Effect of Anions on the Solubility of Zwitterionic Amino Acids

Anjali A. Pradhan and Juan H. Vera*

Department of Chemical Engineering, McGill University, Montreal, Quebec, Canada H3A 2B2

The solubilities of glycine, DL-alanine, DL-valine, and DL-serine in aqueous solutions of $NaNO_3$ and of KNO_3 are reported at 298.2 K. No pH adjustment or buffer was used in the measurements. The effect of the anion of the electrolyte on the solubility of the amino acid is observed by comparing these results with values reported in the literature for the effect of NaCl and KCl. For each cation, the solubilities of the amino acids are higher with nitrate anion than with chloride anion. With the exception of DL-alanine, the solubility of the amino acid is larger with potassium as the cation than with sodium, at the same concentration of the nitrate salt. This behavior of DL-alanine in nitrate solutions contrasts with literature results using chloride as anion. In the case of chloride as anion, the solubility of the amino acid is always larger in the presence of potassium than in the presence of sodium.

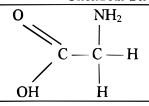
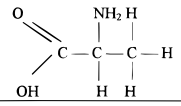
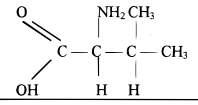
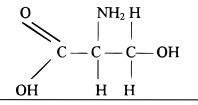
Introduction

The production of amino acids is usually done by synthesis or fermentation. Their separation from the excess reagents, and other impurities in the aqueous solution, is often done by crystallization or precipitation methods. The cost of these separation processes accounts for about 50% of the total production cost (Eyal and Bressler, 1993). The knowledge of the solubilities of amino acids in the presence of different electrolytes is useful for rational design of these processes. In particular, the separate effect of different cations and anions is of potential interest for the separation of amino acids.

There are 20 basic amino acids found in all living organisms. These compounds have an amino group and a carboxyl group, which ionize in aqueous solutions and give rise to zwitterionic amino acid molecules in the neutral pH range. The zwitterionic amino acid molecules have a large dipole moment, which gives rise to important interactions with individual ions.

In a previous publication (Pradhan and Vera, 1998), we reported data on the effect of the cation of a base and of the anion of an acid on the solubility of amino acids. When an acid or a base is added to an aqueous solution of an amino acid, the amino acid passes from its natural zwitterionic form to either a cationic or an anionic form. In this study we focus our attention on the effect of anions on the solubility behavior of the zwitterionic form of the following four amino acids: glycine, DL-alanine, DL-valine, and DL-serine. Table 1 depicts the chemical structures of these four amino acids. Glycine has the simplest chemical structure with only two carbon atoms, DL-alanine and DL-valine have one hydrogen atom of the α-carbon replaced by a methyl and by an isopropyl radical, respectively, and DL- serine is similar to DL-alanine except that it has an OH group replacing a hydrogen of the methyl group.

Table 1. Chemical Structure of Amino Acids Studied

Amino acid	Chemical Structure
Glycine	
DL-alanine	
DL-valine	
DL-serine	

Materials and Methods

Glycine, DL-alanine, DL-valine, and DL-serine, of 99% purity, were obtained from A & C Chemicals Ltd. (Montreal, Quebec, Canada). Sodium nitrate and potassium nitrate were obtained from Anachemia Canada Inc. (Montreal, Quebec, Canada).

The salts were dried for 72 h, and then, prior to use, they were cooled in a desiccator. Electrolyte solutions at five different molalities were prepared using deionized water, with a conductivity of $<0.8\ \mu S\ cm^{-1}$, passed through ion-exchange columns of East pure RF, Compact Ultrapure Water System, Barnstead Thermoline. To compare the effect of the anion, the electrolyte concentrations used were in the same range as those used by Khoshkbarchi and Vera (1997), in their study of the effect of the cation using chloride salts. All solutions were prepared on a mass basis.

Vials of 24-mm outer diameter and 95 mm height were used as sample bottles. The amino acids were added in excess of the amount required for saturation. The electrolyte was then added, as a 20-mL solution, into the sample bottles. The sample bottles were sealed using Parafilm and kept in a thermostatic water bath at 298.2 K. The solutions were agitated for 48 h using Teflon-coated magnetic stir bars. The mixing was then stopped, and the solutions were allowed to settle for 7 h.

Samples were taken of the supernatant liquid phase using a plastic syringe and filtered through a 0.22 μm HPLC MSI disposable filter. An aluminum dish was

* Corresponding author. E-mail: Jvera@chemeng.Lan.McGill.ca.

