J. Chem. Eng. Data 1993, 38, 531-533 531

Protonation Constants of α-Alanine, γ-Aminobutyric Acid, and ϵ-Aminocaproic Acid

I. Brandariz, S. Fiol, R. Herrero, T. Vilariño, and M. Sastre de Vicente*

Departamento de Química Fundamental e Industrial, Facultad de Ciencias, Universidade da Coruña, Campus da Zapateira s/n, E-15071 A Coruña, Spain

In this paper, the stoichiometric acid–base equilibrium constants for the amino acids α-alanine, γ-aminobutyric acid, and ϵ-aminocaproic acid have been determined in KNO_3 aqueous solutions at different ionic strengths and 25 °C. Experimental values of $pK^{app}_{m,i}$ have been plotted as a function of the ionic strength by using the Guggenheim, Scatchard, and Pitzer models for the activity coefficients of the species present at equilibria. Results have been briefly discussed.

Introduction

Amino acids are organic compounds of both biochemical and chemical speciation interest since they are included in one of the best defined fractions of organic matter in natural waters (*1*). As previously stated (2), studies on ionization of amino acids in saline media have been unsystematic and carried out under quite wide conditions, (*3*). Continuing with our work on the application of specific interaction models to the acid–base equilibria (*4*), we have studied the dissociation equilibria (see Appendix) on α-alanine, γ-aminobutyric acid, and ϵ-aminocaproic acid in relatively concentrated saline solutions of aqueous KNO_3 (up to 1.6 mol (kg of water)$^{-1}$). The results have been interpreted according to well-known semiempirical models (Guggenheim, Scatchard, and Pitzer) of the specific interaction theory for electrolytes.

Experimental Section

α-Alanine and γ-aminobutyric acid were from Merck, and ϵ-aminocaproic acid was from Sigma. Experimental details have been previously described (*4*). Background electrolyte solutions were prepared from Merck p.a. grade KNO_3. The water used to prepare the solutions was purified by passage through a Millipore MilliQ system. Titrations were carried out on a glass cell furnished with a thermostating jacket and inlets for the electrode, buret, and a nitrogen stream intended to remove O_2 and CO_2 and, for the meantime, stir and homogenize the solution. The temperature of the cell was 25 °C. Merck p.a. NaOH was added from a Crison microBU 2031 autoburet to the amino acid solution containing Merck p.a. HCl (both 0.01 M). The emf was measured by means of a Crison micropH 2002 pH meter furnished with Radiometer GK2401C combined glass membrane electrodes and Ingold pH electrodes, using Ag/AgCl as the reference. The titration system was operated in a fully automated fashion and was controlled via a Tandon DataPack computer. The electrode was previously calibrated for the proton concentration in order to obtain the reference potential for each experiment (*5*).

Results and Discussion

The apparent equilibrium constants pK^{app}_{m} for the three amino acids (Table I) have been fitted according to different functions of the ionic strength (expressed as $I = {}^1/_2\sum m_i z_i^2$) which can be derived from the equations of the activity coefficients of the species involved in the equilibria as defined by Guggenheim (*6*), Scatchard (*7*), and Pitzer (*8*), respectively.

Guggenheim model (*9*)

$$\log K^{app}_{m,1} = \log K^{\theta}_1 + \epsilon_1 m \tag{1}$$

$$\log K^{app}_{m,2} - 2A'\frac{I^{1/2}}{1 + 1.5I^{1/2}} = \log K^{\theta}_2 + \epsilon_2 m \tag{2}$$

with $A' = 0.5107$.

Scatchard model (*9, 10*)

$$pK^{app}_{m,1} = pK^{\theta}_1 + P_1 I + Q_1 I^2 + R_1 I^3 \tag{3}$$

$$pK^{app}_{m,2} + 2S'\frac{I^{1/2}}{1 + 1.5I^{1/2}} = pK^{\theta}_2 + P_2 I + Q_2 I^2 + R_2 I^3 \tag{4}$$

with $S' = 1.17202/\ln 10$.

