Medium Effect on the Apparent Dissociation Constants of Guanine, Thymine, Uracil, Hypoxanthine, and Cytosine in Various Hydroorganic Media

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The apparent dissociation constants of the nucleobases guanine, thymine, uracil, hypoxanthine, and cytosine were determined at (25.0 ± 0.1) °C and $I = 0.1$ mol·dm^{-3} (KNO_3) by potentiometric pH titration in pure water and different hydroorganic solvent media. The organic solvents used were methanol and ethanol as amphiprotic hydrogen bond acceptor−donor (HBA-D) solvents, *N*,*N*-dimethylformamide (DMF), dimethyl sulfoxide, acetone, and dioxane as hydrogen bond acceptor solvents. A computer program was used to refine the initial estimates of the apparent dissociation constants of the five purine and pyrimidine nucleobases. The results obtained are discussed in terms of average macroscopic properties of the mixed solvents. The effects of organic cosolvents on the acid dissociation equilibria have been interpreted using the solvatochromic quantitative values of Kamlet−Taft hydrogen bond acidity and basicity (α, β) and dipolarity polarizability π^* of the solvent. The free energy of transfer of the protons from water to mixed solvent has been calculated for the nucleobases under investigation.

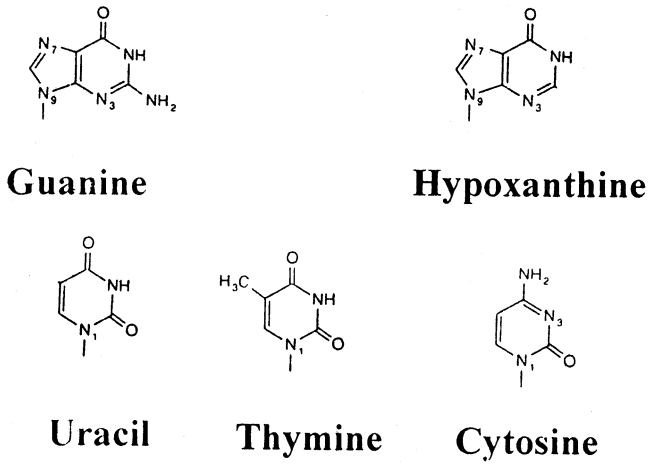
Introduction

The nucleic acids present one of the most instructive examples of ambidentate ligands, because potential nitrogen and oxygen donors occur on the bases, hydroxy groups on the ribose sugar, and negatively charged oxygen atoms in the phosphate residues. Five bases commonly occur in nucleic acids. The proton binding sites of the nucleic bases have been established. Chart 1 shows the predominant tautomeric structures of the purine bases guanine (2-amino-6-oxopurine) and hypoxanthine (6-oxopurine) and the pyrimidine bases uracil (2,4-dioxopyrimidine), cytosine (2-oxo-4-aminopyrimidine), and thymine (5-methyluracil) and the current numbering system. It was concluded that the proton dissociation from protonated cytosine, uracil, or thymine is generated from the N3 H^+ while that of hypoxanthine or guanine is from the N1 H^{+1}.

The literature is yet limited in terms of the acid base properties of guanine, hypoxanthine, cytosine, uracil, or thymine in hydroorganic media. This contribution reports the solvent effect on the acid dissociation constants of the previous nucleobases as a continuation of the authors' work on the dissociation constants of biologically important compounds.[2−9]

It is by now well established that the effective dielectric constants in proteins or active site cavities of metalloenzymes[10] are reduced compared to those in bulk water due to the presence of aliphatic and aromatic amino acid side chains at the protein−water interface. Hence, by employing aqueous solutions that contain different percentages of the organic solvents under investigation (methanol, ethanol, DMF, DMSO, acetone, or dioxane), one may expect to simulate to some degree the actual acid base equilibria of the purine and pyrimidine nucleobases (guanine, hypoxanthine, cytosine, uracil, or thymine) during their interactions in active site cavities.

Chart 1. Structures of the Nucleobases



Experimental Section

Guanine, thymine, uracil, hypoxanthine, and cytosine nucleobases were purchased from Sigma, St. Louis, MO. The organic solvents methanol, ethanol, DMF, DMSO, acetone, and dioxane were from Merck AG, Darmstadt, Germany. A CO_2-free solution of potassium hydroxide (Merck AG) was prepared and standarized against multiple samples of primary-standard potassium hydrogen phthalate (Merck AG) under CO_2-free conditions. KNO_3 was from Merck AG, Darmstadt, Germany. HNO_3 solutions were prepared from HNO_3 (Merck, p.a) and standardized volumetrically with tris(hydroxymethyl)aminoethane. Solutions were prepared by appropriate dilutions of the stock. Hydroorganic solvent mixtures containing different mass

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fractions of the organic solvents were prepared by mixing weighed quantities of water and cosolvent.

