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Potentiometric pK_a determination of water-insoluble compounds: validation study in methanol/water mixtures

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Abstract

The apparent ionization constants (p$_s K_a$) of 25 molecules, based on 431 separate potentiometric titrations, were determined in methanol/water mixtures of 15–65 wt% methanol content. The Yasuda-Shedlovsky extrapolation (p$_s K_a$ + log[H_2O] = $a/\epsilon + b$) was used to obtain the pK_a values in zero methanol%. In the case of water-soluble drugs the extrapolated data were in very good agreement with pK_a values measured in aqueous solutions under the same experimental conditions (average deviation = 0.05). The water-insoluble molecules showed acceptable accordance with spectroscopically measured or with literature pK_a values. Concentration dependence between 1–5 mM was not observed while the range of extrapolation (water-rich: R = 15–35 wt%, or methanol-rich: R = 40–65 wt%) significantly influenced the accuracy of pK_a values. Remarkable changes in the solvation structure of weak acids in methanol-rich mixtures (> 35 wt%) were suggested from analyzing the slopes of Yasuda-Shedlovsky equations. Recommendations for the proper application of a mixed-solvent procedure in order to gain the most reliable aqueous pK_a values are suggested. © 1997 Elsievier Science B.V.

Keywords: Solvent-mixture procedure; Methanol/water mixtures; Validation

1. Introduction

The ionization constant, pK_a, is a very important physico-chemical property of a substance and knowledge of this parameter is of fundamental importance in a wide range of applications and research areas. There are various methods for measuring pK_a values but potentiometry has outstanding importance because it is fast, accurate and reproducible (Albert and Serjeant, 1984). However, the application of pH-metric pK_a measurement is often hindered by the poor water-solubility of the sample. By using a glass electrode of excellent quality, performing proper electrode cal-

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ibration, excluding the presence of ambient carbon dioxide as much as possible and accurately dispensing very small titrant volumes, potentiometry in aqueous solution can be applied to concentrations as low as 10^{-4} M (according to some authors as low as 10^{-5} M). Of course the accuracy and reproducibility of titrations in such dilute solutions is much less than in higher (usually used 1–5 mM) concentration. The best tool of pK_a determination in very dilute (10^{-5}–10^{-6} M) solutions is spectrophotometry, provided that the compound possesses pH-dependent UV absorption due to the presence of a chromophore in proximity to the ionizable group (Albert and Serjeant, 1984). If the molecule suffers from both problems (very poor solubility and lack of an analytical useful chromophore) alternative methods of pK_a determination such as 'mixed-solvent procedure', can be used (Mizutani, 1925). This method is based on the measurement of apparent ionization constants (p_sK_a) in different ratios of organic solvent/water mixtures where the aqueous pK_a is obtained by extrapolation. Organic solvents frequently used are methanol, ethanol, propanol, DMSO, DMFA, acetone and THF. Since most literature data has been accumulated for MeOH/water solvent mixtures and since it is generally accepted that methanol shows a solvation effect closest to water, methanol is normally chosen as the organic solvent of choice (Avdeef et al., 1993) and cited references therein.

Recently Avdeef and coworkers in a series of papers have summarized their activity on development and application of a new, automated analyzer (PCA 101, Sirius, UK) for pK_a and log P determination based on potentiometry (Avdeef, 1992, 1993, 1996; Avdeef et al., 1993, 1995; Slater et al., 1994; Takács-Novák et al., 1994a; Takács-Novák and Avdeef, 1996). Within this series, a paper is devoted to the question of proper electrode calibration in methanol/water mixtures (Avdeef et al., 1993). A new, 'four parameter' calibration approach was proposed which provides very reliable pK_a determination in MeOH/water (and other) solvent systems. The precision of pK_a determination using the Yasuda-Shedlovsky extrapolation method were presented for some examples.

According to this method a linear correlation is established in a plot of $p_sK_a + \log[H_2O]$ versus $a/\epsilon + b$, where $\log[H_2O]$ is the molar water concentration of the given solvent mixture, ϵ is the dielectric constant of the mixture and a and b are the slope and intercept, respectively. Aqueous pK_a values can be obtained for log 55.5 and 1/78.3, the molar concentration and dielectric constant of pure water. The procedure offers many benefits over the traditional plot of p_sK_a versus R (wt% of organic solvent) which often show hockey-stick or bow shaped curves.

It was established by Avdeef et al. (1993) that using the four parameter calibration method, the Yasuda-Shedlovsky extrapolations for values of R wt% up to 60, give errors in pK_a not greater than ± 0.2 for weak acids and ± 0.1 for weak bases. In the case of long distance extrapolation (from the 'methanol-rich' region: 40–60 wt%) a bias correction (-0.34 for acids and 0.12 for bases) has been proposed. Since the primary purpose of the cited work was not a comprehensive validation of the solvent-mixture approach and because a limited number of samples were used, it seemed reasonable to investigate in a systematic study the applicability of this method using the PCA 101 analyzer for pK_a determination of water-insoluble compounds. The aim of the present work is the evaluation of the mixed-solvent procedure.

For the validation two conceptional possibilities can be chosen but each has its own compromise. First, one can select water-soluble compounds, determine the pK_a values in water and in mixtures under identical experimental conditions (method, instrument, temperature, ionic strength, sample concentration, etc.) and compare the measured and extrapolated aqueous pK_a values. Evidently, however, solvent-solute interactions are different for water-soluble and insoluble molecules and real information about the possible specific behaviour of a water-insoluble compound cannot be obtained from this approach.

