# Studies on equilibrium of anthranilic acid in aqueous solutions and in two-phase systems: Aromatic solvent–water

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## ARTICLE INFO

## ABSTRACT

The acid–base equilibria of anthranilic acid have been characterized in terms of macro- and microdissociation constants (dissociation constants $K_{a1}$, $K_{a2}$ and tautomeric constant $K_z$). On the basis of spectrophotometric investigations the values of the distribution ratio $D$ of anthranilic acid in the two-phase systems: aromatic solvent (benzene, ethylbenzene, toluene, chlorobenzene, bromobenzene)–aqueous solution were obtained. Employing the results of potentiometric titration in the two-phase systems: aromatic solvent–aqueous solution the distribution constant $K_D$ and dimerization constant $K_{dim}$ values were calculated. The influence of organic solvent polarity and pH of the aqueous phase on the contents of the particular forms of the acid in the two-phase systems were analyzed.

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## 1. Introduction

Anthranilic acid (2-aminobenzoic acid) is an amino acid that plays very important role in biological and chemical processes. It is widely applied as a starting material for synthesis of biologically active compounds and medicaments, for production of synthetic dyes and for metal ion determination [1–6]. It is the precursor of L-tryptophan in plants, yeast and bacteria [7–9] and is also involved in the biosynthesis of phytohormones (auxins) and their precursors [10,11]. Moreover anthranilic acid stimulates milk secretion in female mammals (it is called vitamin L1). Despite of numerous applications of anthranilic acid, its physicochemical properties and behaviour in solutions particularly in the two-phase systems, have not been examined yet. The data concerning the distribution of this acid in organic solvents are fragmentary, and refer to n-octanol and chloroform [12,13]. These data do not allow prediction of the physicochemical properties and behaviour of this acid in systems with other organic solvents.

One of the fundamental parameters of amino acids in aqueous solution is: an isoelectric point $pH_I$, dissociation constant of cationic form $K_{a1}$ ($H_2R^+ \rightleftharpoons HR + H^+$) and of neutral form $K_{a2}$ ($HR \rightleftharpoons R^- + H^+$). The macroconstant $pK_a$ values are commonly known while the microconstant values (especially tautomerization constant $K_z$) are often not recognized. The molecular interpretation of ionization-dependent phenomena requires the knowledge of the microconstants that describe the proton binding ability of the individual functional groups and are useful in calculating the pH-dependent concentrations of the different protonation isomers (microspeciation) [14–16]. Their accurate determination is important for complete understanding of the chemical behaviour and the biological activity of ampholytes. Especially in a biological context, the fraction of different active species at a particular pH is important and has to be considered in QSAR studies [17]. Literature data concerning zwitterionic constant for 3-aminobenzoic and 4-aminobenzoic acids are available in [18] while no data for 2-aminobenzoic acid have been found. This may be connected with the fact that substitution in the position *ortho* to the amino-group, introduces group interaction absent in the original zwitterionic compound. The most frequently used calculation methods based on the pH dependence of UV or NMR spectra are not suitable, because the protonation of both functional groups may influence on spectral changes. In such cases the extrapolation methods were used [18] but they are insufficiently accurate. Metzler and Snell [19] have worked out the experimental method for this kind of compounds which permits to determine spectrophotometrically the tautomeric constant from mixture of organic solvent and water at the pH of isoelectric point. They applied this method for pyridoxine in dioxane–water system. The method was slightly modified and applied for niflumic acid in methanol–water and dioxane–water systems [20]. The obtained results for both investigated systems were in good agreement. Pagliara et al. stress that both deductive and direct methods are not simultaneously precise and assumption-free, meaning that the results must usually be regarded as mere estimated. However the direct measure of $K_z$ by UV spectrometry is a simple method and gives good results under condition the sharp isosbestic point is present [21]. The proper description of amino acid in two-phase systems requires additionally determination of the distribution constant $K_D$ of acid,

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which participates in extraction equilibrium in a molecular form ($HR_w \rightleftharpoons HR_o$) or the extraction constant $K_{ex}$ involved in the extraction equilibrium when ion-pair form with inorganic anion $X^-$ undergoes an extraction ($H_2R_w^+ + X_w^- \rightleftharpoons (H_2R^+X^-)_o$).

In this study we have determined macroconstant $K_{a1}$, $K_{a2}$ values, isoelectric point $pH_I$, tautomerization constant $K_z$ and microconstants $k_{11}$, $k_{12}$, $k_{21}$, $k_{22}$ values. The distribution of anthranilic acid in two phase systems water–organic solvents was investigated and distribution constant $K_D$, and dimerization constant $K_{dim}$ values were also determined. Moreover, the influence of the selected parameters (pH of the aqueous phase, structure and polarity of the organic solvent) on the equilibria was analyzed with regard of the formation and coexistence of different acid forms in the examined systems.

## 2. Experimental

### 2.1. Materials

Anthranilic acid was purchased from ALDRICH while HCl, KCl and KOH were from POCh Gliwice (Poland). Organic solvents (benzene, toluene, ethylbenzene, chlorobenzene, bromobenzene, nitrobenzene and methanol were purchased from Fluka and POCh and were used without further purification. All reagents were of analytical grade.

