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STUDY OF PH-DEPENDENT DRUGS SOLUBILITY IN WATER*

Abstract

The solubilities of five sparingly soluble drug-compounds in water were measured at constant temperatures (298.2 and 310.2 K) by the classical saturation shake-flask method. All substances presented in this work are derivatives of anthranilic acid: flufenamic acid (FLU), mefenamic acid (MEF) niflumic acid (NIF), diclofenac sodium (DIC) and meclofenamic sodium (MEC). All of them have anti-inflammatory action. Since the aqueous solubility of the ionized drug is significantly higher than the unionized, the experimental conditions that affect equilibrium solubility values such as composition of aqueous buffer were examined. The Henderson-Hasselbalch (HH) relationship was used to predict the pH-dependent solubility profiles of chosen drugs at two temperatures. For this purpose the pK_a values of the investigated drugs were determined using the Bates-Schwarzenbach spectrophotometric method at a temperature of 310.2 K. At temperature of 298.2 K these values were reported previously. Similar values of pK_a were obtained from the solubility measurements.

Keywords: derivatives of anthranilic acid, pH-solubility profile, pKa; shake flask method, Henderson-Hasselbalch approach.

The solubility of a drug is defined as the maximum quantity of a drug dissolved in a given volume of a solvent at chosen temperature, pressure and pH. For ionizable drugs, the solubility can be affected by the pH of the solution, and the intrinsic solubility (S_0) is defined as the concentration of a saturated solution of the neutral form of the drug, in equilibrium with its solid at constant temperature and pressure.

Nowadays, drug design approaches based on a combination of chemistry and quantitative structure–activity relationship led to new active substances that are less water soluble and more lipophilic. Not very lipophilic drugs reveal lower solubility in water and have trouble crossing membranes. The acidic group of a drug molecule becomes negatively charged by losing a hydrogen ion at pH < 7. Research in pharmaceutical chemistry has devoted little attention to the physicochemical properties of the chemical leads and has focused mainly on optimization of the *in vitro* activity [1-3]. The rate at which a drug goes into the solution when it is dissolved in an acidic or a basic medium is proportional to the solubility of the drug. Many drugs have different solubilities at different pHs. These pH-dependent solubility differences lead to pH-dependent dissolution profiles. The solubility-pH profile of drugs or amines has already been reported by many authors [4–13].

The Henderson-Hasselbalch (HH) equation [14] has been used many times for the mathematical description of the solubility-pH profile of drugs or amines existing in the solution as a monomer [4,6,7].

Aqueous solubility has an essential role in the bioavailability of oral drug formulations. There is an established classification, namely, the biopharmaceutical classification system (BCS), which divides drugs into four classes in terms of their solubility and permeability [15]. The BCS classification correlates the *in vitro* solubility and permeability to the *in vivo* bioavailability.

In recent years, the problem of drug solubility in water has become more acute and more common as pharmaceutical companies have improved drugs for certain therapeutic areas [16]. The accuracy of many

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predictive methods can be discussed when it is possible to compare calculated and measured solubility [17,18]. A number of useful experimental methods are reviewed, including the miniaturized shake-flask microtitre plate, the micro-solubility self-calibrating direct UV, the potentiometric and micro-dissolution methods [5] as well as the "new shake-flask method" [19].

The aim of the present study was to measure the solubility of five ionisable drugs: flufenamic acid (FLU), mefenamic acid (MEF), niflumic acid (NIF), diclofenac sodium (DIC) and meclofenamic sodium (MEC) at two temperatures, 298.2 and 310.2 K using the traditional shake-flask solubility method. We have already measured the thermodynamic solubility of these drugs as a function of temperature at natural pH 7 in water, ethanol and 1-octanol [20-22]. These results will be compared to the buffer solutions at the same temperature and pH. The solubility-pH profile of mefenamic acid as well as the pK_a values at two temperatures, 298.2 and 310.2 K have also been presented earlier [5] and will be compared to our new values.

The novelty of the present work is to show the effect of pH on the solubility in buffer solutions at two temperatures and ambient pressure. The intrinsic solubility, S_0 at two temperatures will be developed from the solubility-pH profile measurements. The pK_a values obtained using the Bates-Schwarzenbach spectrophotometric method will be compared with the pK_a values, coming from the solubility-pH profile measurements. The sigmoidal relationship of solubilities of weak acid drugs and two salts will be predicted using the HH equation.

MATERIALS AND METHODS

Chemicals and reagents

The following investigated drugs were obtained from Sigma Aldrich: flufenamic acid (CAS registry No. 530-78-9, ≥0.99), mefenamic acid (CAS registry No. 61-68-7, ≥0.99), niflumic acid (CAS registry No. 4394-00-7; ≥0.99), diclofenac sodium salt (CAS registry No. 15307-79-6; ≥0.99), meclofenamic sodium (CAS registry No. 6385-02-0; ≥0.99). The drugs were used without purification and were used as powder or small crystals. Other chemicals were as follows: methanol for HPLC – super gradient (CAS registry No. 67-56-1, POCH, 99.9%), hydrochloric acid (CAS registry No. 7647-01-0, POCH), sodium hydroxide (CAS registry No. 1310-73-2, POCH), dipotassium hydrogen phosphate (CAS registry No. 7758-11-4, POCH, ≥0.99), disodium tetraborate (CAS registry No. 1303-96-4, POCH, ≥0.999), sodium chloride (CAS registry No. 231-598-3, POCH, ≥0.999),

All solvents were filtrated twice with the Schott funnel with 4 μm pores. They were stored under freshly activated molecular sieves of type 4 Å. Water used as a solvent was twice distilled, degassed and filtered with Milipore Elix 3. The names, abbreviations, systematic (IUPAC) names, molecular formulas and molar mass of the drugs are given in Table 1.