The other possibility is to select water-insoluble substances for validation and to compare the pK_a values obtained from the potentiometric mixed-solvent procedure with aqueous pK_a values measured by another method, e.g. spectroscopy. Of course, here the experimental conditions in the

methods, e.g. concentration, temperature and ionic strength, may differ considerably.

In our work we have combined the two concepts. The 25 molecules examined here can be divided into two groups: (1) set of test compounds (10 water-soluble molecules); and (2) set of insoluble samples (15 compounds). In the group of test compounds (including acids, bases and ampholytes) we compared the potentiometrically measured, highly precise aqueous pK_a values with those extrapolated by the Yasuda-Shedlovsky approach from 15–65 wt% methanol/water mixtures to evaluate the accuracy of the method. The influence of extrapolation range (total: 15–65 wt%; water-rich: 15–35 wt%; methanol-rich: 40–65 wt%) on pK_a values and the relationship between the chemical structure and apparent ionization constants in methanol/water mixtures have also been studied. A further purpose was to investigate how the specific solvation of the water-insoluble compounds (group 2) influences (if at all) their ionization in this solvent mixture.

2. Materials and methods

2.1. Materials

The majority of samples (all of Pharmacopoeia Hung. VII. quality) were purchased from Reanal (Hungary), while the following compounds were generously supplied by the manufacturers: mexiletine by Alkaloida Pharm. Works (Hungary); deramciclane, ibuprofen by EGIS Pharm. Works (Hungary); haloperidol, niflumic acid by Gedeon Richter Chemical Works (Hungary); albendazole sulfoxide, cefalexine, flumequine, KHL-8430, PGE_1, PGE_2, by Chinoin Pharm. Works (Hungary); vancomycin by Eli Lilly (USA). Naltrexone derivatives (HNB-1 and HNB-5) were synthesized at Dept. Medicinal Chemistry, UIC, (Chicago, USA) as published by Nan et al. (1996). Samples were used without further purification. Methanol was of spectroscopic grade (Fluka), all other reagents were of analytical grade.

2.2. Apparatus

The details of the instrument (PCA 101, Sirius, Forest Row, UK) used for pK_a determination were described earlier (Avdeef, 1992, 1993; Avdeef et al., 1993).

2.3. Potentiometric pK_a determination

2.3.1. Electrode calibration

The four-parameter procedure was used for electrode standardization in both aqueous and semi-aqueous medium (Avdeef et al., 1993). HCl solutions of known concentration, containing 0–70 wt% methanol were titrated with standardized NaOH at 25.0 ± 0.1°C, at $I = 0.10$ M, under N_2 atmosphere, in the pH interval 2–12. The operational pH reading was related to the concentration p_cH values by a multiparametric equation.

$$pH = \alpha + Sp_cH + j_H[H^+] + j_{OH}K_w/[H^+]$$

The parameters are determined by a weighted nonlinear least squares procedure. The intercept parameter α in aqueous solution mainly corresponds to the negative logarithm of the activity coefficient of H^+ at the working temperature and ionic strength. The j_H term corrects pH readings for the nonlinear pH response due to liquid junction and asymmetry potentials in moderately acidic solutions, while the j_{OH} term corrects for high-pH nonlinear effect. Factor S accounts that a particular electrode may not have 100% Nernstian slope, K_w is the ionization constant of water. The typical values of the adjustable parameters in aqueous and methanol/water mixtures have been published in Avdeef et al. (1993). The actual calibration parameters of the ORION Ross semi-micro glass electrode used in this work (Table 1) were applied for the calculation of pK_a and p_sK_a values.

2.3.2. Titration in aqueous medium

Ten ml of 1 mM or 5 mM aqueous solutions of the samples were pre-acidified to pH 1.8–2.0 with 0.5 M HCl, and were then titrated alkalimetrically to some appropriate high pH (maximum 12.5). The titrations were carried out at 25.0 ± 0.1°C, at $I = 0.1$ M ionic strength using NaCl, and under

N_2 atmosphere. The initial estimates of pK_a values were obtained from Bjerrum difference plots ($\bar{n}_H$ vs. pH, where $\bar{n}_H$ is the average number of bound protons) and then were refined by a weighed non-linear least-squares procedure (Avdeef, 1992, 1993). For each molecule a minimum of three and occasionally five or more separate titrations were performed and the average pK_a values along with the standard deviations were calculated.

2.3.3. Titrations in solvent mixtures

A series of 1 mM or 5 mM semi-aqueous solutions of the samples, containing 3–70 wt% methanol were titrated under the same experimental conditions as in aqueous titrations. For all the molecules of the validation set (group 1) measurements were carried out at six different R values ranging from 15 to 65 wt%. Titrations at each methanol/water mixture were repeated three times, and then the average of the p_sK_a values was calculated (Table 2). The Yasuda–Shedlovsky procedure was applied to estimate the aqueous pK_a values (Avdeef et al., 1993).

The p_sK_a values of water-insoluble compounds (group 2) were determined at a minimum of three and occasionally five or more different methanol/water mixtures, performing three separate titrations at each R wt% value. The average p_sK_a values (data not shown) were used in the Yasuda-Shedlovsky approach for the extrapolation of the aqueous pK_a (Table 7).

