Solubility of Amino Acids in Pure Solvent Systems

T. E. NEEDHAM, Jr.*, A. N. PARUTA, and R. J. GERRAUGHTY

Abstract □ The effects of various solvents and pH on the solubilities of glycine, L-alanine, L-valine, L-phenylalanine, and DL-aminooctanoic acid were studied in a series of pure aqueous and alcoholic solutions. The aqueous solubility was found to be inversely proportional to the size of the nonpolar portion of the molecule. A low nonaqueous solubility seemed to be due to a dominance of the amino acid by the charged α-amino carboxylic acid portion of the molecule. In aqueous and alcoholic solutions, an isoelectric band of minimum solubility was formed. A distinct increase in solubility proportional to the addition of acid or base was seen as the pH exceeded the limits of the isoelectric band. In the alcoholic solvent systems studied, the addition of either acid or base produced a greater divergence from the isoelectric pH than would be seen in a pure aqueous system

Keyphrases □ Amino acid solubility—pure solvent systems □ Alcoholic, aqueous systems—amino acid solubility □ Isoelectric pH—amino acid minimum solubility □ Acid, base addition effects—amino acid solubility, pure solvents

The importance of amino acids in biological systems is due to the unique properties of this class of chemical compounds. These substances, possessing both acidic and basic properties within the same molecule, are the building blocks leading to complex peptides and proteins which are the basic materials of life.

In 1933, Edsall and Blanchard (1) examined the dissolution and dissociation of several amino acids in aqueous systems. Other studies (2, 3) tabulated the solubility of several amino acids in water and ethanol and presented some theory as to their behavior.

At this time, much research has been done (4, 5) and is being conducted on the selective uptake of several amino acids by various body organs. The emphasis has been placed on qualifying the activity of the *in vivo* receptors. With this in mind, it was felt that a solubility study of a series of amino acids in a series of related solvents at pH above, at, and below the isoelectric point would be useful in predicting solubility patterns which might be related to the nature and properties of these amino acids and their behavior *in vivo*.

EXPERIMENTAL

Equipment—A rotating apparatus[1] capable of holding multiple samples was immersed in a water bath kept at constant temperature by a controlled temperature circulating pump[2]. The pH and apparent pH of nonaqueous systems (pH′) were measured using a combination electrode.

Systems Employed—The amino acids used in this study were glycine, L-alanine, L-valine, L-phenylalanine, and DL-aminooctanoic acid. The solvents used were water (which had been distilled and deionized), anhydrous menthanol, absolute ethanol, *n*-propanol, isopropanol, and tertiary butanol. The label purity of the manufacturer was accepted, and no pretreatment of the chemicals was felt necessary. The water was deionized using a mixed column bed to minimize the possibility of complex formation. Hydrochloric acid and sodium hydroxide were used to adjust pH. All work was performed at a temperature of 25 ± 0.2°.

[1] Menold Rotating Apparatus, Lester, Pa.
[2] Porta-temp., Precision Scientific Co., Chicago, Ill.

Table I—Molar Solubility of Amino Acids in Pure Solvents

Solvent Systems	Amino Acids[a] Glycine	L-Alanine	L-Valine	L-Phenylalanine
H_2O[b]	2.90	1.63	0.474	0.172
Methanol	0.009	0.012	0.013	0.022
Ethanol[b]	0.004	0.002	0.003	0.002
n-Propanol	0.011	0.002	0.003	0.002
Isopropanol	0.004	0.005	0.003	0.001
Tertiary butanol	0.004	0.001	0.007	0.001

[a] Each value is the average of at least three determinations. [b] Solubility in agreement with original data published by Cohn (7).

Procedure—The solubilities of the amino acids in pure solvents were all determined by the same method. At least four samples containing equal volumes of solvent were prepared, an excess amount of the selected amino acid was added, and the sample bottles were rotated in the constant-temperature bath for 24 hr. or until equilibrium was attained. Either 1- or 5-ml. samples were withdrawn from the sample bottles, using a pipet with a pledget of glass wool on the end as a filtering medium. The size of the sample to be withdrawn was determined by preliminary experimentation to minimize error during gravimetric analysis. The sample was immediately transferred to a tared vial, weighed to determine density, placed in an oven, and dried to constant weight. All drying was done at a temperature of 95° or less to avoid decomposition of the amino acids (6). Immediately after the sampling process, the pH or pH′ of each solution was taken.

In the nonaqueous solvents, the relationship between total amino acid solubility and pH′ was the main concern. In the pure aqueous solvent system, the experimental interest included salt formation as well as the difference in total solubility as a function of pH.