The p*K*a measurements

The pK_a measurements were performed with the Bates-Schwarzenbach (BS) method using a UV-Vis spectrophotometer (Perkin Elmer Life and Analytical Sciences, Shelton, USA). The method was described in our previous paper [21]. The UV-Vis spectra for acidity constant measurements at temperature 310.2 K are presented at three conditions: buffer, 0.2 M HCl, and 0.12 M NaOH in Figures 1-5. The pK_a values are also determined from the solubility-pH profiles and are compared to those obtained with BS method.

pH-Dependent solubility studies

The solubility experiment was performed with a new small-scale shake flask method [23] at constant temperatures of 298.2 and 310.2 K. The shake-flask method proposed by Higuchi and Connors [24] is the most reliable and widely used solubility measurement method. This method determines thermodynamic solubility and could be carried out in several steps. Each drug was added in excess to 10 ml of dipotassium hydrogen phosphate (0.15 M) in a test tube. The test tubes were placed on a plate shaker. Using a pH-meter up to a stable pH (solubility equilibrium), the pH of each drug suspension was measured and adjusted if necessary with either diluted 0.2 M HCl, or

Table 1. Basic properties of drugs used in the investigations

Name of compound/ abbreviation	Systematic (IUPAC) name	Molecular formula	Molar mass M / g mol^{-1}
Flufenamic acid/ FLU	2-{[3-(Trifluoromethyl)phenyl]amino}benzoic acid	$C_{14}H_{10}F_3NO_2$	281.23
Mefenamic acid/MEF	2-(2,3-Dimethylphenyl)aminobenzoic acid	$C_{15}H_{15}NO_2$	241.30
Niflumic acid/ NIF	2-{[3-(Trifluoromethyl)phenyl]amino}nicotinic acid	$C_{13}H_9F_3N_2O_2$	282.22
Diclofenac sodium/ DIC	2-(2-(2,6-Dichlorophenylamino)phenyl)acetic sodium	$C_{14}H_{10}Cl_2NO_2Na$	318.13
Meclofenamic sodium/MEC	2-[(2,6-Dichloro-3-methylphenyl)amino]benzoic acid, sodium salt	$C_{14}H_{10}Cl_2NO_2Na$	318.13

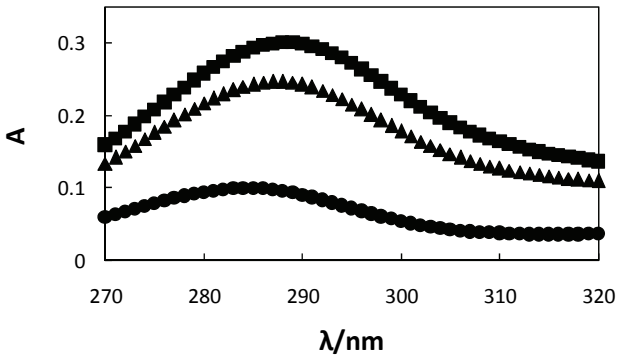

Figure 1. UV-Vis spectra for acidity constant measurement at temperature 310.2 K for flufenamic acid + solvent: (▲) buffer, (●) 0.2 M HCl, (■) 0.2 M NaOH.

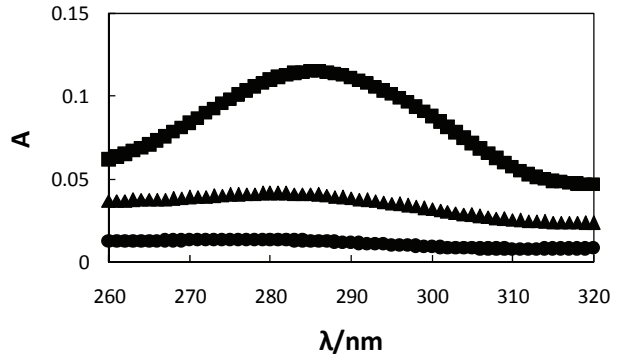

Figure 2. UV-Vis spectra for acidity constant measurement at temperature 310.2 K for mefenamic acid + solvent: (▲) buffer, (●) 0.2 M HCl, (■) 0.2 M NaOH.

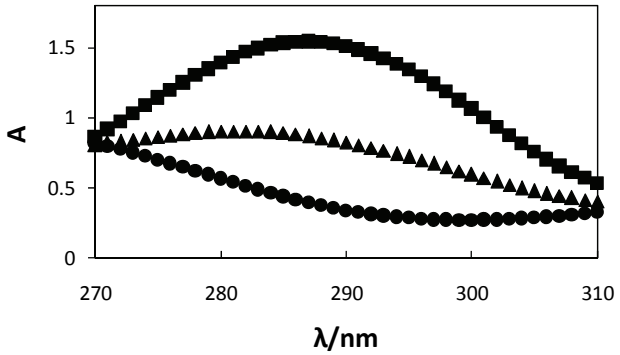

Figure 3. UV-Vis spectra for acidity constant measurement at temperature 310.2 K for niflumic acid + solvent: (▲) buffer, (●) 0.2 M HCl, (■) 0.2 M NaOH.

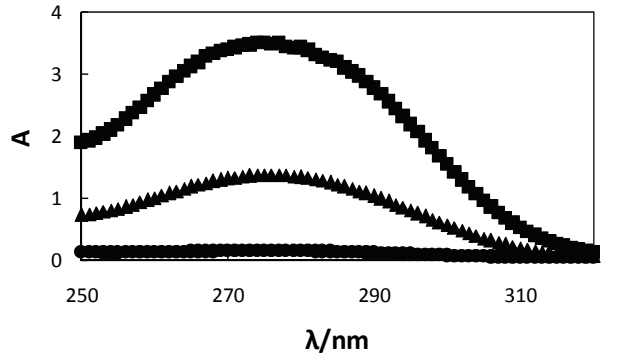

Figure 4. UV-Vis spectra for acidity constant measurement at temperature 310.2 K for diclofenac sodium salt + solvent: (▲) buffer, (●) 0.2 M HCl, (■) 0.2 M NaOH.

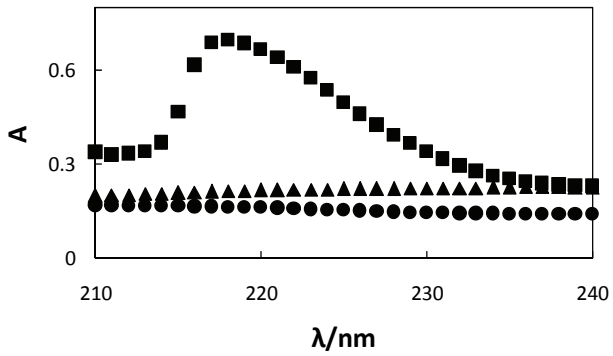

Figure 5. UV-Vis spectra for acidity constant measurement at temperature 310.2 K for meclofenamic sodium salt + solvent: (▲) buffer, (●) 0.2 M HCl, (■) 0.2 M NaOH.

