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The Solubility of Amino Acids in Various Solvent Systems

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THE SOLUBILITY OF AMINO ACIDS

IN

VARIOUS SOLVENT SYSTEMS

BY

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TITLE ABSTRACT

SOLUBILITY OF AMINO ACIDS

ABSTRACT

The effect of varying solvent systems on the solubilities of glycine, L-alanine, L-valine, L-phenylalanine, and DL-amino-octanoic acid at 25° C. was studied. The entire concentration spectrum from pure water to pure semipolar solvent was used for each of the solvent systems of methanol-water, ethanol-water, n-propanol-water, isopropanol-water,, and tertiary butanol-water. Further, the effect of pH variation on the solubilities of the amino acids in each of the solvent systems was studied. Aliquots were withdrawn from each solvent system studied and analyzed gravimetrically to determine the resultant solubility. It was found that the solubility behavior of those amino acids studied was a function of the constant effect of the α-amino carboxylic acid portion of the molecule and the independent interactions of the remaining neutral portion of the molecule. Maximum solubility was found in pure water with a reduction to low solubility in the semipolar solvents in the order of a second degree polynomial equation. To each percent strength of the hydroalcoholic solvent systems, the ratio of water to alcoholic molecules per amino acid molecule remains constant. This would indicate that the lengthening of the nonpolar portion of the chain from the hydrogen of glycine to the methyl

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phenyl of L-phenylalanine does not affect the orientation of the water to alcohol molecules in the solvent system. Each of the amino acids studied demonstrated an ability to differentiate between the hydroalcoholic solvents used. The quantitative order of solubility in the solvent systems used varied for each amino acid, but similar solubility profiles could be seen for the entire solvent series. Variation of pH in pure aqueous solvent systems produced an isoelectric band of invariant solubility with a distinct increase in solubility above and below this band. The increase in total solubility of the amino acids is directly proportional to the number of moles of acid or base added. As the non-polar portion of the amino acid molecule increased, the total resultant solubility per mole of acid or base added also increased.

In an aqueous system, the total solubility is equal to the sum of the original zwitterion solubility plus the solubility of the salt that was found. For those amino acids studied, only single salts were formed. In the hydroalcoholic solvent systems, variation of pH produced minimum solubility at the isoelectric point with no distinct isoelectric band seen. As the percent alcohol increased in those solvent systems studied, similar increments of acid or base added to the system produced a proportionally greater increase in the magnitude of total solubility of the amino acid. This might be attributed to an increase in the importance of the charge species as the polarity of the solvent system decreased as well as the increased affinity of the amino acid for the Na^+ or Cl^- ion present.

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DOCTOR OF PHILOSOPHY THESIS

OF

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UNIVERSITY OF RHODE ISLAND

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I. INTRODUCTION

The importance of amino-acids in viable biological systems is due to the unusual properties of this class of chemical compounds. These substances, possessing both acidic and basic properties within the same molecule, afford the basic linkages leading to complex peptides and proteins, the basic materials of life. Further, ten such amino-acids have been shown to be essential for life processes. Again, due to the basic and acidic character in a given molecule, these substances are dipolar compounds, with either zwitterion species formation or anionic or cationic species formation depending upon the acidity of the fluid environment. The relative concentration of these species, which coexist with each other, depend completely upon the value of pH.

Since there are dissociable groups in these molecules, many studies have been performed to determine the acid and base dissociation constants for the individual amino acids. Cohn and Edsall (1) have tabulated the constants for a large series of these compounds.

In 1933, Edsall and Blanchard (2) postulated that dissociation

1

of the alpha amino acids in aqueous solution was as follows:

$$
\begin{array}{ccc}
& \overset{+}{NH_3} & \\
& R\text{-}C\text{-}COO^- & \\
\end{array}
$$

NH$_3^+$
R-C-COO$^-$

H$^+$ H$^+$

NH$_3^+$ NH$_2$
R-C-COOH   R-C-COO$^-$

H$^+$ H$^+$

NH$_2$
R-C-COOH
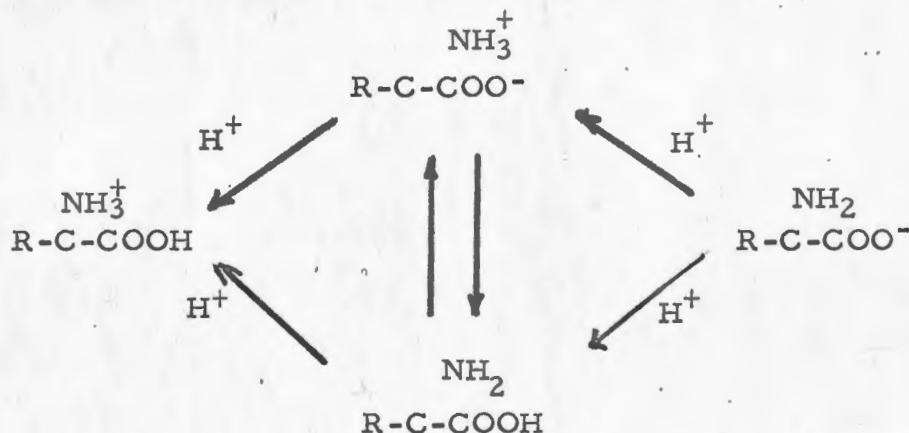
They believed that the charged and uncharged forms were in equili-
brium. Further, they expanded a method developed by Ebert (3) to
calculate the K_z of these systems. This ratio of charged to uncharged
ions in solution was found to be in the range of 10^4 to 10^6 for most
aliphatic amino acids. This theory of the zwitterion or dipolar type
configuration has been further substantiated by Fox and Foster (4).

An important property of the amino acids is that they exhibit an
isoelectric point. This is the point in an electric field at which the
zwitterion form will not migrate to either the anode or cathode (5).
Also, at the isoelectric point, the sum of the net charge is zero. If
we represent the isoelectric point as pI and the dissociation constants
as pK_1 and pK_2, then (6)

$$pI = 1/2\ pK_1 + 1/2\ pK_2$$

Literature values of solubility for amino acids are usually
expressed as the solubility in pure water or in aqueous systems

adjusted to a pH equal to the isoelectric point (7). Since the charged

form of the amino acid would be dominant, it was assumed that this

type of compound would exhibit a high degree of solubility in water.

It was postulated that amino acids would be relatively insoluble

in non-aqueous systems due to the inability of the solvents to interact

with charged molecules. Blanchard and Edsall (8) studied the solubility

of amino acids in several alcohol-water mixtures, and listed the K_z

values of some of the amino acids in concentrated alcoholic solutions.

They theorized that solvent systems having lower dielectric constants

than water should give a lower ratio of charged to uncharged molecules.

Statement of the Problem

It was felt judicious to study the solubility of a series of simple alpha amino acids as glycine, L-alanine, L-valine, L-phenylalanine, and DL-amino-octanoic acid in a series of pure and mixed solvent systems and at varying pH's above, at, and below the isoelectric point.

It was hoped that some discernable solubility patterns might be evidenced which could be related to the nature and properties of either the amino acids used or the solvent systems studied. Further, it was thought desirable to attempt to relate the solubilities observed to the content of the species at any given pH in the solvent systems studied.

II. EXPERIMENTAL

Equipment - A standard rotating solubility apparatus[1] capable of holding multiple samples was immersed in a water bath kept at constant temperature by a heating and circulating pump[2]. The pH and apparent pH's (pH') were measured with a Leeds-Northrup pH meter[3] using a combination electrode. Gravimetric determinations were carried out using a constant temperature drying oven and a Mettler Balance[4]. All water used in pure and mixed solvent systems was deionized using a Barnstead Bantum Demineralizer[5].

---

[1] Menold Rotating Apparatus, Lester, Pennsylvania

[2] Porta-temp, Precision Scientific Co., Chicago, Illinois.

[3] Leeds-Northrup pH Meter Model #7401 with AHT single Electrode #4858-a 15.

[4] Mettler Instrument Corp., Hightstown, New Jersey.

[5] Barnstead Still & Steriline Co., Boston, Massachusetts.

Materials - The amino-acids used in this study were as follows: glycine,[1] L-alanine,[2] L-valine,[3] L-phenylalanine,[4] DL-amino-octanoic acid.[5]

For the purposes of this study, label purity of the manufacturer was accepted and no pretreatment of the solvents was felt to be necessary.

The water used was deionized using a mixed bed column to a concentration of less than 10 ppm of NaCl to minimize the possibility of complex formation with any metals usually present (9). The other solvents used were absolute anhydrous methanol,[6] absolute ethanol,[7] n-propanol,[8] iso-propanol[9] and tertiary-butanol.[10] All solvents had a listed water content of 0.2% or less, and a boiling point range of 2 degrees or less.

The hydrochloric acid[11] and sodium hydroxide[12] used were solutions of analytical reagent grade.

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[1] Lot 070381 - Aldrich Chemical Company, Milwaukee, Wisconsin
[2] Lot 113071 - Aldrich Chemical Company, Milwaukee, Wisconsin
[3] Lot 070381 - Aldrich Chemical Company, Milwaukee, Wisconsin
[4] Lot 102471 - Aldrich Chemical Company, Milwaukee, Wisconsin
[5] Eastman Organic Chemicals, Rochester 3, New York
[6] Mallinckrodt chemicals, New Jersey
[7] U. S. Industrial Chemical Company, New York
[8] Allied Chemical Company, New Jersey
[9] Allied Chemical Company, New Jersey
[10] Mallinckrodt Chemicals, New Jersey
[11] Mallinckrodt Chemicals, New Jersey
[12] Mallinckrodt Chemicals, New Jersey

<u>Methods</u> - Two types of systems were used in this study. The first type of system was the pure and mixed solvents including water, methanol, ethanol, n-propanol, isopropanol and tertiary butanol and mixtures of each with water. The solubility of each of the amino acids used in the study was found in the pure solvent systems and in the mixed solvents systems of 0%, 10%, 30%, 50%, 70%, 90% and 100% (v/v) of methanol-water, ethanol-water, n-propanol-water, isopropanol-water, and tertiary butanol-water. In the second type system, the solubility of each of the series of amino acids was varied by addition of either acid or base.

<u>Procedure</u> - For the first part, the same basic procedure was used for all solvent systems. For each percent strength which was evaluated, three samples of equal volume were prepared from a stock solution. Into each sample bottle, an excess amount of amino acid was added, and the bottle was covered with a cap lined with an inert teflon liner. Since initial studies indicated that the amino acids reached a maximum solubility in one-half to 1 hour, a preliminary rotation period of one hour at room temperature was performed to insure that excess amino acid remained. The samples were then placed into a water bath, which was maintained at a temperature of $25^\circ \pm 0.2^\circ$, and allowed to reach equilibrium. In 24 hours, either one ml. or five ml. samples were withdrawn from the sample bottles using a pipette with a pledget of

glass wool on the end as a filtering medium. The size of the sample

withdrawn from the bottles was determined by preliminary experimen-

tation in order to minimize the possible error during gravimetric

analysis. The withdrawn sample was immediately transferred to a

tared vial which was then weighed to determine density. The series

of tared vials was then placed in an oven to be evaporated and dried to

constant weight. The evaporations were made at a temperature of

95° C. or less to avoid decomposition of the amino acids. (10)

Immediately after sample withdrawal, the pH or pH' of each solvent

system was taken.

In the second part, the interest was twofold and a procedure

was designed to maximize the experimental precision in each case.

Firstly, in the pure aqueous solvent systems, the interest was in

salt formation as well as the variation of total solubility as a function

of pH. A procedure was developed to quantitate the amount of acid or

base present at any given pH. One hundred milliliters of water, a

specific amount of an HCl or NaOH standardized solution, and an excess

of amino acid was mixed in an erlenmeyer flask which was then capped

and placed on a magnetic stirrer and allowed to reach equilibrium. In

twenty-four hours, a series of at least fifteen 5-milliliter samples were

withdrawn from the flask and placed in tared vials. To those samples

previously mixed with HCl, 5 accurately measured different amounts of

NaOH solution were added in sets of 3 each, so that the pH increased to below that of the isoelectric point. After precipitation, these samples were allowed to equilibrate for 24 hours. The amount of the precipitate as well as the amount of the combined amino acids remaining in the solutions and the pH of the solutions were determined using the procedure described in section one for gravimetric analysis. A similar process was followed for the samples mixed with standard NaOH except that HCl was added in incremented amounts to a pH above that of the isoelectric point.

In the non-aqueous solvents, the relationship between total amino acid solubility and pH' was the main concern. Initially, four samples of equal volume were prepared for each strength to be evaluated. Excess amino acids were added to each sample, the preliminary rotation performed, and the samples placed into the water bath at 25° C. and allowed to equilibrate. In 24 hours, one ml. samples were withdrawn, the pH or pH' taken, and the samples were analyzed gravimetrically. Into two of the samples, incremental amounts of acid were added in succeeding 24 hour periods and the pH or pH' as well as a gravimetric analysis performed on the aliquots taken during each period. Into the remaining samples, base was added and a similar analysis prepared. The sample size and number of samples was adjudged sufficient since good agreement was found among succeeding samples for the increase in total solubility of the amino acids.

III. RESULTS AND DISCUSSION

The solubilities of the amino acids studied were determined in
water, methanol, ethanol, n-propanol, isopropanol, and tertiary
butanol at 25° C.

To illustrate the physical and chemical relationships of the
amino acids studied, their structural formulas are shown in Table 1.
It can be seen that all the amino acids studied were alpha amino acids.
The D or L prefixed to the name of the amino acid indicates the con-
figurational relationship of the structural groups about the asymetric
carbon atom. A DL prefix indicates a racemic compound or optically
inactive mixture of the stereoisomers. A (+) or (-) after the initial
letter prefix may be used to show the direction of rotation of polarized
light, but is not usually necessary since no differences in physical or
chemical properties are induced by this property. (11)

It can be seen that variation in solubility characteristics of these
amino acids can be attributed to differences in their chemical structure.
Starting with glycine as the basic unit, L-alanine is α-methyl glycine
and the difference in solubility would then be a result of the special
configuration and polarity effect of the additional methyl group. Further,
in this series, the effect of the following α groups are being studied:
the isopropyl group, the methyl phenyl group, and the six carbon
n-alkyl group.

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TABLE 1.

MOLECULAR STRUCTURE OF THE AMINO ACIDS

Glycine H_2N-CH_2COOH

L-Alanine $CH_3-\overset{NH_2}{CH_2COOH}$

L-Valine $\underset{CH_3}{\overset{CH_3}{}}CH-\overset{NH_2}{CH}COOH$

L-Phenylalanine $-CH_2-\overset{NH_2}{CH}-COOH$

DL-Amino-octanoic Acid $CH_3(CH_2)_5-\overset{NH_2}{CH}-COOH$
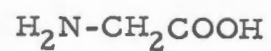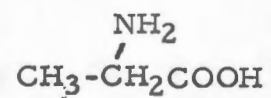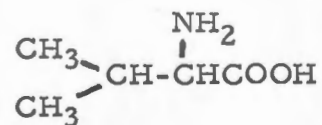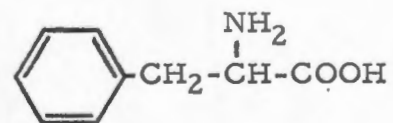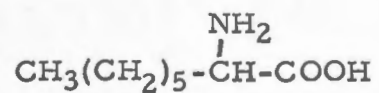
By use of Raman Spectroscopy (12, 13), melting point data (14), changes in molecular volume (15), and dielectric constant measurements (16), the amino acid has been shown to exist as dipolar ions in solution. The zwitterionic character of the amino acid gives it a high aqueous solubility due to its attraction to the dipolar water molecules. Table 2 shows the solubility of the amino acids studied in water at 25° C. These values correspond to a good degree with the range of literature values previously reported. (17, 18)

Since glycine is being considered the basic common structural unit in each of the amino acids, its use as the standard in comparing solubility differences of the other amino acids would logically follow. Table 3 illustrates the ratio of solubility of an amino acid in gm./ml. as compared to the solubility of glycine in water. It can be seen that the addition of a methyl group to the basic glycine molecule reduces solubility by 34 percent, while the addition of the isopropyl group reduces solubility by 73 percent.

The effect on solubility by the addition of CH_2 groups and by aromatic substitution is further illustrated in Figure 1, where the solubility of each amino acid in gm./ml. is plotted against the number of carbons in that structure attached to the basic glycine unit. A smooth non-linear correlation is observed for the measure of decreasing polarity in the alkyl substituted amino acids. In the case of L-phenylalanine, it can be assumed that the solubility of an aromatic

TABLE 2.

SOLUBILITY OF AMINO ACIDS
IN A
PURE AQUEOUS SOLVENT SYSTEM AT 25° C.

Amino Acid	Solubility[a](gm. /ml.)
Glycine	.2178
L-Alanine	.1450
L-Valine	.0559
L-Phenylalanine	.0294
DL-Amino-octanoic acid	.0005

[a]Each solubility is the average of at least 4 determinations.

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TABLE 3.

THE RATIO OF THE AQUEOUS SOLUBILITIES
OF AN AMINO ACID TO THAT OF GLYCINE AT 25° C.

