–	PT	–
0.306 ± 0.0017	–	22.5−24.0	–	19.5−22.4
0.312 ± 0.0013	–	–	–	21.1−24.1
0.352 ± 0.0013	–	27.5−28.9	–	–
0.359 ± 0.0011	–	–	–	26.5−29.0
		transition temperature		
0.395 ± 0.0011	–	PT	–	31.7−33.1[$]
0.402 ± 0.0008	–	–	–	31.1−33.6
0.435 ± 0.0008	–	PT	–	36.5−38.1[$]
0.443 ± 0.0010	–	–	–	36.9−37.9
0.470 ± 0.0010	–	PT	–	39.7−41.0[$]
0.475 ± 0.0008	–	–	–	40.8−42.7
0.498 ± 0.0008	–	PT	–	43.1−44.6[$]
0.506 ± 0.0006	–	–	–	43.5−45.1
0.531 ± 0.0006	–	PT	–	46.2−47.0[$]

[a] Symbol "–": the operational condition was not investigated; symbol "PT" (polymorphic transition): the metastable form transformed into the stable one; symbol "$" : results were obtained for the form II due to the PT of the metastable form I; symbol "*": the dissolution temperature is given both by the temperature determined using the derivate of the reflectance signal and the temperature at which the disappearance of the crystals was visually observed. Error estimations on the concentration and the temperature are clarified in the Supporting Information.

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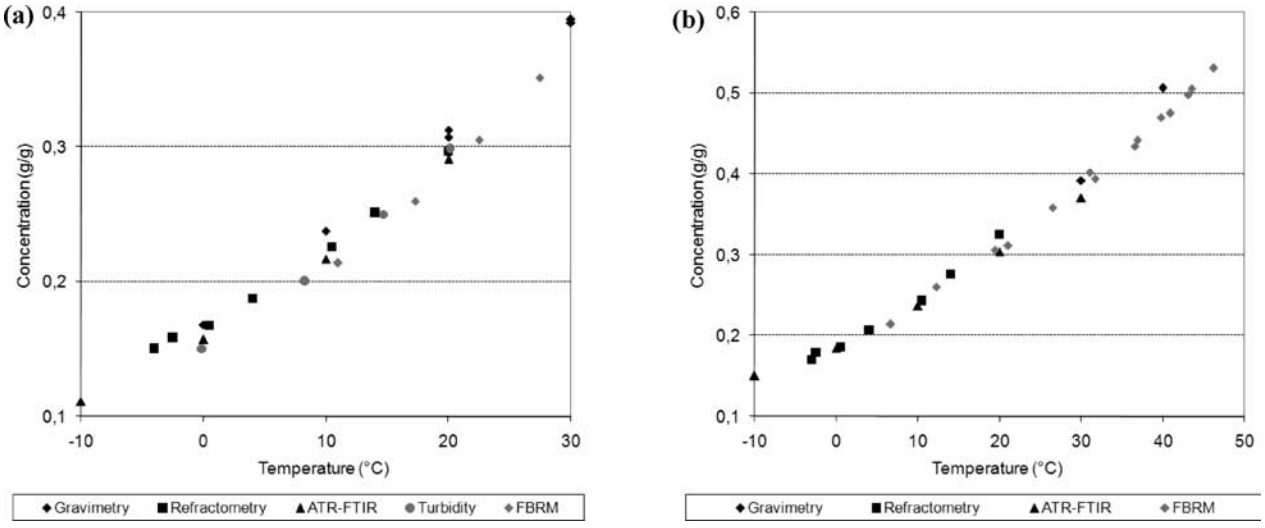

Figure 8. Solubilities of (a) the form I and (b) the form II obtained with the five different experimental methods (the results obtained through isothermal and dynamic methods are presented in black and grey, respectively).

for the turbidity method, average experimental times of 10 h were observed, thus always leading to the polymorphic transition from the metastable form II towards the stable form I (~1 h for II → I at T < 30.5 °C). For temperatures beyond 30.5 °C, even for the short time period spent between the incorporation of seeds in the solution and their disappearance, polymorphic transitions from the metastable form I towards the stable form II were observed (~5 min for I → II at T > 30.5 °C).

