Potentiometric pK_a Determination of Piroxicam and Tenoxicam in Acetonitrile-Water Binary Mixtures

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Abstract: Ionization constant (pK_a) is one among the parameter to be estimated with accuracy, irrespective of solubility constraints. In the present study, acid-base behaviour of the piroxicam and tenoxicam was studied. By using the potentiometric method, pK_a values of piroxicam and tenoxicam have been determined in different percentage of acetonitrile-water binary mixtures (acetonitrile content between 30 and 45% in volume). Aqueous pK_a values of these compounds were calculated by mole fraction and Yasuda-Shedlovsky equations. The experimental pK_a values were compared to the corresponding predicted values derived by SPARC and Marvin Sketch software packages.

Key words: Potentiometric titrations, Acetonitrile, pK_a, Yasuda- Shedlovsky Equation

Asetonitril-Su kili Karı ımlarında Piroksikam ve Tenoksikam'ın Potansiyometrik pK_a Tayini

Özet: yonla ma sabiti (pK_a) çözünebilirlik sınırlaması olmaksızın do ru tahmin edilebilen parametrelerden birisidir. Sunulan bu çalı mada, piroksikam ve tenoksikamın asit- baz davranı ı çalı ılmı tır. Potansiyometrik metot kullanılarak, piroksikam ve tenoksikamın pK_a de erleri asetonitril-su ikili karı ımlarının farklı yüzdelerinde tayin edilmi tir (asetonitril içeri i hacimce % 30 ile 45 arasında). Bu bile iklerin sudaki pK_a de erleri mol kesri ve Yasuda-Shedlovsky e itlikleri kullanarak hesaplanmı tır. Deneysel pK_a de erleri SPARC and Marvin Sketch paket programlarından türetilmi tahmini de erlerle kar ıla tırılmı tır.

Anahtar kelimeler: Potansiyometrik titrasyonlar, Asetonitril, pK_a, Yasuda- Shedlovsky e itli i

1. Introduction

Oxicams belong to a long-acting class of non-sterodial anti-inflammatory drugs (NSAIDs) which displays potent anti-inflammatory and analgesic activity and is effective in the treatment of rheumatoid arthritis, osteoarthtitis, and degenerative joint diseases [1]. These drugs are also found to be very good antioxidants [2]. Piroxicam and Tenoxicam are the representative drugs belonging to the oxicam group. In recommended doses, piroxicam and tenoxicam appear to be the equivalent of aspirin, indomethacin or naproxen for the long-term treatment of rheumatoid arthritis or osteoarthritis [3].

Oxicams are a group of NSAIDs derived from the benzene(tieno)thiazine heterocyclic system, where the group of N-heterocyclic carboxamide include the triazine sulphur, and in position 4 there is an enolic group. Condensation of benzene ring or thiophene with the heterocyclic system as well as substitution of the amide group in position 3 imparts acidic properties to the enolic group [4]. Piroxicam [4-hydroxy-2-methyl-N-2-pyridinyl-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide] and Tenoxicam [4-hydroxy-2-methyl-N-2-pyridinyl-2H-thieno(2,3-e)-1,2-thiazine-3-carboxamide-1,1-dioxide] (Table 1) are enolic acid derivatives that inhibit high levels of COX-2 at the sites of inflammations and thus, has antiinflammatory, analgesic and antipyretic activity. These compounds have enolic, pyridinyl and amidic groups. The enolic acid and the pyridin-2-yl moieties represent the acidic and basic sites, respectively.

