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Journal of Pharmaceutical and Biomedical Analysis 44 (2007) 57–62
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Physicochemical profile of nimesulide Exploring the interplay of lipophilicity, solubility and ionization

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Received 23 October 2006; received in revised form 18 January 2007; accepted 20 January 2007
Available online 30 January 2007

Abstract

The lipophilicity and solubility profile of nimesulide was investigated over a broad pH range. Lipophilicity was assessed by direct partitioning experiments in the octanol–water system using the shake flask method, as well as by reversed-phase HPLC using methanol as organic modifier with or without addition of *n*-octanol. In the latter case the extrapolated retention factors $\log k_w$ were considered as lipophilicity indices. The presence of *n*-octanol in the mobile phase proved to be a crucial factor for the establishment of a $\log k_w$/pH profile very similar to the $\log D$/pH profile of nimesulide. Solubility was determined by the shake flask method using saturated buffer solutions. Both lipophilicity and solubility–pH profiles of nimesulide showed deviations from the theoretically expected behavior as dictated by the Henderson–Hasselbach equation and the usually recorded difference of 4 log units between the corresponding values of the neutral and ionized species in the case of a weak acid. As a consequence the lipophilicity and solubility profiles were found not to be mirror images of each other. However, the pK_a value of nimesulide could be accurately calculated using part of both lipophilicity and solubility profiles since deviations affected mostly the values at increased ionization.

Keywords: Nimesulide; Lipophilicity; Solubility; Ionization; Reversed-phase HPLC; Mobile phase additive

1. Introduction

Permeability and solubility have served as fundamental properties for the establishment of the Biopharmaceutical Classification System [1]. Permeability per se is strongly influenced by lipophilicity, usually expressed by octanol–water distribution coefficients $\log D$ [2,3]. Both lipophilicity and solubility depend on pH in the case of ionizable compounds, therefore, in order for the characterization of highly soluble drugs the physiological relevant pH range 1–8 is considered [4]. The knowledge of the above interrelated physicochemical properties, as well as of the ionization constants provides a better understanding for the efficacy of already approved drugs, while it is essential for the design of new drug molecules.

Nowadays, a number of reliable calculation approaches are available for the prediction of the lipophilicity of the neutral species, designated as $\log P$ [5–8]. Less efficient is the estimation of $\log D$ which further involves the prediction of pK_a values and the effect of counter ions, as well [9]. Methods in order to estimate the intrinsic solubility are also being developed and are based on artificial neural networks or model equations [10,11]. However, their predictive power still needs to be validated by experimental data.

As regards the experimental determination of lipophilicity, reversed-phase HPLC offers several practical advantages compared to the traditional shake flask method. Under suitable conditions that guarantee the predominance of partition mechanism in retention, 1:1 correlation may be obtained between octanol–water $\log D$ values and extrapolated retention factors, $\log k_w$ [12,13]. Standard chromatographic procedures that require the addition of a small amount of *n*-octanol in the mobile phase have been proposed for basic and neutral drugs, referring to the physiological pH 7.4 [14,15]. Recently, the presence of *n*-octanol, as mobile phase additive, proved to be crucial factor for the lipophilicity assessment by HPLC in the case of acidic drugs, as well [16]. Measurements, however, were conducted at low pH at which the ionization of acidic drugs was totally suppressed. To this point it should be noted that only a few studies report a systematic monitoring of retention versus $\log D$ data as a function of pH [17].

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doi:10.1016/j.jpba.2007.01.035

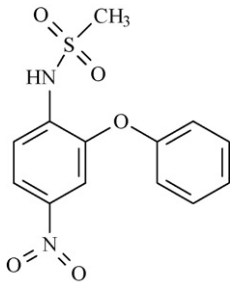

Fig. 1. Chemical structure of nimesulide.

