## Apparent Molar Volumes and Viscosities of Dl-α-Alanine in Water-Ethanol Mixtures

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# APPARENT MOLAR VOLUMES AND VISCOSITIES OF DL-α-ALANINE IN WATER-ETHANOL MIXTURES

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In this work we present a systematic study of the apparent molar volumes and the viscosities of DL-α-alanine in aqueous ethanol solutions at 25,00°C. The molar fractions of ethanol for the solvent mixtures were selected taking into account that thermo-dynamic properties such as partial molar volumes and heat capacities of these mixtures show a transition concentration around $X_{et} = 0,1$ at which its behavior suffers a deep change. Besides, this ethanol concentration is near to that required to produce 50% of protein denaturation.

The results were used to evaluate the limiting partial molar volume $\bar{V}^o$ of the solute, the volume changes associated with its transfer from water to aqueous ethanol solutions and the viscosity $B$ coefficients of DL-α-alanine. The values obtained for the transference molar volumes and the viscosity $B$ coefficients of alanine in the aqueous mixtures show a minimum at $X_{et} = 0,1000$. The results are discussed in terms of changes in the solvent structure (Ref. [1]).

*Keywords:* Amino acid; apparent molar volume; viscosity; mixed solvents; solute-solvent interactions

## 1. INTRODUCTION

Considerable importance has attached, during the last few years, to the study of the physico-chemical properties of amino acid solutions in water and mixed solvents (Ref. [2–24]), because these model compounds can give important information helping the understanding of

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the thermodynamic behavior of proteins and the role of solvent structure in the denaturation process.

Even though alcohols are well known as denaturating agents, few studies on properties of amino acids in water-alcohol mixtures have been made, even though such information contributes to the understanding of the nature of their effect on the native conformation of proteins. The reason is probably related to the complex nature of the alcohol-water mixtures about which there is not yet a definite explanation in terms of the intermolecular forces that determine their particular behavior (Ref. [25–31]).

In this work we present a study of the apparent molar volumes and the viscosities of DL-$\alpha$-alanine in aqueous ethanol solutions. The dependence of the limiting apparent molar volumes, the transfer molar volumes and the viscosity $B$ coefficient of alanine on ethanol concentration do not follow the trend observed in water or other aqueous solvents. This behavior is discussed in terms of solvent structure.

## 2. EXPERIMENTAL SECTION

The materials used in this work were: DL-$\alpha$-alanine Merck analytical reagent which was dried under vacuum for 48 h before use. Ethanol JAC analytical reagent which was distilled and then dried over 3 Å molecular sieves. The water was doubly distilled according to literature (Ref. [32]) and degassed just before use. All the solutions were prepared by weight using a Mettler balance Model AT261.

Densities of the solutions were determined using a magnetic float densimeter described elsewhere (Ref. [33]) with an uncertainty of the order of $\pm 2 \times 10^{-6} \, \mathrm{g \, cm^{-3}}$. The viscosity measurements were done using two Ubbelohde viscometers with efflux times near 350 s for liquid water. All measurements were realized in a constant temperature bath at 25,00°C. The temperature was controlled to $\pm 0,002°C$.

## 3. RESULTS

The densities and efflux times were determined at 25,00°C for DL-$\alpha$-alanine in aqueous ethanol solutions at the following ethanol molar

fractions: $X_{et}$ = 0,0200; 0,0375; 0,1000; and 0,1435. The density and viscosity values measured for the ethanol solutions are given in Table I.

The apparent molar volumes $\Phi_v$ were calculated from Eq. (1).

$$\Phi_v = M/d + 1000(d_0 - d)/md_0d \qquad (1)$$

where $M$ is the molecular weight of alanine, $m$ its molal concentration, $d_o$ is the density aqueous ethanol solvent and $d$ is the solution density. The data obtained in each ethanol solution were fitted by least-squares to a linear equation of the type

$$\Phi_v = \Phi_v^o + S_v m \qquad (2)$$

where $\Phi_v^o$ is the infinite dilution apparent molar volume and $S_v$ is the experimental slope. At infinite dilution $\Phi_v^o$ equals $\bar{V}^o$ the infinite dilution partial molar volume, so the transference molar volume of DL-$\alpha$-alanine $\Delta\bar{V}^o$ from water to the aqueous ethanol solutions was computed as $\Delta\bar{V}^o = \bar{V}_{et}^o - \bar{V}_{water}^o$.