Pitzer model (*11–13*)

$$pK^{app}_{m,1} = pK^{\theta}_1 + A_1 f^{(1)} + B_1 f^{(2)} + C_1 f^{(3)} \tag{5}$$

$$pK^{app}_{m,2} - Df^{(4)} - Ef^{(5)} = pK^{\theta}_2 + A_2 f^{(1)} + B_2 f^{(2)} + C_2 f^{(3)} \tag{6}$$

where the functions of ionic strength are defined as

$$f^{(1)} = 2I$$

$$f^{(2)} = 1 - (1 + 2I^{1/2})\exp(-2I^{1/2})$$

$$f^{(3)} = I^2$$

$$f^{(4)} = -1 + (1 + 2I^{1/2} + 2I)\exp(-2I^{1/2})$$

$$f^{(5)} = -0.392\left[\frac{I^{1/2}}{1 + 1.2I^{1/2}} + \frac{2}{1.2}\ln(1 + 2I^{1/2})\right]$$

In the equations above, ϵ_i, P_i, Q_i, R_i, A_i, B_i, C_i, D_i, and E_i are empirical parameters associated with the specific interactions between the different species (see Appendix) and are listed in Tables II–IV.

The Scatchard and Pitzer models provide a better fit of the experimental data within the conventionally accepted errors (*14*) in pH measurements.

* To whom correspondence should be addressed.

0021-9568/93/1738-0531$04.00/0 © 1993 American Chemical Society

Table I. Experimental pK_m^{app} for the Amino Acids α-Alanine, γ-Aminobutyric Acid, and ϵ-Aminocaproic Acid in Aqueous KNO_3 Solutions at Different Ionic Strengths[a]

$I(KNO_3)$/ [mol (kg H_2O)$^{-1}$]	α-alanine	γ-aminobutyric acid	ϵ-aminocaproic acid
	$pK_{m,1}^{app}$		
	2.34^b	4.03^b	4.43^b
0.30	2.40 ± 0.02	4.09 ± 0.03	4.42 ± 0.03
0.51	2.36 ± 0.07	4.11 ± 0.02	4.43 ± 0.03
0.72	2.42 ± 0.02	4.17 ± 0.02	4.47 ± 0.01
0.93	2.44 ± 0.02	4.17 ± 0.02	4.50 ± 0.03
1.15	2.44 ± 0.03	4.18 ± 0.02	4.49 ± 0.03
1.37	2.47 ± 0.01	4.18 ± 0.02	4.47 ± 0.01
1.60	2.47 ± 0.01	4.24 ± 0.04	4.50 ± 0.001
	$pK_{m,2}^{app}$		
	9.87^b	10.56^b	10.75^b
0.30	9.69 ± 0.02	10.37 ± 0.02	10.73 ± 0.02
0.51	9.66 ± 0.03	10.36 ± 0.02	10.64 ± 0.16
0.72	9.68 ± 0.02	10.38 ± 0.01	10.73 ± 0.02
0.93	9.69 ± 0.02	10.38 ± 0.03	10.73 ± 0.01
1.15	9.693 ± 0.006	10.36 ± 0.04	10.71 ± 0.05
1.37	9.703 ± 0.003	10.33 ± 0.02	10.68 ± 0.02
1.60	9.70 ± 0.01	10.36 ± 0.03	10.69 ± 0.01

[a] Experimental data are the average of at least three runs. The $pK_{m,i}^{app}$ values are given with their standard deviations. [b] pK^θ were taken from ref 3.

