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Potentiometric Study of Acid-base Properties of Thiamine Hydrochloride and Thiamine Mononitrate in Aqueous Medium

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Abstract. A comparative study of thiamine mononitrate and thiamine hydrochloride potentiometric titrations with a strong base in water was performed. The analysis of resulting curves allowed associating particular reactions to each observed pH break point. The corresponding pKa values were calculated by using the program SUPERQUAD and they were subsequently used to simulate titration curves. The pKa values obtained in the conditions of the present work are: $pK_{a1} = 4.80 \pm 0.05$, $pK_{a2} \geq 10.40 \pm 0.04$, $pK_{a3} \leq 8.10 \pm 0.04$. The system behavior mainly depends on parameters such as the time for measuring pH, NaOH concentration and particularly on the stability of one of the two amphotolytes. Comparison of calculated and experimental curves revealed the existence of a dismutation reaction which showed that under steady state conditions and the waiting time between two successive pH measurements, the intermediate “pseudobase (B)”, called like this by several researchers, practically does not exist. The results led to find the optimal conditions to propose quantitative alkalimetric titrations. The end-points for the assay of thiamine hydrochloride and thiamine nitrate in raw material were detected by potentiometry. This involves the use of reagents and solvents that are environment-friendly.

Keywords: Potentiometry, thiamine, dismutation, amphotolyte, acid-base titrations.

Resumen. Se llevó a cabo un estudio comparativo de las titulaciones potenciométricas del mononitrito de tiamina y del clorhidrato de tiamina con una base fuerte en agua. El análisis de las curvas resultantes permitió asociar las reacciones particulares a cada salto de pH observado. Los valores de pK_a se calcularon usando el programa SUPERQUAD y posteriormente fueron usados para simular las curvas de titulación. Los valores de pK_a obtenidos en las condiciones del presente trabajo son: $pK_{a1} = 4.80 \pm 0.05$, $pK_{a2} \geq 10.40 \pm 0.04$, $pK_{a3} \leq 8.10 \pm 0.04$. El comportamiento del sistema depende principalmente de parámetros tales como el tiempo de medida del pH, la concentración de NaOH y particularmente de la estabilidad de uno de los dos anfolitos. La comparación entre las curvas experimentales y simuladas reveló la existencia de una reacción de dismutación la cual mostró que bajo condiciones de estado estacionario y un tiempo de espera entre dos medidas sucesivas de pH, la “pseudobase (B)” intermedia, llamada así por varios autores, prácticamente no existe. Los resultados llevaron a encontrar las condiciones óptimas para proponer titulaciones alcalimétricas cuantitativas. Los puntos de equivalencia para la determinación de clorhidrato de tiamina y nitrato de tiamina en materia prima fueron detectados por potenciometría. Esto implica el uso de reactivos y disolventes de poco impacto al medio ambiente.

Palabras clave: Potenciometría, tiamina, dismutación, anfolito, titulaciones ácido-base.

Introduction

Thiamine, vitamin B1 or aneurin, was the first B vitamin discovered. A deficit of this vitamin causes several diseases in children, such as beri-beri [1,2] which occurs in infants fed with breast milk deficient in this vitamin [3], and epilepsy [4]. These diseases also occur in adults with high intake of white rice or food containing anti-thiamine factors. Malaria [5], Wernicke encephalopathy and Korsakoff syndrome [6-9], and neuronal death [10] are also produced by thiamine deficiency. Excessive consumption of alcohol causes thiamine deficiency [11]. Reductions in thiamine-dependent enzymes have been implicated in multiple neurological disorders including Alzheimer's disease [12].

Many methods have been reported for the determination of thiamine in pure powder and in pharmaceutical forms. Direct titration, which is done with perchloric acid in non-aqueous medium [13,14], indirect complexometric [15,16] and amperometric titrations [17-19]. However, in all these methods strong

interferences among the basic compounds occur. Argentometric [20,21] and mercurimetric [22] titrations also present interferences due to the halide salts of other vitamins, bases and minerals. In some cases, these methods make use of hazardous reagents and solvents.

