Thermodynamic Studies on the Protonation Equilibria of Some Hydroxamic Acids in $NaNO_3$ Solutions in Water and in Mixtures of Water and Dioxane

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The protonation equilibria for nine hydroxamic acids in solutions have been studied pH-potentiometrically via a modified Irving and Rossotti technique. The dissociation constants (pK_a values) of hydroxamic acids and the thermodynamic functions ($\Delta G°$, $\Delta H°$, $\Delta S°$, and δ) for the successive and overall protonation processes of hydroxamic acids have been derived at different temperatures in water and in three different mixtures of water and dioxane (the mole fractions of dioxane were 0.083, 0.174, and 0.33). Titrations were also carried out in water ionic strengths of (0.15, 0.20, and 0.25) mol dm^{-3} $NaNO_3$, and the resulting dissociation constants are reported. A detailed thermodynamic analysis of the effects of organic solvent (dioxane), temperature, and ionic strength on the protonation processes of hydroxamic acids is presented and discussed to determine the factors which control these processes.

Introduction

Hydroxamic acids, the class of naturally occurring and synthetic weak organic acids containing the reactive group RC(=O)N(R′)OH have widespread interest, due to their pharmacological, toxicological, and pathological properties. They fulfill a variety of important roles in biology and medicine, they generally have low toxicities, and they show a wide spectrum of activities in all types of biological systems; several of them have advanced into human clinical trials as pharmaceutical drugs, for the treatment of several diseases.[1] Since their discovery and during the past decades, much experimental work has been done on the chemistry and biology of hydroxamic acids.

The transfer of drugs in solutions of gastrointestinal tract, through a membrane, into solution in the blood is affected by physical−chemical factors. The ultimate goal is to have the drug reach the site of action in a concentration which produces a pharmacological effect. For drugs which are weak acids or bases, the dissociation constant (pK_a) of the drug and the pH of the gastrointestinal tract fluid and blood stream will control the solubility of the drug. When a drug is ionized, it will not be able to get through the lipid membrane. It will only be able to do so when it is nonionized and therefore has higher lipid solubility. Acid dissociation constants (pK_a values) are useful physicochemical parameters describing the extent of ionization of functional groups with respect to pH. These parameters are important in research areas such as pharmaceutical drug discovery and development, where knowledge of the ionization state of a particular functional group is often vital in order to understand the pharmacokinetic and pharmacodynamic properties of new drug substances.[2]

To obtain guidelines for the evaluation of the effectiveness of a given hydroxamic acid drug in a biochemical process, knowledge of its acid−base properties (protonation constants) is essential for understanding the bio-physicochemical properties of the hydroxamic acid reagents which are of considerable importance in their application as analytical and medicinal reagents.

The ability of the hydroxamic acid functionality to form a bidentate chelate with the zinc and nickel ions in the enzyme's active site is considered to be an important functional feature of metalloenzyme inhibition, namely, as the key structural element in many highly potent and selective inhibitors against a variety of metalloprotease enzymes.[3] As the polarity and activity of water are expected to be lower in an active site cavity of an enzyme than in bulk water, the protonation processes of the studied hydroxamic acids in this investigation were examined in water containing organic dioxane solvent, in which the thermodynamic data obtained would be useful to solution research workers in biomedicine.

Hydroxamic acids are acid species but also behave as weak bases due to the NC=O moiety.[4] Despite their recognized importance, there are only a few experimental contributions on their acid−base behavior. A search of literature showed that the studies on the thermodynamic protonation constants of hydroxamic acids using a variety of experimental and theoretical tools have been very few.[5−12] No work seems to have been done on the determination of the dissociation constants of acetohydroxamic acid (Aha); *N*-methyl-acetohydroxamic acid (MAha); *N*-isopropyl-acetohydroxamic acid (iPAha); *N*-phenyl-acetohydroxamic acid (PhAha); propanohydroxamic acid (Pha); hexanohydroxamic acid (Hha); 2-hydroxypyridine-*N*-oxide (PYRha); 2,4-dihydroxy-2H-1,4-benzoxazin-3-(4H)-on-glucoside (DIBOA); and 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-4H-one (DIMBOA) in different $NaNO_3$ solutions and in various (water + dioxane) mixtures at different temperatures.

Generally, the dissociation constants of acids can be estimated by analysis of acid−base titrations. The methods have been critically reviewed:[13−15] besides random errors, the systematic errors arise in instrumental measurements and the dissociation constants are obtained with limited precision and accuracy. Systematic errors are caused by

