Effect of Relative Solubility on Amino Acid Crystal Purity

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The ratio of the pure-component solubility of a primary solute to that of an impurity in the same solvent has a dramatic impact on crystal purity when lattice substitution is the dominant mechanism of impurity incorporation in the crystal. This is demonstrated for a model system of isoleucine and leucine in which leucine is the impurity. The pure-component solubility ratios of isoleucine to leucine in mixed solvents were measured and used to select solvents that could affect the lattice substitution of leucine in isoleucine crystals. Addition of electrolytes and/or cosolvents to aqueous solutions of the two amino acids improved the purity of isoleucine crystals obtained by cooling.

Introduction

The incorporation of impurities in crystals nucleated and grown from impure solutions can occur through a variety of physical and chemical mechanisms. These include inclusions (overgrowth of liquid containing both solute—defined here to be the primary crystallizing species—and impurity), entrapment of liquid in crystal irregularities and agglomerates, adsorption of the impurity on the crystal surface, wetting of filter cakes by residual liquid, lattice substitution, and lattice entrapment. The present work is concerned with lattice substitution, which is controlled by the thermodynamics of the solid-liquid system.

For lattice substitution to be a factor, the molecular structure of the solute and impurity molecules must be similar; in other words, an impurity molecule must fit into the lattice of the solute crystal without causing major stresses on the resulting lattice. Equilibrium between a crystal and the solution from which it is grown provides a limiting condition relative to the purity that can be achieved by crystallization. In the work reported here, a model solute (L-isoleucine) was crystallized from solutions containing a model impurity (L-leucine). The solvents used were mixtures of water with various cosolvents and/or additives selected to affect crystal purity.

The system chosen for study has importance beyond its role as a model for the phenomena being investigated. Specifically, fermentation is used to synthesize biologically active L-isoleucine, and small quantities of the isomorphic impurities L-leucine, L-valine, and α-amino butyric acid are also produced in that process. Evidence from prior experiments has shown that lattice substitution is the dominant mechanism by which the given impurities are incorporated in L-isoleucine crystals (Koolman and Rousseau, 1996; Zumstein and Rousseau, 1989). There is, therefore, an incentive to limit lattice substitution in amino acid systems.

It is well known that many crystallization characteristics are related to solute solubility. Relationships for the solubility of numerous amino acids have therefore been developed with respect to temperature, pH, and electrolyte or co-solvent concentration (Cohn and Edsall, 1943; Nass, 1988; Chen et al., 1989; Orella and Kirwan, 1991; Gupta and Heidemann, 1990; Kuramochi et al., 1996). Bézard et al. (1997) attributed variations in the distribution coefficient of an impurity to the solubility of that species. It is the purpose of the present work to explore the role that some of these variables play in determining crystal purity. The experiments performed were guided by the hypothesis that the pure-component solubility of the impurity in a given solvent relative to the pure-component solubility of the solute in that solvent provides an indicator of crystal purity.

The pure-component relative solubility α° is defined here as

$$\alpha^\circ = \frac{[\mathrm{ILE}]^\circ}{[\mathrm{LEU}]^\circ} \tag{1}$$

where each bracketed term is the pure-component solubility of the indicated species in a given solvent. These quantities

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(solubilities and corresponding $\alpha°$) were determined for each solvent examined and used to identify significant changes in solubility behavior. Effective solution additives were defined as those that gave values of $\alpha°$ significantly different from those in pure water.

Experimental Procedures

Solubility

Care was taken to clean and sterilize all equipment thoroughly to prevent contamination. Solubility experiments were conducted in a 150 mL jacketed glass equilibrium vessel equipped with three ports. One port was used for adding test solutions to the vessel, the second port was used to insert a capillary tube that served as a vent for the air being displaced when the test solution was added, and the third port was wrapped in aluminum foil and sealed with autoclave tape. The contents of the equilibrium vessel were kept at a constant temperature by water circulating through the jacket of the vessel from a programmable water bath. The bath provided temperature control of $\pm 0.01°C$.

Amino acid solutions were prepared in a sterilized glass vessel by adding pre-weighed quantities of the amino acid, pure deionized water, and co-solvent and/or electrolyte (as needed) in order to achieve the desired mol fraction or ionic strength of the components in the solution. The container was then sealed, and the contents were heated and stirred until the solution was homogeneous. The final conditions were held constant for about 30 min to ensure that no solid amino acid was present in the solution.