Table 1
Methanol–water electrode standardization parameters (25°C)

R (wt%)	α	S	j_H	j_{OH}
0	−0.007	1.0037	1.8	−0.8
7.5	0.002	1.0031	1.6	−0.7
15.1	0.019	1.0005	1.6	0.1
23.1	0.055	0.9992	0.5	−1.3
31.1	0.073	0.9970	1.4	−0.1
39.3	0.128	0.9972	1.3	−1.6
47.9	0.170	0.9950	1.6	−0.8
57.4	0.167	1.0021	2.4	−2.8

R is the wt% of methanol in solvent mixtures: the meaning of S, α, j_H and j_{OH} is in the text.

3. Results and discussion

The water-soluble test compounds (listed in Fig. 1a, group 1) have been selected with the intention to represent the different acid/base functional groups that are most frequently present in drugs and to span a wide pK_a range from 2 to 12. These ionizable groups (carboxyl, amide, phenol; aliphatic amine, heteroaromatic N) also appear in the molecules of our second set (group 2), which are insoluble or poorly soluble in water. (Structures of known drugs are not shown except vancomycin, while potential drugs under development are presented in Fig. 1b.)

The apparent ionization constants (p_sK_a) of test compounds obtained in methanol/water mixtures in the interval R wt% 15–65 are summarized in Table 2. The standard deviation (S.D.) of data calculated from three separate titrations at each R value, is indicated in parenthesis as the least significant digit. The measurements were carried out in 5 mM concentration of the sample, while in the case of three compounds (paracetamol, ephedrine, morphine) the p_sK_a values were determined in 1 mM concentration as well. The data prove the excellent reproducibility of the automated titration procedure, the S.D. values vary between 0.001 and 0.006 in 5 mM concentration and 0.005–0.020 in the more diluted solutions but still far less than the usually accepted 0.05 reproducibility of potentiometry. No significant differences can be observed between p_sK_a values obtained at different concentrations (5 and 1 mM), the average of the deviations is 0.074 in absolute value.

The p_sK_a values as a function of methanol wt% are shown in Fig. 2. The points reflect a certain nonlinearity in the plots. This phenomena is found in many mixed solvent systems and was recently explained by Bosch et al. (1995) in 2-propanol/water mixtures. They demonstrated that the solutes (acids) showed preferable solvation with either alcohols (S_1), water (S_2) or an alcohol-water (S_{1-2}) complex depending on the solvent composition.

Fig. 3 contains the Yasuda-Shedlovsky plots $p_sK_a + \log[H_2O]$ vs. $1/\epsilon$ for the test compounds (group 1) and Table 3 summarizes the pK_a values

Table 2
Apparent ionization constants (p_sK_a) in MeOH/water mixtures

Acids:

Salicylic acid		Phenobarbital		Paracetamol		
R (wt%)	p_sK_a (5 mM)	*R* (wt%)	p_sK_a (5 mM)	*R* (wt%)	p_sK_a (5 mM)	p_sK_a (1 mM)
15.4	2.913 (4)	16.3	7.551 (3)	15.7	9.794 (3)	9.720 (9)
23.7	3.014 (3)	25.5	7.745 (3)	24.3	9.950 (3)	9.915 (6)
31.5	3.175 (3)	34.3	7.934 (3)	32.5	10.114 (2)	10.080 (16)
40.3	3.362 (3)	44.2	8.107 (3)	41.6	10.314 (4)	10.187 (10)
48.7	3.520 (3)	53.8	8.304 (4)	50.3	10.427 (5)	10.253 (18)
58.1	3.686 (3)	64.7	8.535 (3)	60.1	10.502 (6)	10.528 (20)

Bases:

Ephedrine			Mexiletine		Papaverine	
R (wt%)	p_sK_a (5 mM)	p_sK_a (1 mM)	R (wt%)	p_sK_a (5 mM)	R (wt%)	p_sK_a (5 mM)
15.7	9.520 (3)	9.488 (6)	16.2	9.021 (3)	16.4	6.066 (5)
24.3	9.460 (3)	9.451 (10)	25.2	8.941 (1)	25.5	5.971 (2)
34.2	9.368 (4)	9.310 (15)	33.9	8.881 (2)	34.3	5.847 (3)
44.1	9.313 (2)	9.240 (5)	43.7	8.757 (1)	44.2	5.683 (3)
53.6	9.173 (3)	9.165 (8)	53.2	8.688 (2)	53.8	5.543 (4)
64.6	9.040 (5)	8.990 (6)	63.9	8.602 (3)	64.7	5.381 (5)

Ampholytes:

Morphine					Cefalexine			Albendazole sulfoxide		
R (wt%)	p_sK_{a1} (5 mM)	p_sK_{a2} (5 mM)	p_sK_{a1} (1 mM)	p_sK_{a2} (1 mM)	R (wt%)	p_sK_{a1} (5 mM)	p_sK_{a2} (5 mM)	R (wt%)	p_sK_{a1} (5 mM)	p_sK_{a2} (5 mM)
15.6	9.587 (4)	8.110 (3)	9.472 (10)	8.029 (10)	14.8	7.125 (3)	2.901 (4)	15.2	10.068 (3)	3.189 (8)
23.5	9.735 (3)	8.053 (3)	9.595 (13)	7.932 (13)	22.8	7.071 (2)	3.068 (3)	23.5	10.157 (3)	3.084 (3)
32.4	9.842 (4)	7.908 (3)	9.727 (10)	7.808 (10)	30.5	7.048 (2)	3.221 (2)	31.3	10.289 (5)	2.959 (5)
41.5	9.992 (4)	7.840 (4)	9.965 (5)	7.727 (6)	38.8	6.971 (2)	3.354 (2)	40.0	10.334 (6)	2.746 (6)
50.2	10.130 (5)	7.649 (4)	10.128 (8)	7.518 (8)	46.8	6.925 (2)	3.561 (2)	48.2	10.393 (6)	2.749 (6)
59.7	10.284 (5)	7.492 (4)	10.306 (17)	7.204 (20)	55.7	6.912 (4)	3.788 (4)	52.8	10.508 (6)	2.619 (6)