### 2.2. Methods

#### 2.2.1. Determination of isoelectric point $pH_I$ and macroconstants $K_{a1}$ and $K_{a2}$ values

Macroconstants were determined by pH-metric titration. Electromotive force variations were measured by means of a CP-501 pH-meter ($E \pm 0.2$ mV) with a OSH 10-00 combined electrode (Metron). The electrode was calibrated with respect to proton activities with standard buffer solutions (pH 4.00, 7.00 and 9.00). The aqueous solutions of 0.01 M anthranilic acid were preliminary acidified by standardized aqueous HCl and titrated by 0.1 M standardized aqueous KOH solution. The titrations were conducted by a CONTINUOUS digital automatic burette (VITLAB). The burette was calibrated by weighing the doubly distilled and purified water with a precision of ±0.02% in added volume over the whole volume range. All measurements were made in 0.10 M KCl solutions (to adjust ionic strength) under argon atmosphere at 25±0.1 °C in a thermostated extraction vessel equipped with a water jacket. During titration the investigated systems were continuously stirred with magnetic stirrer. Titrations were repeated three times. The $pK_{a2}$ value was determined using standard method [18]. The $pH_I$ value was evaluated on the basis of the Hahn method [22]. Subsequently, the $pK_{a1}$ value was determined using Eqs. (1a) and (1b) which are valid for amino acid [23]:

$$pH_I = \frac{pK_{a1} + pK_{a2}}{2} \tag{1a}$$

$$pK_{a1} = 2pH_I - pK_{a2}. \tag{1b}$$

Since, the dissociation constant values were determined at 0.1 M ionic strength (the mixed dissociation constants), Eqs. (2a) and (2b) were used to obtain the thermodynamic dissociation constants [18].

$$pK_{a1}^T = pK_{a1}^M - \frac{0.512\sqrt{I}}{1 + 1.5\sqrt{I}} \tag{2a}$$

$$pK_{a2}^T = pK_{a2}^M + \frac{0.512\sqrt{I}}{1 + 1.5\sqrt{I}} \tag{2b}$$

where

$pK_{a1}^T$ and $pK_{a2}^T$   the thermodynamic dissociation constants;

$pK_{a1}^M$ and $pK_{a2}^M$   the mixed dissociation constants;
$I$          the ionic strength ($I = 0.1$, KCl).

#### 2.2.2. Determination of microconstant values

UV spectroscopy was used to determine the tautomerization microconstant $K_z$ values for the equilibrium $HR^0$ (unionized form) $\rightleftharpoons HR^\pm$ (zwitterionic form). The method is based on the spectral differences between the zwitterionic form of the molecule (found predominantly at the isoelectric pH in aqueous solution) and the unionized form (found predominantly in organic solvent of low dielectric constant, such as methanol). The spectra can be converted from one form to the other by changing the solvent mixture.

A stock solution (0.04 M) of anthranilic acid in methanol was prepared. 1.00 ml of this solution was diluted to 200 ml with methanol or Britton–Robinson buffer at pH 3.51. Subsequently various mixtures of these solutions containing 0 to 100% (in steps of 10%) of methanol were prepared. The absorption spectra of these solutions were recorded by a Beckman DU-640 spectrophotometer in the region of 200–800 nm. The experiment was repeated three times.

#### 2.2.3. Shake-flask determination of the distribution ratio D

The investigations on the distribution of anthranilic acid were performed in the pH interval 1–12 at 25±0.1 °C. Organic and aqueous phases were mutually saturated. Britton–Robinson buffers (acetic, phosphoric and boric acids each at 0.04 M, treated with various amounts of 0.2 M NaOH) were used as the aqueous phase for the pH range 2.0 to 12.0. For pH 1, 0.1 M HCl was used. The compound was dissolved in the organic phase (benzene, ethylbenzene, toluene, chlorobenzene, bromobenzene). A sample (5.0 cm$^3$) of the organic solvent containing anthranilic acid ($1 \times 10^{-4}$ M) and an equal volume of the aqueous phase were agitated vigorously in a stoppered glass tube for 1.0 h to reach the equilibrium and then allowed to stay for 2 h in order to phase separation. A preliminary study had shown that this time was enough to reach the partitioning equilibrium. Subsequently pH of the aqueous phase was measured. The UV absorbance of anthranilic acid in the organic phase, both initial and at equilibrium were measured by a Beckman DU-640 spectrophotometer at $\lambda_{max} = 339$ nm. On the basis of mass balance of the amino acid in the two-phase system the concentration of the amino acid in the aqueous phase was calculated.

#### 2.2.4. Determination of the distribution constant $K_D$ and dimerization constant $K_{dim}$ values

The potentiometric titrations of anthranilic acid in the two-phase systems organic solvent–aqueous solution (KCl, $I = 0.1$) were conducted using benzene, ethylbenzene, toluene, chlorobenzene, bromobenzene and nitrobenzene. Solutions of anthranilic acid of 0.01 M concentration in the investigated organic solvents saturated with aqueous phase were prepared (30 cm$^3$). The aqueous phase (KCl, $I = 0.1$) was initially acidified to pH 2.0 with standardized 0.1 M HCl. Titration was carried out with a standardized KOH solution in a thermostated extraction vessel at 25±0.1 °C. pH was measured continuously with a CP-501 pH-meter (pH±0.01) supplied with a combined electrode OSH 10-00 (Metron). For all systems uniform portions of standardized KOH were added and the electromotive force was recorded as a function of the added volume. The state of equilibrium was controlled on the basis of changes of the electromotive force. When the magnitude of change in the electromotive force after the titrant addition was less than 0.1 mV/ 3 min the state was recognized as stable. The complete titration procedure took between 8 and 10 h depending on the solvent used.

