ВЗАИМОДЕЙСТВИЕ НИКТОТИНОВОЙ КИСЛОТЫ С ФЕНИЛАЛАНИНОМ В БУФЕРНЫХ РАСТВОРАХ: ИССЛЕДОВАНИЕ ТЕПЛОЕМКОСТИ И ОБЪЕМНЫХ СВОЙСТВ

Е.Ю. Тюнина, В.Г. Баделин, В.С. Егоркина

Елена Юрьевна Тюнина *, Валентин Георгиевич Баделин
Лаборатория «Термодинамика растворов незэлектролитов и биологически активных веществ», Институт химии растворов им. Г.А. Крестова РАН, ул. Академическая, 1, Иваново, Российская Федерация, 153045
E-mail: tey@isc-ras.ru *, vgb@isc-ras.ru

Валентина Сергеевна Егоркина
Факультет фундаментальной и прикладной химии, Ивановский государственный химико-технологический университет, Шереметевский просп., 10, Иваново, Российская Федерация, 153000
E-mail: valentina-egorkina@mail.ru

Термодинамические и физико-химические свойства многокомпонентных водных растворов, содержащих биохимически активные соединения, имеют большое значение в различных областях прикладной химии и особенно важны для понимания химических процессов, происходящих в биологических системах. Детальное изучение динамических сил процессов ассоциации и «молекулярного узнавания» между различными активными центрами белков и лекарственных средств затруднено вследствие сложности и разнообразия существующих взаимодействий в жидкостных многокомпонентных средах. Методами дифференциальной сканирующей калориметрии и денситометрии исследованы взаимодействия между модельными соединениями — фенилаланином и никотиновой кислотой в водном фосфатном буферном растворе. Измерения теплоемкости и плотности в системах никотиновая кислота – буфер, фенилаланин – буфер и никотиновая кислота– фенилаланин – буфер выполнены при разных температурах T = (288,15, 298,15, 308,15 и 318,15) К, используя ДСК «SCAL-I» (Pushchino, Russia) и DSA 5000 M (Anton Paar). Оценены значения кажущихся мольных параметров теплоемкости (Cр) и объема (Vр,НА) никотиновой кислоты в буферном растворе и в буферном растворе, содержащем 0,0120 моль/кг аминокислоты. Концентрация никотиновой кислоты изменялась в интервале (0,0079 - 0,036) моль/кг. Определены предельные значения (Cр 0) и (Vр,НА 0) и их производные по температуре. Показано, что взаимодействие NA с Phe сопровождается комплексообразованием. Молекулы NA в водном буферном растворе проявляют структуроразрушающие свойства, тогда как этот эффект уменьшается в буферном растворе с аминокислотой вследствие взаимодействия NA с молекулами Phe в процессе образования молекулярного комплекса между ними. Полученные результаты обсуждены на основе рассмотрения различных взаимодействий, действующих в исследуемых системах.

Ключевые слова: плотность, теплоемкость, кажущиеся мольные объемы и теплоемкости, L-фенилаланин, никотиновая кислота, образование комплекса
INTERACTION OF NICOTINIC ACID WITH L-PHENYLALANINE IN BUFFER SOLUTIONS: HEAT CAPACITY AND VOLUME PROPERTIES STUDY