Then, 100 mL of the hot unsaturated amino acid solution was transferred to the sterilized equilibrium vessel using a heated syringe. Since bacteria and microbial organisms are typically 0.5 μm in size or larger, the solution was filtered through a 0.45-μm filter to remove any contaminants. The equilibrium vessel was also maintained at the elevated temperature of the solution being transferred, and its contents were continuously monitored for any signs of crystallization. The experiment was terminated if crystals were found to be present at the elevated temperature. The temperature set point of the circulating water bath was then lowered until crystals of amino acid could be visually observed. The final temperature was held constant for at least 8 h to ensure equilibrium, after which a sample was withdrawn from the liquid phase using a sterilized syringe fitted with a stylet. The syringe needle and stylet were immersed in the solution for about 10 min prior to sample withdrawal so that temperature equilibrium could be established. The stylet prevented any of the solution from entering the needle and crystallizing inside. After the liquid sample had been withdrawn, the stylet was removed from the core of the needle and a preheated 5-mL plastic syringe was attached to the Luer-Lok fitting of the needle. Approximately 2 mL of solution was withdrawn using this plastic syringe filtered through a 0.45-μm filter, and collected in a high-performance liquid chromatography HPLC vial containing a known mass of an internal standard solution. The entire sampling procedure was typically performed in less than 20 s to minimize the effect of heat losses from the syringe and its contents. The experiments were repeated at several values of the circulating water temperature.

The temperature of the water circulating in the jacket of the equilibrium vessel was measured with a type-T thermocouple calibrated against a standard platinum resistance thermometer (SPRT). A separate series of experiments was performed to determine the difference between the circulating water temperature and the temperature of the solution in the equilibrium vessel. This indirect method of temperature measurement was adopted in order to avoid any contamination of the solution from the thermocouple. The maximum standard deviation between the solution and circulating water temperatures was found to be 0.33°C. After adding the error from the SPRT calibration ($\pm 0.1°C$), a very conservative estimate of the accuracy of the temperature measurements is $\pm 0.5°C$.

Crystallizations and crystal purity

Crystallization experiments were conducted in the same 150-mL jacketed glass vessels that were used in the solubility measurements. Liquid samples were taken from the vessels using the same technique and the same stainless steel needles, syringes, and filters. Since the composition of the solid crystals was desired, a method of extraction, filtration, washing, and drying of the crystals was developed for these studies. In addition, these tests were run in duplicate to obtain estimates of the variability of the crystallization process and experimental errors.

Solutions of the desired concentration were prepared by weighing appropriate quantities of the components in a tared bottle placed on a top-loading balance. These solutions contained 5, 10 or 15 mol % impurity amino acid (on a solvent-free basis). The solutions were heated until all the amino acid was in solution, then cooled until crystals could be observed. After ensuring that the system was at equilibrium, samples of the liquid were withdrawn as described previously. After sampling the liquid, the bath circulator was turned off and the contents of the equilibrium vessel poured through a filter under vacuum. Once all the mother liquor had been drawn through the filter, the wet crystals on the filter paper were rinsed three times with a total of about 100 mL of water in order to wash away any adhering mother liquor. In the washing process, about one-third or more of the mass of amino acid crystals usually was dissolved, which ensured that the adhering mother liquor was entirely removed.

A vacuum was drawn on the funnel and its contents for at least 15 min in order to dry the crystals sufficiently. The filter paper with adhered amino acid crystals was then removed from the funnel and two 0.1-g samples of amino acid crystals were scraped from the filter paper. The samples were collected on weighing paper and added to separate empty 100-mL volumetric flasks. The amino acid samples were then dissolved in approximately 100 mL of purified water. After dissolution, 0.8 mL of each sample was pipetted into a vial and the relative amounts of amino acid were determined by HPLC analysis. Since the crystals consisted of only isoleucine and leucine, the ratio of product to impurity was obtained directly by HPLC analysis without requiring information on the concentration of amino acid in the sample solution. This sampling procedure was then repeated for a second sample.

Analytical procedures

Several analytical techniques were employed to quantify and characterize the various liquid and crystalline samples taken during the solubility and crystal-purity experiments. The concentrations of amino acid in the saturated liquid samples were measured via HPLC analysis. Similarly, initial and final amino acid liquid concentrations from the crystal-purity experiments were measured using HPLC. The degree of incorporation of amino acid impurity in the product crystals was also quantified using this chromatographic technique. Different dilution schemes, however, were used to prepare the liquid and solid samples for HPLC analysis.

GC analysis was used to check for evidence of co-solvent incorporation in the amino acid crystals. Finally, the effect of different electrolytes and co-solvents on the amino acid crystal morphology was verified by optical microscopy.