Thiamine, as hydrochloride or mononitrate (Figure 1), is a molecule that presents acid-base properties. Studies of acid-base titrations generally are contradictory and do not conclusively identify the titration reactions associated with the equivalence points because of the complexity of the deprotonation mechanisms inherent to thiamine structure [23,24].

The aim of the present work is to present a comparative study of the experimental and simulated potentiometric titration curves of thiamine mononitrate and thiamine hydrochloride with a strong base, in water. This analysis permitted us to associate titration reactions for each observed pH break and to find the optimal conditions for a quantitative assay of thiamine hydrochloride in raw material. This method makes use of reagents and solvents more environment-friendly.

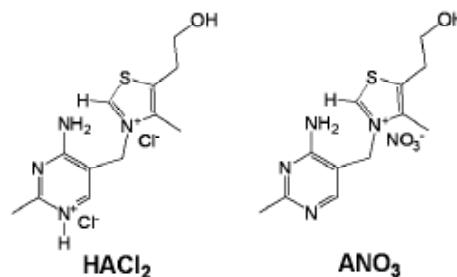


Fig. 1. Structures of thiamine hydrochloride (HACl₂) and thiamine mononitrate (ANO₃)

Results and discussions

Acid-base titrations in water

Figure 2 shows the titration curves of thiamine mononitrate with 0.46 M sodium hydroxide, recording pH as a function of ϕ (ϕ = mmol NaOH/mmol total thiamine). Each curve was obtained using a different waiting time for pH measuring after alkali addition (from 5 to 120 s or more). All curves start from a pH near 8 and a pH break gradually appears as the measuring time increases up to 60 s, where this break is well defined. This pH break corresponds to the consumption of 2 moles of alkali per mole of thiamine nitrate.

These results are in well agreement with those reported by Ogston and Peters [25], who concluded that the global transformation of cation A⁺ to thiol C⁻ requires two equivalents of OH⁻ with an average pKa=9.2 [22,25]. This transformation mechanism implies the existence of a tetrahedral intermediate, “pseudobase B”, where the step A⁺ → B is slow, while the step B → C⁻ is fast, as shown in the reactions of Figure 3 [23, 25, 26].

Figure 4 presents the curves, pH vs ϕ , for the titration of thiamine hydrochloride with 0.46 M NaOH. These curves were obtained for waiting times between successive pH measurements from 5 to 120 s or more. In this case, all the curves initiate at pH about 3.6 and exhibit a common first pH break corresponding to one equivalent of NaOH. As the waiting time for measuring pH increases, the gradual emergence of a second pH break, due to the consumption of two moles of NaOH is observed, just as for the case of thiamine mononitrate titration (Figure 2). The first reaction is the deprotonation of piperidinium ring while the second and third reactions seem to be the same as those observed for thiamine mononitrate. Thus, thiamine hydrochloride is combined with a total of three NaOH equivalents. The described behavior is observed from waiting times of 120 s until 360 s.

These results seem to be the intermediate case between those observed by Hassan and Elnemma [27] who have reported that only one pH break is present using 0.01 M NaOH, but three pH breaks are observed using 1.00 M NaOH.

In order to explore the existence extent of B species, the global formation constants of several chemical models were

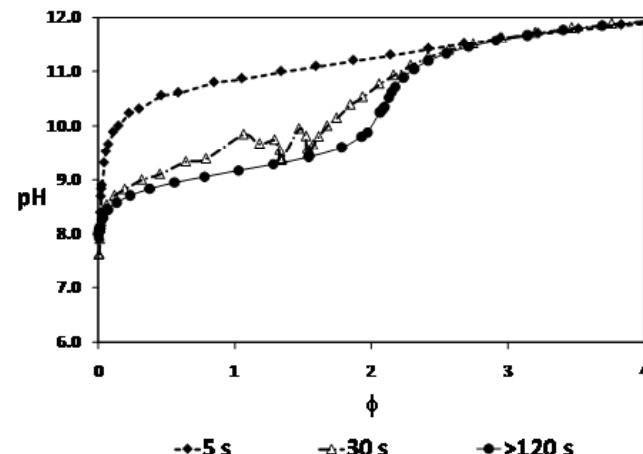


Fig. 2. Titration curves of thiamine mononitrate (A⁺) with 0.46 M NaOH. Graphs of pH as a function of ϕ (ϕ = mmol NaOH/mmol total thiamine) were obtained for different measuring times (t = 5, 30 and 120 s or more) after alkali addition.