0.2 M NaOH to a selected pH value. The experiment was completed when at least three pH measurements performed at an early, an intermediate and a late time-point of the 24 h period resulted in the same pH value. The equilibrium solubility was attained within 24 h. The pH values were also measured for the supernatants obtained after centrifugation, in order to certify that the drug solutions analyzed had the same pH value as the suspensions. Measurements were made using pH-meter (CPC-401 Elmetron) with an associated uncertainty of 0.01. The test-tubes were thermostated by a temperature control thermostat (Lauda A3, Germany) through the jacket of the vessel with uncertainty 0.1 K. Samples were withdrawn after 24 h, since a previous study had shown that a majority of drugs reach their solubility equilibrium within this time-scale [7,23]. Furthermore, this time-scale often is used in drug development settings. Excess solid was equilibrated using a rotating-bottle apparatus (Hettich Zenrifugen, EBA 20) at 300000 rpm for 30 min. Following centrifugation, the supernatant was collected and used for solubility and pH determinations. The concentration of the drug in the supernatant solution was determined by an HPLC procedure with single-wavelength UV detection. All compounds were analyzed in quadruplicates at each investigated pH value, as it was shown in the previous works [23,25,26].

The prerequisites in the selection of the buffer system were that the buffer should display an osmotic pressure comparable to the osmotic pressure of the intestinal fluid (278 mOsm/kg) and that it should have an acceptable buffer capacity. Thus, 150 mM K_2HPO_4 (340 mOsm/kg) was chosen as buffer, and this buffer was mixed with pure drug to obtain the desired pH values. For pH below the pK_a value the HCl was added and for the values above the pK_a values, the NaOH was added [7].

HPLC Analysis

Each sample contained excess of drug and buffer solutions, sodium hydroxide or hydrochloric acid solution. Drug concentration in each sample was measured using an HPLC-UV-Vis apparatus delivered by Agilent Technologies, consisting of: 1200 Series Quat pump, 1200 vacuum degasser, 1200 DAD/ /MWD. A C18 analytical column (4.6 mm×150 mm) with a mean particle size of 5 μm was used. One buffer was prepared for mobile phase: disodium tetraborate (0.01038 M $Na_2B_4O_7$ and 0.01925 M NaCl). As a mobile phase two solutions were used: methanol (A) and borate buffer, pH 9 (B). Injection volumes of 5 μl were used during the analysis. The chromatographic conditions for all the drugs are shown in Table 2.

Data analysis

The modified HH equation may be used for the pH-dependent solubility prediction in two forms, for monoprotic acids:

Table 2. Condition during chromatography separation

Compound	Mobile phase		Flow rate, ml min^{-1}	λ / nm
	Methanol (A), %	Borate buffer (B), %		
FLU	75	25	1.5	290
MEF	75	25	1.5	210
NIF	65	35	1.5	290
DIC	80	20	1.0	280
MEC	70	30	1.0	210

$$\log S = \log S_0 + \log(1+10^{pH-pKa}) \quad (1a)$$

and for monoprotic bases:

$$\log S = \log S_0 + \log(1+10^{pKa-pH}) \quad (1b)$$

where S_0 is the intrinsic solubility, S is the predicted solubility at a given pH, and p*K*a is a pH at which the concentration of unionized and ionized forms of a monoprotic drug in the solution are equal. The HH equation describes solubility as a function of pH. This equation always predicts an increasing solubility of all weak acids as pH increases, and descending solubility of bases when pH increases. In this paper all solubility values, S (mol dm^{-3}) are expressed as log S and are calculated from the HH equation for monoprotic acids (Eq. (1a)). The intrinsic solubility, S_0, was developed from the solubility-pH profile. Using the HH equation, the calculated curves were drawn for all experimentally studied drugs.

RESULTS AND DISCUSSION

The p*K*a of an ionisable compound is an important property, describing the charge state of the drug at a certain pH. However, according to Avdeef [6], it is not recommended to determine p*K*a values from the solubility-pH measurements (pK_a^S). The results listed in Table 3 show a reasonably good agreement between the results obtained by the two different methods at two temperatures: from the solubility-pH measurements (pK_a^S), and with the precise spectrophotometric Bates-Schwarzenbach method (pK_a^{B-S}). The pK_a^S values were obtained from the crossing of two lines interpolated from the experimental points for the unionized and ionized form of the chosen drug. The parameters and correlation coefficients are listed in Table 3. All the literature data of p*K*a values, obtained with different methods (including ours at 298.2 K for comparison) are presented in Table 4. Generally, our values are higher than those from literature with the exception of MEF. In our opinion the Bates-Schwarzenbach method is more precise because it does not use the co-solvent and the extrapolated values to pure aqueous solutions.

The classical shake-flask method was applied to measure the equilibrium solubility at two temperatures for five drugs over a wide pH range from 2 to 8 or 9. The time of stirring was chosen as 24 h at the same temperature and pH to get the repeatable results. After stirring, the two phases (solution and solid material) of the saturated solution were separated, and after 6 h (the necessary time needed for the separation of two phases) the supernatant was taken out for the concentration of drug measurements by HPLC-UV-Vis spectrometry. The theoretical HH solubility, S, was predicted based on the pK_a^{B-S} values and the intrinsic solubilities, S_0 determined by the shake-flask method at two temperatures. Data are listed in Table 4 together with the literature data of S_0. The results are discussed for each drug separately.

