Redetermination of the Thermodynamic Values of the First and Second Dissociation Constants of Glutamic Acid in Aqueous Solutions at 298.15 K

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The thermodynamic values of the first and second dissociation constants ($K_{a,1}$ and $K_{a,2}$) of glutamic acid were determined by a method developed recently. In this method, a simple equation of the Hückel type was used for activity coefficients of ionic species. The dissociation constants of this amino acid and the parameters for the activity coefficient equation were determined from results of potentiometric titrations performed in a glass electrode cell. The ionic strength of the solutions titrated was adjusted either by NaCl or KCl, and ionic strengths up to 0.4 mol kg^{-1} were used. The following thermodynamic values were obtained: $K_{a,1} = 6.2 \times 10^{-3}$ and $K_{a,2} = 4.57 \times 10^{-5}$. Recommended values for these dissociation constants in the previous literature are those of Lumb and Martell (1953), $K_{a,1} = 5.0 \times 10^{-3}$ and $K_{a,2} = 3.09 \times 10^{-5}$, and those of Neuberger (1936), 7.0×10^{-3} and 4.74×10^{-5}, respectively. In the present study, however, the new K_a values are preferred to those in the literature because of experimental evidence: the new titration data can be explained within experimental error by means of these thermodynamic dissociation constants and the Hückel equations obtained for activity coefficients.

Electromotive force (EMF) measurements on cells without a liquid junction have been considered as one of the most accurate methods to determine the thermodynamic values of dissociation constants (K_a) of weak acids. This method was developed in the 1930s to a high precision by Harned and Ehlers using acetic acid solutions in a cell containing a hydrogen electrode and a silver–silver chloride electrode (the cell of this kind is nowadays called the Harned cell). Much experimental work has since been done by this method, and the most reliable K_a values have been obtained for many acids by this method at different temperatures (see e.g. Robinson and Stokes[2]). In amino acid solutions, this method has been applied for example to glycine,[3–5] to alanine, valine, leucine or isoleucine,[6] and to aspartic acid.[7] As far as we know, no detailed glutamic acid data measured by this method are available in the literature.

In more recent studies the dissociation constants of amino acids, particularly at different ionic strengths (I_m or I_c where m refers to the molality and c to the concentration) of some inert electrolytes, have been often determined with glass electrodes. Most of these works

have been reviewed by IUPAC.[8–11] These reviews revealed the absence of systematic studies on the influence of ionic strength on stoichiometric dissociation constants, K_m or K_c. Systematic glass electrode works, however, have been reported, e.g. by the research group of Sastre de Vicente, see for example Refs. 12 and 13. For glutamic acid at 298.15 K, recent glass electrode results have been published by Rey et al.[14] for aqueous KNO_3 solutions ($I_m = 0.025$–0.5 mol kg^{-1}) and by Partanen et al.[15] for aqueous KCl solutions ($I_m = 0.059$–0.37 mol kg^{-1}).

At 298.15 K, $K_{a,1}$ and $K_{a,2}$ values for glutamic acid have been reported, e.g., by Harris[16] (according to him $K_{a,1} = 6.7 \times 10^{-3}$ and $K_{a,2} = 4.1 \times 10^{-5}$), Schmidt et al.[17] ($7.9 \times 10^{-3}$, 8.5×10^{-5}), Miyamoto and Schmidt[18] (6.5×10^{-3}, 5.62×10^{-5}), Neuberger[19] (7.0×10^{-3}, 4.74×10^{-5}), Lumb and Martell[20] (5.0×10^{-3}, 3.09×10^{-5}), Rey et al.[14] (4.8×10^{-3}, 6.61×10^{-5}), and Partanen et al.[15] (5.0×10^{-3}, 4.44×10^{-5}). From the older values, for example, Robinson and Stokes[2] prefer those of Lumb and Martell. The variations of the suggested values for $K_{a,1}$ and $K_{a,2}$ reveal in this list that the thermodynamic dissociation constants for glutamic acid, a constituent of proteins in living organisms, are not known as accurately as desirable. The value suggested

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in our previous study[15] for $K_{a,2}$ was based on the assumption that $K_{a,1} = 5.0 \times 10^{-3}$, as discussed in that paper. In this connection, only dilute glutamic acid solutions were studied where the first dissociation was not very important. Therefore, the value of Lumb and Martell, as most reliable so far, had to be used for $K_{a,1}$ in that study. In the present study, five times stronger glutamic acid solutions with respect to the acid were titrated than earlier. For this reason we are now able to determine $K_{a,1}$ from our own data. In the earlier study,[15] the titrations were carried out only in KCl solutions, but here also NaCl was used to adjust the ionic strength of the solutions investigated. On the basis of the present data, it is possible to recommend new values for $K_{a,1}$ and $K_{a,2}$ of glutamic acid at 298.15 K and additionally to suggest equations for the calculation of the stoichiometric dissociation constants (K_m) for this acid in dilute NaCl and KCl solutions at this temperature.