After initial sample withdrawal and pH′ check of the nonaqueous systems, incremental amounts of acid were added to half of the samples in succeeding 24-hr. periods, the pH′ was taken, and a gravimetric analysis was made on the samples. Base was added to the remaining samples and a similar analysis was performed. For the aqueous systems, the same analysis was performed as well as an additional procedure designed to quantitate the amount of salt present at a given pH. One hundred milliliters of water, a specified

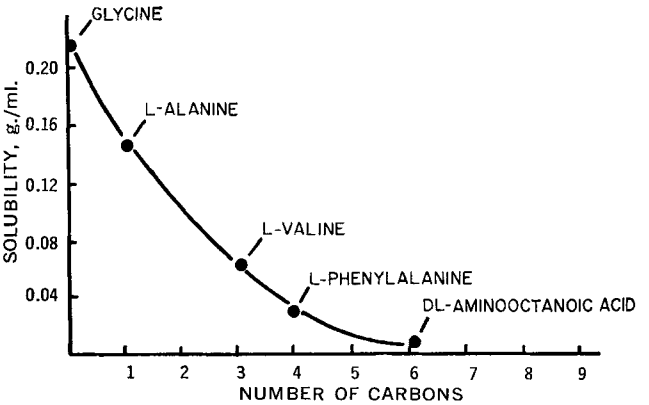

Figure 1—*Solubility of amino acids as a function of the number of carbons attached to the α-amino group.*

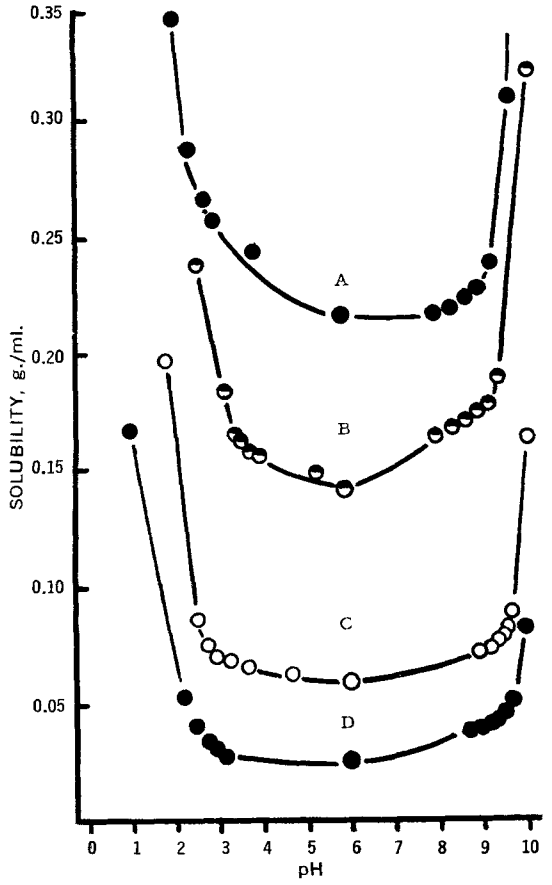

Figure 2—*Change in aqueous solubility of the amino acids as a function of pH. Key: Curve A, glycine; Curve B,* L-*alanine; Curve C,* L-*valine; and Curve D,* L-*phenylalanine.*

amount of HCl or NaOH, and an excess of amino acid were placed in a conical flask, capped, and mixed with a magnetic stirrer until equilibrium was reached. After 24 hr., at least fifteen 5-ml. samples were withdrawn from the flask and examined. To those samples previously mixed with HCl, five accurately measured incremental amounts of NaOH solution were added in sets of three each, so that the pH increased to just below the isoelectric point. Precipitation occurred and the samples were allowed to equilibrate for 24 hr. The amount of precipitate, the amount of combined amino acids remaining in the solutions, and the pH of the solutions were determined using the previously described procedure. A similar process was followed for the samples mixed with NaOH, except that HCl was added in incremental amounts to a pH just above the isoelectric point.

RESULTS AND DISCUSSION

The aqueous solubility of the series of amino acids studied seems to decrease with increasing length of the nonpolar portion of the molecule. If the α-amino carboxylic acid group of glycine is considered to be the primary polar unit of the amino acids, then an increase in the nonpolar portion of the amino acid molecules should produce a solubility decrease (7). The total solubility was due to the combined solubilities of the polar and nonpolar portions of the molecule. In Fig. 1, this expected solubility is shown by a plot of experimental data which shows a nonlinear decrease when the number of carbon atoms attached to the α-amino carboxylic group is increased. The assumption that the solubility of an aromatic ring is approximately equivalent to three CH_2 groups (2) accounts for the inclusion of L-phenylalanine at the fourth carbon, between L-valine and DL-aminooctanoic acid rather than at the seventh carbon, and further substantiates the adoption of glycine as a basic solubility unit.