Table II. Interaction Parameters for α-Alanine[a]

Guggenheim Model

	fit eq 1	fit eq 2
pK^θ	2.35 ± 0.01	9.92 ± 0.02
ϵ	0.08 ± 0.01	−0.16 ± 0.02
σ	0.018	0.03

Scatchard Model

	fit eq 3	fit eq 4
pK^θ	2.35 ± 0.02	9.87 ± 0.01
A	0.05 ± 0.12	0.41 ± 0.07
B	0.05 ± 0.18	−0.24 ± 0.11
C	−0.025 ± 0.073	0.06 ± 0.04
σ	0.022	0.013

Pitzer Model

	fit eq 5	fit eq 6
pK^θ	2.35 ± 0.02	9.87 ± 0.01
A	0.06 ± 0.15	0.02 ± 0.08
B	−0.04 ± 0.35	0.65 ± 0.18
C	−0.018 ± 0.102	0.005 ± 0.054
σ	0.022	0.012

[a] σ = mean square error.

Extrapolated pK ($pK^\theta = K_m/(m^\theta)^{\Sigma\nu}$) depend on the kind of fitting function, and owing to the lack of points at low ionic strengths (<0.3 mol (kg of water)$^{-1}$), extrapolation to zero ionic strength to obtain pK^θ can lead to important divergences; that is the reason why pK^θ from the literature are included along with the experimental data to calculate the interaction parameters from the fit of eqs 1–6.

High errors in the parameters A, B, and C but acceptable values of the error of the fit (σ) have been encountered. It can be explained by taking into account the errors coming from the use of commercial glass pH electrodes (*15*) as well as for the multicollinearity among the predictor variables and the high value of the coefficients (close to unity) in the correlation matrix (Table V). According to ref 16, a value of the VIF (variation inflation factor) greater than 10 is also synonymous with multicollinearity. The resulting VIFs for the predictor variables are stated below: Guggenheim model, (I) 7.3715; Scatchard model, (I) 59.8423, (I^2) 392.6856, (I^3) 171.1934; Pitzer model, ($f^{(1)}$) 408.5740, ($f^{(2)}$) 100.6693, ($f^{(3)}$) 129.8453.

Table III. Interaction Parameters for γ-Aminobutyric Acid[a]

Guggenheim Model

	fit eq 1	fit eq 2
pK^θ	4.05 ± 0.01	10.62 ± 0.03
ϵ	0.11 ± 0.01	−0.13 ± 0.03
σ	0.020	0.044

Scatchard Model

	fit eq 3	fit eq 4
pK^θ	4.03 ± 0.01	10.55 ± 0.02
A	0.29 ± 0.08	0.55 ± 0.09
B	−0.23 ± 0.12	−0.46 ± 0.14
C	0.076 ± 0.049	0.13 ± 0.06
σ	0.014	0.017

Pitzer Model

	fit eq 5	fit eq 6
pK^θ	4.03 ± 0.02	10.55 ± 0.02
A	−0.02 ± 0.12	−0.03 ± 0.14
B	0.26 ± 0.26	0.82 ± 0.32
C	0.03 ± 0.08	0.01 ± 0.09
σ	0.016	0.020

[a] σ = mean square root.

Table IV. Interaction Parameters for ϵ-Aminocaproic Acid[a]

Guggenheim Model

	fit eq 1	fit eq 2
pK^θ	4.39 ± 0.01	10.91 ± 0.05
ϵ	0.08 ± 0.01	−0.17 ± 0.05
σ	0.021	0.074

Scatchard Model

	fit eq 3	fit eq 4
pK^θ	4.37 ± 0.01	10.81 ± 0.05
A	0.21 ± 0.08	0.75 ± 0.25
B	−0.12 ± 0.12	−0.60 ± 0.39
C	0.025 ± 0.051	0.16 ± 0.16
σ	0.015	0.047

Pitzer Model

	fit eq 5	fit eq 6
pK^θ	4.37 ± 0.01	10.80 ± 0.04
A	0.065 ± 0.108	−0.11 ± 0.317
B	0.05 ± 0.25	1.18 ± 0.72
C	−0.05 ± 0.07	0.04 ± 0.21
σ	0.015	0.045

[a] σ = mean square error.

