

Determination of complex 12-grade phytic acid dissociation constants

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The potentiometric method was used to titrate hydrochloric acid with sodium phytate using a combined pH electrode. Four groups of 12-grade dissociation constants, cumulative dissociation constants and ionization degrees of phytic acid were calculated according to the changes in the pH value and the related substance concentration in the titration process. A group of 12-grade relatively reasonable dissociation constants K_a , cumulative dissociation constants β and ionization degrees α of phytic acid was selected, with K_a values between 10^{-10} and 10^{10} , β values in the range of 10^{-12} to 10^{27} , and α values from 10^{-5} to 1. The data are relatively objective and reasonable and provide a theoretical basis for in-depth research on phytic acid.

Keywords: phytic acid; potentiometric titration; dissociation constants; cumulative dissociation constants; ionization degrees

INTRODUCTION

Phytic acid ($C_6H_{18}O_{24}P_6$), also known as myoinositol hexaphosphate, is present in most cereal grains, legumes, nuts, oilseeds, tubers, pollen, spores and organic soils [1-3]. It has been the subject of several reviews [4] owing to the following physical and chemical properties: light yellow or light brown syrupy liquid with relative molecular mass of $660.04 \text{ g mol}^{-1}$, strong acid, soluble in water, 95% ethanol and acetone, insoluble in anhydrous diethyl ether, benzene, hexane, chloroform, etc., decomposed easily by heating, more stable at higher concentrations. Phytic acid in plant sources is natural, non-toxic to human and green to environment, more secure and reliable compared with synthetic food additives. It is a superior example of green food additives because of its unique chemical properties, special physiological, pharmacological and health functions [5, 6]. At present, it covers various fields of industry [7], agriculture, food [8], pharmaceuticals, chemicals, metal corrosion [9, 10],

gradually showing more new features and getting involved in the whole process of human life [11].

The structure of the phytic acid molecule is shown in Figure 1. As can be seen, phytic acid molecule with 12 acidic hydrogen atoms has 12-grade different dissociation constants.

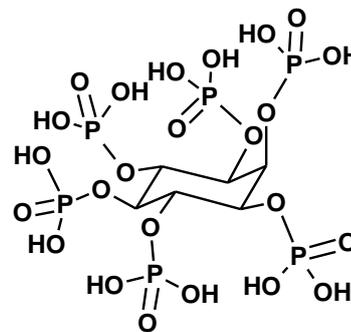


Fig. 1 Structure of phytic acid

On the basis of previous research, 12-grade dissociation constants were obtained by Evans *et al.* [12] in 1982; the results between 10^{12} and 10^{-11} acquired by the application of acid-base titration were relatively objective. However, these results were not accurate according to phytic acid structure, and there are no more accurate reports of phytic acid dissociation constants at all levels in the latest literature.

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The objectives of this study were to apply the method of potentiometric titration of hydrochloric acid with sodium phytate using a combined pH electrode. The dissociation constants, accumulated dissociation constants and ionization degrees of phytic acid at all levels were calculated from the pH titration curve. The reliability of the obtained results is confirmed by the analysis of phytic acid structure, and provides a theoretical basis for further research and development of phytic acid from natural product.

EXPERIMENTAL

Chemical reagents and apparatus

Titration was carried out using a ZD-2A automatic potentiometric titrator (Shanghai Dapu instrument Co., Ltd. China) equipped with 405-60-SC-P-PA- K19/120/3mMETTLER pH/ORP electrode (Shenzhen TRT technology Co., Ltd. China). Weighing was performed on a FA/JA series precision electronic balance (Accuracy: 0.0001 g, Tianjin Tianma hengji instrument Co., Ltd. China). Ultrasound (KQ-100 NC ultrasonic cleaning machine, Kunshan ultrasonic instruments Co., Ltd. China) was used to promote the dissolution of phytic acid in water. DZF-6050B vacuum drying oven (Shanghai Qixin technology instrument Co., Ltd. China) was used to dry sodium borate.

Sodium phytate was purchased from Sigma-Aldrich Corporation. Sodium borate and concentrated HCl were obtained from Shanghai Reagent Co. All reagents were of analytical grade. Deionized water from an ultrapure Milli-Q system (Millipore, Molsheim, France) was used.

Experimental Methods

Sample preparation:

100 mL sodium borate solution was prepared by dissolving 2.8612 g of sodium borate in deionized water. 3.8 mL of concentrated HCl was diluted to 250 mL for calibration. 100 mL standard solution of sodium phytate was obtained by dissolving 9.6584 g of sodium phytate in water.

