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# The Kjeldahl Titrimetric Finish: On the Ammonia Titration Trapping in Boric Acid

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Additional information is available at the end of the chapter

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## Abstract

Kjeldahl method using concentrated boric acid is a common practice in many laboratories. A thorough study of the titration with hydrochloric acid of ammonia trapped in a solution of boric acid is made in an attempt to explain the fundamentals of a widely applied standard method. A new potentiometric method for the determination of the end point in the Kjeldahl titrimetric finish is proposed based on the linearization of the titration curve of the ammonia-boric acid system. The method is strictly based on mole and charge balances, and no approximations are made in deriving the equations. The proposed method has proved very accurate when applied to synthetic titration curves and data. Some problems, however, are experienced in the practice, because the behavior of the experimental system studied is far from the expected one on the basis of the theoretical model. However, a slight modification of the devised method has been applied to the experimental titration of ammonia with hydrochloric acid, in boric acid as trapping solution, to get good results.

**Keywords:** Kjeldahl method, titrimetric finish, ammonia-boric acid system

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## 1. Introduction

Nearly 130 years ago, on March 7, 1883, at a meeting of the Danish Chemical Society, Johan Gustav Christoffer Thorsager Kjeldahl (Head of Chemistry Department of the Carlsberg Foundation Laboratory of the Danish Brewing Carlsberg Company) introduced a method known later under the eponym “Kjeldahl Method” that basically is still in use. Within the same year, the method was published in a German journal *Zeitschrift für Analytische Chemie* [1] and written in French and Danish languages in communications from the Carlsberg Laboratory.

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The Danish brewer Mr. J.C. Jacobsen had a great respect for Pasteur and his work for the French wine industry, and for this reason, French extensive summaries of the Carlsberg papers were also published. As an extended summary of the Kjeldahl paper appeared in Chemical News in August [2], the method was quickly taken up [3]. The Analyst first gave details of the method in 1885 [4, 5], although the method had been briefly mentioned by Blyth [6] though giving the Kjeldahl name incorrectly as Vijeldahl. A surprisingly short period went by between the publication of the Kjeldahl method and the appearance of new publications concerning the topic, both in Europe and America. None of the analytical methods has been as widely chosen, in so short a time [7], as the “Kjeldahl Method” for the estimation of nitrogen.

The Kjeldahl method was originally designed for the brewing industry as an aid in following protein changes in grain during germination. It was Berzelius, who suggested the use of the word “protein” in 1838 in a letter to Mulder because it was derived from the Greek word meaning “to be in the first place” [8]. The Kjeldahl method lacks analytical selectivity because it does not distinguish between protein-based nitrogen from non-protein nitrogen (NPN). Adulteration incidents (i.e., adulteration of protein-based foods with melanine and related non-protein compounds) exploiting this analytical vulnerability have been recently detected and are new examples of a problem that dates back to before the Kjeldahl method was introduced [9]. The presence of NPN compounds in foods (amino acids, ammonia, urea, trimethylamine oxide) overestimates their true protein content as derived from the current nitrogen determination methods. Separation of NPN from true protein nitrogen may be carried out by addition of a protein precipitating agent such as trichloroacetic acid or perchloric acid.

The protein content in a foodstuff is estimated by multiplying the nitrogen content by a nitrogen-to-protein conversion factor, usually set at 6.25, which assumes the nitrogen content of proteins to be 16%. However, pure proteins differ in terms of their nitrogen contents because of differences in their amino acids composition, ranging from 13.4 to 19.3%. So, different multiplying factors are suitable for samples of different kinds.

The protein content determines the market value of major agricultural commodities. In addition, the quantitative analysis of protein content is necessary for quality control, being also a prerequisite for accurate food labeling. In recent years, soy protein products have increased the interest of consumers, especially in Western cultures, due to the high-quality protein of soy foods together with their associated health benefits [10] (according to FDA [11], 25g of soy protein per day may improve cardiovascular health).

**Table 1** [12–32] gives the chemical methods, most commonly used for protein determination. Some of the most significant methods (Dumas, Kjeldahl, and biuret assay) date from the late 1800s. Since the nineteenth century, many other analytical methodologies have been developed to determine the total protein in the field of biochemistry, biology and proteomics, but most of them to address research needs and not necessarily to determine the purity and/or adulteration of food products.

Though there are several experimental approaches to evaluate the nitrogen content in different kinds of samples (Dumas combustion method, NIR methods), the Kjeldahl method still remains as the reference method, being really the “golden standard” for validating other quantifying methodologies in the biopharmaceutical and food industries [9, 33]. The Kjeldahl method is

Technique/eponym	References
Dumas method	[13]
Nessler reagent	[14, 15]
Biuret method	[16–18]
Berthelot's method (Alkali-phenol reagent)	[19, 20]
Kjeldahl method	[1, 2]
Folin-Ciocalteu	[21]
Dye binding	[22]
Lowry method	[23–26]
Direct alkaline distillation	[27]
NIR (Near-infrared reflectance)	[27]
Modified Berthelot reaction	[20]
Modified Lowry method	[25, 28]
Bradford method (Coomassie blue dye-binding method)	[29, 30]
BCA (Bicinchoninic acid method)	[31]
3-(4-carboxy benzyl)quinoline-2-carboxaldehyde	[32]

**Table 1.** Methods for food protein analysis: some key references [12].

applied in official methods [34] to determine the nitrogen content measurement in foods as well as in many other samples, pharmaceutical, agricultural, food products, biological sediments and surface and waste waters. The diversity of papers dealing with Kjeldahl method [12] is attributable to the immense usefulness of the method, to its need for modifications for applications to various types of organic and inorganic compounds, and to the search for catalysts to provide such modifications and to accelerate the digestion [35, 36].

W. Johannsen (1857–1927), a pharmacist, wrote the Kjeldahl obituary, first published in German [37] and then translated to French [38] and English [39]. Johannsen, one of the founders of the science of genetics, was in his beginnings an assistant in the chemistry department at the Carlsberg Laboratory under the chemist Johan Kjeldahl and is well known for coining the term gene in 1909. Kjeldahl was elected to membership in the scientific academies of Denmark and Christiania and received an honorary doctorate from the University of Copenhagen. Kjeldahl (like Nessler) has been verbalized, an honor not usually accorded to a chemist [40]. All chemists understand what it means when it is said that a substance was kjeldahled [5] or that one kjeldahlizes a sample [41]. Kjeldahl was predecessor to S.P.L. Sørensen as Head of the Carlsberg Laboratory in Copenhagen, who introduced the notation of pH [41, 42].

## 2. The Kjeldahl's three steps

Titration analysis is one of the oldest analytical methods, and as a matter of fact, it plays an important role [43–46] in various analytical fields as well as in routine analysis [47–50]. Generally, the quantity of tested components of a sample is determined by adding to the

measured sample an exactly known quantity of the standard titration solution with which the desired constituent reacts in a definite, known proportion. The content of the components is calculated according [45, 46] to the concentration of the standard solution, the consumed volume, the measuring relationship between chemical reactions and the weight of the tested substances, etc. If the tested substance cannot chemically react with the titrant directly, indirect ways of other reactions can be applied to measure its content.

Although some analytical techniques allow the direct determination of species without sample treatment, it is usually necessary to dissolve the sample prior analysis. The analytical adage: *“once the problem is dissolved, the problem is solved”* denotes clearly this fact. Most analyses are preferentially performed on solution samples, a more homogeneous analysis sample representative of the bulk properties of a large solid sample being thus obtained, improving precision and accuracy [51]. Digestion may be defined as the process, in which a complex substance is decomposed into volatile gases and simple salts that are soluble in dilute acid solution [52]. Wet decomposition or acid digestion involves the use of mineral acid, alone or in combination with other acids and oxidizing agents to affect the dissolution of a sample [53].

The three steps [1, 54] of Kjeldahl method includes:

1. Wet acidic (conc.  $\text{H}_2\text{SO}_4$ ) digestion (mineralization) of nitrogen-containing sample, in a long-necked digestion flask, causing its conversion into  $\text{NH}_4^+$  ions. The Kjeldahl flask, which he constructed in 1888 to simplify the method, is still in use today. Traditionally, Kjeldahl flasks with a capacity for 500–800 mL and gas or electric heating have been used for the digestion. The digest must contain residual  $\text{H}_2\text{SO}_4$  to retain the  $\text{NH}_3$  as  $\text{NH}_4^+$ . Water is added manually or automatically to prevent the digest from solidifying, which also may cause bumping, and to avoid mixing concentrated alkali with concentrated acid [55] during the distillation.
2. Transformation of  $\text{NH}_4^+$  ions into  $\text{NH}_3$  (neutralization with alkali) and distillation of the  $\text{NH}_3$ . The flask is heated after the addition of water and alkali to the digested sample, in order to distill a volume of distillate and collect  $\text{NH}_3$  in the acidic distillation receiver. Direct steam distillation drastically decreases the time needed for distillation [55]. Experimental conditions including rate of steam flow, vigor of distillation and volume of solution must be correctly balanced to ensure efficient removal of ammonia without any carry-over of alkaline spray.
3. Titration of the solution from the distillation receiver. The ammonia from the distillation is frequently collected in an excess of standard acid and determined by a back titration with standard alkali solution. A more common practice is the use of boric acid for trapping ammonia. The titration in this case should be carried out as soon as possible after the distillation is complete, ensuring that the temperature of the distillate does not exceed  $25^\circ\text{C}$ . Under these conditions, losses of ammonia are avoided [56] (EN ISO 5983-1, 2009).

Complete details [12] of the three Kjeldahl steps are too lengthy to include here. A series of factors such as sample origin, homogeneity, stability, laboratory skillfulness, sample handling procedures, and composition are of critical importance, as are the size of the test portion taken for

analysis, and concentration of the titrant used in the Kjeldahl analysis [57]. A relatively large analytical sample (1–2 g) was used in the original method, requiring large amounts of acid.

Green alternatives exert considerable pressure to ensure the safe disposal of mercury (when used as catalyst) and, especially, to minimize acid usage [58–60]. The use of Micro-Kjeldahl methods is common practice in order to reduce the amount of acid fumes and also require less acid and catalyst mixture.

The introduction of aluminum blocks (at Tecator, now FOSS) in the early 1970s [61] made possible to improve the speed and accuracy of the digestion procedure, thus saving space, chemicals and energy [60]. The digestion system has since been improved, and the distillation step has been speeded up by the use of the semi-automated systems now available. Block digestions followed by steam distillation are named as rapid Kjeldahl. As a result of technical innovations, there are also available fully automated protein analysis systems that are based on the classical Kjeldahl procedure, for example, the Kjeltex series of Foss Tecator manufactures.

### 3. The ammonia determination: titrimetric finish

Transformation of  $\text{NH}_4^+$  into  $\text{NH}_3$  caused by addition of NaOH (pH growth) into Pregl-Parnas-Wagner results [12] from equation

$$\frac{[\text{NH}_3]}{[\text{NH}_4^+]} = 10^{pH - pK_{1,a}} \quad (1)$$

where  $pK_{1,a} = -\log K_{1,a}$ ;  $K_{1,a}$  refers to reaction  $\text{NH}_4^+ = \text{H}^+ + \text{NH}_3$ ,  $pK_{1,a} = 9.35$  at  $20^\circ\text{C}$ .

The distillation titration method is a standard procedure used by most laboratories to measure ammonium nitrogen in the total Kjeldahl nitrogen digests of various kinds of agricultural and environmental samples [62]. Ammonia may be collected into a solution of a strong acid (HCl or  $\text{H}_2\text{SO}_4$ ). After distillation, the excess of standard acid may be iodometrically determined with starch as indicator, as was done by Kjeldahl, according to consecutive reactions  $\text{IO}_3^- + 5\text{I}^- + 6\text{H}^+ = 3\text{I}_2 + 3\text{H}_2\text{O}$ ;  $\text{I}_2 + 2\text{S}_2\text{O}_3^{2-} = \text{S}_4\text{O}_6^{2-} + 2\text{I}^-$ , though this method is seldom used. The excess of standard acid is instead usually titrated with a strong base. Two standard solutions are then needed: (1) titrant (NaOH solution) and (2) the strong acid in the receiver.

