is given by the dominant chemical element, *e.g.* for the A sample it is Al, for the B one it is Ti and for the C one Nb.

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Influence of the Binary Solvent Methanol—Water on the Values of Protonation Constants in the H^+—Alanine System

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The H^+—alanine system has been studied by the potentiometric method at 25 °C in 1.0 M water—methanol solution of $NaClO_4$ up to methanol mole fraction $x(CH_3OH) = 0.5$.

The values of protonation constants $\log K_n$ were obtained after experimental data evaluation of the function $E = f(V)$ with the assistance of purpose-made computer programs KALIBR.SKE and AC.BAZ, improved with MAGEC and ESAB2M programs.

The effect of various nonaqueous and mixed solvents on the values of equilibrium and rate constants of chemical reactions is known for a long time already [1—5].

For instance, the effect of such solvents on acid-base and transport properties seems to be promising for the application of organic solvents in isotachophoresis [6]. In comparison with organic solvents the mixed aqueous—organic solvents seem to be more advantageous. Trace amounts of water in pure organic solvents can have a great influence on the properties of some solutions and dissolved compounds and the experimental conditions can become hardly reproducible. One of the very advantageous qualities of methanol is its full-range miscibility with water. Further advantage of the water—methanol mixture is its higher boiling point in comparison with pure methanol.

Dey and co-workers [7—9] have systematically studied the protonation constants of amino acids in various mixed solvents. Our work deals with determination of the first and second protonation constant of DL-alanine by the potentiometric method in binary methanol—water solvent at various volume ratios of the two components at 25 °C.

EXPERIMENTAL

All chemicals used in the experiment were of anal. grade. The solutions were prepared with the use of deionized and redistilled water (conductivity < 10^{-6} S) and methanol (Lachema, Brno) dried over molecular sieves (4A) and distilled. The middle fraction was

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collected and checked by the chromatographic method. A carbonate-free solution of sodium hydroxide (Lachema, Brno) was prepared from 50 % solution of sodium hydroxide.

The concentration of NaOH solution was determined by perchloric acid (VEB Laborchemie, Apolda) with methyl orange indicator and potentiometrically. The concentration of $HClO_4$ solution was determined by anhydrous sodium carbonate, with phenolphthalein and bromcresol green indicators and potentiometrically. The concentration of DL-alanine (Merck) was determined using the potentiometric method with the assistance of ESAB2M [10] computer program. Sodium perchlorate hydrate (VEB Laborchemie, Apolda), two times recrystallized from methanol and stored in vacuum with P_2O_5, was used for maintaining the constant value of the ionic strength of the solution. The amount of water in sodium perchlorate was determined by ^{1}H NMR analysis. Solutions were prepared in the usual way. Because of their instability, the solutions of $HClO_4$ in methanol were stored at -10 °C < θ < 0 °C. All solutions were utilized within 24 h after preparation.

Measurements were carried out on an automatic potentiometric titrator, the block-schematic diagram of which is shown in Fig. 1. The measuring system consisted of two digital pH-meters (OP-280/1, Radelkis, Budapest) with the accuracy $s(E) < 0.1$ mV, two automatic burettes (OP-930/1, Radelkis) with the dosage precision $s(V) < 0.01$ cm^3, personal computer PC-AT, printer (NEC P2 Plus), and two titration cells (Metrohm, Herisau), in which the temperature was maintained at (25 ± 0.1) °C by means of water thermostat. The connection between the computer and primary devices was ensured by a block of BIO (TTL I/O) interface circuits. The solution was during the entire experiment stirred and bubbled with pure argon gas. Glass electrode SKE (OP-0718P, Radelkis) and saturated calomel electrode RE (Crytur RCE 102) were used for experiments.

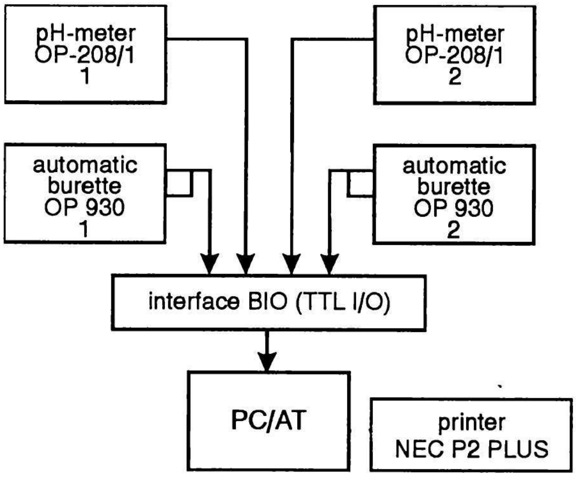

Fig. 1. Block-schematic diagram of the computer-controlled automatic potentiometric equipment.