The viscosity $\eta$ values of the solutions were obtained from the equation:

$$\eta = \alpha dt - \beta d/t \qquad (3)$$

where $\alpha$ and $\beta$ are the viscometer constants, $t$ the efflux time and $d$ the density of the solution. The relative viscosities $\eta_r$ were calculated from the solution and solvent viscosities $\eta_r = \eta/\eta_o$. The values thus obtained were adjusted by least-squares to a second order equation as proposed by Tsangaris-Martin (Ref. [34]).

$$\eta_r = 1 + Bm + Dm^2 \qquad (4)$$

The values obtained for $\Phi_v^o$, $\Delta\bar{V}^o$ the viscosity $B$ coefficients and the relation $B/\Phi_v^o$ are summarized in Table II. The standard deviations of $\Phi_v^o$ are less than $\pm 0,05\,cm^3\,mol^{-1}$.

TABLE I  Density and viscosity of ethanol aqueous solutions at 25,00°C

| $X_{et}$ | $d/g\,cm^{-3}$ | $\eta/cP$ |
|---|---|---|
| 0,0000 | 0,997047 | 0,89025 |
| 0,0200 | 0,988218 | 1,0645 |
| 0,0375 | 0,981843 | 1,2313 |
| 0,1000 | 0,963285 | 1,8242 |
| 0,1435 | 0,950673 | 2,1131 |

TABLE II Apparent molar volumes and viscosity $B$ coefficients of DL-$\alpha$-alanine in aqueous ethanol solutions at 25,00°C

| $X_{et}$ | $\Phi_v^o/cm^3\ mol^{-1}$ | $\Delta\bar{V}^o/cm^3\ mol^{-1}$ | $S_v/cm^3\ mol^{-2}kg$ | $B/kg\,mol^{-1}$ | $B/\Phi_v^o/kg\,cm^{-3}$ |
|---|---|---|---|---|---|
| 0.0000 | 60.35 | 0.00 | 0.87 | 0.2525 | $4.18 \times 10^{-3}$ |
| 0.0200 | 58.71 | $-1.64$ | 0.06 | 0.2175 | $3.70 \times 10^{-3}$ |
| 0.0375 | 57.80 | $-2.55$ | 3.17 | 0.1618 | $2.80 \times 10^{-3}$ |
| 0.1000 | 56.11 | $-4.24$ | 11.68 | 0.1223 | $2.18 \times 10^{-3}$ |
| 0.1435 | 57.82 | $-2.53$ | $-2.24$ | 0.1913 | $3.31 \times 10^{-3}$ |

## 4. DISCUSSION

In the concentration range studied, $\Phi_v$ has a linear dependence on concentration. The limiting apparent molar volumes for alanine in the aqueous ethanol solutions are lower than the corresponding value in water. From Table II it can be observed that $\Phi_v^o$ decreases as the ethanol molar fraction increases until it reaches a minimum at $X_{et} =$ 0,1000. For the most concentrated ethanol solution ($X_{et} = 0,1435$) $\Phi_v^o$ shows an appreciable increase. A similar behavior is shown by the transfer molar volumes. Figure 1 shows the influence of the ethanol concentration on limiting apparent molar volumes of DL-$\alpha$-alanine. The experimental slope $S_v$ for alanine in the more dilute ethanol solution is small and it increases with ethanol concentration up to a maximum at $X_{et} = 0,1000$. The $S_v$ value in the more concentrated alcohol solution is negative.

The dependence of the $B$ viscosity coefficient of alanine solutions on ethanol concentration, can be observed in Figure 2. It also shows a minimum near $X_{et} = 0,1000$.

Properties of ethanol in aqueous mixtures have been exhaustively studied. At present it is believed that the particular and complex behavior of alcohols in water is due to a balance of hydrophilic and hydrophobic interactions with water. This balance is responsible of the abrupt change shown by properties like $\Phi_v, \Delta\bar{V}^o, \Delta H$, etc., at a transition concentration that for ethanol solutions is observed at $X_{et} = 0.1$.