In the present study, we investigated the physicochemical profile of nimesulide over a broad pH range by means of experimental and calculative procedures. Nimesulide is an effective non-steroidal anti-inflammatory drug acting by inhibiting preferentially cycloxygenase-2 [18]. In its neutral form it is sparingly soluble in water (~0.01 mg/ml) and efforts to develop formulations that promote its solubility have been reported [18,19]. Since, however, it possesses a weakly acidic sulfonanilide group both its lipophilicity and solubility are pH dependent (Fig. 1). The aim of the present study was to address the following issues: (a) investigation of the lipophilicity and solubility profile of nimesulide as a function of pH and comparison with theoretical profiles and calculated values, (b) comparison between direct partitioning/pH and retention/pH profiles in presence and absence of *n*-octanol as mobile phase additive and (c) exploration of the appropriateness of the lipophilicity and solubility profiles to generate the pK_a value of nimesulide.

2. Materials and methods

Nimesulide was provided by Elpen A.E. Sodium hydrogen phosphate, potassium dihydrogen phosphate and 3-morpholinepropanesulfonic acid (MOPS), all analytical grades, were purchased from Merck, Darmstadt, Germany. Octanol was extra pure and purchased by Panreac Quimica, Spain. Methanol (MeOH) was HPLC grade and purchased from Lab-Scan Analytical Sciences Ltd., Ireland. Water was deionized and further purified by means of a Milli-Q Plus water purification system (Millipore Co., USA). Metrohm 654 pH-meter was used for pH measurements of the aqueous phase.

2.1. Measurement of partition/distribution coefficients

Octanol–water distribution coefficients were determined by the shaking flask method as in reference [20]. Phosphate buffer in a pH range 2.5–11.6 was used as the aqueous phase. The two phases were mutually saturated before the experiment. Concentration of nimesulide solutions in octanol saturated buffer was in the range 10^{-4}–10^{-5} M. The volume ratio of the two phases was chosen so that adequate amount of the solute remained in the aqueous phase after equilibration. Equilibration time ranged between 1 h for low pH to 3 h at higher pH at which nimesulide is less lipophilic due to ionization. Centrifugation followed for 15 min at 2500 rpm. The aqueous phase was analyzed before and after equilibration by HPLC applying the conditions described under Section 2.2. A linear relationship between peak areas and concentration was established for the concentration range used, which permitted distribution coefficients to be calculated according to Eq. (1).

$$D = \frac{A_o - A_1}{A_1}\frac{V_{aq}}{V_{oct}} \quad (1)$$

A_o and A_1 being the peak area before and after equilibration, respectively.

Each determination was performed at least in triplicate using different initial concentrations and the mean values were used to produce the log D versus pH profile. The standard deviation did not exceed ±0.03 log units. In Table 1 the mean values of log P^N of the neutral form, log P^A of the anion and log $D_{7.4}$ are reported.

2.2. HPLC analysis

The HPLC system consisted of a GBC Model LC1120 pump and a Rheodyne Model 7725i injector with a 20 μl loop, which was coupled to a GBC Model LC1210 UV–vis detector (8 μl flow cell), operated at 254 nm. A BDS C-18 column (250 mm × 4.6 mm i.d., 5 μm particle size) was used as stationary phase. The mobile phase consisted of a mixture of methanol/MOPS buffer 60/40 at pH 7.4. It was filtered through a 0.20 μm nylon membrane before use. Data acquisition and recording of peak areas were performed using WinChrom Chromatography software package, ChemWin v.1.2. Each sample was injected at least three times and the mean peak area was obtained with R.S.D. ranging between 0.95 and 1.10%.

2.3. Determination of extrapolated retention factors by reversed-phase HPLC

The stationary phase was the same described in Section 2.2. Mobile phase conditions were as follows: (A) 20 mM MOPS buffer in a pH range 2.5–7.4/MeOH in a concentration 45–65% and (B) *n*-octanol saturated 20 mM MOPS buffer in a pH range 2.5–7.4/40–60% MeOH + 0.25% *n*-octanol in respect to the volume of MeOH. Retention times t_r were measured at least in triplicate and converted to the logarithm of retention factors log k via Eq. (2):

$$\log k = \log\left[\frac{t_r - t_o}{t_o}\right] \quad (2)$$

t_o being the retention time of methanol.