Calibration of the HCl standard solution:

The concentration of the HCl solution was calibrated by titration. The 10.00 mL standard sodium borate solution was titrated with the HCl solution until the end point of pH = 5.27. The volume of consumed hydrochloric acid was recorded. The above operation was repeated 3 times. The average concentration of the HCl standard solution was calculated to be $0.1250 \text{ mol}\cdot\text{L}^{-1}$. Measurement of 12-grade dissociation constants of phytic acid:

The HCl standard solution and the sodium phytate solution were used for the pH measurements. The temperature of all operations was maintained at $25.0\pm 0.1^\circ\text{C}$.

Data Processing

The experimental data obtained were processed by Excel, statistical analysis using Origin 8.0 software.

RESULTS AND DISCUSSION

Calculation of phytic acid dissociation constants

The results presented in Figure 2 demonstrate that the pH values of the solution varied from 0.74 to 9.67 in the titration process, because the gradually increasing volume of the solution led to a dilution effect, and the concentrations of sodium phytate and HCl changed accordingly (see Figure 3). The hydrogen ion calibration concentration, namely stoichiometric concentration, was denoted as concentration 1. On the other hand, the corrected concentration of hydrogen ion, denoted as concentration 2, includes the activity factors, in other words, this hydrogen ion concentration was calculated according to the electrode potential value. The pH value, the sodium phytate concentration and the HCl concentration were selected in line with the stoichiometric point corresponding to concentration 1 and concentration 2, substituted into the following formula to calculate.

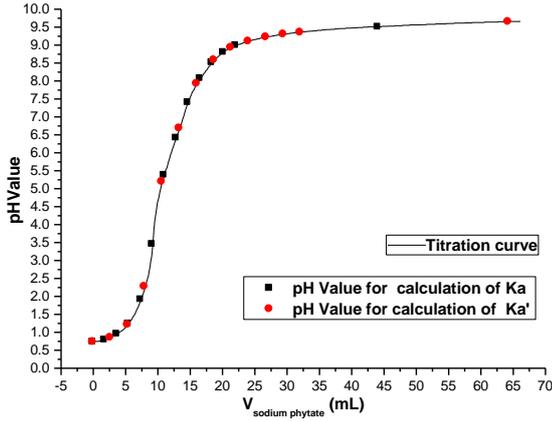


Fig. 2. Curves of sodium phytate titration of HCl

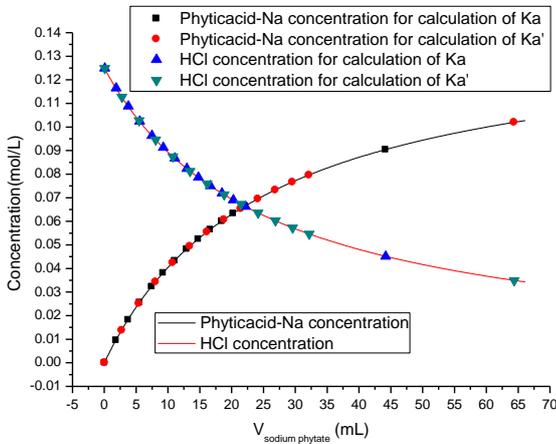
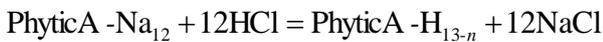


Fig. 3. Concentrations of sodium phytate and HCl

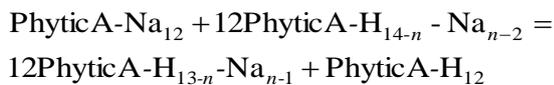
In the titration process, it was assumed that every step of the acid-base titration proceeded completely, the chemical reaction equations being as follows:

When the molar ratio of sodium phytate and HCl was kept at 1:12, the following reaction took place:



Where n is equal to 1.

While the molar ratio of sodium phytate and HCl was kept at 2 : 12, 3 : 12, , 12 : 12, the following reactions occurred:



where n is equal to 2, 3, , 11 or 12.

