The Solubility of Amino Acids in Mixtures of Water and Aliphatic Alcohols

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Crystallization is one of the most frequently utilized separation operations in the pharmaceutical industry as a purification step and for producing a solid product with a desired particle size distribution. This crystallization or precipitation is often effected by the addition of a miscible nonsolvent that drastically reduces the solubility of the solute in the mixture. In order to characterize the important phenomena in the crystallization of molecules that have biological significance, we have studied the fundamentals of the crystallization of biochemicals by nonsolvent addition using a model system: the crystallization of amino acids from mixtures of water and aliphatic alcohols.

Introduction

Peptides, proteins, antibiotics (representative of many pharmaceuticals), and amino acids can be loosely characterized as complex organic molecules that are comprised of various moieties or groups that can be ionic, dipolar, polar, or nonpolar in character. In solution, each of these groups experiences very different interactions with the other system components. As a result, the behavior and physical properties of such molecules in solution are very complex. With their different side chains, the amino acids represent one of the simplest sets of molecules that can be systematically examined in order to determine the importance of the different molecular interactions. In addition, knowledge of the solubility behavior of the solute is a prerequisite to examining the crystal growth and nucleation phenomena that are so important in an operating crystallizer.

Several reports have included the solubility of amino acids in mixed solvent systems, including methanol and ethanol [1, 2, 3]. One of the better known works in the area is that of Tanford, who used solubility data of amino acids in aqueous ethanol mixtures to define a hydrophobicity scale for proteins. The initial purpose of this study was to measure and correlate the solubility limits of amino acids in these mixed solvents. Later work will focus on measurement of the growth and nucleation rates of the amino acids and on modeling of the particle size distribution under various solvent conditions (utilizing the solubility data developed here). In this paper, we report on the solubility of five representative amino acids in systems containing propanol and water, and give a brief background of the thermodynamics governing the solubility.

Materials and Methods

Solubility Measurements

Distilled water and alcohol (Fisher ACS or Mallinkrodt Analytical Reagent) were weighed into an Erlenmeyer flask with ground glass joint with the use of a Mettler PC2000 balance. Excess amino acid (Sigma Chemical) was added, then the flasks were sealed with a stopper and placed in a shaker bath (Eberbach 6250). The shaker operated at 120 minutes⁻¹, while the temperature of the water bath was controlled by a Lauda refrigerating recirculator (RC 20) to within 0.1°C of the setpoint, which was 25°C. The accuracy of the setpoint was determined by comparison of the temperature measured by a thermometer calibrated by the National Bureau of Standards (Fisher). After two days, samples were removed and filtered through a 0.22 micron filter (Gelman Acro LC3A). The amount of solute in the filtrate was determined by the complete evaporation of solvent, HPLC, and in some cases, by both methods.

The pH of the solution was not adjusted after all the components had been added. Thus, the pH of the solution was near the isoelectric pH that was characteristic of the solvent.
composition. As a result, the amino acids were present in the zwitertionic form [4].

**HPLC Assays**

Amino acid concentrations were determined with the use of an HPLC system made up of a Waters model 510 pump, Gibson model 231 autoinjector, and a Waters model 481 variable wavelength UV detector. An isocratic method was used for all assays. Mobile phase (0.1 w % monobasic sodium phosphate, NaH₂PO₄, in distilled water) was pumped through a Whatman C₈ column (12.5 cm Partispher) at a flow rate of 0.5 ml/min. Diluted amino acid solutions were injected using the Gibson autoinjector and the concentration compared with external standards run on the same day. Quantitative results for the unknown samples were determined by using the peak area of the unknown. The concentration range of the unknown was adjusted by adding 0.1% sodium phosphate buffer so that the concentration of the diluted sample was in the range where sample concentration varied linearly with peak area. Solubilities determined by this method or by evaporation to dry solids were generally reproducible to within 5% for solutions containing water and about 20% for solutions that did not contain water.

**Results and Discussion**

An amino acid in the zwitterionic form is a strong dipole in solution. In addition, its side chain can be either hydrophobic or hydrophilic. The solubility of an amino acid can change dramatically as the solvent is switched from water to an alcohol. The relative solubilities of five amino acids in solutions of either 2-propanol or 1-propanol in water at 25°C are presented in Figures 1 and 2. The relative solubility, as defined here, is simply the solubility in a mixed solvent system divided by the solubility of the same amino acid in water. Examination of these figures shows that the relative solubility is reduced by three orders of magnitude for such amino acids as glycine, with no side chain, and L-asparagine monohydrate, with a polar side chain, when the solvent changes from water to either 2-propanol or 1-propanol. For amino acids such as alanine, isoleucine, and phenylalanine with a nonpolar side chain, the magnitude of the change is smaller.