For each pH five different concentrations of methanol were used. Then, isocratic retention factors were used to determine log k_w values by linear extrapolation according to Eq. (3):

$$\log k = -\varphi S + \log k_w \quad (3)$$

φ and log k_w being the slope and the intercept of the regression curve. Correlation coefficients r were >0.9974 in each case. The standard deviation of log k_w did not exceed 0.04. In Table 1 log k_w values corresponding to the neutral species (log k_w^N) and those at pH log $k_{w7.4}$ along with their statistical data are reported.

Table 1
Experimental and calculated lipophilicity data of nimesulide: neutral form ($\log P^N$ or $\log k_w^N$), anionic form ($\log P^A$) and at pH 7.4 ($\log D_{7.4}$ or $\log k_{w7.4}$)

Methods	$\log P^N$ or $\log k_w^N$	$\log P^A$	Diff($\log P^{N-A}$)	$\log D_{7.4}$ or $\log k_{w7.4}$
Octanol/water	2.39 (± 0.04)	−0.29 (± 0.03)	2.68	1.48 (± 0.04)
ADME boxes	2.57	−1.52	4.09	1.05
Pallas	2.68	−0.67	3.35	2.62
HPLC retention	3.11 (±0.02), $r = 0.999$	*	–	2.71(±0.01), $r = 0.999$
HPLC retention (+ *n*-octanol)	2.26(±0.01), $r = 0.9998$	*	–	1.58(±0.02), $r = 0.999$

(*) Not determined due to the column pH limitations.

2.4. Solubility measurements

In respect to the molar absorptivity (ε) measurements, accurately weighed 0.0305 g of nimesulide were dissolved in 10 ml ethanol. Four hundred microlitres of the ethanol solution were diluted to 50 ml by the appropriate phosphate buffer in a pH range 4.5–11.0, so that a concentration of 7.91×10^{-5} M was obtained. The final pH of the nimesulide solutions differed less than 0.02 of the buffer used. Absorption was measured at 393 nm and converted to molar absorptivity (ε). Molar absorptivity changed from 1131 cm^{-1} M^{-1} at pH 4.5 to 15165 cm^{-1} M^{-1} at pH 11. The stability of solutions was examined after staying overnight and no degradation was observed.

Nimesulide saturated phosphate buffer solutions in the pH range 4.5–11.0 were shaken in a thermostatic flask (25 °C) for 4 h and allowed to stand overnight. This time interval guaranteed equilibration. After centrifugation absorption of the supernatant was measured at 393 nm and converted to molar concentration using the molar absorptivity determined above. Each measurement was performed in triplicate and the mean value of the molar concentration was converted to its logarithmic form $\log S$. Standard deviation did not exceed ±0.03. In Table 2 $\log S_o$ values of the neutral form, $\log S_i$ of the ionized form and $\log S_{7.4}$ are reported.

2.5. Calculation of lipophilicity, pK_a and solubility

$\log D$/pH profile was calculated using two softwares:

(A) PrologD module implemented in Pallas 3.1.2.1 (CompuDrug Chemistry Ltd.). PrologD makes use of pKalc to predict pK_a and PrologP to predict $\log P$ of the neutral form [9,21]. For PrologP the default option was used and the calculated $\log P$ value is the weighted average of the values provided by CDR (modified Rekker's system) Atomic6 (modified Ghose–Crippen system) and ANN (Artificial Neural Network option with Ghose–Crippen fragments as input).

(B) ADME Boxes 4.0 by PharmaAlgorithm Inc. [22].

The calculated values corresponding to $\log P^N$ of the neutral form $\log P^A$ of the anion and $\log D_{7.4}$ are included in Table 1.