Appendix

Ionization Equilibria.

$$AH_2^+ \rightleftarrows Z + H^+$$

$$Z \rightleftarrows A^- + H^+$$

AH_2^+ is the protonated amino acid, Z is the zwitterionic species, and A^- is the deprotonated amino acid.

Coefficients in Equations 3 and 4. Subscripts M and X are used for the anion (NO_3^-) and cation (K^+) of the background salt, respectively, and the other subscripts have the same meaning of those in the ionization equilibria.

$$P_1 = \frac{1}{\ln 10}(a_{HX}^{(1)} + b_{MX,HX}^{(0,1)} - a_{AH_2X}^{(1)} - b_{AH_2X,MX}^{(0,1)} + h_0) \quad (7)$$

$$Q_1 = \frac{1}{2\ln 10}(a_{HX}^{(2)} + b_{MX,HX}^{(0,2)} - a_{AH_2X}^{(2)} - b_{AH_2X,MX}^{(0,2)} + h_1) \quad (8)$$

Table V. Correlation Matrix for the Different Models and Amino Acids[a]

Pitzer Model

	$f^{(1)}$	$f^{(2)}$	$f^{(3)}$	$\mathrm{p}K^{\mathrm{app}}_{\mathrm{m},1}$		$f^{(1)}$	$f^{(2)}$	$f^{(3)}$	Y_2
α-Alanine									
$f^{(1)}$	1.0000				$f^{(1)}$	1.0000			
$f^{(2)}$	0.9477	1.0000			$f^{(2)}$	0.9477	1.0000		
$f^{(3)}$	0.9597	0.8243	1.0000		$f^{(3)}$	0.9597	0.8243	1.0000	
$\mathrm{p}K^{\mathrm{app}}_{\mathrm{m},1}$	0.9297	0.8917	0.8805	1.0000	Y_2	0.9623	0.9974	0.8522	1.0000
γ-Aminobutyric Acid									
$f^{(1)}$	1.0000				$f^{(1)}$	1.0000			
$f^{(2)}$	0.9477	1.0000			$f^{(2)}$	0.9477	1.0000		
$f^{(3)}$	0.9597	0.8243	1.0000		$f^{(3)}$	0.9597	0.8243	1.0000	
$\mathrm{p}K^{\mathrm{app}}_{\mathrm{m},1}$	0.9593	0.9738	0.8696	1.0000	Y_2	0.9347	0.9956	0.8059	1.0000
ϵ-Aminocaproic Acid									
$f^{(1)}$	1.0000				$f^{(1)}$	1.0000			
$f^{(2)}$	0.9477	1.0000			$f^{(2)}$	0.9477	1.0000		
$f^{(3)}$	0.9597	0.8243	1.0000		$f^{(3)}$	0.9597	0.8243	1.0000	
$\mathrm{p}K^{\mathrm{app}}_{\mathrm{m},1}$	0.9030	0.9624	0.7702	1.0000	Y_2	0.9003	0.9810	0.7586	1.0000

Scatchard Model

	$f^{(1)}$	$f^{(2)}$	$f^{(3)}$	$\mathrm{p}K^{\mathrm{app}}_{\mathrm{m},1}$		$f^{(1)}$	$f^{(2)}$	$f^{(3)}$	Y_2
α-Alanine									
$f^{(1)}$	1.0000				$f^{(1)}$	1.0000			
$f^{(2)}$	0.9597	1.0000			$f^{(2)}$	0.9597	1.0000		
$f^{(3)}$	0.9049	0.9861	1.0000		$f^{(3)}$	0.9049	0.9861	1.0000	
$\mathrm{p}K^{\mathrm{app}}_{\mathrm{m},1}$	0.9297	0.8805	0.8190	1.0000	Y_2	0.9501	0.8324	0.7470	1.0000
γ-Aminobutyric Acid									
$f^{(1)}$	1.0000				$f^{(1)}$	1.0000			
$f^{(2)}$	0.9597	1.0000			$f^{(2)}$	0.9597	1.0000		
$f^{(3)}$	0.9049	0.9861	1.0000		$f^{(3)}$	0.9049	0.9861	1.0000	
$\mathrm{p}K^{\mathrm{app}}_{\mathrm{m},1}$	0.9593	0.8696	0.8021	1.0000	Y_2	0.8733	0.7185	0.6257	1.0000
ϵ-Aminocaproic Acid									
$f^{(1)}$	1.0000				$f^{(1)}$	1.0000			
$f^{(2)}$	0.9597	1.0000			$f^{(2)}$	0.9597	1.0000		
$f^{(3)}$	0.9049	0.9861	1.0000		$f^{(3)}$	0.9049	0.9861	1.0000	
$\mathrm{p}K^{\mathrm{app}}_{\mathrm{m},1}$	0.9030	0.7702	0.6781	1.0000	Y_2	0.8085	0.6419	0.5438	1.0000

