Determination of Dissociation Constants of Folinic Acid (Leucovorin), 5-Fluorouracil, and Irinotecan in Hydro-organic Media by a Spectrophotometric Method

Nurullah Şanli,*,† Senem Şanli,† and Güleren Alsancak‡

Department of Chemistry, Faculty of Science & Arts, Hitit University, 19040, Çorum, Turkey, and Department of Chemistry, Faculty of Science & Arts, Süleyman Demirel University, 32260, Isparta, Turkey

In this study, a multiwavelength spectrophotometric method was used for the determination of the dissociation constants of drugs used for treatment of colorectal cancer, folinic acid (leucovorin), 5-fluorouracil, and irinotecan, in water and acetonitrile−water binary mixtures up to a 0.50 volume fraction at 25 °C and an ionic strength of 0.1 mol·L^{-1} KCl. The absorbance/pH profiles were assessed and found to conform to those of polyprotic acids. Data evaluation was performed using the STAR software program which calculates stability constants and molar absorbances of the pure species by multilinear regression. A linear relationship between acidity constants and the mole fraction of acetonitrile in the solvent mixture was observed. The effect of solvent properties on acid−base behavior is discussed.

Introduction

The acid−base dissociation constant, pK_a, is an important parameter in absorption, distribution, metabolism, excretion, and toxicity research because it helps to explain chemical phenomena such as absorption, distribution, and elimination of substances. This important parameter has a lot of applications in research areas such as pharmaceutical drug development, solvent extraction, acid−base titration, and ion transport. The toxicity, chromatographic retention behavior, and pharmaceutical properties of organic acids and bases are affected by acid−base properties. In modern organic chemistry, a considerable amount of theoretical foundation is based on the observation of the effect on acid−base equilibrium of changing molecular structure.[1] In addition, the important role of the degree of ionization in the biological behavior of chemical substances, as well as in their ability to function in passive transcellular diffusion and/or in their suitability as substrates for active transport, is well-established.[2]

There are several methods for the determination of dissociation constants. Traditionally, potentiometry and spectrophotometry are methods of choice due to their simplicity, low cost, ease of application, and so on.[3,4] Very often, the main difficulty in the determination of the dissociation constants of drugs is their aqueous insolubility and low values of acidity constants that forces the use of spectrophotometric techniques. This technique is an attractive method for pK_a determination provided that the compound possesses pH-dependent light absorption. Computer programs using data from multiwavelength spectrophotometry are frequently used for the determination of acid dissociation constants.[5,6] These programs often use principal component analysis and target factor analysis to analyze two- and three-component kinetic systems, and the analyte concentrations are calculated by solving the simultaneous equations of mass balance.

The dissociation constants of ionizable analytes have been determined and discussed in terms of solvent characteristics by various authors in mixed solvents, such as methanol−water, acetonitrile (MeCN)−water, and tetrahydrofuran−water mixtures.[7–11] The influence of an organic solvent added to a medium on the dissociation of ionizable analytes is extensive in many cases and must be accounted for. The variation of the pK values with the content of the organic modifier can be explained by consideration of the preferential solvation of electrolytes in organic solvents. To elucidate the influence of a change in the medium on the systems studied and on retention in liquid chromatography, the values of the dissociation constants can be related to macroscopic parameters [cosolvent percentage, the mole fraction of cosolvent (x), and the dielectric constant (ε)] and to microscopic parameters (Kamlet and Taft's solvatochromic parameters: α, solvent hydrogen-bond acidity; β, solvent hydrogen-bond basicity; π^*, dipolarity/polarizability).[12–14]

Colorectal cancer, also called colon cancer or large bowel cancer, includes cancerous growths in the colon, rectum, and appendix. With 639 000 deaths worldwide per year, it is the third most common form of cancer and the second leading cause of cancer-related death in the Western world.[15] Folinic acid (leucovorin, LV), 5-fluorouracil (5-FU), and irinotecan are the drugs used for the treatment of colorectal cancer, and FOLFIRI is the chemotherapy regimen that agents used in combination.