Empirical equations of the Hückel type are here used for ionic activity coefficients as in the previous glutamic acid study.[15] The Hückel method has also been proved to be useful in other weak acid studies.[21–25] Ionic activity coefficients are not accessible by purely thermodynamic methods. However, the equations suggested for them can be tested by means of the existing electrolyte data. In the previous studies, the Hückel equations were tested with the literature data obtained by Harned cells for solutions of aspartic acid,[21] acetic acid,[22] formic, propionic and n-butyric acid,[23] and phosphoric acid.[24] For the Hückel equations of the glutamic acid species, such tests are not possible to perform owing to the lack of Harned cell data, see above. The Hückel equations for the ionic activity coefficients in weak acid solutions can also be tested with the results of potentiometric titrations in these solutions. These tests require a certain assumption for liquid junction potentials in the glass electrode cell used, see below. Also the conventional pH values of the buffer solutions must be used in the calibration of the cell. In the previous studies, the Hückel equations were tested with the potentiometric titration data for solutions of aspartic acid,[21] and acetic acid.[22] In the present study, the Hückel equations were tested only with the titration data. In all previous studies[21–25] and the present study, the suggested Hückel method explains well the data used in the tests. The ionic activity coefficients calculated by the Hückel equations are therefore useful in thermodynamic considerations of dilute weak acid solutions, e.g. in the calculation of stoichiometric dissociation constants or pH values. This is probably the most reliable way to calculate such quantities despite the fact that the ionic activity coefficient equations are always hypothetical because there is no direct thermodynamic way to test them.

Experimental

The titrations of glutamic acid solutions were essentially carried out in a glass electrode cell similarly as those described by Seymour and Fernando[26] and in Refs. 15, 25 and 27. Thus only a brief description of the procedure is given. The following solutions were prepared in RO-filtered water (Millipore) with a conductivity less than 1.0 μS cm^{-1}: 2.00 M NaCl, 2.00 M KCl (both pro analysi, Riedel-de Haën), 0.0100 M L-glutamic acid (>99%, Merck), 0.0500 M L-glutamic acid, 0.100 M NaOH (Dilute-it, J. T-Baker), 0.506 M HCl (Fixanal 1.000 M, Riedel-de Haën) and the pH standard solutions of 4.005 and 6.865 at 298.15 K recommended by IUPAC,[28] i.e. 0.05 mol kg^{-1} potassium hydrogen phthalate (Art. 4878,Merck) and 0.025 mol kg^{-1} KH$_2$PO$_4$ (Art. 4881, Merck) plus 0.025 mol kg^{-1} Na$_2$HPO$_4$ (Art. 6589, Merck).

Four series of glutamic acid solutions were titrated at 298.15 $\pm$ 0.15 K. In two of those, aqueous mixtures of glutamic acid and NaCl were titrated by the NaOH solution. The six titrations in these two series correspond exactly to the base titrations of aqueous mixtures of glutamic acid and KCl described in Ref. 15. The base titrations in KCl solutions of that study were also included in the calculation of the present study. In the other two series of the new titrations, aqueous mixtures of glutamic acid (prepared from the 0.0500 M solution) and NaCl and of glutamic acid and KCl were titrated by using the HCl solution. Otherwise, also these titrations correspond to the titrations of Ref. 15.

Results

In the present study, the following equation (the Hückel equation) was used for the activity coefficient (γ) of ion i on the molality scale:

$$\ln \gamma_i = -\alpha z_i^2 (I_m)^{1/2} / [1 + B_i (I_m)^{1/2}] + b_{i,\text{MX}} I_m / (m^0) \qquad (1)$$

where $m^0 = 1$ mol kg^{-1}, $\alpha = 1.1744$ (mol kg^{-1})$^{-1/2}$ (Archer and Wang),[29] z_i is the charge number of ion i, B_i is a parameter characteristic of ion i, and b_i is a parameter that also is dependent on ion i and additionally on the inert electrolyte (MX) used in the adjustment of the ionic strength I_m. For the present calculations, the following parameters were taken from the literature: $B_H = 1.25$ (mol kg^{-1})$^{-1/2}$,[15] $b_{H,\text{NaCl}} = 0.238$,[15] and $b_{H,\text{KCl}} = 0.178$,[30] where H refers to H$^+$ ions.

The new titration data are presented in Tables 1–4. Tables 1 and 2 show the results of the two parallel titration series (Series OH1N and OH2N) where the titrant was the NaOH solution and where the ionic strength was adjusted by NaCl. Table 3 consists of the results where the titrant was the HCl solution and where the inert electrolyte was NaCl and Table 4 of those where the titrant was the HCl solution and the inert electrolyte was KCl. The base titration results presented in Ref. 15 for KCl solutions were also included in the treatment, and the calculation results of these titrations are presented in Table 5. Glass electrodes cannot be used in the high-precision thermodynamic studies without the elimination of the liquid junction potential problems. All

Table 1. Results of titrations of glutamic acid in NaCl solutions at different ionic strengths (I_m) with a base (NaOH) solution $(c=0.100 \text{ mol dm}^{-3})$ at 298.15 K. Series OH1N.