10.1021/je9902342 CCC: $19.00 © 2000 American Chemical Society
Published on Web 12/16/1999

Table 2. Experimental Data of the Solubility, in mol per kg of water, of Glycine, DL-Alanine, DL-Valine, and DL-Serine in the Presence of the Electrolytes $NaNO_3$ and KNO_3

	m/mol kg^{-1}							
	glycine		DL-alanine		DL-valine		DL-serine	
electrolyte/mol kg^{-1}	$NaNO_3$	KNO_3	$NaNO_3$	KNO_3	$NaNO_3$	KNO_3	$NaNO_3$	KNO_3
0	3.333	3.333	1.891	1.891	0.600	0.600	0.476	0.476
0.05	3.229	3.229	1.916	1.931	0.622	0.694	0.497	0.525
0.1	3.201	3.202	2.040	1.935	0.678	0.712	0.550	0.565
0.2	3.558	3.575	2.175	2.055	0.697	0.750	0.618	0.625
0.3	3.706	3.697	2.290	2.190	0.736	0.750	0.660	0.707
0.5	4.034	4.066	2.530	2.440	0.911	0.960	0.780	0.830
1.0	5.097	5.194	3.115	3.020	1.460	1.560	1.210	1.520
1.5	6.184	6.263	3.628	3.567	2.000	2.240	1.670	2.257

weighed empty and with the filtered solution. The dish and its weighed cap were put into an oven for 48 h at about 308 K and weighed again with the dry sample. The solubility of the sample was calculated from knowledge of the mass of the empty dish, the mass of the cap, the mass of the dish with the solution and the cap, the dry mass of the solid, and the electrolyte concentration of the sample.

To test the accuracy of the above experimental procedure, the solubility of glycine was measured in pure water and compared with literature values (Fasman, 1976). The 95% confidence interval was found to be ±0.009 molal. The values reported in this work are the average of at least three replicates. In the replicates done, different quantities of the amino acid were taken in excess of that at saturation. The results were found to differ by <0.9% molal. The 95% confidence intervals in molalities were found to be ±0.003 for DL-alanine + $NaNO_3$ and DL-alanine + $KN0_3$, ±0.006 for glycine + $NaNO_3$, ±0.008 for glycine + KNO_3, ±0.009 for DL-serine + $NaNO_3$ and DL-serine + KNO_3, ±0.007 for DL-valine + $NaNO_3$, and ±0.009 for DL-valine + $KN0_3$.

To check the possibility of adsorption or incorporation of $NaNO_3$ on the solid-phase amino acid, atomic absorption was used to analyze sodium in the solution. The concentrations of sodium in the electrolyte−water system and in the amino acid−electrolyte−water system were compared. Quantities of amino acid 5%, 10%, and 50% in excess to saturation were added for these comparisons, and the sodium concentration was measured in the supernatant phase. The maximum difference in the results was ±0.009 molal, implying that, even with different quantities of the amino acid added, no appreciable amount of electrolyte was precipitated or adsorbed on the amino acid in the solid phase.

Experimental Results and Discussion

Table 2 presents the experimental data collected in this work. Notably, at high electrolyte molalities, DL-alanine has a higher solubility in the presence of sodium than in the presence of potassium when nitrate is the anion. All the other three amino acids studied here have the opposite behavior. When chloride is the anion, the results reported by Khoshkbarchi and Vera (1997) show that for all the amino acids considered here the solubility is higher with potassium than with sodium.

Figures 1−8 compare the results of this work with literature results (Khoshkbarchi and Vera, 1997) for the same cations with the chloride anion. Figure 1 shows that the solubility of glycine in aqueous solutions of sodium nitrate and sodium chloride first decreases and then increases with an increase in the electrolyte concentration. As seen from Figure 1 at low concentrations, the data points for glycine in the presence of sodium nitrate or sodium chloride overlap. At high concentration of the electrolyte, there is a marked increase in solubility when