It is well known that alanine in water acts as a structure breaker and in mixed solvents such as urea-water, the properties follow a regular change with the concentration of the aqueous urea solutions. However, this is not the case for alanine in water-ethanol mixtures. The apparently strange behavior shown in Figures 1 and 2 in which the
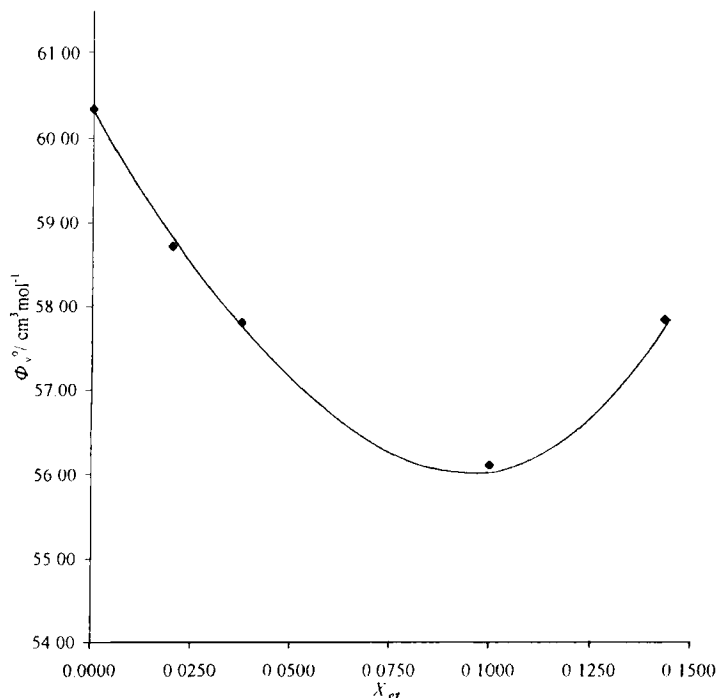
FIGURE 1   Dependence of apparent molar volume of DL-α-alanine with ethanol concentration at 25,00°C.

properties studied suffer an abrupt change around $X_{et} = 0,1000$ do not follow the general trend shown by amino acids in aqueous mixed solvents. Instead, the behavior of alanine in water-ethanol mixtures resembles the volumetric behavior shown by mixed solvent and in both cases the minimum occurs at the same mole fraction of ethanol.

Eventhough only very few studies have been realized with amino acids in ethanol aqueous mixtures, the work of Spink and Duker (Ref. [35]) about transfer entropies of these solutes from water to ethanol aqueous solutions shows for glycine a similar behavior to that presented in this paper. For glycine, the heat of solution behavior changes at an ethanol concentration higher than the presented in this work and for alanine they don't report heat of solution values at ethanol molar fractions beyond 0, 1.

The results of this work show an interesting behavior that may be attributed to induced modifications of water structure caused by the
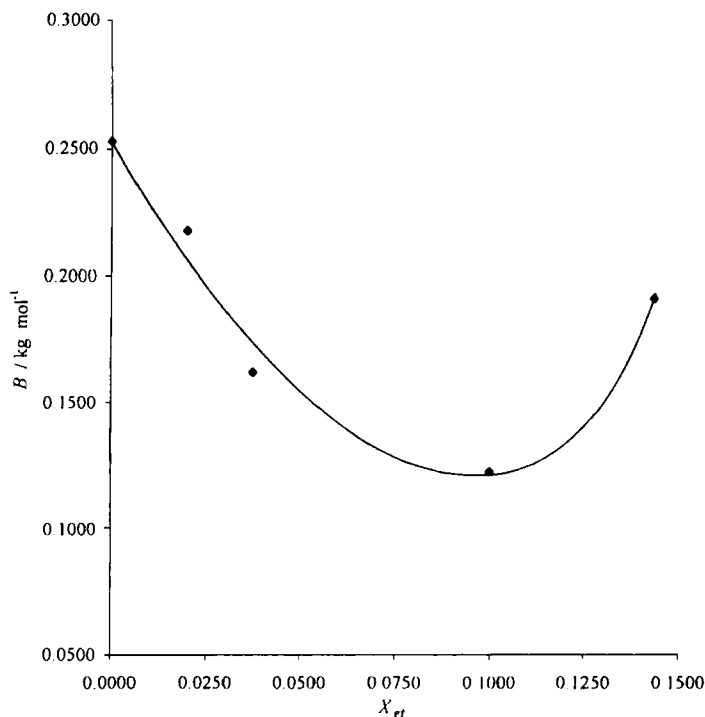
FIGURE 2 Dependence of $B$ coefficients for DL-$\alpha$-alanine in aqueous ethanol solutions at 25,00°C.

addition of ethanol. We think that the decrease of the limiting apparent molar volumes and the $B$ coefficients when compared with respective properties of alanine in water may be due to the less exposed surface of the apolar residue of the aminoacid, owing to the dominant alcohol-water hydrophilic interactions. However, the data for amino acids in alcohol-water mixtures are not enough to attempt a definite explanation about their behavior, which continues to be investigated in our laboratory using other amino acids and alcohol mixtures.

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# *References*

[1] Portions of this paper were presented before the IUPAC International Conference on Chemical and Biological Thermodynamics held at Amritsar, India in January 1997.

[2] Lilley, T. H. (1985). In *Chemistry & Biochemistry of Aminoacids* (Barret, G. C. Ed.), Chapman Hall, London, pp. 591–624.