Amino Acid	Ratio[a] ($\frac{S_x}{S_{glycine}}$)
Glycine	1.0
L-Alanine	0.66
L-Valine	0.27
L-Phenylalanine	0.14
DL-Amino-octanoic Acid	0.002

[a]Each solubility used in calculating the ratio is in gm./ml. and is the average of at least eight determinations.
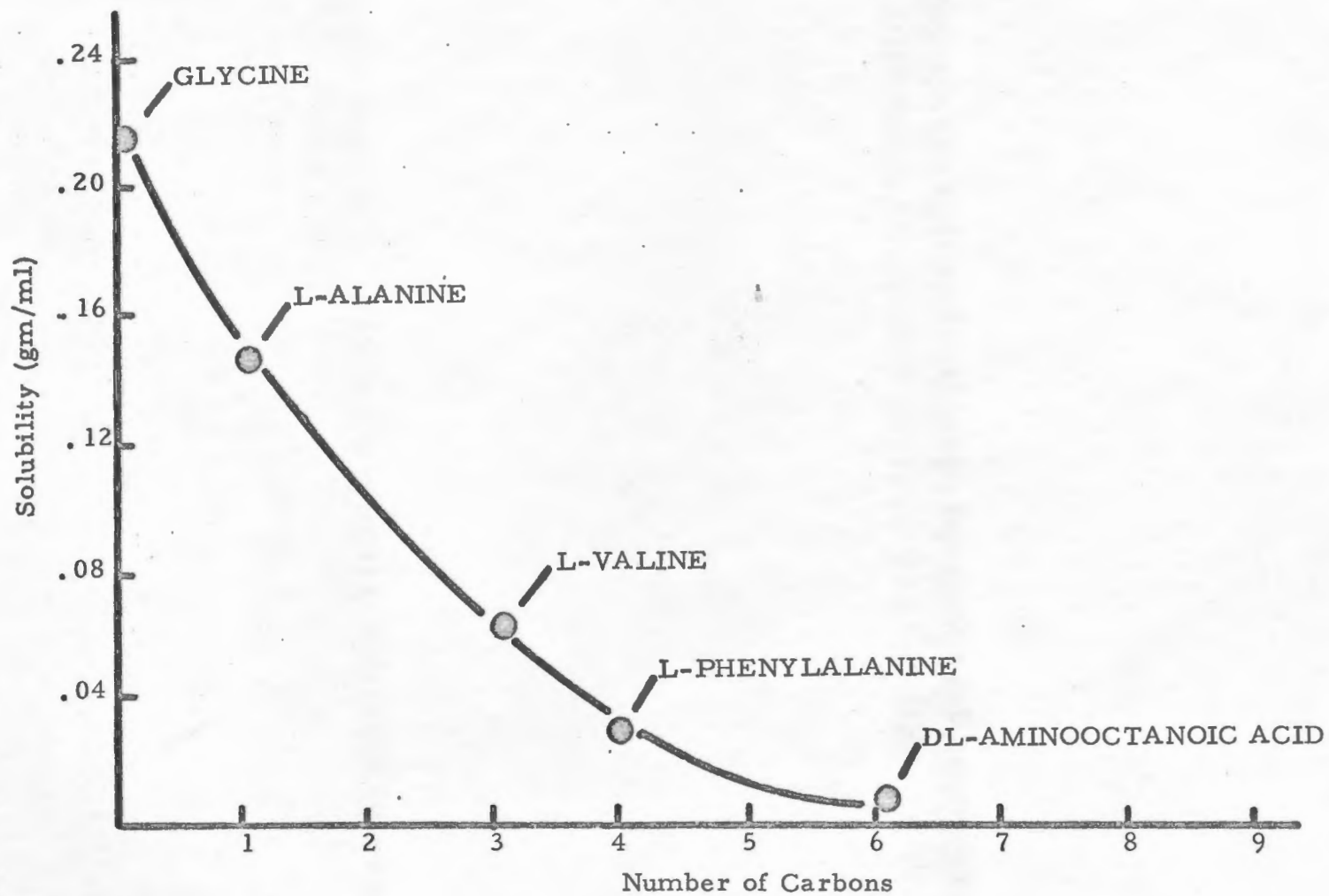
Figure 1. The Solubility of the Amino Acids as a Function of the Number of Carbons Attached to the $\propto$ Amino Group.

ring is approximately equivalent to three CH_2 groups (19). Then

the correlation of this aromatic amino acid into the smooth non-linear

line of decreasing polarity further substantiates the use of glycine as

a basic solubility unit. Now that it has been shown that an increase

in the number of carbon atoms decreases solubility (20, 21) and that

glycine is the basic solubility unit, then it would seem that the total

amino acid solubility is dependent on both the polar and nonpolar

portions of the molecule.

The addition of a semi-polar liquid to the polar aqueous solvent

to form a binary solvent system would be expected to cause a change

in the solubility behavior of the amino acids by providing an environ-

ment of continuously decreasing polarity. As the concentration of

semi-polar liquid is increased, the binary solvent medium would con-

tinually decrease the polarity to the point of the pure semi-polar liquid.

In this way, the polarity of the system is changed in discrete steps by

increasing the alcoholic content.

In a solvent system of decreasing polarity, the differences in

solubility of these amino acids studied would be dependent on the basic

-amino carboxylic acid and the remaining aliphatic or aromatic

portion of the structure. In solution the α-amino carboxylic acid

portion of the molecule is present as a charged species in equilibrium

with an uncharged species. (22)

$$^+H_3N-R-COO^- \rightleftharpoons H_2NRCOOH$$
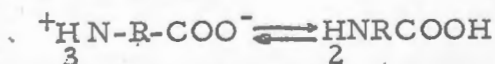
The K_z which is the ratio of the charged to uncharged species, has been shown to decrease as the polarity of the solvent system is reduced. Edsall and Blanchard (23) demonstrated a reduction in K_z of 263,000 to 1000 for glycine as the polarity of a solvent system was changed from pure water to 90 percent ethanol.

It has been shown (24) that the amino acids exist predominately as charged dipoles even in semi-polar solvents. If the attraction which causes the solubility of the amino acid dipoles is defined as being due to the highly polar water molecules, and the semi-polar solvents are assumed to have too little polarity to cause any appreciable solubility of the charged portion of the amino acids, then it would be expected that any reduction in solubility of the amino acids would be directly proportional to a decrease in the aqueous content or polarity of the solvent system.

This assumption of amino acid solubility behavior can be shown in several ways. In Tables 4-8, the molar solubility of the amino acids studied is shown in 0%, 10%, 30%, 50%, 70%, 90%, and 100% (v/v) solutions of methanol, ethanol, n-propanol, isopropanol, and tertiary butanol. A reduction in solubility is seen in each solvent for glycine, L-alanine, L-valine, and L-phenylalanine as the percent of alcohol was increased. Therefore, it would seem in the case of these four amino acids that the charged portion of the molecule dominates the solubility characteristics of the entire molecule. The DL-amino-

TABLE 4.

MOLAR SOLUBILITY OF AMINO ACIDS
IN
METHANOL-WATER SOLVENT SYSTEMS

Percent Methanol (v/v)	Amino Acids[a]			
	Glycine	L-Alanine	L-Valine	L-Phenylalanine
0	2.90	1.63	.474	.172
10	1.74	1.39	.366	.143
30	.658	.743	.207	.099
50	.212	.364	.122	.084
70	.099	.120	.091	.070
90	.017	.026	.025	.036
100	.009	.012	.013	.022

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

TABLE 5.

MOLAR SOLUBILITY OF AMINO ACIDS
IN
ETHANOL-WATER SOLVENT SYSTEMS

Percent Ethanol (v/v)	Amino Acids[a]			
	Glycine	L-Alanine	L-Valine	L-Phenylalanine
0	2.90	1.63	.474	.172
10	2.03	1.28	.362	.143
30	.811	.695	.181	.092
50	.296	.311	.093	.078
70	.077	.076	.044	.050
90	.013	.016	.011	.014
100	.004	.002	.003	.002

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

TABLE 6.

MOLAR SOLUBILITY OF AMINO ACIDS
IN
n-PROPANOL-WATER SOLVENT SYSTEMS

Percent n-Propanol (v/v)	Amino Acids[a]			
	Glycine	L-Alanine	L-Valine	L-Phenylalanine
0	2.90	1.63	.474	.172
10	1.99	1.22	.395	.142
30	1.03	.682	.255	.134
50	.486	.356	.175	.127
70	.122	.110	.056	.078
90	.008	.009	.009	.013
100	.011	.002	.003	.002

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

TABLE 7.

MOLAR SOLUBILITY OF AMINO ACIDS
IN
ISO-PROPANOL-WATER SOLVENT SYSTEMS

Percent Isopropanol (v/v)	Amino Acids[a]			
	Glycine	L-Alanine	L-Valine	L-Phenylalanine
0	2.9	1.63	.474	.172
10	2.06	1.23	.343	.123
30	.857	.627	.178	.093
50	.365	.274	.097	.076
70	.081	.070	.039	.047
90	.008	.006	.006	.009
100	.004	.005	.003	.001

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

TABLE 8.

MOLAR SOLUBILITY OF AMINO ACIDS
IN
TERTIARY-BUTANOL-WATER SOLVENT SYSTEMS

Percent Tertiary-butanol (v/v)	Amino Acids[a]			
	Glycine	L-alanine	L-Valine	L-Phenylalanine
0	2.9	1.63	.474	.172
10	1.93	1.19	.349	.127
30	.941	.632	.180	.094
50	.456	.308	.094	.076
70	.109	.085	.043	.040
90	.005	.005	.009	.005
100	.004	.001	.007	.001

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

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octanoic acid with a low solubility in those solvent systems used, was
not included in the above tables, since it was felt that accurate dif-
ferentiation in solubilities could not be determined. However, for
this amino acid it would seem that the nonpolar-polar ratio has
reached the point where the high charge of the dipolar portion of the
molecule is balanced by the non-polar six carbon n-alkyl chain to pro-
duce an invariant solubility. This would seem to correlate with the
findings of Cohn (25) in which most α-amino acids were found to be
less soluble in ethanol or ethanol-water mixtures than in water, and
as the side chains lengthened a point was reached where the ethanolic
solubility was greater than the aqueous solubility.

Figures 2-6 illustrate the variation in molar solubility of the
amino acids as the percent strength of the semi-polar solvents is
increased. The decrease in solubility can be seen to be a non-linear
curve rather than the proportional linear decrease which would be ex-
pected as the polarity of the solvent system was decreased in discrete
increments. In methanol-water and ethanol-water solvent systems,
glycine shows a greater decrease in solubility than L-alanine as the
percent alcohol is increased. This correlates with the work of Dunn
and Ross (26) which shows the same crossover in solubilities as the
percent ethanol was increased. In the remaining solvents the solubility
decreases in an orderly non-linear fashion with a merging of values at
high alcoholic concentrations. It would seem that as the overall polarity
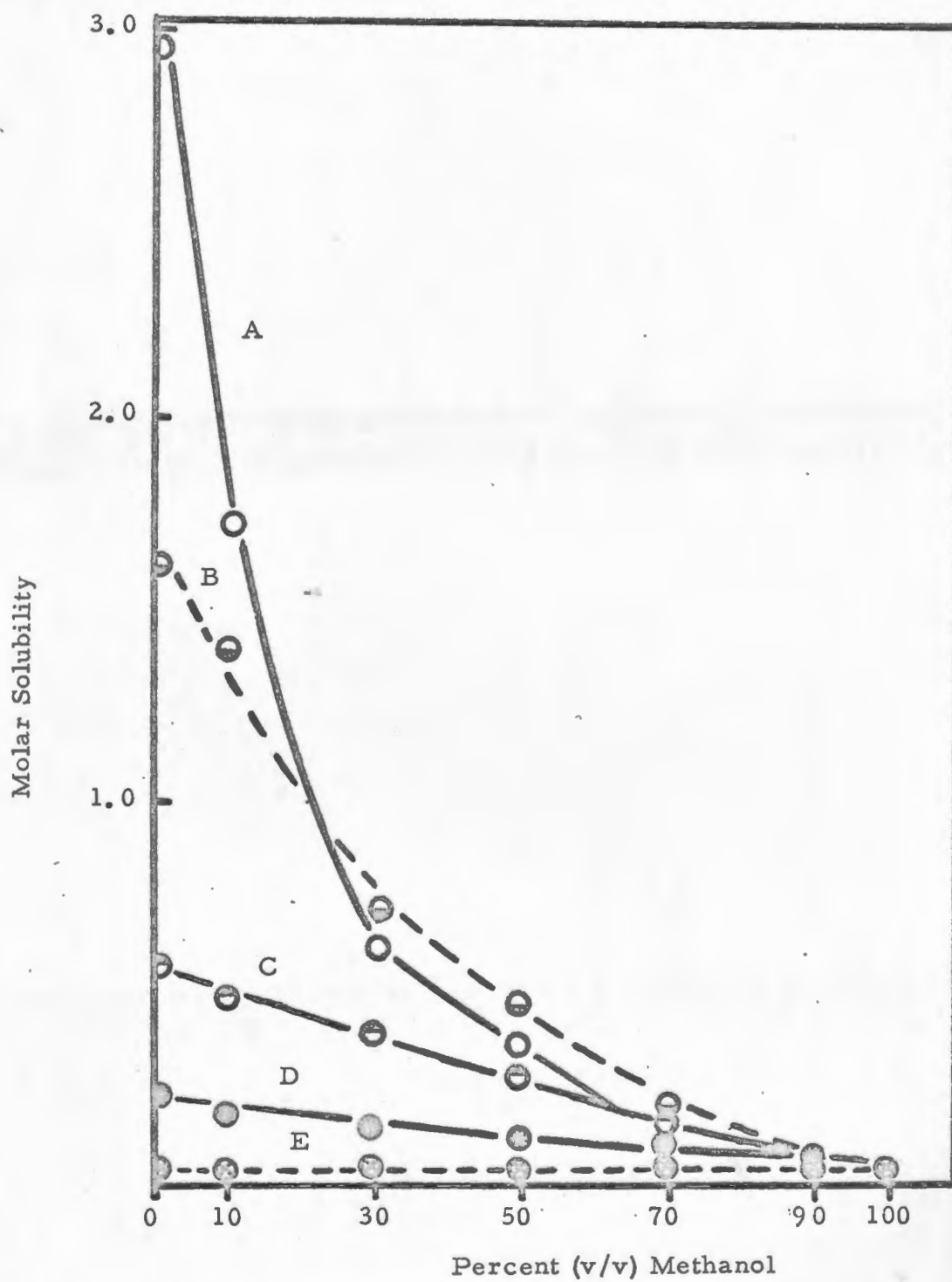
Figure 2. The Molar Solubility of Amino Acid in Methanol-Water
Solvent Systems. Curve A - Glycine, Curve B - L-Alanine,
Curve C - L-Valine, Curve D - L-Phenylalanine, Curve E -
DL-Aminooctanoic Acid.
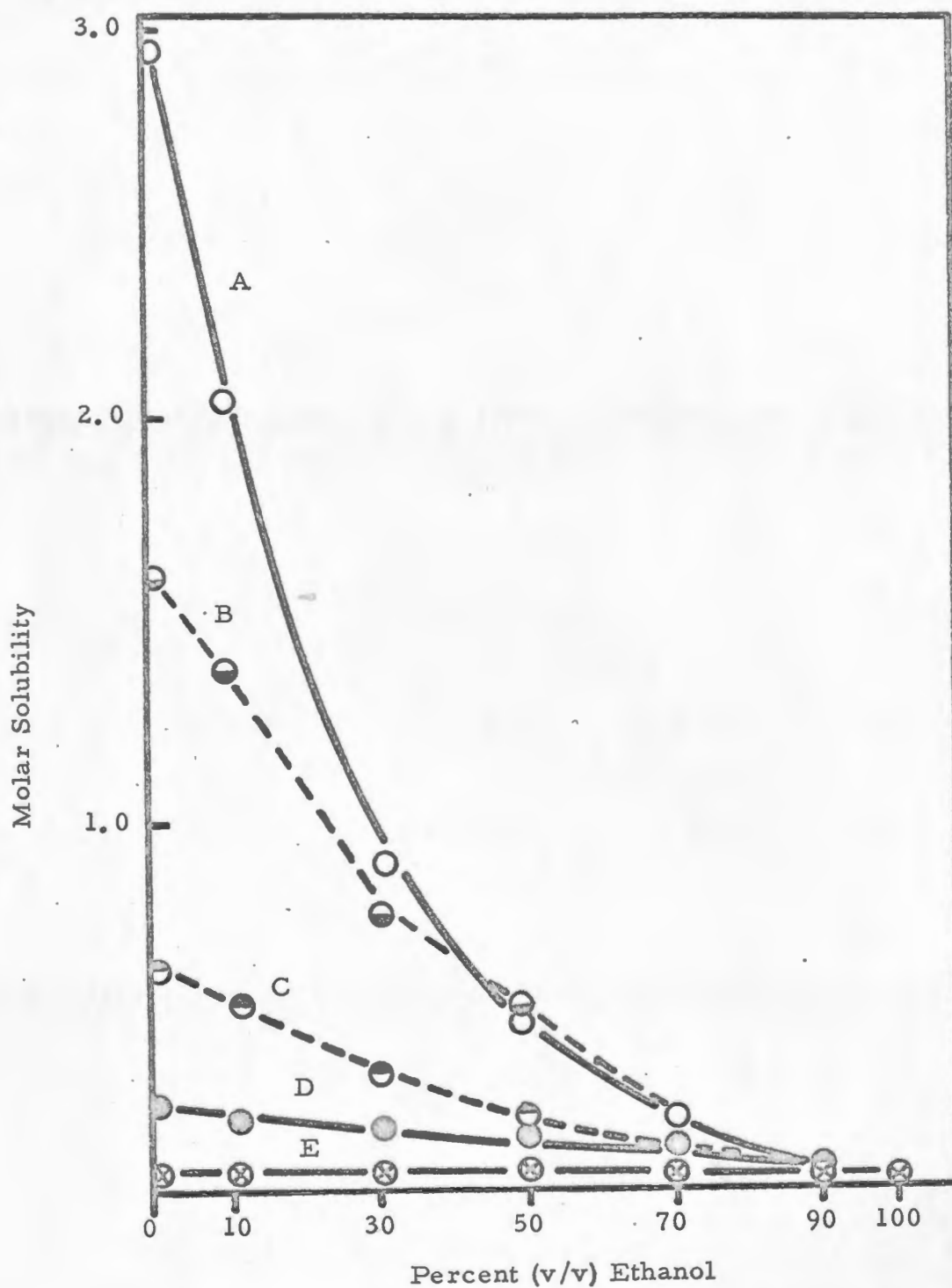
Figure 3.   The Molar Solubility of Amino Acid in Ethanol-Water
Solvent Systems.   Curve A - Glycine, Curve B - L-Alanine,
Curve C - L-Valine, Curve D - L-Phenylalanine, Curve E -
DL-Aminooctanoic Acid.

