Biphasic pK_a Values

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Abstract: A novel approach – termed here as biphasic pK_a values – is presented for quantifying the acidity/basicity of lipophilic compounds embedded in water-immiscible media (membranes or solutions) at equilibrium with aqueous solution. In this approach the hydrogen ion activity is determined in the aqueous phase, while the concentration ratio of protonated and deprotonated forms of acid/base is measured in the lipophilic phase with any suitable technique. The approach has been demonstrated on the example of biphasic pK_a values of some lipophilic indicator bases in the octanol : water system, measured using UV-Vis spectrophotometry. The measurement is straightforward if the counterion identity and concentration and overall ionic strength in the aqueous phase are included in the standard state definition.
Biphasic pK_a values are envisaged to be useful for characterizing lipophilic indicators, synthetic receptors, phase transfer catalysts and other implements that are used in hydrophobic media (e.g. in sensor membranes) in contact with water.
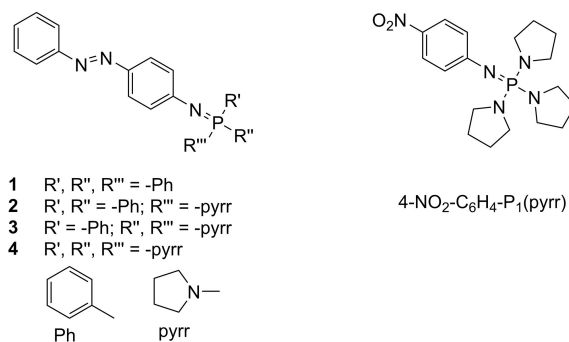
Keywords: acid strength, base strength, nonaqueous solvents, membranes, two-phase equilibria.

INTRODUCTION

ACID-BASE processes in water-immiscible media that are at equilibrium with water, and involving lipophilic acids or bases, are important in many fields: catalysis (phase transfer catalysis,[1] reactions in emulsions[2]), sensors (behaviour of analytes and receptor/ionophore molecules in sensor membranes),[3] bioprocesses (involving cell membranes),[4] etc. pK_a values – the key descriptors of acidity-basicity of molecules[5] – are not easily determined directly in low-polarity media because of the difficulties in quantifying the activity of H$^+$.[6] Aqueous pK_a values are not adequate for description of the solute properties in such biphasic systems, because the involved acids/bases have low aqueous solubility and reside mainly in the lipophilic phase.

In this work an original approach for quantifying acidity/basicity of lipophilic molecules in practically water-immiscible solvents, termed as biphasic pK_a^{ow} values, is proposed for the first time. In this approach the hydrogen ion activity is measured in the aqueous phase, while the concentration ratio of the two forms of the acid/base is measured in the organic phase. We describe the measurement method and present the results of realizing this approach on the example of a set of lipophilic indicator

bases (Scheme 1). The need for such approach has been envisaged by others[7] but to the best of our knowledge it has never been experimentally realized.


1 R', R'', R''' = -Ph
2 R', R'' = -Ph; R''' = -pyrr
3 R' = -Ph; R'', R''' = -pyrr
4 R', R'', R''' = -pyrr

4-NO$_2$-C$_6$H$_4$-P$_1$(pyrr)

Scheme 1. Structures of the lipophilic indicator bases studied in this work.

METHOD

The concept is based on adopting the conventional acid dissociation equilibrium to a situation where both base B and its protonated form BH$^+$ mainly reside in the organic phase, while the pH (for quantifying H$^+$ activity) is measured

in an aqueous phase that is at equilibrium with the organic phase (i.e. the phases are mutually saturated). This concept is realized here on the example of cationic acids BH$^+$ in octanol : water system. It is equally applicable to neutral acids and other solvents immiscible with water. Since solvated H$^+$ ions predominantly reside in water, protonation-deprotonation of the base is likely accompanied by transfer of either H$^+$ ions or bases through the phase boundary. Proton exchange can occur in the aqueous phase as well as the organic phase saturated with water. From the point of view of the pK_a^{ow} the exact mechanism of protonation process is irrelevant because equilibrium constant characterizes the equilibrium state of the system irrespective of how the system arrives into that state. It is important to note that if the aqueous and organic phases are at equilibrium, the thermodynamic activities of the solvated proton (defined via its chemical potential) are equal in both phases, even though the H$^+$ concentrations may be vastly different.[8] The electroneutrality of the phases is preserved by migration of counterions A$^-$ into the lipophilic phase upon protonation of the free bases. It is also expected that in the environment that is eminently less polar than water the ions predominantly reside as ion pairs. The overall equilibrium is:

