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Solvent Effects on Protonation and Complexation of Glutamic and Aspartic Acids with Molybdenum(VI) in Different Aqueous Solutions of Methanol

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The formation constants of the species formed in the systems H^+ + Mo(VI) + aspartic and glutamic acids and H^+ + aspartic and glutamic acids have been determined in different aqueous solutions of methanol [(0 to 40) % v/v] at 25 °C and constant ionic strength (0.1 mol·dm^{-3} sodium perchlorate), using a combination of spectrophotometric and potentiometric techniques. The composition of the complexes was determined by the continuous variations method. It was shown that molybdenum(VI) forms a mononuclear 1:1 complex with the amino acids of the type MoO_3L^{2-} at pH 5.8. The protonation and formation constants in various media were analyzed in terms of Kamlet and Taft's parameters. Single-parameter correlations of the formation constant, K_S, versus α (hydrogen-bond donor acidity), β (hydrogen-bond accepter basicity), and π^* (dipolarity/polarizability) are poor in all solutions, but multiparameter correlations represent significant improvements with regard to the single-parameter models. Linear correlation is observed when the experimental log K_S values are plotted versus the calculated ones, while all the Kamlet and Taft's parameters are considered. Finally, the results are discussed in terms of the effect of solvent on protonation and complexation.

Introduction

Chemists are usually interested to understand solvent effects on overall solution capabilities that depend on all possible intermolecular interactions between solute and solvent molecules. The initial reports dealing with the influence of solvent in a reaction with solute molecules have been documented since the 19th century.[1] The influence of solvent on solute molecules has been intensively studied, but the problem is far from being completely understood. However, the acceptance of a single solvent polarity scale as the most appropriate for interpreting any solvent effect has not yet been achieved. Although the exact definition of solvent polarity is still elusive, it seems reasonable to consider that this property is related to the overall solvation capability of the solvent, encompassing all possible specific and nonspecific intermolecular interactions with solute ions or molecules.[2]

For decades, a great amount of experiments have allowed the proposition of some empirical polarity scales, most of them derived from experiments where single solute models are used to probe (spectrophotometrically, thermodynamically, kinetically, etc.) the interaction with solvents of different polarities. A collection of excellent reviews is available regarding the solvent effect and polarity issues.[3] Solvent effects on transition metal complexes have been reviewed,[4] and more attention has been paid to binary solvent mixtures in this field.[3,5] Solute−solvent interactions are much more complex in mixed solvent systems than in pure solvent due to the possibility of preferential solvation by any of the solvents present in the mixtures. Moreover, the solvent−solvent interactions produced in solvent mixtures can affect the solute−solvent interactions, and therefore they can also affect preferential solvations.[6]

In previous publications,[7–10] we have shown that the dielectric constant alone (as believed for many years) cannot serve as a quantitative measurement of solvent polarity. This approach is often inadequate, since the dielectric constant regards a solvent as a nonstructured continuum, not composed of individual solvent molecules with their own solvent−solvent interaction, and does not take into account specific solute−solvent interactions. In the present work, we have chosen a well understood system, complexation of molybdenum(VI) with aspartic and glutamic acids[11–13] in different aqueous solutions of methanol, to show how the solvents and their mixtures with various polarities affect the formation of such complexes. Further, an attempt is performed to describe the variations of the protonation constants of aspartic and glutamic acids in different aqueous solutions of organic media. The solvation of amino acids that constitute proteins is closely connected with the stabilizing and destabilizing effects of electrolytes on protein structure. Therefore, the study of protonation and solvation processes of amino acids in various organic media is important to elucidate their connection between the chemical ability and biological activity, as the polarity and the activity of water are expected to be lower in an active site cavity of an enzyme than in bulk water.[14]

Experimental Section

Reagents. Methanol was obtained from Merck as an analytical reagent grade material and was used without further purification. L-Aspartic, asp, and L-glutamic, glu, acids (Fluka, analytical reagent grade) were recrystallized from hot water, washed with ethanol, and dried over P_2O_5. Equivalent weights were checked by titration against a standard alkali solution. The NaOH solution was prepared from titrisol solution (Merck), and its concentration was determined by several titrations with standard HCl. Perchloric acid, sodium perchlorate, and sodium molybdate were supplied from Merck (analytical reagent grade) and were used without further purification. A dilute perchloric acid solution

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was standardized against standard NaOH solution. All dilute solutions were prepared from double-distilled water with a specific conductance equal to (1.3 ± 0.1) $\mu S \cdot cm^{-1}$.

