Solubility and Metastable Zone Width of the Methionine Enantiomers and Their Mixtures in Water

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The characterizations of the ternary phase diagram and the metastable zone width are important issues for enantioseparation by crystallization procedures. In this work, a systematic study of the solubility and metastable zone width data of L-methionine, DL-methionine, and different mixtures of the enantiomers in water as solvent was performed. The eutectic composition in the chiral system in the presence of the solvent as an important characteristic for enantioselective crystallization was determined in the temperature range between (1 and 60) °C. A significant shift of this composition was observed.

Introduction

Amino acids are the building blocks of all proteins and therefore vital nutrients for humans and animals. The amino acid methionine is an essential sulfur containing amino acid that is found in nature as the L-enantiomer. In particular, poultry and pigs need considerable amounts of L-methionine in their food, but the L-methionine occurring naturally in the feedstuff (e.g., in soya grains, cereals) shows a significant deficit. Thus, it is necessary to close this gap by adding industrially manufactured methionine. DL-Methionine is manufactured from the primary materials acrolein and mercaptan. Principally, it is not necessary to separate racemic methionine into two enantiomers, since DL-methionine can be transformed into the desired pure L-methionine by the animal organism.[1,2] However, the enantiomer(s) of methionine can be gained by preferential crystallization using methionine derivates or the hydrochloride as feed material.[3]

In the literature, methionine is characterized as a racemic compound forming system with a eutectic composition of the enantiomers at an enantiomeric excess (ee) of 85 % at 25 °C in water.[4] Recently, it was shown that for racemic compound forming systems a hybrid process can be an interesting option to gain directly the desired enantiomer(s). In this case, at first an established separation technique (e.g., chromatography) is applied to provide an enantiomerically enriched solution. Subsequently, classical enantioselective crystallization or preferential crystallization is used to produce the pure enantiomer(s).[5,6] For chromatography and crystallization as the steps involved, the knowledge of the solubility and metastable zone width data is indispensable. Unfortunately, the reported solubility data of methionine are limited to discrete measurements for the enantiomers and the racemic compound.[2,7] Dalton and Schmidt[8] provided a polynomial to approximate the solubility curve of the racemic compound; Fuchs et al.[9] modeled it using the PC-SAFT equation of state. Extensive studies to specify the metastable zone width in the methionine system are not published to the knowledge of the authors.

In the present work, the results of a systematic study of the solubility and metastable zone width are reported. At first, solubilities of the pure substances L-methionine and DL-methionine in water in the temperature range between (1 and 60) °C are shown. Together with results for different mixtures of the enantiomers, the measured data allowed us to create the ternary phase diagram. Finally, the results for the metastable zone width of L-methionine, DL-methionine, and the eutectic mixture of the enantiomers (Eut-methionine) are presented. There, the data cover a temperature range from $t = (20$ to $50)$ °C.

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Experimental Section

Materials. (−)-L-Methionine (CAS no. 63-68-3), (+)-D-methionine (CAS no. 348-67-4), and (±)-DL-methionine (CAS no. 59-51-8) were supplied from Aldrich with purities of > 98 %. In the following, just the D-/L-nomenclature as commonly applied for amino acids is used. Mixtures were always prepared from one of the enantiomers and the racemate. As solvent, methanol (HPLC-grade) and deionized water were used.

Solubility Measurements. For solubility measurements, a classical isothermal method was applied. Calculated amounts of L-methionine or D-methionine, DL-methionine, or different mixtures of both were weighed and filled in small glass vessels of 5 mL total volume. A known amount of solvent, not sufficient to dissolve all the solid, was added. The suspensions were stirred at 400 rpm at constant temperature using thermostatted double jacketed devices. The uncertainty in the determination of the temperature was ± 0.1 °C. After 24 h, the suspensions were filtered. The liquid phases (filtrates) were weighed in a flask (m_{empty}) before ($m_{solution}$) and after evaporation to dryness at room temperature (m_{dry}) using an analytical balance. The resolution of the balance was ± 0.1 mg. The mass fraction solubility (w) is given as

$$w = \frac{m_{dry} - m_{empty}}{m_{solution} - m_{empty}} \quad (1)$$

The standard deviation of the solubility data (SD) was calculated according to eq 2, using the following notation: number of measurements (n), index (i), and solubility (w).