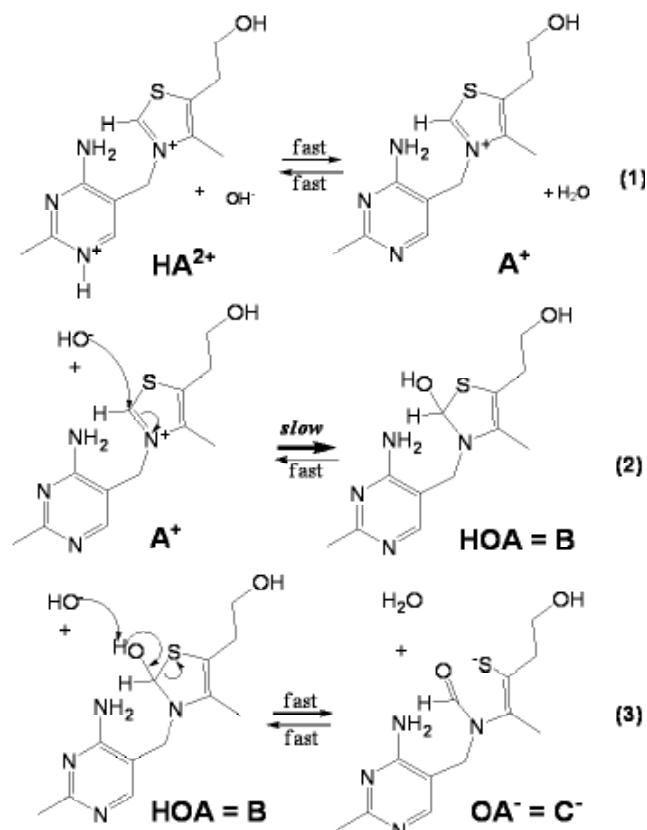


Fig. 3. Acid-base reactions proposed in order to explain the chemical behavior of fully protonated thiamine HA²⁺. The nomenclature B and C⁻, has been introduced for homologation with that of HA²⁺ and A⁺ species, in order to achieve more clarity for explanations.

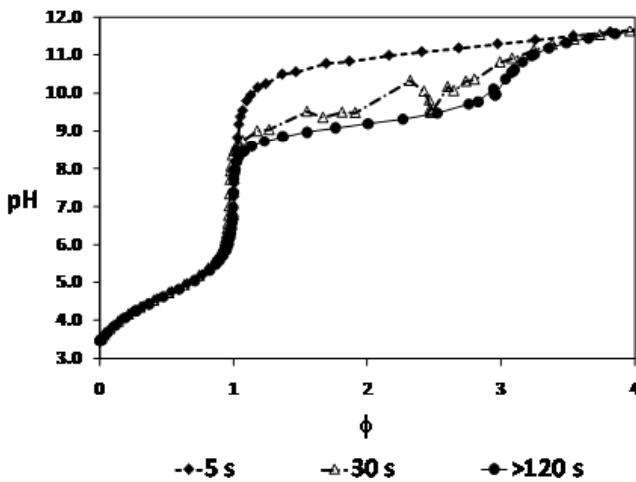


Fig. 4. Titration curves of thiamine hydrochloride (HA^{2+}) with 0.46 M NaOH. Graphs of pH as a function of ϕ were obtained for different measuring times after alkali addition.

refined with the aid of program SUPERQUAD [28], by fitting some of the curves shown in Figure 5. Table 1 summarizes the results obtained for one of these studies. In all cases the first global formation equilibrium is lost if its equilibrium constant is allowed to vary during refining (Model III in Table 1). If the constant of this equilibrium is maintained fixed during refining, the program converges to a fitting a little bit worse (Model II in Table 1), and the dismutation of B maintains only a 2% of this species when it reaches its maximum fraction. In order to have a 15% of B species in the system, as it has been mentioned by El Hage Chahine and Dubois [23] -for the maximum fraction value-, it is necessary that the first two global formation constants remain fixed during refining, but the achieved fitting is still worse (Model I in Table 1).