Apparatus. An Eyela pH meter, PHM 2000, was used for pH measurements. The hydrogen ion concentration was measured with an Ingold UO 3234 glass electrode and an Ingold UO 3236 calomel electrode. Spectrophotometric measurements were performed using a UV−vis Shimadzu 2100 spectrophotometer with a Pentium 4 computer and thermostatically matched 10 mm quartz cells.

Measurements. All measurements were performed at (25 ± 0.1) °C. The ionic strength was maintained at 0.1 $mol \cdot dm^{-3}$ with sodium perchlorate. The pH meter was calibrated for the relevant H^+ concentration with a solution of 0.01 $mol \cdot dm^{-3}$ perchloric acid solution containing 0.09 $mol \cdot dm^{-3}$ sodium perchlorate (for adjusting the ionic strength to 0.1 $mol \cdot dm^{-3}$). For this standard solution, we set $-\log[H^+] = 2.00$, assuming the acid is dissociated 100 %.[15] Junction potential corrections have been calculated from eq 1.

$$-\log[H^+]_{\text{real}} = -\log[H^+]_{\text{measured}} + a + b[H^+]_{\text{measured}} \quad (1)$$

where a and b were determined by measuring the hydrogen ion concentration for two different solutions of $HClO_4$ or NaOH with sufficient $NaClO_4$ to adjust the ionic media.

For each experiment, two solutions of Mo(VI) + asp or glu have been prepared with the same concentration, but the ionic strength of the first was maintained with sodium perchlorate and that of the second with sodium hydroxide, both with the same mole fraction of methanol. In all solutions, the total concentration of the amino acid and the metal ion was kept constant, $[MoO_4^{2-}]$ + [amino acid] = $2.0 \cdot 10^{-3}$ $mol \cdot dm^{-3}$. The first solution was then titrated with the second one. The pH was measured after addition of a few drops of the titrant, and this procedure extended up to the required pH, 5.8. The absorbance of the first solution was then measured when equilibrium was achieved. The procedure was repeated with different solutions of Mo(VI) and the amino acids by varying the mole fraction of Mo(VI) in the range 0 to 1 with a constant total concentration of Mo(VI) and each ligand in solution.

In all cases, the procedure was repeated at least three times, and the resulting average values and corresponding deviations from the average are shown in the text and tables. To exclude carbon dioxide from the system, a stream of purified nitrogen was passed through a sodium hydroxide solution and then bubbled slowly through the reaction solution.

Calibration of the Glass Electrode. The term pH has significance only in aqueous media.[16] The glass electrode potential in an aqueous solution differs from that in a solution of mixed solvents, and a liquid junction potential of uncertain magnitude may affect the results. To overcome this difficulty, it was necessary to calibrate the glass electrode in different solvent mixtures. The experimental method outlined by Van Uitert and Hass[17] was employed for this purpose. The pH meter reading B in methanol + water media was converted into $[H^+]$ using the equation

$$-\log[H^+] = B + \log \mu_H \quad (2)$$

where the concentration factor $\log \mu_H$ was obtained for the ionic strength 0.1 $mol \cdot dm^{-3}$ $NaClO_4$ from the expression $\log \mu_H = \log \mu°_H + \log \gamma_\pm$. The value of $\mu°_H$ is independent of ionic concentration but is dependent on solvent composition, and $\gamma_\pm$ is the mean activity coefficient of perchloric acid in the solvent mixtures. In this work, the values of B were recorded in various solvent mixtures containing a known concentration of perchloric acid and sufficient sodium perchlorate to give a constant ionic strength of 0.1 $mol \cdot dm^{-3}$. The difference between the logarithm of known hydrogen-ion concentrations and the corresponding values of B was used to calculate values of the correction term $\log \mu_H = \log(\mu°_H \gamma_\pm)$.[18]

Results and Discussion

Protonation of Amino Acids. The following species of the amino acids may exist in solution at different pH, L^{2-}, HL^-, H_2L, and H_3L^+, where L^{2-} represents the fully dissociated ligand anion. The protonation constants of asp or glu have been extensively studied in different kinds of background electrolytes, and the results are reported in the literature. The following equilibria were considered.

$$H^+ + H_{2-n}L^{-n} \rightleftarrows H_{3-n}L^{1-n}$$

$$K_{1+n} = [H_{3-n}L^{1-n}]/[H^+][H_{2-n}L^{-n}] \quad (3)$$

where n is 2, 1, or 0 corresponding to K_3, K_2, and K_1 that refer to the protonation of the amino, the side chain carboxylic, and the other carboxylic acid group, respectively.