According to the change in pH values during the titration process, the hydrogen ion concentration

satisfied the following formula:

$$[\text{H}^+]_n = \sqrt{\frac{\prod_{i=n}^{12} K_{ai} \cdot [\text{PhyticA} - \text{H}_{13-n} - \text{Na}_{n-1}]}{[\text{PhyticA} - \text{Na}_{12}]}}$$

From the material balance of the titration process of sodium phytate it follows that:

$$c_{\text{PhyticANa}_{12}} = [\text{PhyticA} - \text{Na}_{12}] + [\text{PhyticA} - \text{H}_{13-n} - \text{Na}_{n-1}]$$

Based on the material balance of the titration process of HCl it follows that:

$$[\text{PhyticA} - \text{H}_{13-n} - \text{Na}_{n-1}] = \frac{c_{\text{HCl}}}{13 - n}$$

$$[\text{PhyticA} - \text{Na}_{12}] = c_{\text{PhyticANa}_{12}} - \frac{c_{\text{HCl}}}{13 - n}$$

Reactions (1) ~ (12) merged, that is:

from (1)+(2)+(3)+.....+(11)+(12), was derived:



When the reactions were completed, and sodium phytate was added dropwise, no chemical reaction occurred, $\text{PhyticA} - \text{Na}_{12}$ and

$\text{PhyticA} - \text{H} - \text{Na}_{11}$ existed only in the buffer

solution, and the hydrogen ion concentration at this time could be expressed by the following relationship:

$$[\text{H}^+]_{13} = \frac{K_{a12}[\text{PhyticA} - \text{H} - \text{Na}_{11}]}{[\text{PhyticA} - \text{Na}_{12}]}$$

$$c_{\text{PhyticANa}_{12}} = [\text{PhyticA} - \text{Na}_{12}] + [\text{PhyticA} - \text{H} - \text{Na}_{11}]$$

$$[\text{PhyticA} - \text{H} - \text{Na}_{11}] = c_{\text{HCl}}$$

$$[\text{PhyticA} - \text{Na}_{12}] = c_{\text{PhyticANa}_{12}} - c_{\text{HCl}}$$

According to the amount of sodium phytate consumed in the titration process, the concentrations of $\text{PhyticA} - \text{Na}$, HCl, $[\text{PhyticA} - \text{H}_{13-n} - \text{Na}_{n-1}]$, $[\text{PhyticA} - \text{Na}_{12}]$, $[\text{H}^+]$ in the reaction (1), (2), (3), (11), (12) are listed in Table 1. No chemical reactions occurred again after the reaction (12) was completed. $\text{PhyticA} - \text{Na}_{12}$

and PhyticA - H - Na₁₁ consisted of buffer solution, but the proportion of the two compositions changed in the buffer solution system with the addition of PhyticA - Na₁₂. Therefore, the concentrations of PhyticA-Na, HCl, PhyticA-H_{13-n}-Na_{n-1}, [PhyticA-Na₁₂], [H⁺] varied correspondingly, the results are shown in Table 1. On the basis of the above formula and the data in Table 1, 12-grade dissociation constants K_a , cumulative dissociation constants β and ionization degrees α of phytic acid were calculated, according to which Figure 4, Figure 5 and Figure 6 were obtained.

*Reasonable analysis of phytic acid K_a , β
 α*

Analysis of K_a

In Figure 4, it is seen that $pK_{a1} \sim pK_{a4}$ were gradually decreasing, and $pK_{a5} \sim pK_{a10}$ increasing according to the stoichiometric concentration. The results were not related to the excessive quantities of sodium phytate in the titration process. When the quantities of sodium phytate in the solution were

excessive, pK_{a12}' and pK_{a11}' , adjacent to pK_{a10}' and pK_{a9}' that were computed according to the concentrations of all species had relatively large errors (Figure 4). In other words, there was no clear downward trend from pK_{a12}' to pK_{a9}' , contrarily, pK_{a12}' was too high, and pK_{a11}' too low. This set of data was unreasonable and was given up. Furthermore, pK_a (corrected) calculated by the corrected concentrations showed a distinct downward trend from pK_{a12} to pK_{a3} , but a sharply upward trend from pK_{a3} (corrected) to pK_{a1} (corrected). So pK_{a3} (corrected) had a minimum value. Compared with pK_a calculated by uncorrected concentrations, pK_{a3} (calibration) was so low that could reach around -24. It was largely different from both pK_{a2} (corrected) = -9 and pK_{a4} (corrected) = -6, so that they could be excluded. Consequently, pK_a in the experiment was more reasonable to have the same variational tendency as pK_a in ref. [12]. Decreasing tendency was displayed from pK_{a1} to pK_{a4} , and increasing from pK_{a5} to pK_{a12} . The changing scale of pK_a was smaller. This set of pK_a values was more reasonable than that reported in the literature. As the difference from pK_{a10} to pK_{a12} reported in the literature was greater than 0, it was concluded that except the last three

