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CONDUCTOMETRIC STUDY OF THE ACIDITY PROPERTIES OF TANNIC ACID (CHINESE TANNIN)

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ABSTRACT

Two tannic acids are studied (H_nT, n = 52), $C_{76}H_{52}O_{46}$, with average molar mass 1701.20 g mol^{-1}. Using their UV and IR spectra it is shown that they have identical composition with respect to their functional groups, while by potentiometric and conductometric titration their structure of chinese tannin is verified and the relations between the acidity constants $K_{a1} > K_{a2} \sim K_{a3} \sim \ldots$ are determined. The absence of gallic acid is proved by HPLC. The conformational flexibility of the tannin molecule is used to measure the stepwise constant K_{a1}. By direct conductometry the acids were studied in the concentration range of 5.00×10^{-4} to 5.00×10^{-2} mol l^{-1}. The latter is determined from the Onsager-Shedlovsky relation. The molar conductivity of the ions $\mathrm{H_{n-1}T^{-}}$ for the infinitely dilute solutions of the two tannic acids is found to be 55.2 and 64.3 S L mol^{-1} cm^{-1}. The degree of dissociation α in the studied concentration range varies from 0.03 to 0.3. The results for the acidity constant exponent pK_{a1} are generalised using variance analysis, yielding $\mathrm{pK_{a1} \pm \Delta pK_{a1} = 4.19 \pm 0.02}$, n = 26. In addition, the approximate value of $pK_{a2} \sim 6.0$ is determined.

Keywords: tannic acid, tannin, conductometry, acidity constant.

INTRODUCTION

Tannic acid (tannin, gallotannin, TA, H_nT) is one of the numerous natural polymers. The subject of our study is tannin, $C_{76}H_{52}O_{46}$, with molar mass 1701.20 g mol^{-1} and structure of chinese tannin, as shown in Fig. 1 [1-4]. It is found in a very pure state in the plant species Chinese galls (Rhus semilata) and Sumac (Rhus typhina) [1]. This TA has valuable characteristics: the conformational flexibility of the molecule [5], complex formation of compounds with proteins and metal ions [4-11]. These complex compounds have anti-bacterial and anti-fungal properties [12]. Well known are also the anti-oxidant, anti-mutagenic and anti-carcinogenic properties of TA [13-15], styptic and shrinking action upon the mucous tissues. Besides in the leather, textile, wine and pharmaceutic industries, gallotannin has applications in medicine, as well. It has inhibitory activity on HIV-1 replication [16] and is being studied with the aim of creating stabilised elastin for cardio-vascular bio-prostheses [5].

The chemical nature of TA is an ester of the D-glucose with gallic acid. Its molecule consists of pentagalloyl D-glucose, PGG, (Fig. 1) and a chain of 5 more galloyl groups on average, $\overline{m} = 5$, which are bonded by hydrolytic depcide linkages to one of the galloyl groups of the PGG [1,5,15]. There are published data from HPLC-MS studies of the tannin 48811 Fluka (with a structure as shown in Fig. 1) which show a presence of tetragalloyl and hexagalloyl glucose and absence of gallic acid [17]. The large number of phenolic groups determines its acidity properties. No analytical data exist in the literature for the acidity constants of TA. In a 1953 textbook about leather tanning [18] the following values for the acidity constants are given: pK_{a1} = 4.23 and pK_{a2} - pK_{a5} = 8.35 - 12.20. The studies were performed using potentiometric titration of 1 % TA with 1 mol l^{-1} KOH, TA being purified by means of dialysis and electro-dialysis. No information was given for the source of the tannin or its structure, the temperature and the exact concentration of the solution, or of the confidence intervals of the constants. The acidity constants of TA cannot be calculated with the computer program ACD/Labs due to the large number of H^+ ions in the phenolic groups.