The pK_a values of these compounds are either not known accurately or not available at all. Only a limited number of studies on the pK values of these compounds are found in the literature.[16–18] This paper focuses on the determination of pK_a values of LV, 5-FU, and irinotecan in water and several MeCN−water mixtures, 0.10, 0.20, 0.30, 0.40, and 0.50 volume fraction at an ionic strength equal to 0.1 mol·L^{-1} with KCl, to overcome the lack of information related to the acid−base equilibria of this kind of compound by means of spectrophotometric measurements.

* Corresponding author. E-mail address: nurullahsanli@hitit.edu.tr. Tel.: +90 364 227 70 00/1635. Fax: +90 364 227 70 05.
† Hitit University.
‡ Süleyman Demirel University.

10.1021/je100072n
Published on Web 04/13/2010

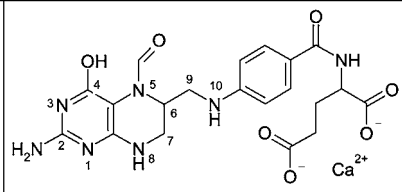
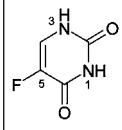
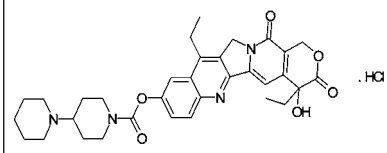
Compound	Chemical Structure
Folinic acid (Leucovorin calcium), N-(4-(((2-Amino-5-formyl-5,6,7,8-tetrahydro-4-hydroxy-6-pteridinyl)methyl)amino)benzoyl)-L-glutamic acid calcium salt CAS No: 1492-18-8	 $C_{20}H_{21}N_7O_7Ca$ 511.51 g mol^{-1}
5-Fluorouracil 5-fluoro-1*H*-pyrimidine-2,4-dione CAS No: 51-21-8	 $C_4H_3FN_2O_2$ 130.08 g mol^{-1}
Irinotecan (CTP-11) (S)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo1H-pyrano[3',4':6,7]-indolizino[1,2-b]quinolin-9-yl-[1,4'bipiperidine]-1'-carboxylate CAS No: 100286-90- 60	 $C_{33}H_{38}N_4O_6$ 677.185 g mol^{-1} (HCl)

Figure 1. Chemical structures of studied compounds.

Experimental Section

Chemicals and Reagents. Analytical reagent grade chemicals were used, unless otherwise indicated. LV, 5-FU, and irinotecan were purchased from Sigma and used without further purification (Figure 1). Ultrapure water, with a conductivity lower than 0.05 μS·cm^{-1}, was obtained from a Human water purification system (Human Corp.).

MeCN was of high-performance liquid chromatography (HPLC) grade and used as the organic modifier. Potassium hydroxide (Titrisol), hydrochloric acid (Titrisol), potassium hydrogen phthalate, and potassium chloride (ionic strength adjuster; 0.1 mol·L^{-1}) were supplied by Merck. While spectrometric measurements were done, solutions of individual compounds were prepared at a concentration of approximately $1\cdot10^{-5}$ mol·L^{-1}. All stock solutions of hydrochloric acid, potassium hydroxide, potassium hydrogen phthalate, and potassium chloride were prepared by dilution in water.

Apparatus. Potentiometric measurements were performed with a Mettler-Toledo MA 235 pH/ion (resolution ± 0.1 mV) analyzer system equipped with an Orion 3 combination glass electrode (Thermo electron Corp.). The UV−vis absorbance spectra were recorded at each pH using a Perkin-Elmer LAMBDA 25 spectrophotometer, equipped with a 1 cm path length cell, controlled by a personal computer. A peristaltic pump was used to circulate the solution from the titration vessel to the spectrophotometer cell, and vice versa, through Teflon or Tygon tubes in a closed loop circuit with continuous flow. All titrations were carried out under N_2 and at (25.0 ± 0.1) °C, which was maintained by circulating water from a constant-temperature thermostat (Selecta Ultra-Therm water bath and temperature control Selecta) through the double-wall Pyrex titration cell of 80 mL capacity.