High Performance Liquid Chromatography. A Shimadzu HPLC system consisting of a SCL-10A controller, S1L-10A autosampler, two LC-10AS high-pressure pumps, and a SPD-10AV UV-visible wavelength detector was used to perform the chromatographic separation on a Rainin Microsorb-MV reverse-phase column. The voltage signal from the detector was interpreted by a Shimadzu CLASS-VP Chromatography Data System version 4.2 software program. The binary gradient used in all HPLC separations allowed the column to equilibrate at an aqueous methanol flow rate of 0.6 mL/min and a pure methanol flow rate of 0.4 mL/min for a total flow rate of 1 mL/min. Conventional amino acid derivatization and analysis procedures were followed to ensure accuracy and reproducibility of the data (Chang, 2000). The reproducibility of the chromatographic technique permitted accurate concentration measurements to ±0.5 g of isoleucine or leucine per kg of solvent. A representative chromatogram is provided in Figure 1 to indicate the separation achieved using this method.

The internal standard, L-valine, was retained in the column for about 3 min, while L-isoleucine and L-leucine were retained for about 5 and 5.5 min, respectively. Unretained components from the ortho-phthalaldehyde (OPA) solution used for derivatization of the amino acids were evident in the few peaks eluting after about 1 min. Additional details regarding the HPLC procedures are given in Givand (1999).

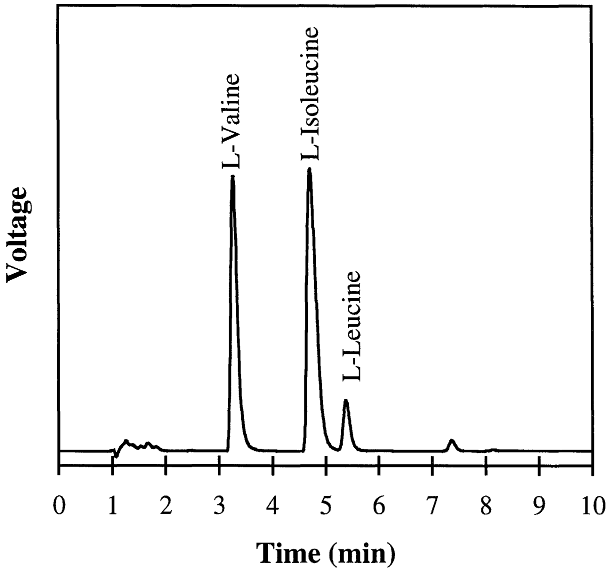

Figure 1. Typical HPLC separation of valine, isoleucine, and leucine.

Gas Chromatography. Torii and Iitaka (1971), Coll et al. (1986), and Koolman and Rousseau (1996) found that water was not part of the L-isoleucine and L-leucine crystal structures. However, the potential for inclusion or retention of other solvent species upon crystal growth from mixed solutions was examined. Gas chromatographic (GC) analyses using a Hewlett-Packard 6890 Series GC System and a phenylmethyl siloxane capillary column (30.0 m long, with a 0.32 mm outer diameter and 0.25 μm thickness) were performed on the crystals recovered from experiments involving a cosolvent. A flame ionization detector (FID) was used to detect the possible presence of *t*-butanol, dimethyl sulfoxide (DMSO), and ethylene glycol. Since quantification was not desired, calibration curves and reproducibility studies were not needed. Each of the components (isoleucine, leucine, *t*-butanol, DMSO, and ethylene glycol) were dissolved or mixed with water and injected into the capillary column to determine their respective retention times. Once the retention time of each component was known, crystal samples from the purity experiments were dissolved in water and analyzed to see if peaks corresponding to the appropriate co-solvent were evident. Details of the GC procedures are given in Givand (1999).

Optical Microscopy. Adverse effects on the morphology or stability are often encountered upon changes to the solvent composition from which a product is crystallized. For example, the morphology may change from cube-like to thin and needle-like, which can lead to filtering, tableting, or other processing difficulties. Optical microscopy was therefore used to study the effect of the chosen co-solvents and electrolytes on the isoleucine crystal morphology.

Crystals of isoleucine were collected by several means to study the morphology. At the completion of selected solubility and crystal-purity studies, samples of the crystals contained in the mother liquor were extracted using a pipette. Crystals were also collected and analyzed by allowing droplets of saturated solution containing the dissolved amino acid to evaporate. Occasionally, the different methods of crystal growth gave rise to different crystal morphologies due to different nucleation and growth kinetics.

The collected crystals were placed or grown on glass slides. The slides were then placed on the stage of the Leica optical microscope, which had a 10× eyepiece and 5 objective lenses for magnifications of 2.5×, 5×, 10×, 20×, and 50×. Transmitted or reflected light sources could be used to illuminate the sample. The light could be polarized if desired and used in a bright-field or dark-field mode for most of the lens objectives. Each combination of operating features allowed different crystal features to be viewed. Dark-field operation was particularly useful in detecting the boundaries and edges of crystals, as well as occlusions of solvent in crystal voids. Polarized transmitted bright field light was used most often for high-quality pictures and the ability to distinguish between overlapping crystals and different crystal faces. An analog video camera was used with the Image-Pro Plus software package and video capture computer card to take digital snapshots of the focused microscope image.

