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Chromatographic Determination of pK_a Values of Some Water-Insoluble Arylpropionic Acids and Arylacetic Acids in Acetonitrile + Water Media

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ABSTRACT: The pK_a values of some arylpropionic and arylacetic acids were determined in acetonitrile + water mixed solvents at 298.15 K. The retention factors of the conjugate acid base species are obtained by fitting the retention factors and pH data by nonlinear regression. The suggested model uses the pH value measured in the hydro-organic mixture used as mobile phase instead of the pH value in water and takes into account the effect of the activity coefficients. The effect of the acetonitrile concentration on the ionization constant was also studied by estimating the pK_a at different acetonitrile volume fraction, ranging from 0.40 to 0.55. The pK_a values were correlated with the Kamlet and Taft solvatochromic parameters of acetonitrile + water mixtures to estimate aqueous pK_a.

INTRODUCTION

The development of new methods capable of sensitive and precise measuring drug active compounds is important. The applicability of liquid chromatography (LC) in the pharmaceutical industry for analysis of drugs has became the mainstay of pharmaceutical research development and of independent research laboratories. Nonsteroidal anti-inflammatory drugs have become one of the most widely used groups of drugs in pharmaceuticals. Nonsteroidal anti-inflammatory drugs, usually abbreviated to NSAIDs, are pharmaceutically active compounds with their analgesic, antipyretic, and anti-inflammatory effects. They are most commonly used for treating low-back pain. NSAIDs are commonly prescribed medications for the inflammation of arthritis and the inflammation of other tissues, such as in tendinitis and bursitis. NSAIDs work to block the effect of an enzyme called cyclooxygenase. This enzyme is critical in the human body's production of prostaglandins. Therefore, by interfering with cyclooxygenase, the human body decreases the production of prostaglandins and decrease pain and swelling associated with these conditions.[1−3] NSAIDs can be broadly classified based on their chemical structure. Most widely used groups of these compounds are propionic acid and acetic acid derivatives. Ibuprofen, naproxen, fenoprofen, ketoprofen, and flurbiprofen are the members of the propionic acid derivative group. Diclofenic acid, etodolac, indomethacin, and sulindac are the members of the acetic acid derivative group.[1−3] The fibrates are a class of lipid-modifying agents that decrease plasma triglycerides; they activate the peroxisome proliferator-activated receptors, belonging to the nuclear hormone receptor super family, inducing alterations in transcription of genes encoding for proteins that control lipoprotein metabolism. Clofibric acid and bezafibric acid are the first members of this group. Clofibric acid and bezafibrate, hypolipidemic agents, are known to induce a number of biochemical changes in the liver. The lipid-modifying profiles of clofibric acid and bezafibrate are characterized by marked decreases in elevated triglyceride levels, increases in high density lipoprotein cholesterol levels, and decreases in total and low density lipoprotein cholesterol levels. They also reduce elevated levels of lipoprotein (a) and fibrinogen, which are independent cardiovascular risk factors.[1−3]

It has long been recognized that the acid−base chemistry of a drug substance plays a pivotal role in the development of a new drug. The acid ionization constant is one of the most important characteristics of a pharmaceutically active compound. Drug compounds and the determination of useful dosage forms and regimes for drugs depend upon an understanding of drug dissociation and the extent of dissociation that will occur in the systems of the body. Also the lipophilicity, solubility, and permeability of drug compounds are pK_a dependent. Knowledge of acid−base dissociation constants of the analytes in hydroorganic mixtures help to improve separation methods and lead to a better understanding of their retention behavior.