An overview of the published data shows that TA has a complex structure and a complex composition, determined by the number of galloyl groups in its molecules. The task of the present work is to study two tannins with identical structure and molar mass. The aim is to test the possibility to determine the first stepwise acidity constant K_{a1} of TA = H_nT, n = 52, with an average galloyl groups number $\overline{m} = 5$. Preliminary experimental and theoretical data showed that the most suitable analytical method for the study is direct conductometry, applied for determination of the equilibrium constant [18-23]. There are three basic advantages of this method. The conformational flexibility of the galloyl chain in the molecule of TA has a positive influence on the conductivity of its ions. For the water solutions of the acid in a wide concentration range, the acidity constant is calculated from the Ostwald dilution law, using the dissociation degree α, namely,

$$K_{a1} = \frac{\alpha^2 C_{TA}}{(1-\alpha)} \quad [20].$$

The ratio, $\alpha = \lambda / \lambda_\infty$ [20], of the molar conductivity (λ) and that at infinite dilution (λ_∞) will best characterise the acid-base properties of the studied solution of TA. An important condition for the polyprotic tannin being studied is that in conductometry the inhibitory influence of the second degree of dissociation begins to manifest itself when there are small differences between the exponents of the acidity constants pK_{a2} and pK_{a1}, namely, for $pK_{a2} - pK_{a1} < 2$ [19]. In comparison, this difference in potentiometry is ~ 3 [20].

EXPERIMENTAL

Reagents

The tannic acids Fluka 48811 TA1 and 403040 (ACS reagent) TA2 with molar mass 1701.20 g mol^{-1} are produced by Sigma-Aldrich (USA). The NaOH solution, 0.1000 mol l^{-1} is prepared from a solution of NaOH, fixanal, 1.000 mol l^{-1}. All solutions of the reagents are prepared with double-distilled water. The carbon dioxide from the solutions was removed by blowing out with 99.99 % nitrogen.

Apparatus

The specific conductivity of the solutions is measured by a conductometer CDM 92, Radiometer, with capability to recalculate the conductivity for a given temperature. The conductometric cell CDC 641, Radiometer, has electrodes made of platinized platinum. The potentiometric measurements are performed using a pH/mV/Temperature Meter, model 3510, Jenway, England and a galvanic cell with pH combined glass electrode, Boeco, Germany. The galvanic cell and the glass vessel for conductometric measurements have orifices for blowing out with nitrogen gas, for the conductometric cell and the combined electrode, respectively, as well as for the burette. The solutions were stirred with an electromagnetic stirrer.

Procedure

The tannic acid is dried for two hours at a temperature of 105°C. From the dried acid an initial solution with concentration 5.00×10^{-2} mol l^{-1} is prepared.

For the direct conductometric measurements, a series of solutions with concentrations 5.00×10^{-5}, 1.00×10^{-4}, 5.00×10^{-4}, 1.00×10^{-3}, 5.00×10^{-3} and 1.00×10^{-2} mol l^{-1} are prepared from the initial solution via consecutive dilution with double-distilled water. The measurement begins with the most diluted solution. A given volume from the studied solution is transferred into the clean and dry glass vessel. The volume of the solution is determined from that of the glass vessel. The conductometric cell is immersed in the vessel. Also attached is a system for blowing out with nitrogen over the surface of the solution. This is followed by blowing out and stirring (200 revolutions min^{-1}) for 10 minutes, stopping the two processes and measurement of the specific conductivity, γ, of the solution in still state at a temperature of 25°C.

For the measurement of the conductivity of the acid at infinite dilution, an initial solution of its sodium salt ($NaH_{n-1}T$) with concentration 5.00×10^{-3} mol l^{-1} is prepared. The amount of NaOH is determined by conductometric titration of TA. The solutions with lower concentrations of up to 2.50×10^{-5} mol l^{-1} are prepared by consecutive dilution with double-distilled water. The specific conductivity of the solutions is measured using the procedure described above at 25°C.

Solutions of TA1 and TA2 with concentrations $1.000x10^{-2}$ mol l^{-1} are prepared for the titration. Potentiometric titration with NaOH solution (0.1000 mol l^{-1}) is applied to 50.00 ml of the solutions of each of the two acids at 20°C, while conductometric – to 40.00 ml of the solutions of each of the two acids at 25°C. The titrations are carried out in absence of CO_2, the solutions in the galvanic cell and the conductometric glass vessel are blown out with 99.99 % nitrogen.

RESULTS AND DISCUSSION

Composition and structure of the studied tannic acids

The choice of tannins is an indispensable part of this study as the companies producing the reagents provide no information about the structure or the plant sources of the acids. Based on the preliminary experiments and contacts with the company (Sigma-Aldrich), the reagents TA1 and TA2 have the structure shown in Fig. 1. The two tannins differ from each other in the intensity of their coloring. TA2 has a more saturated beige-brown color.