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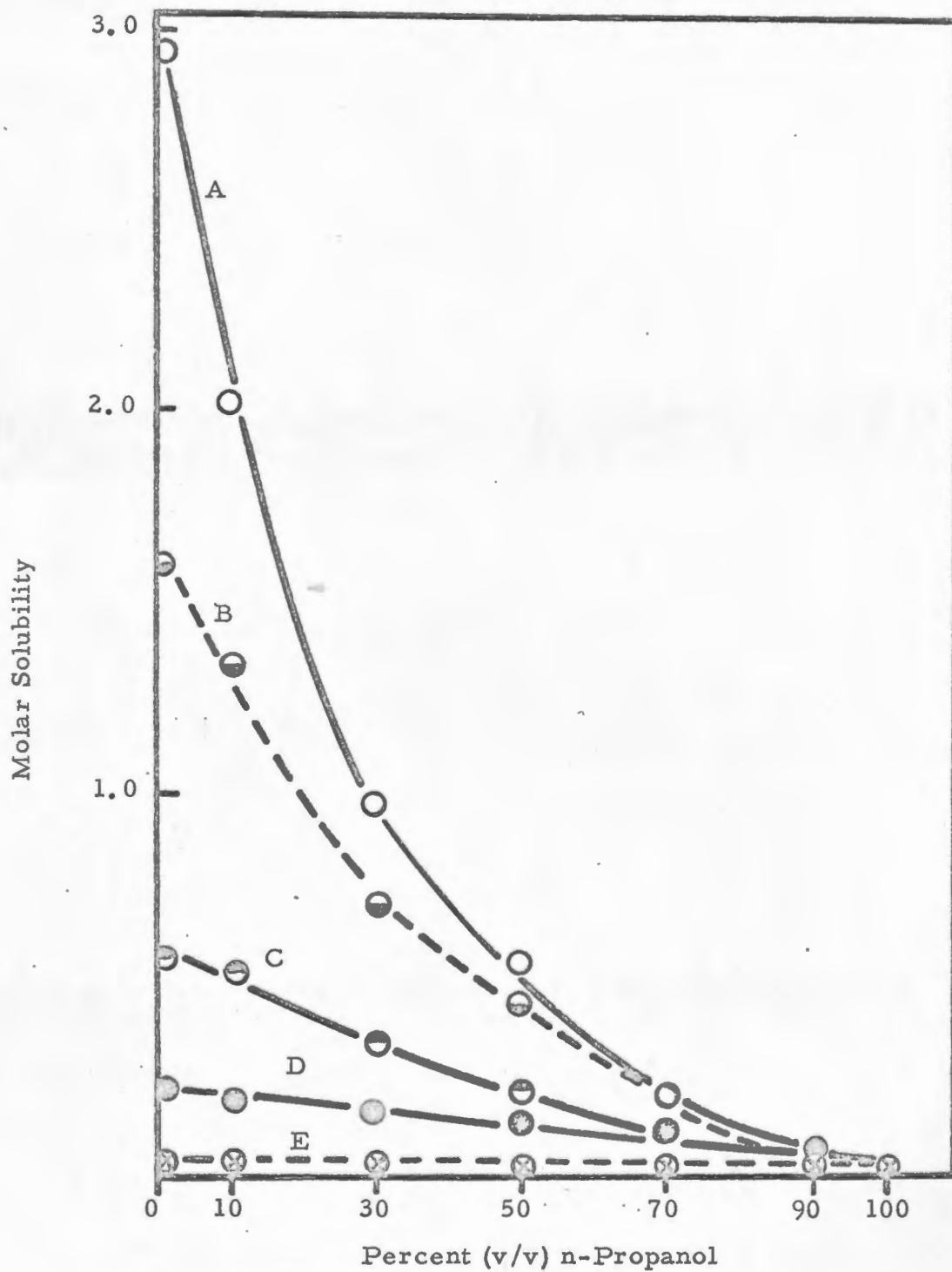
Figure 4. The Molar Solubility of Amino Acid in n-Propanol-
Water Solvent Systems. Curve A - Glycine, Curve B -
L-Alanine, Curve C - L-Valine, Curve D - L-Pheny-
lalanine, Curve E - DL-Aminoocatanoic Acid.
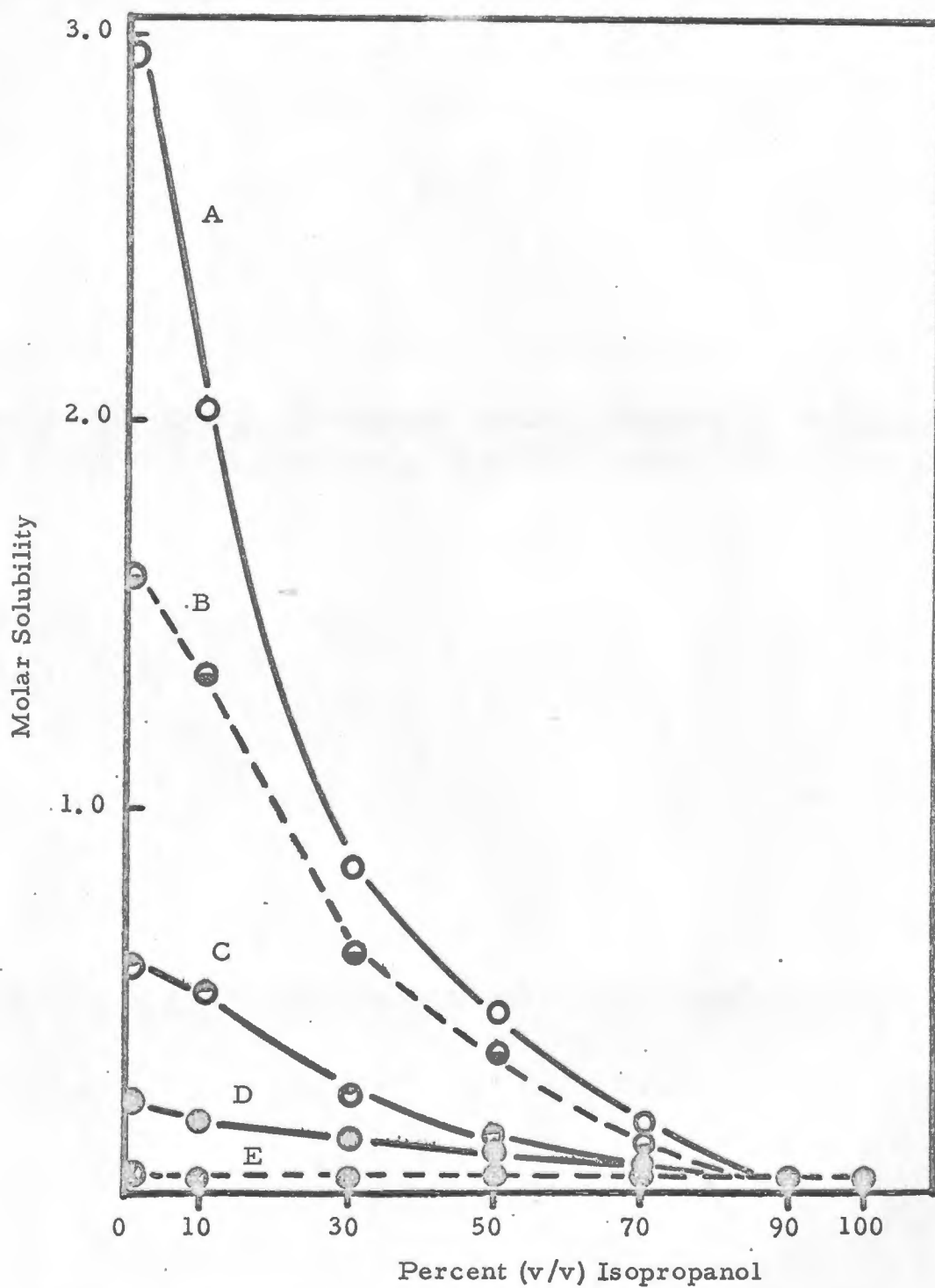
Figure 5. The Molar Solubility of Amino Acid in Isopropanl-
Water Solvent Systems. Curve A - Glycine, Curve B -
L-Alanine, Curve C - L-Valine, Curve D - L-Pheny-
lalanine, Curve E - DL-Aminooctanoic Acid.
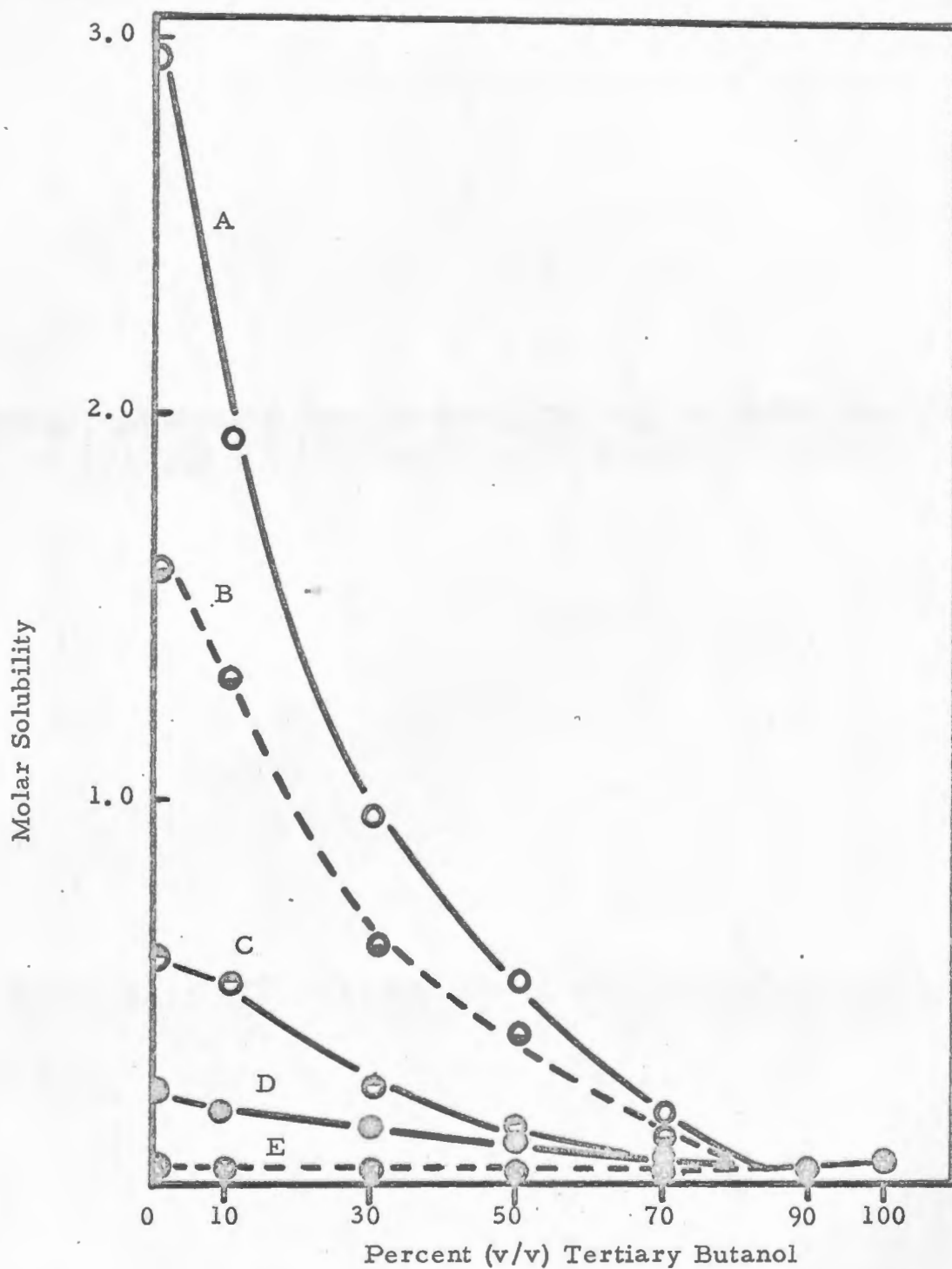
Figure 6.   The Molar Solubility of Amino Acids in Tertiary Butanol-
Water Solvent Systems.   Curve A - Glycine, Curve B -
L-Alanine, Curve C - L-Valine, Curve D - L-Pheny-
lalanine, Curve E - DL-Aminooctanoic Acid.

of the different solvent systems is decreased, the amino acid solubility decreases in a more regular manner.

By calculating the molarity of each component and determining the density, the mole fraction and the average number of solvent molecules per solute molecule can be calculated. For example, in pure water, the solubility of glycine is 2.9 molar and water is 46.8 molar and, using the density to calculate the mole fraction, glycine is found to be equal to 0.058 and water equals 0.942. Dividing through by the mole fraction of glycine, it is found that for each molecule of glycine, there is an average of 16 water molecules present. In this way, the effect of an increase in the nonpolar-polar ration on the solubility characteristics of each of the amino acids can be shown more clearly. In Tables 9-13, the ratio of solvent per solute molecules in 0%, 10%, 30%, 50%, 70%, 90%, and 100% (v/v) is seen for each of the solvents studied. In a pure aqueous system at saturation solubility, each molecule of glycine has 16 molecules of water available for interaction. L-alanine has 30, L-valine has 110, and L-phenylalanine has 308. In columns one and two for each amino acid, the water-solute and semipolar solvent-solute relationship can be seen. This relationship between the solute and the components of the mixed solvent system can be calculated by using the experimentally determined density of the system and the molar solubility of the amino acid. Firstly, the mole fraction of the amino acid as related to the entire solvent system is calculated. Then the mole fraction of each of the remaining solvent

TABLE 9.

THE RATIO OF SOLVENT MOLECULES PER SOLUTE MOLECULE

IN THE METHANOL-WATER SOLVENT SYSTEMS AT 25°C

Percent Methanol (v/v)	Glycine			L-Alanine			L-Valine			L-Phenylalanine		
	Ratio[a] 1	Ratio[b] 2	Ratio[c] 3	Ratio[a] 1	Ratio[b] 2	Ratio[c] 3	Ratio[a] 1	Ratio[b] 2	Ratio[c] 3	Ratio[a] 1	Ratio[b] 2	Ratio[c] 3
0	16.0	—	—	30.0	—	—	110.0	—	—	308.0	—	—
10	26.3	1.3	20.2	32.3	1.6	20.6	135.2	6.8	20.6	338.2	16.7	20.2
30	59.2	11.3	5.23	50.1	9.5	5.27	184.3	35.0	5.26	388.4	73.6	5.28
50	132.6	58.7	2.26	75.3	33.3	2.26	228.9	101.3	2.26	333.0	146.6	2.27
70	175.2	181.0	.97	140.9	145.0	.97	190.5	196.1	.97	243.5	250.5	.97
90	334.2	1332	.25	218.6	867	.25	225.4	898	.25	155.5	618.6	.25
100	—	2499	—	—	2082	—	—	1999	—	—	1123	—

[a]Ratio of water to solute molecules

[b]Ratio of methanol to solute molecules

[c]Ratio of water to methanol molecules per solute molecule

TABLE 10.

THE RATIO OF SOLVENT MOLECULES PER SOLUTE MOLECULE

IN THE ETHANOL-WATER SOLVENT SYSTEMS AT 25° C

	Glycine			L-Alanine			L-Valine			L-Phenylalanine		
Percent Ethanol (v/v)	Ratio[a] 1	Ratio[b] 2	Ratio[c] 3	Ratio[a] 1	Ratio[b] 2	Ratio[c] 3	Ratio[a] 1	Ratio[b] 2	Ratio[c] 3	Ratio[a] 1	Ratio[b] 2	Ratio[c] 3
0	16.0	—	—	30.0	—	—	110.0	—	—	308.0	—	—
10	22.6	.76	29.9	35.7	1.21	29.4	132.6	4.42	30.0	339.6	11.6	29.3
30	45.7	5.95	7.68	53.2	6.96	7.63	213.3	27.9	7.66	420.3	54.8	7.68
50	95.1	29.0	3.28	87.4	26.6	3.29	297.4	90.7	3.28	352.4	107.4	3.28
70	224.2	159.6	1.40	220.0	156.2	1.41	381.1	271.2	1.41	333.4	237.1	1.41
90	445.0	1220	.37	356.0	976	.37	523.5	1435	.37	404.6	1109	.37
100	—	4999	—	—	9090	—	—	5881	—	—	9090	—

[a] Ratio of water to solute molecules
[b] Ratio of ethanol to solute molecules
[c] Ratio of water to ethanol molecules per solute molecule

TABLE 11.

THE RATIO OF SOLVENT MOLECULES PER SOLUTE MOLECULE

IN THE n-PROPANOL-WATER SOLVENT SYSTEMS AT 25°C

	Glycine			L-Alanine			L-Valine			L-Phenylalanine		
Percent n-Propanol (v/v)	Ratio[a] 1	Ratio[b] 2	Ratio[c] 3	Ratio[a] 1	Ratio[b] 2	Ratio[c] 3	Ratio[a] 1	Ratio[b] 2	Ratio[c] 3	Ratio[a] 1	Ratio[b] 2	Ra. 3
0	16.0	—	—	30.0	—	—	110.0	—	—	307.6	—	—
10	22.2	0.5	37.3	38.0	1.0	38.0	119.3	3.1	38.7	344.6	8.9	38.9
30	35.4	3.6	9.84	54.4	5.5	9.81	146.3	14.8	9.90	281.1	28.6	9.84
50	53.1	12.6	4.21	74.8	17.7	4.23	154.2	36.7	4.21	208.2	49.4	4.22
70	133.0	73.4	1.81	151.3	83.5	1.81	289.6	159.9	1.81	209.2	116.0	1.80
90	694.0	1478	0.47	652.2	1308	0.50	631.2	1329	0.48	431.2	919	0.47
100	—	1110	—	—	6666	—	—	4544	—	—	6666	—

[a]Ratio of water to solute molecules
[b]Ratio of n-propanol to solute molecules
[c]Ratio of water to n-propanol molecules per solute molecule

TABLE 12.