Flufenamic acid

During the last few years there were different values of pK_a and S_0 discussed in the literature at temperature 298.2 K for FLU. Our value of p*K*a = 4.62 [20] is close to the presented by Muñoz *et al.* [27], p*K*a = 4.17 and higher than all remaining data. In our opinion, the reason may also be insufficient control of the equilibrium temperature. The same parameters are responsible also for the values of the intrinsic solubility, S_0. The value obtained in this work at T =

Table 3. The linear regression parameters, a_1, b_1 for the unionized form and a_2, b_2 for the ionized form, the corresponding correlation coefficients, R_1 and R_2, the pK_a^S developed from the solubility-pH profile and the pK_a^{B-S} obtained from the Bates-Schwarzenbach method

Compound	Parameter						pK_a^S	pK_a^{B-S}
	a_1	b_1	R_1^2	a_2	b_2	R_2^2		
				298.1 K				
FLU	0.159	-5.835	0.989	1.124	-10.290	0.987	4.62	4.62 [20]
MEF	0.134	-6.698	0.939	1.455	-11.818	0.998	3.87	3.88 [21]
NIF	0.100	-4.465	0.959	0.677	-7.035	0.991	4.45	4.42 [20]
DIC	0.061	-4.322	0.968	1.022	-9.767	0.997	5.66	5.70 [20]
MEC	0.079	-7.138	0.901	1.073	11.543	0.994	4.43	4.39 [22]
				310.2 K				
FLU	0.150	-4.996	0.874	1.143	-10.220	0.997	5.26	5.23
MEF	0.184	-6.159	0.976	1.690	-12.680	0.998	4.33	4.33
NIF	0.142	-4.466	0.939	0.743	-7.234	0.998	4.60	4.60
DIC	0.096	-4.387	0.972	0.768	-7.423	0.998	4.52	4.51
MEC	-0.010	-6.631	0.912	1.036	-10.810	0.999	4.00	3.99

Table 4. The literature values of pKa^{lit}, intrinsic solubility, S_0^{lit}, and experimental intrinsic solubility, S_0^{exp}, developed from solubility-pH profile at two temperatures, 298.2 and 310.2 K

Compound	pKa^{lit}	S_0^{lit}/ mol dm^{-3}	S_0^{exp}/ mol dm^{-3}
FLU	4.62[B-S,a]	4.47×10^{-6} [b]	7.40×10^{-6}
	3.97[b,c]	8.32×10^{-6} [g]	5.20×10^{-5} (310.2 K)
	4.17[d]	3.24×10^{-5} [h]	
	3.84[e]	2.38×10^{-5} [i]	
	3.90[f]		
MEF	3.88[B-S,j]	4.57×10^{-7}[b,c]	5.75×10^{-7}
	4.22[b,c]	8.70×10^{-8} [g,k]	3.55×10^{-6} (310.2 K)
	4.5 [g]	1.70×10^{-4} [i]; 1.66×10^{-6} [l]	(310.2 K)
	4.64 (310.2 K)[g]	3.31×10^{-4} (310.2 K)[l]; 2.45×10^{-7} (310.2 K)[g]	
NIF	4.42[B-S,a]	3.39×10^{-5}[b,m]	7.61×10^{-5}
	4.44[b,m]	1.05×10^{-4} [m]	1.35×10^{-4} (310.2 K)
	4.86[n]		
	2.28[n]		
DIC	5.70[B-S,a]	2.58×10^{-6} [o, p]	1.01×10^{-4}
	3.99[o,p]		1.16×10^{-4} (310.2 K)
	4.00[o]		
	3.90[r]		
MEC	4.39[B-S,s]	1.38×10^{-7}[b]	1.58×10^{-7}
	4.10[b]		2.11×10^{-7} (310.2 K)

[a]Ref. [20]; [b]Ref. [28]; [c]Ref. [29]; [d]Ref. [27]; [e]Ref. [35]; [f]Ref. [36]; [g]Ref. [6]; [h]Ref. [37]; [i]Ref. [30]; [j]Ref. [21]; [k]Ref. [5]; [l]Ref. [31]; [m]Ref. [32]; [n]Ref. [33]; [o]Ref. [34]; [p]Ref. [9]; [r]Ref. [38]; [s]Ref. [22]

= 298.2 K, S_0 = 7.40×10^{-6} mol dm^{-3} is close to that presented by Avdeef [5], S_0 = 8.32×10^{-6} mol dm^{-3}, or by Box *et al*. [28], S_0 = 4.47×10^{-6} mol dm^{-3}. The two other values presented in Table 4 are higher. New values, presented in this work at T = 310.2 K are: pKa = 5.23 and S_0 = 5.20×10^{-5} mol dm^{-3}. As for most of chemical compounds, with an increase of temperature the solubility increases. The pKa also increases with an increase of temperature. Figure 6 shows the pH-equilibrium solubility profile for FLU at 298.2 (Figure 6a) and 310.2 K (Figure 6b). As the pKa values inform below pH 4.6 at 298.1 K and pH 5.2 at 310.2 K, a constant value of the solubility of this unionized form of weak acid is observed. Above these values of pH the compound transformed to the anionic form. At around pH 8 the horizontal plateau due to sodium-salt solubility was observed. The theoretical HH calculated curve from Eq. (1a) closely follows the experimental points.

Mefenamic acid

The mefenamic acid is the only compound for which the solubility-pH profile at two temperatures (298.2 and 310.2 K) was presented in the open literature [6]. The pKa = 4.33 value at 310.2 K is close to the published earlier pKa = 4.64 [6]. The other literature values of pKa and intrinsic solubility are close to ours [28,29] or much higher *i.e.*, S_0 = 1.7×10^{-4} mol dm^{-3} [30] in comparison with our value S_0 = 5.75×10^{-7} mol dm^{-3} at T = 298.2 K. Also the S_0 = 3.31×10^{-4} mol dm^{-3} at T = 310.2 K [31] is far from our data S_0 = = 3.55×10^{-6} mol dm^{-3}. All data are presented in Table 4. The solubility-pH profile for MEF is shown in Figures 7a and 7b for 298.2 and 310.2 K, respectively. Below pH 3.8 and 4.3 at 298.2 and 310.2 K there is a constant value of S of the unionized acid form. Above these two pH values MEF is present in the solution as ionized form. At around pH 6.5 the horizontal plateau due to sodium-salt solubility was observed. A significant deviation from the HH eq. can be observed for the ionized form of MEF (see Figure 7a and 7b). The slope of the linear part of the pH--solubility curve is far from the theoretical HH eq. defined as -1 for monoprotic weak acids. This can be explained as the salting out effect of phosphate counter-ions [4,7]. The pKa and solubility increase with an increase of temperature.