Table 1 Concentrations of each component from the 1st grade to the 12th grade of the titration reactions

n	$c(\text{mol}\cdot\text{L}^{-1})$ PhyticA - Na ₁₂	$c(\text{mol}\cdot\text{L}^{-1})$ HCl	[PhyticA- H _{13-n} -Na _{n-1}]	[PhyticA- Na ₁₂]	[H ⁺]
—	0.000000	0.125000	0.000000	0.000000	1.81972E-01
1	0.010001	0.116171	0.009681	0.000321	1.58489E-01
2	0.018255	0.108885	0.009899	0.008356	1.09648E-01
3	0.025534	0.102459	0.010246	0.015289	5.62340E-02
4	0.032341	0.096451	0.010717	0.021624	1.20230E-02
5	0.038091	0.091374	0.011422	0.026669	3.47000E-04
6	0.043267	0.086806	0.012401	0.030866	4.07000E-06
7	0.048196	0.082454	0.013742	0.034454	3.80000E-07
8	0.052431	0.078715	0.015743	0.036688	3.89000E-08
9	0.056504	0.075120	0.018780	0.037724	8.32000E-09
10	0.060033	0.072005	0.024002	0.036032	3.02000E-09
11	0.063281	0.069137	0.034569	0.028713	1.55000E-09
12	0.066441	0.066348	0.066348	0.000093	1.00000E-09
12 ^a	0.090370	0.045224	0.045224	0.045146	3.09000E-10

^a With PhyticA-Na₁₂ concentration increasing, no chemical reactions occur.

hydrogens in phytic acid which dissociated weakly, the other hydrogens dissociated strongly. This assumption was not proper, too. In our research, pK_{a1} , and pK_{a6} to pK_{a12} were greater than 0. The weak acid dissociation was self-evident including the 1st grade and the 6th grade to the 12th grade.

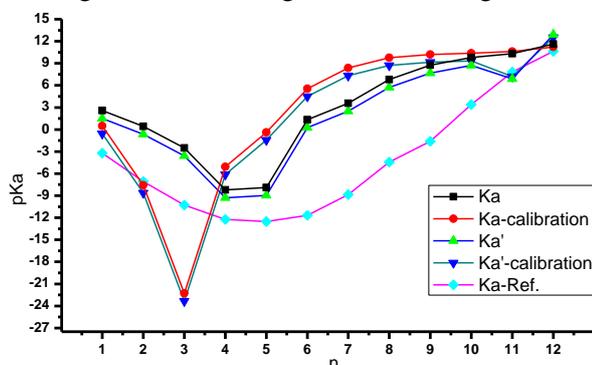


Fig. 4. 1st to 12th dissociation constants ($pK_{a1} \sim 12$) values of phytic acid

Analysis of β

The cumulative dissociation constants β were calculated from the corresponding dissociation constants of pK_a above. The results in Figure 5 show that the values of $p\beta_1$ to $p\beta_9$ in the literature were low, which pointed to strong acid dissociation. The values of $p\beta_{10}$ to $p\beta_{12}$ were greater than those of $p\beta_1$ to $p\beta_9$. The values of $p\beta_1$ to $p\beta_{12}$ were lower than -3, which assumed strong acid dissociation in every grade which contradicts with the phytic acid being a medium strong acid. From the results, it could be seen that the calculated cumulative dissociation constants β are certainly rational by comparison with the calculated results in the literature.

Analysis of α

The ionization degrees α in every grade were calculated from the corresponding dissociation constants of pK_a above. As shown in Figure 6, the values of α_1 to α_9 in the literature [12] were great enough to illustrate the full ionization of phytic acid from the 1st grade to the 9th grade. However, the values of α_{10} were dramatically lower. As strong

