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Studies on the distribution of *N*-phenylanthranilic acid in two-phase system: Aromatic solvent–water

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Abstract

The detailed description of equilibria of *N*-phenylanthranilic acid in the aqueous solutions and in the two-phase systems: aromatic solvent–aqueous solution were obtained. The isoelectric point, pH_I, the dissociation constants of the protonated form, K_{a1}, and the neutral form, K_{a2}, in the aqueous solutions were determined from the apparent ionization constant in methanol–water solutions of various proportions by extrapolation to zero co-solvent using Yasuda–Shedlovsky procedure. On the basis of the spectrophotometric investigations, the values of the distribution ratio, D, of *N*-phenylanthranilic acid in the two-phase systems: aromatic solvent (benzene, ethylbenzene, toluene, chlorobenzene, bromobenzene)–aqueous solution were obtained. Employing the results of the potentiometric titration in the two-phase systems: aromatic solvent–aqueous solution, and using models of singular and multistep equilibria the values of the distribution constants, K_D, dimerization constants, K_{dim}, were calculated. The significant influence of the polarity of the applied aromatic solvents and pH of the aqueous phase on the percentage of the particular forms of *N*-phenylanthranilic acid in the two-phase systems was proved.

Keywords: *N*-Phenylanthranilic acid; Dissociation constant; Liquid–liquid distribution; Two-phase systems equilibria

1. Introduction

N-Phenylanthranilic acid (2-[*N*-phenylamino]benzoic acid) is an amino acid widely applied in medicine, pharmacy and analytical chemistry as starting material for synthesis of biologically active compounds [1–3], comparative medical research [4–6] and for metal ion determination [7–9]. Despite the numerous applications of *N*-phenylanthranilic acid, its physicochemical properties in aqueous solutions and in two-phase systems: aromatic solvent–aqueous solution, have not been determined in details yet. Lack of the fundamental parameters characterizing the chemical and physicochemical properties of this compound makes a prediction and description of its reaction in two-phase systems impossible. Because the water solubility of *N*-phenylanthranilic acid is very low (3.3×10^{-5} mol/dm^3) [1] the classical method recommended for determination of the physicochemical parameters of chemical compounds can hardly be used. That is probably why the properties of this acid in the aqueous solutions and in two-phase systems: organic solvent–aqueous solutions, were not studied yet. In case the compounds are virtually insoluble in water, potentiometric titrations in a mixture of organic solvent (methanol [10–15], ethanol, dimethylsulphoxide, dimethylformamide, dioxane [12], propanol [13], acetone [12,13] izopropyl alkohol [16])–water can be applied for determination of the dissociation constant.

Fundamental parameters for amino acids in water solution are as follows: an isoelectric point (pH_I), dissociation constants of cationic form $K_{a1}(H_2R^+ \rightleftarrows H^+ + HR)$ and neutral form $K_{a2}(HR \rightleftarrows H^+ + R^-)$. The proper description of amino acid in two-phase systems requires an additional determination of acid distribution constant, K_D, which participates in extraction equilibrium in molecular form ($HR_w \rightleftarrows HR_o$) or extraction constant, K_{ex}, involved in the extraction equilibrium when ion-pair form with inorganic anion X^- undergoes an extraction ($H_2R^+_w + X^-_w \rightleftarrows (H_2R^+X^-)_o$).

The detailed description of *N*-phenylanthranilic acid equilibrium in water solution and in two-phase systems: aromatic solvent–aqueous solution, characterized by appropriate constants (dissociation, K_{a1} and K_{a2}, zwitterionic, K_z, distribution,

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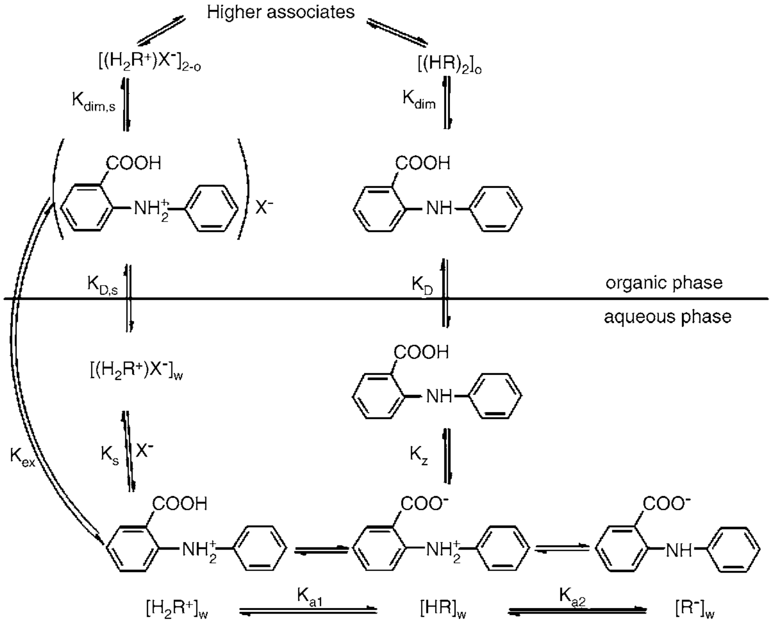