E.Yu. Tyunina, V.G. Badelin, V.S. Egorkina

Thermodynamic and physicochemical properties of multicomponent aqueous solutions containing biologically active solutes are important in various areas of applied chemistry and are essential for understanding the chemistry of biological systems. Interactions between nicotinic acid (NA) and L-phenylalanine (Phe) were studied in aqueous phosphate buffer solutions (pH=7.35) by differential scanning calorimetry and volume methods. Heat capacities and densities of nicotinic acid-buffer, L-phenylalanine-buffer, and nicotinic acid-L-phenylalanine-buffer mixtures were determined at T=(288.15, 298.15, 308.15 and 318.15) K using the microdifferential scanning calorimeter SCAL-1 (Pushchino, Russia) and the density meter DSA 5000 M (Anton Paar). The apparent molar heat capacities ($\phi C_p$) and apparent molar volumes ($V_{\phi,NA}$) of nicotinic acid in buffer solution and in buffer 0.0120 mol kg$^{-1}$ amino acid solutions were evaluated. The concentration of NA was varied from (0.0079 to 0.036) mol kg$^{-1}$. The first and second differentials values were determined for NA in an aqueous buffer solution and for NA in an aqueous amino acid buffer solution. The interaction of NA with Phe is accompanied by complex formation. NA molecules in an aqueous buffer solution are water structure breakers, then the structure breaking effects of NA decrease as a result of interactions with Phe molecules during the complex formation in an aqueous amino acid buffer solution. The results were discussed in terms of various interactions taking place in this system.

Key words: density, heat capacity, apparent molar volume, heat capacity, L-phenylalanine, nicotinic acid, complex formation

Recent advanced in genetic engineering and other biotechnologies have spurred an increased interest in biochemical processes, with insights into the molecular-level interactions of protein and biological active compounds [1-6]. Fundamental thermodynamic data are critical in describing enzymatic active sites and various forms of molecular recognition. However, the study of driving forces for the association process is difficult because of the complexity of interactions in such a large molecule. It is well known, that amino acids are building blocks of proteins. Nicotinic acid, also known as pyridine-3-carboxylic acid, is a member of the B-vitamin family. Nicotinic acid has found important pharmacological applications, particularly, as an antihyperlipidemic agent in the reduction the cholesterol level [7]. Drugs are mostly transported in complexes of serum...
It is a protein consisting of aromatic amino acids residues [8, 9]. The complex formation of the aromatic amino acid, L-phenylalanine (Phe), with the nicotinic acid (NA) in aqueous solution (pH = 7.35) was earlier described [10-12] by calorimetry and UV-vis spectroscopy. No data are available in literature on the heat capacity and volume properties of nicotinic acid in aqueous buffer and aqueous amino acid buffer solutions.

The aim of this work was to study the interaction between the nicotinic acid and L-phenylalanine in aqueous buffer solutions (pH = 7.35) through the determination of heat capacity and volume properties.

**EXPERIMENTAL SECTION**

L-phenylalanine (Phe) (Sigma, ≥ 0.99) and nicotinic acid (NA) (Sigma-Aldrich, ≥ 0.98) were used without further purification after drying for 24 h at 356.15 K in a vacuum until constant weight. All measurements were performed in aqueous phosphate buffer with pH = 7.35. Stock phosphate buffer solutions were prepared using doubly distilled water and sodium phosphate monobasic (Sigma, ≥ 0.99) and sodium phosphate dibasic (Sigma, ≥ 0.99) and used within a few days, after checking the actual pH value with a digital pH-meter Mettler Toledo, model Five-Easy. The buffers were used as solvents for the preparation of the measurement solutions by mass (with an accuracy of 1×10⁻⁵ g) using a Sartorius-ME215S balance.

The density (ρ) of solution was measured using a digital precision vibrating densimeter DMA-5000 M (Anton Paar, Austria). The uncertainty in density measurements was within ±2×10⁻³ kg·m⁻³. The temperature inside the densimeter cell was controlled to ±1×10⁻³ K by built-in Peltier device. The densimeter was calibrated once a day with twice-distilled freshly degassed water and dry air. The densities of pure water at different temperatures were taken from [13].

The specific heat capacity (Cₚ) measurements of NA-buffer, Phe-buffer and NA-Phe-buffer mixtures were performed in a differential scanning microcalorimeter SCAL-1 (SCAL Co. Ltd., Pushchino, Russia [14, 15]) in glass cells of 0.337 cm³ capacity relative to buffer solvent at the scanning rate of 1.0 K/min. The temperature was maintained with accuracy ±0.01 K. The standard uncertainty of the heat capacity was within ±0.03%.