Table II—Effect of Adding Acid on Total Aqueous Solubility of Amino Acids[a]

Amino Acid	*Y*-Intercept	Slope	Correlation Coefficient
Glycine	2.92	0.6583	0.9990
L-Alanine	1.67	0.2738	0.9988
L-Valine	0.514	0.4360	0.9948
L-Phenylalanine	0.208	0.2094	0.8818

[a] Moles/liter HCl as a function of amino acid solubility (moles/liter).

Table III—Effect of Adding Base on Total Aqueous Solubility of Amino Acids[a]

Amino Acid	*Y*-Intercept	Slope	Correlation Coefficient
Glycine	2.90	0.3986	0.9936
L-Alanine	1.75	0.4715	0.9978
L-Valine	0.458	0.5402	0.9968
L-Phenylalanine	0.167	0.5069	0.9910

[a] Moles/liter NaOH as a function of amino acid solubility (moles/liter).

As expected from Cohn's (7) work in ethanolic solvents systems, all alcoholic solvents decreased the solubility of the amino acids to a point considerably below that of water alone. Table I illustrates the magnitude of the reduction in solubility when solvents other than water were used. When the decrease in solubility from aqueous to nonaqueous media was compared (Table I), it was found that the percentage reduction in amino acid solubility was inversely related to the length of the nonpolar portion of the molecule. However, the molar solubilities in the alcoholic solvents were all of the same magnitude, which seemed to indicate that, in alcohols, the α-amino carboxylic acid group dominates the solubility characteristics of those amino acids studied.

The addition of acid or base to an amino acid in aqueous solution at the isoelectric point produced a change in the species present. The addition of HCl to a neutral solution of an amino acid produced cations in proportion to the first dissociation constant (K_1). Conversely, the addition of NaOH to a neutral aqueous solution of amino acid produced anionic species as a function of the K_2 (1).

$$K_1 = \frac{(H^+)\,(^+H_3NRCOO—)}{(^+H_3NRCOOH)} \qquad \text{(Eq. 1)}$$

$$K_2 = \frac{(H^+)\,(H_2NRCOO—)}{(^+H_3NRCOO—)} \qquad \text{(Eq. 2)}$$

The isoelectric point of those amino acids studied was defined (2) as the pH value at which the net charge of the dipolar molecule is equal to zero and pH = $^1/_2(pK_1 + pK_2)$. However, if the solubility is plotted as a function of the pH, as illustrated in Fig. 2, the presence of an invariant band of similar solubility over a range of pH

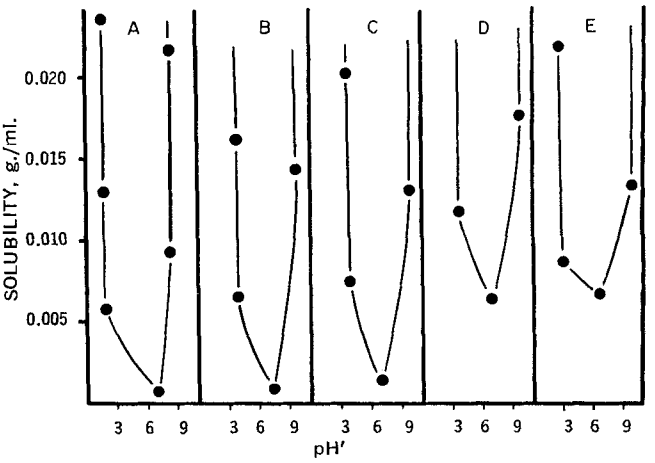

Figure 3—*Solubility of amino acids in pure methanol as a function of pH'. Key: Column A, glycine; Column B,* L-*alanine; Column C,* L-*valine; Column D,* L-*phenylalanine; and Column E,* DL-*aminooctanoic acid.*

Table IV—Effect of Acid and Base Addition on pH and Solubility of Glycine in Methanol and Water[a]

Water			Methanol		
pH	Total Solubility, g./ml.	Acid or Base Added, g./ml.	pH	Total Solubility, g./ml.	Acid or Base Added, g./ml.
2.7	0.3287	0.1417			
3.0	0.3036	0.0631			
3.8	0.2328	0.0080	2.20	0.0260	0.0224
4.4	0.2236	0.0041	3.30	0.0126	0.0074
5.3	0.2182	0.0002	3.25	0.0054	0.0037
6.2	0.2176	0.0000	7.05	0.0009	0.0000
6.8	0.2180	0.0004	9.65	0.0093	0.1053
8.1	0.2212	0.0073	9.75	0.0217	0.0107
8.3	0.2254	0.0142	9.9	0.0458	0.0213
9.0	0.2725	0.0622			
9.3	0.2957	0.1103			