R is the wt.% of methanol in solvent

	compound	acid/base functional group
Group 1		
Acids:	salicylic acid	ar. carboxyl
	phenobarbital	lactam
	paracetamol	phenol
Bases:	ephedrine	aliph.secondary amine
	mexiletine	aliph.primary amine
	papaverine	ar.N
Ampholytes:	morphine	phenol and alic.tert.N
	cefalexine	ar.carboxyl and al.pr.amine
	albendazole sulfoxide	amide and ar.N
	vancomycin	al.carboxyl,phenol(s) and pr. amine,sec.amine (see structure below)
Group 2		
Acids:	flumequine	ar.carboxyl
	PGE_1 and PGE_2	al.carboxyl
	ibuprofen	al.carboxyl
	hydrochlorotiazide	sulfonamide(s)
	hexachlorophene	phenol(s)
Bases:	KHL-8430	al.sec.amine (see struct.below)
	haloperidol	al.tert.amine
	deramciclane	al.tert.amine (see struct.below)
	chlorpromazine	al.tert.amine
	quinine	ar.N,alic.tert.N
Ampholytes:	niflumic acid	ar.carboxyl and ar.sec.amine
	nitrazepam	lactam and =N–
	HNB-1	phenol and alic.tert.N
	HNB-5	phenol and alic.tert.N,ar.N (see structures below)

Fig. 1a. Classification of model compounds. Group 1: water-soluble test compounds. Group 2: water-insoluble samples.

extrapolated to zero methanol content. The parameters of Yasuda-Shedlovsky equations are given in Table 4.

The linearity of the plots is characterized by the regression coefficients (r^2) values which indicate significant linear correlation for the molecules examined ($p<0.001$), except paracetamol and albendazole (amide function). It can be seen from the plots that bases and the basic functional groups of ampholytes all have negative slopes (Fig. 3c) and produce straight lines with randomly scattered points the total interval ($1/\epsilon$: 0.013–0.020)

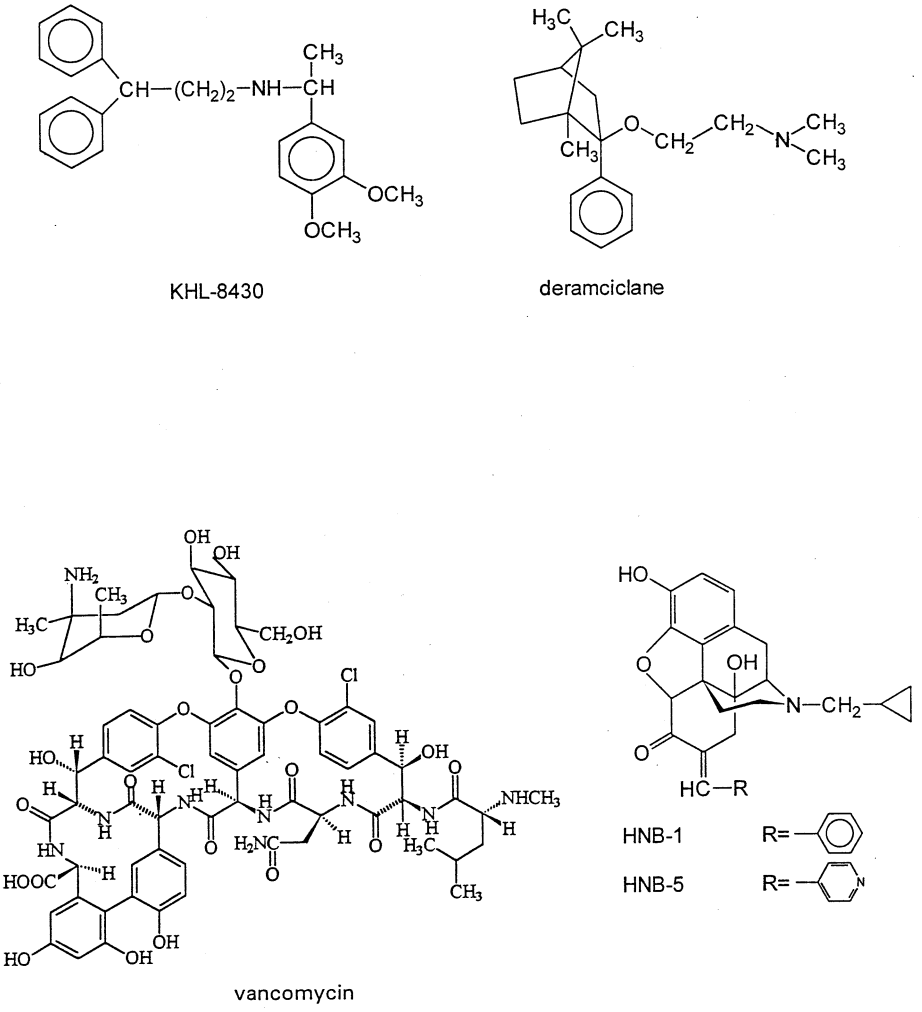

Fig. 1b. Structure of vancomycin and potential drugs under development.

studied. The weak acid plots have positive slopes but the position of the points shows a certain trend, particularly with the phenol functionality. Detailed interpretation of this finding follows later.