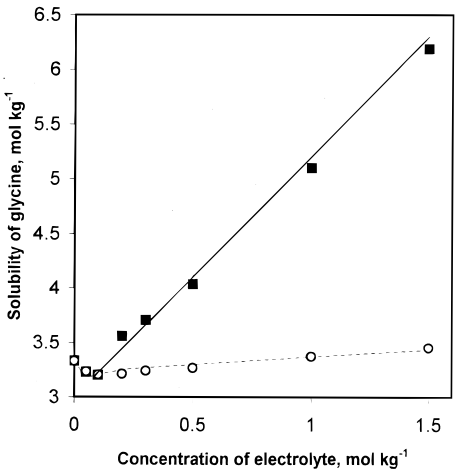


Figure 1. Effect of $NaNO_3$ and NaCl on the solubility of glycine, in mol kg^{-1}, at the same electrolyte concentration: (■) $NaNO_3$; (○) NaCl.

sodium nitrate is present and only a slight increase in solubility with sodium chloride present. Since the experiments described in the previous section showed that no salt precipitates with the amino acid, one concludes that the nitrate anion retains the amino acid in the aqueous phase. The amino acids, existing as zwitterions $^{-}AA^{+}$ in the system, may form complexes with the cation C^{+} and the anion A^{-} of the electrolyte, of the form

$$^{-}AA^{+} + C^{+}A^{-} \Leftrightarrow C^{+}(^{-}AA^{+})A^{-}$$

The comparison of results for glycine in potassium nitrate and in potassium chloride aqueous solutions shown in Figure 2 confirms the strong effect of the anion on the solubility of this amino acid. In Figure 2 we see again the overlap of data points for glycine in the presence of potassium nitrate or potassium chloride at low salt concentrations.

Figure 3 shows the solubility of DL-alanine in sodium nitrate and sodium chloride aqueous solutions. A salting-in effect is observed with the nitrate anion, and a salting-out effect is seen with the chloride anion. This interesting behavior shows the significant effect of the anion of the electrolyte on the solubility of this amino acid. The difference in the solubility trends is probably due to the kind of complexes formed in the aqueous phase by the amino acid with the different anions. Figure 4 shows the solubility behavior of the same amino acid in aqueous solution with the potassium cation and the two anions. With both potassium chloride and potassium nitrate, a salting-in effect is observed.

Figure 5 shows the solubility of DL-valine in aqueous solutions containing sodium nitrate and sodium chloride.

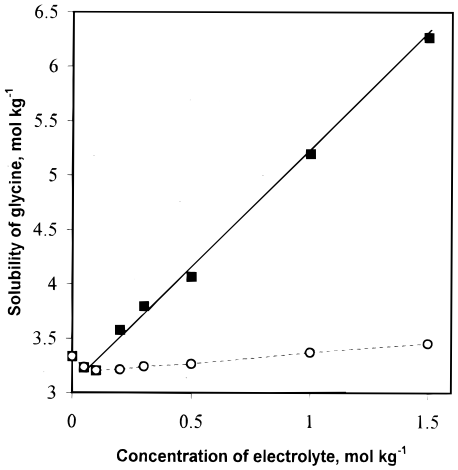


Figure 2. Effect of KNO_3 and KCl on the solubility of glycine, in mol kg^{-1}, at the same electrolyte concentration: (■) KNO_3; (○) KCl.

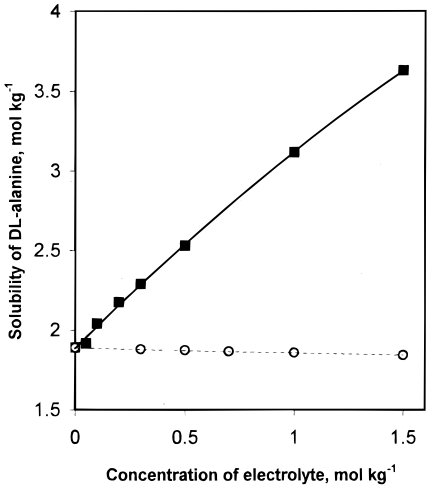


Figure 3. Effect of $NaNO_3$ and NaCl on the solubility of DL-alanine, in mol kg^{-1}, at the same electrolyte concentration: (■)-$NaNO_3$; (○) NaCl.

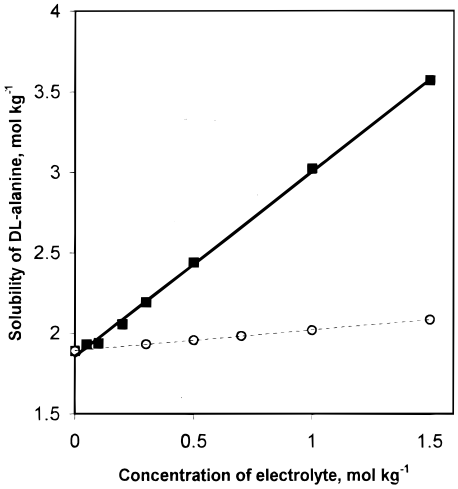


Figure 4. Effect of KNO_3 and KCl on the solubility of DL-alanine, in mol kg^{-1}, at the same electrolyte concentration: (■) KNO_3; (○) KCl.