Procedure

pH potentiometric measurements were made on solutions in a double-walled glass vessel at (25.0 ± 0.1) °C with a commerical Fisher combined electrode. A Fisher Accument pH/ion meter model 825 MP was used. The electrode system was calibrated in aqueous medium in terms of hydrogen ion concentration instead of activities. Thus, all the constants determined in this work are concentration constants. Calibration of the electrode system was done in the working medium by the MAGEC program using the data of titration of nitric acid with potassium hydroxide, both of known concentration, under the same temperature and medium conditions, $I = 0.1$ mol·dm^{-3} (KNO_3). During the MAGEC calculation, the calibration parameters (standard potential of the cell and value of the ionic product of the medium) were used to test the Nernstian response of the potentiometric cell. A Fisher Scientific Isotemp refrigerated circular model 9000 water thermostat controlled the temperature, and it was maintained within ±0.1 °C. Efficient stirring of the solution was achieved with a magnetic stirer. All test solutions (1×10^{-3} mol·dm^{-3} nucleobase + 4×10^{-3} mol·dm^{-3} HNO_3) were prepared in a constant ionic medium, 0.1 mol·dm^{-3} KNO_3, by mixing the appropriate amounts of ligand (nucleobase), nitric acid, and potassium nitrate and the proportion of the different organic solvents studied. For each mixture, at least four titrations were performed. The concentration of free hydrogen ion, $C(H^+)$, at each point of the titration was calculated from the measured emf, E, of the cell RE/TS/GE (RE and GE denote the reference and glass electrodes, respectively, and TS is the test solution) using the Nernst equation.

$$E = E° + Q \log C(H^+) \qquad (1)$$

where $E°$ is a constant that includes the standard potential of the glass electrode.

It is to be assumed that the activity coefficient is constant, an assumption usually justified by performing the experiments with a medium of high ionic strength (0.1 mol·dm^{-3} KNO_3). Values for K_w for water in water + organic solvent systems have been taken from the literature.[11−15]

The protonation constants were then determined by use of the Bjerrum function.[16] The pK_{a2} values were determined from the overall protonation constants calculated by the linearization method of Irving and Rossotti.[17] Initial estimates of pK_{a2} values were refined with the ESAB2M computer program[18] by minimizing the error squares sum

$$U_v = \sum_i W_i(V_i - V_{calcd,i})^2 \qquad (2)$$

where V_i and $V_{calcd,i}$ are the experimental and calculated values of the titrant for every point i of the titration curve. Calculations have been performed with a Gaussian error in V of $S_V = 0.005$.

Since the nature of the solute−solvent interactions of nucleobases could be ascertained from the free energy of transfer (ΔG_t) values, attempts have been made to throw light on this important aspect. Consider reaction 3.