THE RATIO OF SOLVENT MOLECULES PER SOLUTE MOLECULE

IN THE ISOPROPANOL-WATER SOLVENT SYSTEMS AT $25°$ C.

Percent Isopropanol (v/v)	Glycine			L-Alanine			L-Valine			L-Phenylalanine		
	Ratio[a] 1	Ratio[b] 2	Ratio[c] 3	Ratio[a] 1	Ratio[b] 2	Ratio[c] 3	Ratio[a] 1	Ratio[b] 2	Ratio[c] 3	Ratio[a] 1	Ratio[b] 2	Ratio[c] 3
0	16.0	---	---	30.0	---	---	110.0	---	---	308.0	---	---
10	21.7	0.56	38.8	36.8	0.98	38.0	133.3	3.44	38.7	400.1	10.3	38.9
30	42.4	4.2	10.0	58.9	5.9	9.9	213.9	21.5	10.0	417.9	41.9	10.0
50	73.9	17.0	4.35	97.1	22.7	4.37	281.6	65.9	4.30	359.4	84.0	4.30
70	215.0	117.3	1.83	232.9	127.1	1.83	418.6	229.9	1.82	349.0	190.3	1.84
90	735.0	1765	0.42	919.6	1937	0.47	946.2	1994	0.48	619.0	1304	0.48
100	---	3332	---	---	2563	---	---	4347	---	---	14284	---

[a] Ratio of water to solute molecules
[b] Ratio of isopropanol to solute molecules
[c] Ratio of water to isopropanol molecules per solute molecule

TABLE 13.

THE RATIO OF SOLVENT MOLECULES PER SOLUTE MOLECULE

IN THE TERTIARY BUTANOL-WATER SOLVENT SYSTEMS AT 25°C.

Percent T-Butanol (v/v)	Glycine			L-Alanine			L-Valine			L-Phenylalanine		
	Ratio[a] 1	Ratio[b] 2	Ratio[c] 3	Ratio[a] 1	Ratio[b] 2	Ratio[c] 3	Ratio[a] 1	Ratio[b] 2	Ratio[c] 3	Ratio[a] 1	Ratio[b] 2	Ratio[c] 3
0	16.0	—	—	30.0	—	—	110.0	—	—	308.0	—	—
10	23.3	0.5	47.0	38.3	0.84	45.5	133.1	2.9	46.3	385.9	8.3	46.5
30	39.2	3.3	12.0	57.3	4.8	11.96	212.3	17.6	12.1	400.4	33.5	11.96
50	57.2	11.1	5.16	85.1	16.5	5.15	283.0	54.9	5.15	353.8	67.1	5.27
70	144.5	65.6	2.20	185.3	84.1	2.20	371.3	168.1	2.21	394.8	178.7	2.21
90	1070	1871	0.57	1069	1871	0.57	595.0	1043	0.57	1069	1871	0.57
100	—	2499	—	—	11110	—	—	1470	—	—	11110	—

[a]Ratio of water to solute molecules
[b]Ratio of tertiary butanol to solute molecules
[c]Ratio of water to tertiary butanol molecules per solute molecule

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system components is determined as a function of their density.
The number of molecules of the two solvents per amino acid mole-
cule is then calculated by dividing each by the mole fraction of the
amino acid. It can be seen that as the number of semi-polar mole-
cules per solute molecule increases, the ratio of water to semi-polar
solvent molecules decreases and the solubility of the amino acids,
dominated by the charged α-amino carboxylic acid group, decreases.
In the pure semi-polar solvents, the four amino acids show a high
solvent-solute ratio and a low solubility, and substantiate the assump-
tion that this type of solvent does not have the polar attraction to
produce a high solubility in an amino acid dominated by its charged
dipole. However, it should be noted that in any given solvent sys-
tem for any of the amino acids studied, the ratio of water to semi-
polar solvent molecules illustrated in column three is essentially
the same for a given percent strength. For example, in the methanol-
water solvent system, this ratio for each of the amino acids at 10%
(v/v) is 20.2 to 20.6; at 30% it is 5.23 to 5.28; at 50% it is 2.26 to
2.27; at 70% it is .97; and at 90% it is .25. This substantiates the
fact that a given solvent system is composed of a given number of
polar and semi-polar molecules and that the amino acid dipole pro-
duces a similar effect on a given solvent system regardless of the
composition or length of its non-polar portion, and that the
relative difference in solubility between amino acids is due to the

effect of the non-polar portion of the molecule. That these differences

in solubility are due to the non-polar portion of the molecule was men-

tioned by Nozaki,Tanford (27) when they reported that the interaction

of an amino acid side chain is independent of the structure to which it

is attached in ethanol-water and probably in other solvents as well.

The effect of changing a solvent system on the solubility of a

single amino acid can be seen if the molar solubility is plotted as a

function of increasing percent concentration of the solvents used. In

Figures 7 - 9, only four of the solvents are used with isopropanol

eliminated since, in most cases, it gave results which did not vary

significantly from n-propanol. It would be expected that the polar

amino acids would be most soluble in the more polar solvent systems.

Using dielectric constant as a measure of polarity, the pure solvents

can be arranged in this order of decreasing polarity: methanol, ethanol,

n-propanol, and tertiary butanol. These semi-polar solvents in com-

bination with water will produce a series of solvent systems with the

same order of decreasing polarity. For example, at 30% (v/v) of

semi-polar solvent-water, the order of decreasing polarity remains

methanol-water, ethanol-water, n-propanol-water, and tertiary

butanol-water. However, it can be seen in Figure 7 that glycine

throughout the concentration scale shows a decreasing solubility in

the order of n-propanol, tertiary butanol, ethanol and methanol. As

illustrated in Figure 8, the addition of a methyl group to form L-alanine
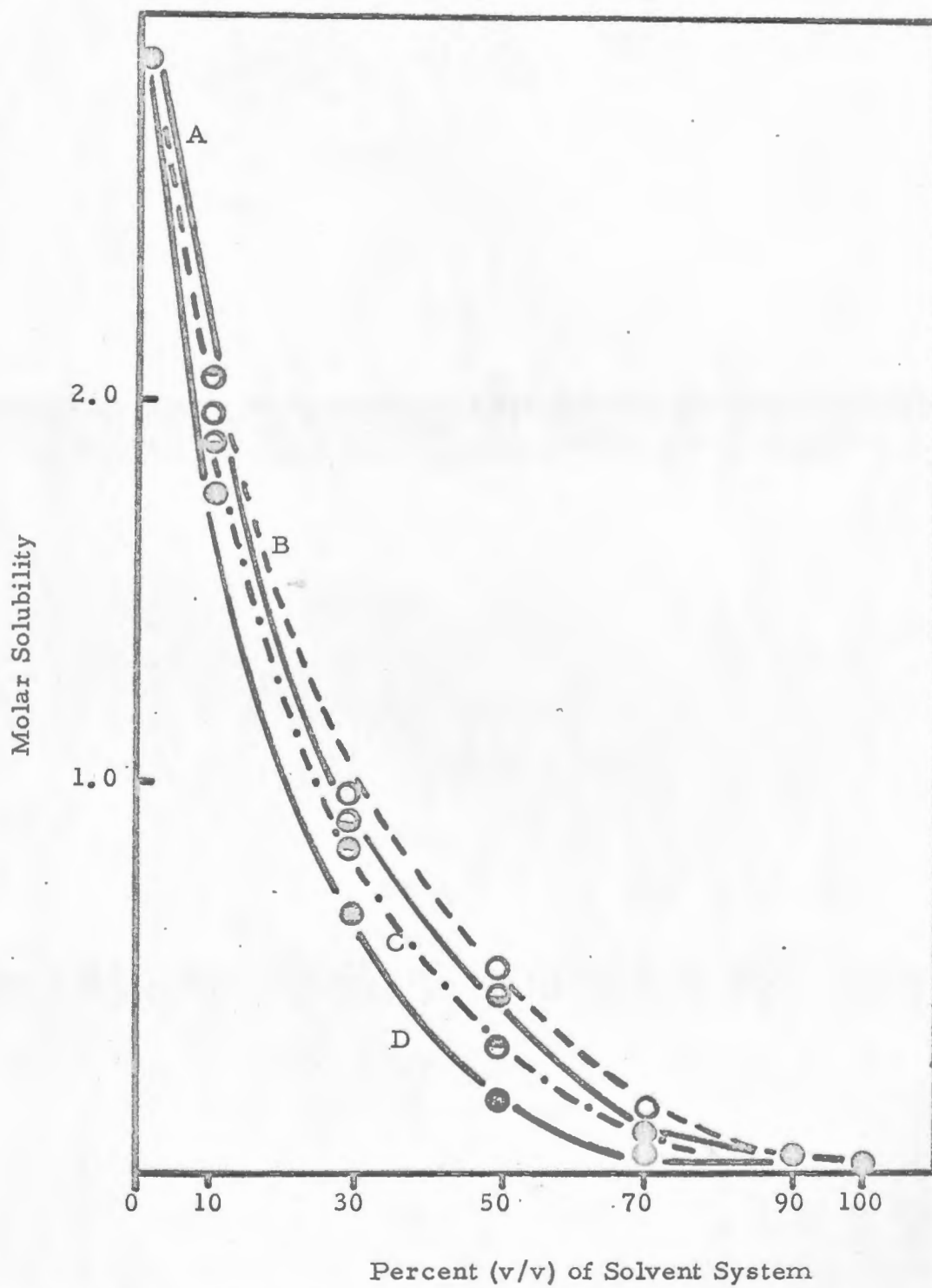
Figure 7. Molar Solubility of Glycine in the Solvent Systems. Curve A - n-Propanol, Curve B - Tertiary-Butanol, Curve C - Ethanol, Curve D - Methanol.
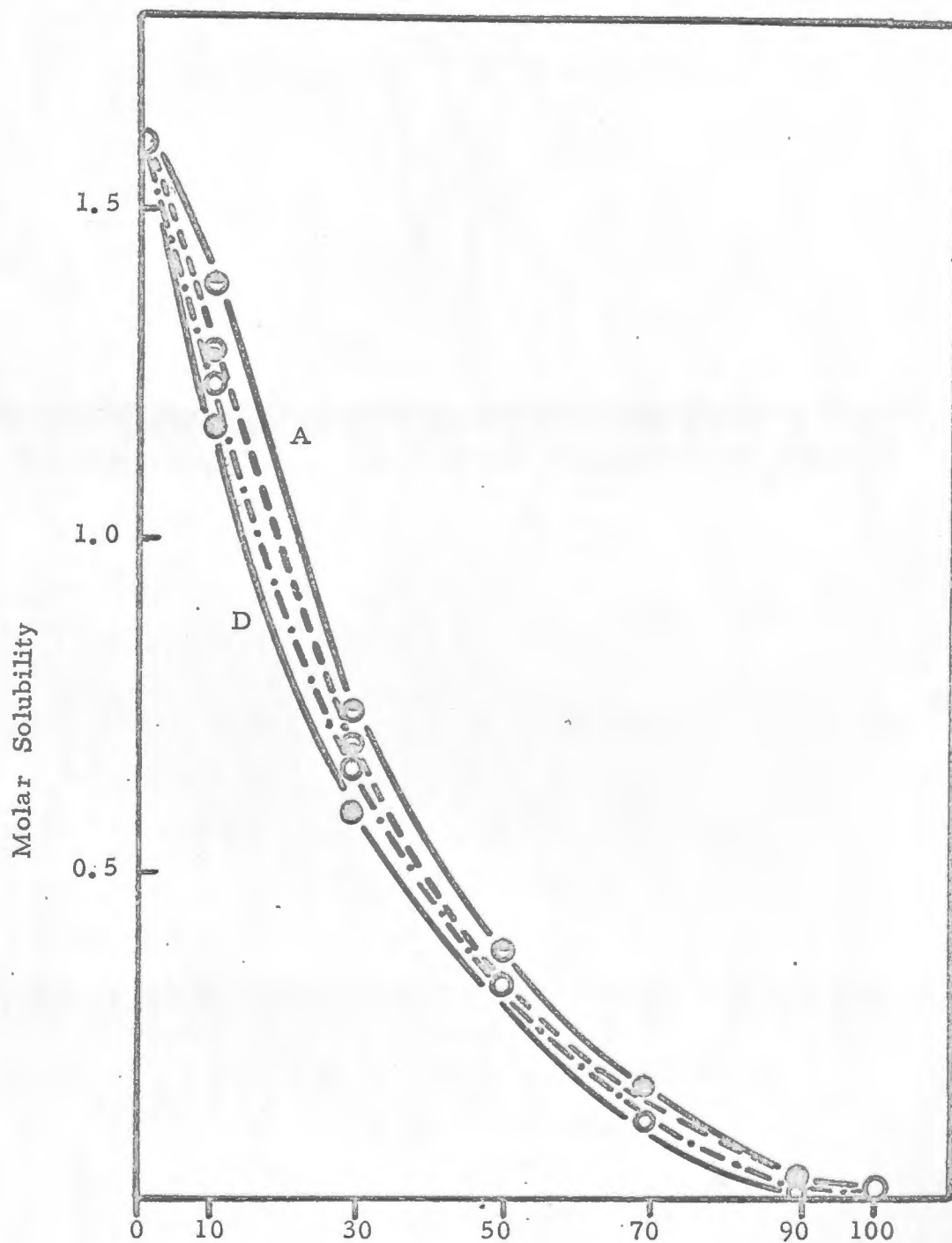
Figure 8. Molar Solubility of L-Alanine in the Solvent Systems.
Curves Identified in Descending Order between 10 and
30% are: Curve A - Methanol, Curve B - Ethanol,
Curve C - n-Propanol, Curve D - Tertiary Butanol.
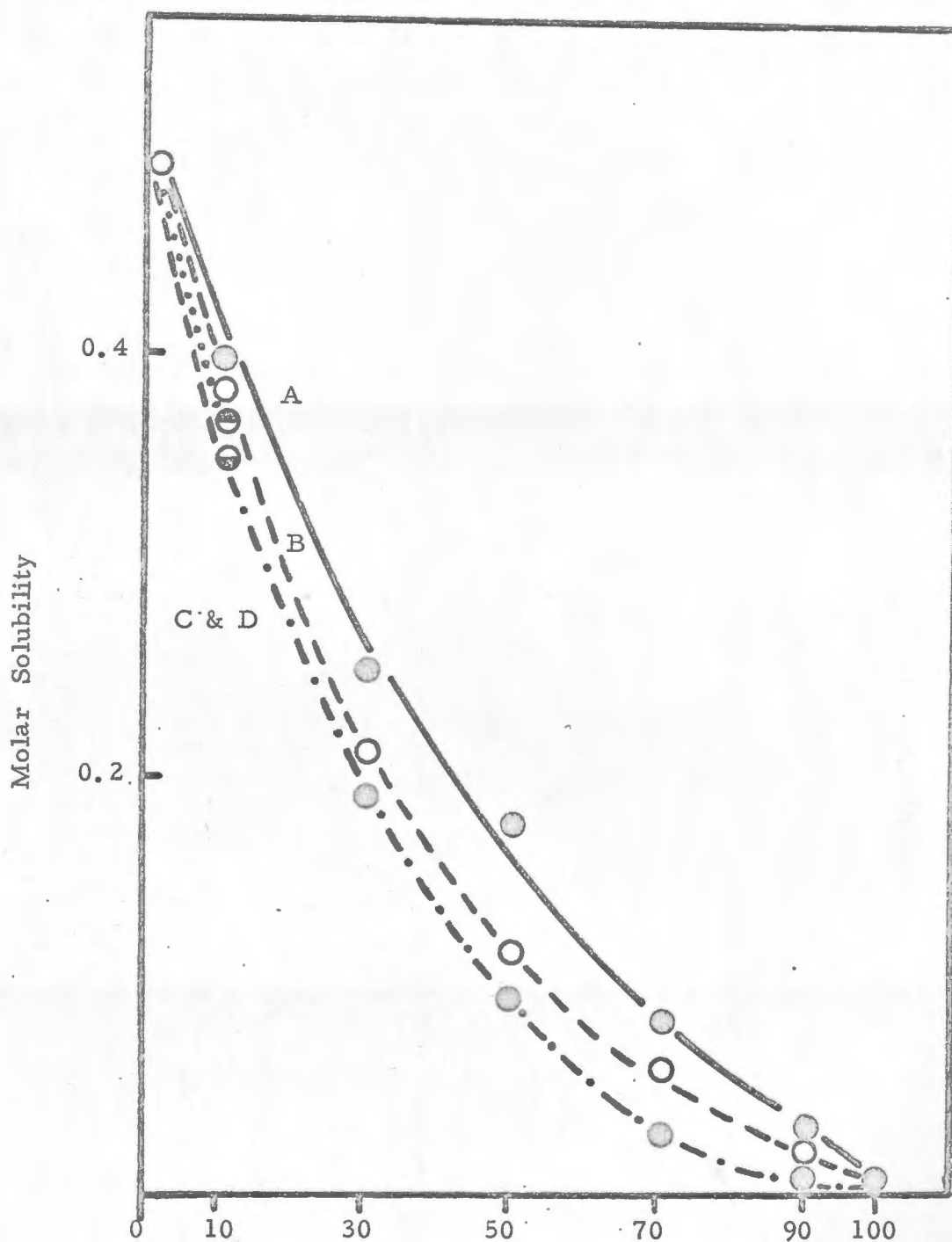
Figure 9.   Molar Solubility of L-Valine in the Solvent Systems.
Curve A - n-Propanol, Curve B - Methanol, Curve C-
Ethanol, Curve D - Tertiary Butanol.

changes the order of binary solubility to that which was originally
expected. In Figure 9, L-valine with an added isopropyl group shows
its greatest solubility in n-propanol followed in decreasing order by
methanol, ethanol, and tertiary butanol. The order of solubility of
L-phenylalanine does not seem to change appreciably with different
solvent systems producing a solid line for any illustrations attempted
on a scale comparative with Figures 7 - 9.