## 3. Results and discussion

### 3.1. Macro- and microdissociation constants of anthranilic acid

Anthranilic acid is not an ordinary amphoteric substance however belongs to the group of zwitterionic compounds [18]. Depending on
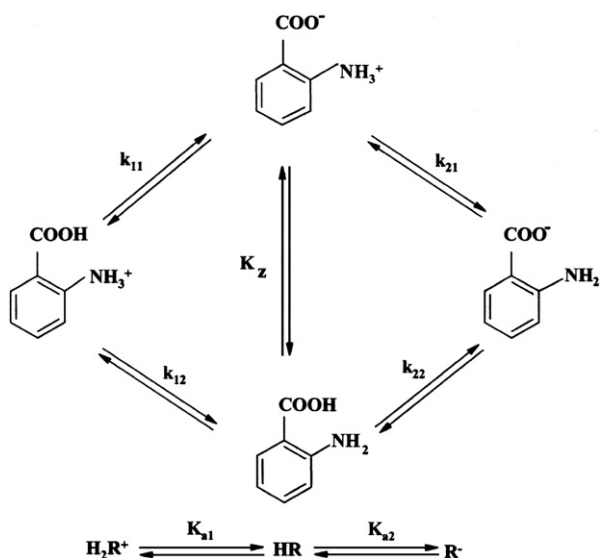
**Fig. 1.** Ionization equilibria of anthranilic acid.

the pH values of aqueous solutions this compound could exist in four different microforms, namely cation ($H_2R^+$), zwitterion ($HR^\pm$), neutral species ($HR^0$) and anion ($R^-$). The equilibria between these forms and the relevant microconstants which characterize the acidity of the individual functional groups are shown in Fig. 1.

In order to determine the ionization microconstant the knowledge of the macroconstants $K_{a1}$ and $K_{a2}$ values and the value of the isoelectric point is necessary. These constants are expressed by use of Eqs. (1a) and (3, 4) [23]:

$$K_{a1} = \frac{\left(\left[HR^\pm\right] + \left[HR^0\right]\right)\{H^+\}}{\left[H_2R^+\right]} \tag{3}$$

$$K_{a2} = \frac{\left[R^-\right]\{H^+\}}{\left[HR^\pm\right] + \left[HR^0\right]} \tag{4}$$

where

$([HR^\pm]+[HR^0])$
the total concentration of zwitterionic and neutral species, which are indistinguishable by acid–base titration;
$[H_2R^+]$, $[R^-]$
the concentrations of cationic and anionic species, respectively;
$\{H^+\}$      the activity of proton.

Literature data concerning p$K_{a2}$ value of anthranilic acid appear to be unreliable and depending on the information source have a value between 4.59 and 4.97 [13,18,24–26]. There are considerably less data concerning p$K_{a1}$ value (p$K_{a1}$=2.15 [13], p$K_{a1}$=2.17 [18] and p$K_{a1}$=2.29 [26]. It makes accurate determination of isoelectric point impossible (knowledge of this point value is necessary for the proper determination of the tautomerization $K_z$ microconstant). Literature data concerning pH$_I$ are in disagreement (pH$_I$ 3.42 [13], pH$_I$=3.44 [26] and pH$_I$ 3.51 [18]). For this reason in the present work the mixed dissociation constants ($I$=0.1, KCl) of anthranilic acid have been determined on the basis of potentiometric titration. In order to correct the effect of ionic strength on p$K_{a1}$ and p$K_{a2}$ determination, the thermodynamic dissociation constants were obtained on the basis of Eqs. (2a) and (2b) [18]. The calculated thermodynamic dissociation constant values are presented in Table 2. The obtained dissociation constants of anthranilic acid are consistent with those reported by Albert and Serjeant [18].

The proper statistical analysis, recommended by many authors [27–31], was conducted in order to demonstrate the reliability of the obtained dissociation constants and the results are presented in Table 1. Sensitive criteria of the reliability of the dissociation constant are the Hamilton $R$-factor and the mean of absolute values of residuals [30]. Here, the mean of absolute values of residuals is 0.12 (in term of $E$) and the standard deviation is 0.16. Both the values are lower than the instrumental noise $s_{inst}(y)$, represented here by $s(E)$=0.2. This confirms that the fitting is good. Moreover, the Hamilton $R$-factor reaches the value of 0.1354%. This is another confirmation of an excellent fit (for a good fitness, the Hamilton $R$-factor reaches the value ≤1%, and for excellent fitness is lower than 0.5% [31]).

The microconstants can be expressed with microspecies concentrations as follows:

$$k_{11} = \frac{\left[HR^\pm\right]\left[H^+\right]}{\left[H_2R^+\right]} \tag{5}$$

$$k_{12} = \frac{\left[HR^0\right]\left[H^+\right]}{\left[H_2R^+\right]} \tag{6}$$

$$k_{21} = \frac{\left[R^-\right]\left[H^+\right]}{\left[HR^\pm\right]} \tag{7}$$

$$k_{22} = \frac{\left[R^-\right]\left[H^+\right]}{\left[HR^0\right]} \tag{8}$$

$$K_z = \frac{\left[HR^\pm\right]}{\left[HR^0\right]}. \tag{9}$$

The relationships between the micro- and macroconstants have been reported in [17,23]:

$$K_{a1} = k_{11} + k_{12} \tag{10}$$

$$\frac{1}{K_{a2}} = \frac{1}{k_{21}} + \frac{1}{k_{22}} \tag{11}$$

$$K_{a1}K_{a2} = k_{11}k_{21} = k_{12}k_{22} \tag{12}$$

$$K_z = \frac{k_{11}}{k_{12}} = \frac{k_{22}}{k_{21}}. \tag{13}$$

These equations can be solved when one of the microscopic constants is known.