Procedures. The p*K* values of the studied antineoplastic agents were determined by means of the data obtained from spectrophotometric titrations in water and in 0.10, 0.20, 0.30, 0.40, and 0.50 volume fraction MeCN−water mixtures at (25.0 ± 0.1) °C and in 0.1 mol·L^{-1} KCl. The spectrophotometric multiple-wavelength pH titration was carried out as follows: in a first step, the electrodic system was calibrated by Gran's method as in the case of potentiometric measurements to obtain the standard electromotive force (emf) values, E^0, of the potentiometric cell. The calibration parameters were checked from the Gran plots.[19,20] The standardization of the electrode system was carried out each time the solvent medium was changed and the constancy of E^0 values ensured by continual surveillance by means of periodic calibrations. In a second step, a solution of fully protonated compounds (50.0 mL containing $1\cdot10^{-5}$ mol·L^{-1} drug) to be analyzed at the required conditions of temperature, ionic strength, and solvent composition was added to the pretitrated background solution, and small amounts of sodium hydroxide or hydrochloric acid solutions were then added. The spectral data were obtained by adding small amounts of acid or base to change the pH in the range of 1.7 to 12.0. After each addition, the potential was allowed to stabilize, and the potential value was used to calculate the pH of the solution using the value of E^0 calculated in the calibration step. These amounts should be high enough to provoke a measurable change in the pH of the test solution, but also small enough to allow

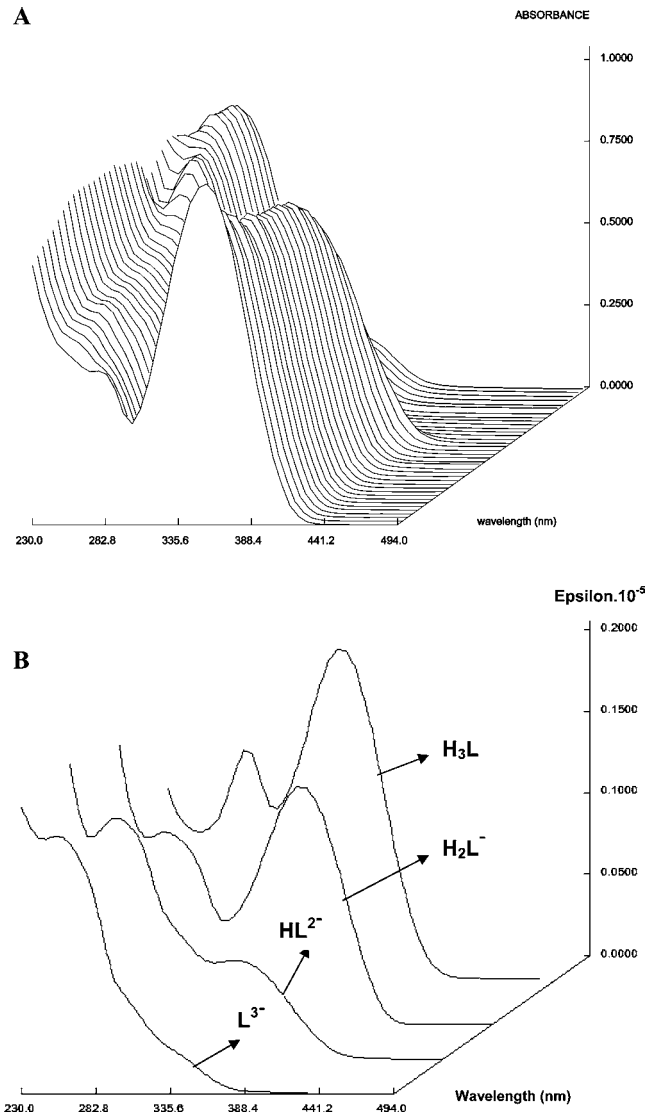


Figure 2. (A) Wavelength (nm) absorbance graphic and (B) molar absorbances of species for $4.9\cdot10^{-5}$ mol·L^{-1} folinic acid as a function of pH in water.