$$(BH^+A^-)_O \rightleftarrows B_O + H^+_W + A^-_W \qquad (1)$$
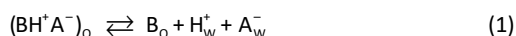
where the subscripts "O" and "W" refer to the organic and aqueous phases, respectively. The corresponding equilibrium constant is expressed via activities of the species as follows:

$$K = \frac{a(B)_O \cdot a(H^+)_W \cdot a(A^-)_W}{a(BH^+A^-)_O} \qquad (2)$$

If the activity of A$^-$ in water and the overall ionic strength of the aqueous phase are kept constant and included in the standard state definition then the equation can be written as follows and the equilibrium constant can be termed as K_a^{ow} :

$$K_a^{ow} = \frac{a(B)_O \cdot a(H^+)_W}{a(BH^+A^-)_O} \qquad pK_a^{ow} = -\log K_a^{ow} \qquad (3)$$

Although it is expected that the ions are predominantly ion-paired in octanol, some amount of BH$^+$ can also be present as free ions. We assume here that the term $a(BH^+A^-)$ accounts for all of the BH$^+$ in octanol.

The nature and activity of the counterion A$^-$ (and its conjugate acid AH) is an important influencing factor in the case of pK_a^{ow} as defined here. According to Eq. (1) higher lipophilicity and activity of A$^-$ both promote formation of BH$^+$A$^-$. For that reason, when using the pK_a^{ow} as defined here, the standard state has to define both the identity and activity of A$^-$. The conjugate acid AH has to be a sufficiently

strong acid to (1) avoid distribution of the neutral AH into the organic phase (the closer the real system is to the simplified model defined by Eq. (1), the easier it is to reproduce the experimental conditions and interpret and compare the results); and (2) ensure that the species [BH$^+$A$^-$] is indeed an ion pair, as opposed to a hydrogen-bonded associate of neutral forms (B···H–A).

Partitioning of the ionized form BH$^+$ (and possibly also the neutral B) into the aqueous phase will inevitably take place to at least some extent and will lead to a decrease of the overall concentration of B in the organic phase. However, the $a(B)_O/a(BH^+A^-)_O$ ratio would not be influenced by that, because it is determined only by the thermodynamic acidity of the organic phase and the pK_a^{ow} of BH$^+$. This is the reason why, at first sight unexpectedly, the log$P$ value of B is not a part of the model equation.

EXPERIMENTAL

Synthesis and characterization of the used lipophilic bases have been described elsewhere.[9] In addition, phosphazene 4-NO$_2$-C$_6$H$_4$-P$_1$(pyrr)[10] was included as a reference compound, having comparatively different structure and directly measured aqueous pK_a value. The rest of the used chemicals were of commercial origin: acetonitrile (Rathburn, HPLC grade, used for preparing stock solutions of base B), octan-1-ol (Sigma-Aldrich, Chromasolv grade), glycine (Reanal, analytical reagent), *p*-toluenesulfonic acid monohydrate (Sigma-Aldrich, ACS reagent, >98.5 %), sodium *p*-toluenesulfonate (Reakhim, "pure"), HBr (Fisher, 48 %, ACS reagent), NaCl (Fisher, ACS reagent) and NaBr (Fisher, ACS reagent). Water was prepared using a MilliQ Advantage A10 setup.

Octanol solutions of each base were prepared and individual aliquots of those solutions equilibrated with equal volumes of aqueous phases having different pH values. Then the phases were separated and their absorption spectra were recorded. With every base the measurements were carried out at 4 different concentrations ranging from 3 to 43 μmol L^{-1}. The experiments were carried out at ambient temperature (23 ± 2) °C.