If the order of solubility of glycine is said to illustrate the be-
havior of the charged α-amino carboxylic acid portion of the molecule,
this variation in the order of solubility as methyl, isopropyl, and
methyl-phen groups were added serves to further substantiate the
independent interactions of each of the non-polar side chains.

The variation of the solvent systems and the effect on the solu-
bility of an amino acid throughout the concentration spectrum can be
further illustrated by generating the polynomial regression for each
solvent. This equation of the line can be computed using a standard
IBM program (28) for an orthogonal polynomial regression. By desig-
nating the molar solubility as the dependent variable and the percent
strength of the alcoholic solution as the independent variable, the
polynomial equation of the standard form $Y = A_0 + A_1X + A_2X^2 +$
$A_3X^3 + \ldots\ldots\ldots A_nX^n$ can be computed. (See Appendix I) The
purpose of generating the equations was to illustrate the relationship
between the solubility profiles of an amino acid in each of the solvent

systems used.

Tables 14 - 17 show the effect ...ch of the amino acids in
the five solvent systems used in the form of a second degree equation.
The second degree polynomial equation was considered most represen-
tative since the sum of the squares of the deviation about the regression
in the analysis of variants showed little improvement when the third
degree or higher polynomial equation was calculated. The Y intercept
in each table is representative of the average solubility in a pure aqueous
solvent. The coefficients of the X and X^2 terms define the curve of the
line. It was not expected that the polynomial regression equation would
precisely define the solubility of the amino acids. In Table 14, the
proximity of the Y intercept values which range from 2.65204 to
2.79148 with a true solubility of 2.9 M. in pure water can be seen.
The X and X^2 values also show a similarity. In Table 15, L-alanine
shows a range of 1.57939 to 1.66396 in the Y intercepts with a true
aqueous solubility of 1.63 M. A range of only 0.08455 as compared
to the range of 0.13944 for glycine, thereby showing a closer approxi-
mation of the true solubility. This is expected since the average of the
sum of the squares of the deviation about the regression for L-alanine
in all solvents is only 0.00538 as compared to 0.11104 for glycine.
The sum of the squares of the deviation about the regression is a
measure of the closeness of fit between the data points calculated
from the computed polynomial equation and those data points observed
experimentally. (29) In Tables 16 and 17 the computed polynomial

TABLE 14.

THE POLYNOMIAL REGRESSION DATA GENERATED FROM THE MOLAR SOLUBILITY OF GLYCINE AS A FUNCTION OF THE PERCENT STRENGTH OF THE SOLVENT SYSTEM

Solvent System[a]	Y Intercept	Coefficients	
		X	X^2
Methanol	2.6520	-0.0743	0.0005
Ethanol	2.7823	-0.0738	0.0005
n-Propanol	2.7591	-0.0564	0.0004
Isopropanol	2.7915	-0.0721	0.0005
Tertiary butanol	2.7303	-0.0676	0.0004

[a] Molar solubility values of Glycine in 0 to 100%(v/v) of each solvent system were used to generate the polynomial equation

TABLE 15.

THE POLYNOMIAL REGRESSION DATA GENERATED FROM THE MOLAR SOLUBILITY OF L-ALANINE AS A FUNCTION OF THE PERCENT STRENGTH OF THE SOLVENT SYSTEM

Solvent System[a]	Y Intercept	Coefficients	
		x	x^2
Methanol	1.6640	-0.0354	0.0002
Ethanol	1.6238	-0.0366	0.0002
n-Propanol	1.5881	-0.0347	0.0002
Isopropanol	1.6019	-0.0378	0.0002
Tertiary butanol	1.5794	-0.0363	0.0002

[a]Molar solubility values of L-alanine in 0 to 100%(v/v) of each solvent system were used to generate the polynomial equation.

TABLE 16.

THE POLYNOMIAL REGRESSION DATA GENERATED FROM

THE MOLAR SOLUBILITY OF L-VALINE AS A FUNCTION

OF THE PERCENT STRENGTH OF THE SOLVENT SYSTEM

Solvent System[a]	Y Intercept	Coefficients	
		x	x^2
Methanol	0.4581	-0.0089	0.00005
Ethanol	0.4629	-0.0104	0.00006
n-Propanol	0.4732	-0.0080	0.00003
Isopropanol	0.4592	-0.0104	0.00006
Tertiary butanol	0.4578	-0.0104	0.00006

[a]Molar solubility values of L-valine in 0 to 100%(v/v) of each solvent system were used to generate the polynomial equation.

TABLE 17.

THE POLYNOMIAL REGRESSION DATA GENERATED FROM

THE MOLAR SOLUBILITY OF L-PHENYLALANINE AS A

FUNCTION OF THE PERCENT STRENGTH OF THE SOLVENT

SYSTEM

Solvent System[a]	Y Intercept	Coefficients	
		x	x^2
Methanol	0.1646	-0.0019	0.00001
Ethanol	0.1659	-0.0022	0.00001
n-Propanol	0.1602	-0.0021	0.00001
Isopropanol	0.1581	-0.0020	0.00001
Tertiary butanol	0.1609	-0.0022	0.00001

[a]Molar solubility values of L-phenylalanine in 0 to 100%(v/v) of each solvent system were used to generate the polynomial equation.

equations for L-valine and L-phenylalanine show an even closer approximation to the experimental points. L-valine shows a range of only 0.01548 for a solubility of 0.474 M. and an average sum of squares of the deviation about the regression of 0.00135. L-phenylalanine has a range of only 0.00788 between its high and low Y intercepts with a true solubility of 0.172 M. and an average sum of the squares of the deviation about the regression of only 0.00055.

The closeness of the Y intercepts and the similarity between the coefficients of the X and X^2 terms seem to indicate the existence of a closely related family of curves when the solubilities of each amino acid in the five solvents are compared. The differences between the curves are quite regular and a function of both dielectric constant of each solvent system and the individual interaction of the amino acid with each solvent.

The addition of acid or base to an amino acid in aqueous solution at the point of neutral solubility produces a change in the species present. The dissociation of the amino acid illustrated below shows the species present and the two dissociation constants, K_1 and K_2. (30)

$$_3^+HNRCOOH \xrightarrow[H^+]{K_1} {}_3^+HNRCOO^- \xrightarrow[H^+]{K_2} {}_2HNRCOO^-$$
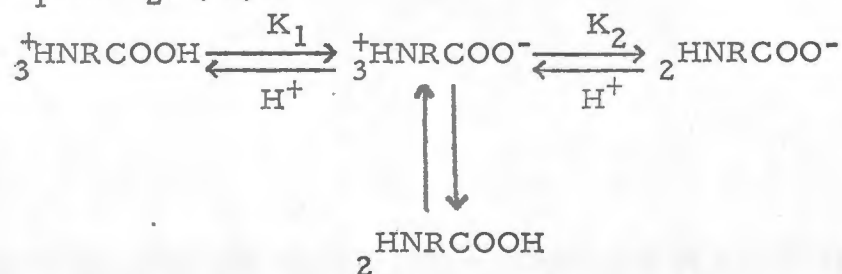
$$_2HNRCOOH$$

$$K_1 = \frac{(H)^+(_3^+HNRCOO^-)}{(_3^+HNRCOOH)} \qquad K_2 = \frac{(H)^+(_2HNRCOO^-)}{(_3^+HNRCOO^-)}$$

Thus it can be seen that in a neutral aqueous solution the zwitterion of the amino acid exists in equilibrium with the uncharged species in a specific ratio as described in the previous section. The addition of acid to the neutral solution will produce the cationic species in proportion to the first dissociation constant, K_1. Conversely, the addition of base to a neutral aqueous solution produces the anionic species as a function of the K_2. (31)

The isoelectric point has been defined as the pH value at which the average net charge of the dipolar molecule is equal to zero. (32) Using the two dissociation constants, K_1 and K_2, the isoelectric point has been shown to be $pH_I = (pK_1 + pK_2)/2$ in the amino acids studied. (33) However, if the solubility is plotted as a function of the

pH, illustrated in Figure 10, the presence of an invariant band of similar solubility for a range of pH variation on either side of the calculated isoelectric point is seen for each amino acid. This is followed by a rapid increase in solubility as the pH is increased or decreased on either side of the invariant band. The presence of an isoelectric band rather than a single isoelectric point was mentioned by Cohn and Edsall. (34, 35) 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 the cation and anion is very low and the dipolar species will dominate.

In Table 18 the effect of adding acid and base on the total solubility of the four amino acids in aqueous solution is illustrated. The solubility range of each amino acid was separated into two portions, that influenced by acid addition and that influenced by base addition, and a least squares computer analysis was calculated for each part. (See Appendix II) In all cases the correlation coefficient was 0.80 or greater. The moles of acid or base added was designated as the independent variable; and the total solubility of the amino acid as the dependent variable. Table 19 shows the calculated first degree equation in the form of $Y = mX + b$ for moles of HCl added. The Y intercept indicated the initial solubility of the amino acid and closely approximated the experimentally observed values with an average error of less than four percent. The slopes of the lines show an increase inversely proportional to the solubility of the amino acids.
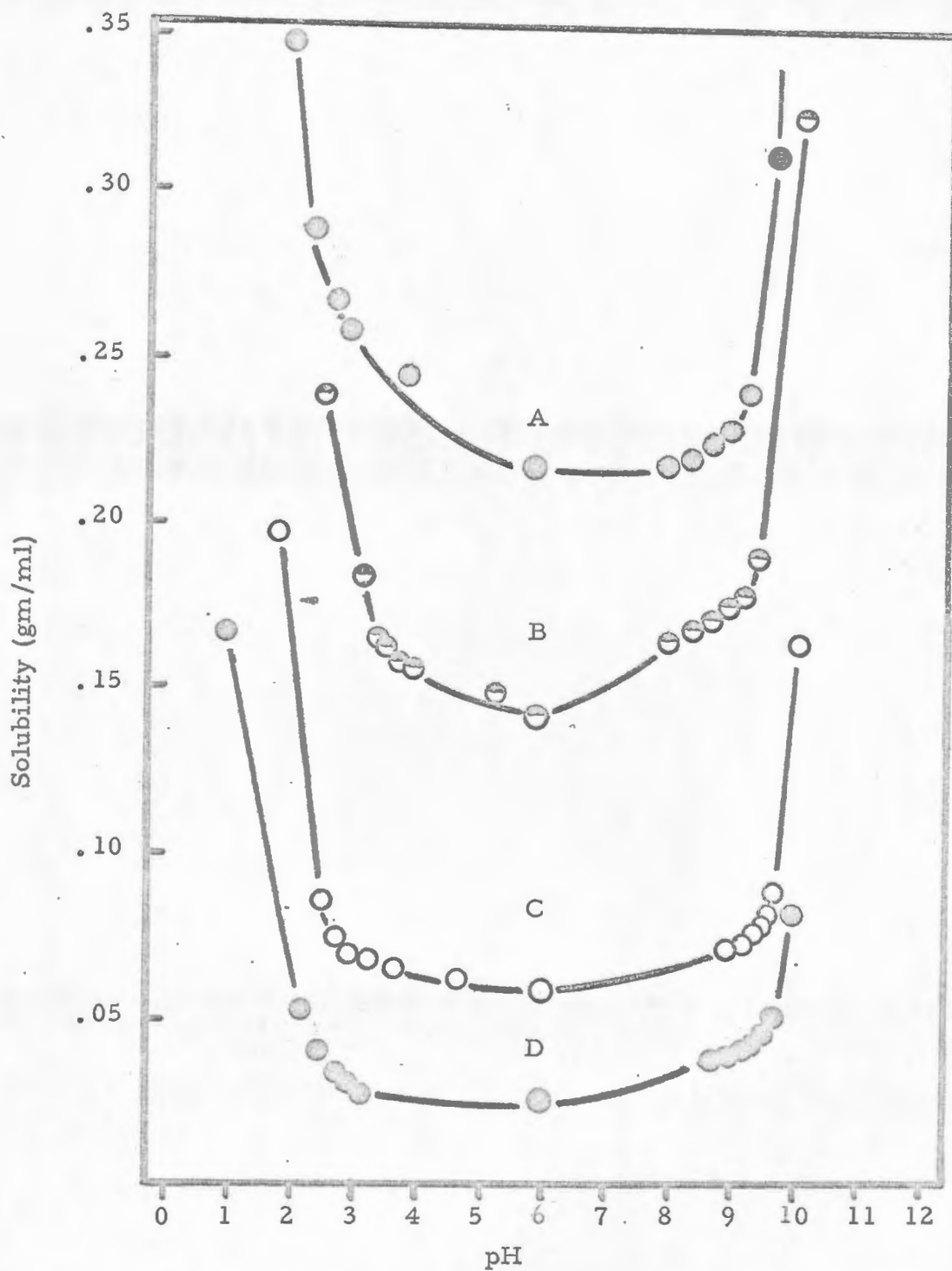
Figure 10. The Change in Aqueous Solubility of the Amino Acids as
a Function of pH. Curve A - Glycine, Curve B - L-Alanine,
Curve C - L-Valine, Curve D - L-Phenylalanine.

TABLE 18.

THE EFFECT OF ADDING ACID OR BASE ON THE TOTAL

SOLUBILITY OF THE AMINO ACIDS IN AQUEOUS SYSTEMS[a]

Glycine			L-Alanine		
Total Acid or Base Added (moles)	Solubility (gm/ml)	pH	Total Acid or Base Added (moles)	Solubility (gm/ml)	pH
—	—	—	0.261	0.1780	3.05
1.090	0.3614	2.40	0.181	0.1652	3.20
0.940	0.2895	2.60	0.151	0.1645	3.40
0.640	0.2661	2.75	0.111	0.1587	3.60
0.490	0.2635	2.85	0.081	0.1575	3.85
0.192	0.2556	3.60	0.031	0.1564	5.40
neutral	0.2178	6.00	neutral	0.1450	6.00
0.321	0.2190	8.20	0.105	0.1721	8.10
0.430	0.2230	8.50	0.155	0.1741	8.40
0.540	0.2266	8.70	0.225	0.1756	8.70
0.651	0.2325	8.90	0.275	0.1766	8.90
0.760	0.2432	9.10	0.345	0.1786	9.10
0.980	0.3088	9.40	0.395	0.1853	9.20

[a]Each solubility value is the average of at least 3 determinations

TABLE 18--Continued

L-Valine			L-Phenylalanine		
Total Acid or Base Added (moles)	Solubility (gm/ml)	pH	Total Acid or Base Added (moles)	Solubility (gm/ml)	pH
0.235	0.0894	2.35	0.293	0.1706	1.15
0.155	0.0806	2.60	0.119	0.0540	2.25
0.125	0.0783	2.70	0.099	0.0478	2.30
0.085	0.0736	3.05	0.089	0.0455	2.40
0.055	0.0707	3.35	0.059	0.0411	2.65
0.005	0.0677	4.60	0.029	0.0368	3.00
neutral	0.0559	6.10	neutral	0.0274	6.00
0.167	0.0826	8.90	0.083	0.0467	8.65
0.217	0.0854	9.10	0.113	0.0495	8.80
0.257	0.0906	9.30	0.123	0.0505	8.90
0.307	0.0951	9.40	0.133	0.0519	9.00
0.337	0.0967	9.45	0.163	0.0560	9.15
0.357	0.0983	9.50	0.183	0.0603	9.20

TABLE 19.

THE EFFECT OF ADDING ACID ON THE TOTAL AQUEOUS

SOLUBILITY OF THE AMINO ACIDS[a]

Amino Acid	Y Intercept	Slope	Correlation Coefficient
Glycine	0.2182	0.1026	0.8967
L-Alanine	0.1482	0.1078	0.9620
L-Valine	0.0624	0.1195	0.9442
L-Phenylalanine	0.0286	0.2042	0.9871

[a]Moles of HCl as a function of amino acid solubility (gm/ml)

Glycine and L-alanine show a similar slope. L-valine displayed an increase of approximately 13 percent over the first two amino acids. L-phenylalanine showed an increase in slope almost twice that of glycine. The first degree equations representing addition of NaOH are shown in Table 20. Here the same general trend is seen as was seen in the case of acid addition. The error in approximating the initial solubility at the Y intercept is on the average less than 6 percent. The glycine and L-alanine show similar slopes with the L-alanine just slightly lower than the glycine. The slopes for L-valine and L-phenylalanine both show an increase over the first two amino acids similar to that produced by acid addition. It would seem that the slope which represents the increase in total solubility as a function of acid or base added is dependent on the structure of the amino acid. As the nonpolar-polar ratio increases the ease of salt formation is enhanced.

In tracing the total solubility of the amino acids as a function of pH and as a function of the acid or base added, it is logical to consider the composition at those points above and below the isoelectric band. It would seem that the total solubility (C_T) is the sum of the solubility due to the zwitterions (C_A) and that due to the anion or cation which would form an associated salt (C_S) with the acid or base added. To prove this postulation the equations:

$$pH = \log (C_A / C_S) + pK_1$$

TABLE 20.

THE EFFECT OF ADDING BASE ON THE TOTAL AQUEOUS

SOLUBILITY OF THE AMINO ACIDS[a]

Amino Acid	Y Intercept	Slope	Correlation Coefficient
Glycine	0.1961	0.0809	0.7973
L-Alanine	0.1554	0.0800	0.8681
L-Valine	0.0587	0.1178	0.9844
L-Phenylalanine	0.0291	0.1738	0.9874

[a]Moles of NaOH as a function of amino acid solubility (gm/ml)

$$pH = \log (C_S/C_A) + pK_2$$

were derived from the appropriate dissociation constant equations previously described. The solubility in the neutral solution was subtracted from the total solubility and this value considered to be equal to the salt formed. The C_A and C_S were converted to molar solubility. In all calculations of salt molarity it was assumed that the amino acid combined in a mole to mole ratio with the acid or base added. The half hydrochloride salts of glycine and L-alanine mentioned in the literature (36) were considered not to form. The log of the ratios of (C_A/C_S) and (C_S/C_A) were considered to be the independent variables, and the pH as the dependent variable. Using the computer program described in Appendix II, the first degree equation and correlation coefficients were calculated. In Tables 21 and 22 the results of this calculation are shown. While the Y intercepts show some deviation from the literature values (37) for the pK_1 and pK_2, the correlation coefficients with a range of 0.8733 to 0.9908 indicate a very high adherance to the first degree equation in all cases. This shows that the initial assumptions are true: that the total solubility of an amino acid in aqueous solution is equal to the sum of the solubility of the ampholyte plus the salt formed, and that the single hydrochloride salts of the amino acids are formed.