Niflumic acid

NIF is a popular drug for which the different values of pK_a and S_0 are presented in the literature at temperature 298.2 K. Our value of pKa = 4.42 [20] is close to the presented by Box *et al*. [28, 32] pKa = = 4.44 and higher than presented by Takács-Novák *et al.* [33], pKa = 2.28. The reason may be the different experimental method and the buffer used. The value of the intrinsic solubility at temperature 298.2 K, S_0 = = 7.61×10^{-5} mol dm^{-3} is close to those presented by

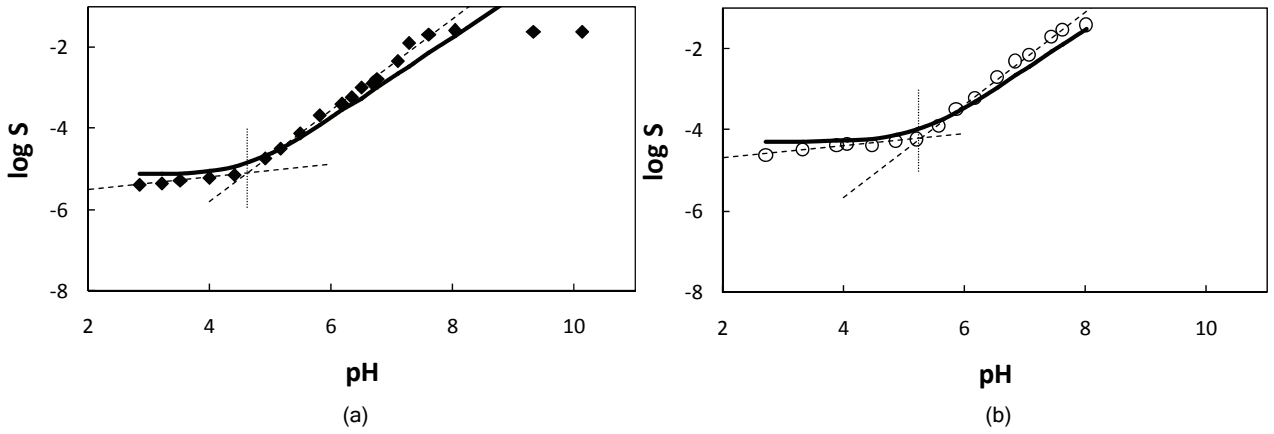

Figure 6. pH-dependent solubility profile of flufenamic acid (points are log S values measured by shake-flask method): a) experimental data at 298.2 K; b) experimental data at 310.2 K; the solid line was calculated with the Henderson-Hasselbalch equation; the dashed line was linear interpolation of the experimental points.

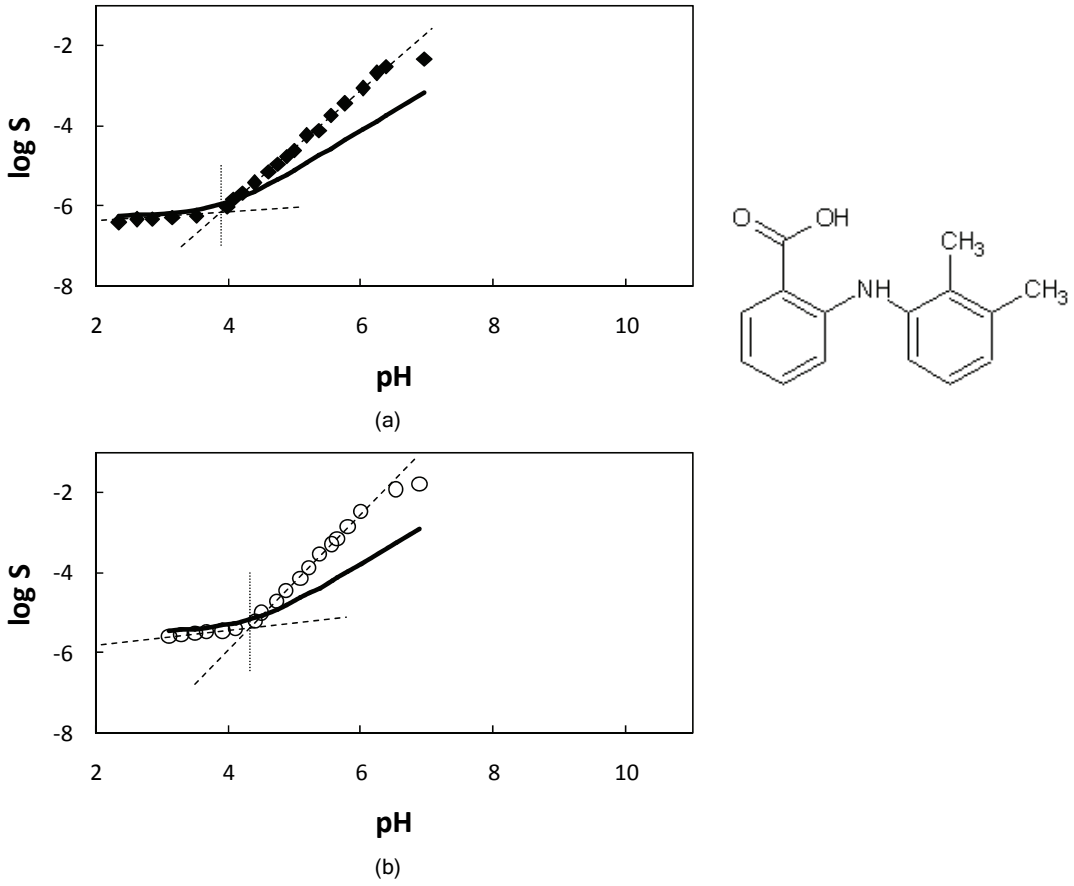

Figure 7. pH-dependent solubility profile of mefenamic acid (points are log S values measured by shake-flask method): a) experimental data at 298.2 K; b) experimental data at 310.2 K; the solid line was calculated with the Henderson-Hasselbalch equation; the dashed line was linear interpolation of the experimental points.