Guggenheim Model

	m	Y_1		m	Y_2
α-Alanine					
m	1.0000		m	1.0000	
Y_1	0.9297	1.0000	Y_2	0.9497	1.0000
γ-Aminobutyric Acid					
m	1.0000		m	1.0000	
Y_1	0.9593	1.0000	Y_2	0.8731	1.0000
ϵ-Aminocaproic Acid					
m	1.0000		m	1.0000	
Y_1	0.9030	1.0000	Y_2	0.8087	1.0000

[a] Y_2 are the functions on the left side of eqs 4 and 6 for the Scatchard and Pitzer models, respectively. Y_1 and Y_2 are the functions on the left side of eqs 1 and 2 for the Guggenheim model.

$$R_1 = \frac{1}{3\ln 10}(a^{(3)}_{\mathrm{HX}} + b^{(0,3)}_{\mathrm{HX,MX}} - a^{(3)}_{\mathrm{AH_2X}} - b^{(0,3)}_{\mathrm{AH_2X,MX}}) \qquad (9)$$

$$P_2 = \frac{1}{\ln 10}(a^{(1)}_{\mathrm{AH}} + a^{(1)}_{\mathrm{MX}} + b^{(0,1)}_{\mathrm{MX,AH}} - h_0) \qquad (10)$$

$$Q_2 = \frac{1}{2\ln 10}(a^{(2)}_{\mathrm{AH}} + 2a^{(2)}_{\mathrm{MX}} + b^{(0,2)}_{\mathrm{MX,AH}} - h_1) \qquad (11)$$

$$R_2 = \frac{1}{3\ln 10}(a^{(3)}_{\mathrm{AH}} + 3a^{(3)}_{\mathrm{MX}} + b^{(0,3)}_{\mathrm{MX,AH}}) \qquad (12)$$

where the a's include the properties of the pure electrolytes, b's appear for mixtures of electrolytes, and h's are the coefficients for the neutral molecules (some researchers (*17*) do not consider the inclussion of h_1 necessary).

Coefficients in Equations 5 and 6.

$$A_1 = -\frac{1}{\ln 10}(\beta^0_{\mathrm{AH_2X}} - \beta^0_{\mathrm{HX}} + \theta_{\mathrm{AH_2M}} - \theta_{\mathrm{HM}} - \lambda) \qquad (13)$$

$$B_1 = -\frac{1}{\ln 10}(\beta^1_{\mathrm{AH_2X}} - \beta^1_{\mathrm{HX}}) \qquad (14)$$

$$C_1 = -\frac{1}{\ln 10}(C^{\phi}_{\mathrm{AH_2X}} + \psi_{\mathrm{AH_2MX}} - C^{\phi}_{\mathrm{HX}} - \psi_{\mathrm{HMX}}) \qquad (15)$$

$$A_2 = \frac{1}{\ln 10}(\beta^0_{\mathrm{AM}} + \beta^0_{\mathrm{HX}} + \theta_{\mathrm{AX}} + \theta_{\mathrm{HM}} - \lambda) \qquad (16)$$