$I_m/(m^0)^a$	0.0595	0.1191	0.1789	0.2388	0.2988	0.3741
$V/(cm^3)^b$	pH^c	pH^c	pH^c	pH^c	pH^c	pH^c
0.05	3.835		3.803	3.791		3.774
0.10	3.883	3.865	3.850	3.838	3.830	3.820
0.15	3.935	3.917	3.901	3.886	3.876	3.871
0.20	3.988	3.966	3.952	3.935	3.925	3.918
0.25	4.044	4.020	4.005	3.988	3.976	3.968
0.30	4.100	4.078	4.061	4.041	4.031	4.024
0.35	4.162	4.133	4.119	4.097	4.088	4.080
0.40	4.223	4.194	4.179	4.156	4.146	4.138
0.45	4.287	4.257	4.240	4.219	4.209	4.199
0.50	4.354	4.323	4.306	4.286	4.272	4.265
0.55	4.427	4.398	4.376	4.357	4.342	4.335
0.60	4.504	4.473	4.452	4.432	4.417	4.410
0.65	4.589	4.556	4.536	4.510	4.497	4.492
0.70	4.680	4.650	4.626	4.602	4.583	4.582
0.75	4.789	4.755	4.731	4.706	4.687	4.684
0.80	4.917	4.883	4.856	4.823	4.806	4.805
Symbol	OH1N1	OH1N2	OH1N3	OH1N4	OH1N5	OH1N6
$10^5 K_{m,2}(\text{obsd})^d$	6.78		7.73		8.18	
$10^5 K_{m,2}(\text{pred})^e$	6.69	7.30	7.67	7.92	8.10	8.24
$(10^4 n_t \text{ mol})^f$	0.980	0.981	0.983	0.990	0.992	0.989
$(pH_{lj})^g$	−0.052	−0.072	−0.086	−0.098	−0.107	−0.114

$^a m^0 = 1$ mol kg^{-1}. $^b V$ is the volume of titrant added. ^{c}The measured pH value. ^{d}Obtained by minimization of the square sum defined by eqn. (A6) in Ref. 21. ^{e}Calculated by eqn. (5) using eqn. (1) and the value of $K_{a,2}=4.57 \times 10^{-5}$. ^{f}The amount of glutamic acid in titration vessel. ^{g}Liquid-junction potential correction, see Appendix of Ref. 21.

Table 2. Results of titrations of glutamic acid in NaCl solutions at different ionic strengths (I_m) with a base (NaOH) solution $(c=0.100 \text{ mol dm}^{-3})$ at 298.15 K. Series OH2N.

$I_m/(m^0)^a$	0.0595	0.1191	0.1789	0.2388	0.2988	0.3741
$V/(cm^3)^b$	pH^c	pH^c	pH^c	pH^c	pH^c	pH^c
0.05	3.833	3.811	3.804	3.792	3.784	3.770
0.10	3.883	3.859	3.852	3.840	3.830	3.816
0.15	3.934	3.910	3.900	3.888	3.877	3.864
0.20	3.988	3.961	3.952	3.939	3.927	3.915
0.25	4.044	4.014	4.003	3.991	3.979	3.966
0.30	4.100	4.068	4.059	4.044	4.034	4.020
0.35	4.156	4.127	4.116	4.100	4.092	4.076
0.40	4.219	4.187	4.173	4.161	4.150	4.136
0.45	4.282	4.250	4.236	4.221	4.211	4.197
0.50	4.348	4.316	4.304	4.289	4.274	4.262
0.55	4.422	4.388	4.372	4.359	4.342	4.330
0.60	4.498	4.462	4.449	4.432	4.416	4.403
0.65	4.581	4.542	4.530	4.513	4.496	4.485
0.70	4.673	4.634	4.622	4.604	4.588	4.576
0.75	4.779	4.734	4.724	4.707	4.690	4.680
0.80	4.906	4.857	4.845	4.830	4.814	4.799
Symbol	OH2N1	OH2N2	OH2N3	OH2N4	OH2N5	OH2N6
$10^5 K_{m,2}(\text{obsd})^d$		7.27		8.01		8.27
$10^5 K_{m,2}(\text{pred})^e$	6.69	7.30	7.67	7.92	8.10	8.24
$(10^4 n_t/\text{mol})^f$	0.986	0.992	0.989	0.988	0.989	0.989
$(pH_{lj})^g$	−0.051	−0.073	−0.084	−0.097	−0.107	−0.118

$^{a-g}$See footnotes to Table 1.

Table 3. Results of titrations of glutamic acid in NaCl solutions at different ionic strengths (I_m) with an acid (HCl) solution $(c=$ 0.506 mol dm^{-3}) at 298.15 K. Series HN.

$I_m/(m^0)^a$	0.0595	0.1191	0.1789	0.2388	0.2988
$V/(cm^3)^b$	pH^c	pH^c	pH^c	pH^c	pH^c
0.30	3.069	3.065	3.062	3.059	3.053
0.35	3.021	3.017	3.014	3.011	3.004
0.40	2.976	2.973	2.969	2.964	2.959
0.45	2.930	2.930	2.926	2.925	2.918
0.50	2.890	2.891	2.887	2.884	2.877
0.55	2.853	2.854	2.849	2.846	2.841
0.60	2.817	2.820	2.817	2.812	2.806
0.65	2.784	2.784	2.784	2.779	2.772
0.70	2.753	2.755	2.752	2.748	2.741
0.75	2.726	2.726	2.724	2.721	2.714
0.80	2.697	2.698	2.697	2.693	2.686
0.85	2.671	2.671	2.671	2.666	2.659
0.90	2.645	2.647	2.645	2.642	2.635
0.95	2.621	2.623	2.621	2.616	2.609
1.00	2.597	2.599	2.597	2.594	2.589
1.05	2.573	2.577	2.575	2.572	2.566
1.10	2.553	2.556	2.554	2.553	2.544
1.15	2.534	2.536	2.534	2.532	2.524
1.20	2.513	2.518	2.515	2.512	2.506
1.25	2.496	2.498	2.496	2.493	2.488
Symbol	HN1	HN2	HN3	HN4	HN5
$10^5 K_{m,2}(pred)^e$	6.69	7.30	7.67	7.92	8.10
$(10^4 n_t/mol)^f$	5.00	5.00	5.00	5.00	5.00
$(pH_{lj})^g$	-0.061	-0.079	-0.092	-0.102	-0.112