Series of potentiometric titrations were carried out at 25 °C, at constant ionic strength $I(NaClO_4) = 1.0$ mol dm^{-3}. The concentration of free H$^+$ ions was measured on the basis of EMF of the following cell

$$- \text{RE}|| \text{ measured solution } |\text{SKE} +$$

where SKE is a glass electrode, RE = Hg, Hg_2Cl_2|saturated NaCl solution||1.0 M-$NaClO_4$ in the mixture H_2O—CH_3OH (x_r = 9/1; 8/2; 7/3; 1/1; 4/6; 3/7; 2/8). At the same time two titrations were performed in 0.001 M DL-alanine in the mixture H_2O—CH_3OH in equal titration cells — one using strong alkali (0.1 M-NaOH in the mixture H_2O—CH_3OH) and the other using strong acid (0.1 M-$HClO_4$ in the mixture H_2O—CH_3OH). The stabilization of the measured potential was controlled by computer.

The function of the glass electrode was checked by calibration titrations strong alkali—strong acid in the binary solvent before and after the experiment. The obtained experimental results (max. 200) were processed by computer programs made for this purpose KALIBR.SKE and AC.BAZ. The evaluation of the calibration titrations with strong acid—strong alkali was done using KALIBR.SKE program in three steps: *1.* The equivalent point of the titration was determined from the evaluation of $E = f(V)$ function by finding its inflection point, where the second derivation has a zero value [11]. *2.* The concentration of hydrogen ions H$^+$ in each point of the titration curve knowing the initial volume of titrate solution V_0 and the concentration of the titrant agent c_T was calculated from the added amounts of titrant agent. *3.* Parameters E^0 and S values of correlation coefficient and standard deviation were calculated by means of linear regression at known hydrogen ion concentration. The determination of the parameters E^0 and S from the set of data $\{E, V\}$ in standard strong acid—strong alkali titration enables to avoid problems with activity coefficient of hydrogen ions γ_H and with liquid-junction potential during the calibration procedure using buffer solutions for particular pH values [12]. The following procedure was used for the evaluation of potentiometric titration curves for the H$^+$—alanine system by means of AC.BAZ computer program:

For each point of the titration curve expressed as $E' = f(V)$ the concentration of H$^+$ ions was calculated using E^0 and S parameters, which were obtained after the evaluation of the potentiometric calibration data using KALIBR.SKE program on the basis of $pH = (E' - E^0)/S$ equation. In the cases of direct measurements of $pH = f(V)$ titration curves, this step is omitted. In the next step the protonation constants calculations for log K_1, ..., log K_n were done by two methods: numerical and Bjerrum's method [13]. The obtained values of E^0, S and log K_n were improved by MAGEC [14] and ESAB2M [10] programs. The values of ion product of water ($-\log K_w$) in the binary water—methanol solvent were taken from the work of *Parsons* and *Rochester* [15] and improved using MAGEC [14] program.

In a view of the properties of the binary solvent used in this experiment, this fact has solved the problem with quantitative expression of acidity of the solution in used solvent at the relevant scale [16]. For the algorithm of the calculation procedure see Fig. 2.

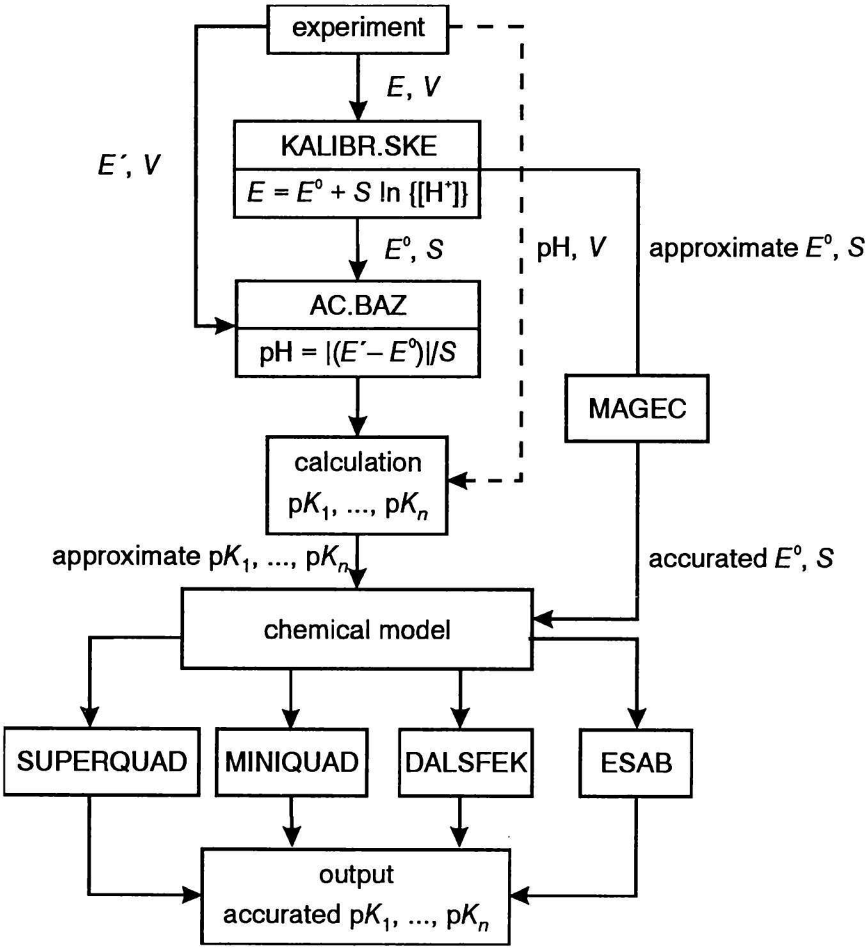

Fig. 2. Algorithm for calculation of the values of protonation constants log K_n.