Titration of ammonia absorbed in  $\text{H}_3\text{BO}_3$  solution was proposed first by Winkler [63] which notes “Boric acid is indeed such a weak acid, that its solution does not cause a noticeable colour change of certain indicators. Ammonia is, however, completely fixed by it, provided that a suitable excess of acid is present.” Boric acid is also commonly used to trap ammonia because only one standardized solution (e.g., HCl or  $\text{H}_2\text{SO}_4$ ) is needed (as titrant).

Mixtures of methyl red and tetrabromophenol blue or methyl red and methylene blue (2:1) (Tashiro indicator) or methyl red alone were suggested as indicators; the latter changes its color at about pH 5.2. After addition of HCl, the Tashiro indicator changes its color from green to violet. Alternatively, a pH titration with end point ( $V_{eq}$ ) at a preset pH value of 5.0 is also

done. A mixed indicator (bromocresol green and methyl red) has been recently used by Beljkaš et al. [64] to locate the end point.

#### 4. Base or acid as titrant: the boric acid trapping choice

The ammonia can be distilled into an excess of standard strong acid (HCl or H<sub>2</sub>SO<sub>4</sub>), and the excess determined by back titration with a strong standard base, since the solution at the equivalence point contains (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, or NH<sub>4</sub>Cl, which hydrolyses,

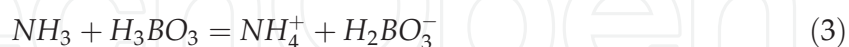
$$pH = -\log\sqrt{K_a C_A} = \frac{pK_a + pC_A}{2} = \frac{9.36 + 1.30}{2} = 5.33 \quad (2)$$

(i.e., 0.05M of ammonium chloride at the end point), methyl red (transition range 4.4–6.2) being used as an indicator [65].

A mistake appears in the (excellent) Bradstreet monograph [66], p. 152, who wrote “*in the case of back titration of a distillate, the equivalence point will occur at pH 7, since this is the point of neutralization of a strong acid by a strong base. Any indicator, therefore, changing colour at or close to pH 7 is suitable.*” The millimoles of NH<sub>3</sub> in the sample are equal to the total millimoles of HCl added minus millimoles of base used in back titration. This method has two disadvantages: i) the amount of NH<sub>3</sub> is obtained as a difference; and ii) two standard solutions are required.

Preferably, the distilled ammonia can be absorbed into a solution of boric acid [63] or other weak acid [67]. Boric acid is sufficiently acid to react with ammonia and prevent loss by volatilization, but it is apparently too weak an acid to interfere with the titration of ammonium borate with diluted hydrochloric acid. When boric acid is used instead of standard acid as the recipient of the distillate, the use of a weak standard alkaline solution is avoided, which suppose a distinct advantage. The alkaline solution is vulnerable to the absorption of atmospheric carbon dioxide with subsequent changes in molarity. Neither the amount nor the concentration (about 4%) of boric acid in the receiving bottle has to be precise.

Then, in the Winkler modification, the NH<sub>3</sub> is caught in an unmeasured excess of boric acid



The borate formed is determined by titration with standard HCl, one mole of HCl being required for each mole of NH<sub>3</sub>



The solution at the equivalence point contains H<sub>3</sub>BO<sub>3</sub> and NH<sub>4</sub>Cl, a mixture of two weak acids,

$$pH = -\log\left(\sqrt{K_{a1}C_{A1} + K_{a2}C_{A2}}\right) \quad (5)$$

so that an indicator transiting in the acid region (pH 5–6) is satisfactory [65].

Note that the acidity constants of boric acid and ammonium ion are very similar. The equilibrium constant of Eq. (3) is given by

$$K_{eq} = \frac{[NH_4^+][^-H_2BO_3]}{[NH_3][H_3BO_3]} = \frac{K_{1,H_3BO_3}}{K_{1,NH_4^+}} = \frac{10^{-9.24}}{10^{-9.35}} = 1.288 \quad (6)$$

Though this equilibrium constant is low, the fraction of ammonia converted into ammonium ion increases with increasing the difference of concentrations between boric acid and ammonia. As we are studying the trapping process (without titrant added), the volume may take as constant and the mass balances are given by

$$C_{H_3BO_3} = [H_3BO_3] + [H_2BO_3^-] \quad (7)$$

$$C_{NH_3} = [NH_3] + [NH_4^+] \quad (8)$$

The following relationship is satisfied in the trapping solution (if solution is not very diluted, the contribution of dissociation of water being in those cases is negligible).

$$[NH_4^+] = [H_2BO_3^-] \quad (9)$$

and then, by combining Eqs. (6) to (9), we get

$$K_{eq} = \frac{[NH_4^+]^2}{(C_{NH_3} - [NH_4^+])(C_{H_3BO_3} - [NH_4^+])} \quad (10)$$

which on rearrangement gives the second degree equation

$$\left(1 - \frac{1}{K_{eq}}\right)[NH_4^+]^2 - (C_{NH_3} + C_{H_3BO_3})[NH_4^+] + C_{NH_3}C_{H_3BO_3} = 0 \quad (11)$$

which may be solved for given concentrations of boric acid and ammonia. Once the value of  $[NH_4^+]$  is known, that is, the fraction of ammonium ion

$$f_{1,\alpha} = \frac{[NH_4^+]}{C_{NH_3}} \quad (12)$$

the *pH* values may be calculated from Eqs. (2) and (12)

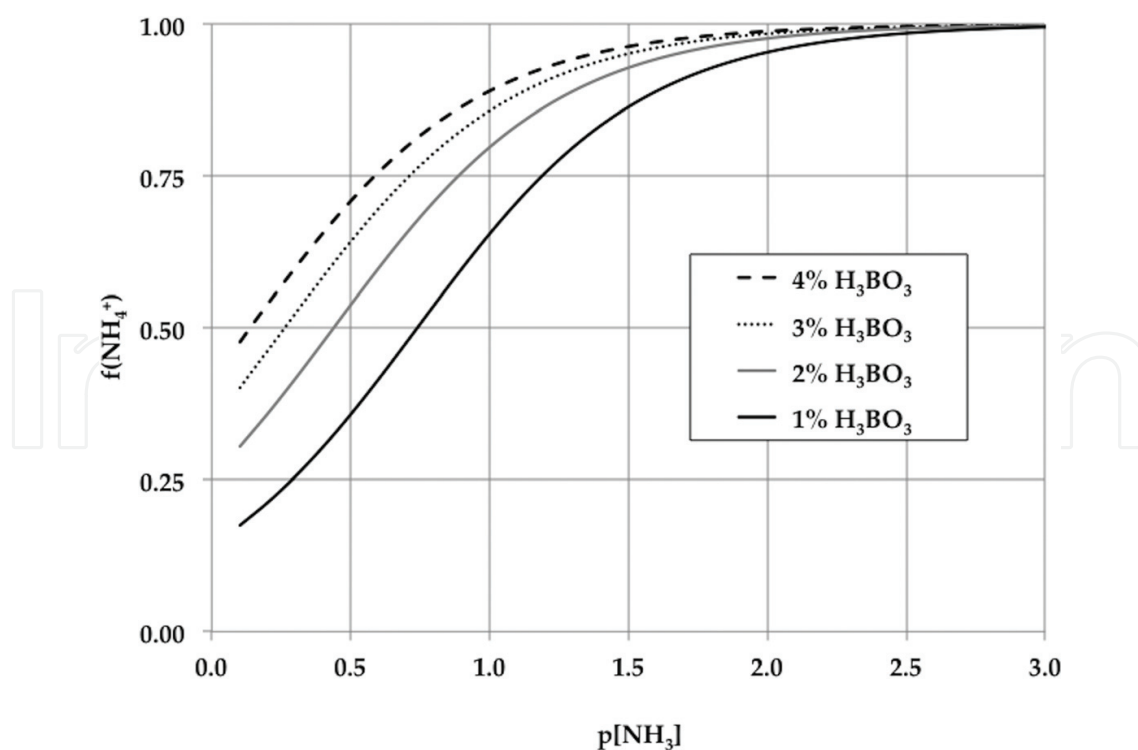
$$[H^+] = K_{1,\alpha} \frac{[NH_4^+]}{[NH_3]} = K_{1,\alpha} \frac{f_{1,\alpha}}{f_{0,\alpha}} = K_{1,\alpha} \frac{f_{1,\alpha}}{1 - f_{1,\alpha}} \quad (13)$$

Hydrochloric acid titrates then the borate ion from the above reaction (Eq. (4)) as well as the ammonia, which is not converted into ammonium ion in Eq. (3), that is,  $H_2BO_3^- + H^+ = H_3BO_3$ ;  $NH_3 + H^+ = NH_4^+$  (the sum of both being equivalent to the ammonia distilled).



The trapping process may be illustrated by means of semilogarithmic diagrams (**Figure 1**). **Figure 1** shows the fraction of ammonium ion (equivalent to the borate ion), at a given  $p[\text{NH}_3]$  for varying concentrations of trapping boric acid. In the absence of other equilibria than those considered previously, this diagram would help to know the concentration of ammonia from the  $pH$  initial of the sample to be titrated, that is, a  $pH$  of about 7 for a 2% boric acid trapping solutions corresponds to a  $p[\text{NH}_3]$  of about 2. However, in numerous cases, things are not an easy business as may appear at first sight and apparently some complications arise in the boric acid-ammonia system associated when high boric acid concentrations are present. When significant quantities of ammonia vapor are delivered into 4% aqueous boric acid containing methyl red-methylene blue indicator, it is necessary to dilute with distilled water so that the expected change in color (purple to green) may occur. Only after considerable dilution had been effected, could the ammonia [68] be satisfactorily titrated with hydrochloric acid. Boric acid forms [68, 69] polymeric borate species (triborate, tetraborate, pentaborate) in concentrated solutions whose acidity constants are greater than the  $K_{1,b}$  of boric acid by a factor between 210 and 440, thus boric acid behaving as a stronger acid in concentrated solution than in diluted solution.

As far as we know, the anomaly behavior of boric acid was revealed first by Prideaux [70] one century ago when studying the titration curve of boric acid, and then by Thygesen [71] through conductivity measurements. In spite of the fact that Kjeldahl titration is one of the titrimetric methods more applied worldwide, scarce mention of this fact has been made in the literature. However, some problems possess the location of the end point in Kjeldahl titration



**Figure 1.** Semilogarithmic diagram molar fraction of ammonium ion versus minus logarithm of the total ammonia concentration ( $p[\text{NH}_3]$ ), at varying percentage of boric acid as trapping solution.

for the indicators recommended, for example, bromophenol blue, methyl red, methyl orange, and congo red, as we have previously indicated.

This fact is another argument in favor to carry out a potentiometric study of the ammonia-boric acid system with the aim of devising a straight-line linearization of titration curve in order to accurately locate the end point of the titration curve. The theoretical background for the new method is outlined in the following sections.

## 5. Titration curves in the ammonia-boric hydrochloric acid titration system

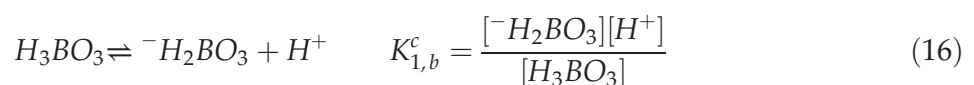
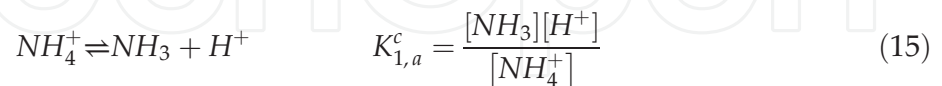
A thorough study of the titration of ammonia trapped in a solution of boric acid is made in this section based on a mathematical approach with equations derived for the titration curves involved. The calculations presented are based on charge and concentration balances, and expressions for equilibrium constants related to acid-base equilibria. This approach can be perceived as the clear confirmation of the statement [72], ascribed to J.C. Maxwell that *“a good theory is the best practical tool.”*

Let us consider the titration of ammonia trapped in boric acid solution with hydrochloric acid as titrant. The initial concentration of ammonia and boric acid is denoted by  $C_{NH_3}$  and  $C_{H_3BO_3}$ , respectively, and the initial volume by  $V_0$ ,  $C_{HCl}$  denotes the concentration of strong acid and  $V$  the volume added. The electroneutrality rule for the solution is

$$[H^+] + [NH_4^+] = [OH^-] + [Cl^-] + [^-H_2BO_3] \quad (14)$$

Eq. (14) should also include concentrations of ions being originated from the neutral salt added to adjust ionic strength (when necessary). As the salts used for this purpose usually potassium nitrate and chloride are completely dissociated, the concentration of the anion and cation is the same and cancel out in the equation. Also, the contribution of the second and third dissociation steps of boric acid is considered negligible at the working  $pH$  range.