Takács-Novák selected the following three types of zwitterionic compounds [32]:

1. Compounds that allow selective monitoring of ionizable group by spectrophotometry (norfloxacin),

**Table 1**
Titration data used for calculation of the dissociation constant and the appropriate statistical parameters

| The initial concentrations of anthranilic acid, $c$ [mol/dm$^3$] | The investigated pH range | The number of experimental point, $n$ |
|---|---|---|
| 0.010 | 3.96–6.61 | 53 |
| 0.010 | 3.80–6.55 | 55 |
| 0.010 | 3.86–6.36 | 53 |

Reliability of parameters estimation by a statistical analysis.
Standard deviation, S.D.=0.16.
Sum of squared residuals as defined in Ref. [30,31], $S$=4.251.
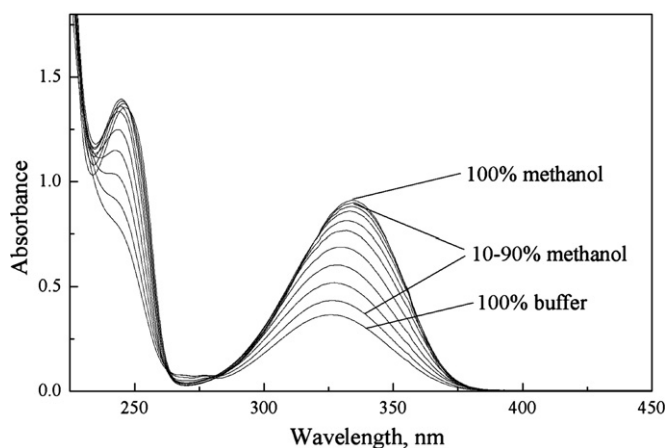Hamilton $R$-factor as defined in Ref. [29,30], $R$-factor=0.001354.

**Fig. 2.** Absorption spectra of $1.0 \times 10^{-4}$ M solutions of anthranilic acid in different methanol–buffer mixtures (0–100% (v/v)).

2. Compounds for which deductive method can be applied because derivative molecules with few ionizable groups are available (piroxicam),
3. Compounds where $K_z$ method can be applied (niflumic acid, piridoxine).

On the basis of UV–VIS spectrum of methyl antranilate and 2-methylaminobenzoic acid it was found that both ionizable groups show the measurable shifts thus the selective spectrophotometric monitoring is not applicable. For this type of molecule with two ionizable groups connected to the chromophores, the microconstant could be determined by measuring the tautomeric ratios spectrophotometrically from mixture of organic compounds and water at the pH of the isoelectric point. This method was found and validated by Takács-Novák and co-workers [20,32,33]. The microconstants of anthranilic acid were determined on the basis of this method with the assumption that the spectrum of anthranilic acid in methanol is identified as that of the unionized form $HR^0$ while the spectrum in aqueous buffer at pH of the isoelectric point (3.51) is assigned to that of the zwitterion $HR^\pm$. The spectra obtained at different methanol–buffer mixtures were presented in Fig. 2.

In the case of the spectral lines series two sharp isosbestic points (at 264 and 282 nm) are observed. The presence of these points indicates that the equilibrium between $HR^0$ and $HR^\pm$ forms occurs in these systems moreover, permit to determine the tautomeric constant $K_z$ accurately [21]. These deductions were based on analogy to pyridoxine and on analysis of other related compounds (niflumic acid, nicotinic acid). The tautomerization microconstant $K_z$ can be calculated from the spectroscopic data using the following Refs. [20,32,34]:

$$K_{z(\%)} = \frac{A_{HR^0} - A_\%}{A_\% - A_{HR^\pm}} \qquad (14)$$

where

$K_{z(\%)}$　　tautomerization constant in a given % solvent mixture;
$A_\%$　　　absorbance of the compound in the given % solvent mixture;
$A_{HR}^0$　　absorbance of the compound in the pure organic solvent;
$A_{HR^\pm}$　　absorbance of the compound in aqueous buffer solution at the isoelectric pH.

From the spectroscopic data $K_{z(\%)}$ values were calculated. The aqueous $K_z$ value was obtained from the intercept of the following equation:

$$\log K_{z\%} = -0.0292R(\% w/w) + 0.9292 (n = 27, R = 0.9960). \qquad (15)$$

**Table 2**
Dissociation macroconstants, microconstants and tautomeric constant of anthranilic acid

| Parameter | Value |
|---|---|
| $pK_{a1}$ | $2.18 \pm 0.05$ |
| $pK_{a2}$ | $4.84 \pm 0.01$ |
| $pH_I$ | $3.51 \pm 0.02$ |
| $\log K_z$ | $0.93 \pm 0.03$ |
| $pk_{11}$ | 2.25 |
| $pk_{12}$ | 3.18 |
| $pk_{21}$ | 4.79 |
| $pk_{22}$ | 3.86 |

The obtained tautomerization $K_z$ constant and microconstant ($k_{11}$, $k_{12}$, $k_{21}$ and $k_{22}$) values calculated according to Eqs. (10)–(13) are summarized in Table 2.

Microconstants concerning to the same dissociation site ($k_{11}$ and $k_{22}$ or $k_{12}$ and $k_{22}$) have significantly different values. This means that the dissociation at one site causes a considerable decrease of the acidity of the other site ($pk_{11} < pk_{22}$ and $pk_{12} < pk_{21}$) [35]. The above observation can be a quantitative measure of the interaction between two sites when dissociation occurs at one of them. The value of this interactivity parameter $\Delta pk$ is 1.61 ($\Delta pk = pk_{22} - pk_{11} = pk_{21} - pk_{12}$). The $\Delta pk$ value of anthranilic acid is higher in comparison with niflumic acid ($\Delta pk = 0.90$) [20]. This fact suggests that in the case of the investigated compound there are strong interactions between the two dissociation sites.