Fig. 1. Reaction scheme for amino acid in the two-phase system: organic solvent–aqueous solution.

K_D, dimerization, K_dim) was the purpose of this study and the results are summarized in Fig. 1.

1.1. Theory

1.1.1. N-Phenylanthranilic acid in water solutions

N-Phenylanthranilic acid belongs to the group of neutral amino acids and consists of one carboxyl group and one amino group in its molecule. Depending on pH of the water solutions this compound can exist as a protonated form, H_2R^+, as a neutral form, HR (as unionized form HR^o or as a zwitterion $HR^\pm$) or as an anionic form, R^- (Fig. 1).

The dissociation constants, K_a1 and K_a2, are equal to Eqs. (1) and (2):

$$K_\mathrm{a1} = [\mathrm{HR}][\mathrm{H}^+][\mathrm{H_2R}^+]^{-1} \tag{1}$$

$$K_\mathrm{a2} = [\mathrm{R}^-][\mathrm{H}^+][\mathrm{HR}]^{-1} \tag{2}$$

Quantitative proportions of the different forms of an amino acid in an aqueous solution at a given temperature mainly depend on pH of the solution and on the initial concentration of amino acid. A special case of equilibrium in the aqueous solution takes place for isoelectric point, $\mathrm{pH_I}$. In this point concentrations of the protonated form, H_2R^+, and anionic form, R^-, are equal. Moreover, for $\mathrm{p}K_\mathrm{a1} \ll \mathrm{p}K_\mathrm{a2}$ amino acid exists mainly in un-ionized form, HR^o, or as zwitterion, $HR^\pm$.

1.1.2. N-Phenylanthranilic acid in two-phase systems

Amino acid in the liquid two-phase systems organic solvent–aqueous solution can undergo distribution between organic and aqueous phase in two ways.

I. If only form capable of entering the organic phase is a neutral form of amino acid, HR, the experimental distribution ratio is described by Eq. (3):

$$D = \frac{c_\mathrm{HR,o}}{c_\mathrm{HR,w}} = \frac{[\mathrm{HR}]_\mathrm{o} + 2[(\mathrm{HR})_2]_\mathrm{o}}{[\mathrm{H_2R}^+]_\mathrm{w} + [\mathrm{HR}]_\mathrm{w} + [\mathrm{R}^-]_\mathrm{w}} \tag{3}$$

II. If the investigated system contains a neutral form, HR, and ion-pair, $H_2R^+X^-$, the distribution ratio is described by Eq. (4):

$$D = \frac{c_\mathrm{HR,o}}{c_\mathrm{HR,w}} = \frac{[\mathrm{H_2R^+X^-}]_\mathrm{o} + [\mathrm{HR}]_\mathrm{o} + 2[(\mathrm{HR})_2]_\mathrm{o} + 2[(\mathrm{H_2R^+X^-})_2]_\mathrm{o}}{[\mathrm{H_2R}^+]_\mathrm{w} + [\mathrm{HR}]_\mathrm{w} + [\mathrm{R}^-]_\mathrm{w}} \tag{4}$$

where $[\mathrm{HR}]_\mathrm{o}$ and $[\mathrm{H_2R^+X^-}]_\mathrm{o}$ are the concentrations of the neutral form of amino acid and the ion-pair, respectively; $[\mathrm{H_2R}^+]_\mathrm{w}$, $[\mathrm{HR}]_\mathrm{w}$ and $[\mathrm{R}^-]_\mathrm{w}$ the concentrations of the cationic, neutral and anionic form, respectively; $[(\mathrm{HR})_2]_\mathrm{o}$ and $[(\mathrm{H_2R^+X^-})_2]_\mathrm{o}$ are the concentrations of the dimer of the neutral form and the ion-pair, respectively.