The law of mass action holds for the reactions



The constants  $K_{1i}$  ( $i=a, b$ ) are concentration constants, useful when the pH-meter is calibrated in term of hydrogen ion concentrations at an ionic strength fixed. If this not the case, mixed constants (at an ionic strength fixed), where the activity of the hydrogen and hydroxide ions (denoted by  $(H^+)$  and  $(OH^-)$ ) are used in conjunction with concentrations of all other species. The activity of the hydrogen ion is related to concentration by the expression

$$(H^+) = [H^+] \gamma_H \quad (17)$$

where  $\gamma_H$  is the activity coefficient of the ion. Then

$$K_{1,a}^B = \frac{[NH_3](H^+)}{[NH_4^+]} = K_{1,a}^C \gamma_{H^+} \quad K_{1,b}^B = \frac{[-H_2BO_3](H^+)}{[H_3BO_3]} = K_{1,b}^C \gamma_{H^+} \quad (18)$$

The total concentrations of the ammonia and boric acid during the titration can be expressed by their respective mass balances

$$[H_3BO_3] + [-H_2BO_3] = \frac{V_0 \cdot C_{H_3BO_3}}{V_0 + V} \quad (19)$$

$$[NH_4^+] + [NH_3] = \frac{V_0 C_{NH_3}}{V_0 + V} \quad (20)$$

The concentration of the chloride ion can be expressed by the following equation

$$[Cl^-] = \frac{V \cdot C_{HCl}}{V_0 + V} \quad (21)$$

The molar fractions of ammonia and borate ions are given by

$$f_{1,a} = \frac{[NH_4^+]}{[NH_3] + [NH_4^+]} = \frac{\frac{[NH_4^+]}{[NH_3]}}{1 + \frac{[NH_4^+]}{[NH_3]}} = \frac{\frac{[H^+]}{K_{1,a}^C}}{1 + \frac{[H^+]}{K_{1,a}^C}} = \frac{10^{pK_{1,a}^C - pH}}{1 + 10^{pK_{1,a}^C - pH}} \quad (22)$$

$$f_{0,b} = \frac{[-H_2BO_3]}{[H_3BO_3] + [-H_2BO_3]} = \frac{1}{1 + \frac{[H_3BO_3]}{[-H_2BO_3]}} = \frac{1}{1 + \frac{[H^+]}{K_{1,b}^C}} = \frac{1}{1 + 10^{pK_{1,b}^C - pH}} \quad (23)$$

and the ammonia and borate concentrations expressed in terms of their respective molar fractions may be substituted into Eq. (14), giving

$$[H^+] + f_{1,a} \frac{V_0 \cdot C_{NH_3}}{V_0 + V} = [OH^-] + \frac{V \cdot C_{HCl}}{V_0 + V} + f_{0,b} \frac{V_0 \cdot C_{H_3BO_3}}{V_0 + V} \quad (24)$$

and then we get

$$\Delta = [H^+] - [OH^-] = \frac{V \cdot C_{HCl} + f_{0,b} V_0 \cdot C_{H_3BO_3} - f_{1,a} V_0 \cdot C_{NH_3}}{V_0 + V} \quad (25)$$

From the Eq. (25), we can, after a simple mathematical manipulation, obtain

$$V = V_0 \frac{f_{1,a} C_{NH_3} - f_{0,b} C_{H_3BO_3} + \Delta}{C_{HCl} - \Delta} \quad (26)$$

thus obtaining a relatively simple, closed-form expression for the titration curve valid at any moment of the titration. It relates the volume of added titrant to the hydrogen ion concentration ( $p[H]$ ) of the solution. Then, by substituting a series of values of  $[H^+]$ , we calculate the corresponding values of  $V$ .

## 6. Graphical method for the determination of the equivalence point

Numerical methods based on the mathematical modeling of the titration curve may be applied for the determination of the end point instead of approximate methods. Gran's linearization known since 1950 [73–75] is one of the examples. In this book chapter, a new method for the determination of the end point is proposed based on the linearization of the titration curve of the ammonia-boric acid system. The method is inspired in a previous method described first by Schwartz [76] and also explained with great detail in two relatively recent analytical chemistry textbooks [77, 78].

Eq. (25) gives

$$(V_0 + V)\Delta = V \cdot C_{HCl} + f_{0,b}V_0 C_{H_3BO_3} - f_{1,a}V_0C_{NH_3} \quad (27)$$

and dividing the left and right members through by  $C_{HCl}$  we have

$$\frac{(V_0 + V)\Delta}{C_{HCl}} = V + \frac{f_{0,b}V_0 \cdot C_{H_3BO_3}}{C_{HCl}} - f_{1,a}V_{eq} \quad (28)$$

The following equation should be valid at the equivalence point

$$V_0 C_{NH_3} = V_{eq}C_{HCl} \quad (29)$$

and then, by combining Eqs. (31) and (30), we get

$$V - \frac{(V_0 + V)\Delta - f_{0,b}V_0 \cdot C_{H_3BO_3}}{C_{HCl}} = f_{1,a}V_{eq} \quad (30)$$

The left member of Eq. (32) is denoted by  $V'$

$$V' = V - \frac{(V_0 + V)\Delta - f_{0,b}V_0 \cdot C_{H_3BO_3}}{C_{HCl}} \quad (31)$$

and taking into account that Eq. (22) is equivalent to

$$f_{1,a} = \frac{[H^+]}{[H^+] + K_{1,a}} \quad (32)$$

we get

$$V' = \frac{[H^+]}{[H^+] + K_{1,a}} \quad (33)$$

By simple manipulation, Eq. (35) gives

$$V'([H^+] + K_{1,a}) = [H^+]V_{eq} \quad V'K_{1,a} = [H^+]V_{eq} - V'[H^+] \quad (34)$$

By multiplying the left and right members of Eq. (36) through by  $1/(K_{1,a} [H^+])$  we finally have

$$\frac{V'}{[H^+]} = \frac{V_{eq}}{K_{1,a}} - \frac{V'}{K_{1,a}} \quad (35)$$

Eq. (35) gives a straight line when  $V'/[H]$  is plotted against  $V'$ . The plot has a slope of  $-1/K_{1,a}$  and intersects the  $V'$  axis at the point  $V_{eq}$ . By use of Eq. (35) the equivalence point can be located with considerable accuracy. Eq. (35) may be considered as a variant (an extension) of the method of Schwartz [76] for the determination of the equivalence point in the titration of a weak base with a strong acid.

The method, which we propose, is based strictly on mole and charge balance equations. No approximations are made in deriving Eq. (35). Both the dissociation of water as well as the dilution effects is precisely accounted for. The only restrictions of the method are, on the one hand, the accuracy, with which the pH-meter and electrodes can be calibrated, and several fundamental assumptions entailed by linear regression that are not always satisfied with data obtained. One, for example, is that the values of  $x$  are free from error but those of  $y$  are drawn from a population having normally distributed errors [79, 80]. When the precision of experimental measurements is very high, no special problems, however, are to be expected.

Note that the use of the complete Eq. (31), which gives the modified volume function  $V'$ , presupposes knowledge of the  $pK_{1,b}$  necessary to obtain the  $f_{0,b}$  value (Eq. (23)). In any case, the  $pK_{1,b}$  value may be extracted from appropriate tables, or from the analytical bibliography [81], though it is not necessary to be known exactly. A trial value of  $pK_{1,b}$  may be assumed and values for  $V_{eq}$  and  $K_{1,a}$  calculated by least squares method on Eq. (35). The procedure is repeated for other assumed values of  $pK_{1,b}$ , and the best value is taken as that for which minimizes  $s_{y/x}$  the standard deviation of the corresponding regression line. The  $s_{y/x}$  values can be easily got using linear regression (method of the least squares), in EXCEL, with the function LINEST.

Taking into account Eqs. (3) and (4), the computation of ionic strength may be made as a first approximation, when  $V < V_{eq}$ , as

$$I = \frac{1}{2} \left( \frac{2C_{NH_3}V_0 - C_{HCl}V}{V_0 + V} + [OH^-] \right) \quad (36)$$

and when  $V > V_{eq}$  as

$$I = \frac{1}{2} \left( \frac{C_{NH_3} V_0}{V_0 + V} + [H^+] \right) \quad (37)$$

As the activity coefficient  $\gamma_H$  will change only to a very small extension during a titration,  $\log \gamma_H$  may be considered constant. A large ratio of sample volume to titrant volume (i.e., 10:1) minimizes errors introduced by variations in activity coefficients due to dilution. The value of most constants has been determined at an ionic strength of  $I=0.1$ . It is thus practical to adjust the ionic strength to this value. However, addition of a basal electrolyte into the solution and the titrant to keep the ionic strength approximately constant and rather high is not usually practiced [82] in the titrations involved with Kjeldahl method.

$\gamma_H \approx 1$  is assumed for simplicity of considerations when the titrations are carried out at low ionic strength. The success of the system of  $pH$  standardization depends on the validity of putting  $-\log(H^+)$  equal to  $pH$ . As  $[H^+]$  normally makes only a small contribution, no sensible error is introduced if  $(H^+)$  is used in its place: in some exceptional cases, when  $[H^+]$  is relatively more important, the quantity may be estimated with sufficient exactness from Eq. (17); on the acid side of  $pH$  (when  $[OH^-]$  is negligible).

If we represent the  $pH$  as  $A \pm \varepsilon$ , where  $\varepsilon$  is the uncertainty, then we have  $[H^+] = 10^{-(A \pm \varepsilon)} = 10^{-A}$  times  $10^{\pm \varepsilon}$ , where  $10^{\pm \varepsilon} = 1$  for  $\varepsilon = 0$  [77]. The resulting uncertainty in  $[H^+]$  is now a multiplicative factor, and therefore a relative uncertainty, which applies regardless of the value of  $pH$ . A relatively small error in the  $pH$  of 0.01, 0.02, or 0.05 corresponds to an uncertainty in  $[H^+]$  of about 2, 5 or 12%, respectively. Consequently, Eq. (35) plot is characterized by extreme sensitivity to small changes in  $pH$ . This problem is compounded by possible activity effects and the requirement to use a precise value of  $pK_{1,b}$ .

Differential (approximate) methods for the determination of end points of titration are based on the presumption that the end point of a titration is the inflection point of the titration curve [83, 84], where the absolute value of the first derivative reaches a maximum (titration of a weak acid with a strong base) or a minimum (titration of a weak base with a strong acid) and the second derivative changes sign. A number of points very closely spaced and preferably of high precision are needed [83, 84] in order that the method is successfully applied. Only the points in the vicinity of the inflection are used for the calculation. This may result in increasing errors as titration data are least accurate right near the end point [85], because buffering is minimal and electrode response is sluggish. The local  $pH$  fluctuations in the inflection region are mostly due [77] to insufficiently rapid mixing of the titrant and the sample, and localized  $pH$  sampling by the glass electrode.

Two advantages may be ascribed to the method devised in this book chapter, based on the Eq. (35). Fewer titration points need to be taken than with conventional methods, and measurements need not be made close to the equivalence point since this point may be obtained by extra(inter)polation. Therefore, problems related to incompleteness of reaction or instability of measurements close to the end point might be avoided. Note that when very weak acids or

bases are titrated, the approximations assumed by Gran when deriving their equations are no longer valid.

Harris [85] have comment in reference to the linearization of titration curve by Gran [74]: “*The beauty of a Gran plot is that it enables us to use data taken before the end point to find the end point.*” This sentence is undoubtedly applied to linear extrapolation methods based [76–78, 86] on an improvement in the methodology proposed by Gran, as it is the case in this book chapter.

