**Melting properties of amino acids and their solubility in water†**

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The state-of-the-art unit operation for separation and purification of amino acids is still crystallization, which requires solubility data and melting properties of pure compounds. Since measuring solubility is time-consuming, prediction tools are desired. Further, melting properties are not yet available due to decomposition of amino acids upon slow heating. In this work, melting properties of twenty amino acids (except Met) were measured by Fast Scanning Calorimetry (FSC) with heating rates up to 20 000 K s\(^{-1}\). PC-SAFT was used to predict interactions in amino acid + water systems. Additionally, solubility, pH, and PXRD was measured. By combining FSC and PC-SAFT, the solubility of 15 amino acids was successfully predicted in a wide temperature range in good agreement with the experimental data. Thus, this work provides melting properties of amino acids for the first time and highlights the usefulness of such data to predict material properties such as aqueous solubility of amino acids.

**Introduction**

Commonly proteins are represented by the set of twenty “standard” \(\alpha\)-amino acids (AA). These either exist as a monomer or they are bound as building blocks in peptides and proteins.\(^{1}\) Since their isolation in the 19\(^{th}\) century the physical and chemical properties of AA have been widely investigated because of their crucial importance in nature and by relevance for industrial processes.\(^{2,3}\) The applied separation unit of fractional crystallization is still state-of-the-art.\(^{4,5}\) This requires basic understanding of the melting temperatures as well as the solubility behavior to further develop and optimize the downstream process.\(^6\)

However, consistent melting temperatures are still not available for the amino acids. Further, aqueous AA solubility studies have not been checked for consistency. Such studies were carried out in the early 20\(^{th}\) century focusing on AA + water.\(^{7–12}\) Many of these works were performed in a narrow temperature range, without pH measurements and analysis of the crystal structure of AA between its pure component and the solid in saturated solutions.

Undoubtedly measuring solubility data is expensive. Hence, prediction of AA solubility in a wide temperature range based on physical properties such as melting properties is highly desired. Unfortunately, conventional methods, e.g. Differential Scanning Calorimetry (DSC), are not applicable to determine the melting properties of AA due to thermal decomposition upon slow heating rates.\(^{13}\) Experimental melting properties is available in literature only for two AA: glycine, \(\alpha\)-alanine\(^{14}\) and \(\alpha\)-arginine.\(^{15}\)

In the current study we continue this work and present the melting properties of twenty proteinogenic AA characterized with Fast Scanning Calorimetry (FSC). FSC with scanning rates up to 20 000 K s\(^{-1}\) has been established as a reliable device to study the melting thermodynamics of thermally labile biomolecules, e.g. bio-polymers,\(^{16,17}\) low molecular mass pharmaceuticals\(^{18}\) and nucleobases.\(^{19,20}\) The experimental melting properties are applied as an input for the thermodynamic framework PC-SAFT to predict the aqueous AA solubility. Additionally, a solid–liquid equilibrium between solid AA and the saturated liquid aqueous phase was assumed. Applying pure solid amino-acid phase the solubility \(x_{i}^{L}\) is determined according to Prausnitz\(^{21}\) as:

\[
\ln(x_{i}^{L} \times y_{i}^{L}) = \frac{\Delta_{le}^{SL}}{RT_{0i}} \left(1 - \frac{T_{\alpha}^{SL}}{T}ight) - \frac{1}{RT} \int_{T_{\alpha}^{SL}}^{T} \Delta_{pe}^{SL}(T) dT \\
+ \frac{1}{R} \int_{T_{\alpha}^{SL}}^{T} \frac{\Delta_{PC}^{SL}(T)}{T} dT \tag{1}
\]

\(\Delta_{le}^{SL}\) and \(\Delta_{pe}^{SL}\) are the liquid and solid phase excess enthalpies of mixing, \(\Delta_{PC}^{SL}\) is the pairwise contribution of the partial molar excess enthalpy of mixing and \(T_{\alpha}^{SL}\) is the melting temperature.
\[ \Delta c_{\text{phi}}^{\text{SL}}(T) = \left( a_{\text{pa}} - a_{\text{ps}} \right) T + \left( b_{\text{ps}} - b_{\text{pa}} \right) \]  

(2)

with \( \gamma_1^* \) as the activity coefficient of AA, \( R \) the universal gas constant, \( \Delta h_{\text{ui}}^{\text{SL}} \) the melting enthalpy at melting temperature, \( T_{\text{ui}}^{\text{SL}} \) the melting temperature, and \( \Delta c_{\text{phi}}^{\text{SL}}(T) \) the temperature-dependent difference between the heat capacities in liquid (L) and solid (S) state of a pure AA. In eqn (2), \( \Delta c_{\text{phi}}^{\text{SL}}(T) \) was assumed to show a linear temperature dependence with \( a_{\text{ps}} \), \( a_{\text{pa}} \) and \( b_{\text{ps}} \), \( b_{\text{pa}} \) as the slope and the intercept of the heat capacities, respectively. The solubility increase (decrease) with decrease (increase) of \( \Delta h_{\text{ui}}^{\text{SL}} \), while increase in \( T_{\text{ui}}^{\text{SL}} \) and \( \Delta c_{\text{phi}}^{\text{SL}}(T) \) reduces the slope of the solubility–temperature curve to less temperature-dependency. The influence of the solvent is accounted by \( \gamma_1^* \), which describes interactions between studied compound and solvent in the liquid phase. The crystal structure of the AA was measured by Powder X-ray Diffraction (PXRD). Eqn (1) is only valid for the neutral form of the molecule, which was confirmed by pH measurements of the saturated solutions.

Detail workflow of this work is illustrated in ESI Fig. S1.† The abbreviation of AA are in ESI Table S1.†

**Methodology**

**Materials and reagents**

Twenty proteinogenic AA investigated in this work are listed in ESI Table S1.† All AA were of commercial origin and used without additional purification. The Millipore-Q grade water for the solubility measurements was directly taken in the lab.