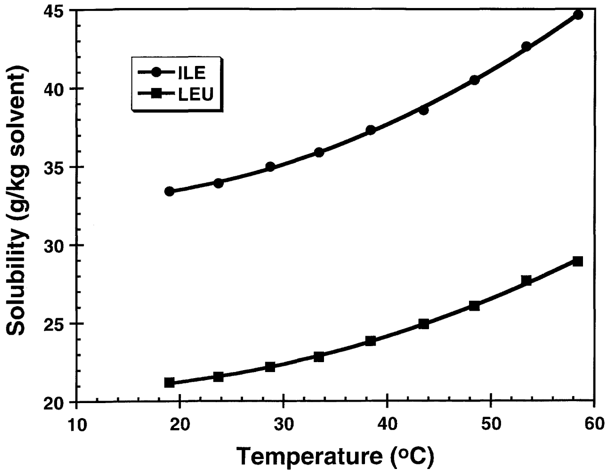

Figure 2. Pure-component solubilities of L-isoleucine and L-leucine in pure water.

Solubilities

Experimentally determined solubilities of L-isoleucine and L-leucine in pure water are shown in Figure 2. The L-isoleucine data correspond closely to those of Hade (1963) and Zumstein and Rousseau (1989), but differ significantly from D-isoleucine solubilities reported by Dalton and Schmidt (1935). The L-leucine solubilities were slightly below those obtained by Dalton and Schmidt (1935) and Budavari (1989). A more detailed presentation of the comparisons is given by Givand (1999). The pure-component solubility ratio [ILE]°/[LEU]° in pure water was determined from the measured solubilities and ranges from about 1.58 at 20°C down to about 1.55 at 60°C.

The effects of three electrolytes (calcium chloride, ammonium sulfate, and cupric chloride) and three cosolvents (*t*-butanol, dimethyl sulfoxide, and ethylene glycol) on the solubilities of L-isoleucine and L-leucine were examined in the present work. Experiments were also performed in which both an electrolyte and a cosolvent was added to the system. All cosolvent compositions are given in mol % while electrolyte concentrations are reported by ionic strength

$$\Gamma = \Sigma_i \frac{m_i z_i^2}{2} \tag{2}$$

where m_i represents the concentration of ion i in mol per kg of solvent and z_i is the valence of ion i.

Electrolytes. Cohn and Edsall (1943) reported a maximum in the solubility of cystine as the concentration of ammonium sulfate in aqueous solutions is increased. Should such a maximum exist for isoleucine and leucine and if the maxima are different, the behavior could provide the basis for selecting a solvent to enhance crystal purity.

Accordingly, isoleucine and leucine solubility data were collected at three different concentrations of ammonium sulfate ($\Gamma/2 = 0.75$, 1.62, 4.01) and are given in Table 1. As shown in Figure 3, both amino acids were salted out dramatically at the intermediate and highest $(NH_4)_2SO_4$ concentrations, but their solubilities were essentially unchanged at the lowest salt concentration. Even at the highest salt concentration, however, the pure-component solubility ratio [ILE]°/[LEU]° is insignificantly changed from the values for pure water over the 20°C to 60°C temperature range.

The solubility of L-leucine in aqueous solutions is reported to diminish upon the addition of simple salts such as NaCl and KCl and to increase with the addition of $CaCl_2$ (Cohn and Edsall, 1943). As shown in Table 2, both L-isoleucine and L-leucine were found to be salted-in by $CaCl_2$ at an ionic strength of 1.27. However, there was only a slight modifica-

Table 1. Solubilities (g Amino Acid/kg Solvent) of L-Isoleucine and L-Leucine in Aqueous $(NH_4)_2SO_4$ Solutions

T (°C)	Isoleucine			Leucine		
$(NH_4)_2SO_4$ ($\Gamma/2$)	0.75	1.62	4.01	0.75	1.62	4.01
19.0	32.46	28.70	20.63	20.77	18.28	13.27
28.7	33.73	30.35	22.17	21.91	19.35	14.13
38.4	37.04	32.92	24.96	23.85	21.28	15.89
48.4	40.11	36.17	26.52	26.08	23.15	17.32
58.4	44.60	40.40	31.18	29.16	26.48	20.47