Ionic strength of the aqueous phase of 0.1 mol L^{-1} was used as the standard state of the aqueous phase and chloride (Cl$^-$), bromide (Br$^-$) or *para*-tosylate (*p*-TsO$^-$) anions were used as the counterion A$^-$. Aqueous phases containing different counter-anions were prepared by mixing 0.1 M solutions of the respective acids, solutions containing 0.1 M of the respective sodium salt and 0.1 M of NaOH, and solutions containing 0.1 M of the respective sodium salt and 0.1 M of glycine. The solutions were combined in such a way as to achieve the desired pH while keeping the counterion concentration in the aqueous phase constant and the overall ionic strength nearly

constant. In the most basic solutions the ionic strength was up to 0.12 M, which is insignificantly different from 0.1 M.

The $a(\text{H}^+)$ values were measured in water using Metrohm 744 pH meter and Mettler Toledo InLab Micro pH electrode, calibrated with Hydrion pH 4.01 and 7.00 buffers (the 0.01 M HCl solution was used as a check solution and its observed pH was always in the range of 1.93 to 2.03). For equilibration, 1.5 mL of the octanol solution and the same volume of the aqueous phase were transferred into 4 mL vials, followed by 1 min agitation after which the mixture was allowed to stand for 20 min to remove the formed emulsion. Confirmation experiments were carried out allowing equilibration for up to 200 min, no difference was found in results. Consistency of the results within a series and between series carried out on different days further confirms that these times were sufficient for equilibration. pH of the aqueous phases was checked after equilibrating and the change was found negligible. Also, it was confirmed that no significant partitioning of the protonated base into the aqueous phase took place.

The absorbances were measured spectrophotometrically (Thermo Spectronic Evolution 300) at a wavelength corresponding to the maximum difference of molar absorptivities of the neutral and protonated forms of the specific base (404/346, 403/347, 404/349 and 405/349 nm for **1**, **2**, **3** and **4**, respectively). Since absorbances reflect concentrations and not activities of the chromophoric species in the solution, first "concentration-based" $\text{p}K_{\text{ac}}^{\text{ow}}$ values (Eq. 4) were obtained.

$$K_{\text{ac}}^{\text{ow}} = \frac{[\text{B}]_{\text{O}} \cdot a(\text{H}^+)_{\text{W}}}{[\text{BH}^+]_{\text{O}}} \qquad \text{p}K_{\text{ac}}^{\text{ow}} = -\log K_{\text{ac}}^{\text{ow}} \tag{4}$$

The $\text{p}K_{\text{ac}}^{\text{ow}}$ values were calculated by least squares fitting of the theoretical absorbance change resulting from ionization of B (expressed by Eq. 5) with the experimental one, varying the $\text{p}K_{\text{ac}}^{\text{ow}}$ values as well as the absorbance values corresponding to B and BH⁺.

$$A_{\text{calc.}} = \frac{A_{\text{BH}^+}}{1 + 10^{\,\text{p}K_{\text{ac}}^{\text{ow}} - \text{pH}}} + \frac{A_{\text{B}} \cdot 10^{\,\text{p}K_{\text{ac}}^{\text{ow}} - \text{pH}}}{1 + 10^{\,\text{p}K_{\text{ac}}^{\text{ow}} - \text{pH}}} \tag{5}$$

For every base B the $\text{p}K_{\text{ac}}^{\text{ow}}$ values were determined at 4 different concentrations C_i (on different days) and it was discovered that their concentration dependence is not negligible. So, the $\text{p}K_{\text{a}}^{\text{ow}}$ values of bases were found as intercepts by extrapolating the $\text{p}K_{\text{ac}}^{\text{ow}}{}_{i}$ values to zero concentration of B according to the following equation:

$$\text{p}K_{\text{ac}}^{\text{ow}}{}_{i} = \text{p}K_{\text{a}}^{\text{ow}} - A\sqrt{C_i} \tag{6}$$

This was carried out using least squares minimization and assuming that the constant A has to be the same for all bases. The value 40 was found for A. The obtained $\text{p}K_{\text{a}}^{\text{ow}}$ values are presented in Table 1. The root mean square deviation (RMSD) of the minimization was 0.15 pK_{a} units.

RESULTS

The obtained $\text{p}K_{\text{a}}^{\text{ow}}$ values together with some other data are presented in Table 1.

The above found RMSD 0.15 can be interpreted as taking into account all within- and between-day random effects. The possible systematic uncertainty of pH measurement was estimated as 0.05 (at standard uncertainty level). These two uncertainty components are expected to account for essentially all uncertainty sources and lead to the combined standard uncertainty of 0.16 $\text{p}K_{\text{a}}^{\text{ow}}$ units.