The solubility variation when acid or base was added to separate samples of each amino acid in the series of decreasing polarity for

TABLE 21.

FIRST DEGREE RELATIONSHIP BETWEEN pH

AND LOG (C_A/C_S) FOR ACID ADDITION

Amino Acid	Y Intercept	Slope	Correlation Coefficient
Glycine	2.09	0.843	0.976
L-Alanine	1.52	1.824	0.938
L-Valine	1.17	3.284	0.943
L-Phenylalanine	1.97	1.900	0.972

TABLE 22.

FIRST DEGREE RELATIONSHIP BETWEEN pH

AND LOG (C_S/C_A) FOR BASE ADDITION

Amino Acid	Y Intercept	Slope	Correlation Coefficient
Glycine	9.83	0.765	0.999
L-Alanine	9.70	3.732	0.887
L-Valine	9.91	2.182	0.996
L-Phenylalanine	9.17	2.294	0.965

10%, 30%, 50%, 70%, 90%, and 100% (v/v) of methanol, ethanol, n-propanol, isopropanol, and tertiary butanol was similar to that seen previously in the pure aqueous solvent systems. The minimum solubility occurred in the neutral solution with the addition of acid or base causing an increase in total solubility. Tables 23 - 27 are representative of the effect of pH' on the total solubility of glycine in each of the solvent systems. Figures 11 - 15 illustrate more clearly the relative solubility between the amino acids and the variation in solubility for each of the amino acids in each of the pure semi-polar solvents. There is an absence of the wide isoelectric band of invariant solubility that was seen in pure aqueous systems, and a distinct increase in total solubility is seen for the change in pH' away from the neutral solubility point. Further, the increase in total solubility in the semi-polar solvents seems to reach a higher magnitude per amount of acid or base added as the polarity of the solvent system is decreased. Using the seven ethanol-water solvent systems in combination with the four most soluable amino acids as an example, the increase in total solubility as a function of acid or base additions as well as decreasing polarity is seen in Figures 16 - 19. Here the ratio of the total solubility (S) to the solubility at the neutral point (S_o) is plotted as a function of grams of acid or base added. The first three strengths, 0, 10, and 30%(v/v), merge as one straight line. Only L-phenylalanine shows any discernable difference between the linear increase at these percentage strengths.

TABLE 23.

THE EFFECT OF ADDING ACID OR BASE ON THE SOLUBILITY OF GLYCINE IN METHANOL-WATER SOLVENT SYSTEMS

Total Acid or Base Added(gms)	10% (v/v)		30% (v/v)		50% (v/v)		70% (v/v)		90% (v/v)		100% (v/v)	
	Solubility (gm/ml)	pH	Solubility (gm/ml)	pH	Solubility (gm/ml)	pH	Solubility (gm/ml)	pH	Solubility (gm/ml)	pH	Solubility (gm/ml)	pH
.282	.2121	3.2	.1160	3.3	.0789	3.1	.0476	3.3	.0278	2.4	.0216	2.4
.094	.1569	3.9	.0708	3.8	.0334	3.4	.0144	3.6	.0150	2.9	.0126	3.3
.047	.1462	4.2	.0622	4.1	.0231	3.7	.0077	3.7	.0075	3.1	.0057	3.2
neutral point	.1308	6.4	.0494	6.6	.0159	6.5	.0076	6.6	.0016	6.6	.0009	7.1
.103	.1575	8.9	.0720	9.0	.0337	9.5	.0183	9.4	.0151	9.2	.0115	9.7
.206	.1755	9.4	.0907	9.6	.0580	9.6	.0282	9.5	.0339	9.3	.0225	9.8
.412	.2234	9.6	.1345	9.6	.1065	9.6	.0668	9.6	.0971	7.4	.0458	9.9

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TABLE 24.

THE EFFECT OF ADDING ACID OR BASE ON THE SOLUBILITY OF GLYCINE
IN ETHANOL-WATER SOLVENT SYSTEMS

Total Acid or Base Added (gms.)	10% (v/v)		30% (v/v)		50% (v/v)		70% (v/v)		90% (v/v)		100% (v/v)	
	Solubility (gm/ml)	pH'	Solubility (gm/ml)	pH'	Solubility (gm/ml)	pH'	Solubility (gm/ml)	pH'	Solubility (gm/ml)	pH'	Solubility (gm/ml)	pH'
.354	.1874	3.6	.1082	3.5	.0561	3.2	.0416	2.9	.0291	2.0	.0073	2.0
.118	.1672	4.1	.0760	3.9	.0309	3.8	.0284	3.5	.0076	3.0	.0022	2.8
.059	.1576	4.8	.0657	4.4	.0263	4.2	.0101	3.9	.0042	3.4	.0014	2.9
neutral point	.1521	6.4	.0609	6.2	.0222	6.3	.0065	6.6	.0010	6.7	.0002	7.1
.096	.1588	8.0	.0689	8.3	.0287	8.8	.0121	9.4	.0073	9.7	.0049	9.5
.192	.1735	8.5	.0830	8.8	.0486	9.2	.0355	9.5	.0116	9.7	.0094	9.3
.384	.1988	8.8	.1159	9.2	.0631	9.5	.0456	9.7	.0326	9.7	.0123	9.5

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TABLE 25.

THE EFFECT OF ADDING ACID OR BASE ON THE SOLUBILITY OF GLYCINE IN n-PROPANOL-WATER SOLVENT SYSTEMS

Total Acid or Base Added (gms.)	10% (v/v)		30% (v/v)		50% (v/v)		70% (v/v)		90% (v/v)		100% (v/v)	
	Solubility (gm/ml)	pH'	Solubility (gm/ml)	pH'	Solubility (gm/ml)	pH'	Solubility (gm/ml)	pH'	Solubility (gm/ml)	pH'	Solubility (gm/ml)	pH'
.354	.2032	3.2	.1568	3.0	.1260	2.9	.0206	2.6	.0064	2.5	.0022	1.3
.118	.1666	3.8	.0960	3.5	.0885	3.6	.0229	2.9	.0050	2.5	.0008	2.6
.059	.1588	4.1	.0867	3.8	.0702	3.8	.0185	3.2	.0046	2.5	.0007	2.6
neutral point	.1492	6.1	.0775	6.2	.0365	6.3	.0089	6.6	.0006	7.0	.0008	5.9
.084	.1583	8.3	.0886	8.6	.0233	9.0	.0133	9.2	.0047	9.7	.0012	7.5
.168	.1798	8.7	.1127	8.8	.0115	9.2	.0122	9.4	.0055	9/7	.0020	7.7
.336	.1853	9.0	.1453	9.2	.0115	9.4	.0081	9.6	.0042	9.5	.0024	7.8

TABLE 26.

THE EFFECT OF ADDING ACID OR BASE ON THE SOLUBILITY OF GLYCINE IN iso-PROPANOL-WATER SOLVENT SYSTEMS

Total Acid or Base Added (gms.)	10% (v/v)		30% (v/v)		50% (v/v)		70% (v/v)		90% (v/v)		100% (v/v)	
	Solubility (gm/ml)	pH'	Solubility (gm/ml)	pH'	Solubility (gm/ml)	pH'	Solubility (gm/ml)	pH'	Solubility (gm/ml)	pH'	Solubility (gm/ml)	pH'
.354	.1967	3.2	.1109	2.9	.0791	2.6	.0447	2.5	.0051	2.4	.0055	1.5
.118	.1647	3.9	.0765	3.7	.0425	3.3	.0200	3.0	.0037	2.3	.0026	2.9
.059	.1565	4.2	.0695	4.0	.0343	3.8	.0132	3.3	.0031	2.6	.0004	3.2
neutral point	.1542	6.2	.0634	6.2	.0274	6.4	.0060	6.6	.0006	6.6	.0003	6.4
.128	.1589	8.9	.0739	8.9	.0402	9.1	.0184	9.5	.0034	9.6	.0009	8.9
.256	.1655	8.9	.0837	9.3	.0498	9.4	.0266	9.6	.0036	9.6	.0017	8.9
.768	.1919	9.1	.1106	9.3	.0812	9.6	.0136	9.5	.0032	9.5	.0019	8.4

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TABLE 27.

THE EFFECT OF ADDING ACID OR BASE ON THE SOLUBILITY OF GLYCINE IN TERTIARY-BUTANOL-WATER SOLVENT SYSTEMS

| Total Acid or Base Added (gms.)	10% (v/v)		30% (v/v)		50% (v/v)		70% (v/v)		90% (v/v)		100% (v/v)	
	Solubility (gm/ml)	pH'	Solubility (gm/ml)	pH'	Solubility (gm/ml)	pH'	Solubility (gm/ml)	pH'	Solubility (gm/ml)	pH'	Solubility (gm/ml)	pH'
.354	.2139	2.9	.1887	3.1	.0189	2.7	.0104	2.7	.0046	2.6	.0062	1.2
.118	.1676	3.5	.1079	3.5	.0185	3.2	.0134	3.2	.0020	2.5	.0002	2.8
.059	.1595	3.9	.0876	3.8	.0180	3.5	.0141	3.2	.0028	2.5	.0004	2.9
neutral point	.1450	6.0	.0707	6.1	.0343	6.3	.0081	6.4	.0004	5.7	.0003	5.0
.09	.1665	2.8	.1070	8.6	.0132	8.8	.0078	9.1	.0013	9.4	.0007	9.3
.18	.1774	8.6	.1370	8.9	.0100	9.1	.0058	9.4	.0003	9.5	.0002	9.3
.36	.1889	9.1	.1698	9.2	.0054	9.3	.0031	9.5	.0009	9.0	.0006	9.3
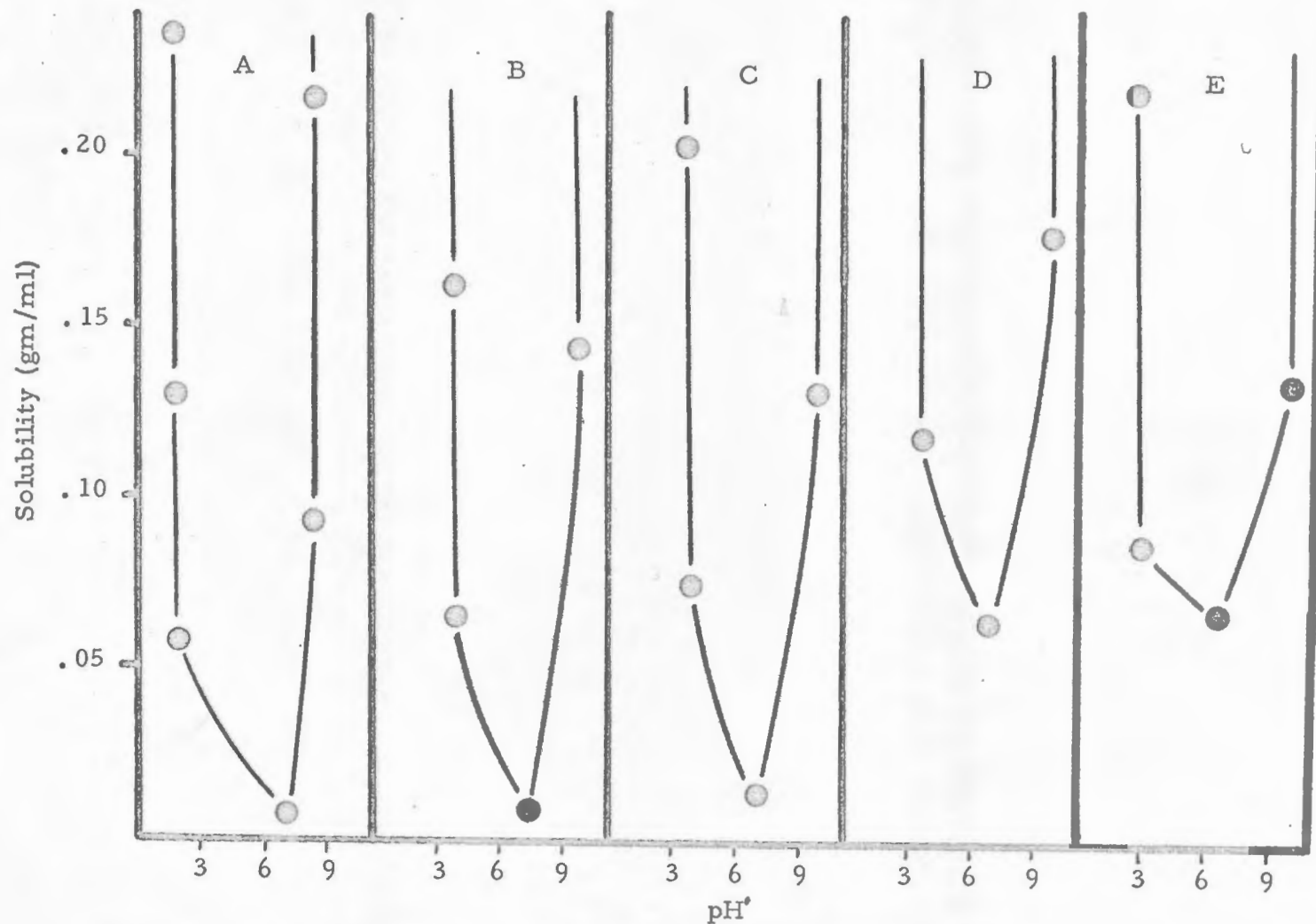
Figure 11. The Solubility of the Amino Acids in Pure Methanol as a Function of pH.
Column A - Glycine, Column B - L-Alanine, Column C - L-Valine, Column D-
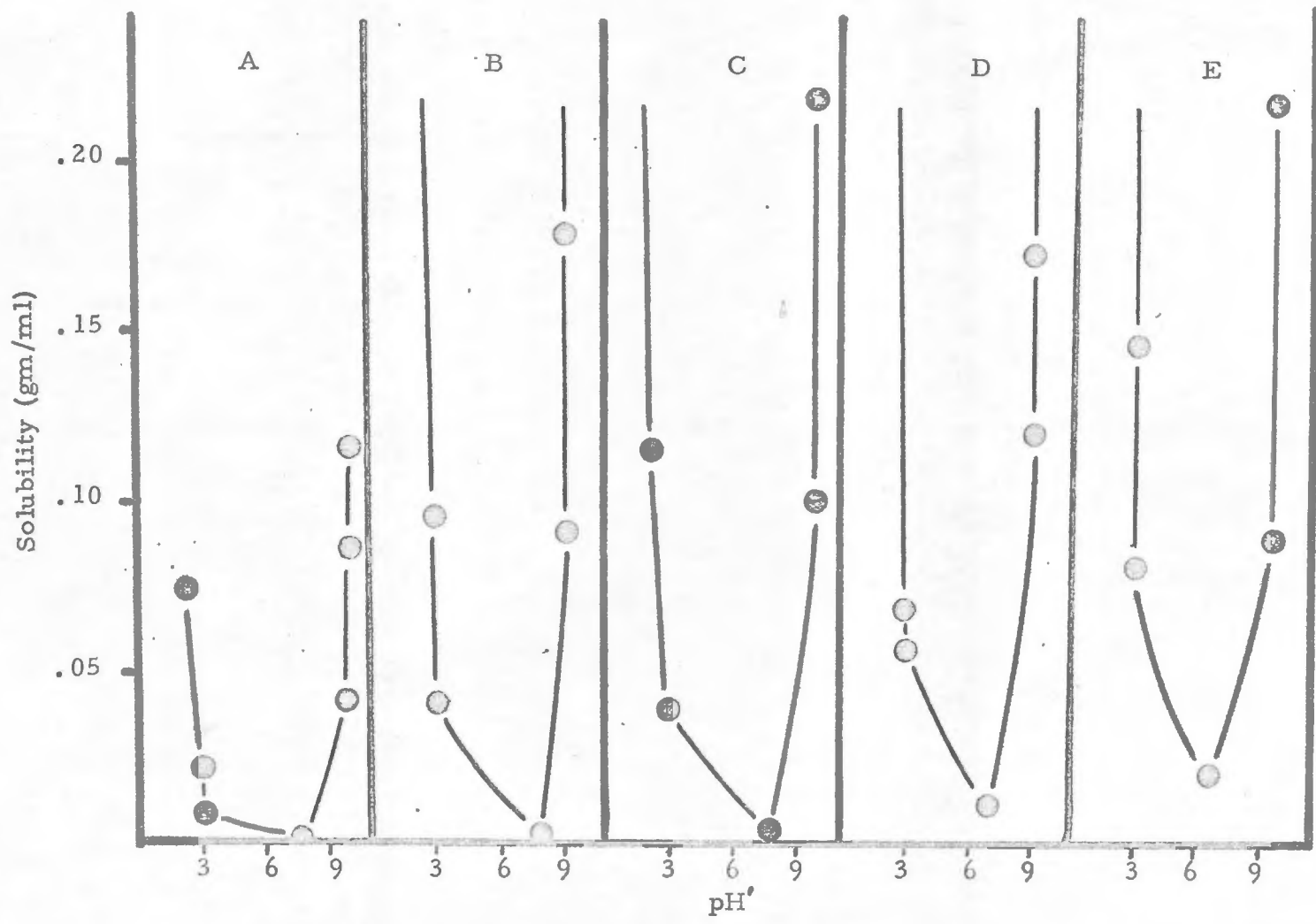L-Phenylalanine, Column E - DL-Aminooctanoic Acid.