Taking into account equilibrium constants, the distribution of amino acid can be described by Eq. (5) obtained on the basis of the models of the singular and multistep equilibria [17,18]:

$$\frac{c_\mathrm{HR,o}}{[\mathrm{HR}]_\mathrm{w}} = K_\mathrm{D} + K_\mathrm{ex}[\mathrm{X}^-]_\mathrm{w}\frac{[\mathrm{H}]^+}{K_\mathrm{a1}} + 2\left(K_\mathrm{D}^2K_\mathrm{dim} + K_\mathrm{dim,s}K_\mathrm{ex}^2[\mathrm{X}^-]_\mathrm{w}\frac{[\mathrm{H}^+]^2}{K_\mathrm{a1}^2}\right)[\mathrm{HR}]_\mathrm{w} \tag{5}$$

where K_D is the distribution constant of the neutral form of amino acid ($K_\mathrm{D} = [\mathrm{HR}]_\mathrm{o}[\mathrm{HR}]_\mathrm{w}^{-1}$); K_ex the extraction constant of the ion-pair ($K_\mathrm{ex} = K_\mathrm{s}K_\mathrm{D,s} = [\mathrm{H_2R^+X^-}]_\mathrm{o}[\mathrm{X}^-]_\mathrm{w}^{-1}[\mathrm{H_2R}^+]_\mathrm{w}^{-1}$); K_s the formation constant of the ion association complex ($K_\mathrm{s} = [(\mathrm{H_2R}^+)\mathrm{X}^-]_\mathrm{w}[\mathrm{H_2R}^+]_\mathrm{w}^{-1}[\mathrm{X}^-]_\mathrm{w}^{-1}$); $K_\mathrm{D,s}$ the distribution constant of the ion association complex ($K_\mathrm{D,s} =$

$[H_2R^+X^-]_o[H_2R^+X^-]_w^{-1}$); $[X^-]$ the concentration of the inorganic anion; K_{dim} the dimerization constant of the neutral form in the organic phase ($K_{dim} = [(HR)_2]_o[HR]_o^{-2}$); $K_{dim,s}$ is 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 of the aqueous phase Eq. (5) can take one of two limiting forms:

I. For pH ≪ pH_I (amino acid is mainly in cationic form) the extraction is possible as the ion-pair with inorganic anion and Eq. (5) has the following form:

$$\frac{c_{HR,o}}{[HR]_w} = K_{ex}[X^-]_w\frac{[H]^+}{K_{a1}} + K_{dim,s}K_{ex}^2[X^-]_w^2[HR]_w\frac{[H^+]^2}{K_{a1}^2} \qquad (6)$$

After some mathematical transformations, a linear dependence (Eq. (7)) can be obtained:

$$\frac{c_{HR,o}}{[HR]_w[X^-]_w[H^+]} = \frac{K_{ex}}{K_{a1}} + 2K_{ex}^2K_{dim,s}[HR]_w[H^+][X^-]_w \qquad (7)$$

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

II. For pH value near to pH_I, amino acid in the aqueous phase occurs mainly in the neutral form and it is the only form able to perform extraction. Under these conditions the extraction of amino acid is described by Eq. (8):

$$\frac{c_{HR,o}}{[HR]_w} = K_D + 2K_D^2K_{dim}[HR]_w \qquad (8)$$

On the basis of the fact that Eq. (8) is a linear dependence the distribution constant, K_D, and dimerization constant, K_{dim}, of the neutral form of amino acid can also be calculated.

2. Experimental

2.1. Materials

N-Phenylanthranilic acid was purchased from ALDRICH. Methanol, benzene, toluene, chlorobenzene, carbon tetrachloride (from POCh, Gliwice, Poland), bromobenzene and ethylbenzene (from Fluka, Switzerland) were used without further purification. All reagents were of analytical grade.

2.2. Detrmination of the values of isoelectric point, pH_I, and the dissociation constants, K_{a1} and K_{a2}

Due to poor water solubility of *N*-phenylanthranilic acid the dissociation constant, pK_{a1} and pK_{a2}, were determined by a mixed-solvent method. Potentiometric titrations were made at 25 ± 0.1 °C. Eight separate semi-aqueous solutions containing 28–65% (w/w) of methanol, 0.7–1.2 mM *N*-phenylanthranilic acid and 0.1 M NaCl (to adjust ionic strength) were prepared. The solutions were preliminary acidified by standardized HCl to pH 2.0 and titrated with standardized aqueous NaOH to pH 10.5 with parallel addition of methanol (in the appropriate volume, dependent on the content of methanol in the investigated system) to maintain the methanol–water composition constant [11]. After each titrant addition the stirrer was turned on and solution was vigorously stirred. Then the stirrer was turned off and pH was measured. The pH values were corrected for the presence of the non-aqueous part of the solvent. For this purpose, the combined glass electrode was calibrated using 0.05 M potassium hydrogen phthalate buffer solution made in 28–65% (w/w) methanol–water mixture [19,20]. The apparent ionization constants in the mixed-solvent were obtained from the difference curve (also called the formation curve or Bjerrum plots) and extrapolated to zero co-solvent by the Yasuda–Shedlovsky procedure [10,14].