The pK_a values were calculated from the above mentioned software. They were also generated from the lipophilicity or solubility/pH profiles by nonlinear fitting according to Henderson–Hasselbach based Eqs. (4)–(6). Lipophilicity in Eqs. (4) and (5) is expressed by $\log D$ and $\log k_w$, respectively.

$$\log D = \log P - \log(1 + 10^{pH-pK_a}) \quad (4)$$

$$\log k_w = \log k_w^N - \log(1 + 10^{pH-pK_a}) \quad (5)$$

$$\log S = \log S_o + \log(1 + 10^{pH-pK_a}) \quad (6)$$

Calculated pK_a values along with their standard deviations and the corresponding correlation coefficients are included in Table 3.

Intrinsic solubility was estimated by AlogpS 2.1, IAlogS and SRC's WsKow programs available in the internet (www.vcclab.org and www.syrres.com, respectively) as well as using the Absolv option of PharmaAlgorithms (Table 2). The first two softwares calculate intrinsic lipophilicity by means of artificial neural networks which have been constructed using 75 electrotopological state indices as input or 238 MOLCONNZ molecular indices, respectively [10]. SRC's WsKow calculates $\log S_o$ from the corresponding $\log P$ value, estimated by the Meylan–Howard calculation procedure and the melting point

Table 2
Experimental and calculated intrinsic and effective solubility values of nimesulide

	M[a]	mg/l
$\log S_o$	−4.25 (±0.03)	17.3
$\log S_i$	−2.99 (±0.01)	315.5
$\log S_{7.4}$(exp)	−3.21	190.1
$\log S_{7.4}$(HH)[b]	−3.68	64.4
AlogpS	−4.24	17.7
IAlogS	−4.77	5.24
$\log S$(SRC)	−4.06	26.9
$\log S$(Absolv)	−3.74	56.1

[a] Molar concentration.
[b] Calculated from $\log S_o$ using Eq. (6).

Table 3
Experimental and calculated pK_a values of nimesulide

Methods	pK_a	r
$\log D$/pH profile	6.49(±0.03)	0.9939
$\log k_w$/pH profile	7.22(±0.02)	0.9963
$\log k_w^{oct}$/pH profile	6.81(±0.03)	0.9945
ADME	5.90(±0.50)	–
Pallas	8.17	–
$\log S$/pH profile	6.43(±0.17)	0.8884
Potentiometry[a]	6.46	–
Spectrophotometry[b]	6.56(±0.01)	–

[a] Taken from [26].
[b] Taken from [27].

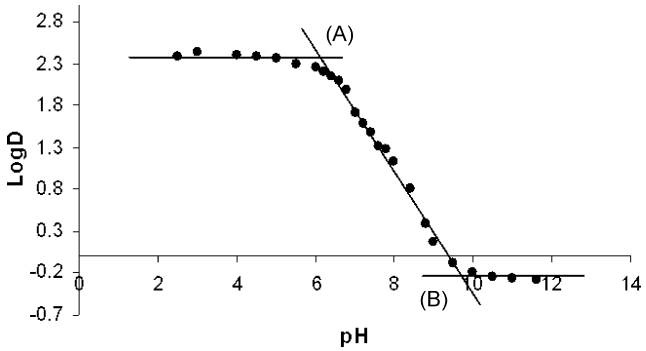

Fig. 2. Experimental log D vs. pH profile of nimesulide. A, pK_a and B, octanolic pK_a.

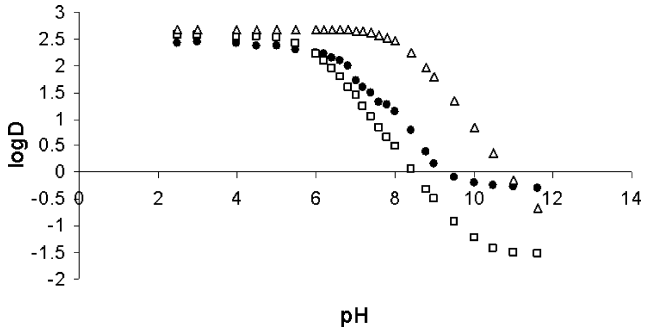

Fig. 3. Graphic comparison between experimental (●) and calculative (Pallas △ and ADME □) log D vs. pH profiles of nimesulide.