Table 1. Chemical Structure of Piroxicam and Tenoxicam

Compounds	Structure
Piroxicam	
Tenoxicam	
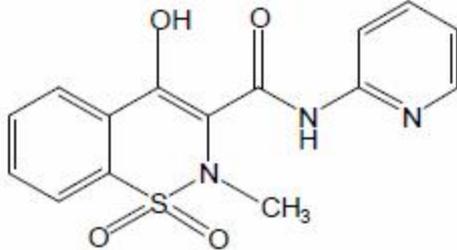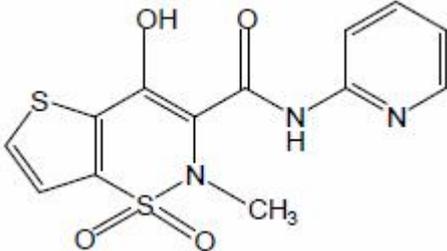
Despite the fact that many drugs are sparingly soluble in water, the evaluation of the aqueous dissociation constants is an unavoidable requirement in drug discovery. In these instances, experimental pK_a determination requires the use of an organic or hydroorganic solvent [5]. The acid-base property of a drug molecule is important physical parameter for evaluation of biological activity in terms of quantitative structure-activity relationships. Several experimental approaches have been employed for the determination of the dissociation constants, including potentiometric titration [6], spectrometry [7], HPLC [8,9] and CE methodology [10]. In addition, relevant software have been developed for the rapid estimation of pK_a values based on the chemical structure [11,12]. Among these techniques, the potentiometric titration is a more general method and it does not require the presence of chromophore groups for pK_a determination. If due care is taken, this technique is accurate and has good reproducibility. Fast and automated instruments are commercially available for potentiometry; however, their disadvantages include the requirements to use milligram

amount of pure substances and the use of carbonate free titrants. Moreover, potentiometry is the preferred technique because of the excellent response of the glass electrode in many pure and binary solvents and the easy automation of the experimental measurements [6,13]. Also, UV- Visible absorption spectrophotometry has still been used widely by the help of improved computer programs for the determination of dissociation constants [7]. In the last decade, a new method has been developed when LC and CE methodoligies are used for determination of dissociation constants in combination with a diode array detector (DAD) for absorbance measurements [8-10].

Traditionally, water has been considered as the solvent which best represents the biological conditions. Poorly water soluble drugs require water miscible cosolvents to solubilise them. However, hydro-organic mixtures such as acetonitrile-water have been found suitable because they show simultaneously a low polar character and a partially aqueous content. Acetonitrile can dissolve a wide majority of organic acids more effectively than water and in many cases it is a more suitable solvent for the determination of the dissociation constants. Acetonitrile behaves as a weaker base and as a much weaker acid than water. It has a relatively high dielectric constant ($\varepsilon = 36$) and a small autoprotolysis constant ($pK_s = 33.6$) [6].

In this work, estimation of the aqueous pK_a of the analyte is predicted from SPARC and Marvin Sketch programs [11-13]. Moreover, for aqueous pK_a ($_w^w pK_a$) determination purposes, two main potentiometric approaches were developed. Both methods employ hydroorganic mixtures as sample solvents and require a suitable extrapolation equation to estimate the $_w^w pK_a$ value, from the experimental constants in the binary solvent ($_s^s pK_a$). The first method involves the determination of $_s^s pK_a$ in several hydroorganic mixtures and further extrapolation by means of the Yasuda–Shedlovsky equation [14,15]. The second approach, linear relationship between the mole fraction of acetonitrile and the $_s^s pK_a$ values.

There are only few systematic report about pK_a values of tenoxicam, piroxicam in different water-organic modifier binary mixtures [8,16-21]. However, some data in literature differ considerably, number of pK_a values and the methods used for their determination are not adequately described (temperature, ionic strength, solvent media, etc.). Only a limited number of studies related with aqueous pK_a values of these compounds are found in the literature [19,21]. In our previous study, pK_a values of these compounds are determined in acetonitrile-water binary mixtures [8]. In this study [8], pK_a values of enolic OH groups were determined but pK_a values of pyridinyl OH groups were not determined. In order to overcome the lack of information related to the acid-base equilibria of tenoxicam and piroxicam, the pK_a values of tenoxicam and piroxicam in acetonitrile-water binary mixtures have been determined by means of fast, accurate and sensitive potentiometric method. The potentiometric determinations were carried out in 30%, 35%, 40% and 45% (*v/v*) acetonitrile content. Aqueous pK_a value of tenoxicam and piroxicam is calculated by Yasuda- Shedlovsky and pK_a-X equations. The parameters obtained allow to study the effect of the preferential solvation of the drugs on the pK_a values and to demonstrate the meaninglessness of the linear extrapolation methods to estimate the aqueous pK_a value from pK_a values obtained in

binary solvents of different compositions. To overcome the frequent lack of $_w^w pK_a$ literature values for tenoxicam and piroxicam needed to estimate the pK_a of the analyte, two different computational programs (SPARC on-line calculator, Marvin Sketch program) are used, and results obtained from $_w^w pK_a$ values are compared with these programs and literature values.