The basic theory of the LC method has been worked out in the 1970s and 1990s.[4−6] A methodological approach of choice is pK_a estimation by isocratic reversed-phase high-performance liquid chromatography (HPLC).[7−12] At a fixed mobile phase composition, acidic compound presents equilibrium between its acidic (HA) and its basic (A^-) forms related by the dissociation constant. The observed capacity factor (k) is an average of the capacity factors of the acid (k_{HA}) and basic forms (k_{A^-}).[13−15]

A widely employed measure of solvent polarity is Dimroth and Reichardt's ET(30).[16,17] This polarity index is often used in the normalized dimensionless form, E_T^N. The relationship between log k and E_T^N has been illustrated experimentally and theoretically.[18] Taking into account the variation of the retention

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factors of acidic compound with E_T^N of the mobile phase, we can write the following equations, which indicate the dependence of the k of the neutral and anionic species with E_T^N.

$$\log k_{HA} = C_{HA} + e_{HA}E_T^N \quad (1)$$

$$\log k_{A^-} = C_{A^-} + e_{A^-}E_T^N \quad (2)$$

The theoretical expression describing the dependence of the k for acidic solutes as a combined function of pH and E_T^N may be expressed as follows:

$$k = \frac{10^{(C_{HA} + e_{HA}E_T^N)} + 10^{(C_{A^-} + e_{A^-}E_T^N)}(K_a/a_{H_m^+}\gamma_{A_m^-})}{1 + (K_a/a_{H_m^+}\gamma_{A_m^-})} \quad (3)$$

where K_a is defined as the dissociation constant of monoprotic acid and HA and A^- represent undissociated acid and fully dissociated acid. Evaluation of the change in k of the acidic analyte versus change in pH of the mobile phase will give an indirect measure of the pK_a. It has been also demonstrated that RP-HPLC can be used to determine the limiting capacity factors. It is known that these factors are very useful to explain the differences between the affinity of the ionized and neutral form to the stationary phase. It has been also shown that the capacity factor of the neutral form of the compounds increases linearly with the partition coefficient (log P_{oct}). From this relation, log P_{oct} of the compounds can be determined easily. Equation 3 also permits the statistical evaluation of the chromatographic method for determination of the pK_a values and limiting capacity factors.[19]

Very often, the main difficulty in the determination of pK_a of drug candidates is their aqueous insolubility that forces the use of water−organic modifier binary mixtures. In recent years the use of HPLC has become the method of choice for the determination of pK_a values of this type of compounds. The determination of pK_a values by LC is based on the relationships between the retention factors and the pH of the mobile phase.[15,18−22] The relationship between analyte retention and mobile phase pH is described by a sigmoidal function of the pK_a value and the limiting retentions of the acid−base forms of the analyte. In this study, pK_a values of water-insoluble arylpropanoic acids and arylacetic acids in an acetonitrile + water binary mixture were determined by using the variation of capacity factors of these drug candidates as a function of the mobile phase pH. Although most of reversed phase liquid chromatography separation procedures are based on water + acetonitrile mobile phase, there are only few systematic reports about pK_a values of flurbiprofen, ibuprofen, diclofenic acid, ketoprofen, fenoprofen, naproxen, indomethacin, bezafibric acid, and clofibric acid in water + acetonitrile binary mixtures and in water−methanol binary mixtures.[18,22,23] To overcome the lack of information related with the acid−base equilibrium of these compounds in water−acetonitrile medium, the pK_a, k_{HA}, and k_{A^-}values of these compounds have been determined by using the variation of the retention factors of these drug candidates as a function of the mobile phase pH in the cosolvent solutions of varying ratios of acetonitrile + water. pH measurements in these medium can be preferred in a manner similar to that in water, taking into account the pH values previously assigned to primary standard buffer solutions in these media. The pK_a values of these compounds, as well as the retention factors of the conjugate acid−base species, are calculated by using through correct pH and activity coefficients of the species in acetonitrile + water. Multiple regression analysis was applied to the pK_a values of these compounds to find the best form of the Kamlet and Taft equation to describe the variation of pK_a values with the Kamlet−Taft solvatochromic parameters for acetonitrile + water mixtures. The aqueous pK_a values (${}^{w}_{w}pK_a$) were calculated using these equations. The aqueous pK_a values of these compounds have been also calculated from pK values determined in several acetonitrile + water mixtures (${}^{s}_{s}pK_a$) by means of the Yasuda−Shedlovsky equation[24,25] and linear relationship between the mole fraction of acetonitrile and the ${}^{s}_{s}pK_a$ values.