On the basis of the determined values of macro- and micro-constants, the distribution diagram for the four microspecies of anthranilic acid was drawn (Fig. 3).

Thus, the protonated form $H_2R^+$ dominates at pH < 2.0. In the pH interval 2.0–5.0 the major form is the zwitterion $HR^\pm$ that pre-dominates over the neutral species $HR^0$ and both reach a maximum at pH around the isoelectric point (pH 3.51). Maximum mole fraction of zwitterion $HR^\pm$ and neutral microform $HR^0$ is about 0.82 and 0.10, respectively. At pH higher than 3.51 the mole fractions of the species $HR^\pm$ and $HR^0$ decrease while the mole fraction of the negative species $R^-$ increases reaching value of 1 at pH ~8. The ratio of the zwitterion to neutral species (equivalent to $K_z$) is identical, irrespective of the pH and is the characteristic feature for the individual amino acid.
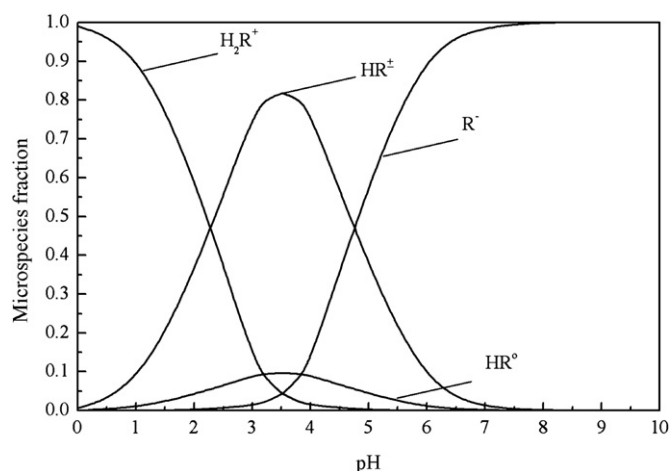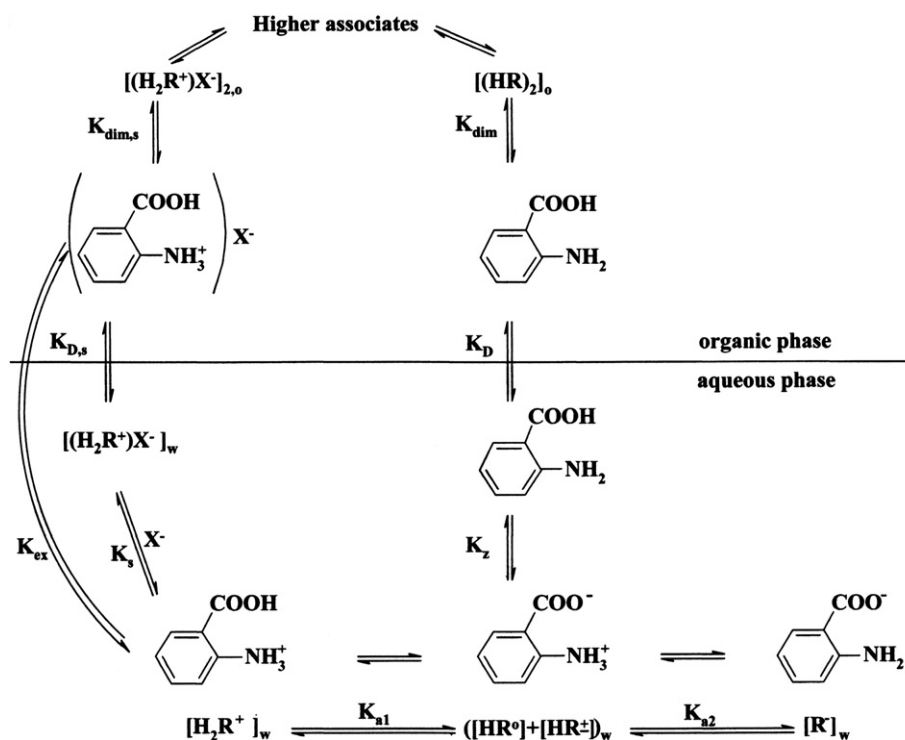


**Fig. 3.** Microspeciation diagram of anthranilic acid.

**Fig. 4.** Reaction scheme for amino acid in two-phase system: organic solvent–aqueous solution (KCl, $I = 0.1$).

### 3.2. Characteristics of anthranilic acid in two-phase systems

The amino acid in the liquid two-phase systems: organic solvent–aqueous solution undergoes distribution between the organic and aqueous phase in two ways as presented in Fig. 4 [36].

I. If the only form capable of entering the organic phase is the neutral form of the amino acid the experimental distribution ratio is described by Eq. (16):

$$D = \frac{c_{HR,o}}{c_{HR,w}} = \frac{[HR]_o + 2[(HR)_2]_o}{[H_2R^+]_w + [HR]_w + [R^-]_w} \quad (16)$$

where:

$c_{HR,o}$    the total concentrations of amino acid in the organic phase ($c_{HR,o} = [HR]_o + 2[(HR)_2]_o$)

$c_{HR,w}$    the total concentrations of amino acid in the aqueous phase ($c_{HR,w} = [H_2R^+]_w + [HR]_w + [R^-]_w$).