**Melting measurements with FSC**

Experimental FSC melting properties measurements were carried out by using Flash DSC 1 (Mettler Toledo) with the calorimetric twin chip sensor UFS1. Experimental FSC study of the AA was given in the previous works, where detailed experimental description of FSC method has been presented.1,2,3,4

All measurements were conducted under inert atmospheres of dry nitrogen (dew point lower than 150 K) with a flow rate of 30 mL min\(^{-1}\). The sensors were conditioned according to manufacturer’s procedure and the temperature was calibrated with recommended calibration metals (indium, bismuth and tin).

The experimental FSC procedure consists of three measurement stages, as presented in the temperature–time profile in ESI Fig. S2.† The starting temperature is set to 303 K to reduce the measuring time, as starting temperature below 303 K requires a cooler and long system equilibration times.

For the first stage (#1 to #4), the temperature range from 303 K to 473 K and constant scanning rate 2000 K s\(^{-1}\) were selected to assure the high reproducibility of the heating and cooling cycles. The reproducibility is indirect proof indicating that sample mass loss due to sublimation and decomposition has not occurred, and that volatile impurities or water were absent. It is also indicating that the sample was measured in its anhydrous form. The sample mass (without silicon oil) is determined in this stage as \( m_0 = M_f [\text{g mol}^{-1}] \times c_{\text{ploi}} [\text{J K}^{-1}][\text{mol}^{-1} K^{-1}] \), where \( c_{\text{ploi}} [\text{J K}^{-1}] \) is the total heat capacity of the sample from the first FSC stage and \( c_{\text{ploi}} [\text{J mol}^{-1} K^{-1}] \) is specific heat capacity obtained DSC measurements (Pyris 1, PerkinElmer, USA).1,4,5,6,24–26

In the second stage, the melting properties were determined in heating step #5. To improve the thermal contact between the sample and the sensor, silicon oil can be added to the sample before heating step #5. All the samples used in FSC measurements were relatively small (less than 100 ng) and for such small samples, the surface-to-volume ratio is rather high, what leads to increase in mass loss due to sublimation or evaporation at higher temperature. This effect is especially prominent for small molecules like AA. Therefore silicon oil not only improves the thermal contact but additionally coats the sample surface and suppresses the mass loss of the sample due to sublimation or evaporation. The heating rates of step #5 typically ranged from 2000 K s\(^{-1}\) to 10 000 K s\(^{-1}\). However for a few extremely thermally labile AA, e.g. Ile, Asn, Cys, higher heating rates up to 20 000 K s\(^{-1}\) were applied together with silicon oil coating to further minimize the sublimation or evaporation processes. Unfortunately even with these methods, sublimation or evaporation of Met cannot be suppressed enough. The melting and evaporation process were overlapping each other which leads to an unsuccessful determination of melting properties.

In the heating step #5 the shaded grey area in Fig. 1(a) in the temperature range of the melting peak was designated as the melting enthalpy, \( \Delta H_{\text{m}}^{\text{SL}} \) [J], while the onset of the melting peak is a scanning rate dependent melting temperature, \( T_{\text{m}}^{\text{SL}}(\beta) \). The specific melting enthalpy, \( \Delta h_{\text{ui}}^{\text{SL}} \), is defined as a ratio \( \Delta h_{\text{ui}}^{\text{SL}} = \Delta H_{\text{m}}^{\text{SL}} \times \frac{M}{m_0} \), where \( M \) is the molar mass of AA and \( m_0 \) is the mass of the sample.

After heating step #5, the molten samples without silicon oil were quenched rapidly to retain the sample in the liquid state below the melting temperature without crystallization. During the heating and cooling cycles (#8 to #11) in third stage a step change in specific heat capacity corresponding to glass transition from amorphous solid of AA to liquid (supercooled) state was observed. Due to complications in avoiding sublimation or evaporation mass loss of the samples at high temperatures in the current state of FSC technique, the glass transition can be determined only for half of the 20 proteinogenic AA.

**Measurement of solubility**

AA are widely investigated and their aqueous solubility data are readily available in literature. Most of the studies are carried out by using the gravimetric method. However, in some cases a discrepancy between literature data and experimental values is observed. In this work an excess amount of solute is added to water till the saturated solution in equilibrium with the solid solute is formed. The compounds were shaken and equilibrated isothermally (at least 72 h) to ensure the solid–liquid equilibrium is reached. After this a defined amount of the saturated liquid phase is withdrawn and weighed. The sample solution is placed in a drying chamber and a vacuum chamber to ensure total evaporation of the water. The remaining solid was weighed again and thus the solubility determined. Additionally often pH values of the saturated solutions and crystal-structure studies of
The area under the melting peak (red area) and 10 000 K s
for glass transition step (blue area) and 6000 K s
—
for solid phase are missing, which are important since the crystal
structure of the pure compound and the solid compound in
solid phase are missing, which are important since the crystal
structure and pH values, AA
solubility model. In order to complete the
equilibrium state is not allowed to change during the solubility
determination and solubility model. In order to complete the
missing information about crystal structure and pH values, AA
solubility (for all 20 AA) was determined gravimetrically at T =
298.15 K in three independent unbuffered aqueous solutions.
The solutions were mixed for 24 h and left without further
shaking for equilibration for 48 h. Then 200 μL of the saturated
liquid phase was withdrawn for the solubility determination.
The pH measurement of the unbuffered saturated solutions in
solid–liquid equilibrium, as well as, the crystal structure of the
initial pure AA (from the supplier), and of the solid phase
equilibrated with saturated liquid phase were determined using
pH meter with a standard uncertainty of ±0.01 and Powder X-
ray Diffractometer (PXRD, Miniflex 600, Rigaku, Japan, operating
temperature (295.15 K) and pressure (1 atm), speed scan
5° min
−1 from 2° to 35° in 0.02° steps, voltage 40 kV, current 15
mA, type of radiation Cu Kα anode), respectively. All the pH
values of the saturated solutions are listed Table 3 and the
PXRD diffractograms are shown in the ESI Fig. S25–S34.†