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Table 1. Dissociation Constants of Hydroxamic Acids (pK_a) in 0.1 mol dm^{-3} $NaNO_3$ Solutions in Water and (Water + Dioxane) at Different Temperatures

	mole fraction of dioxane, x_z (volume fraction, Φ_z)															
	0 (0.00)				0.083 (0.30)				0.174 (0.50)				0.33 (0.70)			
	T/K															
	298.15	310.15	318.15	328.15	298.15	310.15	318.15	328.15	298.15	310.15	318.15	328.15	298.15	310.15	318.15	328.15
cmpd[a]	pK_a	pK_a	pK_a	pK_a	pK_a	pK_a	pK_a	pK_a	pK_a	pK_a	pK_a	pK_a	pK_a	pK_a	pK_a	pK_a
Aha	9.28	9.09	8.94	8.79	9.49	9.28	9.14	9.03	9.88	9.70	9.52	9.38	10.28	10.07	9.88	9.67
MAha	8.80	8.67	8.53	8.37	8.97	8.78	8.64	8.49	9.21	9.03	8.87	8.69	9.48	9.27	9.14	9.03
iPAha	9.28	9.07	8.97	8.78	9.51	9.33	9.27	9.13	9.90	9.71	9.53	9.39	10.25	10.03	9.89	9.73
PhAha	8.51	8.29	8.14	8.03	8.72	8.58	8.43	8.31	8.94	8.76	8.59	8.47	9.23	9.08	8.93	8.84
Pha	9.34	9.17	9.04	8.89	9.53	9.34	9.21	9.14	9.93	9.37	9.18	9.03	10.31	10.15	10.01	9.86
Hha	9.38	9.21	9.13	9.04	9.71	9.48	9.32	9.18	9.98	9.80	9.60	9.42	10.43	10.19	10.08	9.93
PYRha	5.80	5.63	5.50	5.38	5.97	5.84	5.69	5.54	6.23	6.08	5.96	5.81	6.54	6.41	6.33	6.14
DIBOA	6.86	6.64	6.49	6.33	7.02	6.89	6.71	6.58	7.32	7.14	7.02	6.81	7.58	7.39	7.24	7.13
DIMBOA	6.70	6.51	6.36	6.19	6.93	6.79	6.63	6.49	7.19	7.03	6.88	6.73	7.35	7.27	7.15	7.04

[a] Aha = acetohydroxamic acid, MAha = *N*-methyl-acetohydroxamic acid, iPAha = *N*-isopropyl-acetohydroxamic acid, PhAha = *N*-phenyl-acetohydroxamic acid, Pha = propanohydroxamic acid, Hha = hexanohydroxamic acid, PYRha = 2-hydroxypyridine-*N*-oxide, DIBOA = 2,4-dihydroxy-2H-1,4-benzoxazin-3-(4H)-on-glucoside, and DIMBOA = 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-4H-one.