Table 1. pK_a Values of Studied Compounds Obtained by the Spectrophotometric Method in Water and MeCN−Water Media at 0.1 mol·L^{-1} Ionic Strength and 25 °C

compounds	literature	water	0.10 vol. fraction MeCN	0.20 vol. fraction MeCN	0.30 vol. fraction MeCN	0.40 vol. fraction MeCN	0.50 vol. fraction MeCN
folinic acid (leucovorin)	3.10[a]	3.12 (0.11)	3.23 (0.07)	3.33 (0.11)	3.45 (0.08)	3.57 (0.07)	3.70 (0.05)
	4.58	4.60 (0.03)	4.70 (0.08)	4.75 (0.03)	4.83 (0.06)	4.96 (0.06)	5.05 (0.05)
	10.15	10.00 (0.08)	10.08 (0.07)	10.24 (0.07)	10.30 (0.08)	10.37 (0.09)	10.41 (0.08)
5-fluorouracil (5-FU)	7.98[b]	8.05 (0.02)	7.89 (0.07)	7.81 (0.01)	7.69 (0.01)	7.61 (0.01)	7.51 (0.05)
irinotecan (camptosar)	8.79[c] (0.18)	8.71 (0.09)	8.59 (0.07)	8.47 (0.12)	8.42 (0.08)	8.35 (0.05)	8.21 (0.05)

[a] From ref 16. [b] From ref 17. [c] From ref 18.

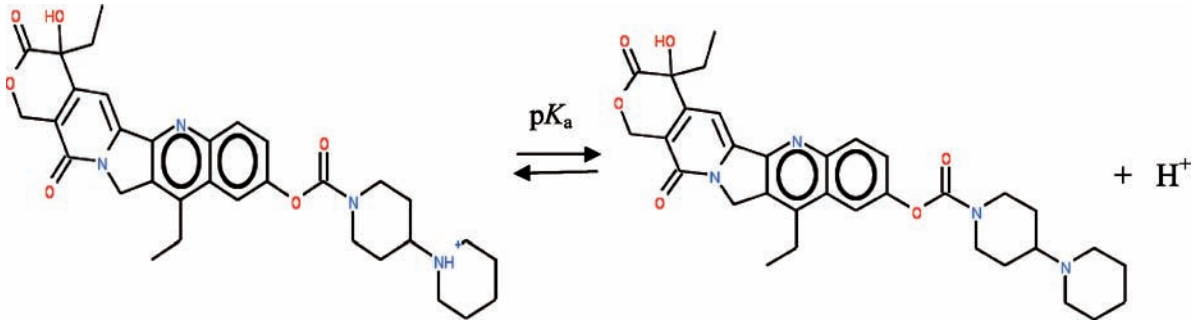


Figure 3. Dissociation equilibrium of irinotecan.

the increase of volume to be neglected. During spectrophotometric titrations, the test solution was pumped to a spectrophotometric flow cell by means of a peristaltic pump. After each addition of titrant, and after waiting for the emf reading to be stable, the spectra were recorded with 1 nm resolution over the (190 to 500) nm interval to obtain different spectra around the maximum λ for each studied compound.

Data Treatment. Spectrophotometric titration data were treated using the program STAR (stability constants by absorbance readings)[21] which calculates stability constants and molar absorptivities of the pure species by multilinear regression. The program requires a previous model of the chemical equilibria, based upon the existence of certain chemical species, to be postulated. The refinement of equilibrium constants is done using the Gauss−Newton nonlinear least-squares algorithm by numerical differentiation, until a minimum in the sum of the squares residual (U) is attained. This function is defined as

$$U = \sum_{i=1}^{n_s} \sum_{j=1}^{n_w} (A_{i,j,\mathrm{exp}} - A_{i,j,\mathrm{calc}})^2 \qquad (1)$$

where n_s and n_w are the number of solutions and the number of wavelengths, respectively, A_{exp} is the experimental data of absorbance, and A_{calc} is obtained by Beer's law from the calculated concentrations of each species and their molar absorptivities. The optimization is performed by means of a nonlinear least-squares procedure. The minimization process is repeated until the relative change of U between two iterations is ≤ 0.01 %.