The concentration of amino acid was fixed at (0.0120±0.0002) mol·kg⁻¹. The concentration of the solutions of nicotinic acid was varied within the range (0.0079-0.0364) mol·kg⁻¹. The uncertainty in the molarities of the solutions was estimated within ±2·10⁻⁴ mol·kg⁻¹.

The densities and specific heat capacities of the solutions were determined at T = (288.15, 298.15, 308.15 and 318.15) K.

**RESULTS AND DISCUSSION**

The densities obtained decrease with increasing temperature and increase with NA concentration. Values of the specific heat capacities obtained decrease with increasing NA concentration and increase with rise in temperature. The apparent molar heat capacities (ΔCₚ) and volumes (Vₚ,NA) of the NA were calculated using respectively eq. 1 and eq. 2:

\[
\Delta C_p = M_{NA}C_p + 1000(c_p^0-c_p^\infty)m_{NA}, \\
V_{p,NA} = M_{NA}\rho - 1000(\rho-\rho_0)m_{NA}\rho_0, 
\]

in which Mₖ is the molecular mass of nicotinic acid (g·mol⁻¹), mₖ is the NA molality (mol·kg⁻¹), cₚ and cₚ₀ are the specific heat capacities of the solution and the solvent (J·K⁻¹·g⁻¹), and ρ and ρ₀ are the densities of the solution and the solvent (g·cm⁻³), respectively.

In aqueous solutions at pH = 7.35, the amino acid and NA exist as zwitterion and anion, respectively, as described in the literature [7, 10, 11]. The interaction of NA with Phe is accompanied by complex formation. This fact has experimental confirmations. The interactions of Phe with NA in buffer solution have been studied by calorimetric method and UV spectroscopy at 298.15 K [10-12] which reveals that the aromatic amino acid form 1:2 complex with NA characterized by an binding constant of middle strength (logKₐ = 3.68±0.03) [10]). The Phe/NA complex formation is an endothermal process [10, 11]. The complex formation between Phe and NA was characterized by positive values of enthalpy and entropy changes. Therefore, the complex is mainly entropically stabilized with a little contribution from the enthalpy factor [11].

The dependences of apparent molar volumes and heat capacities of NA on the NA concentration are represented at different temperatures (Figs. 1-4). As shown in Figs. 2 and 4, the shape of these curves shows that Phe undergoes the binding with NA. The function Vₚ,NA = f(mₖ) has a maximum corresponding to the stoichiometry of complex [16, 17]. The maximal values of Vₚ,NA were observed near mₖ = 0.0249 mol·kg⁻¹ at 298.15 K and near mₖ = 0.0219 mol·kg⁻¹ at 288.15, 308.15 and 318.15 K (Fig. 2), which correspond to the ~1:2 and 1:1.7 Phe/NA molar ratios, respectively. The maximal values of ΔCₚ were observed near mₖ = 0.0199 mol·kg⁻¹, which correspond to the ~1:1.7 Phe/NA molar ratios at the temperatures studied. Thus, the stoichiometry of the complex obtained was found to weakly depend on temperature.
The concentration dependences of the $V_{ϕ, NA}$ in aqueous buffer solutions (Fig. 1) were modeled by linear equations of the form:

$$V_{ϕ, NA} = V_{ϕ, NA}^0 + S, m_{NA},$$

where $V_{ϕ, NA}^0$ corresponds to the apparent molar volume extrapolated to infinite dilution and $S$, is a parameter characterizing the solute–solute interactions. The data on the apparent molar volume of NA in buffer Phe solution (Fig. 2) was fitted to an equation of the form:

$$V_{ϕ, NA} = V_{ϕ, NA}^0 + A, m_{NA} + B, m_{NA}^2.$$
molar volume, which is equal to the partial molar volume at infinite dilution, and A and B are the fitting coefficients. The values of \( V_{\text{p,NA}}^o \) together with standard errors derived by least squares fitting of the \( V_{\text{p,NA}}^o \) values to Eqs. 3-4 are reported in Table 1. The values of \( V_{\text{p,NA}}^o \) of NA in the buffer amino acid solutions are positive and increases with increase in the temperature, whereas a some decrease has been observed in case of the buffer solutions of NA. Since the formation of complex between Phe and NA involves changes in the hydration water of both amino acid and NA molecules, it must be reflected in the volume property. The increase in the values of \( V_{\text{p,NA}}^o \) with increase of temperature can be explained in terms of the desolvation and the overlap of hydration co-spheres of the solutes during the binding [18, 19]. The water molecules from the solvation shells of Phe zwitterions and NA molecules are released into the bulk aqueous solution, resulting in the expansion of the volume of solution at higher temperature.