The acid TA1 is a reagent with a qualification purum. In order to verify its purity the UV and IR spectra of TA1 and TA2 are recorded. The absorption spectra, $A = f(\lambda)$, of water solutions of the acid, registered against distilled water, have identical behavior with two maxima at 225 and 275 nm (see Fig. 2), while the IR spectra, $\%T = f(\bar{\upsilon})$, of TA1 and TA2 are presented in Fig. 3. In these figures all peaks and bands of the functional groups of the tannin appear at one and the same wave numbers. In the IR spectrum of TA1 no bands of groups from other substances are found, i.e. TA1 and TA2 are identical in composition. An interesting result for the acid-base and complex-formation characteristics of the tannin is obtained for the wave number of the OH-groups. It has values from 3372 cm^{-1} to , lower than 3600 cm^{-1}, which shows the presence of hydrogen bonds in the compound [25, 26].

The HPLC study with an UV-detector using the method of Kawamoto et al. [27], confirmed the published data [17] about the absence of gallic acid, $pK_{a1} = 4.41$ [25], in TA1 and TA2. This result is logical because gallic acid has a low solubility in water [29]. The remaining components are not separated and recorded due to nonexistent standards for the higher and lower molecular compounds of the studied tannic acid, as well as due to a low sensitivity due to the fast saturation of the separation column.

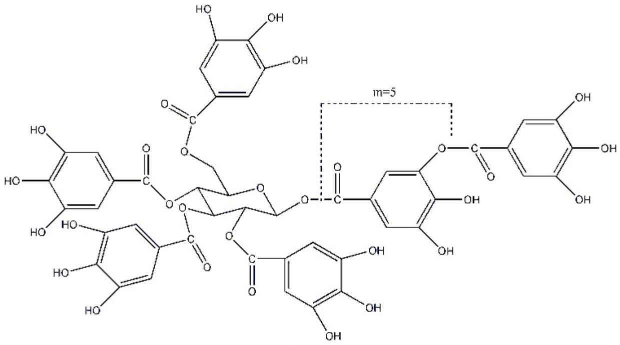

Fig. 1. Structure of tannic acid (Chinese tannin).

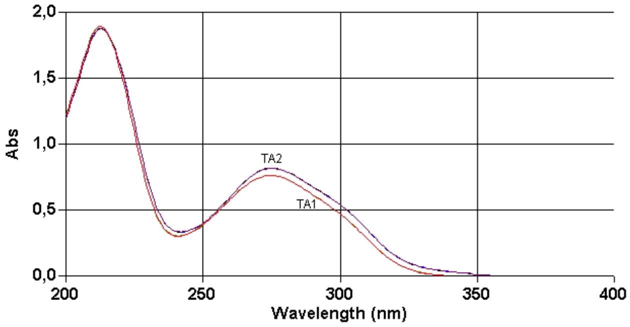

Fig. 2. UV-spectra of $1.04x10^{-5}$ mol l^{-1} TA1 and $1.09x10^{-5}$ mol l^{-1} TA2, registered against distilled water.