## 7. Approximation expressions derived from the complete equation

Eq. (31) may be expressed as

$$V' = V - \frac{(V_0 + V)\Delta}{C_{HCl}} + \frac{f_{0,b}V_0 \cdot C_{H_3BO_3}}{C_{HCl}} \quad (38)$$

Which is in the form

$$V' = V + U + W \quad (39)$$

$$U = -\frac{(V_0 + V)\Delta}{C_{HCl}} \quad (40)$$

$$W = \frac{f_{0,b}V_0C_{H_3BO_3}}{C_{HCl}} \quad (41)$$

**Figure 2** depicts the contributions of the different terms in Eq. (39) to the value of  $V'$ . It is valid for a titration of 150 mL of 0.0096 M ammonia solution and 0.2156 M trapping acid boric solution, titrated with HCl 0.12 M ( $pK_{1,a} = 9.27$ ;  $pK_{1,b} = 9.12$ ;  $pK_w^c = 13.80$ ). It is seen that before the equivalence point, the U term may be considered as negligible whereas beyond the equivalence point it is the W term, which may be neglected.

Thus, before the equivalence point we approximately get

$$V' = V + \frac{f_{0,b}V_0 \cdot C_{H_3BO_3}}{C_{HCl}} \quad (42)$$

Note that the mole fraction of borate varies from about 0.043 at the beginning of the titration to about 0 at the equivalence point (**Figure 3**). Despite the fact that initial volume  $V_0$  and concentrations of HCl and  $H_3BO_3$  are constants, the contribution of the second term of the right hand of Eq. (42) is null only when approximated at the equivalence point. There is no way to use a conventional Gran equation method based on the quotient  $V/[H]$  in the ammonia-boric acid titration case.

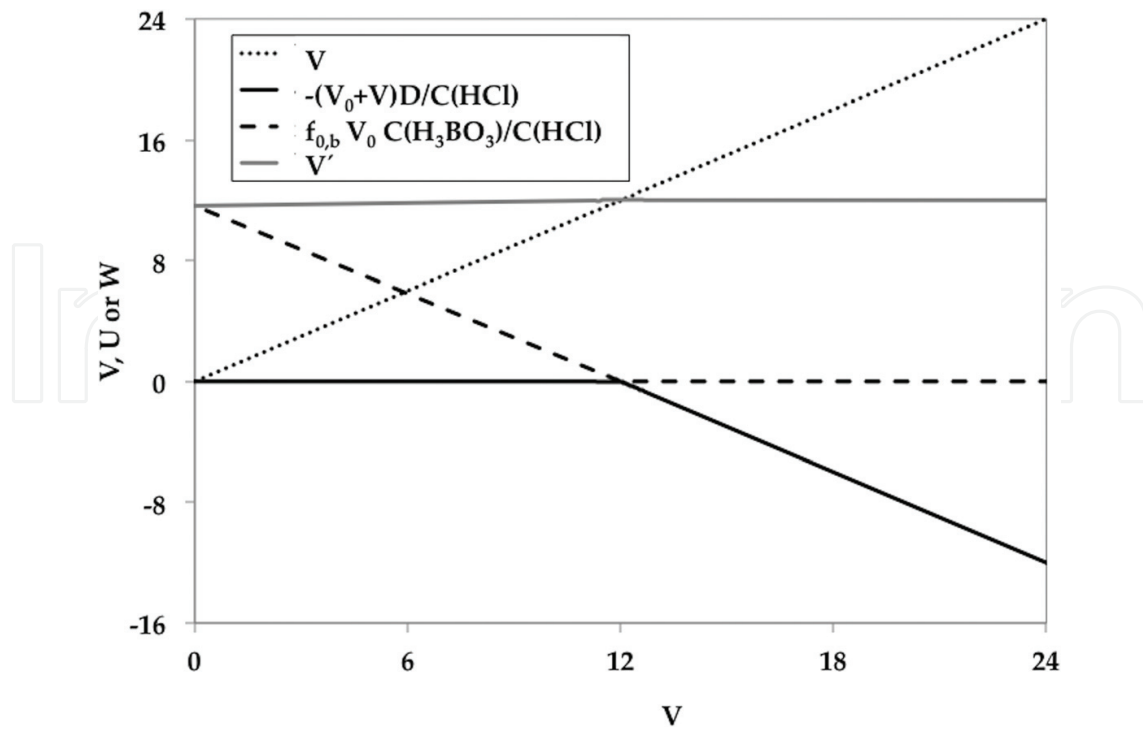


Figure 2. Contributions of the different terms in Eq. (38) to the value of  $V'$ .

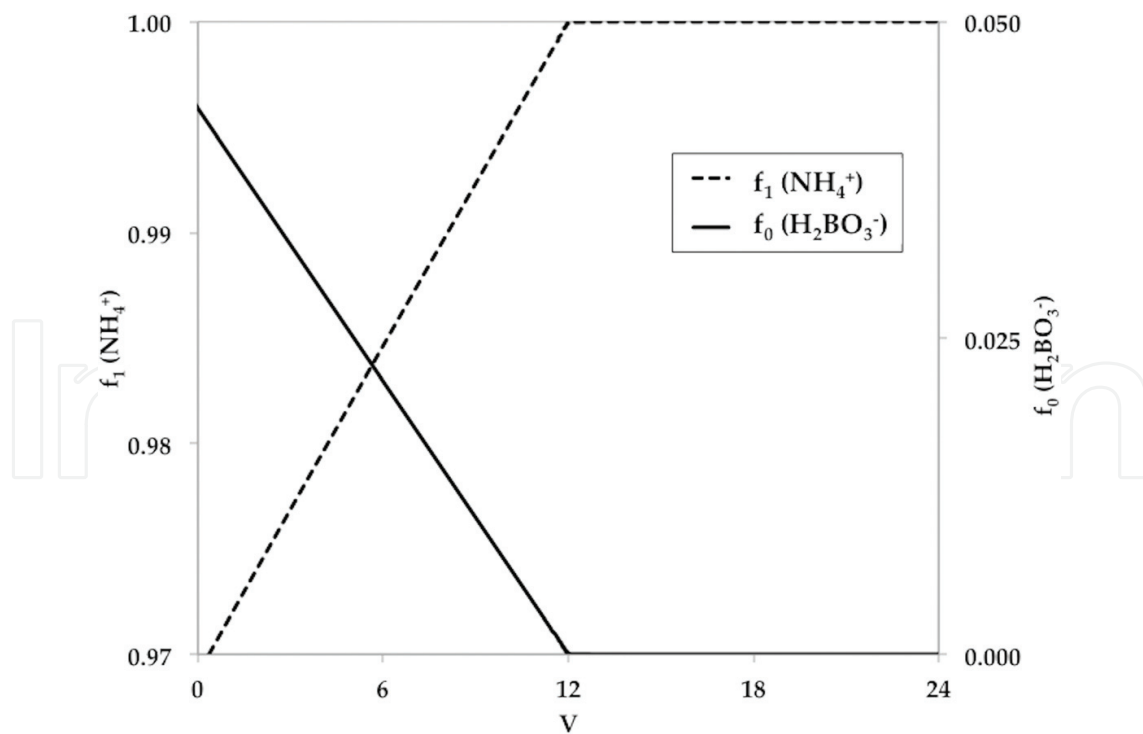


Figure 3. Mole fraction of ammonium ( $f_{1,a}$ ) and borate ( $f_{0,b}$ ) ions as a function of  $V$ .



Beyond the equivalence point we have instead

$$V' = V - \frac{(V_0 + V)\Delta}{C_{HCl}} \quad (43)$$

When  $V > V_{eq}$ ,  $[OH^-]$  is negligible, and

$$\Delta = [H^+] - [OH^-] \approx [H^+] \quad (44)$$

and  $f_{1,a}$  the mole fraction of ammonium ion is virtually equal to the unity, and then by combining Eqs. (43), (44) and (35) we get

$$V' = V - \frac{(V_0 + V)[H^+]}{C_{HCl}} = V_{eq} \quad (45)$$

and then we obtain the Gran expression for the titration of a weak base with a strong acid, beyond the equivalence point

$$(V_0 + V)[H^+] = V C_{HCl} - V_{eq} C_{HCl} \quad (46)$$

By plotting the left hand of Eq. (46) against  $V$ , a straight line is obtained with slope  $C_{HCl}$ , intersecting the  $V$  axis at the point  $V_{eq}$ .

An alternative route to obtain Eq. (46) is from Eq. (27). At  $V > V_{eq}$ ,  $f_{0,b} \approx 0$ ,  $f_{1,a} \approx 1$ ,  $\Delta \approx [H^+]$ , and then,

$$(V_0 + V)[H^+] \approx V C_{HCl} - V_0 C_{NH_3} \quad (47)$$

which is combined with Eq. (29), gives finally Eq. (46).

## 8. Titration error

From Eq. (26), we may calculate the fraction titrated,  $T$ , as

$$T = \frac{C_{HCl}V}{C_{NH_3}V_0} = \frac{C_{HCl}}{C_{NH_3}} \left( \frac{f_{1,a}C_{NH_3} - f_{0,b}C_{H_3BO_3} + \Delta}{C_{HCl} - \Delta} \right) = \frac{f_{1,a} - \frac{f_{0,b}C_{H_3BO_3}}{C_{NH_3}} + \Delta}{1 - \frac{\Delta}{C_{HCl}}} \quad (48)$$

In the vicinity of the equivalence point, the mole fraction of borate ion,  $f_{0,b}$ , is close to zero, and the titration error,  $\Delta T$ , may be approximated as

$$\Delta T = [T - 1]_{end} = \left[ \frac{f_{1,a} + \Delta}{1 - \frac{\Delta}{C_{HCl}}} - 1 \right]_{end} \quad (49)$$

## 9. Checking the proposed linearization method with synthetic data

In order to verify the goodness of the proposed linearization method, based on the use of Eq. (35), a series of theoretical data with variations of 0.05 units of  $pH$ , has been generated with aid of Eq. (26), using the analogous conditions previously published by Cruz [68],  $V_0=150\text{mL}$ ;  $C_{\text{NH}_3}=0.0096\text{M}$ ;  $C_{\text{H}_3\text{BO}_3}=0.2156\text{M}$ ;  $C_{\text{HCl}}=0.12\text{M}$ ;  $pK_{1,a}=9.27$ ;  $pK_{1,b}=9.12$ .

Two sets of data  $[V, pH]$  ( $N=24$  and  $N=11$ ) were selected from the theoretical data in the region prior to the equivalence point ( $V_{eq}=12\text{mL}$ ), ranging from approximately 0.6 to 11.16 mL for the first series, and from 0.6 to 10.8 mL for the second (Table 2). Note that the points selected for study are far from the region of the equivalence point:  $V_{eq} \pm 1\text{mL}$ . A set of data  $[V, pH]$  ( $N=11$ ) was also selected after the equivalence point, between about 13 and 23.5 mL (Table 3).

The proposed linearization method has been applied to data corresponding to  $V < V_{eq}$  (basic zone), the volumes being rounded to 5, 3 and 2 decimal places, respectively. It is proved that the method works well in all cases, correct values for both  $V_{eq}$  and  $pK_{1,a}$  obtained. The same rounding procedure has been applied to the data corresponding to  $V > V_{eq}$  (acid zone).

Applying in this latter case, the Gran method based on Eq. (46) also leads to the correct values of  $V_{eq}$  and  $C_{\text{HCl}}$ . However, a pronounced curvature is observed when the conventional Gran

		$V_0=$	150 mL			$V_{eq}=$	12.000 mL
		$C(\text{HCl})=$	0.12M			$pK_{1,a}=$	9.270
		$pK_a(\text{NH}_4^+)=$	9.27			$a_1=$	-1.864E+09
		$pK_a(\text{H}_3\text{BO}_3)=$	9.12			$2.237E+10$	$=a_0$
		$C(\text{H}_3\text{BO}_3)=$	0.2156M			$s_{(a1)}=$	1.971E+07
		$pK_w=$	13.8			$2.327E+08$	$=s_{(a0)}$
						$R^2=$	0.9990
						$6.386E+06$	$=s_{(y/x)}$
V	V(2)	V(2)/[H]	pH	$\Delta$	$f_{0,b}$	V'(2)	v'(2)/[H]
0.62116	0.62	3.49E+07	7.75	-8.73E-07	4.09E-02	11.65	6.55E+08
1.81368	1.81	9.07E+07	7.70	-7.74E-07	3.66E-02	11.68	5.85E+08
2.88545	2.89	1.29E+08	7.65	-6.86E-07	3.28E-02	11.72	5.24E+08
3.84785	3.85	1.53E+08	7.60	-6.06E-07	2.93E-02	11.75	4.68E+08
4.71136	4.71	1.67E+08	7.55	-5.34E-07	2.62E-02	11.77	4.18E+08
5.48560	5.49	1.74E+08	7.50	-4.70E-07	2.34E-02	11.80	3.73E+08
6.80061	6.80	1.71E+08	7.40	-3.58E-07	1.87E-02	11.84	2.97E+08
7.85420	7.85	1.57E+08	7.30	-2.66E-07	1.49E-02	11.87	2.37E+08
8.69687	8.70	1.38E+08	7.20	-1.88E-07	1.19E-02	11.90	1.89E+08
9.65355	9.65	1.08E+08	7.05	-8.87E-08	8.44E-03	11.92	1.34E+08
10.81924	10.82	6.08E+07	6.75	8.87E-08	4.25E-03	11.96	6.73E+07

**Table 2.** Proposed method applied to theoretical data before the equivalence point and rounded to the second decimal figure in volume (penultimate and last columns).