Box *et al*. [28,32] (see Table 4). The new values, presented in this work at T = 310.2 K are: pKa = 4.60 and S_0 = 1.35×10^{-4} mol dm^{-3}. The pKa and S increase when the temperature increases. Figure 8 shows the pH-equilibrium solubility profile for NIF at 298.2 (Figure 8a) and 310.2 K (Figure 8b). Below pH 4.4 at 298.2 K and pH 4.6 at 310.2 K a constant value of the solubility of the unionized form of NIF is observed. Above these values of pH the compound transformed to the anionic form. At around pH 7.5, the horizontal plateau due to sodium-salt solubility was observed. The theoretical HH calculated curve (Eq. (1a)) closely

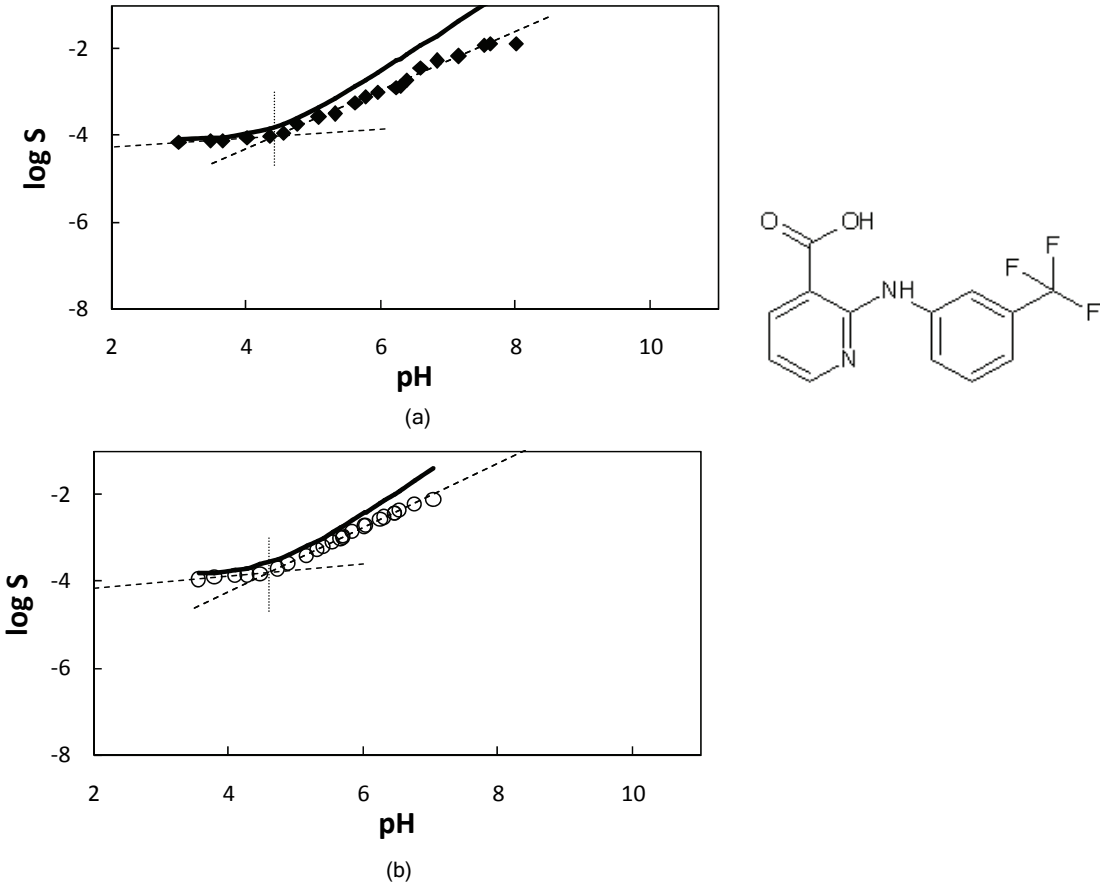

Figure 8. pH-dependent solubility profile of niflumic acid (points are log S values measured by shake-flask method): a) experimental data at 298.2 K; b) experimental data at 310.2 K. The solid line is calculated with the Henderson-Hasselbalch equation; the dashed line is a linear interpolation of the experimental points.

follows the experimental points for the unionized form and slightly deviates for ionized form. The p*K*a and solubility increase with an increase of temperature.

Diclofenac sodium

The sodium salt of diclofenac, DIC was used for the solubility measurements. The pK_a values are 5.70 [20] at T = 298.2 K and 4.51 at T = 310.2 K. All literature values at 298.2 K are much lower, which is shown in Table 4. Its intrinsic solubility is S_0 = = 1.01×10^{-4} mol dm^{-3} at T = 298.2 K, which is much higher than that in literature S_0 = 2.58×10^{-6} mol dm^{-3} [9,34]. The intrinsic solubility obtained by the shake-flak method in this work at T = 310.2 K is S_0 = = 1.16×10^{-4} mol dm^{-3}. Solubility increases as the temperature increases but the p*K*a decreases. In the later the inverse property is observed in comparison with weak acids. Figure 9 show the log *S*-pH profile of DIC. In solution at low pH DIC as an acid predominates; at high pH the ionized form of salt and associates of sodium cation predominate. The HH equation excellent describes the solubility, especially at T = 298.2 K.

Meclofenamic sodium

The meclofenamic sodium salt, MEC was used for the solubility-pH profile measurements. The p*K*a values are 4.39 [22] and 3.99 at T = 298.2 K and T = = 310.2 K respectively. The literature value at T = = 298.2 K is close to that, p*K*a = 4.10 [28] (see Table 4). The intrinsic solubility is S_0 = 1.58×10^{-7} mol dm^{-3} at T = 298.2 K, which is similar to that in literature S_0 = = 1.38×10^{-7} mol dm^{-3} [28]. The intrinsic solubility obtained by the shake-flak method in this work at T = = 310.2 K is S_0 = 2.11×10^{-7} mol dm^{-3}. Solubility increases as the temperature increases but the p*K*a decreases as it was observed for DIC. Figure 10 shows the log *S*-pH profile of MEC. Below the p*K*a values a constant value of the solubility of the unionized form of MEC is observed. In solution at low pH MEC as an acid predominates; at high pH the ionized form of salt and associates of sodium cation predominate. The HH equation fits extremely well to measured solubility values at both temperatures.