Results and Discussion

The UV−vis absorption spectra of the studied compounds in different MeCN−water binary mixtures up to 0.50 volume fraction at various pH values were recorded. Sample UV−vis absorption spectrum and molar absorbances of species of LV over the (230 to 500) nm interval and various pH values in pure water are shown in Figure 2. The data were processed using the STAR program[21] to obtain the pK values for substances using an iterative procedure.

The complete and comparable sets of pK_a values of LV, 5-FU, and CTP-11 in aqueous solution and in MeCN binary mixtures have not yet been reported. The dissociation constant values determined for the equilibria involved for the studied compounds in water, 0.10, 0.20, 0.30, 0.40, and 0.50 volume fraction MeCN−water mixtures at (25.0 ± 0.1) °C, are collected in Table 1, together with respective standard deviations. pK values reported in the literature for water are also shown in Table 1.[16–18]

LV is a 5-formyl derivative of tetrahydrofolic acid (Figure 1). LV behaves as a weak acid owing to the dissociation of the phenolic OH in the pteridine ring moiety and two carboxylic groups in the glutamic acid moiety. Irinotecan has the tertiary amine inside the molecule, and the equilibrium reaction step of this compound is shown in Figure 3.

When elemental fluorine is reacted with uracil, 5-FU is produced. Substitution at the 5-position of uracil can substantially alter the electronic properties of the pyrimidine group by changes in the UV spectra and ionization constants.[22–24] In aqueous solution this can induce significant changes in the physical and biological properties of the pyrimidine. Absorption spectra of 5-FU and a typical acid−base titration curve obtained from absorbance measurements at 265 nm in water at (25 °C) are shown in Figure 4.

Previously,[25] it was demonstrated that electron-withdrawing substituents in the five position of uracil reduces measured pK_a values, while electron-donating substituents have the opposite effect. The ionization of 5-FU could occur at either the N1 or N3 sites, but only the lower value can be observed experimentally. The pK_a values were smaller than those generally observed with uracil in water (e.g., uracil in water has a pK_a = 9.50 and 5-FU has a pK_a = 7.98, respectively).[26] This increase in acidity can be attributed to the electron-withdrawing fluoro group in the five position.

It is known that one of the most important factors determining dissociation constants is the reaction medium and ionic strength. The variation of the pK values of studied compounds versus the mole fraction of MeCN, X_{MeCN}, in the MeCN−water mixtures is presented in Figure 5. The equations between pK_1 and pK_2 values and mole fraction of organic modifier are shown

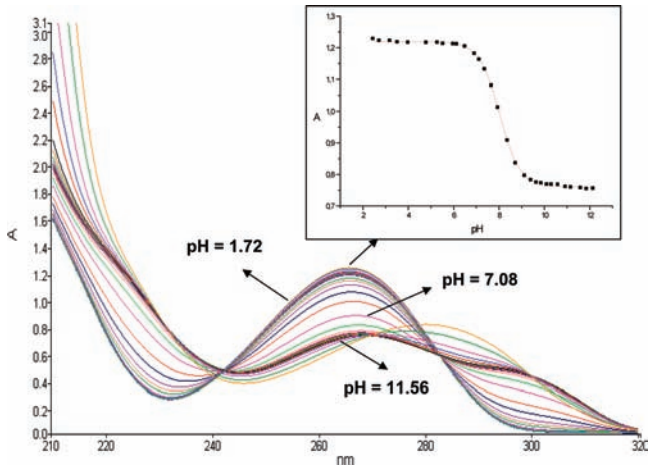


Figure 4. Absorption spectra of $1.6 \cdot 10^{-5}$ mol·L^{-1} 5-FU and a typical acid−base titration curve obtained from absorbance measurements at 265 nm in water at 25 °C.