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$$SD = \sqrt{\frac{1}{n-1} \cdot \sum_{i=1}^{n} (w_i - \bar{w})^2} \quad (2)$$

The enantiomeric compositions of the equilibrated liquid phases were analyzed by HPLC using a Chirobiotic T stationary phase (column: 250 mm × 4.6 mm, 5 μm particles, Astec, USA). As eluent, a mixture of methanol (1) and water (2) ($\varphi_1 = 60$ %) was used. The chromatographic separation was performed at 25 °C and an eluent flow rate of 1 mL·min^{-1}. The wavelength used was 210 nm. To check for phase identity, the solid phases in equilibrium with the saturated solution were analyzed using X-ray powder diffraction (XRPD). The measurements were performed with an X'Pert Pro diffractometer (PANalytical GmbH, Germany).

Metastable Zone Width Measurements. Metastable zone width data for primary nucleation were determined for L-methionine, DL-methionine, and the eutectic mixture of both ((Eut)-methionine) in water using the polythermal method as described by Nývlt et al.[10] The experiments were performed in a magnetically stirred batch crystallizer of 60 mL volume in a temperature range between (20 and 50) °C. Solutions of about 40 g were prepared. After dissolution of all solid at a temperature 10 °C higher than the saturation temperature, the achievable subcooling (ΔT) of the solution was measured at different cooling rates between (2.5 and 15) K·h^{-1}. Nucleation was detected by an inline-turbidity sensor (QR-System; BASF AG, Ludwigshafen, Germany); for temperature measurement, a Pt-100 sensor was used. The uncertainty in the determination of the temperature was ± 0.1 K. The maximum possible subcooling (ΔT_{max}) determined as the desired metastable zone width was obtained by a linear extrapolation of the measured achievable subcooling (ΔT) to a virtual cooling rate "zero". The uncertainty in the determination of the maximum possible subcooling (ΔT_{max}) was ≈ ± 1.5 K.

Results and Discussion

Solubility Data of the Pure Enantiomer and the Racemic Compound. Figure 1 shows the average of the measured solubilities of L-methionine and DL-methionine in water compared to literature values. The obtained data are summarized in Table 1. Further, the calculated standard deviations for the solubility data are presented.

As can be seen, the solubilities determined in this work match well with the literature values available. Generally, methionine shows a low solubility in water. The solubility of the enantiomer L-methionine exceeds that of the racemate, DL-methionine. For both species, an upward trend of the solubilities with increasing temperature is observed. The increase in the mass fraction solubility of L-methionine and DL-methionine in the region from (1 to 60) °C is ≈ 5.4 %. The shape of the solubility curve for the enantiomer is linear (100 $w = 0.0896 \cdot t + 3.1902$, with $r^2 = 0.9961$) and for the racemic compound polynomial (100 $w = 8 \cdot 10^{-4} \cdot t^2 + 0.037 \cdot t + 1.6357$, with $r^2 = 0.9977$). This leads to a different dependence of the solubility on temperature. The standard deviations of the measured data (Table 1) give a first impression of the accuracy of the solubilities. The standard deviations are very small with highest values of ± 0.2 %.

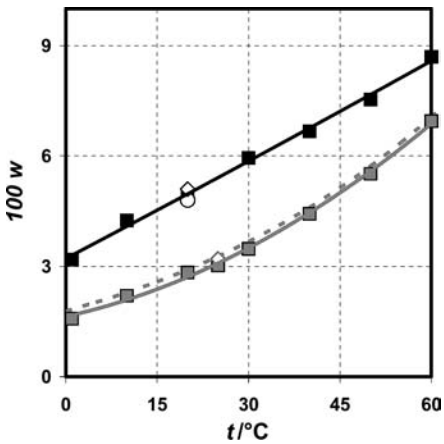

Figure 1. Average of the measured mass fraction solubilities (w) of L-methionine (black) and DL-methionine (gray) in water compared to literature data (□, present work; ○, ref 2; ◇, ref 7), (gray line, black line, present work; gray dashed line, ref 8).

Table 1. Number of Measurements (nr), Average Values, and Standard Deviations (SD) of Mass Fraction Solubility (w) for L-Methionine and DL-Methionine in Water

	L-methionine		DL-methionine	
t/°C	nr	100 w ± SD	nr	100 w ± SD
1	2	3.2	2	1.6
10	4	4.2 ± 0.0	4	2.2 ± 0.0
20	7	5.0 ± 0.0	8	2.8 ± 0.1
30	5	5.9 ± 0.1	5	3.5 ± 0.2
40	4	6.7 ± 0.1	4	4.4 ± 0.0
50	6	7.5 ± 0.1	6	5.5 ± 0.1
60	6	8.7 ± 0.2	6	6.9 ± 0.1

Solubility Data of the Eutectic Mixture. According to their relevance for compound forming systems, the solubility data for the eutectic composition of the enantiomers in the ternary system were measured. The results are shown in Figure 2. There, besides the solubilities, the corresponding eutectic composition of the enantiomers as a function of temperature is considered. The related data and the calculated standard deviations are summarized in Table 2.