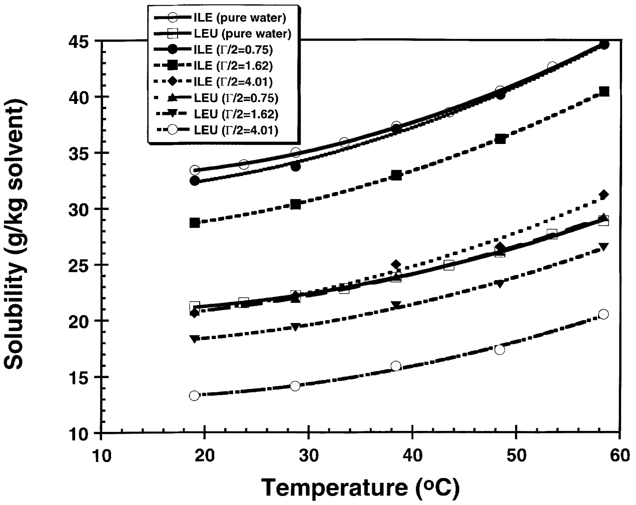

Figure 3. Pure-component solubilities of L-isoleucine and L-leucine in aqueous solutions of ammonium sulfate.

Table 2. Solubilities (g/kg Solvent) of L-Isoleucine and L-Leucine in Water, Aqueous Calcium Chloride ($\Gamma/2 = 1.27$), and Copper Chloride Solutions ($\Gamma = 0.56$)

	Isoleucine			Leucine		
T (°C)	H_2O	$CaCl_2$	$CuCl_2$	H_2O	$CaCl_2$	$CuCl_2$
19.0	33.56	36.50	34.53	33.40	23.60	6.47
23.7	33.85	37.24	—	33.90	24.17	—
28.7	35.28	—	34.10	—	34.97	6.29
33.4	35.89	39.86	—	35.86	26.07	—
38.4	37.29	—	34.55	37.28	—	6.57
43.5	38.25	43.76	—	38.54	28.53	—
48.4	40.22	—	34.56	40.46	—	6.24
53.4	42.13	47.81	—	42.60	31.90	—
58.4	44.58	—	36.87	44.62	—	7.03

tion in α° with the addition of $CaCl_2$. The addition of $CaCl_2$ was not, therefore, expected to have a significant effect on the purity of isoleucine crystallized from aqueous solutions containing both amino acids.

Li and Xu (1992) have shown that copper (II) complexes of the form $Cu \cdot (L\text{-}Ile)_2 \cdot H_2O$ and $Cu \cdot (L\text{-}Leu)_2$ are crystallized from copper-salt containing solutions of the respective amino acids. Furthermore, a means of affecting crystal purity may reside in the fact that L-isoleucine forms a hydrated crystal while leucine crystals are anhydrous.

Experimental solubility data of the two amino acids in $CuCl_2$ solutions of low ionic strength are presented in Table 2. Rather than enhance the ratio of pure-component relative solubilities, the copper complexes changed the system in such a way that the pure-component relative solubilities of the impurity, leucine, were even lower than in the water. Furthermore, isoleucine and leucine molecules exist as copper complexes in the crystalline state, which alters the original case of pure leucine lattice substitution within the pure isoleucine crystal. While transition metals such as copper may serve as effective precipitating agents for several solutes, further solubility and purity studies were not performed since the incorporation of the metal ions does not permit direct comparison to the reference crystalline isoleucine state.

Cosolvents. Isoleucine and leucine are only slightly soluble in water (less than 0.01 mol fraction) and essentially insoluble in organic and other solvents. For these and other reasons, the range of solutions examined in the present work was limited to aqueous mixtures. Furthermore, only those solvents forming miscible solutions were considered, which limited the cosolvents to light alcohols and highly polar organic compounds.

The solubility of several amino acids in aqueous solutions of methanol, ethanol, 1-propanol, and 2-propanol have been reported by Orella and Kirwan (1989, 1991). Leucine and isoleucine were shown to be salted out quite strongly in some of these alcohol solutions, and, therefore, these were not examined in the present research. In addition, larger alcohol species were not considered here because they are only partially miscible with water. The alcohol, *t*-butanol, however, is completely miscible with water and has not been considered by previous investigators.

Two concentrations of *t*-butanol (8 and 15 mol %) in water were used to quantify the impact of this additive on the solubility ratio. The measured solubilities are given in Table 3 and the solubility ratios are given in Figure 4. Both amino acids are salted out by *t*-butanol, and this cosolvent modifies somewhat the dependence of the resulting solubilities on temperature. The addition of *t*-butanol causes the reduction in the solubility of both amino acids with temperature to be much more pronounced. While addition of *t*-butanol favorably increases the yield from a crystallization process, Figure 4 shows that the pure-component solubility ratios $[ILE]^\circ/[LEU]^\circ$ remained relatively unchanged from the reference values in pure water. The scatter in the *t*-butanol data in Figure 4 is troublesome and currently unexplained.