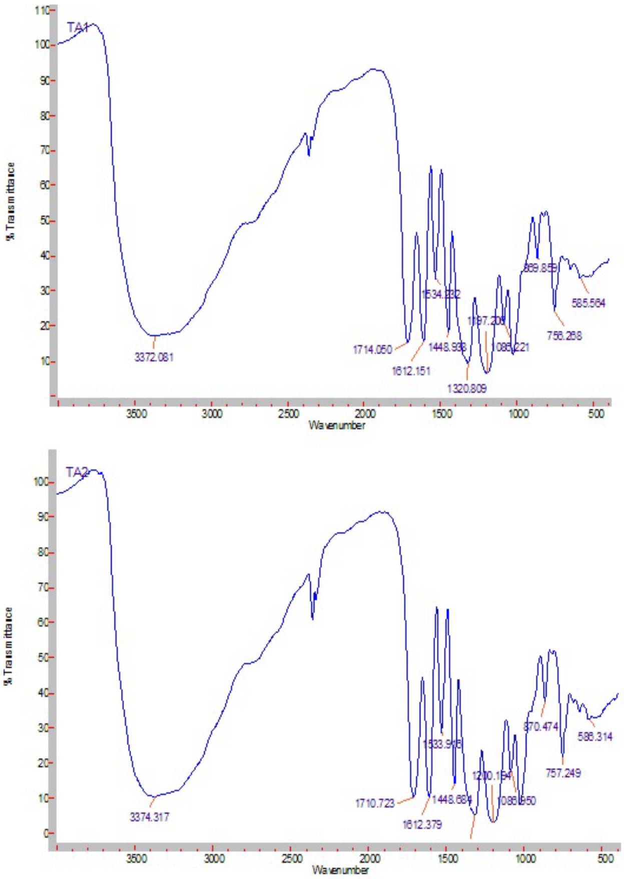

Fig. 3. IR-spectra, $\%T = f(\bar{\nu})$, of the tannic acids TA1 and TA2.

The percentage content of tannic acid in the reagent TA1 – 97.8%, is determined using the method of Slinkard [30] relative to the standard substance TA2 (ACS). This spectro-photometric method uses a reagent of Folin with which polyphenols are analyzed. The structures and compositions of TA1 and TA2, relative to the number of galloyl groups, are compared indirectly – using the potentiometric and conductometric titration curves of equimolar water solutions of the acids. The functions pH = f (V_{NaOH}) and γ = f (V_{NaOH}), γ - specific conductivity, are presented in Fig. 4 and Fig. 5, respectively.

The potentiometric titration curves (Fig. 4) each have one well pronounced jump in the pH interval ~ 4.3 - 6.0, i.e. the protons that take part in the acid-base reactions of TA1 and TA2 are from the phenolic groups in the galloyl groups and are equally and sufficiently separated from PGG along the galloyl chain. These protons determine the first acidity constant of the acid K_{a1}. The second and the third stepwise constants have values that are very close to each other or $K_{a1} > K_{a2} \sim K_{a3} \sim ....$ The latter conclusion is confirmed also by the conductometric curves in Fig. 5. The volumes of NaOH solution used up to the equivalent points (V_{NaOH}^{eq}) of the curves are found by potentiometric titration using the Gran plots [31], while in conductometric titration the equations of the linear parts of the curves are used (Fig. 5). The volumes of the solutions of the titrant at the equivalent points actually obtained (Fig. 4 and Fig. 5) have higher values than 5 ml in potentiometric titration and 4 ml in conductometric titration of TA1 and TA2 (see the procedure in EXPERIMENTAL; 5 ml and 4 ml would be the volumes of NaOH solution used up to the equivalent point for titration of TA with structure and composition of all molecules, as given in Fig. 1). For the

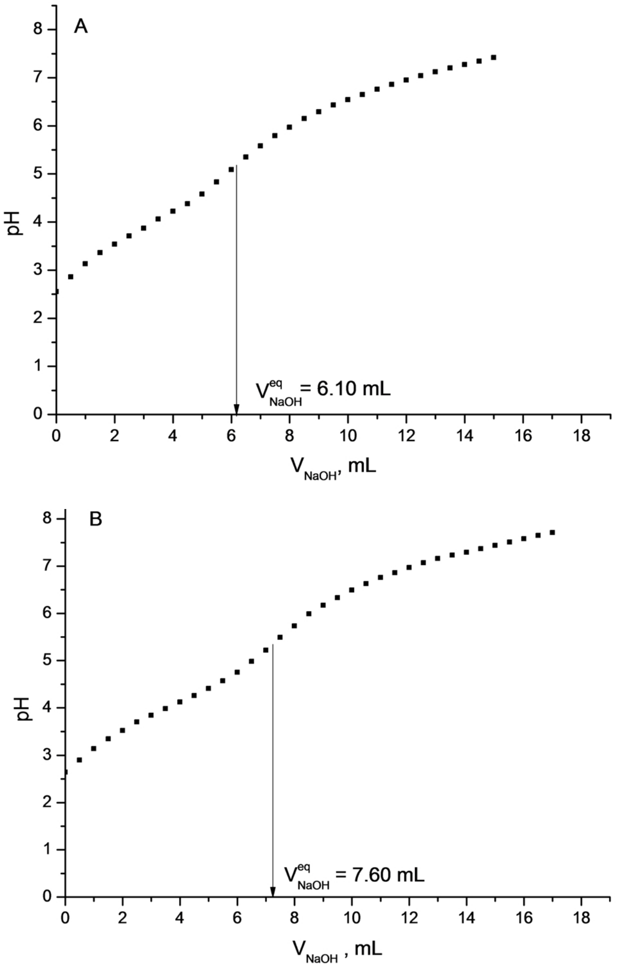

Fig. 4. The functions pH = f (V_{NaOH}), C_{NaOH} = 0.1000 mol l^{-1}, t = 20° C for: A) titration of 50.00 ml 0.01002 mol l^{-1} TA1; B) titration of 50.00 ml 0.009996 mol l^{-1} TA2.