2. Experimental

2.1. Chemicals and Reagents

All chemicals were used without further purification. Antiinflammatory agents used in this study were kindly supplied as follows: piroxicam from DEVA Pharm. Company; tenoxicam from Mustafa Nevzat Pharm. Company. Hydrochloric acid and potassium chloride were obtained from Merck (Darmstadt, Germany). Potassium hydrogen phtalate Merck (Darmstadt, Germany)was dried at 110^oC before use. Stock 0.025 mol/L carbonate-free potassium hydroxide solutions were prepared in each acetonitrile-water mixture from 1 mol/L potassium hydroxide solution (Titrisol, Merck) by dilution. Hydrochloric acid solutions were prepared by dilution from 1 mol/L hydrochloric acid (Titrisol, Merck) in each acetonitrile -water mixture. The ionic strength of all working solutions was adjusted at 0.1 mol/L with potassium chloride (Merck).

The standardization of the electrode system in acetonitrile–water mixtures was carried out by Gran's method [22] and absence of carbonate were periodically checked by pH-meter, using the appropriate Gran function [22] against primary standard oven-dried potassium hydrogen phthalate. Acid solutions prepared from hydrochloric acid were titrated against standardized potassium hydroxide [23].

2.2. Apparatus

In order to obtain the pK_a values using potentiometric technique, measurements of pH and electromotive force were caried out with Mettler-Toledo MA 235 pH/ion analyser and an electrode assembly ($\pm$ 0.1 mV). The cell was thermostated externally at 25^oC $\pm$ 0.1 with a cooler system water bath (HETO CBN 8-30 and temperature control unit HETO HMT 200). Triplicate titrations were carried out in the double-wall Pyrex titration cell of 80 mL capacity.

2.3. Potentiometric procedure

The glass electrode was stored in water when not in use. It was soaked for 20-25 min in acetonitrile-water mixture before potentiometric measurements. Because the glass electrode should be kept to use acetonitrile-water binary mixture. The stabilization criterion for the potential readings was 0.2 mV within 120 s. In all instances, the electrode system gave stable and reproducible potentials within 3 min. The standardization of the electrode system was carried out, each time in acetonitrile -water mixtures (background solution) studied by Gran's method [22]. For this purpose, the electrode was immersed in these mixtures (20 mL and 0.1 mol/L ionic strength). Usually, 10 or 12 additions of hydrochloric acid were enough for E^0 to be accurately

determined. The potential was allowed to stabilize after each addition of acid and the potential values obtained were used to calculate the standard potential of the cell, E^0.

The pK_a values of tenoxicam and piroxicam were determined by titration of solutions of these compounds (2.10^{-3} mol/L for these compounds) in 30%, 35%, 40% and 45% (*v/v*) acetonitrile-water, using KOH solution as titrant, in 0.1 mol/L ionic strength (KCl). Tenoxicam and piroxicam solutions were completely mixed with a magnetic stirrer. The titrations were carried out at constant temperature ($25 \pm 0.1^{\circ}$C) to ensure reproducibility. Drop wise addition of KOH solution (50 μL) was performed by calibrated Nichipet EX (Nichiryo, Japan) and the titrations were continued up to pH 12.