The molecular interactions between the nonpolar side chain of an amino acid and the solvent(s) lead to a relative solubility in solutions with a small mole fraction of water that is higher than that for amino acids with a polar side chain. As a result, these solvents tend to be poorer crystallizing agents for amino acids with nonpolar side chains since there will be a higher yield loss in solution after the crystallization is complete. In fact, the data for phenylalanine illustrate that the interactions between the strongly nonpolar aromatic side chain and the solvents are so strong that the solubility in aqueous 1-propanol can be 25% larger than the solubility in water at the same temperature.

The effect of different alcohols on the solubility can be dramatic, as is illustrated in Figure 3 for phenylalanine. Here, the effect of methanol on the relative solubility [7] is small, while 1-propanol has a very pronounced impact. As a result, the choice of the solvent is important in terms of the yield that can be achieved (and also the rejection of impurities).

It must be recognized that the absolute values of the solubilities of each amino acid are very different at the same ratio of water to alcohol. The absolute solubility of several amino acids in water at 25°C are presented in Table 1. The absolute solubility is affected by the chemical potential of the solid phase and by the solution thermodynamics. However, to understand and characterize the relative power of various agents to reduce the solubility, it is easiest to examine the relative solubility.

The change in the relative solubility reflects a change in the activity coefficient of the solute as the composition of the solv-

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**FIGURE 1.** The solubility of several amino acids in mixtures of water and 2-propanol.

**FIGURE 2.** The solubility of several amino acids in mixtures of water and 1-propanol.
vent changes. If it is assumed that the chemical potential of the solid phase is not affected by the solvent and a single reference state is chosen for all solvent compositions (e.g., infinite dilution of solute in water), then the chemical potential and the activity of the solute in a saturated solution must be independent of the solvent composition. Thus, the relative solubility is equal to the ratio of the activity coefficient in a saturated aqueous solution to the activity coefficient in a mixed solvent.

Correlation and prediction of the relative solubility depends upon the ability to predict the activity coefficient in various solvents. Since the solubility of many amino acids is low in all solvent mixtures, the variation of the activity coefficient with solute concentration is negligible. The activity coefficients calculated from the solubility data therefore, are essentially the infinite dilution activity coefficients for each solvent composition. This makes it impossible to regress activity coefficient data from a set of binary data, as is often done (except for a few of the amino acids that have a significant solubility in water).

There are several possible methods that can be employed in order to correlate the activity coefficients. First, data for a single amino acid solute in mixtures of water and one alcohol were regressed using both a Margules and Wilson form for the activity coefficient of the solute [3]. Parameters were obtained for each set of data that gave excellent agreement with the data. But, data for a single solute in different alcohols could not be represented using a single set of values for the solute-water interaction. These representations are empirical at best, since the binary parameters that are common to more than one set of data do not have a unique value.

A second approach to the activity coefficient problem would be to utilize a group contribution method whereby the interactions could be correlated with the presence of various groups that make up the molecules. We attempted to use this approach by utilizing the UNIFAC [6] group contribution method. We have not been successful in finding a set of parameters that satisfactorily represents the current data base of amino acid solubilities in mixed aqueous solvents.

An attempt was also made to separate the activity coefficient into two terms that represent electrostatic and nonelectrostatic interactions [7]. Since the solute behaves as a strong dipole in solution, there are significant effects when the dielectric constant of the solvent is changed [8]. The nonelectrostatic term was modeled using both a Margules and Wilson form. Again, in order to fit data for a single solute, the parameters for the water-solute interaction were different for each alcohol system.

Finally, another method of considering the nonidealities that the solute exhibits is to define excess quantities. When considering the solubilities of gases in mixed-solvents, O'Connell and Prausnitz [9] calculated Henry's constant for the solute in the mixture in terms of the Henry's constants for the solute in each of the pure solvents. In a similar way, it is possible to define an ideal solubility equal to a mole fraction weighted average of the solubilities of the solute in water and pure alcohol. An ideal solution would be represented by a straight line (on a plot such as Figure 1) that connects the relative solubility in water and the relative solubility in alcohol.

The excess solubility is the ratio of the actual to the ideal solubility, and is unity for an ideal solution. The primary advantage of this method is that while the activity coefficient changes by orders of magnitude, the excess solubility does not exceed five in these systems, and is usually less than three.

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Literature Cited