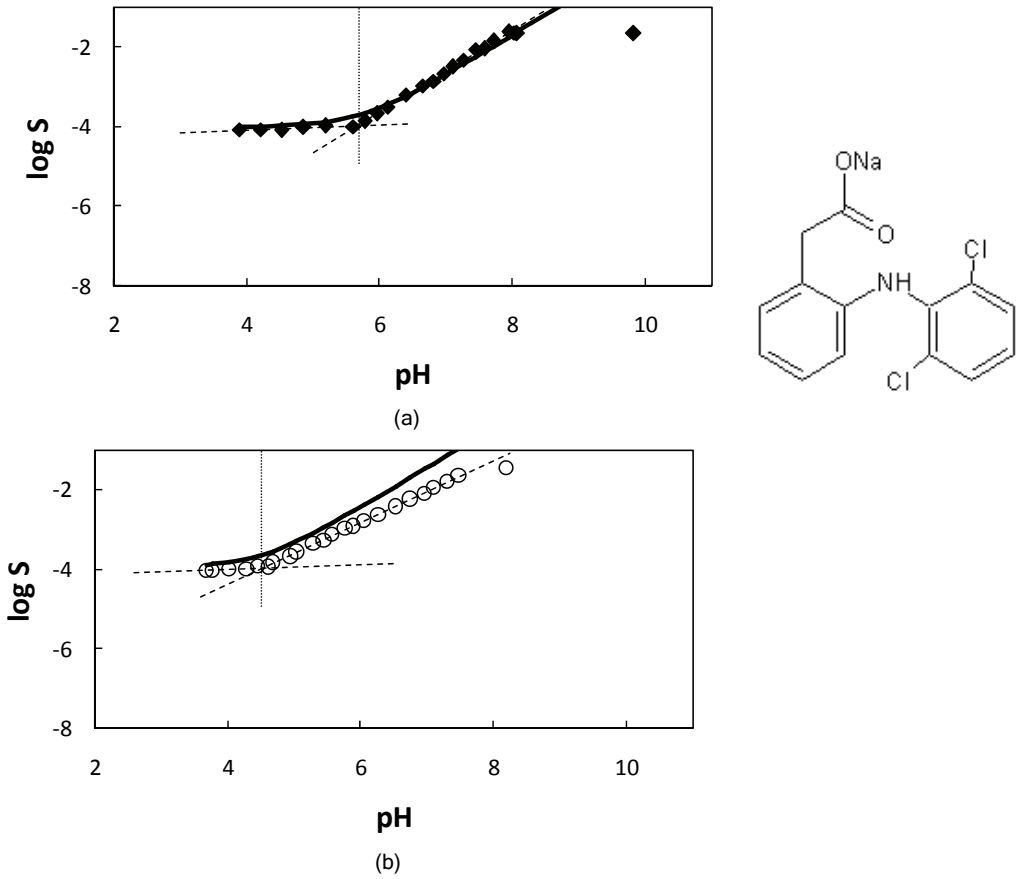

Figure 9. pH-dependent solubility profile of diclofenac sodium (points are log S values measured by shake-flask method): a) ♦, experimental data at 298.2 K; b) ○, experimental data at 310.2 K. The solid line is calculated with the Henderson-Hasselbalch equation; the dashed line is a linear interpolation of the experimental points at 298.2 K.

Some practical comparisons

The values of p*K*a increase as the temperature rises for weak acids and decrease for sodium salts. The dissociation reaction and the corresponding constants for a monoprotic weak acids and other substances were defined in an excellent review paper [6]. The dissociation, dimerization, or aggregation constant of different species in the solution is a temperature dependent constant. The van't Hoff equation shows that for a reaction that is exothermic under standard conditions ($\Delta_r H < 0$) the equilibrium constant K decreases as the temperature rises, which is observed for the measured sodium salts. The opposite occurs in the case of endothermic reactions, as for acids measured in this work.

The shake-flask method, used by pharmaceutical laboratories for the measurement of solubility of drugs in an aqueous buffer solution, assumes the thermodynamic equilibrium between the solid and liquid phase at constant temperature and pressure in saturated solution [4]. The dynamic solubility measurements at natural pH, or shake-flask method at natural pH used in physico-chemical, thermodynamic models/laboratories also assumes the thermodynamic equilibrium between the solid and liquid phase at constant temperature and pressure in saturated solution [22]. The only important difference between these two methods, as we can see, is an aqueous buffer solution in comparison with pure water. The solubility of drug in buffer solution reaches equilibrium at constant pH after few to 24 h. During the first few hours the pH of solution changes due to ionization, salt formation, association of cation, or anion of compound, the salting out effects coming from the buffer used, the common ion effect, the equilibrium formation of different associates, self-association by forming mixed-charge micelles or micelle-like structures and possible aggregation of different species. The influence of the kind of buffer used was recently discussed for the promethazine pH-equilibrium solubility [4]. Table 5 shows the results obtained at the same laboratory for the same drugs with two different methods in pure water solvent, S^T and in an aqueous buffer solution at the same temperature, S, an ambient pressure and the same pH. In general the solubilities of weak acids is higher in an aqueous buffer solution