2.3. Detrmination of the values of the distribution constant, K_D, and dimerization constant, K_{dim}

Potentiometric titrations of *N*-phenylanthranilic acid in the two-phase systems organic solvent–aqueous solution (K_2SO_4, $I = 0.1$) were performed using benzene, ethylbenzene, toluene, chlorobenzene, bromobenzene and nitrobenzene. Solutions of *N*-phenylanthranilic acid of 0.01 M concentration in the investigated organic solvents saturated with aqueous phase were prepared (30 cm^3). The aqueous phase (K_2SO_4, $I = 0.1$) was initially acidified to pH 2.5 with the sulphuric acid. Titration was carried out with a standardized KOH solution in a thermostated extraction vessel at 25 ± 0.1 °C. pH was measured continuously with pH-meter supplied with a Metron combined electrode OSH 10-00.

2.4. Shake-flask determination of the distribution ratio, *D*

The investigations on the distribution of *N*-phenylanthranilic acid were performed at the pH range 1–13, at 25 ± 0.1 °C. Organic and aqueous phases were mutually saturated. The compound was dissolved in the organic phase (benzene, ethylbenzene, toluene, chlorobenzene, bromobenzene). A sample (5.0 cm^3) of the organic solvent containing *N*-phenylanthranilic acid (0.01 mol/dm^3) and an equal volume of the aqueous phase (pH 1–13 obtained using the solutions of HCl or NaOH accordingly) were agitated vigorously in a stoppered glass tube for 0.5 h to reach the equilibrium and then allowed to stay for 2 h in order to phase separation. After then pH of the aqueous phase was measured. The UV absorbance of *N*-phenylanthranilic acid in the organic phase, both initial and at equilibrium was measured by Beckman DU-640 spectrophotometer at 358 nm. The precision of this method in terms of standard deviation (S.D.) was 1.20×10^{-2} ($n = 5$). The concentration of amino acid in the organic phase was determined from the calibration graph. The experimentally obtained calibration parameters with the associated standard deviations are presented in Table 1. On the basis of mass balance of amino acid in the two-phase system the concentration of amino acid in the aqueous phase was calculated.

Table 1
Parameters of the calibration curve ($A = ac_{\mathrm{HR}} + b$) for spectrophotometric determination of *N*-phenylanthranilic acid

Solvent	*a*	*b*	Regression coefficient, *R*	Standard deviation, S.D.	*n*
Ethylbenzene	4.4845×10^{3}	-5.4668×10^{-2}	0.9964	0.03571	6
Benzene	8.0417×10^{3}	-3.0349×10^{-4}	0.9999	0.00298	6
Toluene	7.7455×10^{3}	2.5856×10^{-3}	0.9999	0.00532	6
Chlorobenzene	4.3147×10^{3}	-7.7381×10^{-3}	0.9999	0.00424	6
Bromobenzene	7.5516×10^{3}	8.0104×10^{-3}	0.9993	0.01327	6

A is the absorbance value, c_{HR} the concentration of *N*-phenylanthranilic acid, *a* and *b* the slope and the intercept of the plot, respectively, and *n* is the number of the experimental points.

Besides the UV–vis spectra of the aqueous phase at pH values about 1, 4, 8 were performed for each investigated systems in order to identify of the form of *N*-phenylanthranilic acid that undergo the extraction.

3. Results and discussion

One of the most popular and widespread methods for determination of the dissociation constants of the different compounds is the spectroscopic method. However, the applicability of this method for determination of the value of the dissociation constants of a bifunctional compound, such as amino acids, is limited to the value of the dissociation constant of the carboxylic group only. The protonation of the nitrogen atom does not result in measurable shifts of the UV spectrum [10]. For determining the value of the dissociation constants of amino acids, the best results give, recommended by many authors, the potentiometric method [10,21]. However, for some low water solubility compounds, the applicability of the potentiometric method is difficult because determining the searched constants directly is impossible. In this case, because it is generally accepted that methanol shows a solvation effect close to water, the mixed-solvent (methanol–water systems) method should be taken into consideration [10,13–15]. On the basis of the potentiometric titration, series of *N*-phenylanthranilic acid in the methanol–water systems conducted with methanol weight fraction from 28 to 62%, the values of apparent ionization constant, $\mathrm{p_s}K_{\mathrm{a2}}$, and isoelectric point, $\mathrm{pH_I}$, were obtained from difference Bjerrum plots using Eq. (9) [22,23]:

$$n\mathrm{H} = \{[\mathrm{HCl}] - [\mathrm{KOH}] + nL - [\mathrm{H}^{+}] + (K_{\mathrm{w}}/[\mathrm{H}^{+}])\}/L \qquad (9)$$

where *n* is the number of dissociable protons introduced into the solution by the substance *L*, *L* the total weak acid concentration, [HCl], [KOH] the concentrations of added strong acid and base and K_{w} is the ionization constant of water.

The values of $\mathrm{p_s}K_{\mathrm{a1}}$, were obtained from relationships (10) and (11) valid for a neutral amino acid:

$$\mathrm{p}K_{\mathrm{a1}} + \mathrm{p}K_{\mathrm{a2}} = 2\mathrm{pH_I} \qquad (10)$$

$$\mathrm{p}K_{\mathrm{a1}} = 2\mathrm{pH_I} - \mathrm{p}K_{\mathrm{a2}} \qquad (11)$$

To determine the aqueous $\mathrm{p}K_{\mathrm{a1}}$ and $\mathrm{p}K_{\mathrm{a2}}$ values the Yasuda–Shedlovsky extrapolation procedure (recommended by many authors to obtain precision value of $\mathrm{p}K_{\mathrm{a}}$ [10–15]) was used. The Yasuda–Shedlovsky method was in detail described in [10]. The values of $\mathrm{p_s}K$ and $\mathrm{p_s}K + \log[\mathrm{H_2O}]$, obtained for *N*-phenylanthranilic acid are presented in Table 2. The aqueous $\mathrm{p}K_{\mathrm{a1}}$ and $\mathrm{p}K_{\mathrm{a2}}$ values were obtained by extrapolation to zero co-solvent (for the dielectric constant of the pure water ($\frac{100}{\varepsilon_{\mathrm{H_2O}}} = \frac{100}{78.38} = 1.28$)) using the following linear Eqs. (12) and (13):

$$\mathrm{p_s}K_{\mathrm{a1}} + \log[\mathrm{H_2O}] = 6.375 - \frac{120.24}{\varepsilon} \qquad (12)$$

$$\mathrm{p_s}K_{\mathrm{a2}} + \log[\mathrm{H_2O}] = 7.083 - \frac{13.81}{\varepsilon} \qquad (13)$$

Fig. 2 shows Yasuda–Shedlovsky plot in methanol–water for *N*-phenylanthranilic acid. The $\mathrm{p}K_{\mathrm{a1}}$ and $\mathrm{p}K_{\mathrm{a2}}$ values obtained by this method are 3.10 ± 0.02 and 5.18 ± 0.03, respectively. The isoelectric point, $\mathrm{pH_I}$, was identified at pH 4.14 ± 0.02.

Based on determined values of K_{a1} and K_{a2} as well as $\mathrm{pH_I}$, the distribution diagram of the species of *N*-phenylanthranilic

Table 2
Values of $\mathrm{p_s}K$ and $\mathrm{p_s}K + \log[\mathrm{H_2O}]$ for *N*-phenylanthranilic acid in methanol–water mixtures

Methanol, % w/w	$\mathrm{p_s}K_{\mathrm{a1}}$[a]	$\mathrm{p_s}K_{\mathrm{a2}}$[a]	$\mathrm{p_s}K_{\mathrm{a1}} + \log[\mathrm{H_2O}]$	$\mathrm{p_s}K_{\mathrm{a2}} + \log[\mathrm{H_2O}]$
64.8	2.68 ± 0.02	5.60 ± 0.04	3.90	6.82
54.2	2.79 ± 0.01	5.49 ± 0.04	4.13	6.83
44.1	2.88 ± 0.01	5.40 ± 0.02	4.32	6.84
39.6	2.91 ± 0.03	5.37 ± 0.05	4.39	6.85
36.0	2.92 ± 0.02	5.36 ± 0.04	4.43	6.87
34.4	2.93 ± 0.01	5.35 ± 0.03	4.45	6.87
33.0	2.94 ± 0.03	5.34 ± 0.04	4.47	6.87
28.3	2.96 ± 0.02	5.32 ± 0.02	4.52	6.88
0.0[b]	3.10 ± 0.03[b]	5.18 ± 0.04[b]		

[a] Mean of three determinations.

[b] Extrapolated by the Yasuda–Shedlovsky procedure.