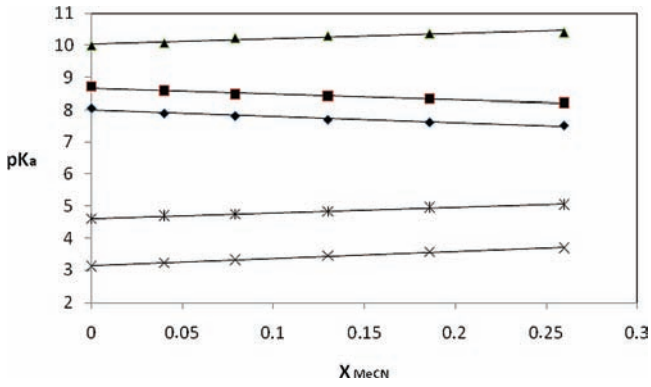


Figure 5. pK_a values versus mole fraction of acetonitrile, X_{MeCN}, in acetonitrile−water mixtures. ×, pK_{a1} values of folinic acid; ∗, pK_{a2} values of folinic acid; ◆, 5-FU; ■, irinotecan; ▲, pK_{a3} values of folinic acid.

Table 2. Equations between pK_a Values of Studied Compounds and Mole Fraction of Organic Modifier[a]

compounds	equations[b]	regression coefficient
folinic acid (leucovorin)	pK_{a1} = 3.14(0.01) + 2.24(0.09)x	0.997
	pK_{a2} = 4.62(0.01) + 1.73(0.09)x	0.995
	pK_{a3} = 10.05(0.04) + 1.60(0.27)x	0.949
5-fluorouracil (5-FU)	pK_a = 7.99(0.03) − 2.00(0.20)x	0.980
irinotecan (camptosar)	pK_a = 8.67(0.03) − 1.80(0.17)x	0.982

[a] Error associated with the values is given in parentheses. [b] x represents the mole fraction of the organic modifier (MeCN).

in Table 2. There is actually a linear relationship between the pK_a values of the studied compounds and X_{MeCN}, in the binary mixtures. We have already observed the same trend for various organic molecules in different solvent mixtures.[27–29] It has been reasonably assumed that preferential solvation of the charged particles by water is mainly responsible for such a monotonic dependence of the acidity constants of studied compounds on the solvent composition.

The variation of the pK values with the mole fraction of MeCN is different for each substance, although, in general, pK_1 and pK_2 values in line with the two carboxylic groups in the glutamic acid moiety and the phenolic −OH in the pteridine ring moiety increase with the mole fraction of MeCN, whereas pK_a values belonging to the protonation of the tertiary amine inside the molecule for irinotecan and the dissociation of pyrimidine ring for 5-FU decrease. The different ways in which pK values change might be explained by the fact that the dissociation process is ruled by electrostatic interactions as well as by specific solute−solvent interactions. It has been found that in several water−organic binary solvent mixtures pK_a values of a given substance show a linear relationship with the mole fraction of the organic solvent.[27] This is indicated by the following expression:

$$pK_{a,\varphi} = pK_{a,w} + \varphi \Delta pK_a \quad (3)$$

where p$K_{a,w}$ indicates the dissociation constant in water, φ the mole fraction of the organic solvent, ΔpK the slope of the linear relationship, and p$K_{a,\varphi}$ the pK_a at the corresponding composition.

As discussed above, the data shown in Table 1 clearly illustrate the important influence of the nature of the solvent on the dissociation reaction. It is obvious that the nature of the solvent plays a fundamental role in acid−base equilibria. It is well-known that the energy required for the separation of charges in acid dissociation, which is inversely proportional to the dielectric constant of the medium, is compensated by the solvation of the resulting ions.[30] It has been shown that the solvating ability[31] (as expressed by the Gutmann donicity scale, DN) and dielectric constant of the solvent play a fundamental role in dissociation reactions. Water is a solvent of high solvating ability (i.e., DN = 33.0 and dielectric constant, ε = 78), which can dissociate the acid and stabilize the produced anion and hydrogen ion. Thus, it is expected that the addition of MeCN with a lower donor number and dielectric constant (DN = 14.0, ε = 36.0) to water decreases the extent of interaction of the acid anion and the proton with the solvent, and this decreases the acidity constant of the acid.