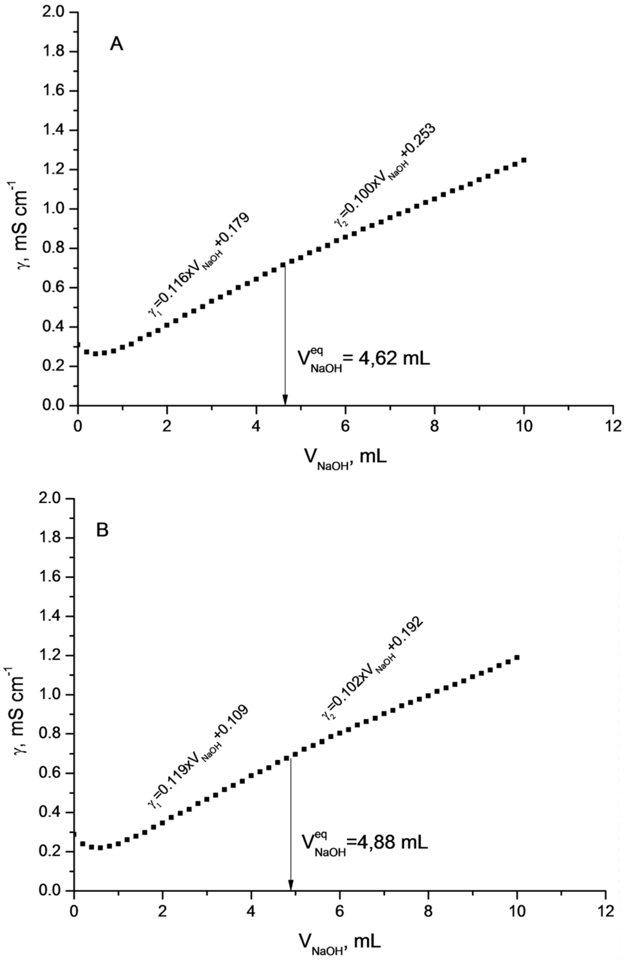

Fig. 5. The functions γ = f (V_{NaOH}), C_{NaOH} = 0.1000 mol l^{-1}, t = 25°C for: A) titration of 40.00 ml 0.009995 mol l^{-1} TA1; B) titration of 40.00 ml 0.01002 mol l^{-1} TA2.

potentiometric titration of TA1, the volume of the titrant is 22 % higher than expected, while for the conductometric titration is 15 %. For TA2 these values are respectively 52 % and 22 %. The percentage increase of the volumes of the titrant is lower for the conductometric titration of the two acids. Two conclusions follow from the data presented above: TA2 contains more molecules with galloyl groups below and above the average of 10 in comparison with TA1. The molecules with more galloyl groups are titrated like polyprotonic acids up to the equivalent point, while those with lower number cannot be titrated. The presence of molecules with nuclei of tetra- and hexagalloylglucose also influences the values of the consumed volumes of the NaOH titrant solution. The second conclusion concerns the difference in the values of the exponents of the stepwise acidity constants pK_{a2} and pK_{a1}, $pK_{a2} - pK_{a1} \approx 2$. The small differences in the slopes of the linear parts of each of the conductometric titration curves, given by their equations in Fig. 5, confirm this conclusion. It is obvious that a part of the acid $H_{n-1}T^{-}$ is also titrated at the potentiometric detection end-point.

Both studied tannic acids are natural products with identical structure and different composition: the first contains predominantly tannins with smaller molar mass than average and the second - tannins with larger molar mass than the average. These tannic acids are also most probably obtained from different plant species. The majority of the tannins TA1 and TA2 consist of molecules containing 10 galloyl groups (Fig. 1).