The protonation constant values have been determined using the potentiometric technique under the same condition of temperature, ionic media, and mole fraction of methanol as mentioned and calculated using the computer program (Microsoft Excel solver) which employs a nonlinear least-squares method.[19] The protonation constant values, expressed in log units, are collected in Table 1 together with the values reported in the literature for comparison.[20–23]

Complexation of Molybdenum(VI). The occurrence of molybdenum polymerization in acidic media complicates the study of molybdenum complexation with the ligands.[24] Due to fact that the stability constants of the polymerization equilibria are not well-known, the region 5 < pH < 7 is useful for spectrophotometric determination. Using the continuous variations method, we determined the absorbances of solutions of Mo(VI) and the amino acids with total concentration of $2.0 \cdot 10^{-3}$ $mol \cdot dm^{-3}$ in the UV range [(260 to 280) nm] at constant pH 5.8. The observed absorbances were corrected for unreacted Mo(VI) from eq 4 and are plotted in Figure 1.

$$A_C = A_{\text{obs}} - \varepsilon_{\text{Mo}}[\text{Mo(VI)}] \quad (4)$$

Raymond et al.[25] synthesized $Na(MoO_3L)H_2O$, among other molybdenum chelate complexes. The IR spectrum of those compounds that were crystallized at pH = 6 show that the tridentate ligands coordinated to a *cis*-trioxo molybdenum core. As well, Cruywagen[26] has demonstrated the acid dissociation of molybdic acid as

$$MoO_4^{2-} + 2H^+ \rightleftarrows H_2MoO_4 \quad (5)$$

Assuming that H_2MoO_4 is equivalent to $MoO_3 + H_2O$, we can write the molybdenum(VI) chelate formation as eq 6. The same conclusion has been obtained before by Lagrange and her coworkers.[27] So, the composition of the complex species indicated by the spectrophotometric measurements at $-\log[H^+]$ 5.8 is MoO_3L^{2-}. The formation of a 1:1 complex with the amino acids thus has Mo:ligand:proton equal to 1:1:2 stoichiometry as

$$MoO_4^{2-} + L^{2-} + 2H^+ \rightleftarrows MoO_3L^{2-} + H_2O \quad (6)$$

with the stability constant, K_S, as

$$K_S = [MoO_3L^{2-}]/[MoO_4][L^{2-}][H^+]^2 \quad (7)$$

Thus, equations can be written for the total concentration of Mo, C_M, and the total concentration of the ligand, C_L, at the

Table 1. Protonation Constants of the First Carboxylic, K_1, the Side Carboxylic, K_2, and the Amino, K_3, Groups of Aspartic and Glutamic Acids (Experimental and Calculated Ones from Equation 11) at 25 °C, Different Aqueous Solutions of Methanol and Ionic Strength 0.1 mol·dm^{-3} ($NaClO_4$), Together with the Values Reported in the Literature