PC-SAFT

The successful prediction of AA solubility using eqn (1) requires the
Corresponding Activity Coefficient and Experimental Melting
Properties. The activity coefficient is the ratio of the fugacity coe-
ficient ϕi at the solubility mole fraction to the fugacity coefficient
ϕ0i of the pure-component. In this work the PC-SAFT (Perturbed-Chain Statistical Associating Fluid Theory) equation
of state is used and the fugacity coefficient is expressed as follows
\[
\ln \phi_i = \frac{\mu_{\text{res}}}{RT} - \ln(Z)
\]
where \(\mu_{\text{res}}\) represents the residual chemical potential and Z the
compressibility. The calculation of \(\mu_{\text{res}}\) and Z requires the residual
Helmholtz energy \(a_{\text{res}}\) which is expressed in this work as
\[
a_{\text{res}} = a_{\text{hc}} + a_{\text{disp}} + a_{\text{assoc}}
\]
where \(a_{\text{hc}}\), \(a_{\text{disp}}\) and \(a_{\text{assoc}}\) are the Helmholtz energy contributions
“hard chain”, “dispersion” and “association”, respectively.
In this work the original PC-SAFT from Gross and Sadowski26
is used, where all required contributions have already been
implemented. For mixtures (here water + AA), the conventional
Berthelot–Lorentz combining rules were applied to describe the
interactions between two components i and j
\[
\sigma_{ij} = \frac{1}{2}(\sigma_i + \sigma_j)
\]
where \(k_{ij}\) is the binary interaction parameter to describe deviations
from the geometric mean of the dispersion-energy parameters of two components i and j (i.e., water and AA).
The interaction parameter \(k_{ij}\) was fitted to osmotic-coefficient
data at \(T = 298.15\) K. For some AA, a linearly temperature-
dependent binary interaction parameter \(k_{ij}(T)\) was available in
the literature, expressed as:
\[
k_{ij}(T) = k_{ij,0} + k_{ij,T}(T - 298.15\text{ K})
\]
In this work \(k_{ij}(T)\) was fitted to solubility data at higher
temperatures.
In the current work the AA were considered as associating
fluids, and each one association site was assigned for the amine

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**Fig. 1** Melting properties of His. (a) Specific heat capacity of His
determined experimentally with FSC (—) and for glass transition step
of ultra-fast quenched melted His (without silicon oil) (—) and DSC
for heat capacity of solid, \(c_p0i\) (—). The area under the melting peak
(■) indicates \(\Delta h_{\text{SL}}\), while onset temperature corresponds to
\(T_m\). \(\Delta h_{\text{SL}}\) is determined at glass transition temperature, \(\Delta c_{\text{p0i}}(T_m)\)
and adjusted to melting temperature, \(\Delta c_{\text{p0i}}(T_m)\). (b) Melting temperature vs.
heating rate diagram. Red line is the linear extrapolation to zero
heating rate. The uncertainty is the standard deviation of multiple
measurements. (c) Enthalpy, \(\Delta H_{\text{SL}}\), of His with respect to sample mass,
\(m_c\), regardless of the scanning rates \(\beta\) [K s
−1]. The slope of the linear fit through zero origin (line) signifies \(\Delta H_{\text{SL}}\).
The applied scanning rates were 2000 K s
−1 (Δ), 4000 K s
−1 (Δ Δ), 6000 K s
−1 (Δ Δ Δ), 8000 K s
−1 (Δ Δ Δ Δ) and 10 000 K s
−1 (Δ Δ Δ Δ Δ). Solid symbols (without silicon oil), empty symbols (with silicon oil). The melting properties of all twenty proteinogenic AA are shown in ESI Fig. S3 and S4.† The
\(T_m\), \(\Delta H_{\text{SL}}\), \(\Delta c_{\text{p0i}}(T_m)\) and \(\Delta c_{\text{p0i}}(T_m)\) for each AA are listed in Table 1. (d)
His aqueous solubility as temperature vs. weight fraction diagram. The red area presents the solubility modeling assuming γ1 = 1 (eqn (1)) in
the range of the uncertainties of the melting properties. \(\Delta r_{\text{SL}} =
(619 ± 7)\) K. Symbols represent literature data (●: Kustov,34 △: Amend). (e) Activity coefficients vs. temperature diagram. (△: Kus-
tov32) uncertainties are based on the uncertainties of the melting
enthalpy. —: PC-SAFT.
group and for the carboxylic group, respectively. In case of specific side chains of the AA, additional association site were added depending on a proton donor (e.g. Glu 1 : 2) or proton acceptor (Gln 2 : 1). The PC-SAFT pure-component parameters for most of the AA are already published\textsuperscript{14} and will be utilized in this work, except for Glu and Asp with improved parameters, and for Trp with completely new parameters (listed in Table 2). The pure-component parameters were fitted to osmotic-coefficient data and density data of aqueous solutions at $T = 298.15$ K. For some further AA new experimental data for osmotic coefficients and mixture density are shown in Fig. S5—S24 in the ESI.\textsuperscript{†} Water was modeled with the 2B association scheme with a temperature-dependent segment diameter as it was used already in previous work.\textsuperscript{14} The PC-SAFT pure-component parameters as well as binary interaction parameters between the AA and water according eqn (6) used in this work are listed in Table 2.

## Results

### Experimental melting properties

The melting properties of 19 proteinogenic AA (except Met) were characterized experimentally with FSC. The FSC experimental results for His as a representation are presented in Fig. 1(a–c), while for all other AA in ESI Fig. S3 and S4.\textsuperscript{†}

Ideally, a direct determination of $\Delta_{\text{pol}}^{\text{ST}}$ at $T_{\text{pol}}^{\text{ST}}$ is preferable from the melting curve. However, this is not possible for some AA due to the mass loss caused by sublimation or evaporation after melting. The mass loss of the sample is indicated by a baseline drop below $c_{\text{pol}}^{\text{ST}}$ after the melting, even though the sample was cooled down rapidly right after the melting to minimize the mass loss at high temperature. If complete mass loss and crystallization are avoided, a glass transition step at $T_{\text{pol}}^{\text{ST}}$ from glassy to supercooled liquid AA is shown as solid green line.