Molar conductivities of the solutions of TA1 and TA2 at infinite dilution and concentration range of protolysis of H_nT to $H_{n-1}T^{-}$

The studies are performed using the procedure described above with sodium salts $NaH_{n-1}T$ of the acids TA1 and TA2. The amounts of NaOH, necessary for the preparation of the salts, are determined from the consumed volumes of NaOH solution at the equivalent point of the conductometric titration curves of TA1 and TA2 (Fig. 5). The studied concentrations of $NaH_{n-1}T$ ($C_{NaH_{n-1}T}$) are in the range from $2.50\text{x}10^{-5}$ to $5.00\text{x}10^{-3}$ mol l^{-1}. The molar conductivities $\lambda_{i(NaH_{n-1}T)}$ are calculated from the measured specific conductivities of the solutions $\gamma_{i(NaH_{n-1}T)}$. The results for $\lambda_{i(NaH_{n-1}T)}$ are processed according to the equation of Onsager-Shedlovsky [32]. The functions

$$\frac{1}{\lambda_{i(NaH_{n-1}T)}} = f(\sqrt{C_{NaH_{n-1}T}})$$

for the sodium salts of the two acids are presented in Fig. 6. The behaviour of the two curves is identical and two clearly pronounced linear regions are visible for each of them. At $C_{NaH_{n-1}T} < 5\text{x}10^{-4}$ mol l^{-1} the values of the molar conductivities of the solutions significantly increase as the concentration of $NaH_{n-1}T$ decreases. This correlation is consistent with the conclusions from the potentiometric and conductometric titration curves in Fig. 4 and Fig. 5, $K_{a1} > K_{a2} \sim K_{a3} \sim \ldots$ Therefore the molar conductivities of the solutions with low concentrations of $NaH_{n-1}T$ are the sums of the conductivities of the ions:

$$\lambda_{(NaH_{n-1}T)} = \lambda_{Na^{+}} + \lambda_{H^{+}} + \lambda_{H_{n-1}T^{-}} + \lambda_{H_{n-2}T^{2-}} + \lambda_{H_{n-3}T^{3-}} + \ldots \quad (1)$$

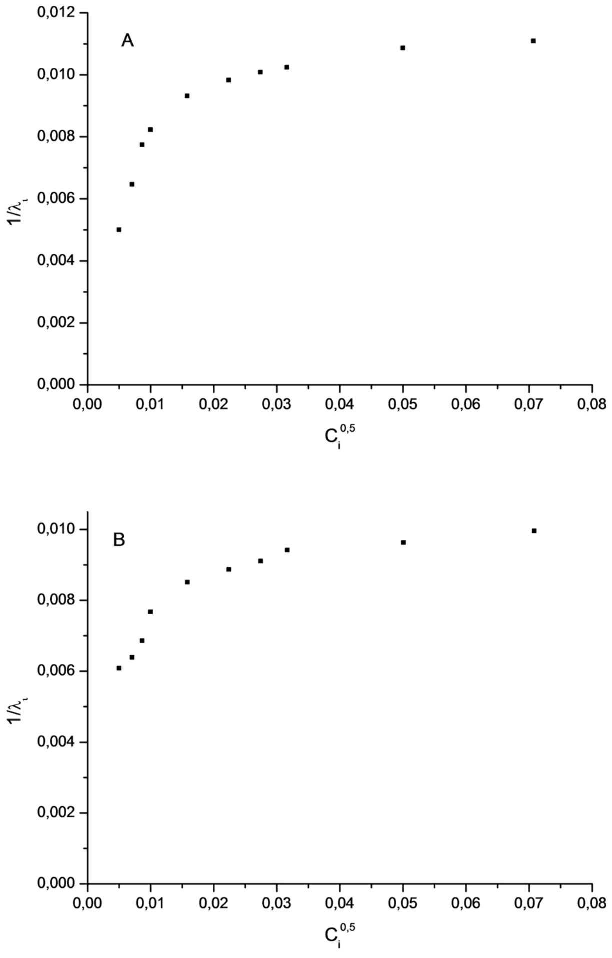

Fig. 6. The functions $\frac{1}{\lambda_{i(NaH_{n-1}T)}} = f\sqrt{C_{i(NaH_{n-1}T)}}$ of $\lambda^{\infty}_{H_{n-1}T}$ determination for: A) TA1; B) TA2.