$^{a-c,e-g}$See footnotes to Table 1.

Table 4. Results of titrations of glutamic acid in KCl solutions at different ionic strengths (I_m) with an acid (HCl) solution $(c=$ 0.506 mol dm^{-3}) at 298.15 K. Series HK.

$I_m/(m^0)^a$	0.0596	0.1792	0.2393	0.2997	0.3755
$V/(cm^3)^b$	pH^c	pH^c	pH^c	pH^c	pH^c
0.35	3.027	3.041	3.039	3.049	3.043
0.40	2.981	2.993	2.995	3.003	2.996
0.45	2.936	2.953	2.952	2.960	2.955
0.50	2.897	2.914	2.914	2.921	2.914
0.55	2.859	2.876	2.875	2.885	2.878
0.60	2.827	2.842	2.842	2.851	2.842
0.65	2.792	2.811	2.810	2.816	2.811
0.70	2.762	2.779	2.779	2.786	2.779
0.75	2.734	2.751	2.751	2.757	2.753
0.80	2.705	2.722	2.722	2.729	2.726
0.85	2.678	2.697	2.695	2.700	2.697
0.90	2.654	2.671	2.669	2.676	2.673
0.95	2.630	2.647	2.647	2.652	2.649
1.00	2.606	2.623	2.623	2.628	2.625
1.10	2.565	2.582	2.582	2.585	2.584
1.20	2.525	2.541	2.542	2.546	2.542
Symbol	HK1	HK3	HK4	HK5	HK6
$10^5 K_{m,2}(pred)^e$	6.68	7.61	7.83	7.98	8.10
$(10^4 n_t/mol)^f$	5.00	5.00	5.00	5.00	5.00
$(pH_{lj})^g$	-0.054	-0.070	-0.078	-0.077	-0.086

$^{a-c,e-g}$See footnotes to Table 1.

Table 5. Results of the recalculation of the titration data presented in Ref. 15 (see Table 1 there) for glutamic acid in KCl solutions at different ionic strengths (I_m) with a base (NaOH) solution at 298.15 K. Series OHK.

$I_m/(m^0)^a$	0.0596	0.1193	0.1792	0.2393	0.2997	0.3755
Symbol	OHK1	OHK2	OHK3	OHK4	OHK5	OHK6
$10^5 K_{m,2}(obsd)^d$	6.65	7.26	7.59	7.80	7.94	8.09
$10^5 K_{m,2}(pred)^e$	6.68	7.26	7.61	7.83	7.98	8.10
$(10^4 n_t/mol)^f$	0.994	0.985	0.988	0.981	0.978	0.978
$(pH_{lj})^g$	−0.035	−0.040	−0.046	−0.052	−0.054	−0.058

$^{a,d-g}$See footnotes to Table 1.

titration data sets were corrected for the difference between the liquid junction potentials in the solution titrated and in those used in the calibration of the pH meter. The correction method has been presented in Appendix of Ref. 21. The titration data were treated thermodynamically as follows.

The dissociation equilibria existing in acidic solutions of glutamic acid are the following:

$$H_3A^+ \rightleftharpoons H^+ + H_2A \qquad (2)$$

$$H_2A \rightleftharpoons H^+ + HA^- \qquad (3)$$

where for example H_2A denotes neutral glutamic acid molecules (or zwitterions). The equilibrium constants for these reactions are given by the following equations:

$$K_{a,1} = a_H a_{H_2A}/a_{H_3A} = (\gamma_H/\gamma_{H_3A})K_{m,1} \qquad (4)$$

$$K_{a,2} = a_H a_{HA}/a_{H_2A} = (\gamma_H \gamma_{HA})K_{m,2} \qquad (5)$$

where a refers to the activity and the new ionic symbols are $H_3A = H_3A^+$ and $HA = HA^-$. In eqns. (4) and (5) the activity coefficients of the neutral species H_2A are assumed to be unity. This assumption can be made because only rather dilute electrolyte solutions (I_m is always smaller than 0.4 mol kg^{-1}) are considered. The departure of γ_{H_2A} from unity is probably not important even for the strongest solutions considered here, because almost the same activity parameters as those suggested below can be obtained by omitting the results of the strongest solutions from the parameter estimation. The stoichiometric or the molality scale dissociation constants $K_{m,1}$ and $K_{m,2}$ in eqns. (4) and (5) are defined by

$$K_{m,1} = m_H m_{H_2A}/(m_{H_3A} m^0) \qquad (6)$$

$$K_{m,2} = m_H m_{HA}/(m_{H_2A} m^0) \qquad (7)$$

The following equation can be derived for the base titration data from eqns. (6) and (7) and from the equations for the material balance for glutamic acid and for the electroneutrality:

$$m_H^3 + (K_{m,1}m^0 + m_b + m_t)m_H^2 + K_{m,1}m^0(K_{m,2}m^0 + m_b)m_H$$
$$+ K_{m,1}K_{m,2}(m^0)^2(m_b - m_t) = 0 \qquad (8)$$