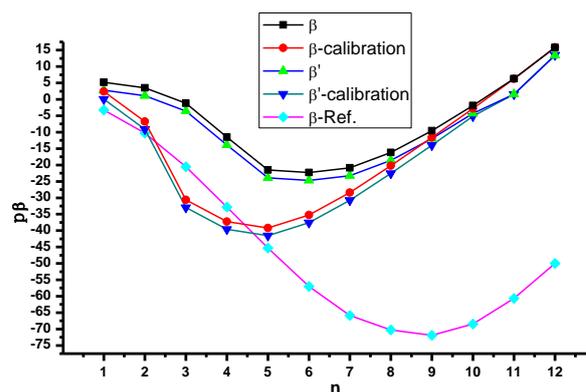


Fig. 5. 1st to 12th cumulative dissociation constants ($\beta_1 \sim 12$ values) of phytic acid

acidity was shown from the former 9 ionizations, and weak from the latter 3 ionizations, the data were not reasonable. The values of α_2 (corrected) to α_5 (corrected) calculated by corrected concentrations showed that phytic acid was almost completely ionized from the 2nd grade to the 5th grade. This statement is opposite to that of ionization from the 6th grade to the 12th grade, and the ionization degrees were close to 0. The unreasonable outcome illustrated that phytic acid molecule was composed of 5 strongly acidic hydrogens and 7 weak hydrogens. There had been a set of reasonable data calculated by uncorrected concentrations, according to which the value of α_1 was quite low, the values of α_2 to α_5 were close to 1, α_6 to α_7 lay between 0 and 1, and $\alpha_8 \sim \alpha_{12}$ were nearly equal to 0. It was concluded that the 1st hydrogen in the phytic acid molecule exhibited weak acidity, the 2nd to 5th hydrogens showed strong acidity, the 6th and 7th hydrogens - medium strong acidity, and the 8th to 12th hydrogens - weak acidity. The data indicated that the ionization degree values of phytic acid increased to 1 and then decreased to nearly 0 gradually, showing a certain regularity.

Analysis of phytic acid structures

The chemical structure of phytic acid is complex, and is different in aqueous solution from that in solid [13, 14]. This is seen in Figures 1 and 7 in the light of the earlier investigation of Johnson *et al.* [15]. Due to the presence of hydrogen bonds in aqueous solution, phosphorus oxygen double bonds ($P=O$) in the phytic acid molecule could bind water

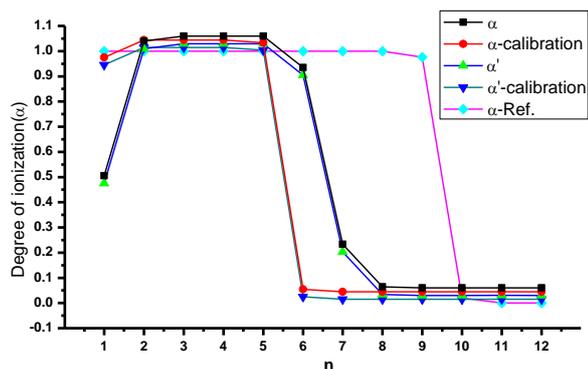


Fig. 6. 1st to 12th ionization degrees ($\alpha_1 \sim \alpha_{12}$ values) of phytic acid

molecules to form trihydrate phytic acid (Figure 7A). In this process, trigonal bipyramidal structure of phosphorus atoms is formed with the transformation from sp^3 hybridization to sp^3d [16], in which 3 hydroxy groups are attached to a phosphorus atom. In aqueous solution, the structure is so unstable that it is easy to dehydrate to form a stable sp^3 hybrid structure [12]. De-trihydrate inositol phosphate (Figure 7B) was obtained by dehydration of three water molecules. It was more appropriate that the structure was called phytic anhydride. Commonly, hydrogen bonds could form easily in strong polybasic acid. On the basis of analyzing the molecular structure, an assumption was proposed: as a hexa-basic acid, phytic anhydride could form intramolecular hydrogen bonds (Figure 7C). Two six-membered rings which were composed of two phosphorus atoms and one oxygen bridge atom were in chair conformation. This conformation similar to the structure of adamantane was chemically relatively stable (hydrogen ion could not easily dissociate).