$$NBH^+ + H_2O \rightleftharpoons H_3O^+ + NB \qquad (3)$$

so

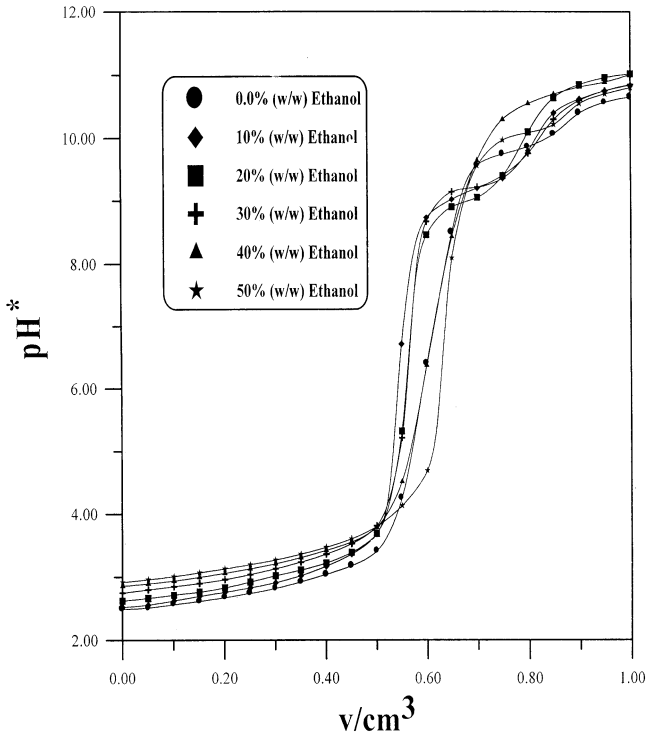

Figure 1. pH* against the volume of 0.0617 mol·dm^{-3} KOH for guanine in ethanol + water mixtures at 25 °C and I = 0.1 mol·dm^{-3} KNO_3.

$$\Delta G_t = \Delta G_t(H^+) + \Delta G_t(NB) - \Delta G_t(NBH^+) \qquad (4)$$

or

$$\Delta G_t - \Delta G_t(H^+) = \Delta G_t(NB) - \Delta G_t(NBH^+) \qquad (5)$$

The left-hand side can be obtained from ΔG_t (calculated using different pK_a values of nucleobases in different hydroorganic media) and $\Delta G_t(H^+)$ values from the literature.[19]

Results and Discussion

The important nucleobase residues studied in this work are shown in Chart 1; they are divided into purines (hypoxanthine and guanine) and pyrimidines (cytosine, uracil, and thymine).

The nucleobase residues of inosine (hypoxanthine) and guanosine (guanine) derivatives may accept a proton at N7; those[20] of cytidine (cytosine), uridine (uracil), and thymidine (thymine) may accept one at N3.[21] Taking into account that these base protonations occur at a pH below the formation of $-P(O)_2(OH)$, the following[20,21] equilibrium for the base deprotonation may be considered,

$$H_2(N)^{(2-n)} \rightleftharpoons H(N)^{1-n} + H^+$$

$$K^H = [H(N)^{(!-n)}][H^+]/[H_2(N)^{(2-n)}]$$

where n = 2, 3, or 4, depending if a nucleoside mono-, di-, or triphosphate is considered, respectively.

Representative titration curves from which the initial estimates of the apparent dissociation constants have been calculated are shown in Figures 1−5. The refined pK_{a2}* values of guanine, hypoxanthine, uracil, thymine, and cytosine in different solvent mixtures are given in Table 1. The values obtained in the present work for the apparent

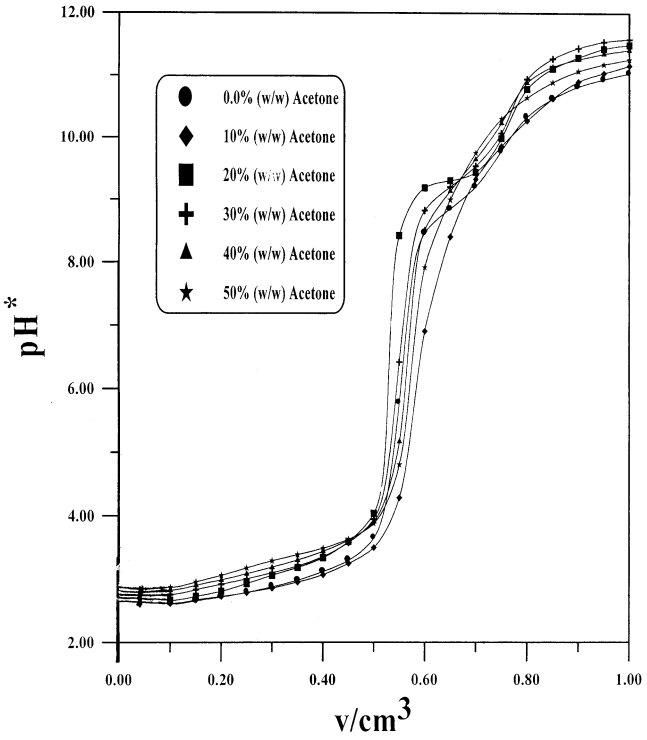

Figure 2. pH* against the volume of 0.0617 mol·dm^{-3} KOH for guanine in acetone + water mixtures at 25 °C and I = 0.1 mol·dm^{-3} KNO_3.