EXPERIMENTAL SECTION

Chemicals and Reagents. Analytical reagent grade chemicals were used, unless otherwise indicated. The compounds used in this study were kindly supplied as follows: fenoprofen calcium (2-(3-phenoxyphenyl)propanoic acid, CAS: 53746-45-5), ibuprofen (2-(4-(2-methylpropyl)phenyl)propanoic acid, CAS: 15687-27-1), flurbiprofen (2-(2-fluorobiphenyl-4-yl)propanoic acid, CAS: 5104-49-4), and diclofenac sodium (2-[2-[(2,6-dichlorophenyl)amino]phenyl]acetic acid, CAS: 15307-86-5) were purchased from Sigma (Steinheim, Germany). Naproxen (2-(6-methoxynaphthalen-2-yl)propanoic acid, CAS: 22204-53-1) and clofibric acid (2-(4-Chlorophenoxy)-2-methylpropanoic acid, CAS: 882-09-7) were purchased from Aldrich (Steinheim, Germany). Ketoprofen (2-(3-benzoylphenyl)propanoic acid, CAS: 22071-15-4) was bought from Sigma-Aldrich (Steinheim, Germany). Bezafibrate (2-(4-{2-[(4-chlorobenzoyl)amino]ethyl}phenoxy)-2-methylpropanoic acid, CAS: 41859-67-0) was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Indomethacin (2-{1-[(4-chlorophenyl)carbonyl]-5-methoxy-2-methyl-1*H*-indol-3-yl}acetic acid, CAS: 53-86-1) was bought from Fluka (Buchs, Switzerland). Acetonitrile (ACN, HPLC grade, Sigma-Aldrich, Steinheim, Germany) was used as an organic modifier. Orthophosphoric acid (Riedel-de Haen Germany; min. 85 %) and sodium hydroxide (Merck, Darmstadt, Germany) were used for the pH adjustment of the mobile phase.

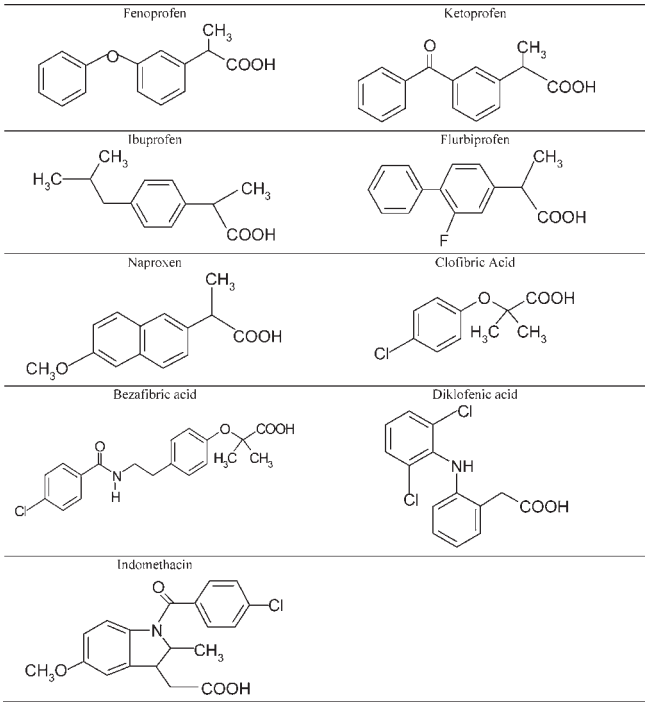
Apparatus. HPLC analyses were performed using a Shimadzu chromatographic system, consisting of a Shimadzu HPLC system (Shimadzu Technologies, Kyoto, Japan), a pump (LC-10AD VP), a UV−visible detector (SPD-10AV VP), column oven (CTO-10AC VP), and a degasser system (DGU-14A). A Gemini C_6 phenyl analytical column (150 mm × 3.0 mm inner diameter (i.d.), 5 μm) provided by Phenomenex (USA) was used for all of the determinations at 25 °C . Results were acquired and processed with the Shimadzu LC Solution data system software (Shimadzu Technologies, Kyoto, Japan).