In eqn. (8) m_b is the molality of the base, NaOH, in the solution titrated and $m_b = c_b V/w_1$, where c_b is the concentration, V is the volume of the base solution added in the titration and w_1 is the mass of water in the solution

titrated, see also eqn. (9) in Ref. 21. m_t in eqn. (8) of the present study is the total molality of glutamic acid in the solution titrated and $m_t = n_t/w_1$ where n_t is the amount of this substance. The equation corresponding to eqn. (8) for the acid titrations can be derived from that by replacing in eqn. (8) m_b by $-m_a$ where m_a is the molality of the acid, HCl, in the solution titrated. In the present calculations, m_H had to be determined by either of these equations for each acid or base titration point using the given values of $K_{m,1}$ and $K_{m,2}$ (see below). It was determined numerically by the Newton–Raphson method.

At first, both $K_{m,1}$ and $K_{m,2}$ were simultaneously determined from all results of each acid titration in NaCl and KCl solutions (Tables 3 and 4). Owing to eqns. (1), (4) and (5), $K_{m,1}$ and $K_{m,2}$ remain constant during each of these titrations performed at an almost constant ionic strength. For each titration, the values of $K_{m,1}$ and $K_{m,2}$ were searched that minimize the square sum of pH errors defined by eqn. (A6) in Appendix of Ref. 21. The predicted pH for each point of the titrations was calculated by

$$pH (predicted) = -\log[\gamma_H m_H/(m^0)] \qquad (9)$$

For this equation γ_H was calculated by eqn. (1) and m_H by the equation corresponding to eqn. (8) in acid titrations, see above. The $K_{m,1}$ values determined by this method for the different titrations (i.e. for the different ionic strengths) seem to vary randomly around 6.2×10^{-3}, and the deviations from the value are small. With $K_{m,1} = 6.2 \times 10^{-3}$, $K_{m,2}$ was then determined from the results of the base titrations for the different ionic strengths in NaCl and KCl solutions (see below). With the final $K_{m,2}$ values [i.e. with the $K_{m,2}(pred)$ values in Tables 1, 2 and 5, see text below], $K_{m,1}$ was again determined from the data obtained in the acid titrations. In this determination, $K_{m,1}$ was calculated from each point for all titrations by the following equation:

$$K_{m,1} = \frac{(m_a - m_t)m_H^2 - m_H^3}{m^0[m_H^2 + (K_{m,2}m^0 - m_a)m_H - K_{m,2}m^0(m_a + m_t)]} \qquad (10)$$

The $K_{m,1}$ values determined in this way for the titration data sets of HN1 and HK6 (Tables 3 and 4) are shown graphically in Fig. 1. The results obtained from these two sets are representative for all results obtained in this determination. According to these results, it seems to be
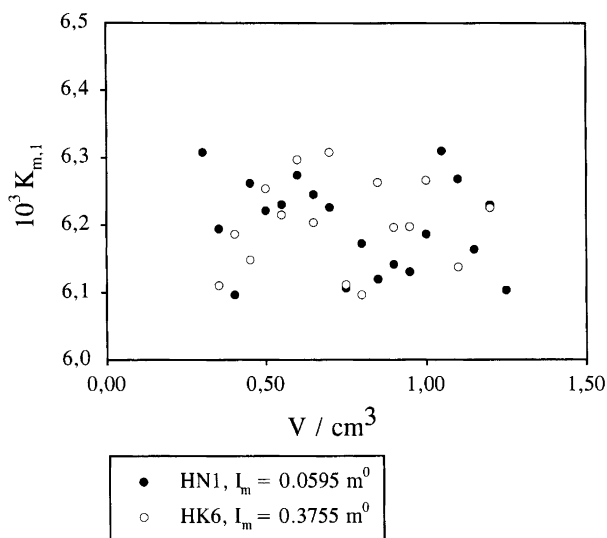
Fig. 1. $K_{m,1}$ estimate, defined by eqn. (6), for the glutamic acid titrations HN1 and HK6 (Tables 3 and 4) as a function of the added volume of the acid titrant. These estimates were calculated by eqn. (10) where the $K_{m,2}$ values of 6.69×10^{-5} (HN1) and 8.10×10^{-5} (HK6) were used. m_H was calculated from the corrected pH (see Appendix of Ref. 21), and the following pH_{lj} values were used -0.061 (HN1, see Table 3) and -0.086 (HK6, see Table 4). Symbols of the two sets are shown at the legend of the figure.

true that $K_{m,1}$ is equal to 6.2×10^{-3} in all ionic strengths between 0.06 and 0.37 mol kg^{-1}, and this value is also the most probable one for $K_{a,1}$ of glutamic acid at 298.15 K.