II. If the investigated system contains the neutral form HR and the ion pair $H_2R^+X^-$, the distribution ratio is described by Eq. (17):

$$D = \frac{c_{HR,o}}{c_{HR,w}} = \frac{[H_2R^+X^-]_o + [HR]_o + 2[(HR)_2]_o + 2[(H_2R^+X^-)_2]_o}{[H_2R^+]_w + [HR]_w + [R^-]_w} \quad (17)$$

where:

$[HR]_o$, and $[H_2R^+X^-]_o$
the concentrations of the neutral form of the amino acid and the ion-pair, respectively
$[H_2R^+]_w$, $[HR]_w$ and $[R^-]_w$
the concentrations of the positive, neutral and negative form, respectively
$[(HR)_2]_o$ and $[(H_2R^+X^-)_2]_o$

the concentrations of the dimer of the neutral form and the ion association complex, respectively.

Taking into account the equilibrium constants, the distribution of the amino acid is described by Eq. (18) obtained on the basis of the models of the singular and multistep equilibria [36,37]:

$$\frac{c_{HR,o}}{[HR]_w} = K_D + K_{ex}[X^-]_w \frac{[H]^+}{K_{a1}} + 2\left(K_D^2 K_{dim} + K_{dim,s}K_{ex}^2[X^-]_w^2 \frac{[H^+]^2}{K_{a1}^2}\right)[HR]_w \quad (18)$$

where:

$K_D$    the partition constant of the neutral form of the amino acid ($K_D = [HR]_o[HR]_w^{-1}$)

$K_{ex}$    the extraction constant of the ion-pair ($K_{ex} = K_s \cdot K_{D,s} = [H_2R^+X^-]_o [X^-]_w^{-1}[H_2R^+]_w^{-1}$)

$K_s$    the formation constant of the ion-pair ($K_s = [(H_2R^+)X^-]_w[H_2R^+]_w^{-1} [X^-]_w^{-1}$)

$K_{D,s}$    the distribution constant of the ion-pair ($K_{D,s} = [H_2R^+X^-]_o [H_2R^+X^-]_w^{-1}$)

$[X^-]$    the concentration of the inorganic anion

$K_{dim}$    the dimerization constant of the neutral form of the amino acid in the organic phase ($K_{dim} = [(HR)_2]_o[HR_o^{-2}]$)

$K_{dim,s}$    the dimerization constant of the ion-pair in the organic phase ($K_{dim,s} = [(H_2R^+X^-)_2]_o[H_2R^+X^-]_o^{-2}$).

Depending on the pH values of the aqueous phase Eq. (18) can take the two limiting forms [36]:

I. For $pH \ll pH_I$ (the amino acid is mainly present as a cation) the extraction is possible in a form of ion-pair with the inorganic anion and then Eq. (18) has the following form:

$$\frac{c_{HR,o}}{[HR]_w} = K_{ex}[X^-]_w \frac{[H^+]}{K_{a1}} + 2K_{ex}^2 K_{dim,s}[X^-]_w^2[HR]_w \frac{[H^+]^2}{K_{a1}^2}. \quad (19)$$
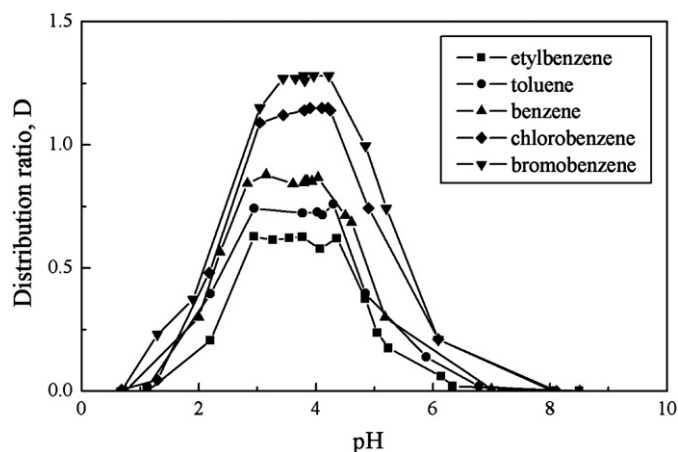
**Fig. 5.** The values of the distribution ratio *D* of anthranilic acid in the two-phase system: organic solvent (ethylbenzene, toluene, benzene, chlorobenzene, bromobenzene)–aqueous solution as a function of the aqueous phase pH.

After some mathematical transformations, a linear dependence can be obtained (Eq. (20)):

$$\frac{c_{HR,o}}{[HR]_w[X^-]_w[H^+]} = \frac{K_{ex}}{K_{a1}} + \frac{2K_{ex}^2 K_{dim,s}}{K_{a1}^2}[HR]_w[X^-]_w[H^+]. \qquad (20)$$

Therefore, for the given ionic strength and the known value of the dissociation constant $K_{a1}$ the determination of both the extraction constant $K_{ex}$ and the dimerization constant of the ion-pair $K_{dim,s}$ in the organic phase is possible.

II. For pH values near to $pH_I$, the amino acid occurs mainly in the neutral form in the aqueous phase and the concentrations of the other forms are low. Under these conditions the extraction of the amino acid is possible only in the neutral form and this process is described by Eq. (21) [38,39]:

$$\frac{c_{HR,o}}{[HR]_w} = K_D + 2K_D^2 K_{dim}[HR]_w. \qquad (21)$$

Taking advantage of the fact that Eq. (21) is linear the partition constant $K_D$ and dimerization constant $K_{dim}$ of the neutral form of amino acid can also be calculated.