For low volatile samples such His or Arg (ESI Fig. S3\textsuperscript{†}), the liquid phase immediately after the melting (solid red line) is in accordance with the $c_{\text{pol}}^{\text{ST}}$ above glass transition. This indicates that the linear extrapolation from $c_{\text{pol}}^{\text{ST}}$ of the glass transition to $T_{\text{pol}}^{\text{ST}}$ is applicable. For consistency reasons this extrapolation was applied for all AA with measured glass transition. For high volatile AA (Gly, Ala, Val, Leu, Ile, Pro, Lys, Phe, Cys) without measurable glass transition, $\Delta_{\text{pol}}^{\text{ST}}(T)$ was estimated as explained in the discussion.

The $c_{\text{pol}}^{\text{ST}}$ of the glass transition was fitted linearly with $a_{\text{lin}}$ as slope and $b_{\text{lin}}$ as intercept, while the $c_{\text{pol}}^{\text{SI}}$ determined from DSC as solid blue line is fitted linearly with $a_{\text{s}}$ and $b_{\text{s}}$. The heat capacity of crystal and glass are assumed to be equal, especially

### Table 1: Molar mass $(M)$, experimental glass and melting properties determined with FSC in this work $(T_{\text{pol}}^{\text{ST}}, T_{\text{pol}}^{\text{SI}}, \Delta_{\text{pol}}^{\text{ST}}, \Delta_{\text{pol}}^{\text{SI}}, \Delta_{\text{pol}}^{\text{ST}(T_{\text{pol}}^{\text{ST}})}$ and $\Delta_{\text{pol}}^{\text{SI}(T_{\text{pol}}^{\text{SI}})})$ of the pure 19 proteinogenic AA. The uncertainties represents the standard deviations of multiple measurements.

| AA with non-polar substituents | M/g mol$^{-1}$ | $T_{\text{pol}}^{\text{SI}}$/K | $T_{\text{pol}}^{\text{ST}}$/K | $\Delta_{\text{pol}}^{\text{SI}}$/kJ mol$^{-1}$ | $\Delta_{\text{pol}}^{\text{ST}}$/kJ mol$^{-1}$ | $\Delta_{\text{pol}}^{\text{SI}(T_{\text{pol}}^{\text{SI}})}$/kJ mol$^{-1}$ K$^{-1}$ | $\Delta_{\text{pol}}^{\text{ST}(T_{\text{pol}}^{\text{ST}})}$/J mol$^{-1}$ K$^{-1}$ |
|-------------------------------|---------------|-----------------|-----------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Gly$^a$                       | 75.07         | —               | 569 ± 9         | 22 ± 3                          | 0.038 ± 0.005                   | —                               | —                               |
| Ala$^a$                       | 89.10         | —               | 608 ± 9         | 23 ± 3                          | 0.038 ± 0.005                   | —                               | —                               |
| Val                           | 117.15        | —               | 529 ± 7         | 44 ± 6                          | 0.083 ± 0.011                   | —                               | —                               |
| Leu                           | 131.18        | —               | 518 ± 8         | 43 ± 5                          | 0.082 ± 0.011                   | —                               | —                               |
| Ile                           | 131.18        | —               | 595 ± 7         | 43 ± 6                          | 0.083 ± 0.011                   | —                               | —                               |
| Pro                           | 115.14        | —               | 527 ± 7         | 19 ± 3                          | 0.036 ± 0.005                   | —                               | —                               |
| AA with polar substituents    |               |                 |                 |                                 |                                 |                                |                                |
| Ser                           | 105.10        | 337 ± 2         | 519 ± 7         | 28 ± 3                          | 0.053 ± 0.006                   | 64 ± 3                          | 50 ± 3                          |
| Thr                           | 119.12        | 355 ± 4         | 587 ± 9         | 34 ± 5                          | 0.058 ± 0.035                   | 69 ± 1                          | 63 ± 9                          |
| AA with acidic substituents   |               |                 |                 |                                 |                                 |                                |                                |
| Asp                           | 133.11        | 386 ± 16        | 610 ± 7         | 35 ± 5                          | 0.057 ± 0.006                   | 93 ± 4                          | 42 ± 4                          |
| Asn                           | 132.12        | 466 ± 11        | 582 ± 7         | 33 ± 4                          | 0.053 ± 0.007                   | 80 ± 2                          | 52 ± 2                          |
| Gln                           | 147.13        | 330 ± 5         | 566 ± 7         | 46 ± 5                          | 0.078 ± 0.006                   | 63 ± 5                          | 25 ± 5                          |
| Gln                           | 146.15        | 323 ± 5         | 589 ± 7         | 50 ± 6                          | 0.076 ± 0.010                   | 79 ± 2                          | 80 ± 2                          |
| AA with basic substituents    |               |                 |                 |                                 |                                 |                                |                                |
| Arg                           | 174.21        | 362 ± 3         | 558 ± 7         | 28 ± 4                          | 0.051 ± 0.007                   | 107 ± 5                         | 35 ± 5                          |
| His                           | 155.16        | 408 ± 9         | 619 ± 7         | 59 ± 6                          | 0.095 ± 0.011                   | 120 ± 3                         | 113 ± 3                         |
| Lys                           | 146.19        | —               | 529 ± 9         | 22 ± 3                          | 0.042 ± 0.004                   | —                               | —                               |
| AA with aromatic substituents |               |                 |                 |                                 |                                 |                                |                                |
| Phe                           | 165.20        | —               | 579 ± 7         | 58 ± 7                          | 0.099 ± 0.013                   | —                               | —                               |
| Tyr                           | 181.20        | 405 ± 3         | 678 ± 7         | 47 ± 6                          | 0.069 ± 0.009                   | 65 ± 1                          | 63 ± 1                          |
| Trp                           | 204.23        | 433 ± 3         | 620 ± 7         | 60 ± 7                          | 0.097 ± 0.012                   | 99 ± 4                          | 22 ± 4                          |
| AA with sulfuric substituents |               |                 |                 |                                 |                                 |                                |                                |
| Cys                           | 121.16        | —               | 604 ± 7         | 45 ± 8                          | 0.074 ± 0.014                   | —                               | —                               |

$^a$ Already published in previous work.\textsuperscript{14}
at temperatures close to \( T^f \). This assumption is commonly accepted, e.g. indomethacin,\textsuperscript{27} saccharides,\textsuperscript{28} o-terphenyl,\textsuperscript{29} selenium,\textsuperscript{30} poly-p-dioxanone.\textsuperscript{31} The heat capacity difference between crystal and glass of such components does not differ by more than 5 to 10%. This difference is also within the uncertainty of our investigation. Nevertheless, we have to acknowledge that there is a difference in the heat capacity, which may influence the result of our investigation. Nevertheless, in sum the difference between heat capacity of solid and glass phases are worst-case assumed to be <10%. Thus, heat capacity of solid was set equal to the glass. This allows indirect determination of melting temperature. The PC-SAFT predicted values of \( \gamma_{\text{SAF}}^{298.15 \, \text{K}} \) for each AA at \( T = 298.15 \, \text{K} \) are listed in Table 2.