The effect of the anion is quite significant in this case, as a salting-in effect is seen with the nitrate anion whereas a salting-out effect is seen with the chloride anion. The difference in the solubility increases as the concentration

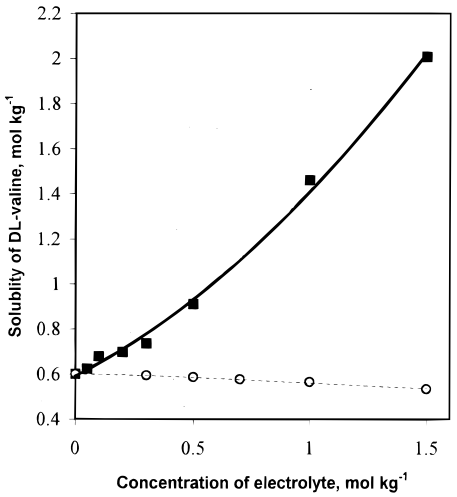


Figure 5. Effect of $NaNO_3$ and NaCl on the solubility of DL-valine, in mol kg^{-1}, at the same electrolyte concentration: (■) $NaNO_3$; (○) NaCl.

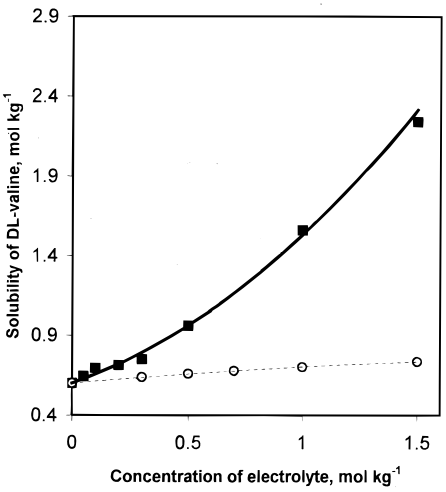


Figure 6. Effect of KNO_3 and KCl on the solubility of DL-valine, in mol kg^{-1}, at the same electrolyte concentration: (■) KNO_3; (○) KCl.

of the electrolyte increases. This can be due to the type of complexes formed by the amino acid in the presence of the different electrolytes and their tendencies to shield the hydrophobic interactions. The comparison of solubility trends in different anions of DL-valine and DL-alanine is similar; DL-valine has two CH_2 groups more than DL-alanine. Hence, in DL-valine and DL-alanine the hydrophobic interactions are larger than those in glycine, leading to a salting-out effect, even with the chloride anion, over the entire range of concentration studied.

Figure 6 shows the solubility of DL-valine in potassium nitrate and potassium chloride aqueous solutions. The chloride and the nitrate anions cause an increase in the solubility of the amino acid. The salting-out effect of chloride ion depicted in Figure 5 is not observed for the case of potassium. This difference in the effect of cations is probably also related to the kind of complexes that the cation forms with the amino acid in aqueous solution.

Figure 7 shows the solubility of DL-serine in sodium nitrate and sodium chloride aqueous solutions. The solubility increases in both sodium nitrate and sodium chloride solutions. With sodium nitrate, in the range of concentration studied, the solubility increases to almost twice the value of the solubility in pure water. The difference

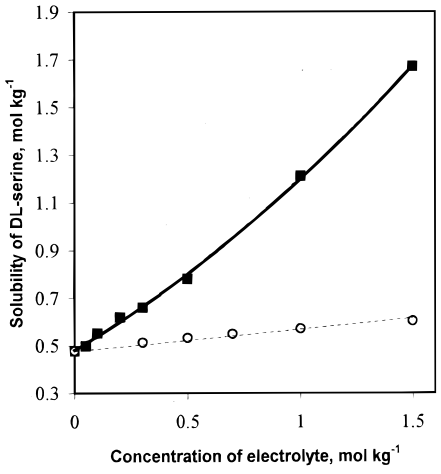


Figure 7. Effect of $NaNO_3$ and NaCl on the solubility of DL-serine, in mol kg^{-1}, at the same electrolyte concentration: (■) $NaNO_3$; (○) NaCl.