On the grounds of the spectrophotometric investigations of the organic phase the values of the distribution ratio *D* of anthranilic acid
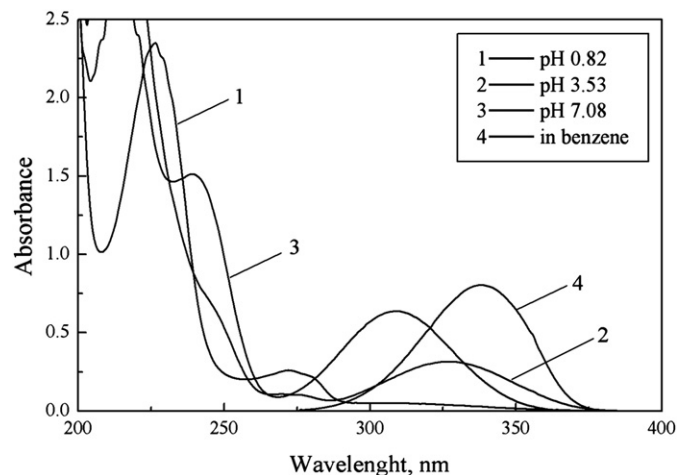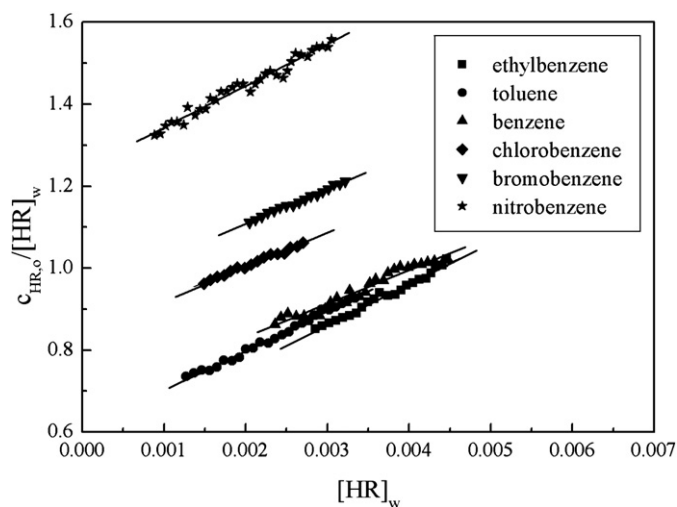


**Fig. 7.** $c_{HR,o}/[HR]_w$ vs. $[HR]_w$ for anthranilic acid in two-phase system: organic solvent (ethylbenzene, toluene, benzene, chlorobenzene, bromobenzene, nitrobenzene)–aqueous solution. The points denotes the experimental data, the solid lines have been determined by linear regression of Eq. (21) using last squares method.

were obtained using Eq. (16). The distribution of anthranilic acid was studied in two-phase systems: organic solvent–aqueous solution (KCl, *I*=0.1) using ethylbenzene, toluene, benzene, chlorobenzene, and bromobenzene as organic phase. Fig. 5 presents the obtained results as *D*=*f*(pH) for all applied solvents. It has been found that distribution ratios significantly depend on the pH of the aqueous phase and the highest values are observed in the pH interval 3.0–4.5 for all studied systems.

Depending on the polarity of the used organic solvent, the values of distribution ratio *D* increase from about 0.62 for ethylbenzene to about 1.28 for bromobenzene in the following order:

$$D_{ethylbenzene} < D_{toluene} < D_{benzene} < D_{chlorobenzene} < D_{bromobenzene}.$$

The obtained results indicate that pH of aqueous phase and polarity of the applied organic solvent has an effect on partition of anthranilic acid in two-phase systems. The partitioning profiles for all studied solvents have approximately parabolic shape with maximum about the isoelectric point. One can conclude that maximum of anthranilic acid extraction efficiency occurs in the pH range 3.0–4.5 and corresponds to the pH range at which the neutral form of this acid $HR^0$ is present. Besides, in order to identify the form of anthranilic acid which undergoes the extraction UV–VIS spectra of the acid in aqueous phase at pH 0.82, 3.52 and 7.08, were assembled and presented in Fig. 6.

The received spectra were significantly different for the cationic, zwitterionic and anionic forms while identical spectra were obtained in organic phases at any pH. On the other hand, the spectra obtained in organic phase are identical with the spectra of anthranilic acid in pure solvent (identified as that of the neutral form) for each investigated



**Fig. 6.** UV spectra of anthranilic acid in benzene and aqueous phase at different pH values.

**Table 3**
The evaluated values of distribution ($K_D$) and dimerization ($K_{dim}$) constants for anthranilic acid in the two-phase system: organic solvent–aqueous solution and the obtained values of statistical criteria

| Solvent | Hildebrand solubility parameter, $\delta$ [kJ]$^{1/2}$ m$^{-3/2}$] | Distribution constant, $K_D$ | Dimerization constant, $K_{dim}$ | Correlation coefficient, $R$ | Number of experimental points, $n$ |
|---|---|---|---|---|---|
| Ethylbenzene | 17.84 | 0.56±0.02 | 160±10 | 0.9916 | 22 |
| Toluene | 18.23 | 0.60±0.01 | 133±5 | 0.9961 | 25 |
| Benzene | 18.72 | 0.67±0.02 | 91±5 | 0.9893 | 27 |
| Chlorobenzene | 19.44 | 0.85±0.01 | 55±3 | 0.9956 | 16 |
| Bromobenzene | 19.74 | 0.94±0.01 | 47±2 | 0.9960 | 17 |
| Nitrobenzene | 20.46 | 1.24±0.01 | 33±2 | 0.9861 | 26 |
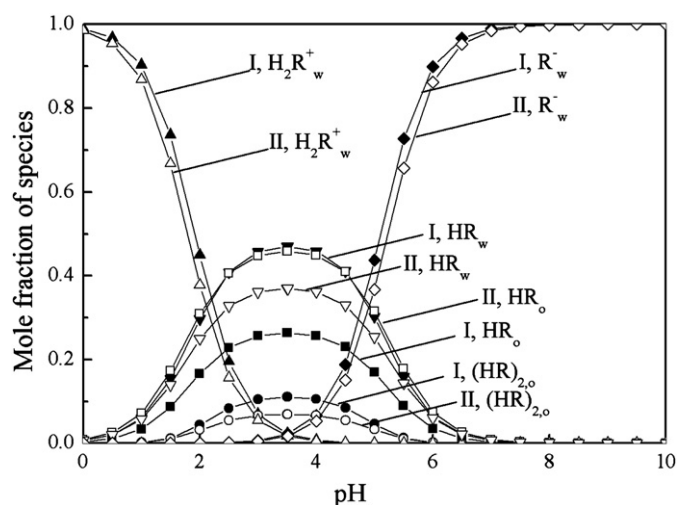
**Fig. 8.** pH dependence of mole fraction of anthranilic acid species in the two-phase systems: organic solvent (I ethylbenzene, II nitrobenzene)–aqueous solution.