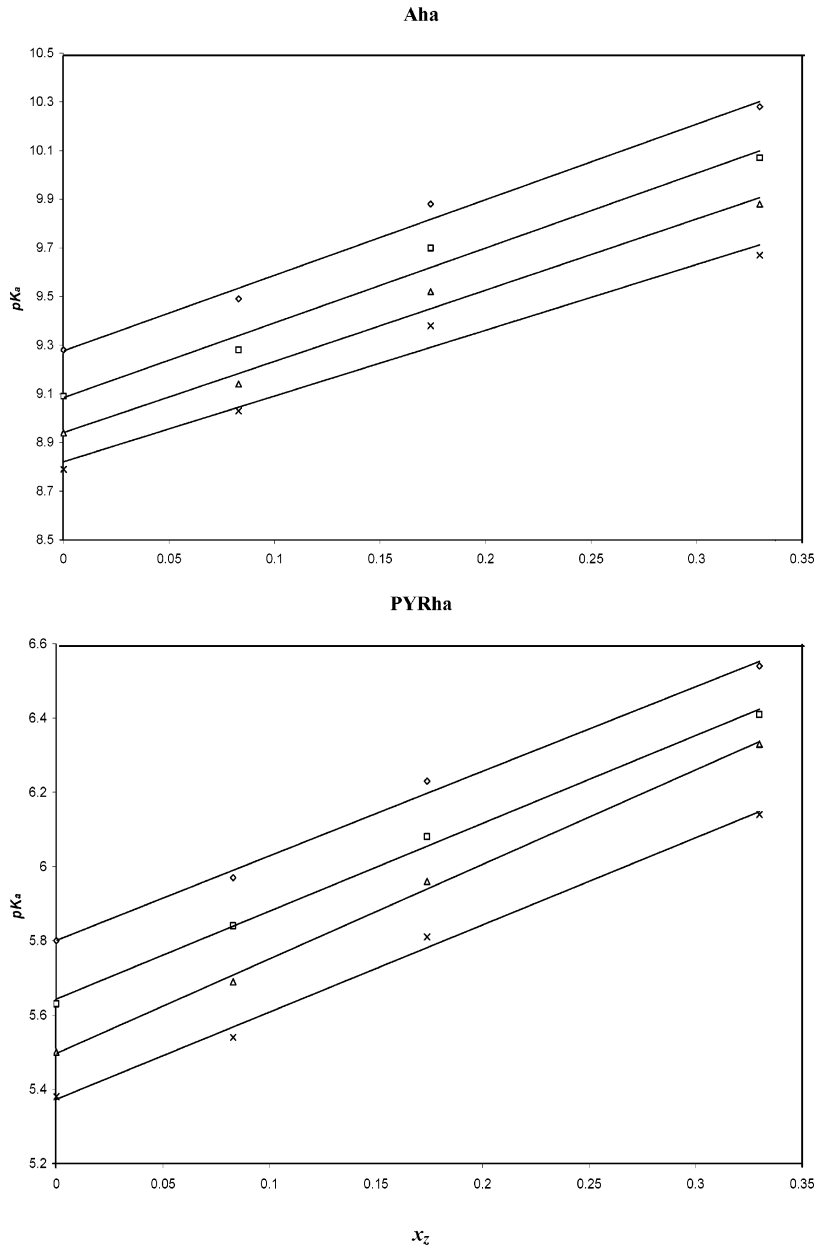

Figure 1. Plot of pK_a values of acetohydroxamic acid (Aha) and 2-hydroxypyridine-*N*-oxide (PYRha) versus x_z of dioxane solvent in 0.1 mol dm^{-3} $NaNO_3$ solution at different temperatures: ◇, 298.15 K; □, 310.15 K; △, 318.15 K; ×, 328.15 K.

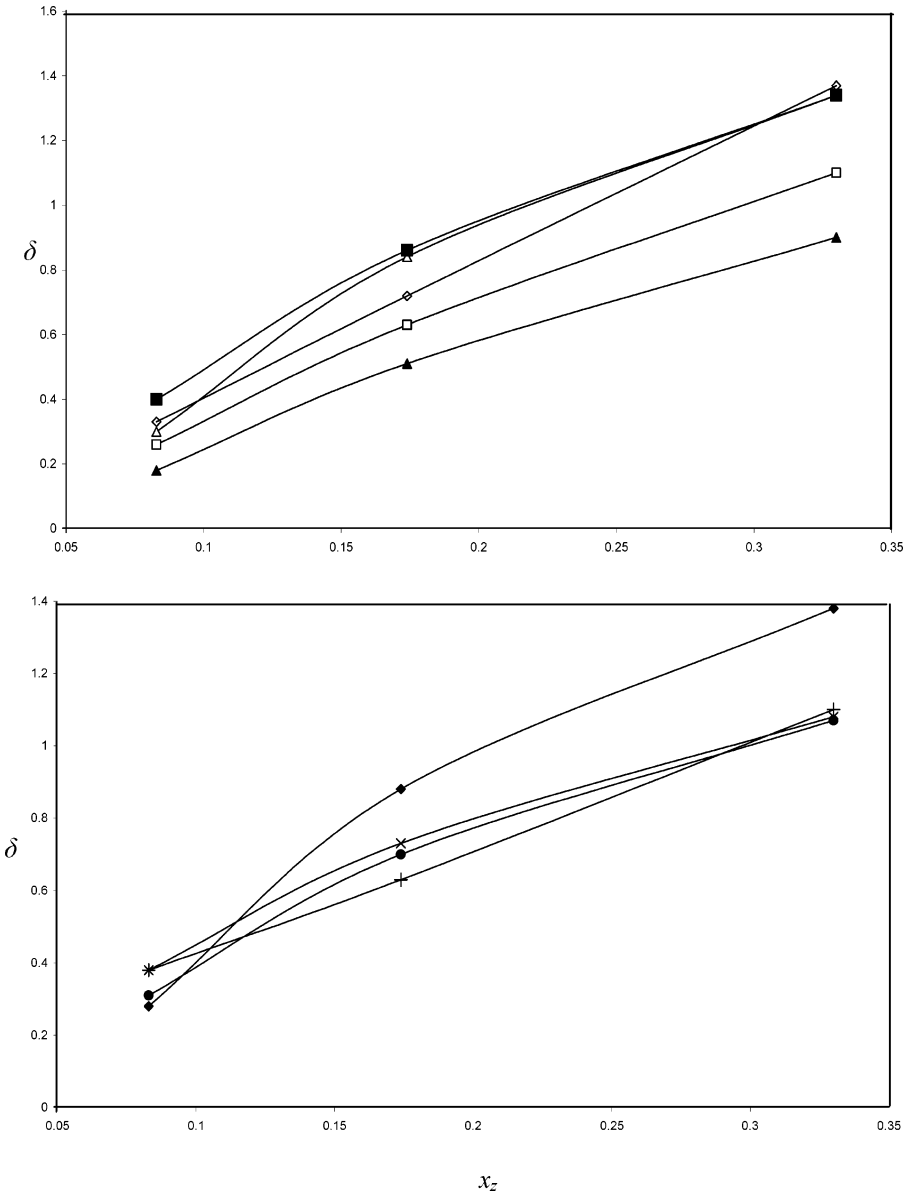

Figure 2. Plot of the free energy change, δ, of the hydroxamic acids vs the mole fraction of dioxane, x_z: ◇, hexanohydroxamic acid (Hha); ■, *N*-isopropyl-acetohydroxamic acid (iPAha); □, 2-hydroxypyridine-*N*-oxide (PYRha); ▲, *N*-methyl-acetohydroxamic acid (MAha); △, acetohydroxamic acid (Aha); ◆, propanohydroxamic acid (Pha); +, *N*-phenyl-acetohydroxamic acid (PhAha); ×, 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-4H-one (DIMBOA); ●, 2,4-dihydroxy-2H-1,4-benzoxazin-3-(4H)-on-glucoside (DIBOA).

limitations of (i) the apparatus and experimental technique and (ii) the procedure of data treatment. Both limitations introduce bias into the quantities of dissociation constants. Besides ESAB,[16,17] which seems to be the most powerful program because it permits refinement of group parameters, another program, PKPOT,[18] will be used.