3. Results and Discussion

The acid dissociation constant (K_a) is important physical parameter for evaluation of biological activity in terms of quantitative structure-activity relationships [24]. Potentiometric titration is a high-precison technique for determining the pK_a values of compounds. In this work, potentiometric pK_a values were determined from several series of independent measurements. The data pairs (emf/titrant volume) obtained were processed by the program PKPOT [25] to determine the pK_a values. This program refines the thermodynamic equilibrium constants in order to minimize the sum of squared differences between the experimental emf values and those calculated on the basis of the mass balances for hydrogen, the equilibrium contants, the cell standard potential and the activity coefficients. One series of measurements for one titration of tenoxicam and piroxicam in 45% (*v/v*) using KOH solution as titrant is shown in Figure 1. Two steps were clearly observed in the potentiometric curve, the first relating to the pyridinyl group, the second to the ionization of enolic group. The third step was not clearly seen as expected and it is related to the amidic group.
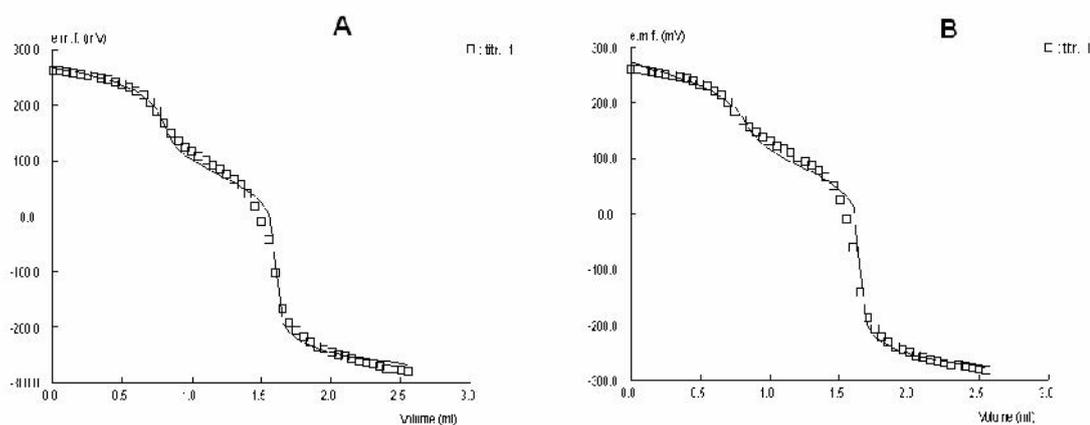

Figure 1. Plot of potentiometric titration of 2.10^{-3} mol/L tenoxicam and piroxicam in 40% (v/v) with 0.025 mol/L KOH. (A) Piroxicam, (B) Tenoxicam. Solid lines indicate the theoretical values calculated by PKPOT program

The values of the pK_a of the oxicams in acetonitrile-water binary mixtures at different compositions (30%, 35%, 40% and 45%, v/v) are given in Table 2. Each pK_a value was calculated as the average of at least three replicates. In all cases the standard deviations are very low and they show good precision of the pK_a values.

Table 2. The pK_{a1} and pK_{a2} values of tenoxicam and piroxicam in acetonitrile-water mixtures (the estimated standard deviations are given in parentheses)

Compounds	Piroxicam		Tenoxicam	
	pK_{a1}	pK_{a2}	pK_{a1}	pK_{a2}
30% ACN	3.211 (0.04)	6.238 (0.03)	3.704 (0.01)	6.276 (0.02)
35% ACN	3.055 (0.02)	6.303 (0.01)	3.533 (0.05)	6.469 (0.08)
40% ACN	2.843 (0.06)	6.345 (0.04)	3.416 (0.05)	6.740 (0.06)
45% ACN	2.674 (0.06)	6.401 (0.09)	3.261 (0.03)	6.974 (0.06)

As is shown in Table 2, these compounds have two pK_a values corresponding to two acid-base functional groups. Values obtained for tenoxicam and piroxicam are consistent with the protolytic equilibrium of pyridinyl and enolic group (Figure 2).
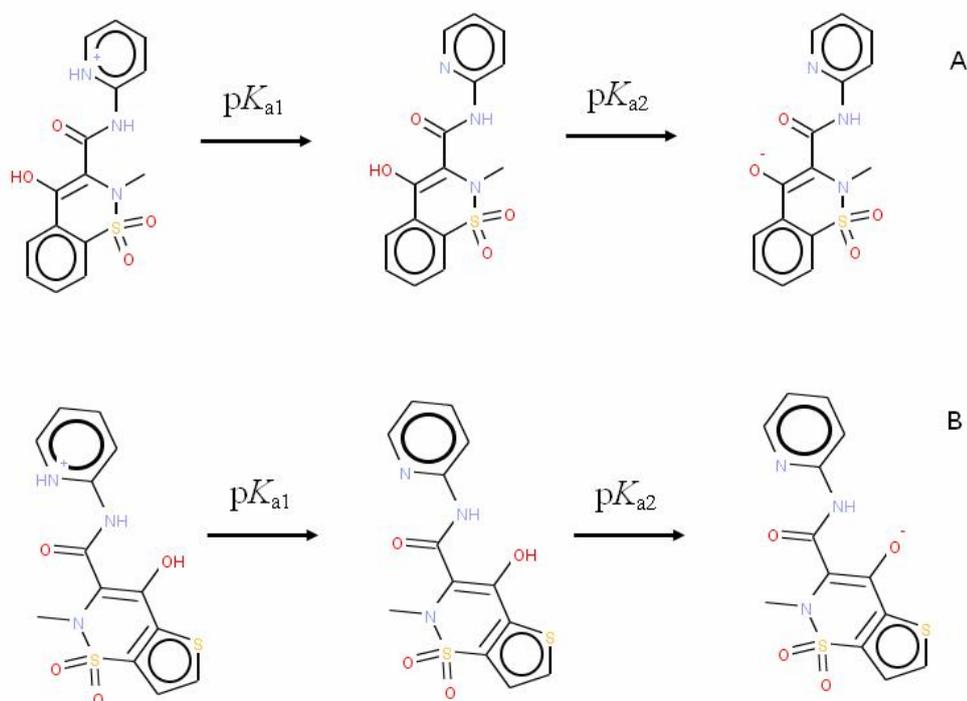