pH measurements of the mobile phase were carried out with a Mettler Toledo MA 235 pH/ion analyzer (GmbH; Schwerzenbach, Switzerland) using M-T combination pH electrode. Potassium hydrogen phthalate was used as primary standard buffer reference solution for the standardization of this apparatus in acetonitrile + water binary mixtures in accordance with the International Union of Pure and Applied Chemistry (IUPAC) rules.[26−28] The activity coefficients, γ, were calculated using the Debye−Huckel expression taking into account the ionic strength, I, of the mobile phases.[29]

Procedure. Throughout this study, the mobile phases assayed were acetonitrile + water at 0.40, 0.45, 0.50, and 0.55 volume fraction, to determine the chromatographic pK_a values. The pH of the mobile phase containing 25.10^{-3} mol·L^{-1} phosphoric acid was adjusted between 3.5 and 7.5 by the addition of sodium

hydroxide. A 20 μL injection of every compound was made in triplicate into the chromatographic system equilibrated with mobile phases at the defined pH value. The dead time (t_o) was measured by injecting uracil solution (Sigma, USA, 0.1 %, in water). The flow rate of the mobile phase was kept constant at 1 mL·min^{-1}. In this study different wavelengths were used for each compounds (for ibuprofen and naproxen 220 nm, for ketoprofen and flurbiprofen 260 nm, for diclofenic acid and fenoprofen 270 nm, for clofibric acid and bezafibric acid 225 nm, for indomethacin 280 nm).

Table 1. Structure of Arylpropionic Acids and Arylacetic Acids



Preparation of Standard Solutions. Stock solutions (10 μg·mL^{-1}; 100 μg·mL^{-1}) of each compound were prepared in the mobile phase. All of the subsequent dilutions for working standards were made with the mobile phase.

RESULTS AND DISCUSSION

In this study, the dependence of retention of arylpropionic and arylacetic acids (Table 1) on pH of the mobile phase was investigated by using LC methodology, and the experimental results were applied for the determination of pK_a values and intrinsic capacity factors. The experimental region was selected in such a way that the k of arylpropionic acids and arylacetic acids would stay within the limits $1 < k < 10$ except indomethacin. The pK_a values associated with the carboxylic acid function were determined from k/pH data pairs by means of the NLREG program.[30] pK_a, k_{HA}, and k_{A^-} values for these compounds were summarized in Table 2. In compliance with IUPAC rules,[26,27] the activity coefficients of the species in acetonitrile + water mixtures have been calculated from the ionic strength through the Debye−Hückel equation.[29] All of the pK_a values calculated for the compound studied are closer to those of benzoic acids than to the ones of the aliphatic carboxylic acids. This points out that the carboxylic group is sensitive to the effect of the aromatic part of the molecule.

The correlation between the theoretical and experimental retention factors of the drug candidates over the pH range investigated was good, as shown in Figure 1. The example of

Table 2. pK_a Values and the Retention Factors of These Compounds Calculated by Using Activity Coefficients in Acetonitrile + Water Mixtures