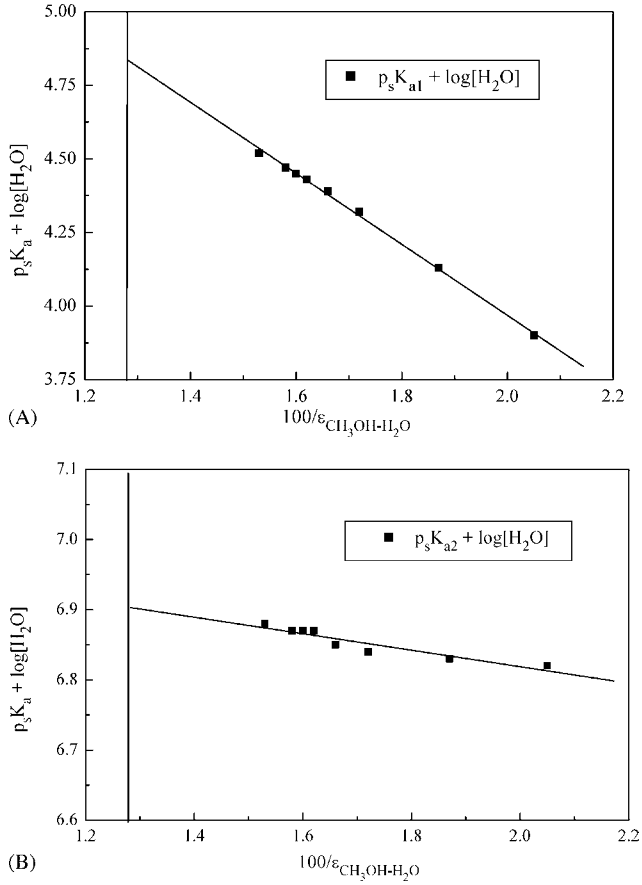

Fig. 2. Yasuda–Shedlovsky plot for *N*-phenylanthranilic acid in methanol–water mixture for determination of the values of pK_{a1} (A) and pK_{a2} (B).

acid in aqueous solutions (K_2SO_4, $I = 0.1$) depending on pH was prepared (Fig. 3). It was found that the protonated form of amino acid, H_2R^+, significantly dominates at $pH < 3.0$ whereas at pH 3.0–5.0 the major form is the neutral species, HR, and the anionic form, R^-, is the dominant at $pH > 5.0$.

On the basis of spectrophotometric investigations of the organic phase the values of the distribution ratio, *D*, of *N*-phenylanthranilic acid for all the solvents studied were calculated and presented in Fig. 4 as a function of pH of the aqueous phase. Depending on the organic solvent used, the values of the distribution ratio, *D*, increase from about 70.0 for ethylbenzene to about 145.0 for bromobenzene at pH about 3–5 in the following order:

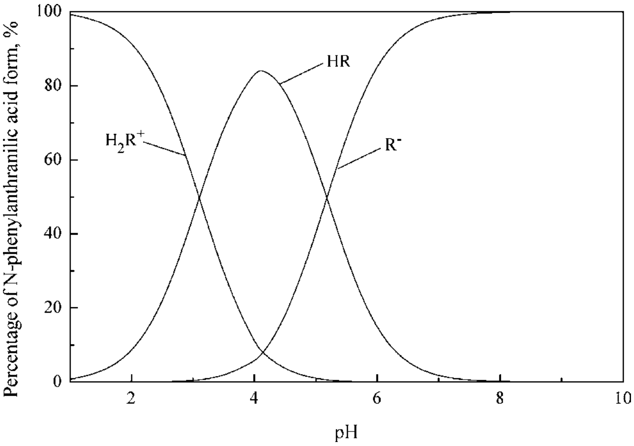

Fig. 3. The percentage of particular forms of *N*-phenylanthranilic acid in the aqueous solution as a function of pH.

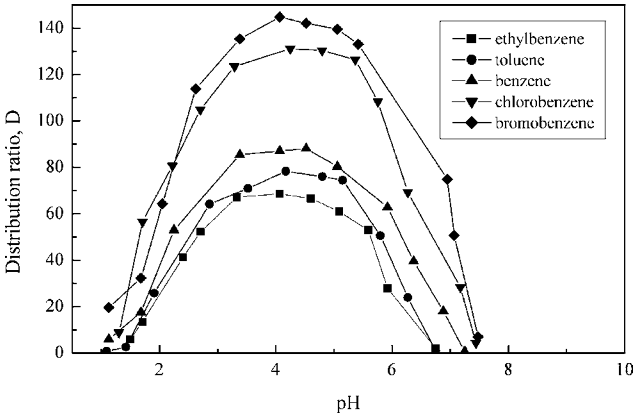

Fig. 4. The values of the distribution ratio, *D*, for *N*-phenylanthranilic acid in the two-phase system: organic solvent–aqueous solution as a function of pH of the aqueous phase.