Figure 2. Protolytic equilibria of piroxicam (A) and tenoxicam (B)

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. In order to evaluate the ${}_{w}^{w}pK_a$ of tenoxicam and piroxicam, ${}_{s}^{s}pK_a$ values were plotted against to acetonitrile mole fraction (Figure 3). These linear equations obtained from this approach are the aqueous pK_a values of these compounds (Table 3). The intercepts of these linear equations are the aqueous pK_a values of these compounds (Table 4). The variation of the pK values with the mole fraction of acetonitrile is different for these compounds. pK_{a1} values in line with the pyridinyl groups decrease with the mole fraction of acetontrile, whereas pK_{a2} values in line with the enolic acid groups increase with the mole fraction of acetontrile. Values of mole fraction for acetonitrile-water mixtures at 25°C are obtained from the work of Barbosa et.al [26].
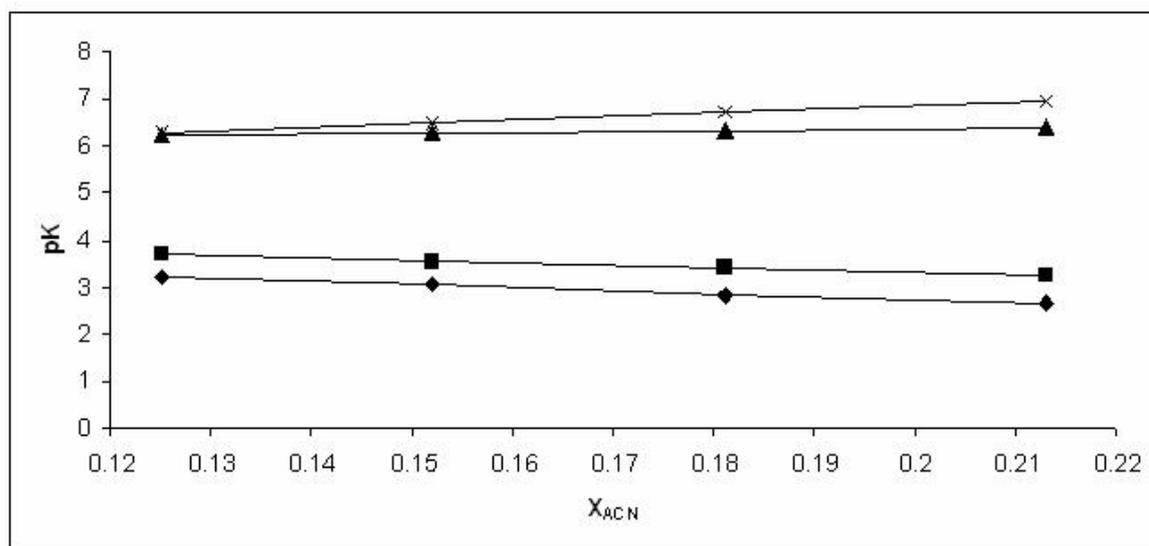

Figure 3. pK values versus mole fraction in acetonitrile- water mixtures. ( ), pK_{a1} values of tenoxicam; ( ), pK_{a1} values of piroxicam; (x), pK_{a2} values of tenoxicam; ( ),pK_{a2} values of piroxicam