The experimental $K_{m,2}$ values at different ionic strengths were determined from the base titration results of Tables 1 and 2 in the present study (in NaCl solutions) and of Table 1 in the previous study[15] (in KCl solutions). $K_{m,2}$ for each titration set was obtained by searching the minimum of the pH square sum defined by eqn. (A6) in Appendix of Ref. 21 when both $K_{m,2}$ and n_t were varied and $K_{m,1}$ was kept constant ($=6.2 \times 10^{-3}$). The amount of glutamic acid (n_t) was used, as previously, as an adjustable parameter. A very accurate determination of this parameter was especially required by the most basic titration points. The experimental $K_{m,2}$ values determined by this method are shown in Tables 1, 2 and 5.

The experimental $K_{m,2}$ values were used for the determination of $K_{a,2}$ for glutamic acid. For this purpose the following equation was derived from eqns. (1) and (5):

$$\ln K_{m,2} - \alpha(I_m)^{1/2}\{1/[1 + B_H(I_m)^{1/2}] + 1/[1 + B_{HA}(I_m)^{1/2}]\}$$

$$= y = \ln K_{a,2} - (b_{H,MCl} + b_{HA,MCl})I_m/(m^o) \qquad (11)$$

The value of $B_{HA} = 2.5$ (mol kg^{-1})$^{-1/2}$, determined previously,[15] applies to this equation, and eqn. (11) was used to solve the quantities of $\ln K_{a,2}$ and $b_{H,MCl} + b_{HA,MCl}$ by means of linear regression analysis. From the NaCl data (the experimental $K_{m,2}$ values of sets OH1N1, OH2N2, OH1N3, OH2N4, OH1N5 and OH2N6 in Tables 1 and 2 were included in the determination), the

following results were obtained: $K_{a,2} = 4.60 \times 10^{-5}$ [$pK_{a,2} = (4.337 \pm 0.007)$, the confidence interval is given at a level of 0.95] and $b_{HA,NaCl} = 0.035$. From the KCl data (Table 5), on the other hand, the following results were obtained: $K_{a,2} = 4.56 \times 10^{-5}$ [$pK_{a,2} = (4.341 \pm 0.003)$] and $b_{HA,KCl} = 0.143$. The $K_{a,2}$ estimates obtained from the NaCl and KCl solutions agree well with each other and the following value can, therefore, be suggested for the thermodynamic value of the second dissociation constant of glutamic acid at 298.15 K: $K_{a,2} = 4.57 \times 10^{-5}$ ($pK_{a,2} = 4.340$). This value is not very different (but significantly) from that (i.e. from $K_{a,2} = 4.44 \times 10^{-5}$) determined in Ref. 15 on the basis of the assumption $K_{m,1} = K_{a,1} = 5.0 \times 10^{-3}$.

To tests the new K_a values and activity parameters for eqn. (1), the results of all titrations were predicted by using these values. The $K_{m,2}$ values predicted by eqns. (1) and (5) are also given in Tables 1–5 and these values were used in the calculation of the pH predictions. Also the liquid junction corrections were made for the predicted sets (see Appendix of Ref. 21) and the resulting values of pH_{lj} are shown in these tables. The results of these tests are shown as error plots in Figs. 2 and 3. The three graphs of Fig. 2 show the pH errors obtained from the base titration data in the two NaCl and one KCl series, and the two graphs of Fig. 3 those of the acid titration data in the NaCl and KCl series. In the error plots of these graphs, the pH errors are presented at each ionic strength as a function of the volume of the titrant added. These errors were calculated by

$$e_{pH} = pH(\text{corrected}) - pH(\text{predicted}) \qquad (12)$$

where pH(corrected) was obtained by

$$pH(\text{corrected}) = pH(\text{measured}) - pH_{lj} \qquad (13)$$

where pH_{lj} is the liquid junction correction (see Appendix of Ref. 21).

Discussion

According to Figs. 2 and 3, all titration data obtained from glutamic acid solutions can be predicted accurately by means of the new K_a values for this acid and the new parameters for eqn. (1). The predicted pH values agree with the experimental ones almost always within ± 0.003, and the pH errors form for most titrations sets a random pattern. According to these figures, therefore, the new $K_{a,1}$ and $K_{a,2}$ values are strongly supported by experimental data. The K_a values proposed earlier in the literature for glutamic acid at 298.15 K (shown above) do not agree well with each other, and the $K_{a,1}$ and $K_{a,2}$ values recommended by no earlier research group agree very closely with those suggested here. Because of the experimental evidence presented in Figs. 2 and 3, we believe that the present values are the most reliable.