$V_0=$	150 mL			$V_{eq}=$	11.999 mL	
$C(HCl)=$	0.12M			$C(HCl)=$	0.120M	
$pK_a(NH_4^+)=$	9.27					
$pK_a(H_3BO_3)=$	9.12	$a_1=$	0.120	-1.440	$=a_0$	
$C(H_3BO_3)=$	0.2156M	$s_{(a1)}=$	4.471 E-05	8.219E-04	$=s_{(a0)}$	
$pK_w=$	13.8	$R^2=$	0.9999	4.778E-04	$=s_{(y/x)}$	
<b>V</b>	<b>(V<sub>0</sub>+V) [H]</b>	<b>V(3)</b>	<b>(V(3)+V<sub>0</sub>) [H]</b>	<b>V(2)</b>	<b>(V(2)+V<sub>0</sub>) [H]</b>	<b>pH</b>
13.07922	0.129538429	13.079	0.130	13.08	0.13	3.10
14.16181	0.260178937	14.162	0.260	14.16	0.26	2.80
15.07963	0.369567257	15.080	0.370	15.08	0.37	2.65
15.89625	0.467559159	15.896	0.468	15.90	0.47	2.55
16.93586	0.592310783	16.936	0.592	16.94	0.59	2.45
18.26332	0.751604642	18.263	0.752	18.26	0.75	2.35
19.06089	0.847311598	19.061	0.847	19.06	0.85	2.30
19.96482	0.955782421	19.965	0.956	19.96	0.96	2.25
20.99062	1.078877875	20.991	1.079	20.99	1.08	2.20
22.15642	1.218774118	22.156	1.219	22.16	1.22	2.15
23.48355	1.37802882	23.484	1.378	23.48	1.38	2.10

**Table 3.** Gran method applied to theoretical data after the equivalence point and rounded to the second decimal figure in volume (antepenultimate and penultimate columns).

function ( $V/[H^+]$ ) is applied to the data obtained in the basic region ( $V < V_{eq}$ ), which makes it impossible to apply this method to values of the ammonia-boric acid system away from  $pH_{eq}$ .

From the data obtained and using linear regression (method of the least squares), in EXCEL, with the function LINEST, the volume at the equivalence point is obtained as

$$V_{eq} = -\frac{a_0}{a_1} \quad pK_{1,a} = -\log\left(-\frac{1}{a_1}\right) \quad (50)$$

to data corresponding to  $V < V_{eq}$ , and

$$V_{eq} = -\frac{a_0}{a_1} \quad C_{HCl} = a_1 \quad (51)$$

to data corresponding to  $V > V_{eq}$ . The data corresponding to the statistic parameters of the straight line using the least squares method are displayed in **Tables 2** and **3**. The parameters obtained using linear regression by the function LINEST in EXCEL are: intercept, slope and coefficient of determination (correlation coefficient squared):  $a_0$ ,  $a_1$  and  $R^2$ , respectively; and standard deviations of the intercept, slope and regression:  $s_{(a0)}$ ,  $s_{(a1)}$  and  $s_{(y/x)}$ , respectively.

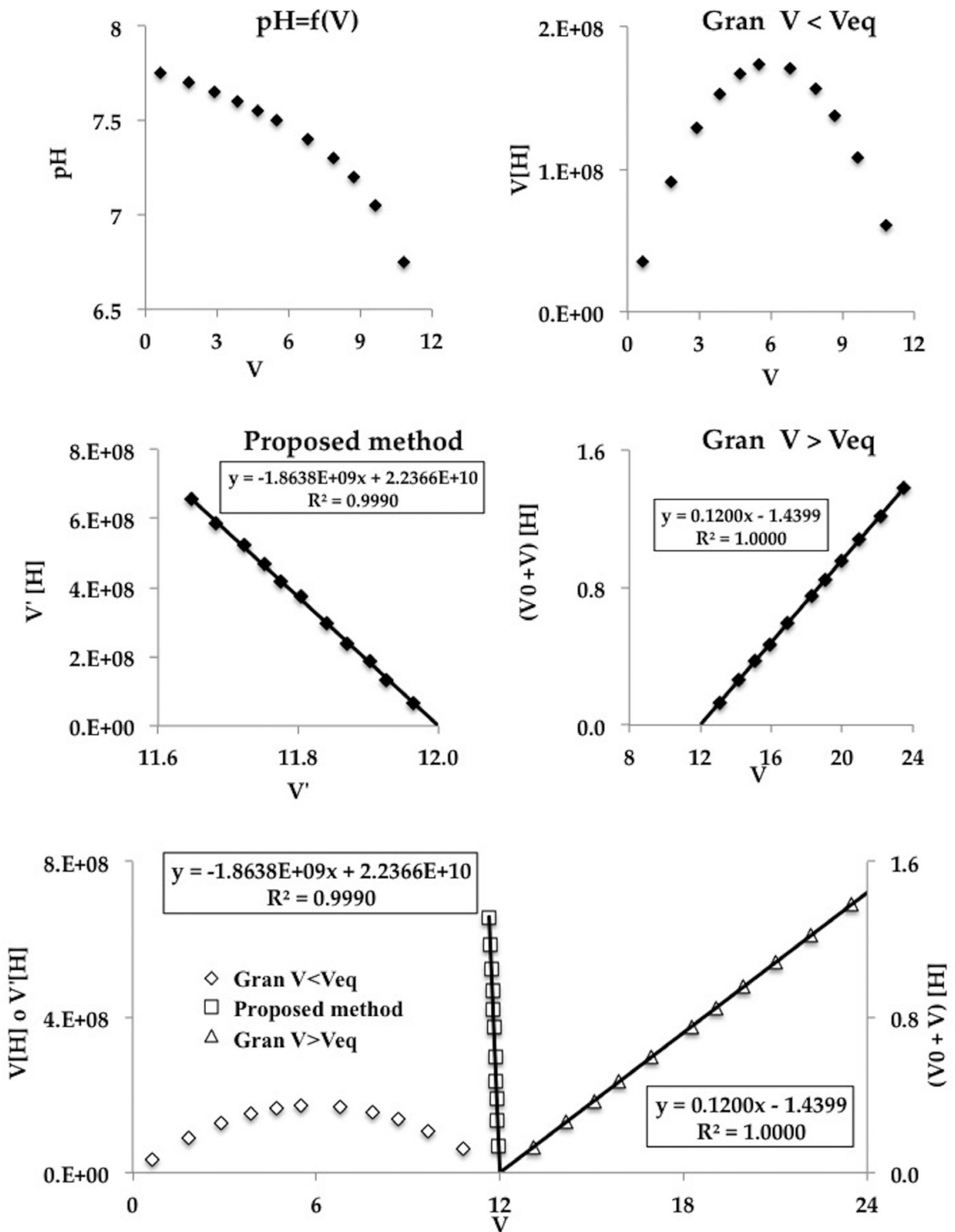
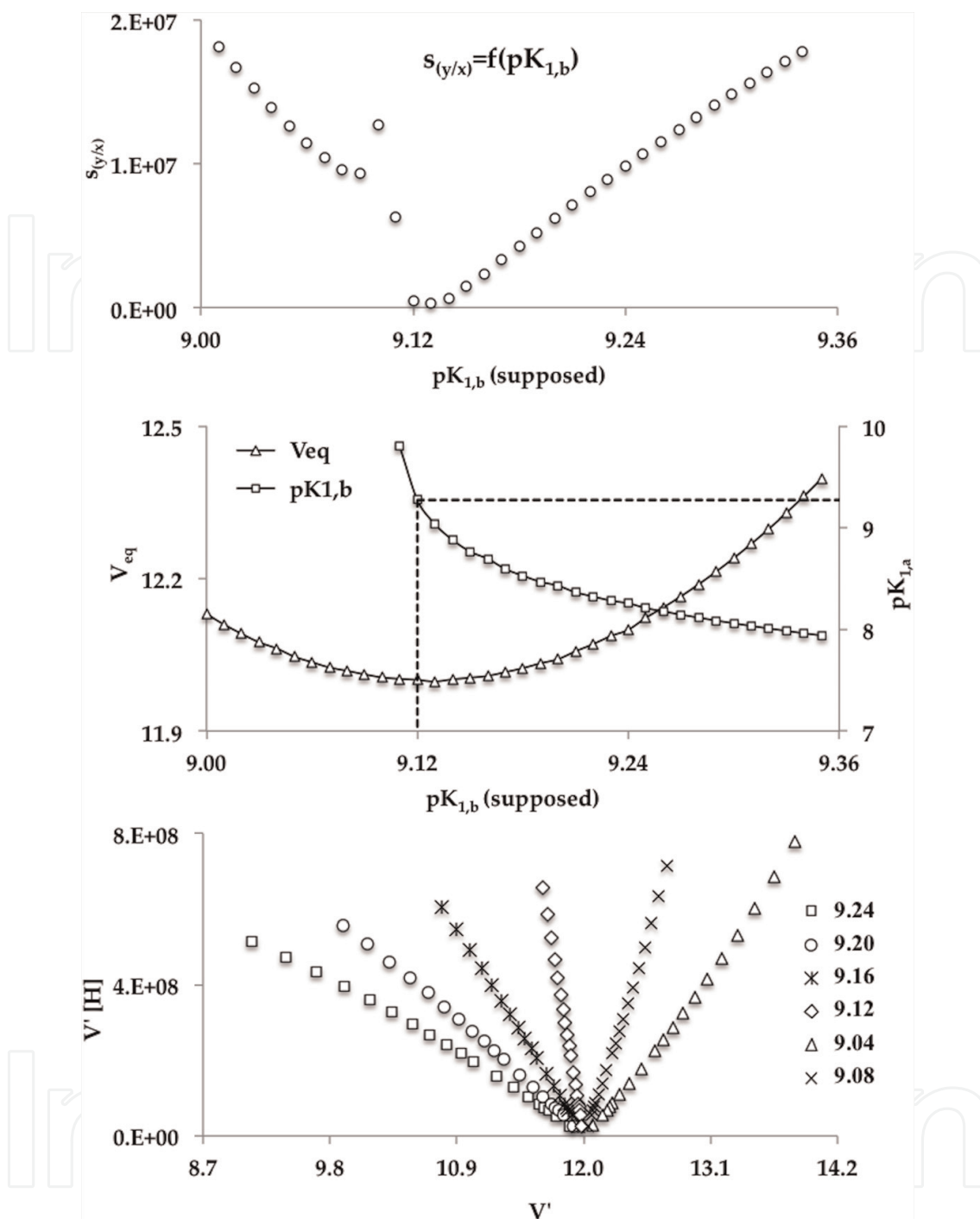


Figure 4. Top: ammonia titration curve ( $pH=f(V)$ ) in boric acid (LEFT) and Gran method  $V < V_{eq}$  (right). Middle left: modified Schwartz method. Middle right: Gran method  $V > V_{eq}$ . Bottom: comparative study.



**Figure 5.** Top: Standard deviation of the regression as a function of  $pK_{1,b}$  supposed. Middle: equivalence volume and  $pK_{1,a}$  values as a function of  $pK_{1,b}$  supposed. Bottom: plots for a series of  $pK_{1,b}$  values selected. Data  $[V; pH]$ : [0.62; 7.75] [1.81; 7.70] [2.89; 7.65] [3.85; 7.60] [4.71; 7.55] [5.49; 7.50] [6.18; 7.45] [6.80; 7.40] [7.36; 7.35] [7.85; 7.30] [8.30; 7.25] [9.05; 7.15] [9.65; 7.05] [10.13; 6.95] [10.52; 6.85] [10.68; 6.80] [10.82; 6.75] [11.06; 6.65] [11.53; 6.35].