**PXRD measurements**

The hydration of AA has been widely reported in literature.\textsuperscript{44–49} In this work the PXRD measurement lead to further investigations in terms of possible polymorphs or formation of hydrates. Unfortunately, some AA were found to form hydrates (Ser,\textsuperscript{12} Lys,\textsuperscript{29} Asn, Pro), which does not allow the application of eqn (1) since the solid crystal in solution as well as for the melting properties must be the same. All the PXRD measurements were performed for the saturated solutions at \( T = 298.15 \, \text{K} \) and are shown in ESI Fig. S25–S34.†

**PH measurements**

The pH measurement in aqueous solution of AA was conducted in order to ensure that only one neutral species (>99%) was present in the saturated solution. Asp (≈95%), Arg (≈90%), Glu (≈88%) have less neutral species present in the saturated solutions, but this is still sufficient for PC-SAFT modeling, unfortunately not for Lys (≈70%), for which Lys was excluded from the PC-SAFT modeling. The pH values for all AA solutions are listed in Table 3.

**Solubility predictions with PC-SAFT**

The solubility of all AA was predicted with PC-SAFT based on the experimental melting properties measured with FSC. Prediction means that all PC-SAFT pure-component parameters were fit to non-solubility properties such as osmotic coefficients and mixture densities at \( T = 298.15 \, \text{K} \) in water. The deviations between PC-SAFT values and the experimental solubility were quantified with the absolute relative deviations (ARD) according to eqn [8]

\[
\text{ARD} = 100 \frac{1}{\text{NP}} \sum_{i=1}^{\text{NP}} \left| \frac{x_i^{\text{PC-SAFT}} - x_i^{\text{exp}}}{x_i^{\text{exp}}} \right|
\]

where NP is the number of the available experimental solubility points, \( x_i^{\text{PC-SAFT}} \) and \( x_i^{\text{exp}} \) are the PC-SAFT predicted and the experimental solubility, respectively.

As shown recently\textsuperscript{42} the \( \Delta h_{\text{Gli}}^{\text{SL}} \) has the highest influence on the solubility prediction. Unfortunately, \( \Delta h_{\text{Gli}}^{\text{SL}} \) values from FSC have rather high uncertainty up to 20%, in comparison to the \( \Delta h_{\text{Gli}}^{\text{SL}}(T_{\text{Gli}}) \) (up to 5%) and \( T_{\text{Gli}}^{\text{SL}} \) (up to 2%). Therefore, FSC experimental results of \( \Delta h_{\text{Gli}}^{\text{SL}}(T_{\text{Gli}}) \) and \( T_{\text{Gli}}^{\text{SL}} \) were utilized as input for solubility predictions with PC-SAFT directly, i.e. without varying within the experimental uncertainty. In contrast, the \( \Delta h_{\text{Gli}}^{\text{SL}} \) was adjusted [within the range of uncertainty of the FSC results] to experimental solubility data at 298.15 K. As a result, the FSC data for \( \Delta h_{\text{Gli}}^{\text{SL}} \) in Table 1 and the PC-SAFT fit for \( \Delta h_{\text{Gli}}^{\text{SL}} \) (Table 3) are nearly identical, which proves the general suitability of PC-SAFT method for the mixtures considered in the present work, where the predicted PC-SAFT solubility is in good agreement with experimental solubility Table 2.

Most of the PC-SAFT parameters were already available in the literature.\textsuperscript{8} These are listed in Table 2 together with binary
interaction parameters between water and AA. The parameter $k_{ij}(T)$ was applied for AA with a rather low temperature dependence of solubility. Therefore, the solubility ratio between $T = 323.15 \text{ K}$ and $T = 298.15 \text{ K}$ should indicate the necessity of a temperature-dependent interaction parameter. Ratio lower than (greater than) 1.5 increases (decreases) the probability of using two such parameters (one parameter).

**AA with non-polar substituents**

From Fig. 2(a) it was observed that the solubility decreases in the following order Gly > Ala > Val > Leu for $T < 450 \text{ K}$. However at higher temperatures, this order is disarranged. This new finding becomes possible only due to the availability of the new experimental melting data from FSC in this work. All non-polar aliphatic AA show a high tendency for sublimation/evaporation after the melting, so no glass transition step could be measured. However, even small values for differences of heat capacities moderately influence the slope of the solubility line. Therefore, heat capacity differences were fit to experimental solubility-temperature curves.

The aqueous AA solubility of Ile and Pro are shown in Fig. 2(b). Apparently Pro is most soluble in water among the twenty proteinogenic AA. In this case the PXRD results from the present work showed a change in the crystal structure which was referenced to the formation of a hydrate. The exact hydration is at least below $T_{\text{hydration}} \leq 298.15$. As the melting properties belong to the anhydrous form, eqn (1) cannot be applied.