	0.40 volume fraction of ACN			0.45 volume fraction of ACN			0.50 volume fraction of ACN			0.55 volume fraction of ACN		
	pK_a	k_{HA}	k_{A^-}	pK_a	k_{HA}	k_{A^-}	pK_a	k_{HA}	k_{A^-}	pK_a	k_{HA}	k_{A^-}
ketoprofen	5.494	4.817	0.210	5.646	3.280	0.202	5.782	2.081	0.169	5.984	1.578	0.098
	(0.053)*	(0.017)	(0.010)	(0.032)	(0.040)	(0.012)	(0.037)	(0.026)	(0.011)	(0.056)	(0.026)	(0.008)
naproxen	5.801	5.389	0.500	6.043	3.253	0.333	6.274	2.278	0.260	6.536	1.626	0.151
	(0.057)	(0.099)	(0.023)	(0.034)	(0.029)	(0.016)	(0.094)	(0.049)	(0.017)	(0.047)	(0.015)	(0.011)
fenoprofen	5.854	9.952	0.624	5.992	5.848	0.494	6.144	3.603	0.451	6.260	2.901	0.375
	(0.040)	(0.127)	(0.026)	(0.022)	(0.035)	(0.023)	(0.038)	(0.033)	(0.018)	(0.087)	(0.057)	(0.013)
flurbiprofen	5.495	12.216	0.997	5.822	6.635	0.763	6.116	4.026	0.627	6.421	2.472	0.415
	(0.052)	(0.256)	(0.041)	(0.029)	(0.059)	(0.055)	(0.059)	(0.057)	(0.027)	(0.068)	(0.033)	(0.027)
diclofenic acid	5.445	15.343	1.270	5.620	8.429	1.015	5.782	5.078	0.809	5.963	3.553	0.551
	(0.041)	(0.262)	(0.036)	(0.039)	(0.117)	(0.022)	(0.041)	(0.063)	(0.036)	(0.023)	(0.022)	(0.011)
ibuprofen	6.010	15.852	0.945	6.149	9.108	0.561	6.329	5.532	0.405	6.549	3.899	0.278
	(0.035)	(0.159)	(0.042)	(0.039)	(0.093)	(0.016)	(0.058)	(0.073)	(0.015)	(0.051)	(0.039)	(0.008)
indomethacin	5.290	20.712	2.012	5.428	8.659	0.910	5.612	5.378	0.638	5.814	3.801	0.523
	(0.042)	(0.394)	(0.012)	(0.069)	(0.244)	(0.016)	(0.062)	(0.119)	(0.023)	(0.053)	(0.061)	(0.006)
clofibric acid	4.622	5.386	0.302	4.748	3.631	0.283	4.844	2.424	0.204	4.931	1.760	0.176
	(0.067)	(0.225)	(0.015)	(0.033)	(0.071)	(0.004)	(0.046)	(0.063)	(0.004)	(0.032)	(0.030)	(0.011)
bezafibric acid	4.789	5.813	0.719	4.916	3.817	0.439	5.090	2.235	0.336	5.267	1.495	0.215
	(0.054)	(0.172)	(0.027)	(0.031)	(0.063)	(0.016)	(0.048)	(0.051)	(0.014)	(0.040)	(0.026)	(0.010)

* The values between parentheses are the standard deviation.

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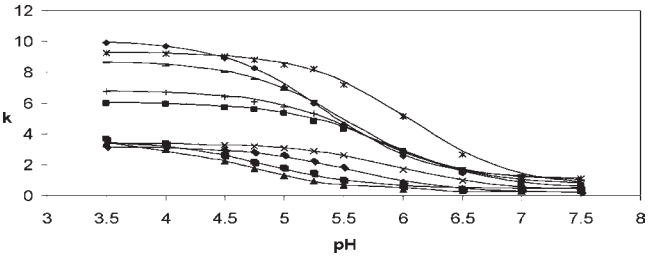

Figure 1. Plots of k values of arylpropionic acids and arylacetic acids vs the pH of the mobile phase for 0.45 volume fraction of acetonitrile (activity coefficient was used in these calculations): ■, fenoprofen; ×, naproxen; ▲, clofibric acid; ♦, indomethacin; +, flurbiprofen; black rectangle, ketoprofen; −, diclofenic acid; ∗, ibuprofen; ▰, bezafibric acid. The theoretical results calculated using eq 3 are indicated as continuous lines, and the plotted points are experimental results.