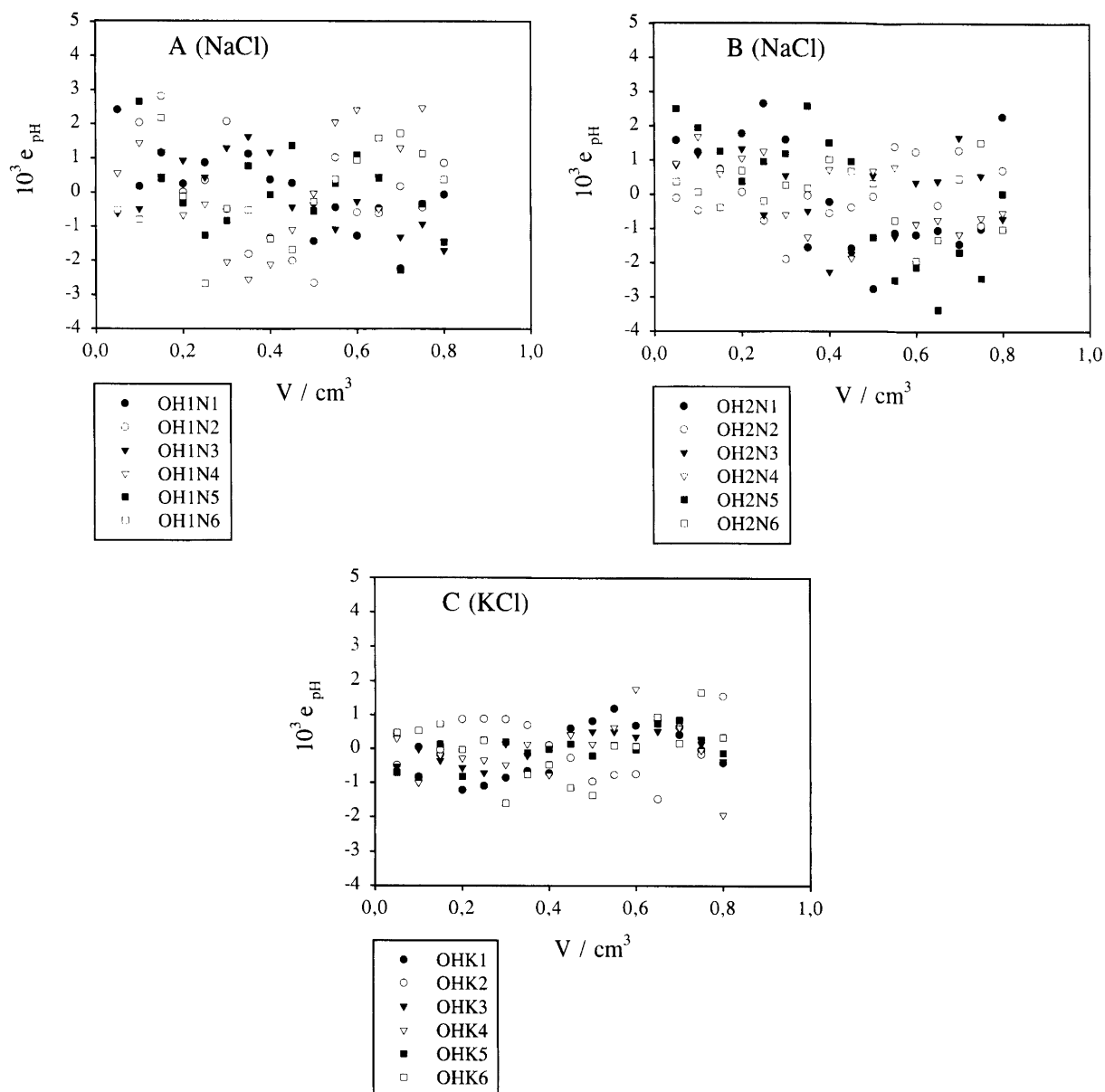By means of eqns. (1) and (5), it is possible to calculate

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Fig. 2. The difference between the observed and predicted pH values, e_{pH} in eqn. (12), as a function of the titrant volume in the titrations of glutamic acid solutions by the base (NaOH) solution. Graphs A and B show the results of the titrations obtained for NaCl solutions (Series OH1N, graph A, and OH2N, B) and graph C shows those obtained for KCl solutions (OHK). The observed pH was corrected as described in Appendix of Ref. 21, and the predicted pH was calculated by means of the new $K_{a,1}$ and $K_{a,2}$ for glutamic acid and new parameters for eqn. (1), see text. Symbols of the different sets are shown at the legends of the graphs (Tables 1, 2 and 5).

$K_{m,2}$ for glutamic acid solutions at any ionic strength up to about 0.4 mol kg^{-1} when I_m is adjusted by NaCl or KCl. The calculated $K_{m,2}$ values at rounded ionic strengths are shown in Table 6. $K_{m,2}$ for glutamic acid in the isotonic saline media of $I_m = 0.16$ mol kg^{-1} is biochemically interesting. According to Table 6 in these media, $K_{m,2}$ is close to 7.6×10^{-5} and thus the dissociation constant is almost two times larger than $K_{a,2}$. In general in biological fluids, the K_m values of amino acids for the isotonic saline solution are much more important than the thermodynamic ones.