			log K_2		log K_3		
methanol % (v/v)	species	log K_1	exptl	calcd	exptl	calcd	ref
0.0	asp	2.05 ± 0.02	3.81 ± 0.03	3.82 ± 0.05	9.99 ± 0.05	9.99 ± 0.06	this work
	glu	2.14 ± 0.03	4.24 ± 0.02	4.24 ± 0.02	9.67 ± 0.06	9.68 ± 0.05	
10	asp	2.12 ± 0.03	3.71 ± 0.02	3.70 ± 0.04	9.83 ± 0.04	9.83 ± 0.07	this work
	glu	2.18 ± 0.04	4.12 ± 0.03	4.12 ± 0.03	9.55 ± 0.05	9.54 ± 0.06	
15	asp	2.17 ± 0.02	3.65 ± 0.04	3.65 ± 0.02	9.76 ± 0.06	9.76 ± 0.07	this work
	glu	2.24 ± 0.04	4.06 ± 0.03	4.06 ± 0.04	9.48 ± 0.05	9.48 ± 0.06	
20	asp	2.24 ± 0.04	3.59 ± 0.06	3.59 ± 0.05	9.68 ± 0.05	9.68 ± 0.05	this work
	glu	2.31 ± 0.03	4.00 ± 0.04	4.00 ± 0.05	9.41 ± 0.04	9.41 ± 0.03	
25	asp	2.28 ± 0.01	3.53 ± 0.04	3.53 ± 0.06	9.60 ± 0.06	9.60 ± 0.05	this work
	glu	2.37 ± 0.04	3.94 ± 0.02	3.94 ± 0.04	9.34 ± 0.07	9.34 ± 0.06	
30	asp	2.39 ± 0.02	3.47 ± 0.05	3.47 ± 0.07	9.52 ± 0.04	9.52 ± 0.03	this work
	glu	2.48 ± 0.04	3.88 ± 0.03	3.88 ± 0.06	9.27 ± 0.06	9.27 ± 0.05	
35	asp	2.52 ± 0.02	3.41 ± 0.04	3.41 ± 0.05	9.44 ± 0.03	9.44 ± 0.04	this work
	glu	2.59 ± 0.05	3.82 ± 0.04	3.82 ± 0.06	9.21 ± 0.02	9.21 ± 0.03	
40	asp	2.64 ± 0.03	3.35 ± 0.05	3.35 ± 0.07	9.36 ± 0.05	9.36 ± 0.05	this work
	glu	2.72 ± 0.02	3.75 ± 0.02	3.76 ± 0.04	9.14 ± 0.04	9.14 ± 0.06	
0.0	asp	–	3.70		9.62		20
	glu	–	4.21		9.54		
0.0	asp	–	3.71		9.63		21
	glu	–	4.05		9.46		
0.0	asp	–	3.79		9.75		22
	glu	–	4.15		9.61		
0.0	asp	–	2.88		9.77		23
	glu	–	4.15		9.49		

Table 2. Average Values of the Experimental and Calculated log K_S for Mo(VI)−Aspartic and −Glutamic Acid Systems at −log[H^+] 5.8, Different Aqueous Solutions of Methanol, and Ionic Strength 0.1 mol·dm^{-3} ($NaClO_4$)

methanol %	log K_S (asp)	log K_S (asp)	log K_S (glu)	log K_S (glu)	
(v/v)	exptl	calcd	exptl	calcd	ref
0.0	18.70 ± 0.10	18.69 ± 0.06	17.54 ± 0.12	17.53 ± 0.05	this work
10	18.99 ± 0.11	19.00 ± 0.06	17.85 ± 0.11	17.85 ± 0.08	this work
15	19.16 ± 0.13	19.16 ± 0.08	18.01 ± 0.14	18.02 ± 0.07	this work
20	19.31 ± 0.09	19.31 ± 0.07	18.17 ± 0.10	18.18 ± 0.06	this work
25	19.47 ± 0.08	19.47 ± 0.10	18.34 ± 0.12	18.34 ± 0.09	this work
30	19.63 ± 0.12	19.63 ± 0.11	18.50 ± 0.14	18.50 ± 0.08	this work
35	19.78 ± 0.11	19.78 ± 0.08	18.66 ± 0.11	18.66 ± 0.05	this work
40	19.94 ± 0.08	19.94 ± 0.11	18.82 ± 0.10	18.82 ± 0.10	this work
0.0	18.20	–	–	–	28
0.0	16.79	–	16.78	–	29

maximum point on the plot, as described previously[11,12] (Figure 1)

$$C_M = [Mo] + [C] \tag{8}$$

$$C_L = [L] + [C] \tag{9}$$

Combining eqs 4 and 8 and 9 in eq 7 and solving for K_S gives the stability constant of eq 6, and its average values at different wavelengths are shown in Table 2 together with the values reported before.[28,29]

With some differences, the stability constant values resulting in this work are in agreement with those reported before. The differences are possibly due to the different experimental method and the fact that a different background electrolyte has been employed to determine the values. Further, the structure of sodium molybdate is believed to be octahedral in solution,[12] with three positions to form its complexes with a tridentate ligand like aspartic or glutamic acids. These complexes usually have great stability constants to confirm this speculation. As discussed before,[12] aspartic acid coordinates with Mo(VI) as a six-membered ring between Mo(VI) and the β-carboxylic oxygen atom (more stable) and a seven-membered ring between Mo(VI) and the γ-carboxylic oxygen atom in the glutamic acid complex (less stable). The latter complex would probably be more strained and less favored, and a smaller K_S value would be expected, that agrees with the present results.