**AA with polar substituents**

Fig. 2(c) shows that solubility of Ser is higher than of Thr. For Thr the melting properties were taken as measured and the solubility prediction is in good agreement with the literature. For Ser a crystal change was found during the solubility measurement. The crystal change can be referenced to Luk et al.\textsuperscript{12} which shows the formation of a hydrate ($T_{\text{hydration}} < 312.15 \text{ K}$). At higher temperatures the anhydrous Ser was formed (confirmed by PXRD), which allows the application of eqn (1).

| $m_1^{\text{seg}}$ | $\sigma_1/\AA$ | $u_1/k_B/\text{K}$ | $r_{\text{AI}1}/k_B/\text{K}$ | $r_{\text{AI}1}$ | $N$ | $k_{ij,298.15 \text{ K}}/10^4$ | $k_{ij,T \text{ K}}/10^4$ | $\omega_{123.15 \text{ K}}/\omega_{298.15 \text{ K}}$ | ARD/% | $N_{\text{dp}}$/ref. | $T_{298.15 \text{ K}}$ | PXRD trans. |
|------------------|--------------|-----------------|-----------------|---------------|--------|-----------------|-----------------|-----------------|--------|---------------|-----------------|-----------------|
| H$_2$O | 1.2047 | $a$ | 353.94 | 2425.67 | 0.045 | — | — | — | — | — | — | — |

**AA with non-polar substituents**

Gly$^a$ 4.850 2.327 216.960 2598.060 0.039 2 $-5.85^d$ | — | 1.392 | 3.84 | 10/36 | 0.305 | — |

Val$^b$ 7.485 2.589 306.410 3183.800 0.039 2 $-7.57^b$ | 3.85$^b$ | 1.223 | 2.07 | 7/37 | 0.059 | — |

Leu$^b$ 8.304 2.700 330.000 3600.000 0.020 2 $-6.39^d$ | 5.00$^d$ | 1.245 | 3.63 | 19/11 | 0.129 | — |

Ile$^b$ 8.241 2.586 281.884 2207.529 0.001 2 $-8.75^d$ | 2.70$^d$ | 1.199 | 4.60 | 8/38 | 0.043 | — |

Pro$^b$ 6.981 2.548 289.720 5527.750 0.036 2 $-6.99^b$ | — | 1.192 | — | — | — | — |

**AA with polar substituents**

Ser$^b$ 7.024 2.284 236.920 2671.930 0.039 3 $-2.57^b$ | 4.00$^d$ | 1.526 | 0.76 | 5/12 | 0.193 | x |

Thr$^b$ 6.329 2.606 325.370 2519.410 0.039 3 $-2.78^b$ | 1.25$^d$ | 1.388 | 0.44 | 8/39 | 0.465 | — |

**AA with acidic substituents**

Asp$^d$ 5.827 2.522 287.625 2544.234 0.041 3 $1.43^d$ | — | 1.889 | 8.17 | 16/40 | 5.825 | — |

Asn$^b$ 3.000 3.367 280.000 3265.670 0.044 3 $0.09^b$ | — | 2.879 | — | — | — | x |

Glu$^d$ 6.831 2.560 227.192 2544.234 0.041 3 $-4.45^d$ | — | 2.501 | 4.52 | 23/41 | 0.324 | — |

Gln$^d$ 9.289 2.360 273.555 2657.341 0.020 3 $-5.18^b$ | — | 1.992 | 3.33 | 8/42 | 0.114 | — |

**AA with basic substituents**

Arg$^b$ 9.908 2.657 349.710 2555.450 0.039 4 $-1.45^d$ | — | 1.848 | 10.1 | 11/37 | 0.969 | — |

His$^a$ 9.088 2.473 281.954 2640.981 0.078 3 $-3.89^d$ | 0.91$^d$ | 1.517 | 6.62 | 11/32 | 0.205 | — |

Lys$^b$ 11.673 2.378 301.210 3787.310 0.033 3 $-7.07^b$ | — | 1.358 | — | — | — | x |

**AA with aromatic substituents**

Phe$^d$ 9.310 2.690 391.827 3206.094 0.010 2 $-5.18^d$ | — | 1.502 | 14.2 | 17/7 | 1.755 | — |

Tyr$^b$ 8.139 2.280 289.370 2500.000 0.040 3 0.0227 | — | 1.934 | 18.8 | 11/36 | 11.17 | — |

Trp$^d$ 10.577 2.825 260.641 2563.249 0.024 3 $-7.68^d$ | 1.78$^d$ | 1.493 | 1.66 | 11/36 | 0.021 | — |

**AA with sulfuric substituents**

Cys$^b$ 7.739 2.384 322.910 1964.000 0.010 3 $-2.35$ | — | 1.755 | — | — | — | x |

Met$^b$ 16.026 2.150 220.370 1964.000 0.010 3 $-1.43$ | 1.57 | 1.416 | — | — | — | — |

$^a$ Temperature-dependent segment diameter $\sigma = 2.7927 + 10.11 \exp(0.01775 T) - 1.417 \exp(-0.01146 T)$.

$^b$ Pure-component parameters from Held et al.\textsuperscript{14} $^c$ Pure-component parameters from Chua et al.\textsuperscript{14} $^d$ Pure-component parameters from this work.
This might explain the slight kink in the solubility curve observed for Ser in Fig. 2(c). The melting properties can only be determined for the anhydrous form. For the PC-SAFT predictions melting temperature and difference in heat capacity was taken from the FSC measurements. The melting enthalpy was adjusted within FSC uncertainty to the only available experimental solubility value at $T = 315.15$ K. A good agreement between PC-SAFT and experimental solubility-temperature data supports the proposed procedure.

**AA with acidic substituents**

The acid AA (Asp, Glu) are characterized by a carboxyl group in the side chain and the amides (Asn, Gln) have a primary amide group. These additional polar groups also affect the pH value of the saturated solutions, which corresponds to their isoelectric point ($pI$). In general, these four AA show very low solubility in water, the amide AA are slightly more soluble than their acidic pendants at their $pI$ (Fig. 2(e)). However, for Asn a hydrate has formed upon equilibration in water. Unfortunately, solubility literature data of the anhydrous Asn was not available. Hence eqn (1) could not be applied for temperatures below $T_{Hydration} \leq 298.15$ and Asn solubility cannot be predicted. For Glu, Gln and Asp eqn (1) was applied and the results are in good agreement with the literature.