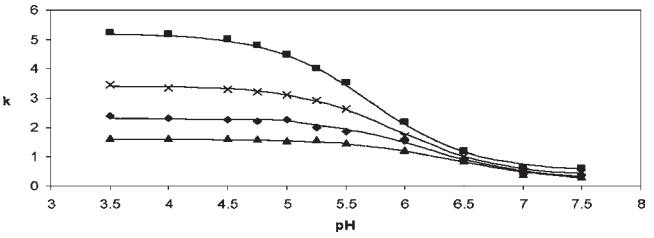

Figure 2. Plots of the retention factors of naproxen vs the pH of the mobile phase for 0.40, 0.45, 0.50, and 0.55 volume fraction of acetonitrile (activity coefficient was used in these calculations): ■, 0.40; ×, 0.45; ♦, 0.50; ▲, 0.55.

Table 3. Results of the Application of the Retention Model Proposed to All Experimental Retention Data Available for Each Solute Considered (Coefficients Describe Chromatographic Retention in Terms of eq 3; the Activity Coefficient Was Used in These Calculations)

compounds	C_{HA}	e_{HA}	C_{A^-}	e_{A^-}	SSQ[a]	RRMSD[b]
ketoprofen	−10.04	12.824	−7.471	8.187	0.386	4.386
naproxen	−10.435	13.338	−11.075	12.898	0.132	2.139
ibuprofen	−12.096	15.887	−11.316	13.488	3.277	3.670
fenoprofen	−10.853	14.148	−4.779	5.462	3.157	5.822
flurbiprofen	−13.841	17.845	−7.917	9.482	1.622	3.810
diclofenic acid	−12.654	16.532	−7.540	9.162	3.594	4.614
indomethacin	−14.492	18.844	−12.246	14.936	7.523	6.093
clofibric acid	−9.864	12.671	−5.961	6.533	0.406	5.809
bezafibric acid	−12.200	15.518	−11.109	13.105	0.331	4.415

[a] SSQ, sums of squares of the residuals = $\Sigma(k_{obs} - k_{pred})^2$. [b] RRMSD, relative root mean squared differences $=100(\Sigma(k_{obs} - k_{pred})^2)^{1/2}/\Sigma(k_{obs})^2$.

dependence of the retention behaviors on acetonitrile volume fraction of mobile phase (0.40, 0.45, 0.50, 0.55) is given for naproxen in Figure 2. It can be concluded that shifts of sigmoidal curves of k versus pH of the mobile phase are related to the influence of an organic modifier on the dissociation behavior of the weak acidic solutes.

The chromatographic data were evaluated statistically to indicate the quality of the description of these retention data. In this way, a complete description of the retention behavior of each solute in the space defined by the pH of the mobile phase and E_T^N was modeled. The coefficients found to describe the chromatographic behavior of these analytes using eq 3 are listed in Table 3. The sums of squares of the residuals (SSQ) and the relative root-mean-squared differences (RRMSD) are shown in this table. In all cases the SSQ and the RRMSD are calculated for the 44 experimental data points. The results shown in Table 3 demonstrate the adequate performance of the liquid chromatographic method for determination of pK_a and k_{HA} and k_{A^-} values of the compounds studied.

Table 4. Relationships between pK_a for Some Carboxylic Acids and α, β, and π^* Solvatochromic Parameters of Acetonitrile + Water Mixtures in the Interval Studied

compounds	multiparametric equation	r
ketoprofen	$pK_a = 13.435 - 8.030\pi^*$	0.998
naproxen	$pK_a = 17.856 - 12.180\pi^*$	0.999
fenoprofen	$pK_a = 12.639 - 6.850\pi^*$	0.999
flurbiprofen	$pK_a = 20.709 - 15.360\pi^*$	0.999
diclofenic acid	$pK_a = 13.939 - 8.580\pi^*$	0.999
ibuprofen	$pK_a = 14.885 - 8.985\pi^*$	0.997
indomethacin	$pK_a = 13.965 - 8.780\pi^*$	0.998
clofibric acid	$pK_a = 9.697 - 5.115\pi^*$	0.998
bezafibric acid	$pK_a = 12.734 - 8.040\pi^*$	0.998