Solvent Effect

Protonation Constant. The three protonation constants of both amino acids in water−methanol mixed solvents have different behavior (Table 1). The protonation constant of the amino, K_3, and the side chain carboxylic acid, K_2, groups of the amino acids decreased as the solvent became enriched in the organic component, but the protonation constant of the other carboxylic acid group, K_1, increased as methanol increased in the mixtures. It is very difficult to interpret the variation of the protonation constant values of the amino acids with respect to the percentage of methanol in the mixtures using the dielectric constant of the solutions as a single parameter.

In general, the standard free energy of protonation equilibria consists of two terms: an electrostatic term, which can be estimated by the Born equation,[16,30] and a nonelectrostatic term, which includes specific solute−solvent interaction. When the electrostatic effects predominate, then in accordance with the Born equation, eq 10, the plot of log K versus reciprocal of dielectric constant of the media, ε_r, should be linear.

$$\Delta \log K = (121.6n/r)(1/\varepsilon_r - 0.0128) \tag{10}$$

where r is the common radius of the ions and n is the square summation of the charges involved in the protonation equilibria. For example, $n = 4$ for the charge type L^{2-} Φ HL^-, $n = 2$ for

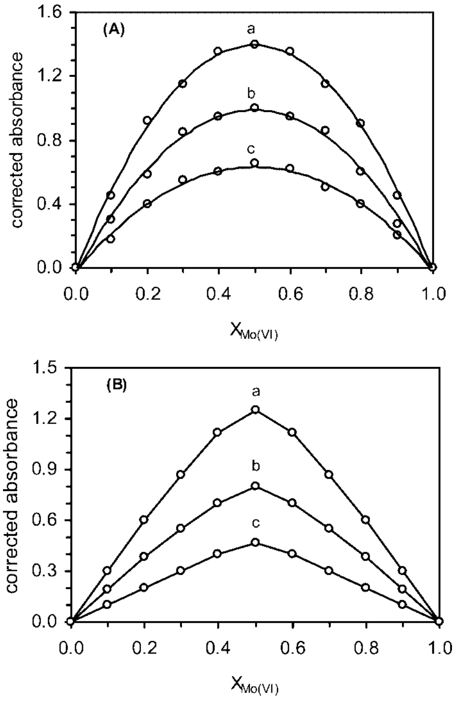

Figure 1. Continuous variations plots of corrected absorbances of (A) $MoO_3(glu)^{2-}$ and (B) $MoO_3(asp)^{2-}$ in aqueous solution versus mole fraction of Mo(VI) at 25 °C, ionic strength 0.1 $mol \cdot dm^{-3}$, $NaClO_4$ and various wavelengths (a) 260 nm, (b) 270 nm, and (c) 280 nm.

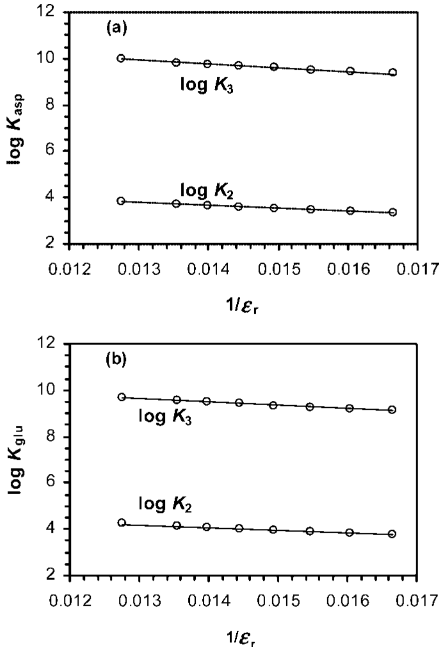

Figure 2. Plots of the experimental values of log K_2 and K_3 versus the reciprocal of dielectric constant of different mixed solvents at 25 °C and ionic strength 0.1 $mol \cdot dm^{-3}$ $NaClO_4$: (a) aspartic acid, (b) glutamic acid.

Table 3. Kamlet and Taft's Solvatochromic Parameters and the Dielectric Constants of Different Methanol−Water Solvent Mixtures

methanol % (v/v)	α^a	β^a	π^{*a}	ε_r^a
0.0	1.17	0.47	1.09	78.36
10	1.15	0.49	1.04	73.79
15	1.14	0.50	1.02	71.51
20	1.13	0.51	0.99	69.22
25	1.12	0.52	0.97	66.94
30	1.11	0.53	0.94	64.65
35	1.10	0.54	0.92	62.36
40	1.09	0.55	0.89	60.08

[a] The values α, β, π^*, and ε_r for pure methanol and water have been obtained from ref 3.

the charge type HL^- Φ H_2L, and $n = 0$ for the charge type H_2L Φ H_3L^+.