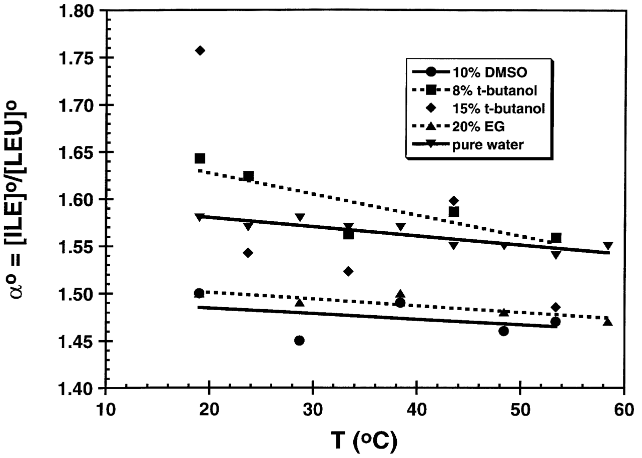

Figure 4. Pure-component solubility ratios in water and aqueous solutions of DMSO, of *t*-butanol, and of ethylene glycol.

A number of researchers have shown that the solubility of organic species in different solvents is influenced by the dielectric constant of the species (Hampe, 1986; Takei, 1988; Treivus, 1994). Because isoleucine and leucine show such different solubilities in pure water (Figure 2), cosolvents with dielectric properties similar to that of water were considered as candidates for modification of the relative solubility of isoleucine and leucine.

The dielectric constant of water varies from about 80 to 67 over the temperature range of 20°C to 60°C. The dielectric constants of dimethyl sulfoxide (DMSO) and ethylene glycol (EG) are about 50 and 40, respectively, at 20°C. This compares to a value of about 20 for methanol, ethanol, 1-propanol, and 2-propanol at that temperature. Both DMSO and EG are completely miscible with water.

Measured solubilities of the amino acids are given in Table 4 for solutions of 10 mol percent DMSO and for solutions of 20 mol percent EG in water. Both amino acids were substantially salted out by DMSO. Importantly, DMSO reduced the

Table 3. Solubilities (g Amino Acid/kg Solvent) of L-Isoleucine and L-Leucine in Aqueous *t*-Butanol Solutions

T (°C)	Isoleucine			Leucine		
t-Butanol (mol %)	0	8.0	15.0	0	8.0	15.0
19.0	33.56	15.41	13.02	33.40	9.38	7.41
23.7	33.85	17.38	13.05	33.90	10.70	8.46
33.4	35.89	21.67	17.21	35.86	13.87	11.30
43.5	38.25	27.73	23.14	38.54	17.48	14.48
53.4	42.13	33.48	26.97	42.60	21.48	18.16

Table 4. Solubilities (g Amino Acid/kg Solvent) of L-Isoleucine and L-Leucine in Aqueous 10 mol % DMSO and 20 mol % Ethylene Glycol Solutions

Cosolvent	DMSO		Ethylene Glycol	
T (°C)	ILE	LEU	ILE	LEU
19.0	9.88	6.59	13.44	8.98
28.7	11.02	7.62	15.86	10.65
38.4	13.34	8.97	18.80	12.57
48.4	15.91	10.88	22.31	15.07
53.4	17.51	11.91		
58.4			26.47	18.06

pure-component solubility ratio [ILE]°/[LEU]° to about 1.47; in other words, leucine is more soluble, relative to isoleucine, in the DMSO solution than it is in pure water. According to the hypothesis presented earlier, this is expected to reduce the inclusion of leucine impurity in the isoleucine crystal. It is also interesting to note that the dependence of the solubilities on temperature was essentially the same in DMSO solutions as they were in pure water.

The results with EG differ from those with DMSO. The solubilities of isoleucine and leucine are slightly stronger functions of temperature than with either pure water or aqueous solutions of DMSO. (Again, this property can be used to increase the yield in a crystallization process.) In addition, the reduction in absolute solubility of the two amino acids was not as dramatic with 20 mol % EG as was observed with 10 mol % DMSO. The reductions in solubility ratio were similar for both DMSO and EG. On a molar basis, however, the amount of DMSO required to reduce the solubility ratio from about 1.56 in pure water to about 1.47 was half that of EG.

Combined Additives. A significant reduction in the pure-component solubility ratio [ILE]°/[LEU]° was realized only with addition of either DMSO or ethylene glycol to aqueous solutions. High dielectric properties were common to both of these additives. DMSO has a higher dielectric constant and was more effective, on a molar basis, than ethylene glycol. However, the absolute solubilities of the amino acids were suppressed further with DMSO than ethylene glycol.