4. DISCUSSION

4.1. Equilibrium Solubility vs Kinetic Solubility. Solubilities of the form I and the form II determined using the five different methods are presented in Figure 8a and 8b, respectively. The results presented in black are related to the isothermal methods (Table 1), while those presented in grey are related to the dynamic methods (Table 2).

As shown in Figure 8, the results of the five methods are in good agreement with one another. For the isothermal methods, it has been shown that a 30-min hold before measurement was sufficient for equilibrium to be reached, either thermodynamic or metastable. The solubilities determined by these methods are therefore considered as *equilibrium solubilities*. Although a relative precise model could be created, one should not forget that the PLS model of the ATR-FTIR probe was mainly constructed in the under-saturated domain. Moreover, if very fine particles would be present in suspension, a possible diffraction of the evanescent wave could further influence the exactness of the obtained results. As the results obtained by the ATR-FTIR method are similar to those obtained using the two other isothermal methods, this effect is not expected to play a major role for the compound studied. For the dynamic methods, dissolution temperatures (i.e. temperatures at which all of the crystals were dissolved when increasing the suspension temperature) were determined. Rigorously, these temperatures are different from the thermodynamic solubility temperatures, as the crystals do not dissolve instantaneously in suspension. These methods can therefore not be applied to compounds that are characterized by a very slow dissolution rate, unless very low heating rates are applied. As the dissolution rate of etiracetam in methanol is very fast, the dissolution temperatures determined by the two dynamic methods compare well with the thermodynamic solubility temperatures. In other words, the *kinetic solubilities* determined by the two dynamic methods can be considered as *equilibrium solubilities*.

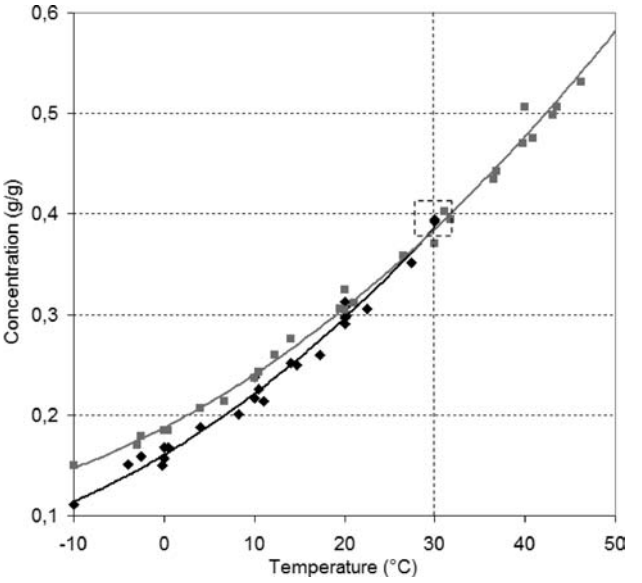

Figure 9. Experimental solubilities and polynomial regressions related to form I and form II in black and grey, respectively. The transition temperature is estimated at 30 °C.

4.2. Solubility Phase Diagram. Combining the data obtained from the five methods, second order polynomial regression curves are determined using the ensemble of points for respectively the form I (black points) and the form II (grey points). Figure 9 clearly shows the difference in solubility between the two crystallographic forms below 30.5 °C, with the difference in solubility increasing with a decrease in temperature. Moreover, the enantiotropic relationship of the two crystallographic forms of etiracetam is confirmed in Figure 9. The temperature at which the solubilities of the two forms are equal is found to be 30 °C, and comparable to the transition temperature of 30.5 °C determined previously.[13]