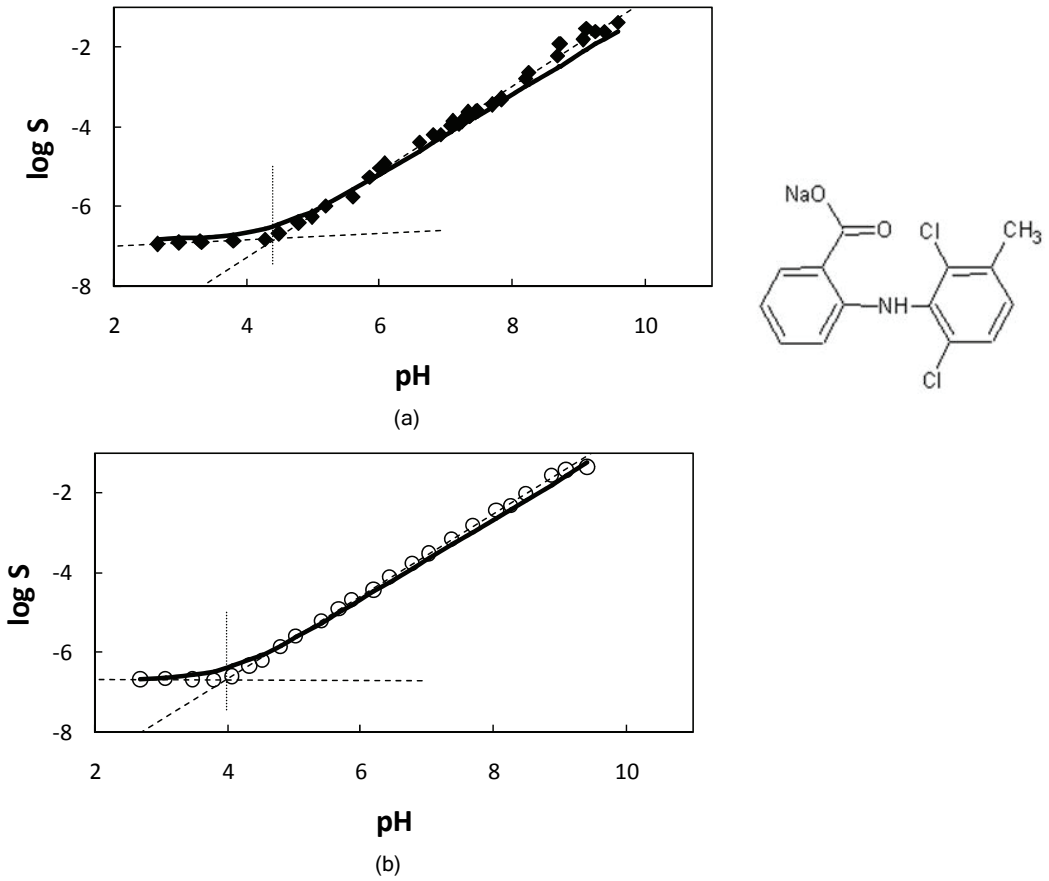

Figure 10. pH-dependent solubility profile of meclofenamic sodium (points are log S values measured by shake-flask method): a) experimental data at 298.2 K; b) experimental data at 310.2 K. The solid line is calculated with the Henderson-Hasselbalch equation; the dashed line is a linear interpolation of the experimental points at 298.2 K.

Table 5. Solubility of drugs derivatives of anthranilic acid in water obtained by dynamic method in natural pH 7, S^T, and by shake-flask method in buffer solutions, S, at pH 7

Compound	S^T / mol dm^{-3}	S / mol dm^{-3}
	298.2 K	
FLU	3.38×10^{-5}[a]	3.81×10^{-3}
MEF	$<5.55\cdot10^{-6}$[b]	4.87×10^{-3}
NIF	9.11×10^{-5}[a]	5.11×10^{-3}
DIC	2.89×10^{-2}[a]	2.47×10^{-3}
MEC	0.903[c]	9.31×10^{-5}
	310.2 K	
FLU	1.49×10^{-4}[a]	6.00×10^{-3}
MEF	$<5.55\times10^{-6}$[b]	1.62×10^{-2}
NIF	1.39×10^{-4}[a]	7.30×10^{-3}
DIC	0.202[a]	9.19×10^{-3}
MEC	2.09[c]	2.75×10^{-4}

[a]Ref. [20]; [b]Ref. [21]; [c]Ref. [22]

but that of the sodium salts are lower. However, MEF can exists at pH 7 as a sodium salt, the solubility is three ranges of order higher than that coming from the dynamic method. It is evident that weak acids drugs tend to be more soluble in an aqueous buffer solution than would be measured in pure water, or predicted by the thermodynamic models. The number of polar groups and hydrogen bond donors and acceptors always tend to have influence on the solubility [32].

CONCLUSIONS

This paper presents a systematic study of the p*K*a (at 310.2 K) and equilibrium solubility-pH profile measurements by the saturation shake-flask method of five drugs at 298.2 and 310.2 K. The solubility results depend on temperature and pH. Each of the investigated compounds reveals a specific pH-dependent solubility profile. It was shown that for some drugs as mefenamic acid or niflumic acid the HH equation has a limited applicability in phosphate buffer. The solubility of all drugs increases with an increase of pH and temperature. For all solutions above certain pH the characteristic plateau was observed. The p*K*a increases with an increase of temperature for weak acids and decreases for sodium salts.

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NAUČNI RAD

ISPITIVANJE RASTVORLJIVOSTI LEKOVA U VODI NA RAZLIČITIM pH VREDNOSTIMA

Rastvorljivost pet slabo rastvornih lekova u vodi je merena pri konstantnim temperaturama (298,2 i 310,2 K) klasičnom metodom zasićenja u erlenmajeru. Sve testirane supstance su derivati antranilne kiseline: flufenaminska kiselina (FLU), meklofenaminska kiselina (MEF), nifluminska kiselina (NIF), diklofenak-natrijum (DIC) i meklofenamik-natrijum (MEC), i poseduju anti-inflamatorno dejstvo. Pošto je rastvorljivost u vode jonizovanog leka znatno veća nego nejonizovanog, ispitani su eksperimentalni uslovi koji utiču na vrednosti ravnotežne rastvorljivosti, kao što je sastav vodenog pufera. Henderson-Hasselbalch jednačina (HH) je korišćena za predviđanje zavisnosti rastvorljivosti izabranih lekova od pH na pomenutim temperaturama. Za ovu svrhu su pKa vrednosti ispitivanih lekova utvrđene Bates-Schvarzenbach spektrofotometrijskom metodom na temperaturi 310,2 K, s obzirom na to da su vrednosti dobijene pri temperaturi od 298,2 K ranije publikovane. pKa dobijene ovim metodama i merenjem rastvorljivosti imaju slične vrednosti.

Ključne reči: derivati antranilne kiseline, rastvorljivost, pKa, metoda erlenmajera, Henderson-Hasselbalchova jednačina.