To evaluate the mixed-solvent procedure for pK_a determination, the extrapolated pK_a data were compared to pK_a values measured in aqueous solution. The differences expressed as $\Delta = pK_a$ extrapolated $- pK_{a\,measured}$ are collected in Table 5. The extrapolated pK_a values were calculated in three different ways (Table 3): using p_sK_a values from the total range ($N = 6$ methanol/water mixtures, $n = 18$ titrations), using p_sK_a values from the water-rich ($N = 3$, $n = 9$) and from the methanol-rich region ($N = 3$, $n = 9$). This latter group shows the reliability of the method for drugs soluble only in mixtures of high methanol content ($R > 40$ wt%).

Data in Table 5 indicate the good agreement of the measured and extrapolated pK_a values. The mean error of total-range extrapolation is as low as 0.05, similarly good are produced by water-rich extrapolation (0.07), however the average error of the extrapolation from the methanol-

rich region is considerable higher, 0.17 (each in absolute value). The pK_a values of bases extrapolated to zero methanol are always underestimated (negative Δ values) independently from the R wt% region used, contrary to the acids, where the water-rich region gives underestimated while the methanol-rich region produces overestimated ionization constants. Analyzing the parameters of the Yasuda-Shedlovsky equations (Table 4) the following conclusions can be drawn. The slope is highly dependent on the chemical structure of bases, e.g. primary and secondary aliphatic amines (mexiletine and ephedrine) have closely identical slopes but they differ remarkably from papaverine which contains an aromatic N functionality. At the same time, for a given base the slopes calculated for the three regions (using different R wt% ranges) do not exhibit considerable differences, at least no significant trend can be observed. Contrary to this, the slopes of acids containing carboxyl, and phenol functions are rather similar by total range extrapolation (86.6, 85.7, 75.2 for salicylic acid, phenobarbital

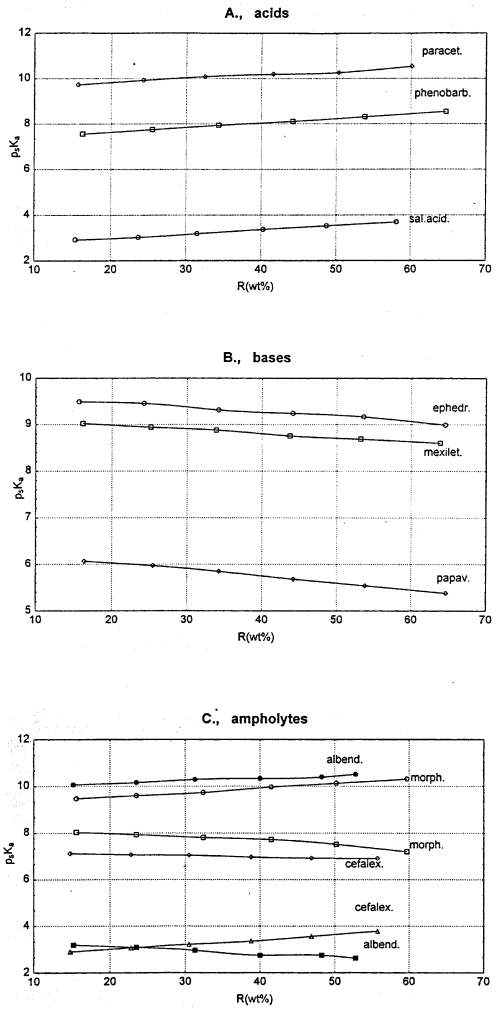

Fig. 2. The p$_s$$K_a$ values of test compounds in methanol/water mixtures as a function of weight percent of methanol.

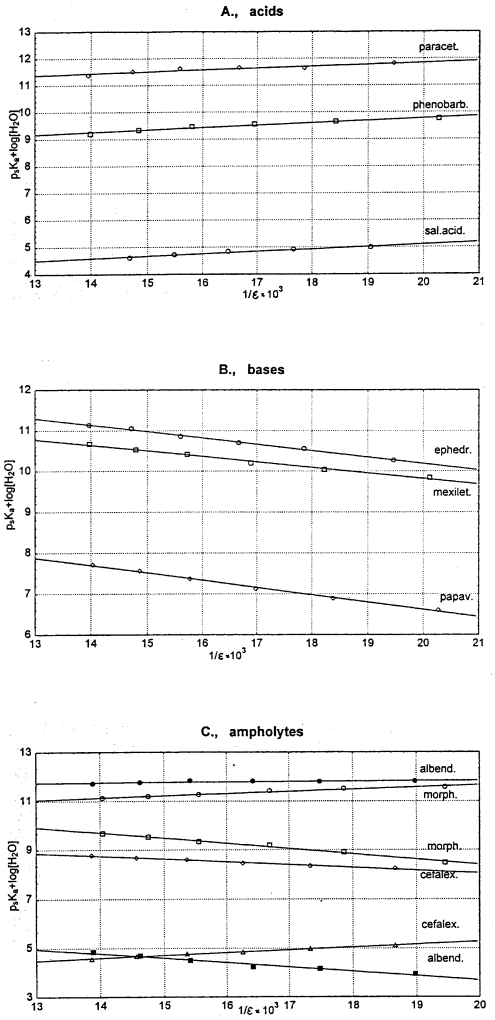

Fig. 3. The Yasuda-Shedlovsky plots of test compounds.

