Solubility and Induction Time of the L-Enantiomer of Amino Acid
Arginine Determination with in situ 3D-ORM

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Three-dimensional optical reflectance (3D-ORM) technique [1] is used to measure the important properties of the L-enantiomer of arginine (L-Arg). This advance technique is fully capable to follow particle processes in line and in real-time, for instance applications in milling, homogenization, fermentation, etc. In this work, another application of 3D-ORM in combination with selective multi depth focus (SMF) is introduced for crystallization process. L-arginine was selected as a model compound. Solubility and induction time are interested properties which are objectives of this work and will be validated via 3D-ORM SMF technique. Those parameters are essential for a sustainable enantioseparation design via crystallization processes. Herewith, solubility is determined via the polythermal method with a suitable heating rate. Consequently, solubility curve is correlated with experimental data via a three parameter model proposed by Grant et. al. Indeed, L-Arg is quite good soluble in water due to polar-polar interaction as well as hydrogen bonding formation between solute and solvent. In induction period investigation, clear supersaturated solutions are kept at constant temperatures and well agitation to detect homogeneous nucleation. The results reveal that 3D-ORM SMF is a sensitive process analytical tool (PAT) which can be used to detect the nucleus formation from homogeneous supersaturated solutions.

1. Introduction

Arginine is an important amino acid for animal growth. Its derivatives with organic and inorganic acids gained attention in the field of nonlinear optical materials. They are frequently used in optical devices such as optical switches and modulators [2]. Arginine can further be used as an additive to control the crystal shapes of other amino acids, for instance glutamic acid [3]. The pure enantiomers of Arginine possess also the potential to be applied as pharmaceutical ingredients [4]. However, knowledge about several important properties of arginine is still limited. There is a lack of reliable solid-liquid equilibrium (SLE) as well as metastable zone width (MSZW) or induction period data required for a rational design of crystallization processes. In the present work, based on laser multi capture signal analysis of Sequip S + E GmbH, determination of SLE and induction period of L-Arg in aqueous solution will be carried out under suitable conditions.

The principle of those determinations is schematically described in Fig. 1. A suitable heating program is applied for know-composition suspensions to detect the saturated temperatures where all particles dissolving into solutions and then remaining clear mediums. For example, a suspension at point A in Fig. 1 is slowly heated up to point B on solubility curve. The saturated temperature T_clear is the target of measurement. On the other hand, clear solutions in stable region, e.g. point C, are cooled down and kept in metastable zone width, for instance point D, under isothermal and well mixing condition until first nuclei forming. The time for that event is recorded as induction time.
Fig. 1: Schematically of solubility and induction time determination. A know-
composition suspension at A is slowly heated with a 0.5 Kmin⁻¹ to observe a clear so-
lution at B. Corresponding temperature at B is the saturated temperature. The MSZW 
can be determined from different cooling rate R, R', R'', etc. Then the metastable 
zone limit can be obtained by an extrapolation at cooling rate equal to zero. To 
measure induction time, a system was kept in MSZW for a certain time until nuclea-
tion.

3D-ORM SMF [5] uses a laser source with energy of 5mW. A laser beam goes 
though a single mode optical fiber, which allows detecting accurately only particles in 
focal regime close to sapphire window. Spiral focal is generated from fast movements 
of the focus point in both circular and axial pathways as depicted in Fig. 2. Thus the 
focus is able to detect all particles within a three-dimension volume inside medium. 
This technique is able to detect exposed particle surface area [6] instead of only 
chord length of particles. This feature is a remarkable advance comparing to conven-
tional methods in cases of challenging measured particles due to their intricate 
shape, needle-like shape, and large dimension particles. Besides, the advantage of 
in situ analytical 3D-ORM SMF technique for particle process characterization is also 
in terms of high accuracy and good repeatability of experimental data since no sam-
pling and diluting steps required.

Fig. 2: Illustration of 3D-ORM SMF probe [7]

2. Experimental Methods

2.1 Materials
The anhydrous enantiomer of arginine (L-arg, purity > 98%) was obtained from Sig-
ma-Aldrich, (Steinheim, Germany). Distillated water (Millipore machine, Schwalbach, 
Germany) was used as a solvent. All these substances were used without further pu-
rification.
Experimental determinations were operated with: Ф18 mm 3D-ORM SMF probe (Sequip S + E GmbH), thermostat (Julabo ME), Pt-100, glass crystallizer 500 mL, Stirrer KIA eurostar.