The $\text{p}K_{\text{a}}^{\text{ow}}$ values of compounds **1–4** obtained with Cl⁻ as counterion are linearly correlated with the aqueous pK_{a} values:

$$\text{p}K_{\text{a}}^{\text{ow}} = \left(1.57 \pm 0.02 \right) \cdot \text{p}K_{\text{a}} - \left(7.8 \pm 0.1 \right)$$
$$R^2 = 0.9998 \quad S = 0.03 \tag{7}$$

DISCUSSION

The main findings from the data presented in Table 1 are:

(1) The $\text{p}K_{\text{a}}^{\text{ow}}$ values are markedly – on an average by 2.7 pK_{a} units – lower than the conventional aqueous pK_{a} values. This is not unexpected. On one hand, in octanol, relative to water, the neutral B is more stabilized compared to its protonated form BH⁺, even if the latter is ion-paired with A⁻. On the other hand, transfer of the anion A⁻ from water to octanol is also energetically disfavored.

(2) The $\text{p}K_{\text{a}}^{\text{ow}}$ values within the compound series follow the trend of aqueous pK_{a} values. The within-series correlation between the two parameters (Eq. 7) is very good. The slope of the correlation is higher than unity, indicating somewhat better differentiating ability of the biphasic $\text{p}K_{\text{a}}^{\text{ow}}$ values as opposed to the conventional aqueous pK_{a} values.

(3) The biphasic pK_{a} value is strongly dependent on the anion A⁻. The more lipophilic is A⁻ the smaller is the difference between $\text{p}K_{\text{a}}^{\text{ow}}$ and pK_{a}.

The main advantage of the $\text{p}K_{\text{a}}^{\text{ow}}$ values over customary pK_{a} values is the possibility to characterize the acid-base behavior of molecules in non-aqueous media, such as polymer membranes, lipid bilayers, etc., while at the same time using the conventional aqueous pH scale. This approach is very relevant in all those applications where the hydrophobic medium is at equilibrium with water, such as sensors, phase-transfer catalysis, etc. The correlation of biphasic pK_{a} values with their aqueous counterparts can in principle provide means for estimating aqueous pK_{a} values of weakly soluble compounds. However, the dependence of the correlation parameters on the type of compound needs to be further investigated.

Table 1. The pK_a^{ow} values obtained in this work together with the conventional aqueous pK_a values for comparison

Compound	Aqueous pK_a[a]	pK_a^{ow}	Counterion
1	7.7[9]	4.3	Cl^-
2	8.4[9]	5.4	Cl^-
3	9.2[9]	6.7	Cl^-
4	10.2[9]	8.2	Cl^-
4-NO_2-C_6H_4-P_1(pyrr)	9.22[10]	6.4	Cl^-
1	7.7[9]	4.5[b]	Br^-
1	7.7[9]	6.1[b]	p-TsO^-

[a] Estimated values, see Ref [9].

[b] Tentative values obtained from a measurement series at one concentration and applying Eq. (6).

Recently the novel approach to evaluation of pH, the concept of unified pH (pH$_{abs.}$), defined via the chemical potential of solvated proton in a respective medium, has been introduced.[8] The pH$_{abs.}$ values are most conveniently expressed as pH$_{abs.}^{H_2O}$ values, whereby the pH$_{abs}$ scale has been shifted in such a way that the acidities accessible in water match the aqueous pH values.[11] As mentioned above, the definition via chemical potential automatically means that the pH$_{abs.}^{H_2O}$ values of a low polarity medium and an aqueous solution at equilibrium with it are equal. Thus, the pK_a^{ow} values can also be interpreted as pK_a values in octanol saturated with water whereby the pH in octanol is quantified as pH$_{abs.}^{H_2O}$.

CONCLUSIONS

In this work an original approach for quantifying acidity/basicity of lipophilic molecules in water-immiscible solvents is proposed for the first time. Its advantages are: (1) possibility to rigorously quantify acid-base equilibria in low-polarity media; (2) aqueous pH measurements are used, making the acidities expressed via biphasic pK_a values in different media comparable to the aqueous pH scale; (3) the obtained values are easily linked to the recently introduced pH$_{abs}$ scale (unified pH scale).

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