Table 3
Measured and extrapolated pK_a values of test compounds

Compound	$pK_{a(meas.)}$	$pK_{a(extrap.)}$		
		Total range	Water-rich region	MeOH-rich region
Salicylic acid	2.75 (1)	2.73 (3)	2.69 (2)	2.88 (1)
Phenobarbital	7.41 (3)	7.41 (5)	7.29 (1)	7.55 (1)
Paracetamol	9.63 (1)[a]	9.67 (8)	9.55 (1)	10.03 (3)
	9.60 (2)[b]	9.60 (7)	9.45 (2)	9.71 (6)
Ephedrine	9.64 (3)[a]	9.60 (2)	9.62 (1)	9.63 (3)
	9.64 (3)[b]	9.59 (2)	9.60 (3)	9.57 (2)
Mexiletine	9.14 (1)	9.07 (3)	9.08 (2)	8.93 (1)
Papaverine	6.28 (3)	6.18 (3)	6.23 (1)	6.05 (1)
Morphine				
(Phenol)	9.34 (1)[a]	9.46 (3)	9.42 (3)	9.56 (1)
(Amine)	8.18 (1)	8.24 (2)	8.25 (2)	8.27 (3)
(Phenol)	9.32 (6)[b]	9.27 (4)	9.26 (1)	9.44 (2)
(Amine)	8.23 (6)	8.21 (5)	8.22 (1)	8.43 (1)
Cefalexine				
(Amine)	7.14 (2)	7.15 (2)	7.15 (2)	7.04 (2)
(Carboxyl)	2.53 (2)	2.72 (2)	2.67 (1)	2.69 (2)
Albendazole sulfoxide				
(Amide)	9.93 (1)	9.99 (4)	9.88 (2)	10.06 (1)
(Ar. N)	3.28 (1)	3.32 (8)	3.36 (1)	2.87 (5)

[a] Measured in 5 mM concentration.
[b] Measured in 1 mM concentration.

and paracetamol, respectively) but slopes of acids studied here (including the acid function of ampholytes as well) have been tendentiously changed with the extrapolation range. The high slope for the equations in the water-rich region dramatically decreased in the methanol-rich one. These findings are in line with previous experiences (Avdeef et al., 1993) where benzoic acid and alkoxyphenols exhibited similar behaviour.

Since it is known that the slope of the Yasuda-Shedlovsky plots is inversely proportional to the average ionic diameter of the solvated molecule (Shedlovsky, 1962) our above results indicate a change in the solvation structure at about 35–40 wt% methanol content. The ionic diameters of the solvated acids increase with increasing methanol fraction showing the enhanced role of methanol in the solvation of the acidic functional group by hydrogen bonds. Theoretical studies on the solvation structure of nicotinic acid in methanol/water mixtures using Monte Carlo simulations (Nagy and Takács-Novák, 1997) found a comparable role of the solvent components in the solvation process via forming strong hydrogen bonds. The calculated coordination numbers suggested the dominance of methanol molecules in solvation above 40% methanol.

The plots and pK_a data of vancomycin which has six ionizable groups are shown in Fig. 4 and Table 6. The acid/base properties and proton speciation of this complicated structure have been described first by Takács-Novák et al. (1993). In the present work the molecule is used as a test compound to demonstrate that even in the case of multiple overlapping protonation processes, the solvent-mixture procedure of the PCA 101 can be successfully applied.

Table 7 contains the pK_a values of water-insoluble samples (group 2) obtained by the mixed-solvent approach. These compounds could not be

Table 4
Parameters of the Yasuda-Shedlovsky equations ($p_sK_a + \log[H_2O] = a/\epsilon + b$)