The purpose of this investigation was to determine the dissociation constants of the above hydroxamic acids (Aha, MAha, iPAha, PhAha, Pha, Hha, PYRha, DIBOA, and DIMBOA) and study their equilibria at various temperatures in water and in different aqueous dioxane media and at different ionic strengths.

Experimental Section

Materials and Solutions. Acetohydroxamic acid (Aha) and 2-hydroxypyridine-*N*-oxide (PYRha) were commercially available chemicals (ICN Biochemical (U.S.A.) and Aldrich, Sigma). The propanohydroxamic acid (Pha), hexanohydroxamic acid (Hha), *N*-phenyl-acetohydroxamic acid (PhAha), *N*-methyl-acetohydroxamic acid (MAha), and *N*-isopropyl-acetohydroxamic acid (iPAha) were prepared by standard procedures from the corresponding carboxylic esters and hydroxylamine.[19] The isolation of 2,4-dihydroxy-2H-1,4-benzoxazin-3-(4H)-on-glucoside (DIBOA-g1) was carried out as reported previously by Pethö.[20] 2,4-Dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-4H-one, a hydroxamic acid from maize (DIMBOA), was isolated from the seedings by a modification of the method described previously.[21,22] Due to the thermal decomposition of DIMBOA,[22] a stock solution to be diluted for titrations could not be used and fresh solutions were prepared each time.

B. D. H. "AnalaR" *p*-dioxane was purified by the procedure of Weissberger.[23] It was refluxed over pellets of KOH for about (8 to 10) h and distilled, and the middle fractions of the distillate were refluxed over metallic sodium for (5 to 6) h and distilled. The middle fraction was used. Its purity was established by determining the freezing point which varied from 11.60 °C to 11.80 °C (uncorrected) against the reported range of 11.65 C° to 12 C°.[24,25]

The levels of purity of the hydroxamic acids used in this investigation and the hydroxamic acid concentrations were determined using Gran's plot method.[26] Carbonate free sodium hydroxide pellets (titrant, prepared in 0.10 mol dm^{-3} $NaNO_3$ solution) were standardized potentiometrically with KH-phthalate solution (Merck AG). Nitric acid, sodium hydroxide, and sodium nitrate were from Merck P. A. Deionized water was used throughout the experiments.

Apparatus. The pH-potentiometric titrations were performed using a Metrohm 796 titroprocessor with a 685 dosimate and a 728 magnetic stirrer, coupled with a dosino buret model 700. The pH titrations were carried out in an 80 cm^3 commercial double-walled glass vessel. The ionic strength of the solutions are maintained at a constant level by using the desired concentration of $NaNO_3$ solution as supporting electrolyte, and the temperature was adjusted inside the cell at the desired temperature, by circulating thermostated water using an oil-thermostated setup. During the course of titrations, a stream of oxygen free nitrogen was passed through the reaction cell to eliminate the adverse effect of the atmospheric carbon dioxide.

Calibration of a Glass Electrode Cell. A computer program (GLEE, glass electrode evaluation)[27] has been used for the calibration of a glass electrode by means of a strong acid−strong base titration. This program provides an estimate of the carbonate contamination of the base, the pseudo-Nernstian standard potential and slope of the electrode, and, optionally, the concentration of the base and pK_W.

Procedure for Equilibrium Titration. To determine the dissociation constants of protonation equilibria of hydroxamic acids, the following solutions were prepared (total volume of 50 cm^3) and titrated potentiometrically against standard carbon dioxide free NaOH (0.10 mol dm^{-3}) solution: (a) HNO_3 (0.003 mol dm^{-3}) + $NaNO_3$ (0.10 mol dm^{-3}) and (b) solution a + hydroxamic acid (0.001 mol dm^{-3}).

Each of the above solutions was left to stand for about 5 min before titration. Each titration was repeated at least four times under carefully controlled experimental conditions.

Calculations. To account for the differences in acidity, basicity, dielectric constant, and ion activities for partially aqueous solutions relative to pure aqueous ones, the pH values of the former solutions were corrected by making use of the procedure described by Douheret.[28]

$$pH^* = pH_{(R)} - \delta \qquad (1)$$

where pH* = corrected value and $pH_{(R)}$ = meter readings. The value of δ for the various proportions of solvent was determined as described below.