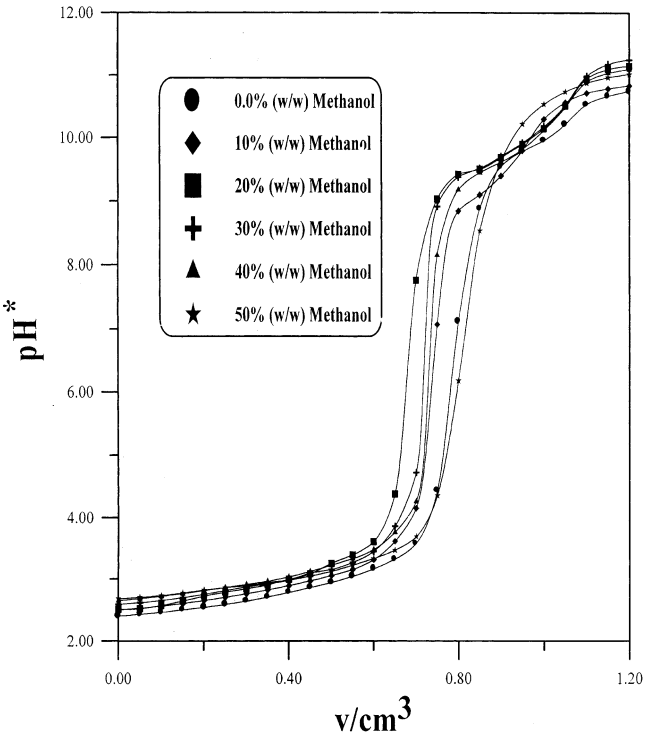

Figure 3. pH* against the volume of 0.0617 mol·dm^{-3} KOH for uracil in methanol + water mixtures at 25 °C and I = 0.1 mol·dm^{-3} KNO_3.

dissociation constant (pK_{a2}) values of the nucleobases studied in pure water agree with the literature data.[22]

Examination of the results revealed that there is no systematic variation in the pK_{a2}* values of the nucleobases under investigation with the organic solvent (methanol and ethanol) content. In the case of methanol it is slightly decreased (guanine and cytosine) while it is slightly

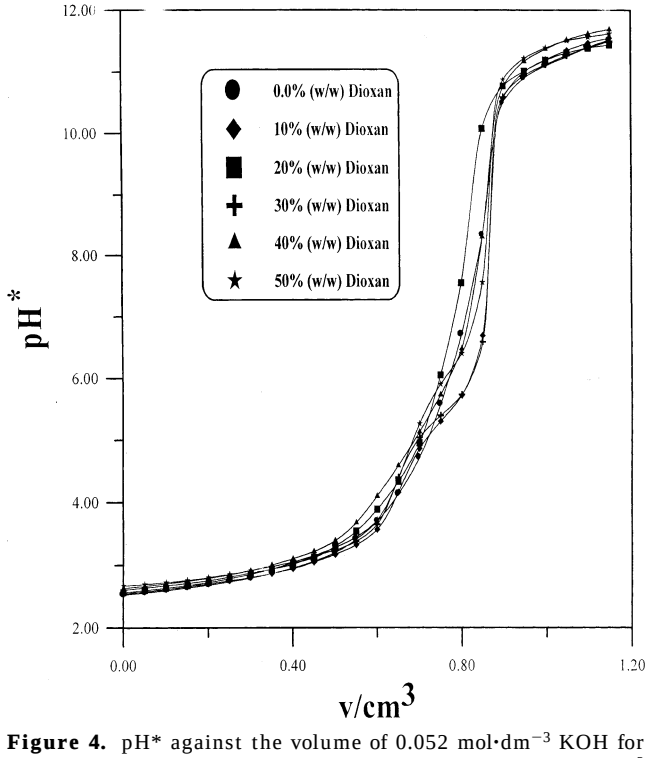

Figure 4. pH* against the volume of 0.052 mol·dm^{-3} KOH for cytosine in dioxan + water mixtures at 25 °C and I = 0.1 mol·dm^{-3} KNO_3.