Figure 5 shows the fitting achieved with these models while Figure 6 shows the distribution diagrams for the several cases described above, related with the refinements presented in Table 1. As it can be seen in those figures, B species is practically nonexistent in aqueous solutions in the experimental conditions explored in the present paper and this information

Table 1. Results obtained by the refinements achieved with the program SUPERQUAD for the titration curve of 100 mL of 0.006 M thiamine hydrochloride with 0.46 M NaOH, waiting time 360 s between two successive pH measurements. 61 points of the titration curve were introduced to SUPERQUAD and a chemical model of three global formation equilibria.

Model	Equilibria	Regression parameters	Global formation constants	Acidity constants*
I	$\text{OA}^- + \text{H}^+ \rightleftharpoons \text{HOA}$ $\text{OA}^- + 2\text{H}^+ \rightleftharpoons \text{A}^+ + \text{H}_2\text{O}$ $\text{OA}^- + 3\text{H}^+ \rightleftharpoons \text{HA}^{2+} + \text{H}_2\text{O}$ $\text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{OH}^-$ $\text{HA}^{2+} + \text{H}^+ \rightleftharpoons \text{A}^+ + \text{H}^+$ $\text{A}^+ + \text{H}_2\text{O} \rightleftharpoons \text{HOA} + \text{H}^+$ $\text{HOA} \rightleftharpoons \text{OA}^- + \text{H}^+$	$\sigma_{\text{reg}} = 2.16$ $\chi^2 = 30.67$	$\log \beta_1 = 8.8^{**}$ $\log \beta_2 = 18.4^{**}$ $\log \beta_3 = 23.11 \pm 0.03$ $\text{pK}_w = 13.80 \pm 0.03$	$\text{pK}_{a1} = 4.71 \pm 0.03$ $\text{pK}_{a2} = 9.6$ $\text{pK}_{a3} = 8.8$
II	$\text{OA}^- + \text{H}^+ \rightleftharpoons \text{HOA}$ $\text{OA}^- + 2\text{H}^+ \rightleftharpoons \text{A}^+ + \text{H}_2\text{O}$ $\text{OA}^- + 3\text{H}^+ \rightleftharpoons \text{HA}^{2+} + \text{H}_2\text{O}$ $\text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{OH}^-$ $\text{HA}^{2+} + \text{H}^+ \rightleftharpoons \text{A}^+ + \text{H}^+$ $\text{A}^+ + \text{H}_2\text{O} \rightleftharpoons \text{HOA} + \text{H}^+$ $\text{HOA} \rightleftharpoons \text{OA}^- + \text{H}^+$	$\sigma_{\text{reg}} = 1.31$ $\chi^2 = 18.08$	$\log \beta_1 = 8.1^{**}$ $\log \beta_2 = 18.50 \pm 0.03$ $\log \beta_3 = 23.26 \pm 0.04$ $\text{pK}_w = 13.84 \pm 0.02$	$\text{pK}_{a1} = 4.80 \pm 0.05$ $\text{pK}_{a2} = 10.40 \pm 0.04$ $\text{pK}_{a3} = 8.1$
III	$\text{OA}^- + 2\text{H}^+ \rightleftharpoons \text{A}^+ + \text{H}_2\text{O}$ $\text{OA}^- + 3\text{H}^+ \rightleftharpoons \text{HA}^{2+} + \text{H}_2\text{O}$ $\text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{OH}^-$ $\text{HA}^{2+} + \text{H}^+ \rightleftharpoons \text{A}^+ + \text{H}^+$ $\text{A}^+ + \text{H}_2\text{O} \rightleftharpoons \text{HOA} + \text{H}^+$	$\sigma_{\text{reg}} = 1.07$ $\chi^2 = 9.16$	$\log \beta_2 = 18.36 \pm 0.03$ $\log \beta_3 = 23.26 \pm 0.03$ $\text{pK}_w = 13.82 \pm 0.01$	$\text{pK}_{a1} = 4.70 \pm 0.04$ $\text{pK}_{a2} = 9.18 \pm 0.03$

* Calculated by the combination of global equilibria.