The pK_a values were calculated adopting the Irving and Rossotti technique as described in our previously work.[29] Computations related to the estimation of dissociation constants were performed by regression analysis of titration curves using the least-squares computer ESAB[16,17] and PKPOT programs.[18] The adequacy of a proposed regression chemical model with experimental data and the reliability of parameter estimates (pK_a) may be examined by the goodness-of-fit test.[15]

The thermodynamic quantities (Gibbs free energy, $\Delta G°$, enthalpy, $\Delta H°$, and entropy, $\Delta S°$, changes) associated with the protonation equilibria of hydroxamic acids were calculated by the following equations

$$\Delta G° = 2.303RTpK_a \qquad (2)$$

Table 2. Thermodynamic Quantities for the Dissociation Processes of Hydroxamic Acids in 0.1 mol dm^{-3} $NaNO_3$ Solutions in Water and in (Water + Dioxane) Mixtures at Different Temperatures

	mole fraction of dioxane, x_2 (volume fraction, Φ_2)														
	0 (0%)			0.083 (0.30)				0.174 (0.50)				0.33 (0.70)			
compd[a]	$\Delta G°$/ kJ·mol^{-1}	$\Delta H°$/ kJ·mol^{-1}	$-\Delta S°$/ J·mol^{-1}·K^{-1}	$\Delta G°$/ kJ·mol^{-1}	$\Delta H°$/ kJ·mol^{-1}	$-\Delta S°$/ J·mol^{-1}·K^{-1}	δ/ kJ·mol^{-1}	$\Delta G°$/ kJ·mol^{-1}	$\Delta H°$/ kJ·mol^{-1}	$-\Delta S°$/ J·mol^{-1}·K^{-1}	δ/ kJ·mol^{-1}	$\Delta G°$/ kJ·mol^{-1}	$\Delta H°$/ kJ·mol^{-1}	$-\Delta S°$/ J·mol^{-1}·K^{-1}	δ/ kJ·mol^{-1}
Aha	12.77	10.73	6.84	13.07	10.73	7.85	0.30	13.61	10.94	8.96	0.84	14.11	12.83	4.30	1.34
MAha	12.16	8.73	11.50	12.34	10.52	6.10	0.18	12.66	10.94	5.77	0.51	13.06	10.52	8.52	0.90
iPAha	12.77	10.73	6.84	13.17	8.41	15.97	0.40	13.63	11.25	7.98	0.86	14.11	11.57	8.52	1.34
PhAha	11.66	11.25	13.75	12.04	8.83	10.77	0.38	12.30	10.52	5.97	0.63	12.76	8.83	13.18	1.10
Pha	12.89	9.67	10.80	13.17	9.46	12.44	0.28	13.27	13.24	0.10	0.88	14.27	9.57	17.40	1.38
Hha	13.00	7.99	16.80	13.33	12.10	4.13	0.33	13.73	11.78	6.54	0.72	14.37	11.46	9.76	1.37
PYRha	7.89	9.36	4.93	8.15	8.83	2.30	0.26	8.51	8.83	1.10	0.63	8.99	7.78	4.06	1.10
DIBOA	9.31	11.78	8.28	9.62	9.25	1.24	0.31	10.01	10.41	1.34	0.70	10.38	10.31	0.23	1.07
DIMBOA	9.11	10.94	6.14	9.49	9.25	0.81	0.38	9.84	9.78	1.20	0.73	10.19	6.20	13.39	1.08

[a] Aha = acetohydroxamic acid, MAha = *N*-methyl-acetohydroxamic acid, iPAha = *N*-isopropyl-acetohydroxamic acid, PhAha = *N*-phenyl-acetohydroxamic acid, Pha = propanohydroxamic acid, Hha = hexanohydroxamic acid, PYRha = 2-hydroxypyridine-*N*-oxide, DIBOA = 2,4-dihydroxy-2H-1,4-benzoxazin-3-(4H)-on-glucoside, and DIMBOA = 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-4H-one.