### Table 1

<table>
<thead>
<tr>
<th>T, K</th>
<th>( V_{\text{p,NA}}^o ), cm(^3)·mol(^{-1})</th>
<th>( (\partial V_{\text{p,NA}}^o / \partial T)_{\text{b}} ), cm(^3)·mol(^{-1})·K(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>288.15</td>
<td>94.8±0.2</td>
<td>-0.47±0.10</td>
</tr>
<tr>
<td>298.15</td>
<td>91.0±0.3</td>
<td>-0.58±0.11</td>
</tr>
<tr>
<td>308.15</td>
<td>87.2±0.3</td>
<td>-0.67±0.15</td>
</tr>
<tr>
<td>318.15</td>
<td>76.9±0.4</td>
<td>-0.79±0.21</td>
</tr>
</tbody>
</table>

**Notes:** \( \gamma_{\text{m,m}} = 0.0120 \) mol·kg\(^{-1}\), \( \beta(\partial C_p^o / \partial T)_b \) was calculated by equation: \( (\partial C_p^o / \partial T)_b = \alpha + \beta T + \gamma T^2 \), where \( \alpha, \beta, \gamma \) are constants from Eq. (6).

The concentration dependences of the reported apparent molar heat capacities (Figs. 3, 4) were modeled using equations of the general form:

\[
\Delta C_p = \Delta C_p^o + A_{\text{NA}} + B_{\text{NA}}^2.
\]  

Here, \( \Delta C_p^o \) is the limiting value of apparent molar heat capacity, which is equal to the partial molar heat capacity at infinite dilution, and A, B are the fitting coefficients. The estimated values for \( \Delta C_p^o \) and their associated uncertainties are reported in Table 2.

The values of \( \Delta C_p^o \) for the NA in the binary (NA – buffer) and the ternary (NA – Phe – buffer) systems are positive and increase with increase in the temperature from 288.15 to 318.15 K. This behavior is indicative of hydrophilic character of the solutes molecules as described in the literature [20]. The increase of \( \Delta C_p^o \) with increase in temperature indicates that interactions between solutes molecules are stronger than intermolecular hydrogen bonding between water molecules. The higher positive \( \Delta C_p^o \) values for NA in 0.0120 mol·kg\(^{-1}\) Phe compared to the aqueous buffer solution at each temperature generally suggest that the interactions between Phe zwitterions and NA molecules are predominant.

### Table 2

**Limiting apparent molar heat capacities, \( \Delta C_p^o \), of nicotinic acid and \( (\partial \Delta C_p^o / \partial T)_b \) values in aqueous buffer solutions and in an aqueous buffer of L-phenylalanine at different temperatures**

<table>
<thead>
<tr>
<th>T, K</th>
<th>( \Delta C_p^o ), J·K(^{-1})·mol(^{-1})</th>
<th>( (\partial \Delta C_p^o / \partial T)_b ), J·K(^{-2})·mol(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>288.15</td>
<td>292±4</td>
<td>4.85±0.10</td>
</tr>
<tr>
<td>298.15</td>
<td>331±5</td>
<td>3.75±0.11</td>
</tr>
<tr>
<td>308.15</td>
<td>363±4</td>
<td>2.65±0.15</td>
</tr>
<tr>
<td>318.15</td>
<td>386±4</td>
<td>1.15±0.21</td>
</tr>
</tbody>
</table>

**Notes:** \( \gamma_{\text{m,m}} = 0.0120 \) mol·kg\(^{-1}\), \( (\partial \Delta C_p^o / \partial T)_b \) was calculated by equation: \( (\partial \Delta C_p^o / \partial T)_b = \beta + \gamma T^2 \), where \( \beta, \gamma \) are constants from Eq. (6).