**Figure 4** (top) shows the titration curve corresponding to the series of eleven pairs of data (left side) and the application of the conventional Gran method (right side). **Figure 4** (middle) shows the linearization method proposed in this chapter (left side) and the Gran method for data beyond the equivalence point (right side). Finally, at the bottom of **Figure 4**, a comparative

study of the three methods is shown together. Gran and modified Schwartz (proposed method) on the same scale, showing the superiority of the latter transforming the whole curve in a straight line. The scale corresponding to the application of the Gran method in acid medium ( $V > V_{eq}$ ) is shown on the secondary axis.

On the other hand, the proposed method, based on the use of Eq. (35), has been applied to a series of 19 data pairs (compiled in the legend of **Figure 5**) in the first instance, assuming the value of  $pK_{1,b}$  unknown and varying its in 0.01 units of  $pK$  in the vicinity of  $pK_{1,b}$  true value. The standard deviation of the corresponding regression lines ( $s_{y/x}$ ) obtained as a function of the assumed  $pK_{1,b}$  value is shown in **Figure 5** (top). The standard deviation of the regression is minimal when the value of  $pK_{1,b}$  agrees on the true value (or differs in less than 0.01 unit). The values obtained for the equivalence volume and for  $pK_{1,a}$  (ammonium ion), as a function of the assumed value of  $pK_{1,b}$ , are plotted in the central part of **Figure 5**.

Two conclusions can be drawn from the study:

1. The proposed method is sufficiently robust regarding to variations in the supposed value for  $pK_{1,b}$ . Values of  $pK_{1,b}$  between 9.09 and 9.16 give rise to an error in the determination of the equivalence volume  $< 0.1\%$ .
2. The proposed method does not work to estimate, simultaneously, the value of the acidity constant of the ammonium ion, as shown in the central part of **Figure 5** by plotting  $pK_{1,a}$  obtained (secondary axis) versus the supposed value of  $pK_{1,b}$ . Small variations in the supposed value of  $pK_{1,b}$  lead to large variations in the value obtained for  $pK_{1,a}$ . Moreover, in case of  $pK_{1,a}$  is not applicable a minimization criterion because a minimum or maximum value is not reached when  $pK_{1,b}$  agrees on the true value. When  $pK_{1,b} < 9.11$ , the slope of the line changes from negative to positive, so Eq. (50b) is not applicable, since the value of the constant obtained is negative, which has no physical meaning.

## 10. Checking the proposed linearization method with experimental data

Once tested that our proposed method works well, the theory devised has been applied to the experimental data recently reported by Cruz [68] in the Journal of Chemical Education in a study about the determination of ammonia with HCl using concentrated (4% w/v) and diluted (1.3% w/v) boric acid to reproduce the final-Kjeldahl titration when two different volumes of ammonia distillate are collected (data  $[V, pH]$  appear as supporting information).

**Table 4** shows the data  $[V, pH]$  corresponding to the titration curves of ammonia in solutions of  $H_3BO_3$  at 4% w/v (0.6469M) and 1.33% w/v (0.2156M). The shape of titration curves and the application of the conventional Gran method in the region prior to equivalence point are shown in **Figure 6**. A curvilinear shape is obtained with both solutions (being the curve most flattened when  $[H_3BO_3]=0.6469M$ ), so that, in its original philosophy, the Gran method is not applicable.

In order to apply our proposed linearization method, data with  $V_{HCl} < 11\text{mL}$  are selected (region prior to  $V_{eq}$ , which is around 12mL). **Tables 5** and **6** show the calculations performed

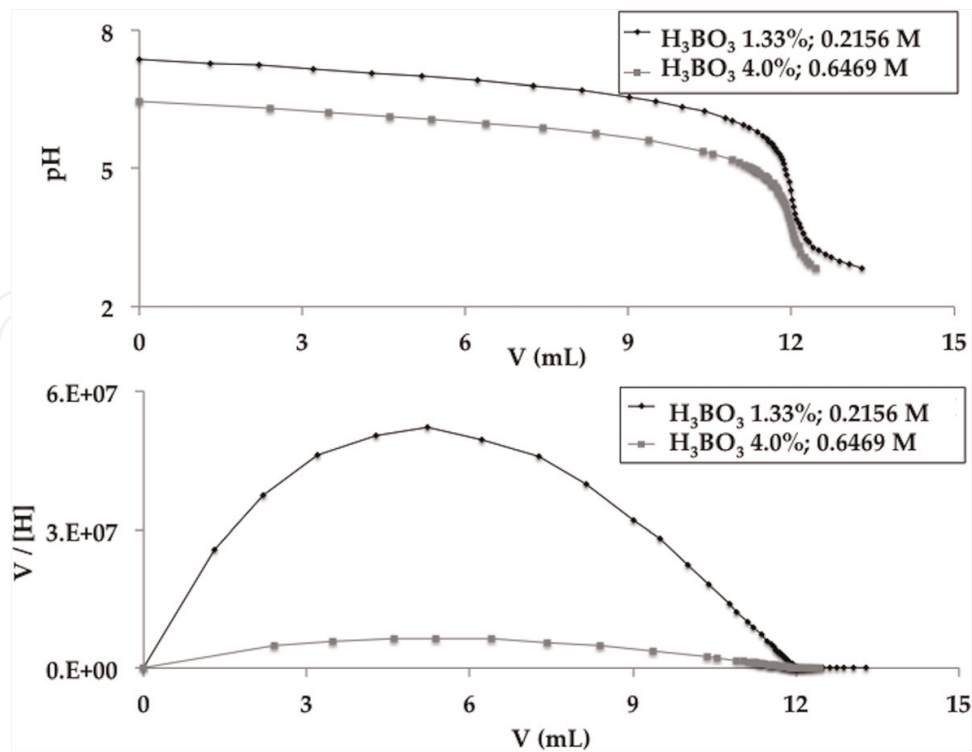
V <sub>0</sub> =50 mL; C(HCl)=0.12M						V <sub>0</sub> =150 mL; C(HCl)=0.12M					
C(H <sub>3</sub> BO <sub>3</sub> )=0.6469M (4% w/v)						C(H <sub>3</sub> BO <sub>3</sub> )=0.2146M (1.33% w/v)					
V	pH	V	pH	V	pH	V	pH	V	pH	V	pH
0.00	6.45	11.35	4.95	11.91	4.12	0.00	7.38	11.37	5.80	12.01	4.32
2.41	6.31	11.38	4.94	11.93	4.02	1.31	7.29	11.48	5.70	12.04	4.15
3.48	6.23	11.43	4.89	11.96	3.91	2.21	7.23	11.54	5.65	12.07	4.00
4.60	6.14	11.49	4.84	11.99	3.79	3.20	7.16	11.59	5.59	12.10	3.88
5.38	6.08	11.52	4.82	12.01	3.67	4.28	7.07	11.62	5.55	12.13	3.79
6.39	5.99	11.55	4.79	12.04	3.57	5.21	7.00	11.65	5.51	12.16	3.71
7.42	5.88	11.58	4.76	12.07	3.46	6.22	6.90	11.69	5.47	12.21	3.58
8.40	5.76	11.63	4.69	12.10	3.38	7.26	6.80	11.71	5.43	12.27	3.48
9.37	5.61	11.66	4.66	12.13	3.31	8.14	6.69	11.74	5.37	12.33	3.40
10.38	5.38	11.69	4.67	12.18	3.18	9.02	6.55	11.78	5.32	12.41	3.30
10.56	5.32	11.72	4.58	12.24	3.08	9.50	6.47	11.80	5.25	12.50	3.23
10.92	5.18	11.75	4.53	12.30	3.00	10.00	6.35	11.83	5.18	12.62	3.14
11.04	5.13	11.77	4.48	12.35	2.93	10.40	6.24	11.86	5.09	12.74	3.07
11.15	5.07	11.80	4.42	12.44	2.84	10.78	6.11	11.89	4.99	12.88	2.99
11.21	5.04	11.83	4.36			10.92	6.05	11.92	4.87	13.06	2.92
11.27	5.01	11.85	4.29			11.11	5.96	11.95	4.72	13.29	2.84
11.32	4.97	11.88	4.21			11.22	5.89	11.98	4.52		

**Table 4.** Data  $[V, pH]$  corresponding to the titration curves of ammonia in solutions of  $H_3BO_3$  at 4% w/v (0.6469M) and 1.33% w/v (0.2156M) [68].

when applying the proposed linearization method for the optimum  $pK_{1,b}$  (value shown in the box) in the studies of the ammonia solutions diluted (0.2156M) and concentrated (0.6469M), respectively.

The most diluted solution ( $[H_3BO_3]=0.2156M$ ) is firstly studied. **Figure 7** (top) shows the plots obtained for different supposed values of  $pK_{1,b}$  in the range from 9.04 to 9.24. The best straight line corresponds to a value of  $pK_{1,b}$  around 9.12, as shown in the bottom left side of **Figure 7** plotting the standard deviation of the regression obtained for each line versus the supposed value of  $pK_{1,b}$  which reaches a minimum when  $pK_{1,b}$  equals to 9.12. The supposed  $pK_{1,b}$  is then varied around 9.12 in 0.001 units (**Figure 7**, bottom right), which minimizes the standard deviation of the regression to 9.117.

In the study of  $[H_3BO_3]=0.6469M$ , the results obtained indicate it is necessary to explore the values for supposed  $pK_{1,b}$  in a wider range toward lower values of pK, consistent with the fact that the boric acid strength increases with its concentration [68, 70, 71]. It should be noted that the  $pK_{1,b}$  value used with the proposed method is an adjustment parameter, and in fact



**Figure 6.** Ammonia titration curve ( $pH=f(V)$ ) in boric acid (top) and conventional Gran method (bottom).

apparent, without a specific physical meaning. From **Figure 8** (top), it can be observed that when the apparent  $pK_{1,b}$  reaches a value around 7.5, the representation of  $V/[H]$  versus  $V'$  has the form of a straight line. This parameter is varied in 0.001 units around this value, being verified that the standard deviation of the regression is minimum (**Figure 8** bottom) when it reaches the value of 7.509 corresponding to a  $V_{eq}$  of 11.827 mL.

**Figure 9** shows the application of the proposed method for the ammonia solutions diluted (0.2156 M) and concentrated (0.6469 M) using the optimum value for  $pK_{1,b}$  in each case (9.117 and 7.509, respectively). Note how the response of the diluted sample in boric acid, in a relation to 1:3, is more sensitive (greater slope) than that of the sample more concentrated in boric acid.

An empirical parameter,  $\Gamma$ , can be defined, which take into account the variation in the value of  $pK_{1,b}$  optimum when the boric acid concentration increases, reflecting the increase in acidity motivated by the appearance of polynuclear species. The complexity of such treatment far exceeds the objective of this book chapter.

$$\Gamma = \frac{[K_{1,b}]_c}{[K_{1,b}]_d} = 10^{([K_{1,b}]_c - [K_{1,b}]_d)} = 10^{(9.117 - 7.509)} = 40.55 \quad (52)$$

The determination of the various polyborate species in solution has proved to be difficult because they appear at fairly high concentrations of boric acid and involve the addition of no more than one  $\text{OH}^-$  ion per  $\text{B}(\text{OH})_3$  group [87]. The  $pK$  values of triborate



$V_0=$	150 mL			$V(\text{eq})=$	12.024 mL	
$C(\text{HCl})=$	0.12M			$\text{pK}^*=$	7.179	
$\text{pK}_a(\text{H}_3\text{BO}_3)=$	9.117					
$C(\text{H}_3\text{BO}_3)=$	0.2156M	$a_1=$	-1.510E+07	1.816E+08	$=a_0$	
$\text{pK}_w=$	13.8	$s_{(a1)}=$	1.353E+05	1.189E+06	$=s_{(a0)}$	
		$R^2=$	0.9990	1.015E+06	$=s_{(y/x)}$	
V	V/[H]	pH	$\Delta$	$f_{0,b}$	V'	V'/(H)
1.31	2.554E+07	7.29	-2.577E-07	1.468E-02	5.265E+00	1.027E+08
2.21	3.753E+07	7.23	-2.103E-07	1.281E-02	5.661E+00	9.614E+07
3.20	4.625E+07	7.16	-1.599E-07	1.092E-02	6.143E+00	8.880E+07
4.28	5.029E+07	7.07	-1.011E-07	8.894E-03	6.677E+00	7.845E+07
5.21	5.210E+07	7.00	-5.849E-08	7.580E-03	7.253E+00	7.253E+07
6.22	4.941E+07	6.90	0.000E+00	6.031E-03	7.845E+00	6.232E+07
7.26	4.581E+07	6.80	5.849E-08	4.796E-03	8.553E+00	5.396E+07
8.14	3.987E+07	6.69	1.265E-07	3.727E-03	9.144E+00	4.479E+07
9.02	3.200E+07	6.55	2.256E-07	2.703E-03	9.748E+00	3.459E+07
9.50	2.804E+07	6.47	2.921E-07	2.249E-03	1.011E+01	2.982E+07
10.00	2.239E+07	6.35	4.112E-07	1.707E-03	1.046E+01	2.342E+07
10.40	1.807E+07	6.24	5.479E-07	1.326E-03	1.076E+01	1.869E+07
10.78	1.389E+07	6.11	7.558E-07	9.830E-04	1.104E+01	1.423E+07
10.92	1.225E+07	6.05	8.735E-07	8.563E-04	1.115E+01	1.251E+07

**Table 5.** Linearization method applied to the titration data lower than 11 mL ( $V_{eq} \approx 12$  mL): diluted boric acid series (1.33%, 0.2156M).