As expected, the solubility of the eutectic mixture ((Eut)-methionine) exceeds the solubility values of L-methionine and DL-methionine and also rises with increasing temperature. The shape of the solubility curve of the eutectic mixture is polynomial (100 $w = 5 \cdot 10^{-4} \cdot t^2 + 0.0684 \cdot t + 3.8321$, with $r^2 = 0.9988$). Generally, for ideal systems, the composition of the enantiomers in their eutectic mixtures does not depend on the presence of the solvent and is independent of temperature. In the literature, up to now there is only the example of Tröger's base[11] reported where unequivocally the eutectic composition of the enantiomers shifts significantly with increasing temperature in the solvent ethanol and therefore differs from the binary case of the two enantiomers. In the case of the methionine enantiomers, this composition decreases from ≈ 88 % ee at 1 °C to ≈ 70 % ee at 60 °C and thus indicates a significant nonideality in the ternary system. The shape of the eutectic composition as a function of the temperature is polynomial (100 ee $= -2.6 \cdot 10^{-3} \cdot t^2 - 0.1393 \cdot t + 87.907$, with $r^2 = 0.9899$). The direction of the shift corresponds to Tröger's base case.[11] However, also the other direction is possible from the theoretical point of view. The published value for the enantiomeric excess of 85 % for a saturated eutectic solution at 25 °C in water[4] corresponds well to the result determined in this work.

Ternary Phase Diagram. Figure 3 comprises all measured solubility data of the methionine enantiomers, the racemic compound, and different mixtures of both in the solvent water in the ternary phase diagram.

It can clearly be seen that the solubility isotherms are of the typical shape of the racemic compound forming systems. Five solubility isotherms between (1 and 60) °C are considered. Solubility data measured exemplarily for the 20 °C isotherm

Table 2. Number of Measurements (nr), Average Values, and Standard Deviations (SD) of Mass Fraction Solubility (w) for Eutectic Mixtures of the Enantiomers ((Eut)-methionine) in Water and the Corresponding Eutectic Composition of the Enantiomers (ee) Measured

		(Eut)-methionine	
t/°C	nr	100 w ± SD	100 ee ± SD[a]
1	7	3.9 ± 0.0	87.9 ± 2.4
20	4	5.4 ± 0.1	83.8 ± 3.7
30	3	6.4 ± 0.1	81.3 ± 0.3
40	5	7.5 ± 0.1	79.3 ± 0.8
50	5	8.4 ± 0.1	73.7 ± 2.2
60	3	9.9 ± 0.1	70.4 ± 0.7

[a] ee - enantiomeric excess (ee = $[w_L - w_D]/[w_L + w_D]$).

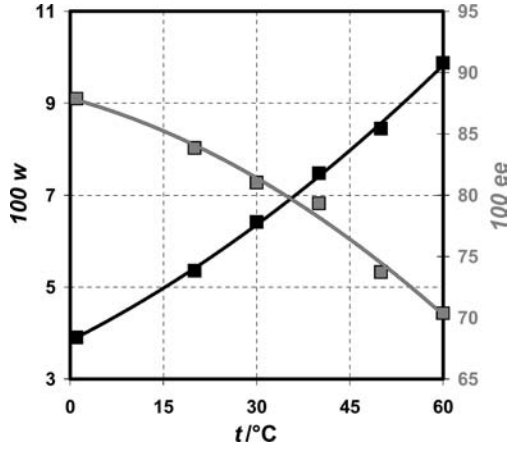

Figure 2. Average of the mass fraction solubilities of (Eut)-methionine in water (black square, w, gray) and the corresponding eutectic composition of the enantiomers (gray square, ee, black) as a function of the temperature (t).