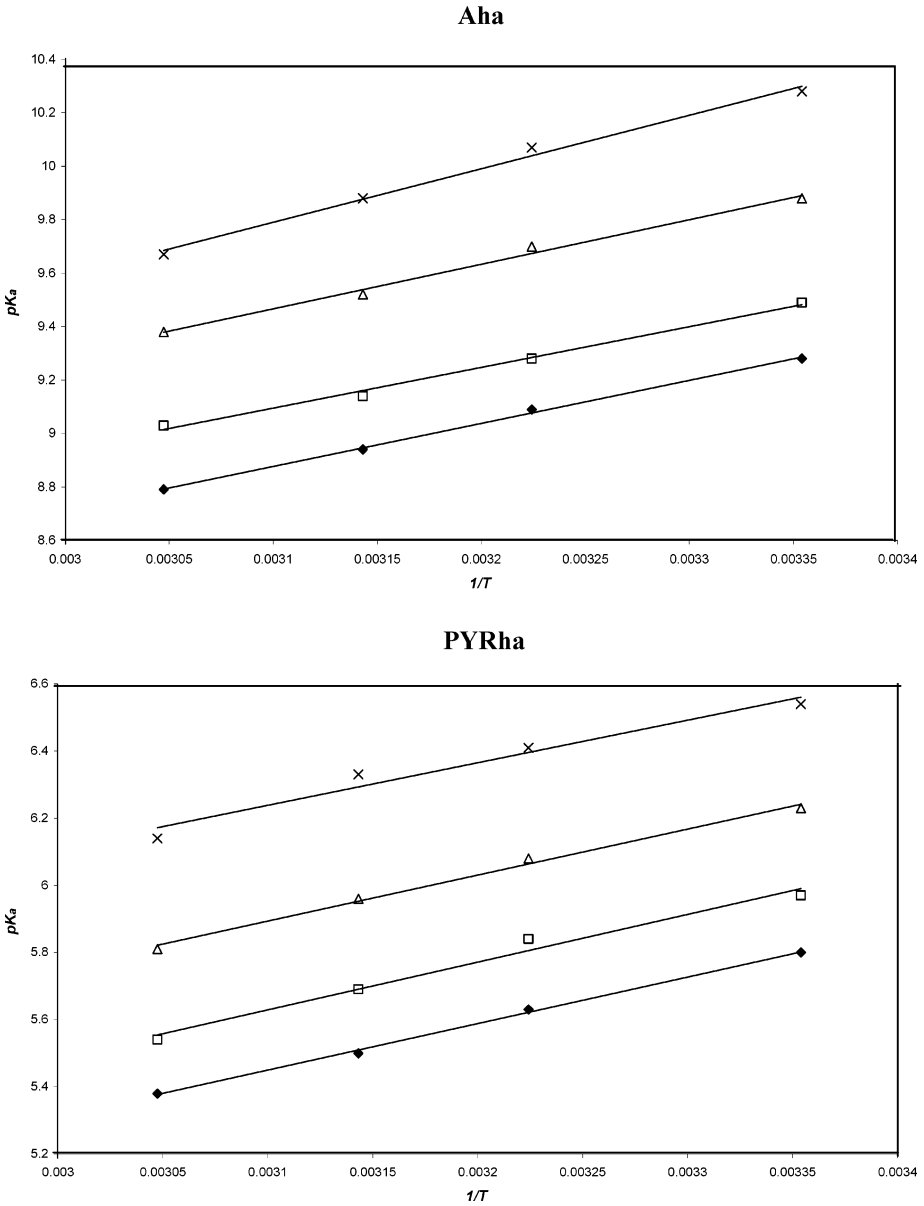

Figure 3. Variation of the dissociation constants (pK_a) of acetohydroxamic acid (Aha) and 2-hydroxypyridine-*N*-oxide (PYRha) studied vs 1/*T* in 0.1 mol dm^{-3} $NaNO_3$ solutions at different water−dioxane mixtures: ×, $x_z = 0.33$ and $\Phi_z = 0.70$; △, $x_z = 0.174$ and $\Phi_z = 050$; □, $x_z = 0.083$ and $\Phi_z = 030$; ◆, $x_z = 0$ and $\Phi_z = 0.0$.

and

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (3)$$

The change in Gibbs free energy from mixed aqueous media, δ, can be calculated as following from the equation

$$\delta = \Delta G^\circ_s - \Delta G^\circ_w \quad (4)$$

where ΔG°_w and ΔG°_s refer to the standard Gibbs free energy change from pure water as solvent and in nonaqueous mixtures, respectively.

Results and Discussions

The titration of nitric acid (HNO_3) in the presence and absence of the hydroxamic acids showed that the protonation of these acids is very low. The acidity of hydroxamic acids may be attributed essentially to the −OH group, and the basic character of the nitrogen atom is suppressed as it is in amides. Hydroxamic acids are very weak, though several times stronger than phenol. The suppression of acidic character may be attributed to intramolecular hydrogen bonding, as shown by infrared studies.[30]

As can be seen in Table 1, the hydroxamic acids (Aha, MAha, iPAha, PhAha, Pha, Hha, PYRha, DIBOA, and DIMBOA) can release one proton in the measurable pH range. The values in Table 1 are consistent with hydroxamic acid acidity in general, and they are also in good agreement with the related literature data.[31−38] Evaluation of the effects of C- and N-substituents on the acidity of the hydroxamic acid group leads to the conclusion that the pK_a increases slightly in the series Aha > Pha > Hha. The differences in the electronics result in more definite changes in the acidity of the secondary hydroxamic acids. As expected, there is a significant increase in the acidity of the hydroxamic acid moiety if the C−N bond is involved in a delocalized ring system.[33] The −OH in the hydroxamic acid like PYRha is the most acidic.