$$D_{\text{ethylbenzene}} < D_{\text{toluene}} < D_{\text{benzene}} < D_{\text{chlorobenzene}} < D_{\text{bromobenzene}}$$

The obtained partitioning profiles for all studied solvents have approximately parabolic shape with maximum at the isoelectric point. The obtained results show that maximum of the extraction efficiency of *N*-phenylanthranilic acid occurs at pH 3.0–5.0 and the pH range of the extraction of *N*-phenylanthranilic acid corresponds to the pH range at which the neutral form of this acid, HR, is the dominant.

On the other hand, analyses of the UV–vis spectra in the aqueous (at pH about 1, 4, 8) and organic phases showed significantly different spectra for the cation, zwitterion and anion, while identical spectra were obtained in organic phases at any pH. Moreover, the spectra obtained in organic phase are identical with the spectra of *N*-phenylanthranilic acid in pure solvent (identified as that of the neutral form) for each investigated systems. This method was recommended by Takács-Novák et al. as very simple but exact experimental evidence for the partitioning of the neutral form even when it is the minor component [24]. Moreover, the literature data indicate that ion-pair formation as sulphate or hydrogen sulphate ammonium salts does not occur in that pH range between 2 and 8 [25]. These findings show that not the ion-pair but only the neutral form of the acid, HR, is transferred from aqueous to organic phase.

Taking advantage of the results of the potentiometric titration in two-phase systems: organic solvent–aqueous solution and using models of singular and multistep equilibria [17,18], the graphs presented dependence of $cHR_w/[HR]_w$ as a function of $[HR]_w$ for *N*-phenylanthranilic acid in the two-phase systems organic solvent–aqueous solution were prepared and presented in Fig. 5. These were a linear dependence (the values of the regression coefficients, *R*, were in the range of 0.9899–0.9993) so determination of the values of the distribution constants, K_D,

Table 3
The values of the distribution constant K_D and dimerization constant K_{dim} in the two-phase system: organic solvent–aqueous solution

Solvent	Hildebrand solubility parameter δ ($kJ^{1/2}\ m^{-3/2}$)	Distribution constant, K_D	Dimerization constant, K_{dim}
Ethylbenzene	17.84	58.79 ± 0.69	14.49 ± 0.78
Toluene	18.23	64.35 ± 0.34	12.05 ± 0.45
Benzene	18.72	75.12 ± 0.46	9.41 ± 0.54
Chlorobenzene	19.44	100.58 ± 0.77	6.34 ± 0.63
Bromobenzene	19.74	126.20 ± 0.74	4.02 ± 0.45
Nitrobenzene	20.46	258.05 ± 0.52	1.33 ± 0.15

and dimerization constants, K_{dim}, was possible. The calculated values of K_D and K_{dim} are presented in Table 3. The values of K_D and K_{dim} depend on the polarity of the organic solvent used. It has also been found that the values of distribution constants, K_D, increase while the values of dimerization constants, K_{dim}, decrease along with increasing of Hildebrand solubility parameter δ of the organic solvents studied.

The lowest value of K_D was found for the system with ethylbenzene ($K_D = 58.79$, $\delta = 17.84$), while the highest was observed for the system with nitrobenzene ($K_D = 258.05$, $\delta = 20.46$). At the same time, the dimerization constant, K_{dim}, decreases from 14.49 (ethylbenzene) to 1.33 (nitrobenzene).

Based on the determined values of dissociation constants, K_{a1} and K_{a2}, distribution constants, K_D, dimerization constants, K_{dim}, as well as the molar balance of amino acid in the studied systems the content of the particular forms of *N*-phenylanthranilic acid in both phases was calculated as a function of pH of the aqueous phase. The results obtained for the systems with extremely different K_D and K_{dim} are presented in Fig. 6. The obtained results show that for $pH < 1.5$ *N*-phenylanthranilic acid exists in the aqueous phase mainly as the protonated form, H_2R^+. The increase of pH causes that concentration of the acid in the organic phase increases too. For the pH range between 2.5 and 5.5 the percentage of monomeric form of the acid, HR_o, in organic phase is equal to about 97% for the system with nitrobenzene and about 80% for the system with ethylbenzene. The percentage of the dimeric form, $HR_{2,o}$, in those systems is lower and equal to about 1.3% for nitrobenzene and about 9.2% for ethylbenzene. Under the same conditions, the percentage of monomeric form of the acid in aqueous phase, HR_w, is very low—0.4% for the system with nitrobenzene and 1.4% for the system with ethylbenzene. The increase of pH of the aqueous phase (pH 5.5–8.0) results in decrease of the concentration of the neutral forms, HR_o, $HR_{2,o}$ iHR_w, and simultaneous increase of the concentration of the anionic form, R_w^-. At the higher value of pH ($pH > 8.0$), *N*-phenylanthranilic acid occurs only in the aqueous phase as the anionic form, R_w^-.