[11]. log S (Absolv) was derived by Abraham's sovatochromic equation for solubility implemented in ADME Boxes 4.0 [23].

2.6. Statistical analysis

For linear and nonlinear regression SPSS for windows 10.0 software package was used.

3. Results and discussion

3.1. Experimental log D/pH profile

Octanol water distribution coefficients were determined at a pH range 2.5–11.6. The log D/pH profile is illustrated in Fig. 2. At pH < 4 the flat region of the curve corresponds to the partitioning of the neutral form log P^N, while at pH > 9 the flat region corresponds to the partitioning of the anion in the form of an ion pair log P^A (Table 1). The difference between log P^N and log P^A values, diff(log P^{N-A}) equals 2.68, while for weak acids a difference of 4 log units is usually recorded at standard conditions of 0.15 M KCl. In absence of electrolyte an even larger difference should be anticipated. However, deviations from this rule are also observed [24]. The presence of a nitro group in para position to the sulfonamide may result in a charge delocalization which according to Scherrer could be one of the reasons for lower diff(log P^{N-A}) [25]. The slope between these two flat regions is assumed to be −1 for a monoprotic acid. The experimental slope ($\check{S}_{exp}$) was established by regression analysis considering the log D/pH profile in the pH range 6.6–8.0. As reported in Table 4, it was found considerably lower than |1|.

Table 4
Slopes of the linear part of the experimental and calculated lipophilicity vs. pH profile of nimesulide

Profile	$\check{S}$	r
log D_{exp}/pH	0.76(±0.02)	0.9958
log D_{ADME}/pH	0.94(±0.01)	0.9997
log D_{Pallas}/pH	0.93(±0.01)	0.9995
log k_w/pH	0.44(±0.34)	0.9939
log k_w^{oct}/pH	0.68(±0.03)	0.9975
log S/pH	1.15(±0.08)	0.9924

In Fig. 2 points A and B, where the horizontal asymptote lines intersect with the line of the linear part of the curve, correspond to the pK_a value of nimesulide and to its octanolic pK_a^{oct}, respectively. The pK_a of nimesulide was calculated from the log D/pH profile in the pH range 2.5–7.4 using Eq. (3). The value estimated by this procedure (p$K_a = 6.49 \pm 0.03$) is in agreement with the experimental values reported in literature [26,27] (Table 3). The octanolic pK_a, firstly introduced by Scherrer [28] is a conditional constant associated with the ion pairing and depending on the ionic strength and the buffer constituents. It can be calculated via the interrelation of the four equilibrium constants reflected in Eq. (7) [29]:

$$\text{diff}(\log P^{N-A}) = \log P^N - \log P^A = |pK_a^{oct} - pK_a| \quad (7)$$

Using Eq. (7) the pK_a^{oct} for nimesulide under the applied conditions was found equal to 9.23.

3.2. Comparison between experimental and calculated log D/pH profiles

The calculated log D/pH profiles are illustrated in Fig. 3. Both calculation procedures provide acceptable prediction concerning the log P^N (Table 1). Upon ionization large differences are obtained due to poor prediction of the pK_a value, especially in the case of Pallas 3.2.1.1 (Table 3), as well as to the lower slope of the experimental log D/pH profile, already discussed. In addition, the calculated log P^A values are considerably lower than the experimental one, so that the corresponding diff(log P^{N-A}) are higher than 3 log units (Table 1).