When the ionization of an acid gives a net increase of ions, a decrease in the dielectric constant of the solvent should be accompanied by a decrease in the protonation constant (increase of pK_a) of a weak acid dissolved in it. A

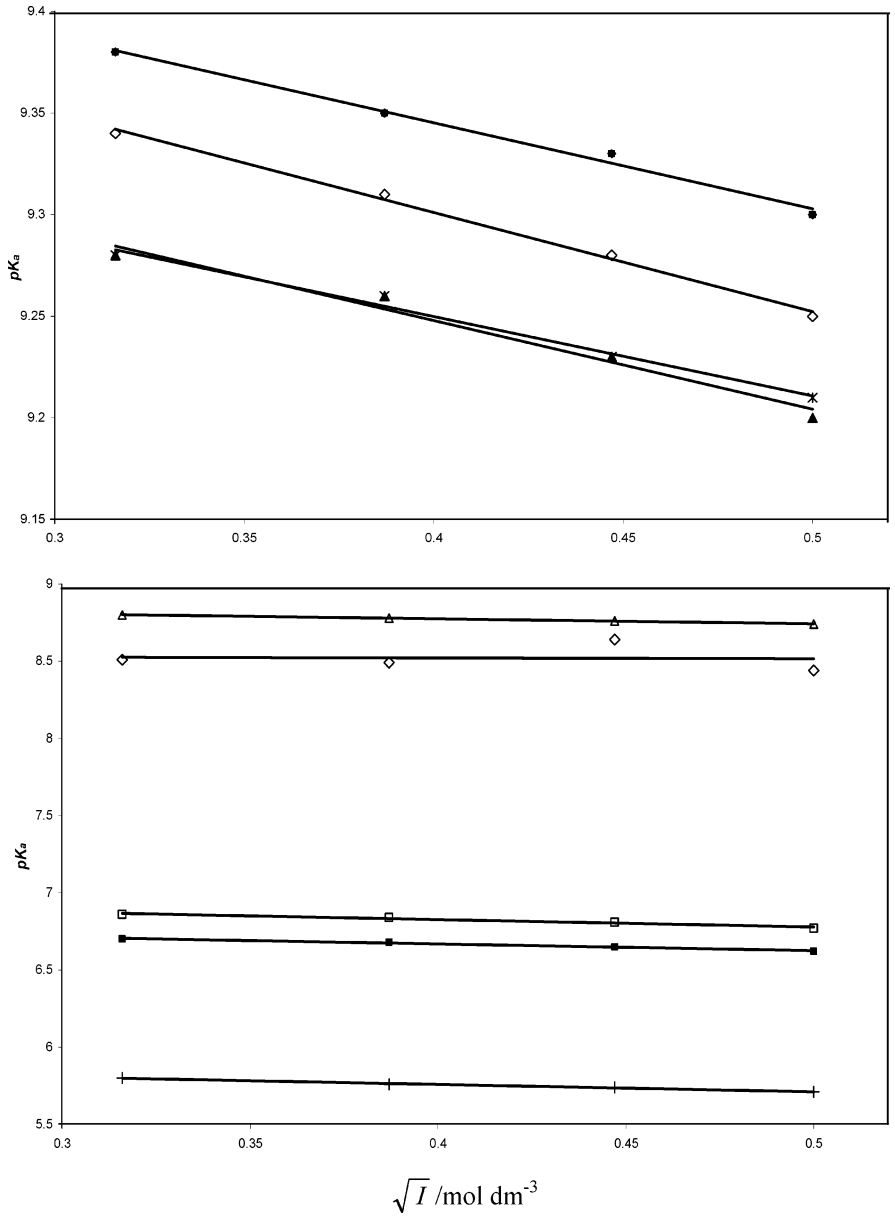

Figure 4. Variation of the dissociation constants (pK_a) of the hydroxamic acids studied vs $\sqrt{I}$ in aqueous water solutions: ◆, acetohydroxamic acid (Aha); ▲, *N*-methyl-acetohydroxamic acid (MAha); △, *N*-isopropyl-acetohydroxamic acid (iPAha); ×, *N*-phenyl-acetohydroxamic acid (PhAha); ◇, propanohydroxamic acid (Pha); ●, hexanohydroxamic acid (Hha); +, 2-hydroxypyridine-*N*-oxide (PYRha); □, 2,4-dihydroxy-2H-1,4-benzoxazin-3-(4H)-on-glucoside (DIBOA); ■, 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-4H-one (DIMBOA).

solvent of low dielectric constant increases the electrostatic forces between the ions, facilitates the formation of molecular species, and should increase the pK_a; as borne out for hydroxamic acids by Table 1, the pK_a values increase with an increase in dioxane content (mole fraction of dioxane) because of the decrease in the dielectric constant of bulk solvent.

As the dielectric constant decreases, the ion interaction involving the proton and anionic oxygen on the acid decreases to a greater extent than the ion dipole interaction between the proton and the solvent molecule. A plot of pK_a versus mole fraction of dioxane, x_z (Figure 1), shows a linear relationship of the form $pK_a = mx_z + c$, where m, x_z, and c represent the slope, mole fraction of dioxane, and intercept, respectively. A similar behavior is found for several other acids such as benzoic, acetic, propionic, and formic acids[39] and for a number of β-diketones in aqueous dioxane.[40] Likewise, linear plots are also obtained for some other mixed water solvents, for example, acetic, propenoic, butyric, and benzoic acids in a methanol−water mixture.[41]

Further, the plot of change in Gibbs free energy from mixed aqueous media, δ, versus x_z is shown in Figure 2. In this plot, we have observe a distinct curvature between aqueous to 0.70 volume fraction of dioxane medium at various temperatures.