2.2 Method
SLE measurements are performed with the conventional polythermal method [8]. A heating rate (0.5 K.min⁻¹) is used to dissolve known-composition suspensions of L-Arg into “clear” solutions in a suitable temperature range. Optical density of solution is observed by 3D-ORM SMF probe. The clear solution is defined as number of count close to zero and remained constantly in 30 min. In all measurements, a lab-scale crystallizer of 500 mL is designed with an adjustable angle of the sensor to ensure the proper position configuration, thus increasing quality of measurement. Systems are well agitated at a speed of 350 rpm. Temperature is well controlled by a Julabo thermostat, version ME. Obtained solubility data are used for the next part. Parameterization of Grant’s model [9] was done by optimizing the objective function. Event thought the Grant’s model is relative simple, it can correlate quite well SLE of many systems. Another variation of this method, so-called Apenblat, uses the similar form and gained relative good agreement with many organic and inorganic compounds [10,11].

Three terms in Grant’s model involve enthalpy and entropy of dissolution process, see the follow equation:

\[ \ln x_i^c(T) = a_i \frac{1}{T} + b_i \ln T + c_i \]  \hspace{1cm} \text{and} \hspace{1cm} \Delta H_i = a_i + b_i T \tag{2} \]

where: \( a_i, b_i \) and \( c_i \) are empirical experimental fitted parameters. \( x_i \) is mole fraction of solute \( i \) in solution and \( T \) is absolute temperature. \( \Delta H_i \) is apparent enthalpy.

Fig. 3: Illustration of crystallization and dissolution process of L-Arg in water. First, a clear saturated solution L-Arg at 35 °C is cooled down. The temperature is kept constantly at 10 °C within 100 min. Then, the temperature is increased again to 35 °C.

Induction time are determined at isothermal conditions [8]. Different supersaturated degrees are applied to detect homogenous primary nucleation within metastable zone limit. 3D-ORM SMF is a sensitive PAT sensor, which is able to recognize the
nucleation event (the number of particle significantly increasing). The period remaining “clear” solution is recorded as induction time. In induction time experiments, the onset of nuclei formation was determined based on the total number of particles measured by the 3D-ORM MSF within the size range of 0-10 µm, other ranges are also recorded to follow crystal growth or agglomeration.

An illustration of solubility and induction time determination is represented in Fig. 3. Herein, several important phenomena of crystallization process can be derived from the laser back reflection spectra. In Fig. 4, one can compare the intensity and shape of reflections relating to different events. Obviously, distinguished signal from single nuclei formation, crystal growth, agglomeration and breakage of L-Arg are observed. From Fig. 3, laser back reflection spectra are derived to analyze important events occurring during crystallization process. 3D-ORM SMF is a crystal-track™ PAT which is able to detect not only particle size but also morphology variation.

![Graphs showing different events](image)

**Fig. 4:** Back reflection spectra corresponding to different events during crystallization of the L-enantiomer of amino acid arginine.

In clear solution, the transmit laser beam goes in solution without any interrupt from liquid medium and the base line is linear like Fig. 4a. Then the nucleation occurs at a certain supersaturated degree and period of time that result tiny particles so-called nuclei with detectable diameter in a range less than 5 µm. This spectrum contains reflection of fine particles as Fig. 4b. Consequently, those fine particles growth under supersaturated conditions, Fig. 4c shows bigger particles since stronger reflections are observed. The reflective spectrum in Figure 4d indicates strong agglomeration behavior of L-Arg in water. They form various flocculates which change the surface state and detectable diameter of L-Arg. Here it is necessarily to notice that a single
perfect spherical particle will provide a reflection spectrum similar to Gauss-shape. The complexity of reflection spectrum is resulted from integration of all individual reflection behaviors of particles e.g. in agglomerates.

3. Results and Discussion

3.1 SLE of L-Arg in water

At first in water as solvent, solubility curve is a monotonically increase function of temperature. Fig. 5 showed relative high solubilities of the enantiomer. For instance, the solubilities of the enantiomer at 25 °C is about 15.21%. The explanation might relate to particular hydrogen bond network in the crystal structure of L·2H2O [12]. In the dissolution processes, arginine was also able to create the hydrogen bonds with water molecules resulting in high solubilities. Besides, strong polar-polar interactions between L-Arg and water also play an important role. Solute and solvent in this case are both polar compounds.