Compound	R (wt%) range	a	b	r^2	N	n
Salicylic acid	15–60	86.6	3.371	0.9723	6	18
	15–32	103.6	3.109	0.9604	3	9
	40–60	58.6	3.876	0.9906	3	9
Phenobarbital	15–65	85.7	8.058	0.9592	6	18
	15–34	142.4	7.212	0.9966	3	9
	44–65	62.4	8.495	0.9948	3	9
Paracetamol	15–60[a]	75.2	10.450	0.7746	6	18
	15–60[b]	73.8	10.401	0.8626	6	18
	15–33[a]	128.0	9.659	0.9994	3	9
	15–33[b]	160.1	9.146	0.9899	3	9
	40–60[a]	4.2	11.716	0.0127	3	9
	40–60[b]	48.6	10.829	0.7522	3	9
Ephedrine	15–65[a]	143.5	13.177	0.9980	6	18
	15–65[b]	159.5	13.371	0.9959	6	18
	15–35[a]	−156.6	13.364	0.9964	3	9
	15–35[b]	−164.7	13.449	0.9798	3	9
	40–65[a]	−149.0	13.278	0.9955	3	9
	40–65[b]	−155.9	13.307	0.9919	3	9
Mexiletine	15–64	−141.7	12.620	0.9922	6	18
	15–34	−145.2	12.681	0.9936	3	9
	43–64	−115.5	12.154	0.9994	3	9
Papaverine	15–65	−183.1	10.267	0.9958	6	18
	15–34	−200.0	10.527	0.9992	3	9
	40–65	−158.5	9.813	0.9991	3	9
Morphine	15–60[a]	59.3	10.450	0.9575	6	18
p_sK_{a1} (phenol)	15–60[b]	98.5	9.757	0.9651	6	18
	15–32[a]	80.6	10.133	0.9187	3	9
	15–32[b]	92.4	9.826	0.9998	3	9
	40–60[a]	40.3	10.791	0.9904	3	9
	40–60[b]	62.1	10.391	0.9773	3	9
p_sK_{a2} (amine)	15–60[a]	−184.0	12.338	0.9978	6	18
	15–60[b]	−204.6	12.563	0.9917	6	18
	15–32[a]	−188.8	12.407	0.9890	3	9
	15–32[b]	−224.5	12.833	0.9999	3	9
	40–60[a]	−188.4	12.421	0.9942	3	9
	40–60[b]	−251.4	13.383	0.9997	3	9
Cefalexine	15–60	−119.5	10.422	0.9868	6	18
p_sK_{a1} (amine)	15–32	−117.1	10.401	0.9822	3	9
	40–60	−94.9	9.997	0.9887	3	9
p_sK_{a2} (carboxyl)	15–60	111.5	3.044	0.9926	6	18
	15–32	140.1	2.624	0.9924	3	9
	40–60	119.0	2.910	0.9952	3	9
Albendazole sulfoxide	15–60	19.9	11.480	0.4477	6	18
p_sK_{a1} (amide)	15–32	75.9	10.657	0.9517	3	9
	40–60	0.5	11.801	0.1838	3	9
p_sK_{a2} (ar. N)	15–60	−206.8	7.700	0.9677	6	18
	15–32	−223.2	7.952	0.9974	3	9
	40–60	−105.5	5.963	0.9565	3	9

R is the wt% of methanol in solvent mixtures.
[a] In 5 mM.
[b] In 1 mM.

measured in aqueous solution (in 0.5–1 mM concentration) due to precipitation during the potentiometric titration. Solvent-mixtures with the lowest possible methanol content in which precipitation was not observed were used as the first R wt% value for p_sK_a measurements. Thus some compounds could be measured in the water-rich region (e.g. flumequine, prostaglandins, ibuprofen, quinine) while the others were soluble and measurable only in mixtures of $R > 30$ wt%. The least soluble drug examined here was hexachlorophene where the extrapolations could only be made from high methanol concentrations and over a rather narrow region (R: 42–60 wt%). The extrapolated pK_a data of water-insoluble samples were compared to pK_a values obtained by spectroscopy (our work) and to literature data measured by various methods (spectroscopy, conductivity, pH-dependent solubility measurements, etc). Considering the remarkably different experimental conditions in these methods the agreement of pK_a values is acceptable. The pK_a values of some potential drugs under development were determined by us and first reported here.

Table 5
3Differences between extrapolated and measured pK_a values ($\Delta = pK_{a\ extrapolated} - pK_{a\ measured}$)

Compound	Δ		
	Total range	Water-rich	MeOH-rich
Salicylic acid	−0.02	−0.06	0.13
Phenobarbital	0	−0.12	0.14
Paracetamol	0.04[a]	−0.08	0.40
	0[b]	−0.15	0.11
Ephedrine	−0.04[a]	−0.02	−0.01
	−0.05[b]	−0.04	−0.07
Mexiletine	−0.07	−0.06	−0.21
Papaverine	−0.10	−0.05	−0.23
Morphine			
(Phenol)	0.12[a]	0.08	0.22
(Amine)	0.06	0.07	0.09
(Phenol)	−0.05[b]	−0.06	0.12
(Amine)	−0.02	−0.01	0.21
Cefalexine			
(Amine)	0.01	0.01	0.10
(Carboxyl)	0.19	0.14	0.16
Albendazole sulfoxide			
(Amide)	0.06	−0.05	0.13
(Ar. N)	0.04	0.06	−0.41
$\lvert\Delta pK_a\rvert$	0.05	0.07	0.17

[a] In 5 mM.
[b] In 1 mM.

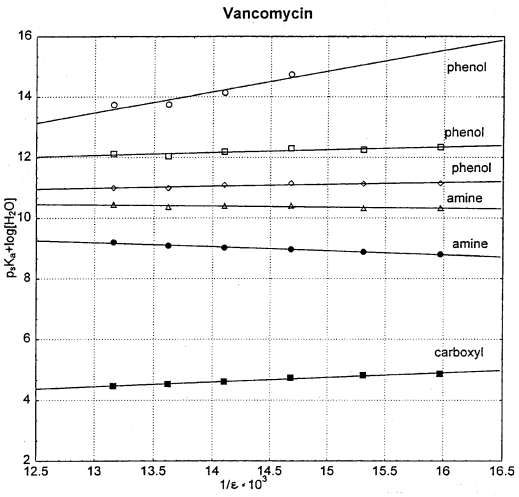

Fig. 4. The Yasuda-Shedlovsky plots of vancomycin.

Results of molecules in group 1 and 2 revealed the mostly anomalous behaviour of compounds having a phenol functionality (except morphine) in the mixed-solvent procedure. The Yasuda-Shedlovsky plots and the parameters of the relevant equations indicate very low slopes (almost horizontal straight lines) for paracetamol at 5 mM concentration in the methanol-rich region, at two of the phenol functions of vancomycin, at one phenol function of hexachlorophene and for naltrexone derivatives. The small changes of p_sK_a values with an increase of methanol content may be due to the less hindered dissociation in higher methanol %, because of the favourable solvation of phenolate by methanol molecules. However the role of ionpair formation should not be excluded.