3.3. Comparison between octanol–water partitioning and retention/pH profile

Retention was expressed by the extrapolated retention factors log k_w^{oct} and log k_w corresponding to the presence and absence of n-octanol as mobile phase additive, respectively. The retention/pH profile was established in the entire pH range permitted by the column limitations (2.5–7.4) and is illustrated in Fig. 4. In Fig. 4, the corresponding experimental log D/pH profile is also presented for comparison reasons. In absence of n-octanol, enhanced retention was observed with log k_w much higher than the corresponding log D values. Moreover, the absolute value of slope of the linear part of the log k_w/pH relationship is consid-

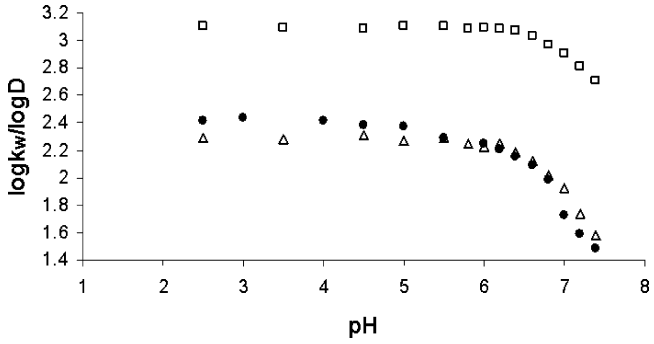

Fig. 4. Graphic comparison between chromatographic log k_w (□), log k_w^{oct} (△) indices and log D (●) values as a function of pH.

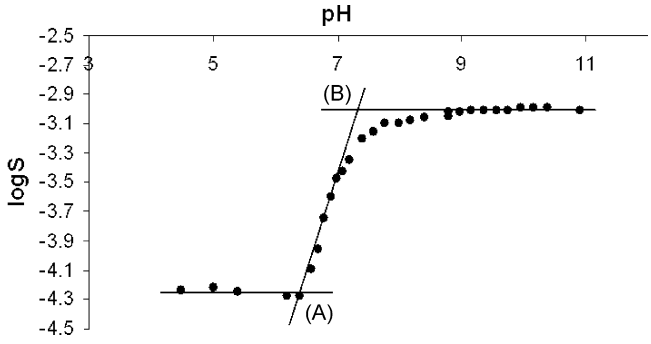

Fig. 5. Experimental logs vs. pH profile of nimesulide.

erably lower compared to the expected value of |1| as well as to the experimental slope of the log D/pH relationship (Table 4). This behavior may be attributed to secondary silanophilic interactions which become more pronounced as the pH increases due to the ionization of the free silanols including in the stationary phase. Indeed, it is well known that silanophilic interactions strongly increase the retention of basic compounds but also, to a lesser extent, of compounds containing strong hydrogen acceptor groups like nimesulide [30]. Addition of *n*-octanol resulted in a substantial decrease of the extrapolated log k_w values (Table 1, Fig. 4). At the same time, the slope of the linear part of the log k_w^{oct}/pH relationship increased to the level of the slope of the log D/pH relationship (Table 4, Fig. 4). The precise mechanism through which *n*-octanol influences the retention is not fully clarified. However, the above findings support the assumption that it may act like a masking agent covering the free silanol groups. As a result of the alterations in retention induced by *n*-octanol the log k_w^{oct}/pH showed a close similarity to the log D/pH profile (Fig. 4). Nevertheless, at the limit of pH 7.4 the degree of ionization of nimesulide is still not very high, so that this behavior cannot be generalized for drugs with a stronger acidic function.

The retention/pH profiles were further used to derive the pK_a value of nimesulide.

Nonlinear fitting of log k_w and log k_w^{oct} applying Eq. (5) led to very good correlation coefficients in both cases (Table 3). However, the pK_a value derived by the retention/pH profile in absence of *n*-octanol was considerably larger indicating a reduced acidic function, most probably as a result of the secondary silanophilic interactions mentioned above. In presence of *n*-octanol, the pK_a derived by the retention/pH profile was still higher but quite close to the experimental value (Table 3).