systems. This method was recommended by Takács-Novák et al. as very simple but exact experimental evidence for the distribution of the neutral form even when it is the minor component [33]. It was confirmed based on the investigations of mefenamic and N-phenylanthranilic acid distribution [36,37]. These findings show that not the ion pair but only the neutral form of the acid $HR^0$ is transferred from aqueous to organic phase.

Taking advantage of the potentiometric titration results in the two-phase systems, organic solvent–aqueous solution (KCl, $I=0.1$), and using models of the singular and multistep equilibria [36,37], the graphs presented dependence of $c_{HR,o}/[HR]_w$ as a function of $[HR]_w$ for anthranilic acid were prepared and shown in Fig. 7.

As can be seen, the linear dependences were obtained for all tested systems, so determination of the values of the distribution constants $K_D$ and the dimerization constants $K_{dim}$ were possible by linear regression of Eq. (21). The accuracy of the determination of these constants was assessed for the 95% confidence interval of Student's test. The results of calculations were presented in Table 3.

It has been found, that the distribution constant $K_D$ and the dimerization constant $K_{dim}$ values depend on the polarity of the used organic solvent. Moreover, the distribution constants $K_D$ values increase along with increase of the Hildebrand solubility parameter $\delta$ of the studied organic solvent. The lowest value of $K_D$ was found for the system with ethylbenzene ($K_D=0.56$, $\delta=17.84$ kJ$^{1/2}$m$^{-3/2}$), while the highest one was noted for the system with nitrobenzene ($K_D=1.24$, $\delta=20.46$ kJ$^{1/2}$m$^{-3/2}$). In the case of the dimerization constants $K_{dim}$ an inverse dependence was observed. The values of $K_{dim}$ decrease in the examined solvents from 160 for ethylbenzene to 33 for nitrobenzene. The obtained results show that distribution and dimerization constants have comparable values with benzoic and 2-chlorobenzoic acid [40,41]. In comparison with N-phenylanthranilic acid the distribution constants of anthranilic acid demonstrate significantly higher values while the dimerization constant values are substantially lower [36].

Based on the determined values of the dissociation constants $K_{a1}$ and $K_{a2}$, distribution constants $K_D$ and dimerization constants $K_{dim}$ as well as the mass balance of the amino acid in the studied systems, the contents of the particular forms of anthranilic acid in the organic and aqueous phase were calculated as a function of pH of the aqueous phase. The results obtained for two systems with extremely different $K_D$ and $K_{dim}$ are presented in Fig. 8.

The obtained results show, that in the two phase systems the protonated form $H_2R^+$ dominates at pH <2.0. At pH 2.0–5.0 the major forms are: the monomeric form $HR_o$, and the dimeric form $(HR)_{2,o}$ in

organic phase and the monomeric form $HR_w$ in aqueous phase. In the range of pH 2.5–4.5 the mole fraction of the acid monomeric form $HR_o$ in the organic phase is equal to about 0.45 and 0.25 for nitrobenzene and ethylbenzene, respectively. The mole fraction of the dimeric form $(HR)_{2,o}$ in those systems is lower and equal to about 0.07 for nitrobenzene and 0.10 for ethylbenzene. On the other hand, under the same conditions, the mole fraction of the acid monomeric form $HR_w$ in the aqueous phase is significant and results about 0.35 and 0.45 for the systems with nitrobenzene and ethylbenzene, respectively. Further increase in pH of the aqueous phase (pH >5) results in decrease of the concentration of the neutral forms $HR_o$, $(HR)_{2,o}$ and $HR_w$, and simultaneous increase in the concentration of the anionic form $R_w^-$.

## 4. Conclusions

Due to the fact that anthranilic acid is widely applied in many fields, it is indispensable to characterize its properties in terms of the microconstants. These give information about submolecular dissociation states and may be very useful by the proper determination of the lipophilicity of ampholytic compounds. Moreover, the physicochemical behaviour of this compound is determined by the zwitterionic form, which exists predominantly in aqueous solution.

The application of the UV–VIS analysis permitted us to come to the conclusion that only the neutral form of anthranilic acid undergoes the extraction. We have demonstrated that the potentiometric method is suitable for determination of the distribution and dimerization constant values. The obtained distribution constant values increase in the series of aromatic hydrocarbons from ethylbenzene to nitrobenzene while for the dimerization constant values the trend is opposite. Using the obtained values of $K_D$ and $K_{dim}$ we have described the physicochemical properties of anthranilic acid in the two phase systems. We have also demonstrated that the influence of the aqueous phase pH on the content of acid forms is particularly evident in the pH range 2.5–5.0. Moreover, distribution of anthranilic acid in two phase system practically proceeds in the same pH range.

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