** This value was maintained fixed during refining.

*** The first global equilibrium ($\text{OA}^- + \text{H}^+ \rightleftharpoons \text{HOA}$) is lost if the introduced value is allowed to vary during refining.

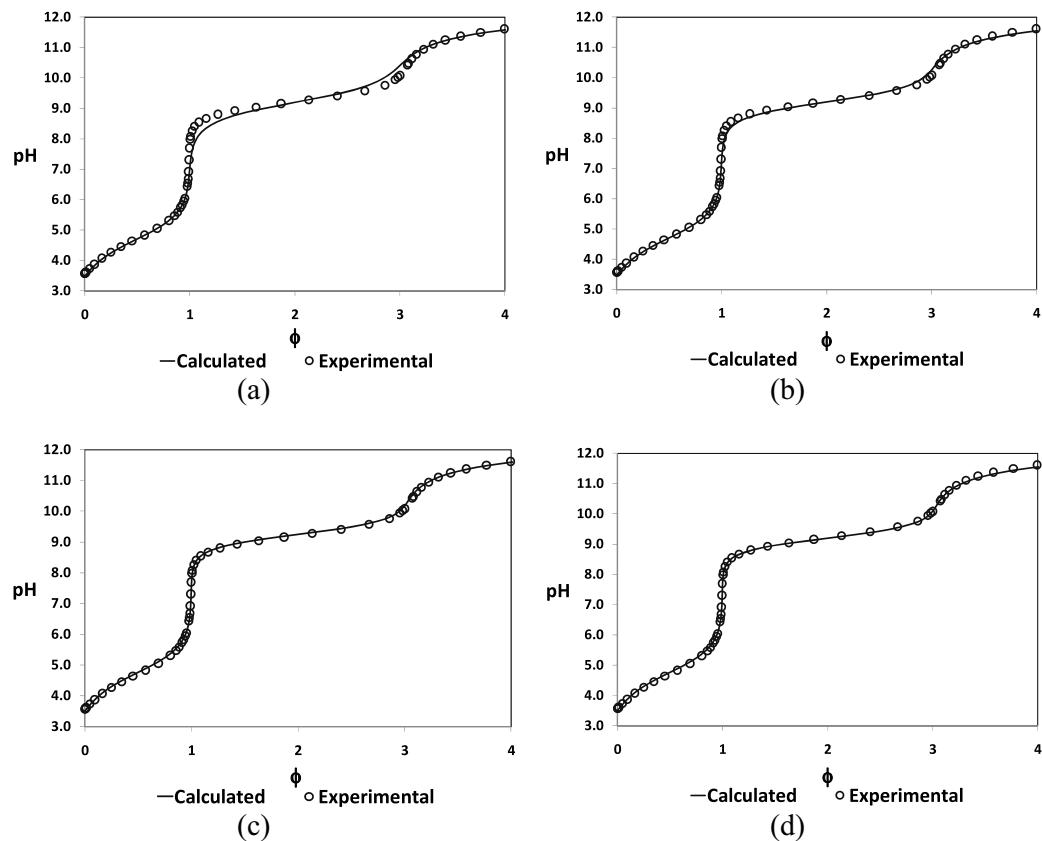


Fig. 5. Comparison of calculated and experimental titration curves of thiamine hydrochloride. a) $pK_{a1} = 4.70$, $pK_{a2} = pK_{a3} = 9.20$, b) $pK_{a1} = 4.70$, $pK_{a2} = 9.60$, $pK_{a3} = 8.80$, c) $pK_{a1} = 4.76$, $pK_{a2} = 10.40$, $pK_{a3} = 8.10$, d) $pK_{a1} = 4.76$, $pK_{a2} = 18.40$, $pK_{a3} = 0.00$.

agrees well with other studies reported in the scientific literature [22,23,26,29]; nevertheless these results are different to those reported by Hassan and Enlemma [27] but, unfortunately, they do not report the waiting time they have used for pH measurements. Model II in Table 1 may be considered as the limit case for the stability of B species and from the equilibrium constants of this model a lower limit of $K_{dism}=10^{2.3} \approx 200$ may be established for the dismutation of B, as shown in Figure 7.