The \( V_{\text{p,NA}}^o \) values for the NA studied in the aqueous buffer solutions and in the aqueous Phe buffer solutions at different temperatures were fitted by the method of least squares using the equation:

\[
V_{\text{p,NA}}^o = \alpha + \beta T + \gamma T^2,
\]  

where \( Y = V_{\text{p,NA}}^o \) or \( \Delta C_p^o \), \( \alpha, \beta, \gamma \) are constants and \( T \) is the temperature. The \( (\partial Y_{\text{p,NA}}^o / \partial T)_b \) and \( (\partial \Delta C_p^o / \partial T)_b \) parameters were then determined from the above Eq. 6. The calculated values of \( (\partial V_{\text{p,NA}}^o / \partial T)_b \) for NA are given in Table 1 at different temperatures. The values of \( (\partial V_{\text{p,NA}}^o / \partial T)_b \) decrease with increase in temperature in
case of NA – buffer solutions whereas in case of NA – Phe – buffer solutions the values of ($\partial^2 V_p/\partial T^2)_p$ increase with increase in temperature. The positive values of ($\partial^2 V_p/\partial T)_p$ indicate the release of electrostricted water from the solvation layers of NA and Phe and hence favoring amino acid – nicotinic acid interactions.

The second differential, ($\partial^2 V_p/\partial T^2)_p$ has been used to classify solutes on the basis of their effect on water – water interactions as described in the literature [21]. According to Hepler [21], the structure-breaking solutes possess negative ($\partial^2 V_p/\partial T^2)_p$ values. The positive values of ($\partial^2 V_p/\partial T)_p$ should be associated with the structure-making solutes. In our case, the values of ($\partial^2 V_p/\partial T)_p$ are -0.0099 cm$^6$·mol$^{-2}$·K$^{-2}$ for NA in aqueous buffer solution and 0.0107 cm$^6$·mol$^{-2}$·K$^{-2}$ for NA in aqueous amino acid buffer solution. Thus, the negative value of ($\partial^2 V_p/\partial T)_p$ suggests that NA in aqueous buffer solution is structure breaker. The positive value of the term can be indicated an obvious structure-making tendency of NA in aqueous Phe buffer solutions.

The ($\partial^2 C_p^o/\partial T)_p$ and ($\partial^2 C_v^o/\partial T)_p$ parameters were determined for NA in aqueous buffer solution and for NA in aqueous amino acid buffer solution using Eq. 6. The values of ($\partial^2 C_p^o/\partial T)_p$ at different temperatures are given in Table 2. The values of ($\partial^2 C_v^o/\partial T)_p$ are -0.11 J·K$^{-3}$·mol$^{-1}$ for NA in aqueous buffer solution and -0.08 J·K$^{-3}$·mol$^{-1}$ for NA in aqueous amino acid buffer solution. If one identifies NA molecules in aqueous buffer solution as water structure breakers [22, 23], then the structure breaking effects of NA decrease as a result of interactions with Phe molecules during the complex formation in aqueous amino acid buffer solution.

**CONCLUSIONS**

The heat capacities and densities measurements of nicotinic acid in aqueous buffer solutions and aqueous 0.0120 mol·kg$^{-1}$ L-phenylalanine buffer solutions have been carried out at various temperatures. Apparent molar heat capacities ($\partial C_p^o$) and volumes ($V_{\phi NA}$) and partial molar heat capacities ($\partial C_p^o$) and volumes ($V_{\phi NA}$) at infinite dilution have been calculated from the experimental data. The results obtained in this work, along with the data reported earlier [10-12], support that the hydrophilic–hydrophilic interactions of the zwitterionic centers (COO$^-$/NH$_3^+$) of Phe with the charged carboxyl group of NA molecule are predominant.

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