4. Conclusions

The results presented here show the good applicability of the mixed-solvent procedure for pK_a determination in methanol/water mixtures. The reproducibility and reliability of this method, based on 431 separate titrations in the interval of 15–65 wt% methanol content using 25 model

Table 6
Measured and extrapolated pK_a values of vancomycin

Functional group	$pK_{a(meas.)}$	$pK_{a(extrap.)}$	MeOH range (wt%)	*a*	*b*	r^2	*N*	*n*
Phenol	11.88(1)	11.77(8)	6–24	444.32	7.843	0.8064	4	12
Phenol	10.15(1)	10.33(7)	6–37	82.08	11.022	0.7636	6	18
Phenol	9.28(1)	9.27(3)	6–37	46.12	10.420	0.7752	6	18
Amine	8.62(1)	8.71(4)	6–37	−42.20	10.995	0.6577	6	18
Amine	7.48(1)	7.46(3)	6–37	−126.21	10.812	0.9809	6	18
Carboxyl	2.64(1)	2.68(5)	6–37	140.40	2.634	0.9817	6	18

Table 7
Extrapolated pK_a values of water-insoluble substances

Compound	$pK_{a(extrap.)}$	MeOH range (wt%)	r^2	*N*	*n*	Literature pK_a
Flumequine	6.38 (4)	3–44	0.9682	5	15	6.35[a]
PGE_1	4.85 (7)	13–34	0.9461	5	15	5.02[b]
PGE_2	4.77 (9)	9–25	0.7669	6	18	4.94[b]
Ibuprofen	4.51 (7)	16–51	0.9768	3	9	5.2[f]
Hydrochlorotiazide						
$-SO_2NH_2$	10.22 (2)	23–37	0.8165	3	9	11.2[a]; 9.2[c]
$-SO_2NH-$ in ring	8.57 (4)	23–37	0.8461	3	9	8.9[a]; 7.0[c]
Hexachlorophene						
Phenol	10.83 (2)	42–60	0.9914	5	15	10.05[d]
Phenol	4.89 (2)	49–60	0.0003	4	12	4.21[d]
KHL-8430	10.60 (5)	29–40	0.9935	3	9	–
Haloperidol	8.65 (5)	40–60	0.9964	5	15	8.3[c]
Deramciclane	9.61 (3)	34–53	0.9944	5	15	–
Chlorpromazine	9.24 (2)	34–50	0.9975	3	9	9.30[c]
Quinine						
Quinuclidine N	8.55 (4)	15–69	0.9912	7	21	8.5[c]
Quinoline N	4.24 (9)	15–69	0.9745	7	21	4.1[c]
Niflumic acid						
Carboxyl	4.44 (3)	30–55	0.9606	5	15	5.14[e]
Ar. amine	2.26 (8)	30–55	0.6243	5	15	2.11[e]
Nitrazepam						
Lactam	10.51 (5)	10–64	0.8886	6	12	10.66[a]
=N–	2.84 (20)	10–64	0.9364	6	12	2.94[a]
HNB-1						
Phenol	9.20 (5)	33–60	0.4083	4	12	–
Piperidine N	8.49 (2)	33–60	0.6939	4	12	–
HNB-5						
Phenol	9.42 (4)	24–44	0.2581	6	18	–
Piper. N	8.64 (5)	24–44	0.9860	6	18	–
Ar N	4.31 (5)	24–44	0.9722	6	18	–

[a] Measured by spectroscopy (Takács-Novák et al., 1994a,b; Takács-Novák and Avdeef, 1996).
[b] Uekama et al. (1978).
[c] Craig (1990).
[d] Freese et al. (1979).
[e] Asuero (1989).
[f] Foye (1981).

compounds, were found to be acceptable. The pK_a data extrapolated to zero methanol content from the total interval examined or from the water-rich region agree well with the measured aqueous values (average error: 0.05 and 0.07). The extrapolations from methanol-rich regions give errors in pK_a not greater than ± 0.2 for weak acids and ± 0.1 for weak bases.

Remarkable changes in the solvation structure of weak acids could be concluded from the slopes of the Yasuda-Shedlovsky equations in mixtures with methanol content higher than 35–40 wt%. The greatest deviation from linearity was shown by phenols which draws attention to the specific solvation interactions of this functional group in methanol/water mixtures.

Based on our experiences in the above validation work, we propose the following recommendations:

(i) Proper electrode calibration using 'four-parameter' approach for each methanol/water mixture is essential; calibration must be made at the same constant temperature and ionic strength as to be used for pK_a measurements.

(ii) Selection of the lowest possible R (wt% methanol) value in which the compound is dissolved and measurements at a minimum of 3, advisably 5 or 6, different methanol/water mixtures in increments of 5–10% are recommended; the highest R value must be below 70%.

(iii) An appropriate concentration for accurate work is believed to be 1–5 mM; usage of inert gas atmosphere (nitrogen or argon) to avoid carbon dioxide absorption is also recommended.

(iv) A greater number of parallel measurements will increase the reliability of pK_a determination, particularly for phenols where small changes in p_sK_a values with increasing methanol content hardly exceed the experimental error.

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