![Fig. 5: Solubility of L-Arg in water as a monotonically increasing function of temperature. Symbol and line represent experimental determination and model correlation, respectively.](image)

The parameters of the modified model (Eq. 1) are estimated from above solubility data. The results are presented in Tab. 1. While $a_i$ and $b_i$ possess positive values, $c_i$ is found as negative number. The obtained parameters are replaced in to Eq. 1. The results show a good agreement with experimental determination as Fig. 5.

Dissolution of arginine was an endothermic process, apparent enthalpy of dissolution of the enantiomer in pure water is $+29.4 \text{ kJ.mol}^{-1}$ at 25 °C (Eq. 2).

![Tab. 1: Three parameters in the Grant's model for SLE of L-Arg in water.](image)

<table>
<thead>
<tr>
<th>Grant's model parameters</th>
<th>Term</th>
<th>Value</th>
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<tbody>
<tr>
<td></td>
<td>$a_i$</td>
<td>9.7925e+003</td>
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<tr>
<td></td>
<td>$b_i$</td>
<td>65.8878</td>
</tr>
<tr>
<td></td>
<td>$c_i$</td>
<td>-45.2458</td>
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This method is practical application in case of compound decomposition before melting, which occurs with almost amino acids. In Grant’s equation, thermal properties such as melting temperature and enthalpy of fusion do not need. Other SLE models such as NRTL, UNIQUAC, etc. always use those data as input parameters.

3.2 Induction time determination

Kinetic information including MSZW and induction time is important parameters for crystallization design. In pre-experiments, MSZW of arginine are known as relative broad, i.e. more than 30 °C. Induction time determination is performed within the MSZW.
The induction time of arginine in water is strongly impacted by supersaturated degree. The relationship is seen as a monotonically decrease function as depicted in Fig. 6. Induction time is a logarithms function of supersaturated degree. Obviously, in high concentration solution, the nucleus is easier to form due to collision theory comparing to diluted solution. From those data, the increasing tendency of interfacial tension versus degree of supersaturation is observed. However, details of critical size or free energy of nucleus formation needed to study in more details.

Recently, nucleation behavior is proved that depending on reactor size [13]. The good quality of obtained data in this work reveals that 500 mL crystallizer is sufficient enough to eliminate the volume effect on detection of nucleation. That is also an advantage of 3D-ORM SMF probe which can be flexible immersed in any lab-scale or industrial-scale crystallizers.

Apparently, induction time of L-Arg is quite suitable for further application such as preferential crystallization. This method is based on kinetic control and designed within MSZW or induction period. Hereby, the nucleation period can be considered sufficiently to resolute two enantiomers of arginine. For instance, in moderate degree of supersaturation about 1.5, solution can remain as a clear solution longer than 30 min without homogeneous nucleation.

4. Conclusions

3D-ORM in combination of SMF is able to detect important parameters for crystallization such as solubility and induction time. In real-time and inline, this technique gains many advantages such as no sampling needed, measuring in original concentration without dilution, eliminating of air bubble effect. Especially, reflection spectra can give useful extra information about several important events e.g. agglomeration, crystal brakeage. For further application, this function is also able to detect different morphology of particles. That allows to follow polymorphic or solvate transformations which are extremely important for many applications, e.g. pharmaceuticals.

Solubility of L-arginine is detected as relative high values due to the fact that polar-polar interaction between solute and solvent. Hydrogen bond formation is also a reason to result good solubility of arginine in water. High solubility will support for crystal-
lization process in terms of productivity, reducing size of crystallizer. Furthermore, induction time of L-arginine in water is sufficient to perform crystallization. Thus, MSZW of L-arginine is relatively broad. That strongly assists crystallization design to produce single pure enantiomers from bio- or chemical processes.

Further works will focus in different mechanism of homogeneous nucleation. The obtained data in this work can be coupled with several mathematic models to have deeper understanding about this complicated phenomenon.

List of Symbols and Abbreviation

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>(a_i, b_i, c_i)</td>
<td>Parameters in Eq.1</td>
</tr>
<tr>
<td>L-Arg</td>
<td>L arginine</td>
</tr>
<tr>
<td>PAT</td>
<td>Process analytical tool</td>
</tr>
<tr>
<td>MSZW</td>
<td>Metastable zone width</td>
</tr>
<tr>
<td>SLE</td>
<td>Solid-liquid equilibrium</td>
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<tr>
<td>3D-ORM SMF</td>
<td>Three-dimensional optical reflectance measurement with selective multi depth focus</td>
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References