$$B_2 = \frac{1}{\ln 10}(\beta^1_{\mathrm{AM}} + \beta^1_{\mathrm{HX}}) \qquad (17)$$

$$C_2 = \frac{1}{\ln 10}(C^{\phi}_{\mathrm{AM}} + C^{\phi}_{\mathrm{MX}} + C^{\phi}_{\mathrm{HX}} + \psi_{\mathrm{AMX}} + \psi_{\mathrm{HMX}}) \qquad (18)$$

$$D_2 = \frac{2\beta^1_{\mathrm{MX}}}{\ln 10} \qquad (19)$$

$$E_2 = \frac{2}{\ln 10} \qquad (20)$$

where β's and θ's are the coefficients for double interactions between ions of different and the same charge signs, respectively, C^{ϕ}'s include all the triple interactions between the two specified ions and ψ's the triple interactions among three different ions (anion–anion–cation and cation–cation–anion), and λ is the salting coefficient for the neutral species.

Literature Cited

(1) Thurman, E. M. *Organic Geochemistry of Natural Waters*; Martinus Nijhoff/Dr. W. Junk Publishers: Dordrecht, 1985.
(2) Rey, F.; Varela, A.; Antelo, J. M.; Arce, F. *J. Chem. Eng. Data* **1989**, *34*, 35–37.
(3) Martell, A. E.; Smith, R. M. *Critical Stability Constants: Vol. 1, Aminoacids*; Plenum Press: New York, 1982.
(4) Herrero, R.; Armesto, X. L.; Arce, F.; Sastre de Vicente, M. E. *J. Solution Chem.* **1992**, *21*, (11), 1185–1193.
(5) Fiol, S.; Armesto, X. L.; Arce, F.; Penedo, F.; Sastre de Vicente, M. E. *Fresenius' Z. Anal. Chem.* **1992**, *343*, 469–472.
(6) Pitzer, K. S.; Brewerd, L. *Revision of Lewis Randall's Thermodynamics*; McGraw-Hill: New York, 1961; Chapter 23.
(7) Scatchard, G. *J. Am. Chem. Soc.* **1961**, *83*, 2636–2642.
(8) Pitzer, K. S. *J. Phys. Chem.* **1973**, *77* (2), 268–277.
(9) Brandariz, I.; Arce, F.; Armesto, X. L.; Penedo, F.; Sastre de Vicente, M. E. *Monatsh. Chem.* **1993**, *124*, 249–257.
(10) Brandariz, I.; Herrero, R.; Sastre de Vicente, M. E. *J. Chim. Phys. Phys.-Chim. Biol.* **1993**, *90*, 63–77.
(11) Herrero, R.; Brandariz, I.; Sastre de Vicente, M. E. *Ber. Bunsen-Ges. Phys. Chem.* **1993**, *97* (1), 59–63.
(12) Izaguirre, M.; Millero, F. J. *J. Solution Chem.* **1987**, *16* (10).
(13) Herrero, R.; Brandariz, I.; Fiol, S.; Sastre de Vicente, M. *Collect. Czech. Chem. Commun.*, in press.
(14) Albert, A.; Serjeant, E. P. *The determination of ionization constants*, 3rd ed.; Chapman and Hall: New York, 1984; Chapter 3.
(15) Herrero, R.; Brandariz, I.; Fiol, S.; Vilariño, T.; Sastre de Vicente, M. E. *An. Quím.*, in press.
(16) Wetherill, G. B.; Dumombe, P.; Kenward, M.; Köllerström, J.; Paul, S. R.; Vowden, B. J. *Regression Analysis with Applications*; Chapman and Hall: London, 1986; Chapter 4.
(17) Long, F. A.; McDevit, W. F. *Chem. Rev.* **1952**, *51*, 119.

Received for review December 7, 1992. Revised manuscript received April 5, 1993. Accepted May 10, 1993. The authors wish to acknowledge the financial support of the Xunta de Galicia (Project XUGA10303B92).