The reduction in the amino acid solubilities by DMSO could have an adverse effect on the usefulness of the solvent. On the other hand, at an ionic strength of about 1.27, $CaCl_2$ had little effect on the relative solubility, but increased the solubility of the amino acids by about 10 to 15 percent. In hopes of obtaining the best characteristics of each additive, solubilities of the amino acids in solutions containing both DMSO and $CaCl_2$ were determined.

Aqueous DMSO solutions were prepared containing $CaCl_2$ concentrations equivalent to solution ionic strengths of approximately 1.2 and 3.0. The solubilities of isoleucine and leucine were measured in these solutions and are given in Table 5. Clearly, calcium chloride enhanced the solubility of both amino acids in these solutions.

The [ILE]°/[LEU]° solubility ratio depends on the concentrations of DMSO and $CaCl_2$ in the aqueous solutions. Table 6 shows interpolated values of the pure-component solubility ratio at 25°C. Clearly, the impact of $CaCl_2$ on the pure-component solubility ratio is slight in comparison to the effect of DMSO. The objective of the solubility experiments was satisfied by identifying a solution in which the pure-component solubility ratio [ILE]°/[LEU]° was significantly lower than in pure water. On this basis, the aqueous 20 mol % DMSO solution containing $CaCl_2$ in a solution ionic strength of about 3.0 was proposed as a mother liquor from which to crystallize isoleucine to achieve improved purity.

Table 5. Solubilities (g Amino Acid/kg Solvent) of L-Isoleucine and L-Leucine in Aqueous Mixtures of DMSO and $CaCl_2$

	10 mol % DMSO		20 mol % DMSO			
$CaCl_2$(Γ/2) *T* (°C)	1.2 ILE	1.2 LEU	1.2 ILE	3.0 ILE	1.2 LEU	3.0 LEU
19.0	12.59	8.61	5.73	9.01	4.21	6.69
28.7	14.49	9.99	7.53	11.50	5.43	8.57
38.4	17.88	12.45	9.28	14.65	6.45	10.75
48.4	20.95	14.30	11.93	18.73	8.63	13.94
58.4	26.00	17.81	15.47	24.20	11.09	17.72

Table 6. Pure-Component Solubility Ratio $\alpha°$ in Solutions of DMSO and $CaCl_2$ at 25°C (Interpolated from Data)

	$CaCl_2$ Content (Γ/2)			
DMSO (mol %)	0	1.2	1.2	3.0
0	1.56	—	—	—
10	1.47	1.45	—	—
20	—	—	1.39	1.35

Effect of Amino Acid Impurities on Solubility. The presence of one amino acid in solution has been reported to affect the solubility of another amino acid (Jin and Chao, 1992). This effect also was observed for the isoleucine-leucine system in the present work. Two cases were examined: one in which the leucine content was about 4 mol % and another at 18 mol %. There was an insignificant increase in isoleucine solubility in the 4%-leucine solutions. At 20°C, however, the isoleucine solubility was about 10% greater in the 18%-leucine solution than in pure water. The salting-in effect diminished substantially with an increase in solution temperature, essentially disappearing at 55°C.

Crystal Purity

Crystallizations of isoleucine from aqueous solutions containing varying concentrations of leucine were conducted and established reference purities for resulting crystals. For comparison, crystallizations of the complementary system also were performed, that is, leucine was crystallized from aqueous solutions containing varying concentrations of isoleucine. The results of both sets of these experiments, which are given in Figure 5, show that the leucine-to-isoleucine ratio in isoleucine crystals is 26% greater than in the equilibrium

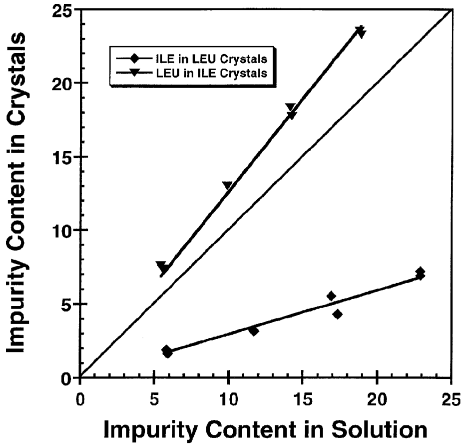

Figure 5. Impurity content of L-isoleucine and L-leucine crystals.

Compositions are in mol % on a solvent-free basis and *T* = 25°C.