[a] Each value is the average of at least three determinations.

on either side of the calculated isoelectic point is seen for each amino acid. This invariance is followed by increases in solubility as the pH is increased or decreased on either side of the band. The presence of an isoelectric band rather than a single isoelectric point was described by Cohn and Edsall (2). They postulated that for a simple amino acid, whose K_1 and K_2 are sufficiently separated, an isoelectric band is produced, throughout which the concentration of cations and anions is very low and the dipolar species dominates. This behavior can be further explained if Eqs. 1 and 2 are rearranged in terms of the Henderson–Hasselbach buffer equation:

$$\text{pH} = pK_1 + \log (C_A/C_S) \quad \text{(Eq. 3)}$$

$$\text{pH} = pK_2 + \log (C_S/C_A) \quad \text{(Eq. 4)}$$

where C_A is the solubility attributed to the dipolar ionic form of the amino acid, and C_S is the solubility due to the anion or cation that forms an associated salt with the added acid or base. It can be seen that when the pH is equal to the pK, the ratio of the C_S to C_A or C_A to C_S will be one, and the maximum buffer effect will be present. As expected, total solubility shows an increase upon addition of acid or base without significant change in pH.

It would seem that the total solubility of an amino acid is the sum of the solubility due to the zwitterion plus that due to the anion or cation. To examine this hypothesis quantitatively, the solubility range of each amino acid was separated into two parts: that influenced by acid addition and that influenced by base addition. A computer program was prepared to calculate, using a least-squares analysis, the first-degree equation and the correlation coefficients for two variables. The moles of acid or base added was designated as the independent variable, and the total molar solubility of the amino acid was designated as the dependent variable. In all calculations of total solubility, the assumption was made that the amino acid combined in a mole-to-mole ratio with the acid or base.

Tables II and III show the calculated first-degree equation parameters when X moles of either HCl or NaOH was added. The Y-intercepts indicate the initial solubility of the amino acid, and these closely approximate the experimentally observed values. In the general first-degree equation, $Y = mX + b$, the slope should be one if total solubility was equal to the solubility due to the salt form plus the initial solubility of the zwitterionic species. However, the calculated slopes show a deviation from the expected values. Since the intercepts closely approximate their intended values, these differences are probably due to a combination of acid and/or base with amino acid in other than the expected 1:1 ratio. It would seem that the half HCl salts mentioned in the literature (3) are not formed but that the reciprocal is true because more moles of acid are required per mole of salt formed. Since there is a rather wide deviation of slope from one amino acid to another, it would also seem that the ratio of the association salt formed depends on the individual characteristics of each amino acid, including the affinity of each amino acid for protons and the possible solute–solute interactions. This can be seen in Figs. 2 and 3 by examining the reciprocal behavior of the slopes upon addition of acid and base. This behavior may be due in part to the ability of the amino acid to accept or release hydrogen ions.

It can be seen from Fig. 3 that the addition of acid or base to a neutral solution of the alcoholic solvents produced a solubility profile similar to that seen in water. This figure illustrates the relative solubility of the amino acids in neutral solutions of methanol and the subsequent increase in solubility as the pH′ was varied. This illustration is representative of the solubility changes observed with variation of pH′ in each of the semipolar solvents tested. Note, for example, the increase in the pH′ at minimum solubility in neutral solution as compared to that seen in Fig. 2 for aqueous solvents. This change in pH′ was expected since methanol has a lower autoprotolysis constant than water. In ethanol, *n*-propanol, isopropanol, and tertiary butanol, the shift in the pH′ at minimum solubility was also attributable to difference in autoprotolysis.

Table IV illustrates the difference in solubility and pH′ as a function of acid or base added for glycine in water and methanol. In all semipolar solvents studied, the addition of either acid or base produced a greater divergence from the isolectric pH than would be seen in a similar aqueous system. It would seem that this change in behavior is mainly due to a lower buffer capacity produced in these less solubilizing solvents.

In conclusion, for the amino acids studied in aqueous solvent systems, a band of minimum solubility was found at pH values above and below the isoelectric point and a distinct increase in total solubility was seen as the pH exceeded the limits of the isoelectric band. Further, the increase in total solubility of the amino acids was linearly related to the moles of acid or base added. In the alcoholic solvents, a similar solubility–pH profile was found; but due to the lower solubility of the amino acids in these solvents, the addition of acid or base produced a greater divergence from the isoelectric pH than was seen in aqueous systems. The isoelectric region in the alcoholic systems seemed to be shifted as a function of the autoprotolysis constant of the specific solvent.

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* To whom all requests for reprints should be directed.