Table 5. Linear Equations between Experimental pK_a Values and the Mole Fraction of Acetonitrile

compounds	equation	r
ketoprofen	$pK_a = 4.548(0.042)X + 4.670(0.178)$[a]	0.998
naproxen	$pK_a = 6.883(0.150)X + 4.564(0.035)$	0.999
fenoprofen	$pK_a = 3.861(0.244)X + 5.165(0.058)$	0.996
flurbiprofen	$pK_a = 8.667(0.382)X + 3.950(0.090)$	0.999
diclofenic acid	$pK_a = 4.846(0.140)X + 4.576(0.033)$	0.999
ibuprofen	$pK_a = 5.096(0.201)X + 5.075(0.047)$	0.997
indomethacin	$pK_a = 4.975(0.142)X + 4.380(0.033)$	0.998
clofibric acid	$pK_a = 2.877(0.269)X + 4.118(0.063)$	0.991
bezafibric acid	$pK_a = 4.554(0.122)X + 3.957(0.029)$	0.999

[a] The values between parentheses are the standard deviations.

The linear solvation energy relationship (LSER) methodology was used to correlate pK_a values with solvent dipolarity/polarizability (π^*), solvent hydrogen bond donating acidity (α), and solvent hydrogen-bond accepting basicity (β).[31] An appropriate equation (eq 4) was applied to correlate pK_a values in acetonitrile + water mixture with solvatochromic parameters.

$$pK_a = (pK_a)_0 + s\pi^* + a\alpha + b\beta \quad (4)$$

The coefficients of π^* in the Kamlet and Taft equations summarized in Table 4 are negative in all instances, which means that an increase in the polarity of the cosolvent mixture decreases the pK_a values. This agrees with the fact that acetonitrile is a poor solvate solvent with a low hydrogen-bond donor and acceptor capability. These equations confirm the main dependence of the pK_a values of these compounds on the solvent dipolarity/polarizability ($r > 0.99$). The dissociation process in acetonitrile + water is governed by electrostatic interactions as well as specific solute−solvent interactions. In the dissociation of the carboxylic acid group, charges are created, and the dissociation process is disturbed when the dielectric constant of the mixed solvents decreases with the increase in acetonitrile content. The electrostatic interaction overwhelms solvation effects, and this fact is

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Table 6. Aqueous pK_a Values ${}^{w}_{w}pK$ of Arylpropionic and Arylacetic Acids Obtained from Different Approaches

compounds	literature ${}^{w}_{w}pK_a$							this study		
	a	*b*	*c*	*d*	*e*	*f*	*g*	Yasuda−Shed.	p$K_a - X$	p$K_a - \pi^*$
ketoprofen	4.35	4.09	4.36	4.32	3.98	3.95	-	4.638	4.670	4.281
naproxen	4.50	4.26	4.57	4.76	4.18	-	4.39	4.515	4.564	3.971
fenoprofen	4.18	-	-	-	-	-	-	5.137	5.165	4.830
flurbiprofen	4.24	4.13	4.35	4.36	4.03	4.45	4.24	3.887	3.950	3.199
diclofenic acid	4.12	3.97	4.16	4.17	3.99	4.33	4.05	4.542	4.576	4.158
ibuprofen	4.53	4.30	4.52	4.64	4.45	4.32	4.40	5.041	5.075	4.642
indomethacin	4.66	-	-	-	4.42	-	-	4.346	4.380	3.956
clofibric acid	3.61	-	-	-	-	-	-	4.097	4.118	3.866
bezafibric acid	3.73	-	-	-	-	-	-	3.926	3.957	3.568

[a] SPARC program.[32] [b] Pure tert-butyl alcohol[33] (potentiometric method). [c] Pure isopropyl alcohol[33] (potentiometric method). [d] From ${}^{s}_{s}pK$ values in different isopropyl alcohol + water mixtures[34] (potentiometric method). [e] From ref 35. [f] From ref 36 (I = 0.117), (spectrophotometric method). [g] From ref 37 (I = 0) (potentiometric method).

reflected in the greater magnitude of the π^* coefficients in the Kamlet and Taft equations for the dissociation of carboxylic acid group.