**AA with basic substituents**

His, Arg and Lys and increase the pH value in unbuffered aqueous solution, resulting in high $pI$ values (Table 3). For Lys the experimental solubility re-measured in this work was higher than the only available literature data, see Fig. 2(d). The PXRD diffractograms might hint Lys-hydrate formation in aqueous solutions. Williams et al. observed Lys monohydrate, depending on the relative humidity level to which Lys was exposed. The anhydrous form can only be attained after vacuum drying. Thus, eqn (1) was not applied to Lys. The surprisingly low values for the melting properties ($T_{Hydration}^0_{Lys} = 529 \pm 9$ K, $\Delta h^{SL}_{Hydration} = 22 \pm 3$ K) indicate possible high solubility of Lys.

For His and Arg no change in crystal structure was detected and the conventional approach was applied. The solubility prediction is in good agreement with the literature data.

### Table 3 Solubility $m_{SAF}^{sol}$, pH values of saturated solutions under study (uncertainties represents the standard deviations of multiple measurements, isoelectric point (pI) from literature and melting properties used in PC-SAFT: melting temperature $T_{0i}$, melting enthalpy $\Delta h_{0i}$ of the heat capacity of liquid and solid, and difference in the heat capacity at melting temperature $\Delta c_{0L}(T_{0i})/c_{0S}$)

| AA with non-polar substituents | $m_{SAF}^{sol}$ 298.15 k g$^{-1}$ | $pH_{SAF}^{sol}$ 298.15 K | $T_{0i}$ K | $\Delta h_{0i}$ kJ mol$^{-1}$ | $a_{pI}$ J mol$^{-1}$ K$^{-2}$ | $b_{pI}$ J mol$^{-1}$ K$^{-2}$ | $a_{pI}$ J mol$^{-1}$ K$^{-2}$ | $b_{pI}$ J mol$^{-1}$ K$^{-2}$ | $\Delta c_{0L}(T_{0i})/c_{0S}$ J mol$^{-1}$ K$^{-1}$ |
|-------------------------------|-----------------|-----------------|----------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Gly                           | 0.2019 ± 0.0020 | 6.32 ± 0.04     | 5.97     | 569             | 24.96           | 0.225           | 62.681          | 0.266           | 21.033          | 18.59          |
| Ala                           | 0.1415 ± 0.0015 | 6.33 ± 0.02     | 6.00     | 608             | 25.99           | 0.267           | 64.148          | 0.324           | 24.225          | 5.26           |
| Val                           | 0.0553 ± 0.0006 | 6.08 ± 0.07     | 5.96     | 529             | 46.72           | 0.351           | 106.488         | 0.453           | 32.573          | 20.00          |
| Leu                           | 0.0237 ± 0.0003 | 5.68 ± 0.15     | 5.98     | 518             | 49.09           | 0.525           | 71.622          | 0.577           | 24.322          | 10.15          |
| Ile                           | 0.0329 ± 0.0003 | 6.22 ± 0.14     | 6.02     | 595             | 47.11           | 0.459           | 87.228          | 0.512           | 35.624          | 20.39          |
| Pro                           | 0.6365 ± 0.0154 | 7.26 ± 0.07     | 6.30     | —               | —               | —               | —               | —               | —               |
| **AA with polar substituents** |                               |                 |          |                 |                 |                 |                 |                 |                 |
| Ser                           | 0.2867 ± 0.0123 | 6.01 ± 0.02     | 5.68     | 519             | 32.98           | 0.267           | 121.318         | 0.346           | 31.028          | 49.38          |
| Thr                           | 0.0894 ± 0.0008 | 5.87 ± 0.01     | 5.60     | 587             | 36.64           | 0.379           | 125.276         | 0.406           | 47.019          | 62.18          |
| **AA with acidic substituents** |                               |                 |          |                 |                 |                 |                 |                 |                 |
| Asp                           | 0.0057 ± 0.0002 | 3.05 ± 0.01     | 2.77     | 610             | 35.73           | 0.176           | 213.341         | 0.397           | 37.182          | 41.37          |
| Asn                           | 0.0267 ± 0.0016 | 5.13 ± 0.05     | 5.41     | —               | —               | —               | —               | —               | —               |
| Glu                           | 0.0088 ± 0.0003 | 3.28 ± 0.04     | 3.22     | 566             | 48.24           | 0.321           | 147.115         | 0.481           | 32.014          | 24.33          |
| Gln                           | 0.0405 ± 0.0002 | 5.01 ± 0.04     | 5.65     | 589             | 51.96           | 0.474           | 129.528         | 0.500           | 34.849          | 79.19          |
| **AA with basic substituents** |                               |                 |          |                 |                 |                 |                 |                 |                 |
| Arg                           | 0.1639 ± 0.0034 | 11.45 ± 0.02    | 10.8     | 558             | 28.64           | 0.326           | 265.689         | 0.690           | 27.698          | 34.83          |
| His                           | 0.0414 ± 0.0003 | 7.75 ± 0.05     | 7.59     | 619             | 56.01           | 0.507           | 152.902         | 0.537           | 21.854          | 112.80         |
| Lys                           | 0.5197 ± 0.1256 | 10.66 ± 0.10    | 9.74     | —               | —               | —               | —               | —               | —               |
| **AA with aromatic substituents** |                               |                 |          |                 |                 |                 |                 |                 |                 |
| Phe                           | 0.0291 ± 0.0007 | 5.99 ± 0.20     | 5.48     | 579             | 60.66           | 0.496           | 280.823         | 0.635           | 15.731          | 184.37         |
| Tyr                           | 0.0006 ± 0.0001 | 5.77 ± 0.34     | 5.66     | 678             | 49.77           | 0.664           | 93.511          | 0.681           | 19.229          | 62.74          |
| Trp                           | 0.0138 ± 0.0001 | 5.08 ± 0.11     | 5.89     | 620             | 65.55           | 0.351           | 289.570         | 0.758           | 15.771          | 21.82          |
| **AA with sulfuric substituents** |                               |                 |          |                 |                 |                 |                 |                 |                 |
| Cys                           | 0.1419 ± 0.0060 | 6.14 ± 0.03     | 5.74     | —               | —               | —               | —               | —               | —               |
| Met                           | 0.0536 ± 0.0014 | 5.25 ± 0.03     | 5.74     | —               | —               | —               | —               | —               | —               |