From Figure 7, it can be explicitly seen that the dismutation involves the formation of water, where a hydroxide ion is given by one molecule of B and reacts with one proton of other molecule of B. Maybe the break of the oxygen-carbon bond is the reason for the observed kinetic behavior and the variety of results reported in other papers.

Purity determination of thiamine hydrochloride

Titration curves in Figure 4 may be used to propose the best pH break for determination of thiamine hydrochloride purity by potentiometry in aqueous solutions. Table 2 present the purity calculated from the different titration curves shown in this figure.

As shown in Table 2, the percent purity obtained from the first break point is nearer to the nominal purity of the analyzed product, and the position of this break point is practically independent of the waiting time for pH measuring.

Conclusions

A potentiometric study for the acid-base titrations of thiamine mononitrate and thiamine hydrochloride with an automatic titrator, at 25°C and under an inert nitrogen atmosphere, has been described in this paper. It has been demonstrated that the waiting time between two successive pH measurements should be considered as an important parameter to achieve experimental results reproducibility. The values of equilibrium constants of thiamine in aqueous medium, obtained from potentiometric data in this work, were consistent with previously reported values obtained by other methods, but it seems that the stability of the “pseudobase B” was overestimated in those papers. This kind of study should be extended to other solvents and other NaOH concentrations (>0.5 M), in order to compare the obtained results with those reported in other potentiometric studies.