( $3\text{B}(\text{OH})_3 = \text{B}_3\text{O}_3(\text{OH})_4^- + \text{H}^+ + 2\text{H}_2\text{O}$ ) and pentaborate ( $5\text{B}(\text{OH})_3 = \text{B}_5\text{O}_6(\text{OH})_4 + \text{H}^+ + 5\text{H}_2\text{O}$ ) are of the order of 6.60 and 6.90, respectively [68].

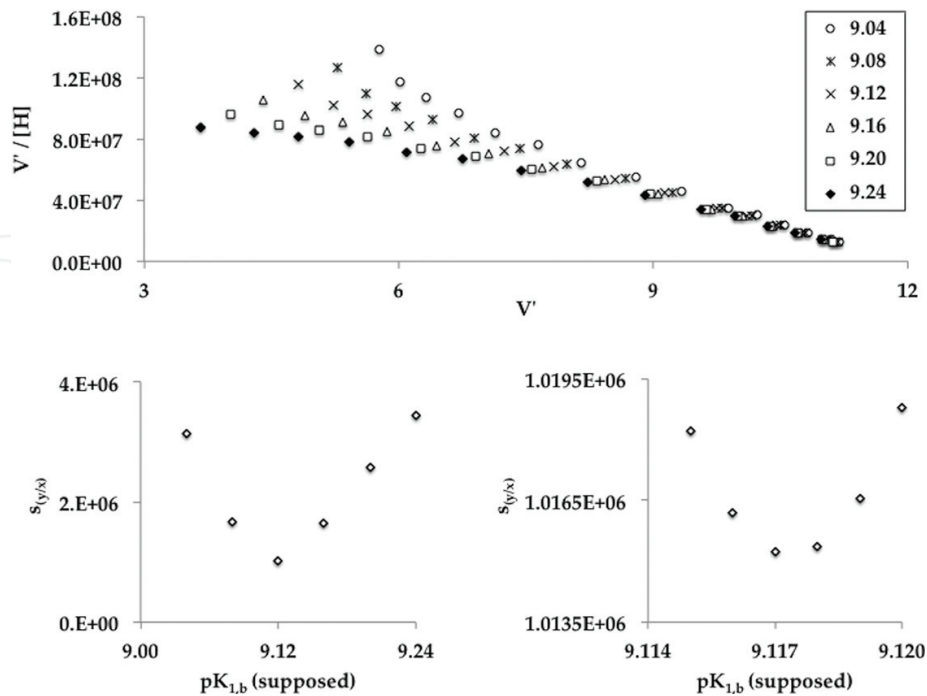
The titration with  $\text{H}_3\text{BO}_3$  0.2156M results in a wider *pH* range and a steeper slope, contrary to that predicted by the theoretical models of the titration curves. This is for the concurrent presence of other polymer species more abundant in  $\text{H}_3\text{BO}_3$  0.6469M, which disappear progressively as the dilution increases. The *pH* value at the equivalence point is variable depending on the initial concentration of boric acid, which can lead to systematic errors when using colored indicators in the detection of the end point.

**Figure 10** (top) shows the conventional Gran method (I) to the data compiled in **Table 4**. Despite the precision of the data, the application of this method has difficulties in the appreciation of the equivalence volume. Cruz [68] applied the Gran method (II) to the titration data between 11 and 12mL, which could be questioned from the methodological point of view. The results obtained by Cruz are shown in **Figure 10** (bottom).

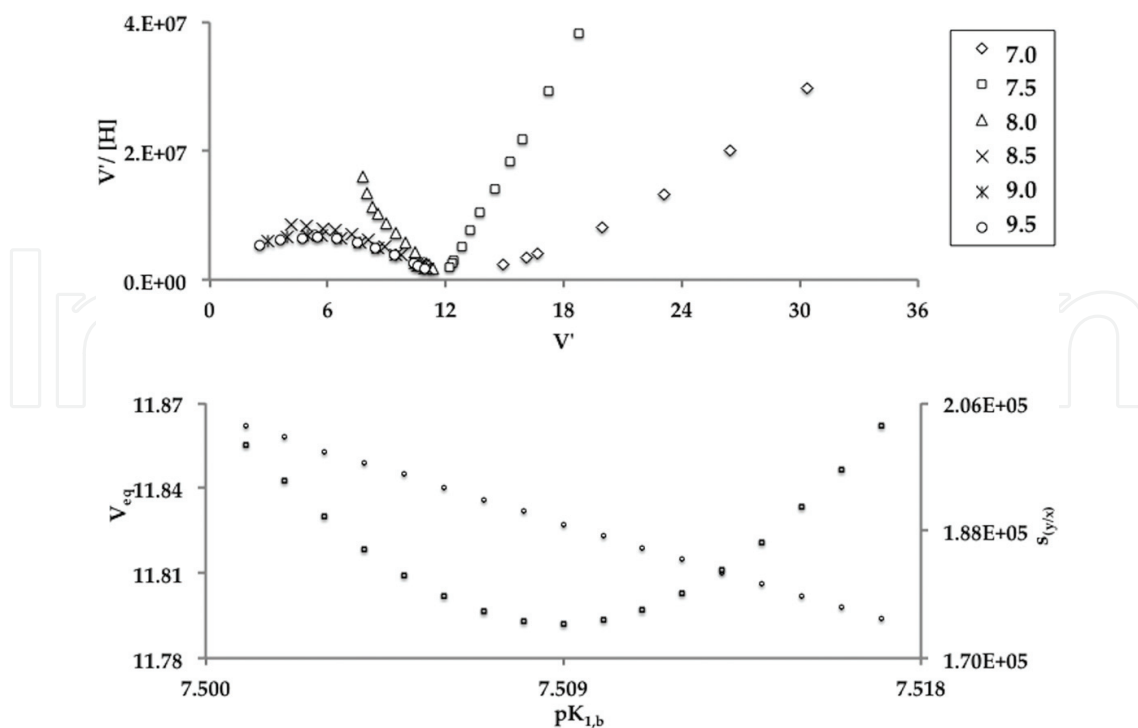
**Table 7** summarizes a comparative study of the results obtained in the calculation of the equivalence point using the proposed method described in this book chapter and those

$V_0=$	50 mL			$V(\text{eq})=$	11.827 mL		
$C(\text{HCl})=$	0.12M						
$\text{pKa}(\text{H}_3\text{BO}_3)=$	7.509						
$C(\text{H}_3\text{BO}_3)=$	0.6469M		$a_1=$	5.662E+06	-6.697E+07	$=a_0$	
$\text{pKw}=$	13.8		$s_{(a1)}=$	2.662E+04	3.828E+05	$=s_{(a0)}$	
			$R^2=$	0.9998	1.749E+05	$=s_{(y/x)}$	
V	V/[H]	pH	$\Delta$	$f_{0,b}$	V'	V'/(H)	
2.41	4.921E+06	6.31	4.574E-07	5.948E-02	1.844E+01	3.765E+07	
3.48	5.910E+06	6.23	5.619E-07	4.997E-02	1.695E+01	2.878E+07	
4.60	6.350E+06	6.14	7.026E-07	4.100E-02	1.565E+01	2.161E+07	
5.38	6.468E+06	6.08	8.127E-07	3.590E-02	1.506E+01	1.810E+07	
6.39	6.245E+06	5.99	1.008E-06	2.938E-02	1.431E+01	1.398E+07	
7.42	5.629E+06	5.88	1.306E-06	2.296E-02	1.361E+01	1.032E+07	
8.40	4.834E+06	5.76	1.729E-06	1.751E-02	1.312E+01	7.549E+06	
9.37	3.817E+06	5.61	2.448E-06	1.246E-02	1.273E+01	5.185E+06	
10.38	2.490E+06	5.38	4.165E-06	7.375E-03	1.237E+01	2.966E+06	
10.56	2.206E+06	5.32	4.783E-06	6.430E-03	1.229E+01	2.568E+06	
10.92	1.653E+06	5.18	6.605E-06	4.666E-03	1.217E+01	1.843E+06	

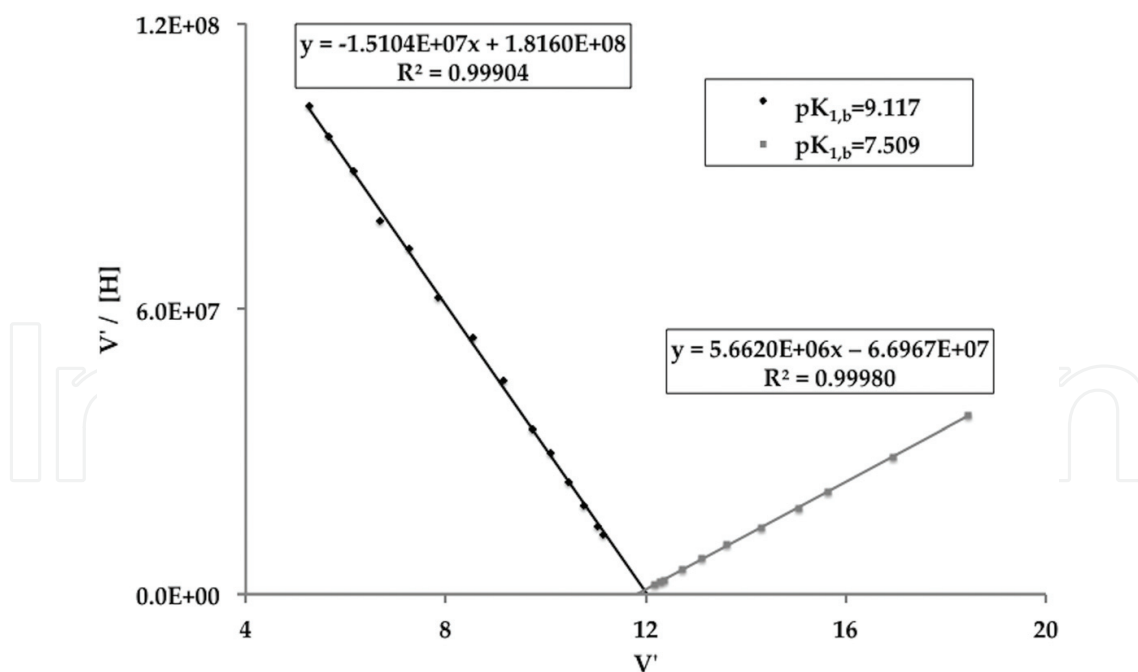
**Table 6.** Linearization method applied to the titration data lower than 11 mL ( $V_{eq} \approx 12$  mL): concentrated boric acid series (4% w/v, 0.6469M).



**Figure 7.** Top: proposed linearization method as a function of  $\text{pK}_{1,b}$  supposed. Data  $[V, \text{pH}]$  are shown in Table 4. Bottom: standard deviation of the regression as a function of  $\text{pK}_{1,b}$  supposed.