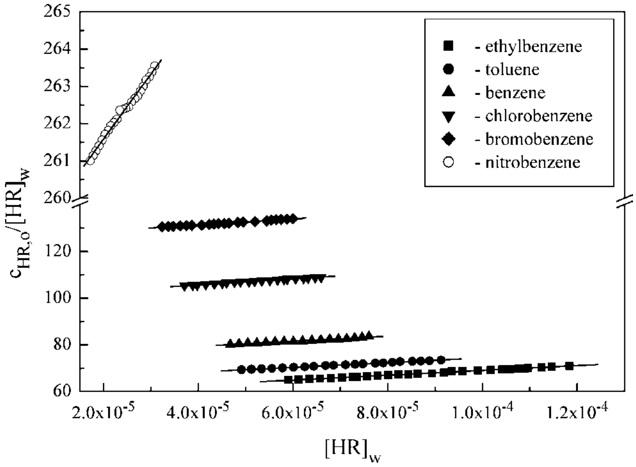

Fig. 5. $c_{HR,w}/[HR]_w$ vs. $[HR]_w$ for *N*-phenylanthranilic acid in the two-phase system: organic solvent (ethylbenzene, toluene, benzene, chlorobenzene, bromobenzene, nitrobenzene)–aqueous solution; the solid lines are the calculated curves based on the last squares method.

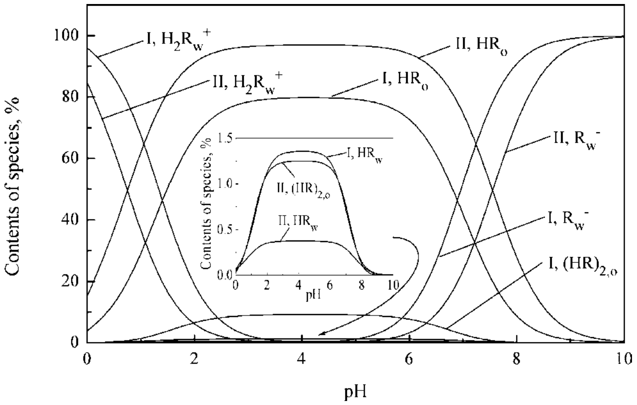

Fig. 6. The percentage of species of *N*-phenylanthranilic acid in the two-phase systems: organic solvent (I ethylbenzene, II nitrobenzene)–aqueous solution as a function of pH of the aqueous phase.

4. Conclusions

Knowledge of physical and chemical properties of *N*-phenylanthranilic acid is essential for the interpretation of structure–activity relationships. For this reason in this paper the detailed analysis of *N*-phenylanthranilic acid equilibria in aqueous solution and in two-phase systems: aromatic solvent–aqueous solution, characterized by appropriate constants (dissociation, K_{a1} and K_{a2}, distribution, K_D, dimerization, $K_{dim}K_{dim}$) were performed.

It was demonstrated that a suitable method to the characterization of *N*-phenylanthranilic acid in aqueous solutions was the potentiometric titration in methanol–water system. The application in our research of the Yasuda–Shedlovsky extrapolation procedure permitted to determine the aqueous pK_{a1} and pK_{a2} values.

Analyse of the UV–vis spectra in the aqueous and organic phases showed that the partitioning profiles obtained for all studied solvents have approximately parabolic shape with maximum at the isoelectric point and only the neutral form of *N*-phenylanthranilic acid undergo the extraction. The extraction of *N*-phenylanthranilic acid as ion-pair does not occur.

The application of the models of singular and multistep equilibria permitted to calculate the values of the distribution constants, K_D, and dimerization constants, K_{dim}. It was demonstrate that the values of K_D and K_{dim} depend on the polarity of the organic solvent used. It was found that the values of distribution constants, K_D, increase while the values of dimerization constants, K_{dim}, decrease along with increasing of Hildebrand solubility parameter δ of the organic solvents studied.

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