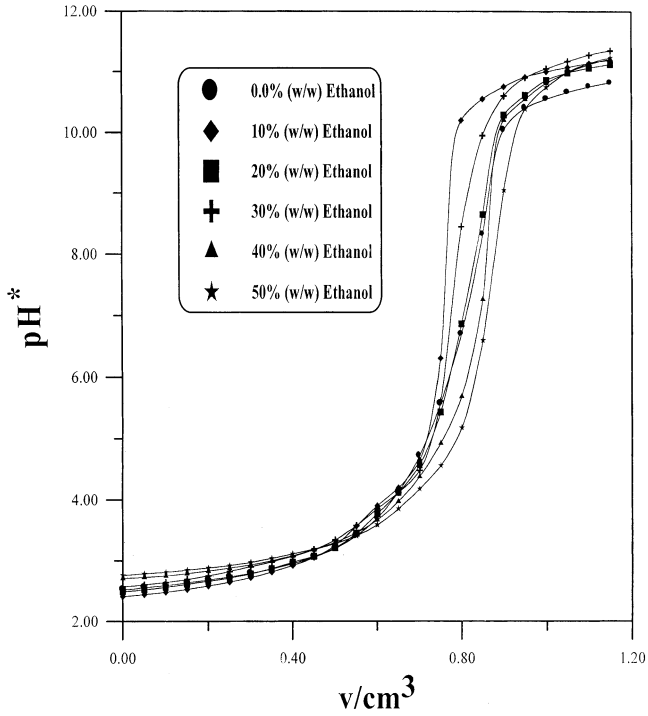

Figure 5. pH* against the volume of 0.052 mol·dm^{-3} KOH for cytosine in ethanol + water mixtures at 25 °C and I = 0.1 mol·dm^{-3} KNO_3.

increased in most cases for thymine, uracil, and hypoxanthine.

The effects of coorganic solvents on the pK_{a2}* values of the nucleobases can be interpreted using the solvatochromic quantitative values of Kamlet−Taft hydrogen bond acidity and basicity (α, β) and dipolarity polarizability π* of the solvent.[23,24] These solvatochromic parameters may be used to quantify and rationalize multiple interacting solvent effects on the dissociation equilibria of the different

Table 1. Refined pK_{a2}* (Apparent Dissociation Constant) Values[a] for Nucleobases in Different Mass Fraction (w)Organic Solvent + (1 − w)Water Mixtures at (25.0 ± 0.1) °C and I = 0.10 mol·dm^{-3} KNO_3