Figure 12. The Solubility of the Amino Acids in Pure Ethanol as a Function of pH. Column A - Glycine, Column B - L-Alanine, Column C - L-Valine, Column D - L-Phenylalanine, Column E - DL-Aminooctanoic Acid.
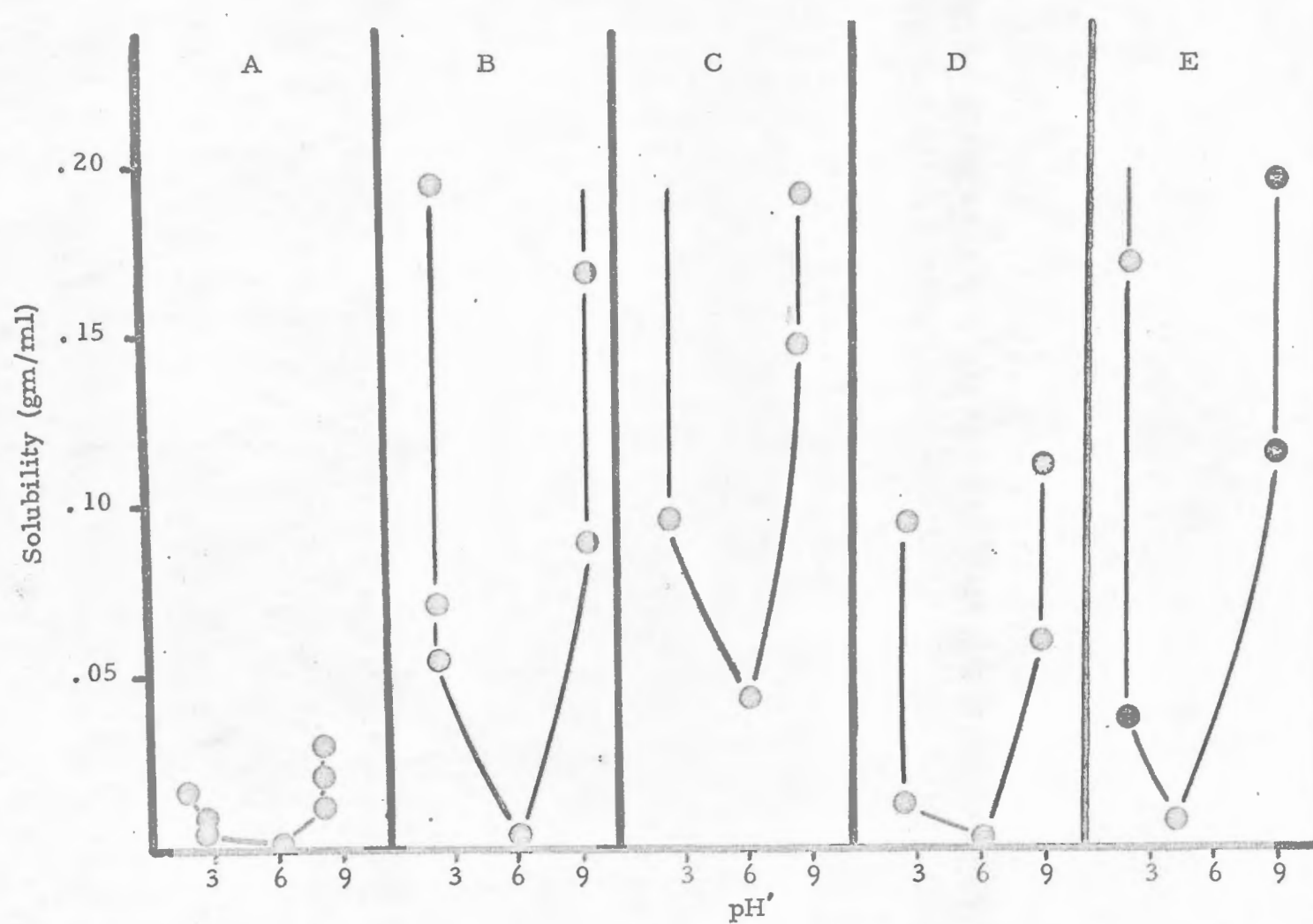
Figure 13. The Solubility of the Amino Acids in Pure n-Propanol as a Function of pH.
Column A - Glycine, Column B - L-Alanine, Column C - L-Valine, Column D-
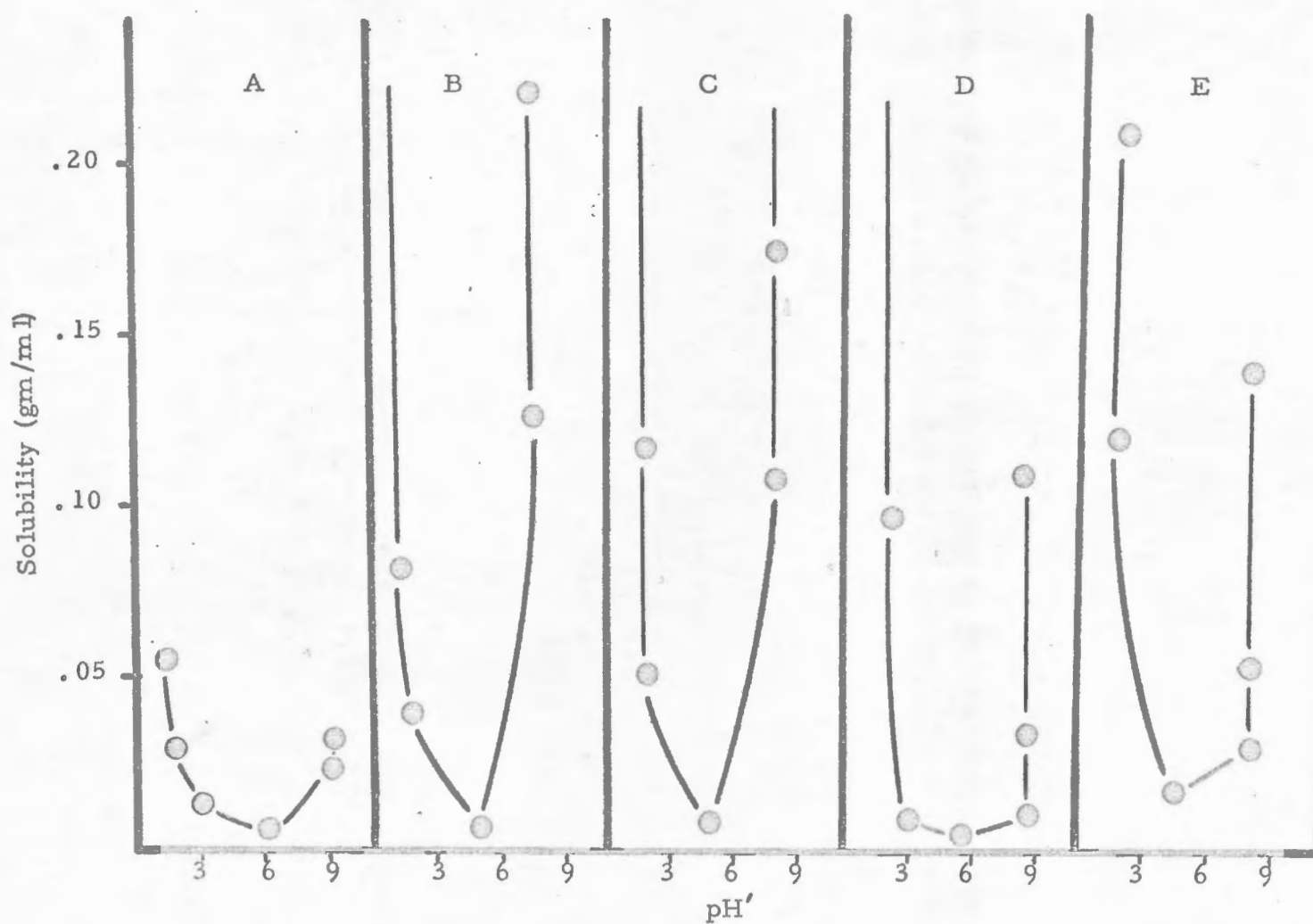L-Phenylalanine, Column E - DL-Aminooctanoic Acid.

Figure 14. The Solubility of the Amino Acids in Pure Isopropanol as a Function of pH.
Column A - Glycine, Column B - L-Alanine, Column C - L-Valine, Column D-
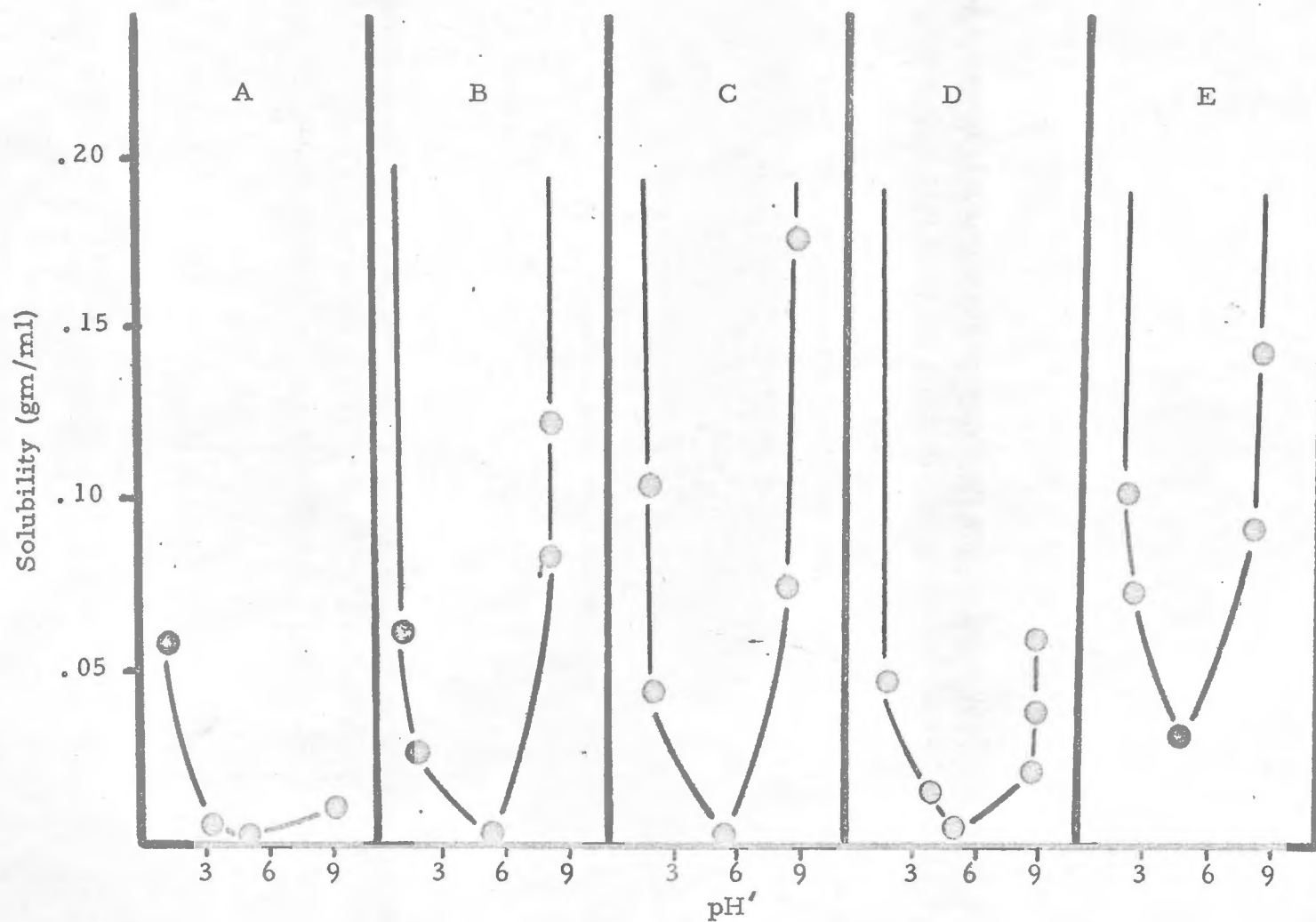L-Phenylalanine, Column E - DL-Aminooctanoic Acid.

Figure 15. The Solubility of the Amino Acids in Pure Tertiary Butanol as a Function of pH'.
Column A - Glycine, Column B - L-Alanine, Column C - L-Valine, Column D-
L-Phenylalanine, Column E - DL-Aminooctanoic Acid.

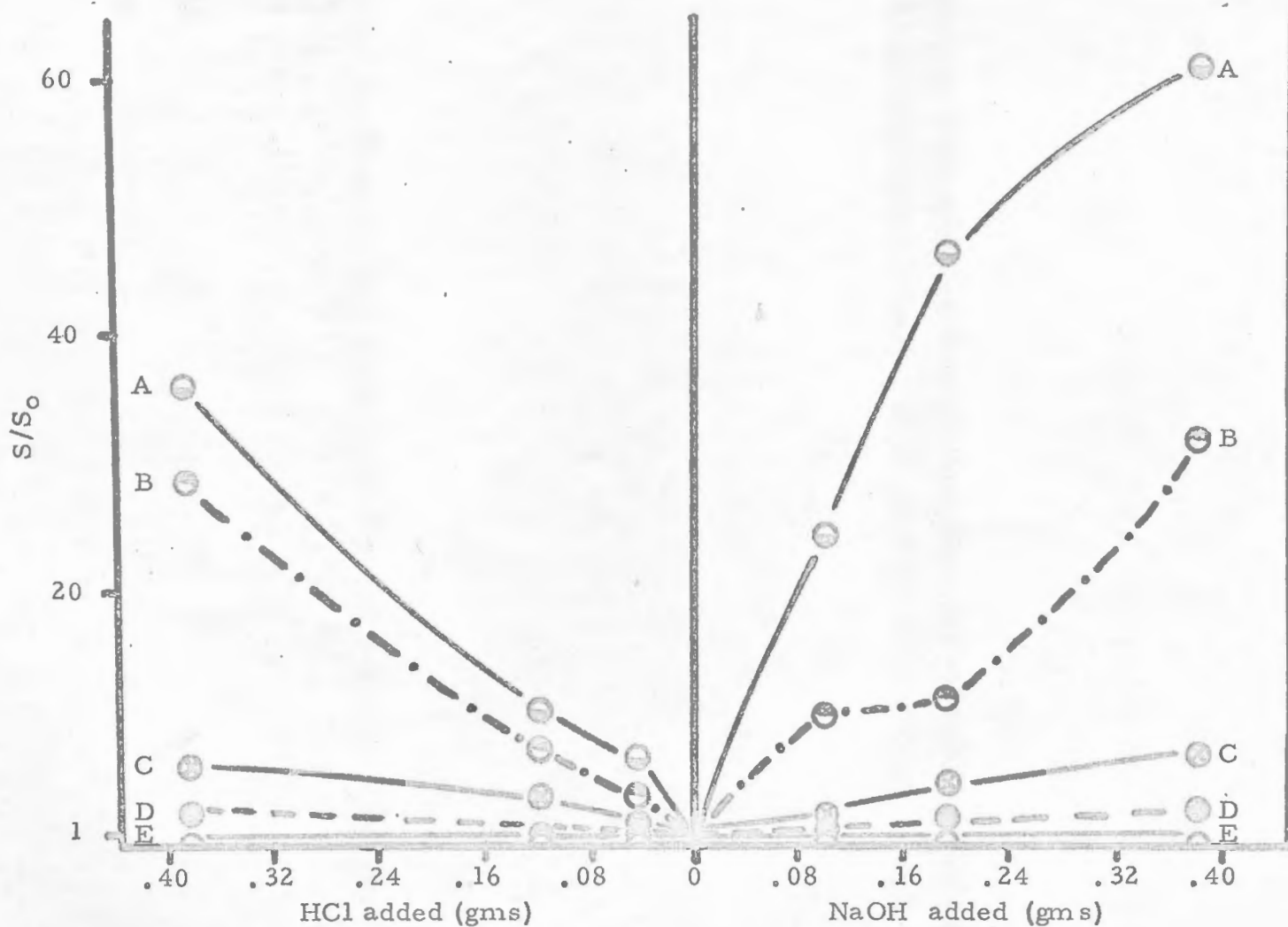Figure 16. The Ratio of the Total Solubility (S) to the Solubility at the Neutral Point (S_o) for Glycine as a Function of HCl and NaOH added in an Ethanol-Water System. Curve A-100%, Curve B-90%, Curve C-70%, Curve D-50%, Curve E-0,10,30% (v/v) of Ethanol

Figure 17. The Ratio of the Total Solubility (S) to the Solubility at the Neutral Point (S_0) for L-Alanine as a Function of HCl and NaOH added in an Ethanol-Water System. Curve A-100%, Curve B-90%, Curve C-70%, Curve D-50%, Curve E-0,10,30% (v/v) of Ethanol.