[3] Lilley, T. H. (1993). *Pure & Appl. Chem.*, **65**, 2551.

[4] Shahidi, F. and Farrell, P. G. (1978). *J. Chem. Soc. Faraday Soc. 1*, **74**, 858.

[5] Shahidi, F. (1980). *J. Chem. Soc. Faraday Trans. 1*, **76**, 101.

[6] Millero, F. J., Lo Surdo, A. and Shin, C. (1978). *J. Phys. Chem.*, **82**, 784.

[7] Jolicoeur, C., Riedl, B., Desrochers, D., Lemelin, L. L., Zanojska, R. and Enea, O. (1986). *J. Solution Chem.*, **15**, 109.

[8] Kharakoz, D. P. (1989). *Biophys. Chem.*, **34**, 115.

[9] Mishra, A. K. and Ahluwalia, J. C. (1984). *J. Phys. Chem.*, **88**, 86.

[10] Cabani, S., Contin, G. and Lepori, L. (1974). *J. Phys. Chem.*, **78**, 1030.

[11] Belibagli, K. B. and Ayranchi, E. (1990). *J. Solution Chem.*, **19**, 867.

[12] Abrosimov, V. K. and Sibrina, S. V. (1989). *Izv. Akad, Navk. SSSR, Ser Khim.*, **6**, 1256.

[13] Hakin, A. W., Duke, M. M., Klassen, S. A., McKay, R. M. and Preuss, K. E. (1994). *Can. J. Chem.*, **72**, 262.

[14] Chalikian, T. V., Sarvazyan, A. P. and Breslauer, K. J. (1993). *J. Phys. Chem.*, **97**, 13017.

[15] Barone, G., Castronuovo, V., Elia, V. and Puzziello, S. (1989). *J. Solution Chem.*, **18**, 1105.

[16] Castronuovo, V., Elia, V. and Magliulo, M. (1991). *Can. J. Chem.*, **69**, 794.

[17] Bhat, R., Kishore, N. and Alhuwalia, J. (1988). *J. Chem. Soc. Faraday Trans. 1*, **84**, 2261.

[18] Romero, C. M., Munar, R. E. and Rojas, J. E. (1993). *Rev. Col. de Quim.*, **22**, 27.

[19] Romero, C. M., Lastra, R. E. and Rojas, J. E. (1993). *Rev. Col. de Quim.*, **22**, 37.

[20] Romero, C. M. and Munar, R. E. (1998). *Phys. and Chem. of Liquids*, **36**, 83.

[21] Tyrrell, H. J. V. and Kennerly, M. (1968). *J. Chem. Soc. (A)*, 2724.

[22] Mason, L. S., Kampmeyerand, P. M. and Robinson, A. L. (1952). *J. Am. Chem. Soc.*, **74**, 1287.

[23] Sandhu, J. S. and Kashyap, U. (1986). *J. Electrochem. Soc. India*, **35**, 283.

[24] Ogawa, T., Mizutani, K. and Yasuda, M. (1984). *Bull Chem. Soc. Jpn.*, **57**, 2064.

[25] Franks, F. and D. J. G. (1966). *Ives. Quart. Rev.*, **20**, 1.

[26] Lama, R. F. and Lu, B. C.-Y. (1965). *J. of Chem. Eng. Data*, **10**, 216.

[27] Sakurai, M., Nakamura, K. and Takenaka, N. (1994). *Bull Chem. Soc. Jpn.*, **67**, 352.

[28] Sakurai, M., Nakamura, K. and Nitta, K. (1994). *Bull Chem. Soc. Jpn.*, **67**, 1580.

[29] Friedman, M. E. and Scherage, H. A. (1965). *J. Phys. Chem.*, **69**, 3795.

[30] Ott, J. B., Sipowska, J. T., Gruszliewicz, M. S. and Woolley, A. T. (1993). *J. Chem. Thermodynamics*, **25**, 307.

[31] Liew, K. Y., Seng, C. E. and Ng, B. H. (1992). *J. Solution Chem.*, **21**, 1177.

[32] Weissberger, A. (1972). *Techniques of Chemistry.* (Wiley Interscience, New York), **1**, Part 4.

[33] Romero, C. M. and Munar, R. M. (1997). *Revista de la Academia Colombiana de Ciencias Exactas, Fisicas y Naturales.*, **21**, 535.

[34] Tsamgaris, J. M. and Martin, R. B. (1965). *Arch. Biochem. Biophys.*, **112**, 267.

[35] Spink, C. H. and Duker, M. (1970). *J. Phys. Chem.*, **74**, 1742.