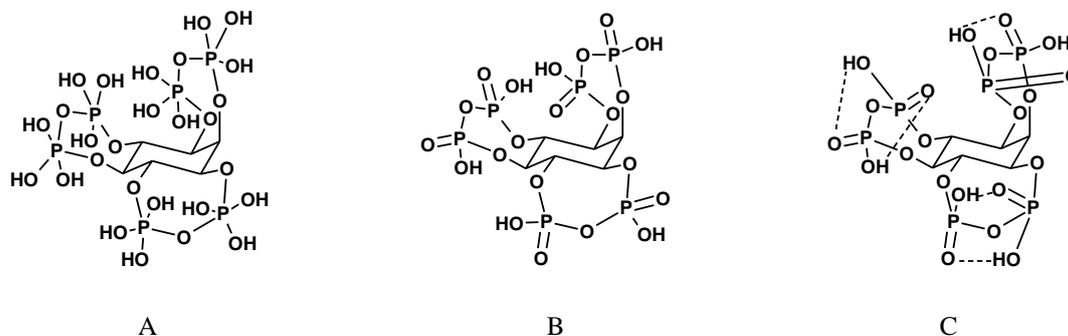


Fig. 7. Structures of phytic acid. A: Trihydrate phytic acid; B: Phytic acid anhydride; C: Phytic acid anhydride with intra-molecular hydrogen bonds

Therefore, the first-grade ionization degree α_1 of phytic acid is relatively smaller, with a value about 0.5. When one hydrogen ion from phytic acid anhydride is ionized, the presence of hydrogen ions would promote the destruction of the intramolecular hydrogen bonds, resulting in a relatively larger ionization degree between the second-grade to the sixth-grade $\alpha_2 \sim \alpha_6$ near to 1.0, and displaying strong acid ionization. At present, the six hydrogen in phytic acid anhydride ionized completely, and the framework structure of hexavalent phytic acid anhydride anion was formed (Figure 7B). In the process of further ionization of phytic acid anhydride, each molecule needs to combine three water molecules to form phytic acid (Figure 1), while phytic acid would further combine water molecules to generate trihydrate phytic acid (Figure 7A). Phytic acid and trihydrate phytic acid, which are weak acids, coexist in the equilibrium solution system. So, phytic acid of the 7th-grade to the 12th-grade ionization degrees $\alpha_7 \sim \alpha_{12}$ which were all below 0.2 was relatively smaller. Therefore, it was concluded that the ionization degrees in our works were more reasonable than those of the other 4 groups (including α) according to the assumed molecular structure and reported in the literature.

CONCLUSION

Potentiometric titration was used for titrating sodium phytate with hydrochloric acid using a combined pH electrode. Four groups of 12-grade dissociation constants, cumulative dissociation constants and ionization degrees of phytic acid

were calculated according to the changes of the pH value and the related substance concentrations in the titration process. A group of 12-grade relatively reasonable dissociation constants K_a , cumulative dissociation constants β and ionization degrees α of phytic acid was obtained compared with the corresponding literature values. The results shown in Table 2 are consistent with phytic acid structure. The data are relatively objective and reasonable and provide a theoretical basis for in-depth research on natural phytic acid.

Table 2 Dissociation constants, cumulative dissociation constants and ionization degrees at all levels of phytic acid

n	K_{an}	β_n	α_n
1	3.57735E-01	3.57735E-01	0.445415
2	4.92753E+01	1.76275E+01	0.980490
3	4.45596E+04	7.85476E+05	0.999978
4	2.17067E+10	1.70501E+16	1.000000
5	1.05257E+10	1.79464E+26	1.000000
6	6.12137E+00	1.09857E+27	0.874942
7	3.64539E-02	4.00470E+25	0.173570
8	2.16032E-05	8.65143E+20	0.004637
9	2.32527E-07	2.01169E+14	0.000482
10	2.07516E-08	4.17458E+06	0.000144
11	6.45879E-09	2.69628E-02	0.000080
12	3.08492E-10	8.31779E-12	0.000018

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ОПРЕДЕЛЯНЕ НА СЛОЖНА, ДВАНАДЕСЕТОСТЕПЕННА ДИСОЦИАЦИОННА КОНСТАНТА НА ФИТИНОВА КИСЕЛИНА

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(Резюме)

Използван е потенциометричен метод за титруване на солна киселина с натриев фитат с комбиниран рН-електрод. Изчислени са четири групи от 12-степенни дисоциационни константи, кумулативни дисоциационни константи и йонизационни степени на фитиновата киселина според измененията на рН и съответните концентрации на титрувания субстрат. Подбрана е група от 12-степенни дисоциационни константи K_a , кумулативни дисоциационни константи β и степени на йонизация α за фитиновата киселина със стойности на K_a между 10^{-10} и 10^{10} , стойности на β в интервала от 10^{-12} до 10^{27} и стойности на α от 10^{-5} до 1. Данните са относително обективни, разумни и предлагат теоретична основа за по-задълбочени изследвания.