Figure 18. The Ratio of the Total Solubility (S) to the Solubility at the Neutral Point (S_o) for L-Valine as a Function of HCl and NaOH added in an Ethanol-Water System. Curve A-100%, Curve B-90%, Curve C-70%, Curve D-50%, Curve E-0,10,30% (v/v) of Ethanol.
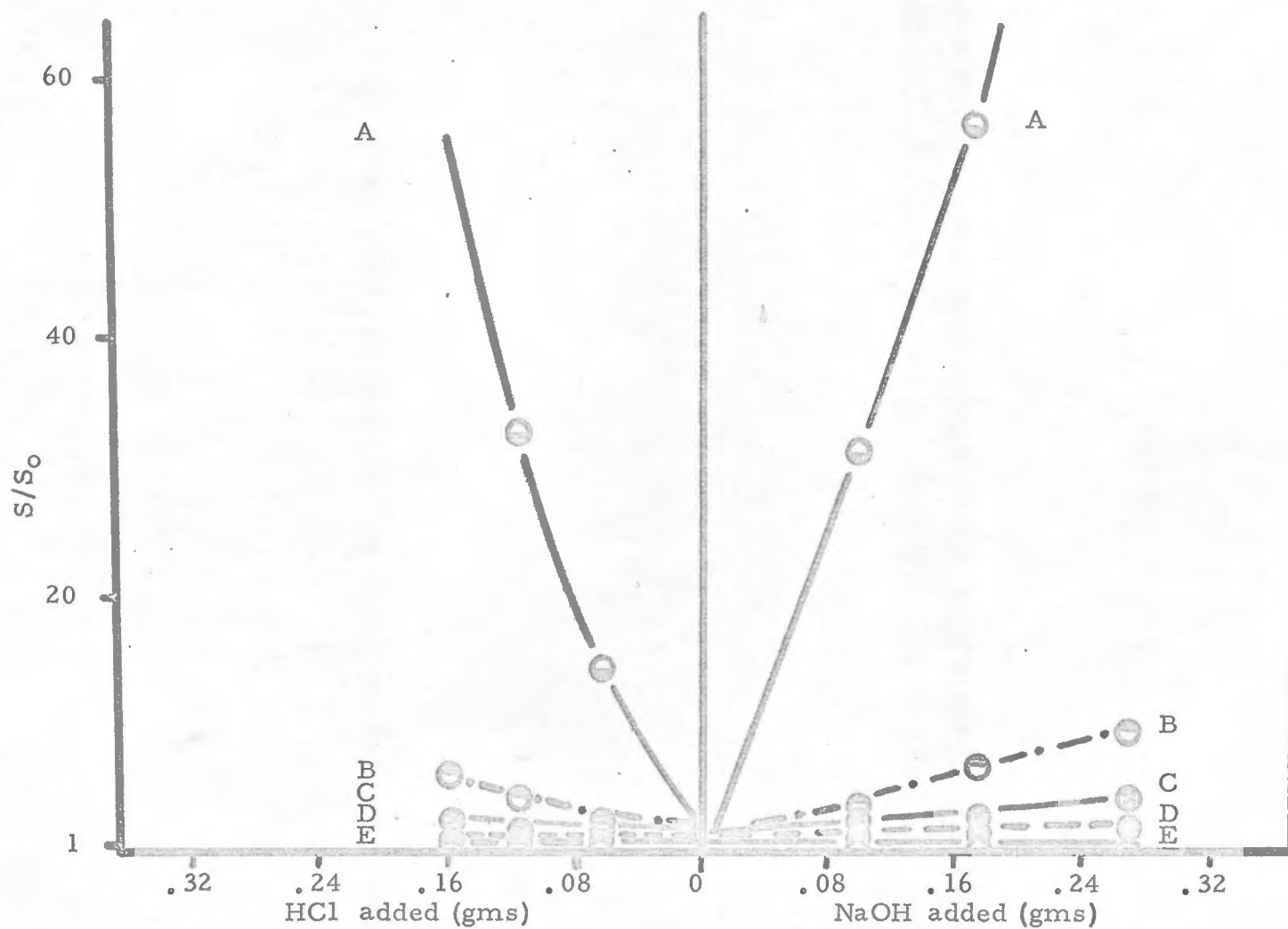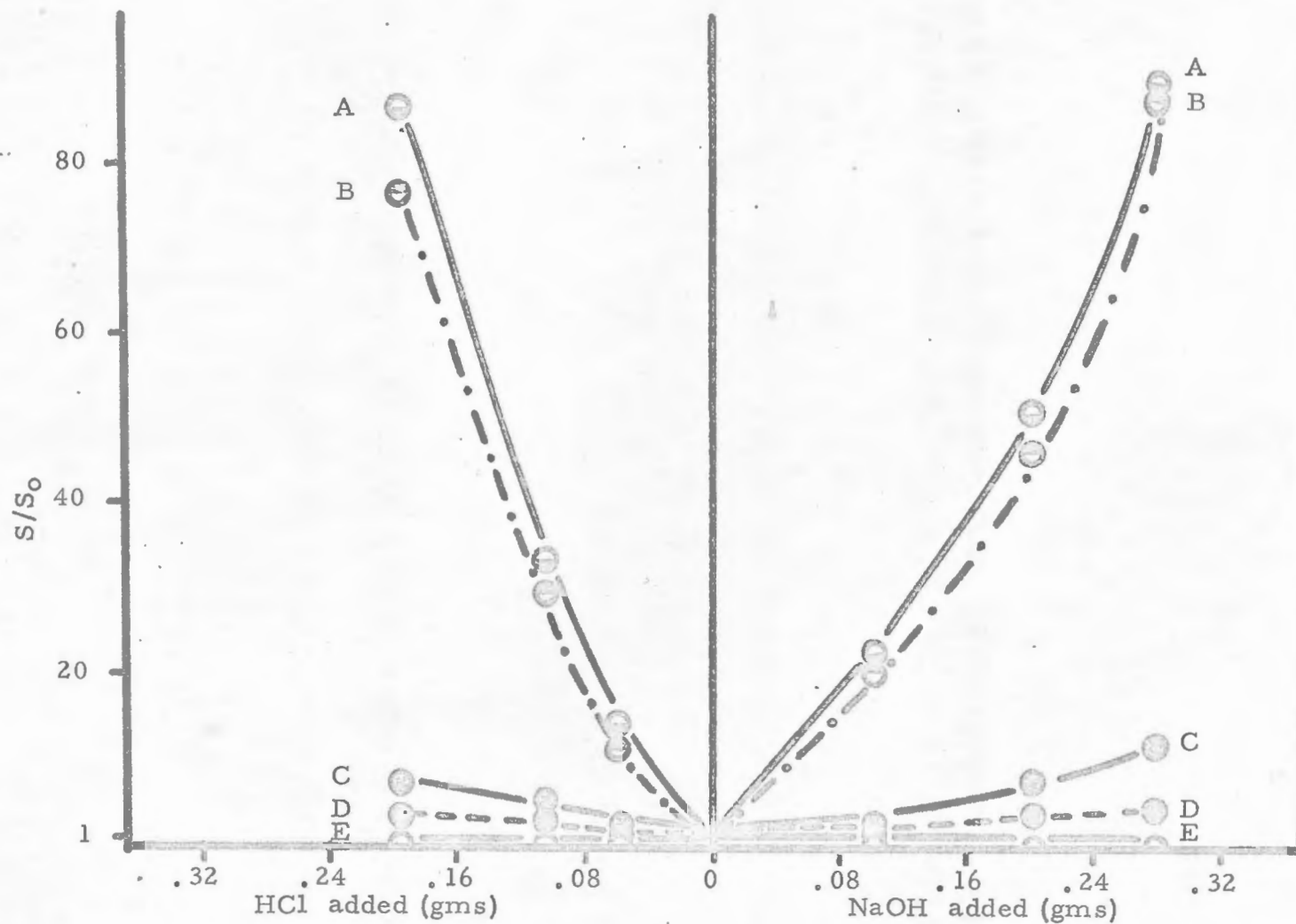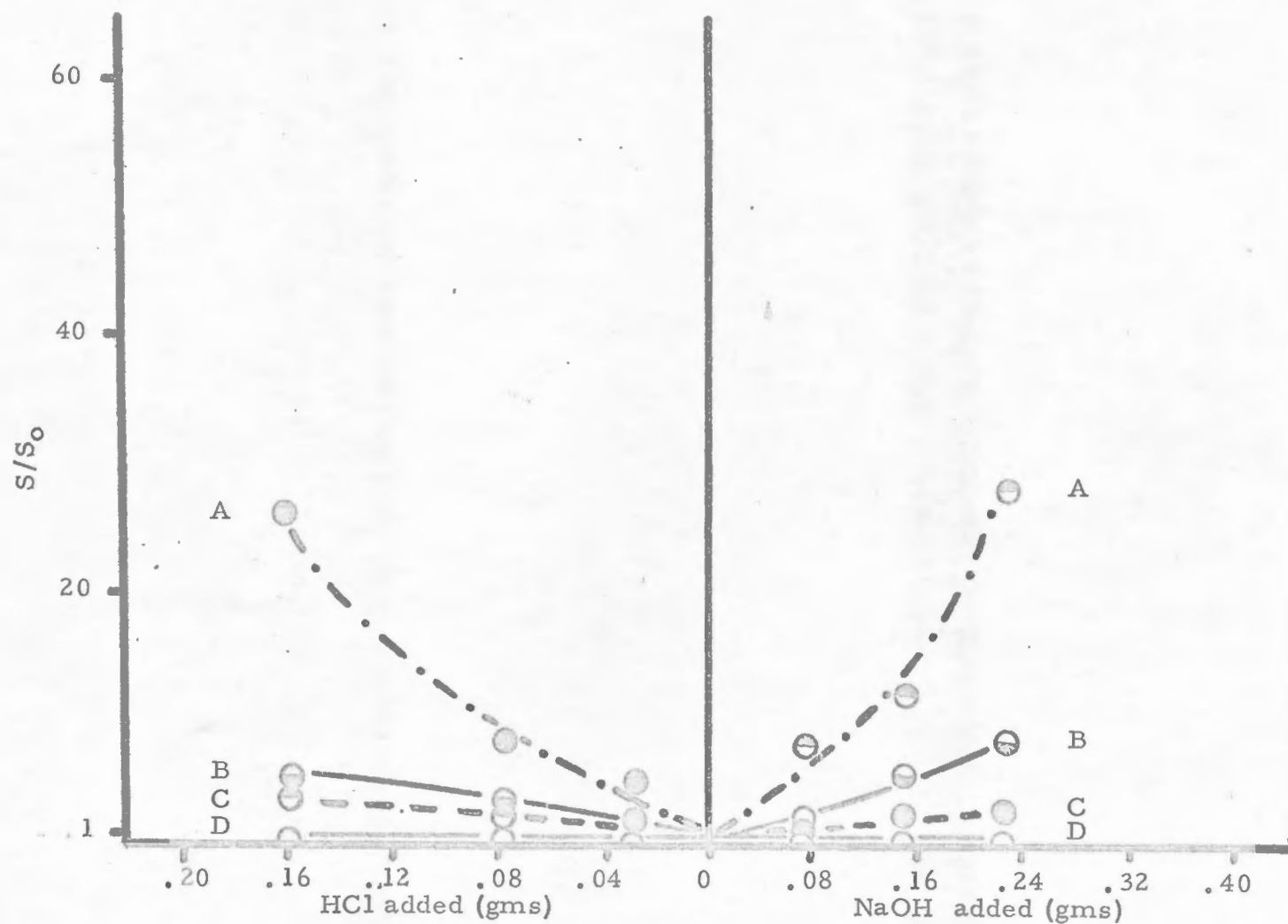
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Figure 19. The Ratio of the Total Solubility (S) to the Solubility at the Neutral Point (S_o) for L-Phenylalanine as a Function of HCl and NaOH added in an Ethanol-Water System. Curve A-100%, Curve B-70, 90%, Curve C-30, 50%, Curve D-0, 10%, (v/v) of Ethanol.

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The 50% and 70%(v/v) begin to show a slight increase in salt forma-
tion as evidenced by an increase in the slope of the line. The 90%
and 100%(v/v) solutions of ethanol show a distinct increase in the
efficiency of salt formation except in the case of L-phenylalanine
which shows a distinct break only at 100%. This increasing effi-
ciency of salt formation can be correlated with mole fraction or
essentially the number of molecules of water present as compared
to the number of molecules of the semi-polar solvent. Note that
up to 70% ethanol the number of water molecules is greater or equiv-
alent to the number of ethanol molecules. (See Table 10) However,
between 70% and 90% the ethanol molecules begin to outnumber the
water molecules and this in turn corresponds to the increased effi-
ciency of salt formation in the solvent systems. In a solvent system
as the dielectric constant of the medium is reduced, the interionic
forces become more important since they compose an increasingly
greater portion of the attractive charge between oppositely charged
ions. These ions of Na or Cl which are added separately to different
solvent systems, as HCl or NaOH, can be attracted to the dipolar
ions in the form of cations or anions to form the amino acid salts or
can form associations with the hydrogen or hydroxyl ions with which
they were added. However, in considering the activity coefficients
for pure HCl (38) and pure NaOH (39) solutions at the molar concen-
trations of acid or base which were added to the amino acids in the
semi-polar solvents; it was found that at the highest concentration of

acid or base added the activity coefficients were reported to be approximately 0.86 and 0.85 respectively. Therefore it would seem that at the low concentrations of acid or base added in solutions of low dielectric constant, the Cl^- and Na^+ ions would provide the major attractive force for the predominately charged amino acids. This increased attraction as the polarity of the solvent system is reduced would seem to explain the greater efficiency of salt formation.

Further, the comparison of the ratio of S to S_0 with the quantity of acid or base added seems to provide an indication of a reduction in the isoelectric band. Since by definition the species present within the isoelectric band are predominately dipolar and produce an invariant solubility, it would seem that an increase in total solubility could be attributed to salt formation. The increase in the S/S_0 ratio as the percent alcohol was increased would indicate a reduction in the isoelectric band as a function of decreasing polarity. This reduction was due to an increased affinity of the amino acids for the Na^+ and Cl^- ions.

IV. CONCLUSIONS

1. The solubility behavior of the amino acids studied is a function of the constant effect of the α-amino carboxylic acid portion of the molecule, and the independent interaction of the remaining portion of the molecule.

2. In the hydroalcoholic solvent systems studied, the solubility of glycine, L-alanine, L-valine, and L-phenylalanine is dominated by the α-amino carboxylic portion of the molecule. Maximum solubility is shown in pure water with a reduction to very low solubility in the semi-polar solvents in the order of a second degree polynomial equation.

3. Each of the amino acids studied demonstrated an ability to differentiate between the hydroalcoholic solvents used throughout the concentration spectrum. A family of second degree polynomial curves could be generated from the solubility data as a function of percent (v/v) of each alcohol. The quantitative order of solubility of each amino acid varied in the solvent systems studied. This would indicate that the structural differences in the alcohols produce slight changes in solubility of each amino acid, but that an overall similarity

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in the solubility profile will be seen. Further, structural differences in the non-polar portion of the amino acid molecules will produce different interactions with molecules of the solvent systems and produce an order of solubility which is characteristic of the specific amino acid.

4. For those amino acids studied in aqueous solvent systems, a band of minimum solubility is found from a pH above and below the isoelectric point. A distinct increase in total solubility is seen as the pH exceeds the limits of this isoelectric band.

5. In aqueous systems, the increase in total solubility of those amino acids studied is directly proportional to the moles of acid or base added.

Total solubility = (slope)(moles of acid or base added) + initial solubility of the amino acid.

The slope which represents the increase in total solubility as a function of acid or base added shows an increase as the non-polar to polar ratio of the amino acids increases. This would indicate that as the non-polar portion of the amino acid molecule increases, the anion or cation species is more readily available for salt formation.

6. The total solubility of the amino acids studied at various pH's in aqueous solutions is the sum of the original zwitterion

solubility plus the salt formed by acid or base addition. Under the experimental conditions observed, the amino acids studied form the single salts.

7. In the hydroalcoholic solvent systems studied, the amino acids show a minimum solubility at a pH' corresponding to the iso-electric point. However, the isoelectric band seen in aqueous systems was not seen.

8. As the percent of alcohol increased in those solvent systems studied, similar increments of acid or base added to the system produced a proportionally greater increase in the magnitude of the total solubility of the amino acids. This was attributed possibly to an increase in the importance of the charged species as the polarity of the solvent system was decreased as well as the increased affinity of the amino acids for the Na^+ and Cl^- ions present.

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(39)   Lewis, G. N., and Randall, M., _Thermodynamics and Free Energy of Chemical Substances_, (McGraw Hill Book Co., New York, 1923) p. 302.

VI. APPENDIX

I. Program Fitting a Set of Data to a Polynomial Equation

A successive series of polynomial equations are calculated from a set of data consisting of two variables, X and Y. The Y intercept and the coefficients for each degree of X is given. A standard analysis of variance table is displayed for each degree of the polynomial calculated. The sum of the squares of the deviation about the regression for the polynomial equation if compared with that of the preceeding equation. The program is terminated when no improvement in the fit of the equation is shown. A table of residuals is calculated to show the difference between the input data and the data computed from the final polynomial equation. A graph is produced showing the calculated and observed values of the dependent variable as a function of the independent variable for the highest polynomial equation computed.

The actual program written in Fortran IV computer language as well as related theoretical references may be found in references (28, 29).

II.   Program Computing Least Squares of a First Degree

Equation with Correlation Coefficient.

```
        DIMENSION X(20), Y(20), XD(20), YD(20), XDS(20), YDS(20),
        XY(20), ALF(15)
        READ(5,1)ALF
    1   FORMAT(15A4)
        READ(5,2)N
    2   FORMAT(I2)
        DO 4 I=1, N
        READ(5,3)X(I), Y(I)
    3   FORMAT(2F6.4)
    4   CONTINUE
        XSM=0
        YSM=0
        DO 5 I=1, N
        XSM=XSM+X(I)
        YSM=YSM+Y(I)
    5   CONTINUE
        XA=XSM/N
        YA=YSM/N
        XYS=0
        XDSS=0
        YDSS=0
        DO 6 I=1, N
        XD(I)=X(I)-XA
        YD(I)=Y(I)-YA
        XDS(I)=XD(I)**2
        YDS(I)=YD(I)**2
        XY(I)=XD(I)*YD(I)
        XYS=XYS+XY(I)
        XDSS=XDSS+XDS(I)
        YDSS=YDSS+YDS(I)
    6   CONTINUE
        SLOPE=XYS/XDSS
        R=XYS/SQRT(XDSS*YDSS)
        WRITE(6,10)
   10   FORMAT(///, T20, 'CORRELATION COEFFICIENT OF
                AMINO ACID VS. ACID OR BASE ADDED')
        WRITE(6,11)ALF
   11   FORMAT(//, T25, 15A4)
        WRITE(6,12)R, SLOPE
   12   FORMAT(//, T35, 'R = ', T45, F6.4, //, T35, 'SLOPE = ',
```

```
          T45, F6.4)
      B=YA-(SLOPE*XA)
      WRITE(6,100)B
100   FORMAT(//, T30,'Y INTERCEPT = ', T42, F6.4)
      STOP
      END
```