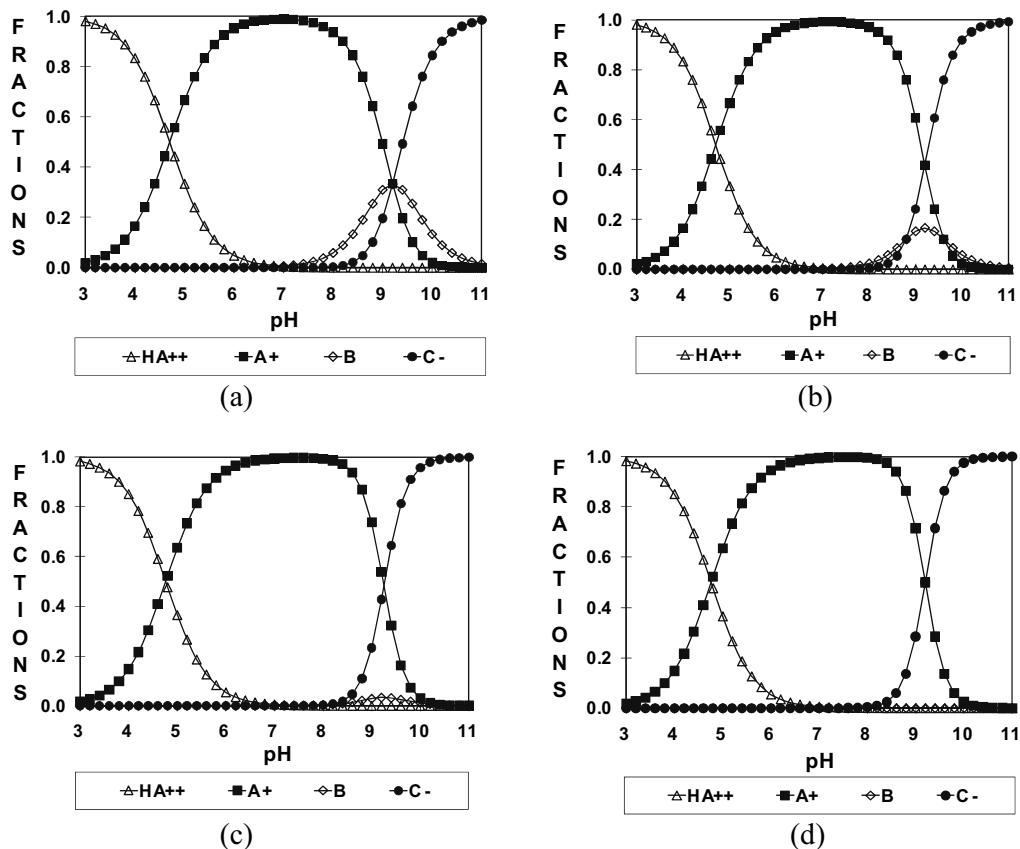


Fig. 6. Normalized distributions of thiamine species versus pH. a) $pK_a_1 = 4.80$, $pK_a_2 = pK_a_3 = 9.20$, b) $pK_a_1 = 4.80$, $pK_a_2 = 9.60$, $pK_a_3 = 8.80$, c) $pK_a_1 = 4.80$, $pK_a_2 = 10.40$, $pK_a_3 = 8.10$, d) $pK_a_1 = 4.80$, $pK_a_2 = 18.40$, $pK_a_3 = 0.00$.

Experimental

Apparatus: An automatic Metrohm 785 DMP TITRINO titrator was used for pH measurements.

Chemicals and reagents: 99% thiamine hydrochloride (Sigma) and 99% thiamine mononitrate (Sigma) were used; all

other chemicals were of analytical reagent grade. Deionized water with a resistivity of 18 MΩ cm was used throughout.

Sodium hydroxide (0.5 M): About 0.2 g of pure NaOH (Merck) was weighed and dissolved in water, the solution was made up to 100 mL with water. This solution was titrated with standardized HCl and was diluted when it was necessary.

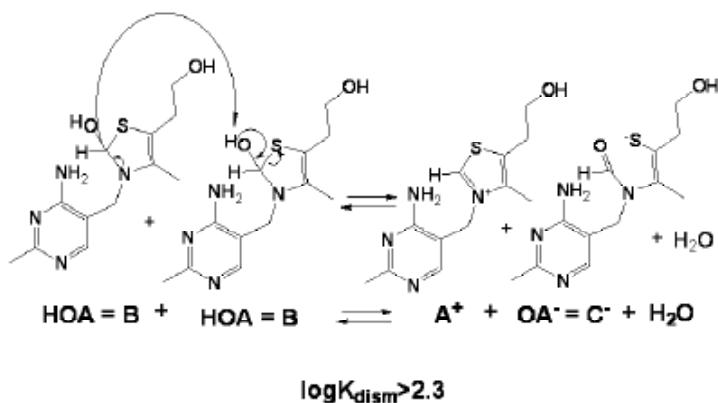


Fig. 7. Dismutation process involved with the stability of B species. $\log K_{\text{dism}}$ has been estimated from Model II of Table 1.

Table 2. Percent purity of thiamine hydrochloride as calculated from first and second breakpoints observed in Figure 4.

Waiting time for measuring pH (seconds)	Purity from first break point (%)	Purity from second break point (%)
5	100.13	not observed
15	98.94	not observed
120	99.74	not determined
240	98.31	101.71
300	98.83	101.12
360	98.84	103.40
Mean: 99.1 ($\sigma = 0.68$)		Mean: 102.1 ($\sigma = 1.16$)

pH measurements: Approximately 208 mg of thiamine hydrochloride was dissolved and diluted with deionized water to 100 mL in a volumetric flask. Each solution was titrated with standardized 0.5 M NaOH at 25°C and under an inert nitrogen atmosphere. The titration curves, pH as a function of NaOH added volume, were obtained at variable time intervals, measuring pH at different waiting times after alkali addition, using a combined glass-reference electrode. The potentiometer was previously calibrated with three standard buffers of pH 4, 7, and 10. The cell efficiency was periodically checked and used to correct the pH values.

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