		pK_{a2}*				
organic solvent	w	guanine	thymine	uracil	hypoxanthine	cytosine
methanol	0.00	9.21 ± 0.02	9.79 ± 0.02	9.55 ± 0.02	8.89 ± 0.01	4.72 ± 0.02
	0.10	9.14 ± 0.01	9.82 ± 0.02	9.57 ± 0.02	9.08 ± 0.01	4.61 ± 0.02
	0.20	8.99 ± 0.01	9.89 ± 0.02	9.59 ± 0.02	9.18 ± 0.01	4.56 ± 0.02
	0.30	8.95 ± 0.02	9.95 ± 0.01	9.61 ± 0.02	9.27 ± 0.02	4.52 ± 0.01
	0.40	8.94 ± 0.01	10.03 ± 0.02	9.63 ± 0.01	9.37 ± 0.02	4.47 ± 0.01
	0.50	8.92 ± 0.02	10.07 ± 0.02	9.64 ± 0.02	9.46 ± 0.02	4.42 ± 0.02
ethanol	0.00	9.21 ± 0.02	9.79 ± 0.01	9.55 ± 0.02	8.89 ± 0.01	4.72 ± 0.02
	0.10	9.29 ± 0.01	9.84 ± 0.02	9.62 ± 0.02	9.21 ± 0.02	4.65 ± 0.01
	0.20	9.36 ± 0.01	9.90 ± 0.02	9.69 ± 0.02	9.40 ± 0.02	4.57 ± 0.02
	0.30	9.42 ± 0.02	9.96 ± 0.01	9.74 ± 0.01	9.60 ± 0.01	4.48 ± 0.02
	0.40	9.50 ± 0.03	10.02 ± 0.02	9.80 ± 0.01	9.81 ± 0.02	4.38 ± 0.02
	0.50	9.56 ± 0.03	10.08 ± 0.02	9.84 ± 0.03	10.01 ± 0.03	4.18 ± 0.03
acetone	0.00	9.21 ± 0.02	9.79 ± 0.01	9.55 ± 0.02	8.89 ± 0.01	4.72 ± 0.02
	0.10	9.33 ± 0.02	9.90 ± 0.02	9.72 ± 0.02	9.23 ± 0.02	4.84 ± 0.02
	0.20	9.44 ± 0.02	10.01 ± 0.02	9.88 ± 0.02	9.47 ± 0.02	4.96 ± 0.01
	0.30	9.54 ± 0.01	10.12 ± 0.01	10.05 ± 0.01	9.71 ± 0.02	5.09 ± 0.01
	0.40	9.65 ± 0.02	10.23 ± 0.02	10.21± 0.02	9.86 ± 0.03	5.21 ± 0.03
	0.50	9.75 ± 0.03	10.34 ± 0.03	10.37 ± 0.03	10.18 ± 0.03	5.33 ± 0.03
DMF	0.00	9.21 ± 0.02	9.79 ± 0.01	9.56 ± 0.02	8.89 ± 0.01	4.72 ± 0.02
	0.10	9.38 ± 0.02	9.86 ± 0.02	9.68 ± 0.01	9.10 ± 0.02	4.94 ± 0.03
	0.20	9.5 ± 0.01	9.92 ± 0.02	9.77 ± 0.02	9.31 ± 0.02	5.02 ± 0.02
	0.30	9.72 ± 0.02	10.00 ± 0.02	9.86 ± 0.02	9.50 ± 0.02	5.08 ± 0.03
	0.40	9.88 ± 0.02	10.07 ± 0.01	9.99 ± 0.02	9.71 ± 0.01	5.13 ± 0.03
	0.50	10.05 ± 0.03	10.14 ± 0.03	10.10 ± 0.02	9.90 ± 0.03	5.18 ± 0.03
DMSO	0.00	9.21 ± 0.02	9.79 ± 0.01	9.55 ± 0.02	8.89 ± 0.01	4.72 ± 0.02
	0.10	9.41 ± 0.02	9.91 ± 0.02	9.66 ± 0.02	9.21 ± 0.02	7.81 ± 0.02
	0.20	9.59 ± 0.02	10.03 ± 0.02	9.76 ± 0.03	9.53 ± 0.02	4.90 ± 0.02
	0.30	9.79 ± 0.03	10.16 ± 0.03	9.88 ± 0.03	9.85 ± 0.02	4.99 ± 0.02
	0.40	9.97 ± 0.04	10.28 ± 0.03	9.99 ± 0.03	10.17 ± 0.03	5.09 ± 0.03
	0.50	10.15 ± 0.04	10.40 ± 0.04	10.10 ± 0.04	10.50 ± 0.04	5.18 ± 0.04
dioxane	0.00	9.21± 0.02		9.55 ± 0.02	8.89 ± 0.01	4.72 ± 0.02
	0.10	9.31± 0.01		9.89 ± 0.01	9.54 ± 0.02	4.86 ± 0.03
	0.20	9.44 ± 0.03		10.10 ± 0.02	9.37 ± 0.02	4.95 ± 0.03
	0.30	9.79 ± 0.02		10.39 ± 0.03	9.36 ± 0.01	5.04 ± 0.02
	0.40	10.21 ± 0.01		10.77 ± 0.04	9.23 ± 0.02	5.13 ± 0.02
	0.50	10.68 ± 0.04		10.54 ± 0.04	9.16 ± 0.03	5.26 ± 0.03

[a] pK_{a2}* = corrected pK_{a2} values.

purine and pyrimidine bases studied. The results presented in Table 1, with respect to the amphiprotic hydrogen bond acceptor−donor (HBA-D) solvent ethanol (π^* = 0.54, β = 0.77, and α = 0.83) can conveniently be discussed in terms of ΔG(protonation), the difference between the standard free energies of ionization in the mixed solvent and in water.[25] Protonations of the solvent (SH) by the nucleobase can be represented by the general equation

$$NBH^+ + SH \rightleftharpoons SH_2^+ + NB$$

Since the solvents involved in the ionization are charged, only charge transfer will be dominant.[25] Therefore, although the difference in the dipolarity polarizability solvatochromic parameter π^* between pure water and the water + ethanol mixture is appreciable, it will have little effect on the protonation constants of the solutes. Also, the solvation in mixed ethanol + water as solvent should not differ much from that in water, since the structures of the two are similar. This behavior can be quantitatively attributed to the small difference in the solvatochromic parameter α between pure water and methanol solvents.

The observed slight changes in the pK_{a2}* values of guanine, thymine, uracil, hypoxanthine, and cytosine as the solvent is enriched in methanol can be mainly interpreted as resulting from the following two factors.