Conclusion

The pK_a values of drugs used for FOLFIRI chemotherapy regimens for the treatment of colorectal cancer have been determined precisely with spectrophotometric titrations in water and a wide range of water−organic solvent binary mixtures. pK_a is a key parameter, especially for understanding and quantifying the reaction rates, biological activity, biological uptake, biological transport, and environmental fate as well as knowledge of pK_a values as a function of solvent composition is also useful in the application of reversed-phase LC and capillary electrophoresis for the separation of these ionizable compounds.

Acknowledgment

The authors greatly acknowledge Dr. Jose L. Beltran from Universitat de Barcelona for kindly providing the spectral data processing software, STAR.

Literature Cited

(1) Zhao, Y. H.; Yuan, X.; Yang, L. H.; Wang, L. S. Quantitative Structure-Activity Relationships of Organic Acids and Bases. *Bull. Environ. Contam. Toxicol.* **1996**, *57*, 242–249.
(2) Ghasemi, J.; Ahmadi, S.; Kubista, M.; Forootan, A. Determination of Acidity Constants of 4-(2-Pyridylazo)resorcinol in Binary Acetonitrile + Water Mixtures. *J. Chem. Eng. Data* **2003**, *48*, 1178–1182.
(3) Polster, J.; Lachmann, H. *Spectrometric Titrations: Analysis of Chemical Equilibria*; VCH: Weinheim, 1989.
(4) Meloun, M.; Havel, J.; Högfeldt, E. *Computation of Solution Equilibria: A Guide to Methods in Potentiometry, Extraction and Spectrophotometry*; Ellis Horwood Limited: Chichester, 1988.
(5) Hilal, S. H.; El-Shabrawy, Y.; Carreira, L. A.; Karickhoff, S. W.; Toubar, S. S.; Rizk, M. Estimation of the Ionization pK_a of Pharmaceutical Substances Using the Computer Program Sparc. *Talanta* **1996**, *43*, 607–619.