In the present study, the following thermodynamic values were determined for the first and second dissociation constants of glutamic acid at 298.15 K: $K_{a,1} = 6.2 \times 10^{-3}$ and $K_{a,2} = 4.57 \times 10^{-5}$. With the precision of the present titrations, no ionic-strength dependence of the first stoichiometric dissociation constant [$K_{m,1}$ in eqn. (6)] was observed up to I_m of 0.4 mol kg^{-1} in both NaCl and KCl solutions. Therefore in these conditions, the following approximation can be made: $K_{m,1} = K_{a,1} = 6.2 \times 10^{-3}$. For the second stoichiometric dissociation constant [$K_{m,2}$ in eqn. (7)] in NaCl or KCl solutions, the
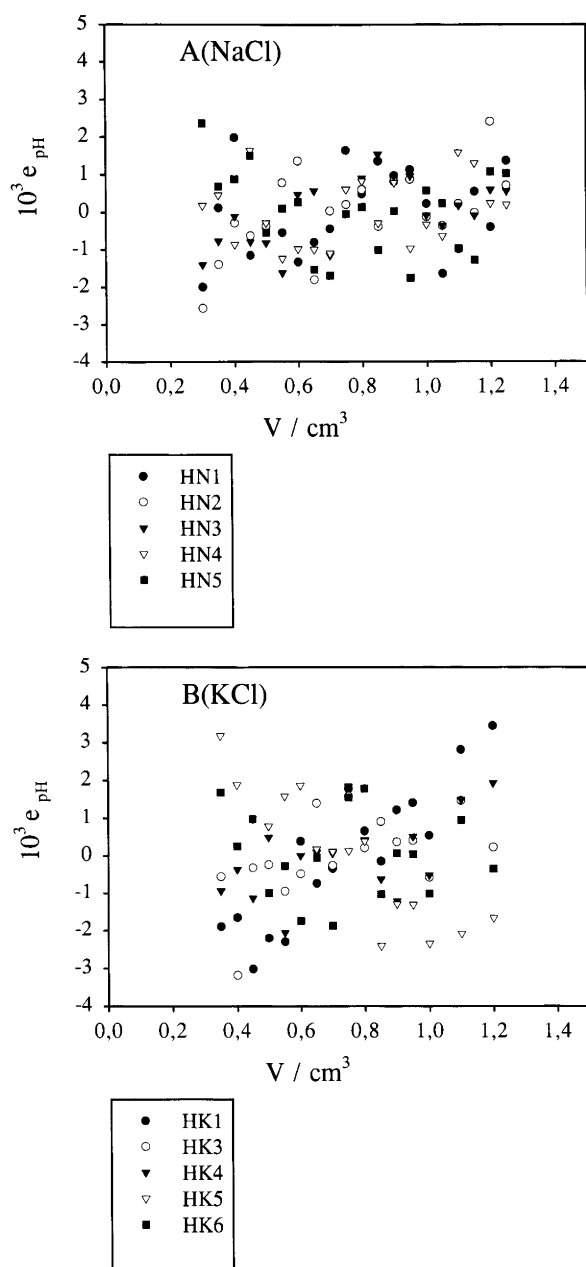
Fig. 3. The difference between the observed and predicted pH values, e_{pH} in eqn. (12), as a function of the titrant volume in the titrations of glutamic acid solutions by the acid (HCl) solution. Graph A shows the results of the titrations obtained for NaCl solutions (Series HN) and graph B those obtained for KCl solutions (HK). The observed pH was corrected as described in Appendix of Ref. 21, and the predicted pH was calculated by means of the new $K_{a,1}$ and $K_{a,2}$ for glutamic acid and new parameters for eqn. (1), see text. Symbols of the different sets are shown at the legends of the graphs (Tables 3 and 4).

following equation was here determined:

$$\ln K_{m,2} = \ln K_{a,2} + \alpha(I_m)^{1/2}$$

$$\times \{1/[1 + B_H(I_m)^{1/2}] + 1/[1 + B_{HA}(I_m)^{1/2}]\}$$

$$- (b_{H,MCl} + b_{HA,MCl})I_m/(m^0) \tag{14}$$

Table 6. Stoichiometric values for the second dissociation constant $(K_{m,2})$ of glutamic acid at 298.15 K as a function of the ionic strength (I_m) in aqueous NaCl and KCl solutions.

$I_m/(\text{mol kg}^{-1})^a$	$10^5 K_{m,2}(\text{NaCl})$	$10^5 K_{m,2}(\text{KCl})$
0	4.57	4.57
0.01	5.56	5.56
0.02	5.92	5.91
0.03	6.17	6.17
0.04	6.38	6.37
0.05	6.55	6.53
0.07	6.83	6.80
0.10	7.14	7.11
0.15	7.51	7.46
0.16	7.57	7.51
0.20	7.77	7.69
0.25	7.95	7.86
0.30	8.09	7.98
0.35	8.20	8.06
0.40	8.27	8.11

$^a I_m$ is the same as m_{NaCl} or m_{KCl}.

where H refers to the protons, HA to the hydrogen glutamate ions and where $\alpha = 1.1744 \,(\text{mol kg}^{-1})^{-1/2}$, $B_H = 1.25 \,(\text{mol kg}^{-1})^{-1/2}$, $B_{HA} = 2.50 \,(\text{mol kg}^{-1})^{-1/2}$, $b_{H,NaCl} = 0.238$, $b_{H,KCl} = 0.178$, $b_{HA,NaCl} = 0.035$ and $b_{HA,KCl} = 0.143$. This equation applies to the cases where NaCl or KCl alone fixes the ionic strength at least up to I_m of 0.4 mol kg^{-1}.

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