The first is the relatively high stabilization of the conjugate bases by donor hydrogen bonds in a pure aqueous medium relative to that in the presence of methanol. This is due to the greater tendency of water molecules to donate a proton in a solvent-to-solute hydrogen bond (α = 1.17). Considering only this effect, an increase in the methanol proportion in the aqueous medium will result in an increase in the activity coefficient of the conjugate base, thereby causing a slight increase in the pK_{a2}* values.

The second is the greater stabilization of the proton in methanol + water through ion−solvent interaction.[26] This effect will generate a low activity coefficient of the proton, therefore causing a slight decrease in pK_{a2}* values. This factor may predominate in the case of guanine and cytosine while the reverse is true for thymine, uracil, and hypoxanthine.

In the presence of hydrogen bond acceptor (HBA) solvents, dimethyl formamide and dimethyl sulfoxide with high values of the solvatochromic parameter β (β = 0.69 for DMF and 0.76 for DMSO), deprotonation of the different purine bases (guanine and hypoxanthine) or pyrimidine bases (thymine, uracil, and cytosine) is rendered more difficult because the solvent mixture solvates the nucleobase more than the proton.

The β scale of HBA (hydrogen bond acceptor) basicities provides a measure of the solvent's ability to accept a proton (donate an electron pair) in a solute-to-solvent hydrogen bond. Thus, the pK_{a2}* values increase with increasing content of the dipolar aprotic solvents with high donicity DMF and DMSO. The observed small increase in the pK_{a2}* values of guanine, thymine, uracil, hypoxanthine, and cytosine when the amount of the organic cosolvent

Table 2. Free Energy of Transfer (ΔG_t(nucleobase) − $\Delta G_t(H^+)$) of Nucleobases in Organic Solvent + Water at (25.0 ± 0.1) °C and I = 0.1 mol·dm^{-3} KNO_3

			(ΔG_t(nucleobase) − $\Delta G_t(H^+)$) (kJ·mol^{-1})				
organic solvent	w	$-\Delta G_t(H^+)$	guanine	thymine	uracil	hypoxanthine	cytosine
methanol	0.10	3.50	3.10 ± 0.03	3.67 ± 0.03	3.16 ± 0.03	4.58 ± 0.03	2.87 ± 0.02
	0.20	5.60	4.34 ± 0.04	6.17 ± 0.05	5.82 ± 0.02	7.25 ± 0.04	4.68 ± 0.03
	0.30	6.80	5.31 ± 0.04	7.71 ± 0.05	4.17 ± 0.03	8.97 ± 0.05	5.66 ± 0.04
	0.40	7.50	5.96 ± 0.04	8.86 ± 0.04	7.95 ± 0.04	10.24 ± 0.04	6.07 ± 0.05
	0.50	7.60	5.94 ± 0.05	9.19 ± 0.05	8.11 ± 0.03	10.85 ± 0.04	5.88 ± 0.03
ethanol	0.10	1.80	2.14 ± 0.02	2.08 ± 0.02	2.20 ± 0.02	3.63 ± 0.03	1.40 ± 0.02
	0.20	4.70	5.44 ± 0.03	5.32 ± 0.03	4.49 ± 0.03	7.61 ± 0.02	3.84 ± 0.02
	0.30	6.40	7.48 ± 0.04	7.37 ± 0.04	7.48 ± 0.05	10.45 ± 0.04	5.03 ± 0.03
	0.40	6.70	8.24 ± 0.05	8.01 ± 0.03	8.12 ± 0.05	11.95 ± 0.05	4.76 ± 0.03
	0.50	5.80	7.68 ± 0.04	7.45 ± 0.04	7.45 ± 0.04	12.19 ± 0.04	2.72 ± 0.02
acetone	0.10	5.40	6.08 ± 0.02	6.02 ± 0.03	6.37 ± 0.03	7.34 ± 0.03	6.08 ± 0.03
	0.20	9.20	10.51 ± 0.04	10.45 ± 0.04	11.08 ± 0.03	12.51 ± 0.04	9.88 ± 0.04
	0.30	11.50	13.38 ± 0.05	13.38 ± 0.05	14.35 ± 0.04	16.18 ± 0.05	13.61 ± 0.05
	0.40	12.30	14.81 ± 0.05	14.81 ± 0.05	16.06 ± 0.05	17.83 ± 0.05	15.26 ± 0.05
	0.50	11.40	14.48 ± 0.05	14.53 ± 0.04	16.07 ± 0.05	18.76 ± 0.04	14.88 ± 0.04
DMF	0.10	5.00	5.97 ± 0.04	5.39 ± 0.03	5.69 ± 0.02	6.20 ± 0.05	6.25 ± 0.03
	0.20	10.70	12.64 ± 0.05	11.44 ± 0.04	16.10 ± 0.05	13.10 ± 0.04	12.41 ± 0.04
	0.30	14.90	17.81 ± 0.05	16.09 ± 0.05	16.61 ± 0.04	18.38 ± 0.05	16.95 ± 0.04
	0.40	15.60	19.42 ± 0.04	17.19 ± 0.04	18.06 ± 0.05	20.28 ± 0.04	17.94 ± 0.03
	0.50	16.20	20.99 ± 0.05	18.19 ± 0.03	19.28 ± 0.04	21.96 ± 0.03	18.82 ± 0.04
DMSO	0.10	4.40	5.54 ± 0.04	5.08 ± 0.02	5.02 ± 0.02	6.23 ± 0.02	4.91 ± 0.03
	0.20	9.50	11.66 ± 0.05	10.86 ± 0.04	10.69 ± 0.03	13.15 ± 0.03	10.52 ± 0.04
	0.30	14.80	18.11 ± 0.05	16.91 ± 0.05	16.68 ± 0.04	20.28 ± 0.04	16.34 ± 0.05
	0.40	18.80	23.13 ± 0.04	21.59 ± 0.04	21.31 ± 0.05	26.10 ± 0.05	20.91 ± 0.05
	0.50	21.30	26.66 ± 0.04	24.78 ± 0.04	24.43 ± 0.05	30.49 ± 0.05	23.92 ± 0.05
dioxane	0.10	2.10	2.67 ± 0.03		4.04 ± 0.02	5.81 ± 0.03	2.90 ± 0.02
	0.20	1.70	3.01 ± 0.02		4.83 ± 0.03	4.44 ± 0.02	3.01 ± 0.02
	0.30	−0.80	2.51 ± 0.02		3.99 ± 0.02	1.88 ± 0.02	1.02 ± 0.02
	0.40	−5.30	0.40 ± 0.01		1.66 ± 0.02	−3.36 ± 0.02	−2.96 ± 0.02
	0.50	−11.60	−3.22 ± 0.02		−5.96 ± 0.02	−10.06 ± 0.03	−5.82 ± 0.03