(6) Citores, M. J.; Alonso, R. M.; Fernandez, L. A. Applications of Computers in the Study of Solution Equilibria V. APH, a Program for the Graphical Evaluation of pK_a Values From Spectrophotometric Data. *TrAC, Trends Anal. Chem.* **1992**, *11*, 310–314.
(7) Paabo, M.; Bates, R. G.; Robinson, R. A. Dissociation of Ammonium Ion in Methanol-Water Solvents. *J. Phys. Chem.* **1966**, *70*, 247–251.
(8) Subirats, X.; Roses, M.; Bosch, E. On the Effect of Organic Solvent Composition on the pH of Buffered HPLC Mobile Phases and the pK_a of Analytes - A Review. *Sep. Purif. Rev.* **2007**, *36*, 231–255.
(9) Janos, P. Determination of Equilibrium Constants from Chromatographic and Electrophoretic Measurements. *J. Chromatogr., A* **2004**, *1037*, 15–28.
(10) Jimenez-Lozano, E.; Marques, I.; Barron, D.; Beltran, J. L.; Barbosa, J. Determination of pK_a Values of Quinolones from Mobility and Spectroscopic Data Obtained by Capillary Electrophoresis and a Diode Array Detector. *Anal. Chim. Acta* **2002**, *464*, 37–45.
(11) Barron, D.; Barbosa, J. Acid-Base Behaviour of Substituted Phenolic Substances and Resolution of Acid Strength in Tetrahydrofuran. *Anal. Chim. Acta* **2000**, *403*, 339–347.
(12) Barbosa, J.; Berges, R.; Toro, I.; Sanz-Nebot, V. Protonation Equilibria of Quinolone Antibacterials in Acetonitrile-Water Mobile Phases Used in LC. *Talanta* **1997**, *44*, 1271–1283.
(13) Kamlet, M. J.; Taft, R. W. The Solvatochromic Comparison Method. I. The Beta-Scale of Solvent Hydrogen-Bond Acceptor (HBA) Basicities. *J. Am. Chem. Soc.* **1976**, *98*, 377–383.
(14) Kamlet, M. J.; Abboud, J. L.; Taft, R. W. The Solvatochromic Comparison Method. The π^* Scale of Solvent Polarities. *J. Am. Chem. Soc.* **1977**, *99*, 6027–6038.
(15) Cancer Knowledgebase (2009) World Health Organization. http://www.who.int/mediacentre/factsheets/fs297/en/index.html (accessed Apr 30, 2009).
(16) Avdeef, A. *Absorption and Drug development: Solubility, Permeability, and Charge State*; John Wiley & Sons: Hoboken, NJ, 2003.
(17) Jang, Y. H.; Sowers, L. C.; Cagin, T.; Goddard, W. A. First Principles Calculation of pK_a Values for 5-Substituted Uracils. *J. Phys. Chem. A* **2001**, *105*, 274–280.
(18) Anilanmert, B.; Ozdemir, F. A.; Erdinc, N.; Pekin, M. Potentiometric Determination of the Dissociation Constants of Epirubicin HCl and Irinotecan HCl. *Mendeleev Commun.* **2006**, *16*, 97–98.
(19) Gran, G. Determination of the Equivalence Point in Potentiometric Titrations. Part II. *Analyst* **1952**, *7*, 661–671.
(20) Marcus, Y. Preferential Solvation. Part 3. Binary Solvent Mixtures. *J. Chem. Soc., Faraday Trans.* **1989**, *85*, 381–388.
(21) Beltran, J. L.; Codony, R.; Prat, M. D. Evaluation of Stability Constants from Multi-Wavelength Absorbance Data: Program STAR. *Anal. Chim. Acta* **1993**, *276*, 441–454.
(22) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1983.
(23) Sowers, L. C.; Shaw, B. R.; Veigl, M. L.; Sedwick, W. D. DNA Base Modification: Ionised Base Pairs and Mutagenesis. *Mutat. Res.* **1987**, *177*, 201–218.
(24) Yu, H.; Eritja, R.; Bloom, L. B.; Goodman, M. F. J. Ionization of Bromouracil and Fluorouracil Stimulates Base Mispairing Frequencies with Guanine. *Biol. Chem.* **1993**, *268*, 15935–15943.
(25) Eric, J.; Sowers, P. C. A Proposed Mechanism for the Mutagenicity of 5-Formyluracil. *Mutat. Res.* **1996**, *354* (2), 151–156.
(26) Niedzwicki, J. G.; Niedzwicki, M. H.; El Kouni, S. H.; Cha, S. Structure-Activity Relationship of Ligands of the Pyrimidine Nucleoside Phosphorylases. *Biochem. Pharmacol.* **1983**, *32*, 399–415.
(27) Erdemgil, F. Z.; Sanli, S.; Sanli, N.; Ozkan, G.; Barbosa, J.; Guiteras, J.; Beltran, J. L. Determination of pK_a Values of Some Hydroxylated Benzoic Acids in Methanol-Water Binary Mixtures by LC Methodology and Potentiometry. *Talanta* **2007**, *72*, 489–496.
(28) Sanli, S.; Altun, Y.; Sanli, N.; Alsancak, G.; Beltran, J. L. Solvent Effects on pK_a values of Some Substituted Sulfonamides in Acetonitrile-Water Binary Mixtures by the UV-Spectroscopy Method. *J. Chem. Eng. Data* **2009**, *54*, 3014–3021.
(29) Aktaş, H.; Şanli, N.; Pekcan, G. Spectrometric Determination of pK_a Values for Some Phenolic Compounds in Acetonitrile-Water Mixtures. *Acta Chim. Slov.* **2006**, *53*, 214–218.
(30) Bates, R. G. *Determination of pH, Theory and Practice*; Wiley: New York, 1973.
(31) Gutmann, V. *The Donor-Acceptor Approach to Molecular Interactions*; Plenum Press: New York, 1978.

Received for review November 7, 2009. Accepted April 3, 2010. The authors are thankful for the financial aid received from The Scientific and Technological Research Council of Turkiye (TUBITAK), Project No. 108T637.

JE100072N