RESULTS AND DISCUSSION

Six titrations were performed for each mixture $x_r(H_2O—CH_3OH)$ = 9/1; 8/2; 7/3; 1/1; 4/6; 3/7; 2/8 in order to find values for 0.001 M DL-alanine — three using 0.1 M-NaOH and three using 0.1 M-HClO$_4$.

The results obtained by the evaluation of experimental data in a view of two equilibria taking place in the H$^+$—alanine system

$$HL^{\pm} \underset{\log K_1}{\rightleftharpoons} L^- + H^+ \qquad (1)$$

$$HL^{\pm} + H^+ \overset{\log K_2}{\rightleftharpoons} H_2L^+ \qquad (2)$$

are shown in Table 1.

As it is shown in Fig. 3, the value of the protonation constant log K_2 increases with the increasing methanol mole fraction $x(CH_3OH)$. The following equation was obtained by means of the linear regression method: $y = 2.44(\pm 0.11) + 1.25(\pm 0.42)x$. The value of the standard deviation is $s = 0.08$ and the correlation coefficient is $R = 0.947$. The increase of the protonation constant log K_2 values with the mole fraction of methanol $x(CH_3OH)$ can be caused by solvation of the reaction product — H_2L^+ cation (see eqn (2)) by protolytic solvent in acid medium.

Beginning at the methanol mole fraction $x(CH_3OH) = 0.41$ a deviation from the linear course can be seen and the difference between the experimental and calculated values of log K_2 increases. When evaluating only the values of the protonation constants log K_2 up to the mole fraction of methanol

Table 1. Values of Protonation Constants of DL-Alanine (c = 0.001 mol dm^{-3}) in Water—Methanol Solvent at 25 °C and I(NaClO$_4$) = 1.0 mol dm^{-3}

Mole ratio x(CH$_3$OH)	log K_1	log K_2
0	9.83 ± 0.01	2.40 ± 0.02
0.04	9.85 ± 0.02	2.53 ± 0.02
0.09	9.86 ± 0.02	2.62 ± 0.02
0.14	9.87 ± 0.02	2.62 ± 0.02
0.26	9.87 ± 0.02	2.72 ± 0.02
0.34	9.87 ± 0.03	2.83 ± 0.02
0.41	9.84 ± 0.02	2.83 ± 0.03
0.50	9.83 ± 0.02	3.19 ± 0.04

x(CH$_3$OH) = 0.41, the value of standard deviation s = 0.047 and the correlation coefficient R = 0.9607 were obtained by the method of linear regression and the following equation was found: $y = 2.47(\pm 0.07) + 0.97(\pm 0.32)x$. With the increasing mole ratio of methanol x(CH$_3$OH) the value of the protonation constant log K_1 is unchanged (within the interval of experimental error). The standard deviation is s = 0.017 and the average value of the dependent variable is 9.854. The solvation process has in fact no influence on the equilibrium of bipolar ion HL$^{\pm}$ protonation process in alkali solutions (see eqn (*1*)).

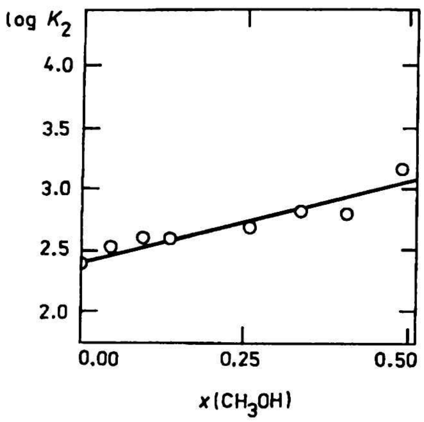

Fig. 3. Dependence of the values of protonation constants log K_n on the methanol mole fraction x(CH$_3$OH).

A comparison of the available literature data on protonation constants in aqueous solution is given in Table 2.

Table 2. Literature Data on DL-Alanine Protonation Constants Values in Aqueous Solvents Obtained by Potentiometric Methods

Ref.	log K_1	log K_2	I / mol dm^{-3}	θ / °C
[17]	9.84	2.31	1.0(?)	20
[18]	9.68 ± 0.005	2.38 ± 0.001	0.6(NaCl)	25
[19]	9.311	2.297	0.15(NaCl)	37
This paper	9.83 ± 0.01	2.40 ± 0.02	1.0(NaClO$_4$)	25

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