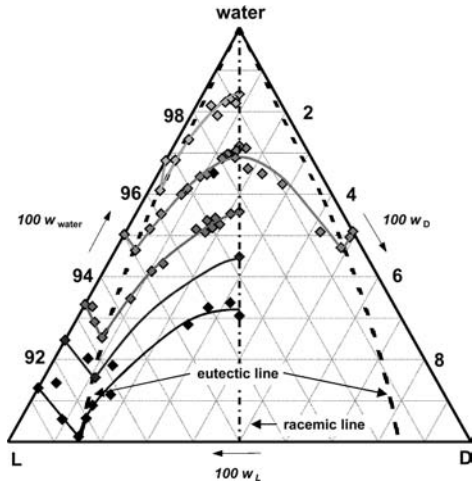

Figure 3. Ternary phase diagram of L-methionine (L) + D-methionine (D) + water. Just the upper part (upper 10 %) of the phase diagram is shown here for isotherms at 1 °C, 20 °C, 40 °C, 50 °C, and 60 °C (from top to bottom). The isotherm lines are just guides to the eyes.

prove the symmetry in the system. As discussed before, the solubilities increase with increasing temperature with maximum values for the eutectic mixtures. The shift of the eutectic composition of the enantiomers with temperature is reflected in the curved eutectic line, bended convex to a lower enantiomeric excess at higher temperature. No further polymorph or hydrate phase of the methionine enantiomers or the racemate were observed during the performed study.

Metastable Zone Width Data. In Figure 4, the metastable zone width data together with the solubilities of L-methionine and DL-methionine in water are presented as a function of temperature. The determined maximum possible subcooling ΔT_{max} and maximum nucleation-free supersaturation Δw_{max} for L-methionine, DL-methionine, and also their eutectic mixtures ((Eut)-methionine) in water are summarized in Table 3.

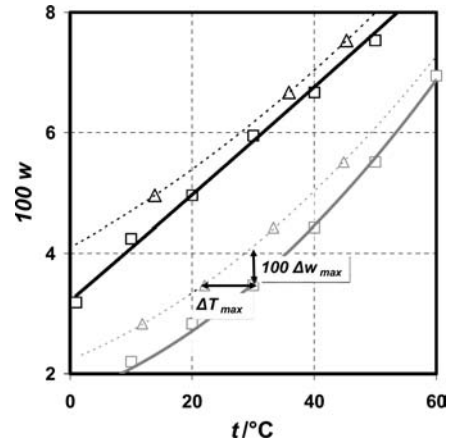

Figure 4. Metastable zone width with regard to primary nucleation for L-methionine (black) and DL-methionine (gray) in water as a function of temperature (t). black outlined square and line, gray outlined square and line, mass fraction solubility; black outlined triangle and dashed line, gray outlined triangle and dashed line, metastable limit.

Table 3. Maximum Possible Subcooling ΔT_{max} and Maximum Nucleation-Free Supersaturation Δw_{max} of L-Methionine, DL-Methionine, and (Eut)-methionine in Water

	L-methionine		DL-methionine		(Eut)-methionine	
t/°C	100 Δw_{max}	ΔT_{max}/K	100 Δw_{max}	ΔT_{max}/K	100 Δw_{max}	ΔT_{max}/K
25	0.4	4.7	0.7	8.9	0.7	7.4
35	0.3	3.4	0.6	7.0	0.7	6.7
45	0.3	3.2	0.5	5.4	0.6	5.1

It can be seen that in all cases the metastable zone width decreases with increasing temperature. In the case of DL-methionine and (Eut)-methionine, the measured ΔT_{max} and Δw_{max} values are very close to each other. For L-methionine, just about half of the maximum possible subcooling and the maximum possible nucleation-free supersaturation of DL-methionine is found. This different subcooling behavior might be explained with the differences in formation of the nuclei. Assuming the racemic compound exists only in the solid state, a recombination of heterochiral molecules is required previous to nucleation of the racemic compound in the solution. Further, it is known that impurities or also tailor-made additives can widen the metastable zone[12,13] which could here be the role of the counter-enantiomer. However, the results correspond to observations made in the racemic compound forming system mandelic acid + water.[14] Generally, the metastable zone in the methionine + water system is very tight.

Conclusion

In this contribution, the solubilities and metastable zone width for the chiral system of the methionine enantiomers in water have been studied. On the basis of the solubilities and the resulting ternary phase diagram, the methionine + water system could be clearly verified as a racemic compound forming system. It is characterized by a significant shift of the eutectic composition in the ternary phase diagram. With increasing temperature, the eutectic composition moves toward the racemic composition, i.e., to lower enantiomeric excesses. Thus, methionine is a further example for such a behavior next to Tröger's base. The exact knowledge of the eutectic composition is of particular

importance for designing crystallization-based enantioseparations. Generally, the low solubilities and the tight metastable zone observed in the system allow just for moderate yields of the desired crystallized product. First results for a crystallization based enantioseparation will be published soon.

Acknowledgment

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