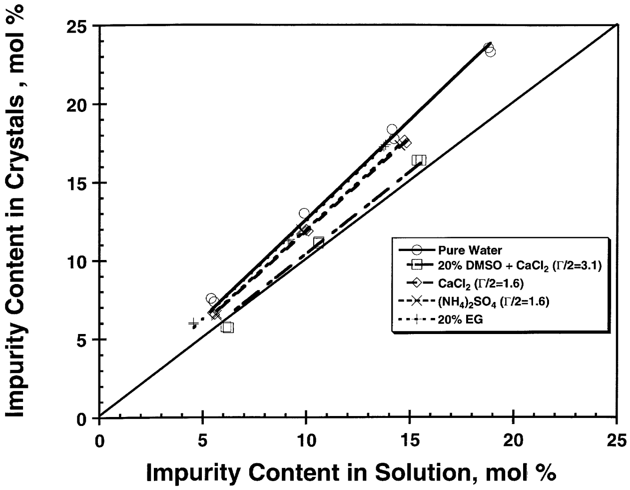

Figure 6. Effects of solution composition on the purity of L-isoleucine crystals.

Compositions are in mol % on a solvent-free basis and $T = 25°C$.

mother liquor; on the other hand, the isoleucine-to-leucine ratio in leucine crystals is about 30% lower in the leucine crystals than in the equilibrium mother liquor. Both data sets follow linear relationships between the impurity content of product crystals and the equilibrium mother liquors, and the lines through the data are linear fits through the origin. Finally, the limited scatter in the experimental data indicates precise and reproducible experimental techniques for liquid and crystal sampling and crystal washing.

It is interesting to note that the purities of isoleucine and leucine crystals plotted in Figure 5 satisfy the hypothesis upon which this research was formulated. Namely, since isoleucine has a higher solubility in pure water than does leucine (see Figure 2), the tendency of leucine to contaminate isoleucine crystals is greater than the tendency of isoleucine to contaminate leucine crystals. Subsequent experimentation tested the hypothesis and focused on the incorporation of leucine in isoleucine crystals, since this was considered to be the more severe problem.

The sensitivity of the lattice substitution mechanism to the solubility ratio was tested by performing a series of experiments in which isoleucine was crystallized from aqueous solutions containing various additives and was contaminated with initial concentrations of roughly 0.05, 0.10 and 0.15 mol of leucine/total mol total amino acids. The solutions were prepared at 65°C, cooled at a fixed rate to 25°C, and were held at that temperature for a 24 h period prior to sampling.

The effect of solvent composition is shown in Figure 6, which plots the impurity (leucine) content of the recovered crystals as a function of the final impurity content of the mother liquor. The solutions tested included calcium chloride in water with $\Gamma/2 = 1.6$, ammonium sulfate in water with $\Gamma/2 = 1.6$, 20 mol % ethylene glycol in water, and calcium chloride with $\Gamma/2 = 3.1$ in a 20 mol % DMSO in water solution. The effect of the solvent on resulting crystal purity qualitatively follows what is predicted from the hypothesis; as the solubility of the impurity relative to the primary species increases (because of a change in the solvent), the purity of the resulting crystals increases. This is most apparent for the solvent giving the largest change in the relative solubility: namely, the aqueous $CaCl_2$ solution containing 20 mol % DMSO.

To Sum Up

Prediction of the purity of crystals recovered from mother liquors containing small concentrations of nearly isomorphic impurities is difficult. The present work has shown that the solvent plays a significant role in determining crystal purity. Furthermore, a basis for adjusting the solvent composition to enhance crystal purity has been identified. Specifically, it has been shown that the ratio of the pure-component solubility of the impurity [imp]° to the pure-component solubility of the product species [prod]° is a key variable.

This behavior can be expressed in the following terms: define the mol fraction of the impurity in the recovered crystals as y_{imp} and the mol fraction of the impurity in the final mother liquor as x_{imp}. If it is now assumed that the crystals and the final mother liquor are in equilibrium, a distribution coefficient may be defined as

$$y_{\text{imp}} = K_{\text{imp}} x_{\text{imp}} \tag{3}$$

K_{imp} is the distribution coefficient for the impurity in the solvent-crystal system, and it has been shown to depend upon the pure-component solubilities of the system species.

$$K_{\text{imp}} = f\left(\frac{[\text{imp}]^\circ}{[\text{prod}]^\circ} \right) \tag{4}$$

The solubilities of both isoleucine and leucine were measured in different aqueous solutions of electrolytes and cosolvents. However, few solution additives were capable of substantially changing the [ILE]°/[LEU]° solubility ratio. Based on considerations of polarity and dielectric properties, dimethyl sulfoxide was identified as an effective cosolvent in modifying the solubility ratio. However, DMSO also substantially reduced the absolute solubility of the two amino acids. The observed salting-in behavior of calcium chloride was therefore exploited to enhance the absolute amino acid solubilities in aqueous DMSO solutions while sustaining the reduction in solubility. Isoleucine crystals obtained by cooling solutions of isoleucine and leucine in aqueous DMSO and $CaCl_2$ showed dramatic improvements in crystal purity. The present work therefore establishes a direct link between the relative solubility ratio (primary solute to impurity) and the resultant crystal purity.

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