The correlation between log K_3 and log K_2 with the reciprocal of the dielectric constant of methanol−water mixtures is linear, with correlation coefficients more than 0.99 (Figure 2). However, there is no change in the number of charges involved in the protonation equilibria of the zwiterionic form of the amino acids, K_1. In this case, the protonation depends only on the solute−solvent interaction of the different species in the mixtures,[31] and the correlation between log K_1 values and $1/\varepsilon_r$ is poor. Therefore, it is essential to elucidate the nature of solute−solvent interactions for a better understanding of solvent effects.

Log K_1 values of the amino acids show small changes in the range 0 % to about 20 % (v/v) of methanol and a larger increase when the mixture is richer in methanol. This variation with the percentage of the organic solvent is due to solute−solvent interaction effects. This effect possibly changes the structure of the mixtures.[32] In fact, the water structure remains intact on the water-rich region, and the methanol molecules occupy the cavities between water molecules without changing the water structure.[33] In this region, there are small changes in log K_1 values of the amino acids. However, log K_1 values change in larger amount when the percentage of methanol increases to higher values. In this region, the influence of methanol on water structure is high, and the solute−solvent interactions cause a greater variation in log K_1 values. This discussion is in accordance with previous results for other aqueous−organic solvent mixtures and in agreement with the present results.[31,32,34,35]

To obtain a quantitative method for evaluation of the solute−solvent interaction on protonation or other equilibrium constants, during the last two decades, many empirical solvent scales have been devised. [3,5] Among these scales (more than 40), the most comprehensive are the solvatochromic ones, but only a few of them have found a wider application in the correlation analysis of solvent effects. A quantitative measurement of the solvent polarity has been introduced by Kamlet, Abboud, and Taft (KAT).[36,37] The KAT equation contains nonspecific as well as specific solute−solvent interactions separately, and the latter should be subdivided into solvent Lewis acidity interactions (hydrogen-bond accepter, HBA solute, and hydrogen-bond donor, HBD solvent) and solvent Lewis basicity interactions (HBD solute−HBA solvent). In general, all of these parameters constitute more comprehensive measures of solvent polarity than the dielectric constant or any other single physical characteristic because they reflect more reliably the complete picture of all intermolecular forces acting between solute and solvent molecules. In general, this approach has been widely and successfully applied in the correlation analysis of all kinds of solvent-dependent processes.[3] Using the solvatochromic solvent parameters, α, β, and π^* which have been introduced in previous reports,[6−10] the multiparametric equation, eq 11, has been proposed for use in the so-called Linear Solvation Energy Relationship.

$$\log K = A_0 + a\alpha + b\beta + p\pi^* \qquad (11)$$

where A_0 represents the regression value and π^* is the index of the solvent dipolarity/polarizability, which is a measure of the ability of a solvent to stabilize a charge or a dipole by its own dielectric effects. The π^* scale was selected to run from 0.0

for cyclohexanone to 1.0 for dimethylsulfoxide. The α coefficient represents the solvent hydrogen-bond donor (HBD) acidity; in other words, it describes the ability of a solvent to donate a proton in a solvent to a solute hydrogen bond. The α scale extends from 0.0 for non-HBD solvents to about 1.0 for methanol. The β coefficient is a measure of a solvent hydrogen-bond acceptor (HBA) basicity and describes the ability of a solvent to accept a proton in a solute to solvent hydrogen bond. The β scale was selected to extend from 0.0 for non-HBA solvents to about 1.0 for hexamethylphosphoric triamide. The empirical values of the solvatochromic solvent parameters (α, β, and π^*) for pure methanol and water were obtained from the literature.[4] However, the solvatochromic parameters for the different binary aqueous mixtures of methanol were calculated with the procedure proposed in the literature, eq 12[38]

$$P_{\text{mixture}} = P_{\text{MeOH}}\Phi_{\text{MeOH}} + P_{\text{H2O}}\Phi_{\text{H2O}} \tag{12}$$

where P is the property of interest and Φ is the volume fraction of the component in the solution. The calculated values of solvatochromic parameters used for different aqueous mixtures of methanol are listed in Table 3.