3.4. Comparison of lipophilicity and solubility profiles

Solubility/pH profile of nimesulide was established in the pH range 4.5–11 and is illustrated in Fig. 5. At pH < 4 the flat region of the curve corresponds to intrinsic solubility of the neutral form log S_o, whereas at pH > 9 the flat region corresponds to the solubility of the anion log S_i. The values of log S_o, log S_i, as well as, of the effective solubility at pH 7.4, log $S_{7.4}$, are presented in Table 2 along with the corresponding values expressed as mg/l. The available calculated values for log S_o are also included in Table 2. AlogPS, based on artificial neural networks using electrotopological state indices as the input, provided a very accurate estimation of intrinsic solubility. Less precise but still acceptable are the estimates of IAlogS and log S(SRC), while log S(Absolv) calculated by the solvatochromic equation provided an overestimation of solubility.

The difference diff(log S^{o-i}) equals to 1.28 log units, much lower compared to the corresponding lipophilicity difference diff(log P^{N-A}). It should be noted that according to the general rule a difference diff(log S^{o-i}) of 4 log units is expected for weak acids [29]. Exceptions to this rule, however, have been reported in literature indicating that the counter ion effect is substance specific and this behavior is difficult to generalize [31]. The slope between these two regions is assumed to be +1 for a monoprotic acid. The experimental slope ($\check{S}_{exp}$) established by regression analysis considering the linear part of the log S/pH profile in the pH range 6.4–7.4 was found slightly steeper (Table 4). Bergström et al. suggested that steeper slopes may be the result of low molecular weight aggregates, like dimers [31]. As a result of the steeper slope, estimation of log $S_{7.4}$(HH) from intrinsic solubility by means of the Henderson–Hasselbach type Eq. (6) led to a lower value than the experimental log $S_{7.4}$ (Table 2).

In Fig. 5 points A′ and B′, where the horizontal asymptote lines intersect with the line of the linear part of the curve, correspond to the pK_a value and to the Gibbs pK_a of nimesulide, respectively. The latter relates to the pH at which precipitated salt is in equilibrium with the free acid [32] and is estimated by Eq. (8) which reflects the interrelationship of the tetrad equilibrium constants.

$$\text{diff}(\log S^{o-i}) = \log S_o - \log S_i = |pK_a^{Gibbs} - pK_a| \quad (8)$$

Estimation of the pK_a value of nimesulide from the solubility/pH profile using the pH range 4.5–7.4 provided pK_a = 6.43 ± 0.17. A moderate correlation coefficient r = 0.888 was obtained in this case. The estimated pK_a value, although with larger error, is in agreement with the experimental values reported in literature [26,27], as well as with the pK_a value derived by the log D/pH profile (Table 3). Using Eq. (8) the Gibbs pK_a could be calculated and was found to be equal to 7.78. This finding in combination with the difference in the absolute value of the slopes in the lipophilicity and solubility–pH relationships of nimesulide indicate that the two profiles are not mirror images of each other.

4. Conclusions

In the present study, nimesulide, a noncarboxylic weak acidic drug, was used as a template to investigate the three interrelated physicochemical properties, essential in passive diffusion: lipophilicity, ionization and solubility. The presence of *n*-octanol in the mobile phase proved to be a crucial factor for the establishment of a $\log k_w$/pH profile very similar to the $\log D$/pH profile of nimesulide. The role of *n*-octanol was assumed to be associated with its potential to reduce silanophilic interactions, acting as a masking agent. This finding supports the advantages of HPLC in lipophilicity assessment of weak acidic compounds over a broad pH range and contributes to the standardization of the relevant chromatographic conditions. Both lipophilicity and solubility/pH profiles of nimesulide showed deviations from the theoretically expected behavior as dictated by the Henderson–Hasselbach equation and the general rule of 4, concerning the difference between the corresponding values of the neutral and ionized species in the case of a weak acid. Analogous results are reported in literature for basic drugs, indicating that predictions based on HH equation should be considered only as rough estimates [31]. Nevertheless, since deviations affected mostly the values at increased ionization, the pK_a value of nimesulide could be accurately calculated using part of both lipophilicity and solubility profiles. The above results on the interplay between the three physicochemical properties indicate the boundaries within which knowledge of one of them can be safely used to obtain information on the other two.

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