*Published in ref. 14. * Measured in this work. * Melting properties of anhydrous Ser.
Fig. 2. The temperature-dependent solubilities of AA: triangles represent literature data; empty circles represent the solubility measurements in present study; lines represent PC-SAFT predictions. (a) AA with non-polar substituents: Gly ▲: Lundblad,36 ▼: Amend,9 ▪: PC-SAFT. Ala ▲: Daldrup25, Amend,9 ▪: PC-SAFT. Val ▲: Lundblad,36 ▼: Amend,9 ▪: PC-SAFT. Leu ▲: Daldrup,25 ▼: Amend,9 ▪: PC-SAFT. (b) AA with non-polar substituents: Ile ▲: Zumstein,38 ▼: Amend9 ▪: PC-SAFT. Pro ▲: Lundblad,36 ▼: Amend,9 No PC-SAFT modeling due to a crystal change (ESI Fig. S26†). (c) AA with polar substituents: Thr ▲: Lundblad,36 ▼: Amend,9 ◆: Ferreira,43 ▪: PC-SAFT. Ser ▲: Luk,42 ▼: Amend,9 ▪: PC-SAFT. (d) AA with basic substituents: His ▲: Kustov,32 ▼: Amend,9 ▪: PC-SAFT. Arg ▲: Yalkowsky,37 ▼: Amend,9 ▪: PC-SAFT. Lys ▲: Amend,9 No PC-SAFT modeling due to a crystal change (ESI Fig. S32†). (e) AA with acidic substituents: Asn ▲: Dalton,7 ▼: Amend,9 ▪: PC-SAFT. Asp ▲: Apelblat,40 ▼: Amend,9 ▪: PC-SAFT. Gln ▲: Yu,42 ▼: Amend,9 ▪: Yalkowsky,37 ▪: PC-SAFT. Glu ▲: Matsuo,43 ▼: Amend,9 ◆: Yalkowsky,37 ▪: PC-SAFT. (f) AA with aromatic substituents: Phe ▲: Dalton,7 ▼: Amend,9 ◆: PC-SAFT. Tyr ▲: Yalkowsky,37 ▼: Amend,9 ◆: Lundblad,36 ▪: PC-SAFT. Trp ▲: Lundblad,36 ▼: Amend,9 ◆: Dalton,7 ▪: PC-SAFT.
AA with aromatic substituents

The aqueous solubility of the aromatic AA are very low (Fig. 2(f)) with order of Phe > Trp > Tyr. The solubility measurements from this work are in good agreement with the literature data. No crystal structure change is detected in the PXRD in aqueous solutions, which allows modeling by application of eqn (1).

Due to high sublimation/evaporation, the glass transition of Phe was unattainable, subsequently the heat capacity difference could not be determined. The heat capacity was estimated to be \( \Delta C_p^\text{sol}(T_\text{ref}) = 184.37 \, \text{J mol}^{-1} \, \text{K}^{-1} \) in order to maintain the FSC determined \( \Delta h^\text{sol}_\text{f} \) within its experimental uncertainty. Modeling solubility without taking into account of \( \Delta C_p^\text{sol} \) would predict a very low \( \Delta h^\text{sol}_\text{f} \), which is inconsistent with FSC data. This shows that the heat capacity difference is a very important property, which is unfortunately often neglected in thermodynamic modeling.

For Trp and Tyr the experimental melting properties applied in PC-SAFT are within the uncertainties of the FSC measurement. The predicted solubility of Phe, Trp and Tyr are in good agreement with the experimental solubility data.

AA with sulfuric substituents

The solubility order for sulfuric AA is Cys > Met (ESI Fig. S35†). A crystal structure change for Cys during the measurement was observed. Hence, solubility modeling with eqn (1) was not performed.

The experimental solubility for Met is consistent with the literature data.31 Unfortunately no melting properties could be measured using FSC. Thus solubility modeling is also not possible. No crystal change was observed for both Cys and Met.

Comparison to literature

The classical way of thermodynamic solubility model for components with inaccessible experimental melting properties are performed as follows: different \( g^\text{SAFT} \) models or equations of state were used to calculate the activity coefficients for eqn (1), while simultaneously fitting the melting properties to experimental solubility data. This procedure is still state-of-the art in the literature; however, the results of this approach differ strongly from the FSC-determined melting properties. Additionally, often applied in the literature solubility model differs from the eqn (1) used in this work. For example, the modified Apelblat equation

\[
\ln x^i_\text{f} = \exp \left[ A + \frac{B}{T} + C \ln(T) \right]
\]  

which fits the solubility with three independent parameters \( A, B \) and \( C \). In this case it is not possible to distinguish the proper melting properties and therefore the comparison to the FSC melting properties is not possible. For this reason the “right side” of each solubility model can be treated as the solubility product \( K_{SP} \), which consist of the solubility \( x^i_\text{f} \) and activity coefficient \( \gamma^i_\text{f} \).

\[
x^i_\text{f} \times \gamma^i_\text{f} = K_{SP} = f(T)
\]

\( K_{SP} \) depends only on the absolute temperature \( T \). This allows the comparison of different solubility models without accounting for the fitting to physically meaningful melting properties or purely adjustable fitting parameters (Fig. 3).

In Fig. 3 the solubility product of each AA is shown at \( T = 298.15 \, \text{K} \) and \( T = 323.15 \, \text{K} \). In some literature studies the melting properties were calculated by using group contribution methods without further applying it on solubility modeling.37,55 In this case, we applied eqn (1). However, regardless of how the melting properties/adjustable parameter was achieved, it is clear that the literature data differ to the solubility product determined in the current work. The predicted solubility based on the experimental melting properties is in good agreement with the experimental solubility, therefore the solubility product is more precise in comparison to other models in literature.

Conclusions

In this work nineteen proteinogenic AA (except Met) were characterized using FSC and the melting properties were successfully determined. It was shown that the experimentally determined melting properties are indispensable parts of solubility predictions using PC-SAFT. The access to the melting properties not only allows solubility prediction but also the quantification of the activity coefficients, which will give access to future model validation. The combination of FSC and PC-SAFT opens the door to predict solubility of solid compounds that decompose before melting.

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Conflicts of interest

There are no conflicts to declare.

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