In eq 11, the discontinuous polarizability correction term is omitted because of the solvent used in this work containing no chlorine atom. The regression coefficients a, b, and p measure the relative susceptibilities of the solvent-dependence of log K to the indicated solvent parameters. To explain the obtained log K values through the KAT solvent parameter, the protonation constants were correlated with solvent properties by means of single and multiple regression analysis by the same computer program.[19] We used the Gauss−Newton nonlinear least-squares method in the computer program to refine the log K by minimizing the error squares sum from eq 13. Single-parameter correlations of log K_1, log K_2, and log K_3 individually with α, β, or π^* did not give a good result in all cases. As a typical example, the single-parameter correlations of log K_1 are shown by eqs 14a to 14f.

$$S = \Sigma(\text{p}K_{\text{exptl}} - \text{p}K_{\text{calcd}})^2 \tag{13}$$

$$\log K_1\ (\text{glu}) = 11.23 - 7.85\alpha \tag{14a}$$

$$\log K_1\ (\text{glu}) = -1.64 + 7.85\beta \tag{14b}$$

$$\log K_1\ (\text{glu}) = 5.36 - 3.04\pi^* \tag{14c}$$

$$\log K_1\ (\text{asp}) = 11.34 - 8.03\alpha \tag{14d}$$

$$\log K_1\ (\text{asp}) = -1.83 + 8.03\beta \tag{14e}$$

$$\log K_1\ (\text{asp}) = 5.34 - 3.11\pi^* \tag{14f}$$

(n = 8, $r^2 \cong 0.90$ to 0.92 from eqs 14a to 14f).

So, we thought it interesting to correlate log K versus a multiparametric equation involving α, β, and π^*. However, the result presented from a multiparametric eq 11 indicates significant improvement with regard to the single-parameter models, eqs 15a to 15f.

$$\log K_1\ (\text{glu}) = 0.95 + 1.26\alpha + 3.87\beta - 2.01\pi^* \tag{15a}$$

$$\log K_2\ (\text{glu}) = 0.83 + 0.34\alpha + 0.51\beta + 2.54\pi^* \tag{15b}$$

$$\log K_3\ (\text{glu}) = 4.85 + 0.65\alpha + 1.52\beta + 3.07\pi^* \tag{15c}$$

$$\log K_1\ (\text{asp}) = 0.94 + 1.25\alpha + 3.78\beta - 2.02\pi^* \tag{15d}$$

$$\log K_2\ (\text{asp}) = 0.38 + 0.48\alpha + 0.54\beta + 2.40\pi^* \tag{15e}$$

$$\log K_3\ (\text{asp}) = 1.57 + 2.96\alpha + 3.04\beta + 3.24\pi^* \tag{15f}$$

(n = 8, r^2 = 0.93, 0.9997, 0.9993, 0.94, 0.9988, and 0.9998, respectively).

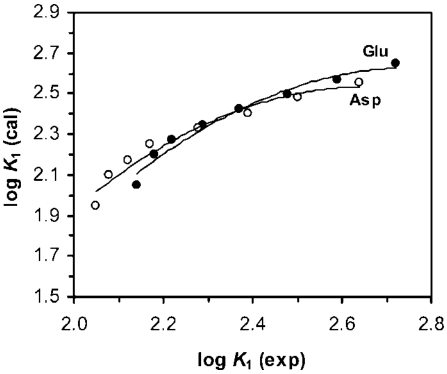

Figure 3. Plots of the experimental values of log K_1 versus the calculated values for both amino acids at 25 °C and ionic strength 0.1 mol·dm^{-3} NaClO$_4$.

Table 4. Percentage Contribution of Kamlet and Taft's Parameters on the Effect of Different Media on Protonation and Complexation at 25 °C and Ionic Strength 0.1 mol·dm^{-3} (NaClO$_4$)

species	α	β	π^*
log K_1 (glu)	17.6	54.2	28.2
log K_2 (glu)	10.0	15.0	75.0
log K_3 (glu)	12.4	29.0	58.6
log K_1 (asp)	17.7	53.6	28.6
log K_2 (asp)	14.0	15.8	70.2
log K_3 (asp)	32.0	32.9	35.1
log K_S (glu)	11.8	33.3	54.9
log K_S (asp)	2.5	30.0	67.5

The coefficients α, β, and π^* in eqs 15a to 15f are different from each other and are in the order of $\pi^* > \beta > \alpha$ for K_2 and K_3 and $\beta > \pi^* > \alpha$ in the case of K_1 for both amino acids. This indicates the hydrogen-bond accepter basicity parameter of the solvent is the most important for K_1, but in the case of K_2 and K_3, the polarity parameter plays a major role. Finally, the hydrogen-bond donor acidity parameter has less significance in the variation of protonation constant values of the amino acids in the proposed various aqueous solutions of methanol.