Table 3. The linear equations between experimental pK_a values and the mole fraction of acetonitrile

Compounds	Equation	S.E. of Slope	S.E. of Intercept	r
Piroxicam	$pK_{a1} = -6.201X + 3.986$	0.274	0.047	0.998
	$pK_{a2} = 1.803X + 6.019$	0.130	0.022	0.995
Tenoxicam	$pK_{a1} = -4.913X + 4.303$	0.305	0.052	0.996
	$pK_{a2} = 8.052X + 5.264$	0.297	0.051	0.999

The most important equation is Yasuda-Shedlovsky to estimate $_w^w pK_a$ from $_s^s pK_a$ [14,15].

$$pK_a + \log[H_2O] = a_v \vee^{-1} + b_v$$

It is known that Yasuda- Shedlovsky extrapolation treatment was found to be useful, particularly in the water rich region, in order to predict aqueous pK_a values from the $_s^s pK_a$ values. The experimental $_s^s pK_a$ values obtained from the various acetonitrile percentages were used in Eq 1 for calculation the aqueous dissociation constants of the compounds studied (Table 4). Table 4 summarizes aqueous pK_a values predicted by the program SPARC and Marvin Sketch [11,13]. In this study, $_s^s pK_a$ values were determined in microheterogenity region of acetonitrile-water mixtures. $_s^s pK_a$ values of these compounds and 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 microheterogenetiy 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 microheterogenity region of acetonitrile -water binary mixture.

Table 4. Aqueous pK_a values ($_w^w pK$) of piroxicam and tenoxicam

	pK_a values	SPARC	Marvin	Yasuda-Shedlovsky	pK_a-X
Piroxicam	pK_{a1}	2.17	3.79	3.95	3.99
	pK_{a2}	7.91	8.08	5.98	6.02
	pK_{a3}	10.31	11.73	-	-
		SPARC	Marvin	Yasuda-Shedlovsky	pK_a-X
Tenoxicam	pK_{a1}	1.90	4.78	4.27	4.30
	pK_{a2}	6.88	7.21	5.19	5.26
	pK_{a3}	9.99	13.63	-	-

The difference between the pK_{a2} values predicted by computer programs and the pK_{a2} values calculated by our approach is very high. But, Augustin-Nowacka et al. [27] and Chmurzy ski [28] demonstrated that pK_a value of pyridinyl group is close to about 5.0. As shown in Table 4, aqueous pK_a values estimated by equations for studied compounds good agreement with those obtained literature values for pyridinyl group .

The aqueous pK_a values in Table 5 in some instances with values reported here, but in other cases are quite different because of the different methods and different solvents employed. In the present study, we have used a single method, temperature, ionic strength and solvent system, so our data are self consistent and suitable for our purpose.

Table 5. Aqueous pK_a values ($^w_w pK$) of piroxicam and tenoxicam obtained from different approaches

Oxicam	pK_a	Solvent	Method	Temp.
Piroxicam	pK_{a1} : 1.86 pK_{a2} : 5.46	DMSO : buffer solution pH 2	UV-spectrophotometric	Not given
	pK_{a1} : - pK_{a2} : 4.58	Methanol : water	LC	25 ^{o}C
Tenoxicam	pK_{a1} : 1.07 pK_{a2} : 5.34	DMSO : buffer solution pH 2	UV-spectrophotometric	Not given
	pK_{a1} : - pK_{a2} : 5.29	Methanol : water	LC	25 ^{o}C

4. Conclusions

This paper presents the first study dealing with the determination of pK_a values of tenoxicam and piroxicam by potentiometric method in acetonitrile-water binary mixtures and gives a possibility for deeper analysis of the processes which take place during LC analysis of these compounds. Acetonitrile-water mixtures ranging from 10 to 70% (*v/v*) are solvents for acid-base titrations of sparingly water soluble compounds. Like many other drug compounds, tenoxicam and piroxicam are practically insoluble in water. In this study, the acid–base dissociation constants of tenoxicam and piroxicam containing enolic and pyridinyl groups were determined in acetonitrile-water binary mixtures. The $^s_s pK_a$ values were obtained using PKPOT program and these values were used to calculate the $^w_w pK_a$ of each drug. The potentiometric method proposed here, proved to be convenient and rapid in determining the pK_a values of these compounds.

Acknowledgements

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