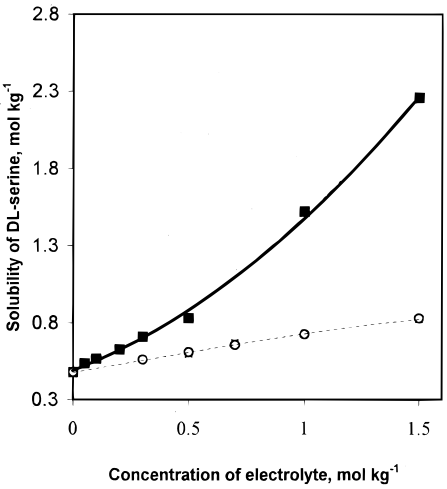


Figure 8. Effect of KNO_3 and KCl on the solubility of DL-serine, in mol kg^{-1}, at the same electrolyte concentration: (■) KNO_3; (○) KCl.

between DL-serine and DL-valine is that DL-serine has an OH group. This group obviously causes a salting-in effect with both anions. Figure 8 shows the solubility of DL-serine in potassium chloride and potassium nitrate aqueous solutions. The solubility of DL-serine increases with both the chloride and the nitrate ions when the cation is potassium.

The experiments described in the previous section indicate that the electrolyte does not participate in the solid amino acid phase. However, as previously reported by Khoshkbarchi and Vera (1997), it was observed that the amino acid crystals obtained in the presence of an electrolyte are different in appearance from the crystals obtained in pure water. In pure water and at low salt concentrations, the crystals are small, but as the concentration of salt increases, the crystals grow in size and become needle-like. In addition we observed that the length of the amino acid crystals formed in the presence of potassium nitrate at high concentration was up to 1 mm whereas in the presence of sodium nitrate, at the same molality, the length of the crystal was shorter. Further crystallographic study was considered to be beyond the scope of the present study.

Conclusions

Experimental data for the solubility of four amino acids in $NaNO_3$ and KNO_3 solutions were measured. Comparison of these results with literature data (Khoshkbarchi and Vera, 1997) showed the effect of the two anions Cl^- and NO_3^-. For all cases studied, it was observed that the solubilities are always higher in the presence of NO_3^- than in the presence of Cl^-. These systems also show a difference between solubilities in the presence of sodium and potassium. Thus, it is demonstrated that the nature of both the cation and the anion affects the solubility of the amino acids in electrolyte solutions. The behavior of DL-alanine with $NaNO_3$ and KNO_3 seems to be anomalous. While for all other amino acids studied here the solubility is higher in the presence of potassium, when the anion is NO_3^-, for DL-alanine the reverse holds. We verified the results repeatedly for this system, since with the Cl^- as anion all amino acids, including DL-alanine, present higher solubility with potassium than with sodium (Khoshkbarchi and Vera, 1997). Recently reported results for aminobutyric acid with $NaNO_3$ and KNO_3, however, also show a higher solubility in the presence of sodium (Soto et al., 1998).

Literature Cited

Eyal, A. M.; Bressler, E. Industrial Separation of Carboxilic and Amino Acids by Liquid Membranes: Applicability, Process Considerations, and Potential Advantages. *Biotechnol. Bioeng.* **1993**, *41*, 287−295.

Fasman, G. D. Physical and Chemical Data. *Handbook of Biochemistry and Molecular Biology*, 3rd ed.; CRC Press: Cleveland, OH, 1976; Vol. 1.

Khoshkbarchi, M. K.; Vera, J. H. Effect of KCl and NaCl on the Solubility of Amino Acids at 298.2 K: Measurement and Modeling. *Ind. Eng. Chem. Res.* **1997**, *36* (6), 2445−2451.

Pradhan, A. A.; Vera, J. H. Effect of Acids and Bases on the Solubility of Amino Acids. *Fluid Phase Equilib.* **1998**, *152*, 121−132.

Soto, A.; Arce, A.; Khoshkbarchi, M. K.; Vera, J. H. Effect of the Cation and the Anion of an Electrolyte on the Solubility of DL-Aminobutyric Acid in Aqueous Solutions: Measurement and Modelling. *Biophys. Chem.* **1998**, *73*, 77−83.

Received for review August 20, 1999. Accepted November 2, 1999. The authors are grateful to the Natural Sciences and Engineering Research Council of Canada for financial support.

JE9902342