Table 3. Synthesis of the average experimental errors on the temperature and the concentration for the five experimental methods investigated

experimental method	ΔT °C	Δc g/g_{sol}
Isothermal Methods		
gravimetry	0.1	10^{-4}
refractometry	0.5	10^{-2}
ATR-FTIR Method	0.1	10^{-2}
Dynamic Methods		
turbidity	1.5	10^{-3}
FBRM Method	1	10^{-3}

4.3. Precision, Advantages, and Limitations. Table 3 summarizes the precision of the temperature and the concentration obtained using the five methods. The error noted for the temperature mostly depends on the thermometer used and hence is equipment dependent explaining the difference between the methods. Table 3 clearly shows that the dynamic methods are less precise than the isothermal methods in terms of temperature. In terms of concentration, Table 3 shows that the less precise methods are the isothermal methods that required calibration (refractometry and ATR-FTIR method). The errors on the concentration ($\Delta c \approx 10^{-2}$ g/g_{sol}) for the refractometry and the ATR-FTIR methods are comparable to the difference between the solubilities of both forms at a given temperature. Using only one of these two methods to determine the solubility curves of both forms is therefore not recommended. However, although the calibration of the ATR-FTIR probe is quite time consuming, the experimental solubility measurements by the ATR-FTIR method occurrs online, therefore, ultimately being less time consuming compared to refractometry. Furthermore, online in situ analysis allows avoiding errors due to sample contamination or offline analysis. Methods that do not require any calibration (gravimetry, turbidity, and FBRM method) allow determining the solubility with a 10-fold or more increased precision ($\Delta c \leq 10^{-2}$ g/g_{sol}), as the only errors induced are those due to weighing. Nevertheless, for the concentration of approximately 0.4 g/g_{sol}, dynamic methods show a difference between the dissolution temperatures of the two forms of the same order of magnitude as the experimental error ($\Delta T \approx 1$ °C). The major advantage of the dynamic methods therefore mostly lies with the in situ analysis, hereby avoiding offline errors. Due to the experimental set up used in this work, the turbidity method is found to be quite time consuming, while the FBRM method is very fast and precise, requiring a reduced effort. Ultimately, gravimetry is found to be the most precise method (Table 3), although limited to a restricted temperature range for each form, and rather time consuming, with samples left to dry for almost one week. From this discussion it is clear that considering the data of only a single specific method is insufficient to determine the solubility curves of two enantiotropically related forms. Moreover, if only one method is used, the uncertainty on the determination of the transition temperature substantially increases. For an accurate determination of the complete solubility phase diagram of an enantiotropic system, it is therefore recommended to use a combination of different experimental methods. Furthermore, combining the data from the different methods consolidates the reliability of the results, whereas these may be questioned when using only a single method.

5. CONCLUSIONS

Determination of the thermodynamic and the metastable solubilities of two enantiotropically related forms can be quite challenging, especially around the transition temperature, where the difference in solubility of both forms becomes fairly small. In this paper, five different methods are used to determine the solubility of the two forms of the etiracetam compound in methanol, showing a transition temperature of about 30 °C. The results of the five methods are in good agreement with one another, confirming the exactness of the obtained results. While most of the methods allow determining the solubility of the metastable form II below 30.5 °C, no information is obtained about the solubility of the metastable form I beyond 30.5 °C, as the polymorphic transition towards the stable form II occurs before experimental measurements can be taken. While the use of one specific method can introduce a relatively large uncertainty on the transition temperature, the findings presented in this paper show that a combination of the results obtained using different methods leads to an accurate estimation of this temperature. On the basis of these results, it is therefore recommended to use a combination of different methods to increase the quality and the reliability of solubility phase diagrams for enantiotropically related crystallographic forms.

■ ASSOCIATED CONTENT

Supporting Information. Calibration curve of the refractometer (Figure SI.1), principle of the ATR technology (Figure SI.2), calibration model of the ATR-FTIR probe (Figure SI.3), and principle of the FBRM probe (Figure SI.4). Clarification of the error estimations on the concentration and the temperature, presented in Tables 1 and 2. This material is available free of charge via the Internet at http://pubs.acs.org.

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