acetone (low basic aprotic solvent) in the medium is increased can be mainly attributed to a low stabilization of the free conjugate bases of these nucleobases by hydrogen bonding interaction. The observed increase in the pK_{a2}* of the nucleobases as the medium is enriched in the aprotic nonionizing dioxane solvent may be attributed to the fact that the release of the proton from N7 of guanine and hypoxanthine and from N3 of uracil, thymine, and cytosine is rendered more difficult in the presence of this cosolvent. This behavior is probably attributed to the lower β values of dioxane ($\beta = 0.37$).

The solvent medium effect is a measure of changes in the total solvation energy (chemical potential) of a solute i when it is transferred from one solvent (S1) to another (S2). The magnitude of this effect defines the relative stability of the solute in the two solvents and thus the consequences of changing the solvent on the redox, acid−base, and complexation characteristics of the solute. The medium effect is directly related to the standard molar Gibbs energy of transfer of solute i, $\Delta G°_t(I)_{S1\rightarrow S2}$.

Thus, the effects of different organic solvents on the acid dissociation equilibria of the five nucleobases under investigation may be interpreted using the free energy of transfer of the proton,[19] the neutral nucleobase, and the protonated nucleobase from water (w) to w(S) (mixed solvent) in relations 6 and 7

$$p\mathit{K}_{a2} - p\mathit{K}_{a2}^* = \Delta G°_t(H^+)_{w\rightarrow w(S)} + \Delta G°_t(NB)_{w\rightarrow w(S)} - \Delta G°_t(NBH^+)_{w\rightarrow w(S)} \quad (6)$$

$$\Delta G_t(\text{nucleobase}) - \Delta G_t(H^+) = \Delta G°_t(NB) - \Delta G_t(NBH^+) \quad (7)$$

Values of relation 7 are given in Table 2.

The $\Delta G°_t(H^+)_{w\rightarrow w(S)}$ values change with increasing content of the solvent mixture, leading to changes in pK_{a2}* values of the different nucleobases guanine, thymine, uracil, hypoxanthine, and cytosine according to eq 6.

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