Table 1. Data for the equations of the second linear parts of the functions in Fig. 6 (at $C_{NaH_{n-1}T} > 5\times10^{-4}$ mol l^{-1}).

TA	$\frac{1}{\lambda_{NaH_{n-1}T}} = a + b\sqrt{C_{NaH_{n-1}T}}$	$\lambda^{\infty}_{H_{n-1}T^-}$, S L cm^{-1} mol^{-1}	$\lambda^{\infty}_{H_nT}$, S L cm^{-1} mol^{-1}
TA1	a=0.00950; b=0.0237 corr=0.97	55.2	405.0
TA2	A=0.00874; b=0.0174 corr=0.97	64.3	414.1

To determine the dissociation degree α and the first stepwise acidity constant K_{a1}, the second linear parts of the functions in Fig. 6 when > $5x10^{-4}$ mol l^{-1} are relevant. The slopes of the straight lines are small (has a small value, is a weak acid) and the molar conductivities are found from the sum:,

$$\lambda_{NaH_{n-1}T^-} = \lambda_{Na^+} + \lambda_{H_{n-1}T^-} \tag{2}$$

where $H_{n-1}T^-$ denotes the mean composition of the anion of TA1 and TA2. According to the equation of Onsager – Shedlovsky [30, 31], the linear segment, *a*, takes the form:

$$a = \frac{1}{\lambda^{\infty}_{NaH_{n-1}T}} = \frac{1}{\lambda^{\infty}_{Na^+} + \lambda^{\infty}_{H_{n-1}T^-}} \tag{3}$$

Using the value $\lambda^{\infty}_{Na^+}$ = 50.1 S L cm^{-1} mol^{-1} at 25^{o}C [19, 32] allows us to calculate $\lambda^{\infty}_{H_{n-1}T^-}$ for the salts of TA1 and TA2.

The limiting conductances of the acids TA1 and TA2 are found from the sums:

$$\lambda^{\infty}_{H_nT} = \lambda^{\infty}_{H^+} + \lambda^{\infty}_{H_{n-1}T^-} \tag{4}$$

$\lambda^{\infty}_{H^+}$ = 349.8 S L cm^{-1} mol^{-1} at 25^{o}C [19,32].

The processing of the experimental results is performed in the following order: the equations of the linear parts for $C_{NaH_{n-1}T}$ > $5x10^{-4}$ mol l^{-1} are derived using regression analysis and the values of $\lambda^{\infty}_{H_{n-1}T^-}$ and $\lambda^{\infty}_{H_nT}$ are successively calculated from the equations (2) and (3), respectively.

The obtained values are presented in Tab. 1. The results for $\lambda^{\infty}_{H_nT}$ of the acids TA1 and TA2 are different because their content of molecules with larger and smaller number of galloyl groups is different. The relatively large values of $\lambda^{\infty}_{H_{n-1}T^-}$ for molecules with large molar mass are due to the flexibility of the galloyl chain, in which the charge of the ion is present as well. On the basis of the functions

$$\frac{1}{\lambda_{(NaH_{n-1}T)}} = f(\sqrt{C_{NaH_{n-1}T}}),$$

the experimental studies of the constant K_{a1} should be performed at C_{TA1} and C_{TA2} > $5x10^{-4}$ mol l^{-1}.

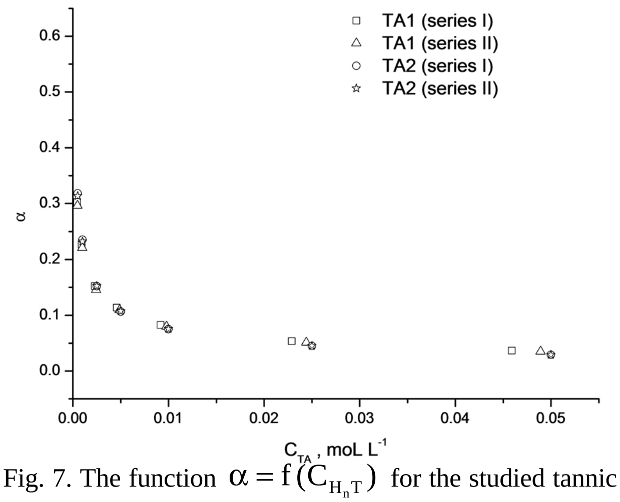

Fig. 7. The function $\alpha = f(C_{H_nT})$ for the studied tannic acids TA1 and TA2.