If the dielectric constant of the media was the only factor for the solvent effect on the protonation, it may be expected that the log K in a solution with higher dielectric constant should be greater than those of all the other aqueous solutions of methanol. It can be seen from Tables 1 and 3 that the dielectric constant of the solvent mixtures decreases as the solutions are enriched in methanol. The values of log K_2 and log K_3 decrease with decreasing dielectric constant of the media, but this is not true in the case of log K_1 values. It is impossible to explain this variation using the dielectric constant approach as a single parameter. However, a multiparametric approach according to the KAT equation was applied to find out which parameter is responsible for this behavior. The positive π^* coefficients in the correlation analysis of log K_2 and log K_3 by the KAT equation imply that a decrease in the polarity of the mixed solvent decreases the protonation constant values of the amino and the side chain carboxylic acid groups. According to the above discussion, the negative π^* coefficient obtained for log K_1 of both amino acids represents a decrease in polarity of the solvent mixtures that causes an increase in the protonation constant values of α-carboxylic acids. In the latter case, the positive coefficient β (with a large difference with the other coefficients (Table 4) suggesting the basicity of the solvent mixtures) has a major role.

To show the efficiency of the suggested multiparameter correlations, the calculated log K_2 and log K_3 are listed in Table 1 for comparison, and the experimental values of log K_1 were plotted versus their calculated ones for both systems from eqs 15a to 15f, for the different aqueous solutions of methanol (Figure 3). It is observed that the experimental and the calculated values of log K_2 and log K_3 are in good agreement with each other, $r^2 > 0.999$, in both cases. However, as mentioned, the correlation of log K_1 is poor (Figure 3). From the values of the regression coefficients, the contribution of each parameter on a percentage basis was calculated and is listed in Table 4.

Complexation Constant. To explain the obtained log K_S values through the KAT equation, the formation constants were correlated with the solvent properties by means of single and multiple linear regression analysis using the same computer program. We again used the Gauss−Newton nonlinear least-squares method in the computer program to refine the log K_S by minimizing the error squares sum from eq 13. Single-parameter correlations of log K_S individually with α, β, or π* did not give a good result. However, the result presented in eqs 16a to 16b, multiparametric equations, indicates significant improvement with regard to the single-parameter models.

$$\log K_S\,(\text{asp}) = 23.30 + 0.20\alpha + 2.44\beta - 5.49\pi^* \quad (16a)$$

$$\log K_S\,(\text{glu}) = 20.68 + 1.23\alpha + 3.46\beta - 5.70\pi^* \quad (16b)$$

($n = 8$, $r^2 = 0.9999$ and 0.9998, respectively).

In this, case the solvent polarity parameter of the media, π*, has a major role and increases with increasing mole fraction of water in aqueous solutions of methanol. If the π* of the media was the only factor for describing the solvent effect on complexation, it may be expected that the log K_S in water should be greater than those of all the other aqueous solutions of methanol. However, the formation constant of both complexes increases with an increase in the solvent hydrogen-bond acceptor basicity parameter, β, and decreases with increasing solvent polarity π*. As well, they increase with a decrease in the hydrogen-bond donor acidity parameter, α, of the solvents. The coefficients of π*, α, and β in eqs 16a and 16b are in the order of $\pi^* > \beta > \alpha$. This suggests that the polarity parameter power of the solvent is the most important; the hydrogen-bond acceptor basicity parameter plays a relatively small role; and finally the hydrogen-bond donor acidity parameter nearly has no significance in changing the formation constants of Mo(VI) + asp and glu systems in the proposed various aqueous solutions of methanol. From the magnitude of the coefficients *a*, *b*, and *p*, the percentage contributions of the Kamlet and Taft solvatochromic parameters on the effect of different media on complexation were calculated and are given in Table 4. To show the efficiency of suggested multiparameter correlations, the calculated values of formation constant are listed in Table 2. It can be seen that the experimental values of log K_S and calculated ones are in good agreement.

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