It is known that an aqueous dissociation constant is an unavoidable requirement in routine drug development. There are different approaches to estimate aqueous pK_a from the drug's pK_a in the hydroorganic mixtures. To evaluate the ${}^{w}_{w}pK_a$ of arylpropionic and arylacetic acids, ${}^{s}_{s}pK_a$ values were plotted against to acetonitrile mole fraction (Table 5). The intercepts of these linear equations are the aqueous pK_a values of these compounds (Table 6).

The most important equation is Yasuda−Shedlovsky to estimate ${}^{w}_{w}pK_a$ from ${}^{s}_{s}pK_a$.[24,25]

$$pK_a + \log[H_2O] = a_\varepsilon \varepsilon^{-1} + b_\varepsilon \qquad (5)$$

The experimental ${}^{s}_{s}pK_a$ values obtained from the various acetonitrile percentages were used in eq 5 for calculation of the aqueous dissociation constants of the compounds studied (Table 6).

Table 6 gives the pK_a values reported in the literature, together with those predicted by the program SPARC.[32] As shown in Table 6, aqueous pK_a values estimated by equations (in Table 4) for these compounds are good agreement with those obtained using different approaches.[33−37] It is known that the Yasuda−Shedlovsky extrapolation treatment was found to be useful, particularly in the water-rich region, to predict aqueous pK_a values from the ${}^{s}_{s}pK_a$ values. In this study, ${}^{s}_{s}pK_a$ values were determined in the microheterogeneity region of acetonitrile + water mixtures. ${}^{s}_{s}pK_a$ values of these compounds and the mole fraction of acetonitrile correlate linearly over the whole experimental range of acetonitrile contents studied. It is known that the slopes of the straight lines for microheterogeneity region are different from the slopes of the straight lines obtained from water-rich compositions. Aqueous pK_a values of these compounds have been determined from ${}^{s}_{s}pK_a$ values obtained from the microheterogeneity region of the acetonitrile + water binary mixture. Herrador and Gonzáles[38] investigated the medium effects on the ketoprofen in acetonitrile + water mixtures. The aqueous pK_a of ketoprofen was calculated by using the relationships between ${}^{s}_{s}pK_a$ and mole fraction of acetonitrile. The ${}^{w}_{w}pK_a$ and ${}^{s}_{s}pK_a$ values of ketoprofen obtained from this study are in good agreement with those obtained by Herrador and Gonzáles. The correlations of pK_a values with the solvatochromic parameters π^*, α, and β of acetonitrile + water mixtures permit the determination of pK_a values in any condition. As shown in Table 6, the aqueous pK_a values obtained by using the Yasuda−Shedlovsky extrapolation are generally consistent with those determined using the equations in Table 5.

CONCLUSION

This paper presents the first study dealing with the chromatographic determination of ${}^{s}_{s}pK_a$ values of arylacetic and arylpropionic acids at four different acetonitrile percentages in microheterogeneity region of acetonitrile + water mixtures. Equation 3 can be effectively used to calculate the pK_a values of studied compounds and the intrinsic retention factors of the neutral acids and the anionic base from the linear correlations between the retention factors with E_T^N values and the pH of the mobile phase. Equation 3 shows that the retention of the weak acids thus not only depends on pH of the mobile phase and constants of the solute but also depends on the activity coefficients. Also, eq 3 permits the statistical evaluation of the chromatographic method for the determination of the pK_a values and limiting capacity factors.

The chromatographic procedure is a very popular approach to determine pK_a values of ionizable compounds; taking into account the variation of the retention factors of weak acidic compounds with E_T^N of the mobile phase, k_{HA}, k_{A^-}, and pK_a values of any compounds studied at any mobile phase composition can be calculated. A similar procedure can be used to predict the retention of any ionizable solute in any buffer and mobile phase composition after investigating the retention behavior of the solute in appropriate buffer at different mobile phase compositions.

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