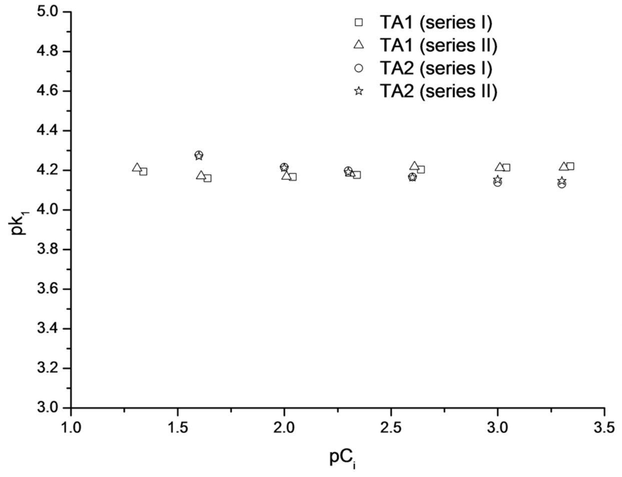

Fig. 8. Results for the acidity constant pK_{a1} of TA.

Table 2. Data for the average values of pK_{a1} in the studied series, standard deviations and number of results n.

TA	TA1		TA2	
series	I	II	I	II
pK_{a1} mean	4.190	4.197	4.188	4.190
s	0.02354	0.02156	0.05505	0.04695
n	7	7	6	6

Degree of dissociation α and acidity constant pK_{a1} of the tannic acid

The analysis of the dissociation degree α of the acids TA1 and TA2 is performed according to the procedure given in EXPERIMENTAL. The specific conductivities of four series of water solutions of the acids TA1 and TA2 (two for TA1 and two for TA2) are measured in the concentration range from $5.00x10^{-4}$ to $5.00x10^{-2}$ mol l^{-1}. The molar conductivities are calculated and using the obtained values for λ^{∞}_{TA1} and λ^{∞}_{TA2}, Table 1, the dissociation degree of each concentration of TA1 and TA2 under analysis in each performed experiment are found. The results for α are presented in Fig.7. They vary in the range 0.03 - 0.3 and display a well-shaped hyperbolic function $\alpha = f(C_{TA})$. For each solution the value of the acidity constant K_{a1} is calculated as a function of the respective values for the dissociation degree and the concentration of TA1 and TA2 according to Ostwald dilution law [20]. The 26 results for pK_{a1} obtained in this way are given in Fig. 8, however they belong to the four studied series. A check for outliers using the criterion of Dean and Dixon is performed for each series, the average values of pK_{a1} and the standard deviations *s* are shown in Table 2. The average values of pK_{a1} in the series are compared using an analysis of variance. The χ^2-test and the subsequent F-test show that the differences in the standard deviations and the average values for pK_{a1} are due to random errors:

$$\chi^2 = 7.09 < \chi^2\ (\alpha = 0.05;\ f = 3) = 7.81$$

$$F = 0.072 < F\ (\alpha = 0.05;\ f_1 = 3;\ f_2 = 22) = 3.05\ [25, 33].$$

The following results are obtained for the acidity constant and its confidence interval: $pK_{a1} \pm \Delta pK_{a1} = 4.19 \pm 0.02$, consistent with the result given in [18], $pK_{a1} = 4.23$. The value of pK_{a1} defines TA as a moderately strong acid [25, 34].

CONCLUSIONS

Tannic acid is a complex natural product and the choice of two reactive agents, TA1 and TA2, with the same molar mass and structure requires preliminary research. The result of the first stepwise acidity constant, $pK_{a1} \pm \Delta pK_{a1} = 4.19 \pm 0.02$, n=26, achieved from the experiments on TA1 and TA2, proves that direct conductometry is a suitable method of research of its acidity properties in a wide concentration range. In addition, computer simulations have been performed on the structural models of TA with a lower number galloyl groups in the chain, m < 5. They showed that the first protolysis reaction is most likely due to a proton from the phenolic group in the next-to-last galloyl residue in the chain. This is further influenced by the hydrogen bonds proven in the IR spectrums of the acids.

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