Since equilibrium constants in general are temperature dependent, a variation in temperature during measurements would naturally have a deleterious effect on the quality of recorded data. Of much higher significance, however, is the fact that the measuring electrode itself is highly temperature sensitive, with respect to its response to changes ("slope") in [H^+]. The sum of these effects is usually of the order of 1 mV C^{-1}, and it is therefore vital that the experiments are performed with a temperature control of at least ±0.05 K.[42]

In this investigation, the effect of the medium on the protonation processes of hydroxamic acids in water solution and in various aqueous dioxane media have been evaluated at (298.15, 310.15, 318.15, and 328.15) K and the ionic

Table 3. Dissociation Constants of Hydroxamic Acids (pK_a) in Water in Different Ionic Strengths of $NaNO_3$ Solutions at 298.15 K

	I/mol dm^{-3}			
	0.10	0.15	0.20	0.25
cmpd[a]	pK_a	pK_a	pK_a	pK_a
Aha	9.28	9.26	9.23	9.21
MAha	8.80	8.78	8.76	8.74
iPAha	9.28	9.26	9.23	9.2
PhAha	8.51	8.49	8.46	8.44
Pha	9.34	9.31	9.28	9.25
Hha	9.38	9.35	9.33	9.30
PYRha	5.80	5.76	5.74	5.71
DIBOA	6.86	6.84	6.81	6.76
DIMBOA	6.70	6.68	6.65	6.62

[a] Aha = acetohydroxamic acid, MAha = *N*-methyl-acetohydroxamic acid, iPAha = *N*-isopropyl-acetohydroxamic acid, PhAha = *N*-phenyl-acetohydroxamic acid, Pha = propanohydroxamic acid, Hha = hexanohydroxamic acid, PYRha = 2-hydroxypyridine-*N*-oxide, DIBOA = 2,4-dihydroxy-2H-1,4-benzoxazin-3-(4H)-on-glucoside, and DIMBOA = 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3-4H-one.

strength of the medium was held constant using the desired concentration of $NaNO_3$ (0.10 mol dm^{-3}).

The dissociation constant of a weak acid is a function of temperature, and generally, it has a maximum value, K_a(max) or pK_a(min), near room temperature. An examination of the data given in Table 1 reveals that the pK_a values of all hydroxamic acids studied decrease as the temperature is raised. Their heats of protonation, $\Delta H°$, are positive.

From the data listed in Tables 1 and 2, we can conclude that the positive values of $\Delta H°$ found for all dioxane media and hydroxamic acids studied here indicate that the protonation process of hydroxamic acids at temperatures up to 298.15 K is endothermic, and will be exothermic only above T_{max}. It is observed that there is a general tendency for $\Delta S°$ to increase with an increase in dioxane content of the solvent medium. Further, the change in $\Delta H°$ with the change in medium is relatively small and within the experimental error. For this reason, the plots of pK_a versus x_z give practically the same slopes at different temperatures (Figure 1). It is therefore justifiable to assume that $\Delta H°$ is independent of solvent composition over the range studied. The positive values of $\Delta G°$ for the dissociation processes of the hydroxamic acids studied denote that the processes are not spontaneous. In addition, the positive values of entropy changes point toward increased ordering due to association. The plot of pK_a versus $1/T$ gives a straight line (Figure 3).

The errors induced in the determination of pK_a are reflected in all the values of all thermodynamic functions. Therefore, an estimate of error is necessary to show how reliable these results are. The pK_a values were determined generally with a precision of ±0.02 and did not exceed ±0.03, and hence, the error in $\Delta G°$ is estimated to be between (0.03 and 0.04) kJ·mol^{-1}.

A high electrolyte concentration is used to be able to keep variations of the activity coefficients at a minimum. Precise thermodynamic data can only be obtained provided an inert electrolyte of fairly high concentration (0.10 mol dm^{-3} and above) is used.

The main differences between the different procedures for the study of ionic equilibria in aqueous (water solutions) and in nonaqueous solutions are due to the activity coefficients; as in most equilibria in aqueous media, a background electrolyte is added to maintain constant the ionic strength (the concentration ranges from about (0.1 to 3.0) mol dm^{-3} $NaNO_3$); this is allowed in some (water + ethanol) or (water + dioxane) mixtures, but in solvents of low dielectric constants where the solubility of electrolytes is very low.

The dissociation constants of hydroxamic acids studied were determined at different ionic strengths of I = (0.1, 0.15, 0.2, and 0.25) mol dm^{-3} $NaNO_3$ and are listed in Table 3. The linear square-root dependence is observed according to the Debye–Hückel or Davies equations, as shown in Figure 4.

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