**Figure 8.** Top: proposed linearization method in the titration with  $[H_3BO_3] = 0.6469$  and  $V_0 = 50$  mL when  $V < V_{eq}$ . Bottom: equivalence volume and  $s_{(y/x)}$  values as a function of  $pK_{1,b}$  supposed;  $[H_3BO_3]=0.6469$  M.



**Figure 9.** Left: proposed linearization method in the titration with  $[H_3BO_3]=0.2156$  and  $V_0=150$  mL. Right: proposed linearization method in the titration with  $[H_3BO_3]=0.6469$  and  $V_0=50$  mL.

obtained by Cruz [68]. The differences range from 0.15% for  $[H_3BO_3]=0.2156$  M, and  $-1.6\%$  for  $[H_3BO_3]=0.6469$  M. The values of the standard deviation of the volume at the equivalence point are equal to 0.036 and 0.017 volume units for the proposed method for  $[H_3BO_3] = 0.2156$  M and

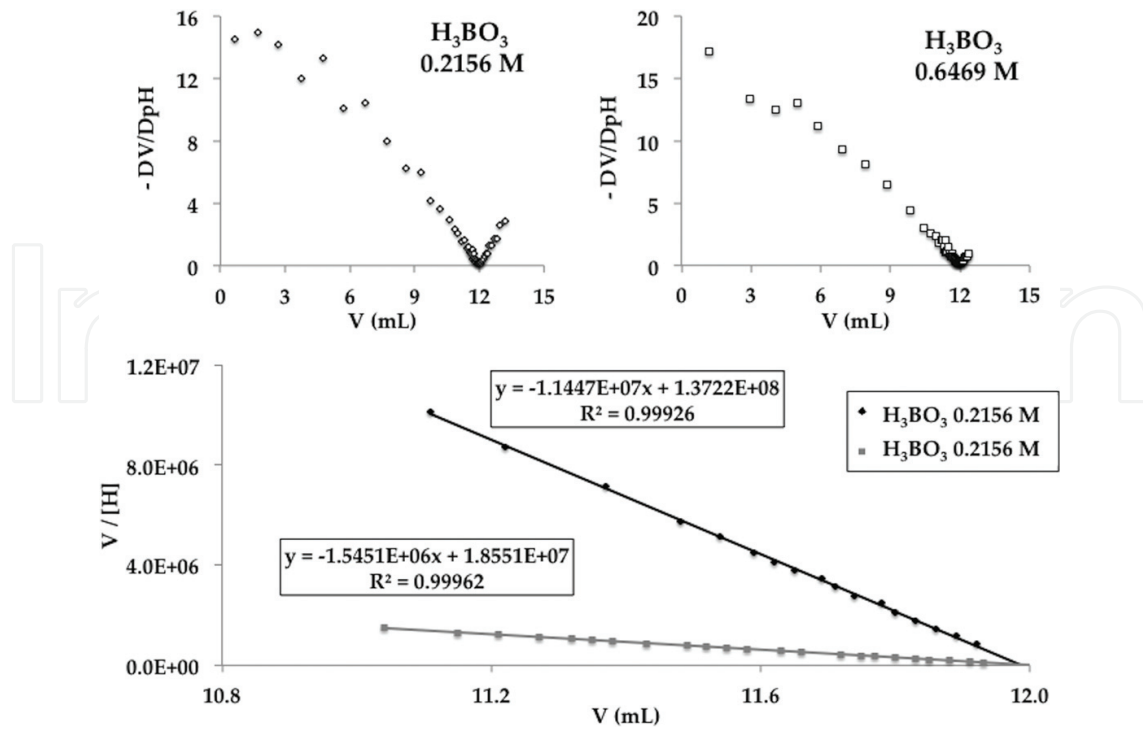


Figure 10. Top: Gran I method. Bottom: Gran II method (conventional) for  $11 < V < 12$  mL (as applied by Cruz (2013)).

$[H_3BO_3]$	pH range	N	Method	$pK_{1,b}$	V(eq)	s[V(eq)]	s[V(eq)]*
0.2156	1.31–10.92	14	Proposed	9.117	12.024	0.036	0.133
0.6164	2.41–10.92	11	Proposed	7.509	11.794	0.017	0.102
0.2156	11.04–11.93	23	Gran II	-	12.006	-	-
0.6164	11.11–11.92	17	Gran II	-	11.987	-	-

Table 7. Comparison results obtained in the calculation of the equivalence point using the proposed method and the Gran method [68].

0.6469 M, respectively, and 0.3 and 0.15% in terms of relative standard deviation, correspondingly. If the covariance is not taken into account, these values would increase in absolute terms to 0.133 and 0.102 units of volume corresponding to 1.11 and 0.86%, respectively (an increase of the relative error between four and six times more). So, the covariance of measurements can be important as the variances and both contribute significantly to the total analytical error.

Finally, the proposed method has been applied to experimental data carried out in our laboratory reproducing the conditions reported by Cruz [68] in the case of diluted boric acid.

### 10.1. Reagents

All reagents were analytical grade unless otherwise specified:

- Boric acid ( $H_3BO_3$ )  $M=61.83$  g/mol (Merck>99.5%), Ammonia (30%)  $M=17.03$  g/mol. (Panreac); Trishydroxymethyl aminomethane (TRIS;  $C_4H_{11}NO_3$ )  $M=121.14$  g/mol.

- Buffers pH=3, 5 (Riedel-de Häen), 4.01, 7 (Crison).
- Phial of HCl 1 M (Riedel-de Häen).

## 10.2. Instruments

Analytical balance (Metler AE200) (4 decimals), Granatario (Metler PJ 400) (2 decimals), pH-meter Crison GLP 21, with a combined Ag/AgCl glass electrode were used. The pH-meter was calibrated using *pH* buffers 3, 4.01, 5 and 7, using a two-point calibration method. Burette of 5 mL (Brand) ( $\pm 0.01$  at 20°C) was used.

## 10.3. Experimental

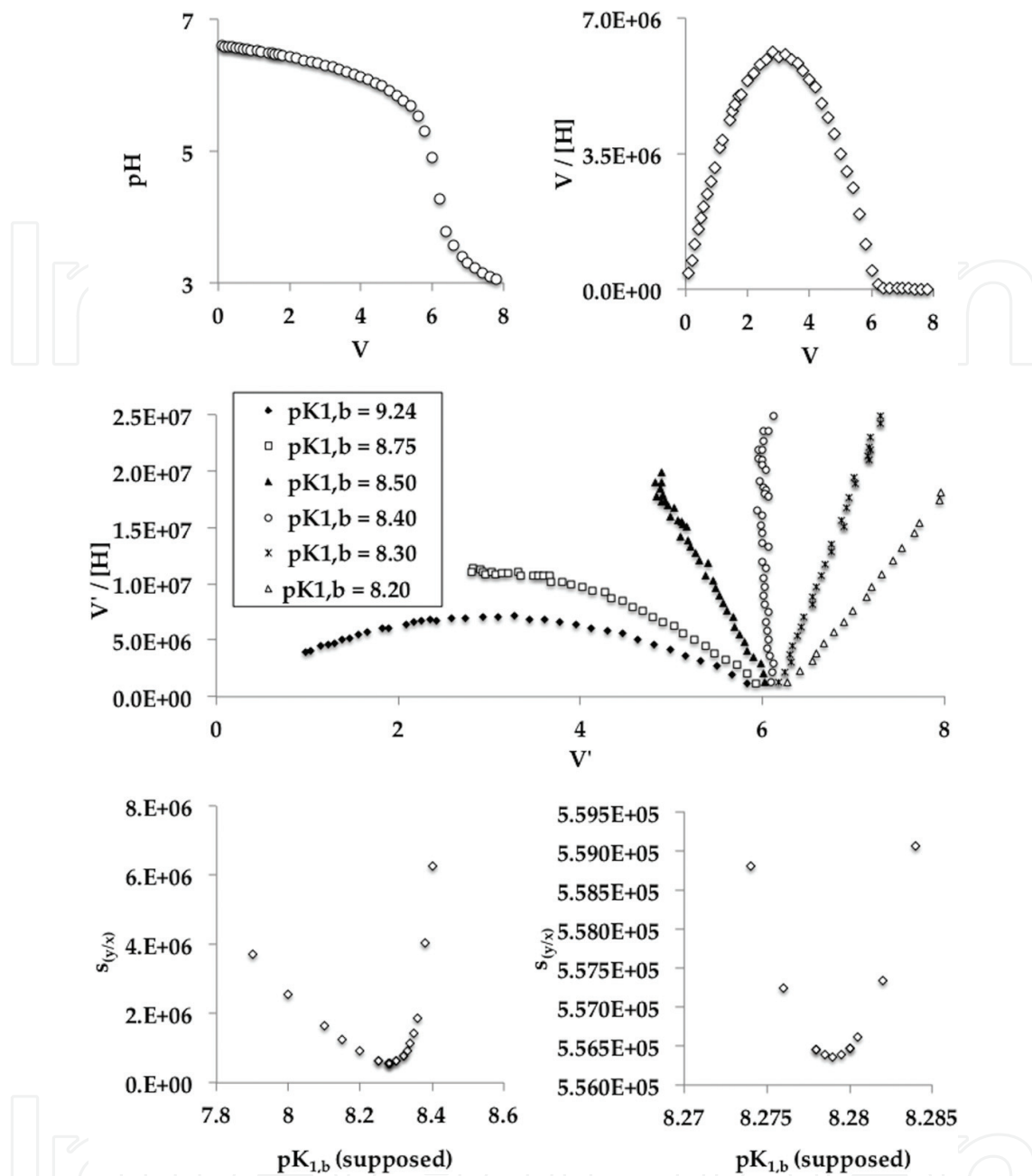
About 5 mL of ammonia solution (0.12M) was pipetted into a 50 mL volumetric flask and made up to the mark with a boric acid solution (4% w/v). The contents of this solution were transferred to a 200 mL Erlenmeyer flask containing 100 mL of distilled water. Then, the ammonium borate solutions were titrated potentiometrically with HCl (0.077M) (previously standardized with TRIS) using the glass *pH* electrode.

**Table 8** shows the data [*V*, *pH*] corresponding to the titration curve of ammonia in solution of H<sub>3</sub>BO<sub>3</sub> (0.1941M). The shape of the valuation curve is shown in the upper part of **Figure 11**. All data prior to the equivalence point, before the jump in the titration curve, have been taken to apply the calculus by the proposed method, which leads to an equivalence volume equal to 6.17 mL. The equivalence volume obtained by the first derivative method is equal to 6.20 mL (although not many points are available in this case).

If the first points of the titration in which the solution is not sufficiently buffered, are neglected, a slightly lower volume (6.14 mL) is obtained. An analogous result is obtained by using only

V	pH	V	pH	V	pH	V	pH
0.10	6.609	1.20	6.506	2.80	6.340	4.81	5.922
0.19	6.594	1.42	6.489	3.00	6.302	5.00	5.847
0.30	6.590	1.51	6.483	3.20	6.278	5.20	5.769
0.41	6.577	1.60	6.474	3.40	6.243	5.40	5.687
0.50	6.565	1.71	6.466	3.60	6.210	5.60	5.537
0.59	6.562	1.81	6.444	3.80	6.171	5.80	5.303
0.70	6.549	2.00	6.429	4.00	6.131	6.00	4.907
0.80	6.544	2.19	6.406	4.20	6.095	6.20	4.275
0.92	6.534	2.40	6.383	4.40	6.038	6.40	3.787
1.10	6.522	2.60	6.358	4.60	5.985	6.60	3.574

**Table 8.** Data [*V*, *pH*] corresponding to the titration curves of ammonia in solutions of H<sub>3</sub>BO<sub>3</sub> (0.1941M) with HCl (0.077M).



**Figure 11.** Top left: ammonia titration curve ( $pH=f(V)$ ) in boric acid (0.1941M) with HCl (0.077M). Top right: conventional Gran method. Middle: proposed linearization method as a function of  $pK_{1,b}$  supposed. Bottom:  $s_{(y/x)}$  values as a function of  $pK_{1,b}$  supposed.

the last 9 points prior to the equivalence point. However, the great contribution of this method is the complete linearization.

**Figure 11** (middle) shows the plots obtained for different supposed values of  $pK_{1,b}$ , in the range from 8.20 to 9.24. The best straight line corresponds to a value of  $pK_{1,b}$  around 8.279, as shown in the bottom of **Figures 11** and **12**.

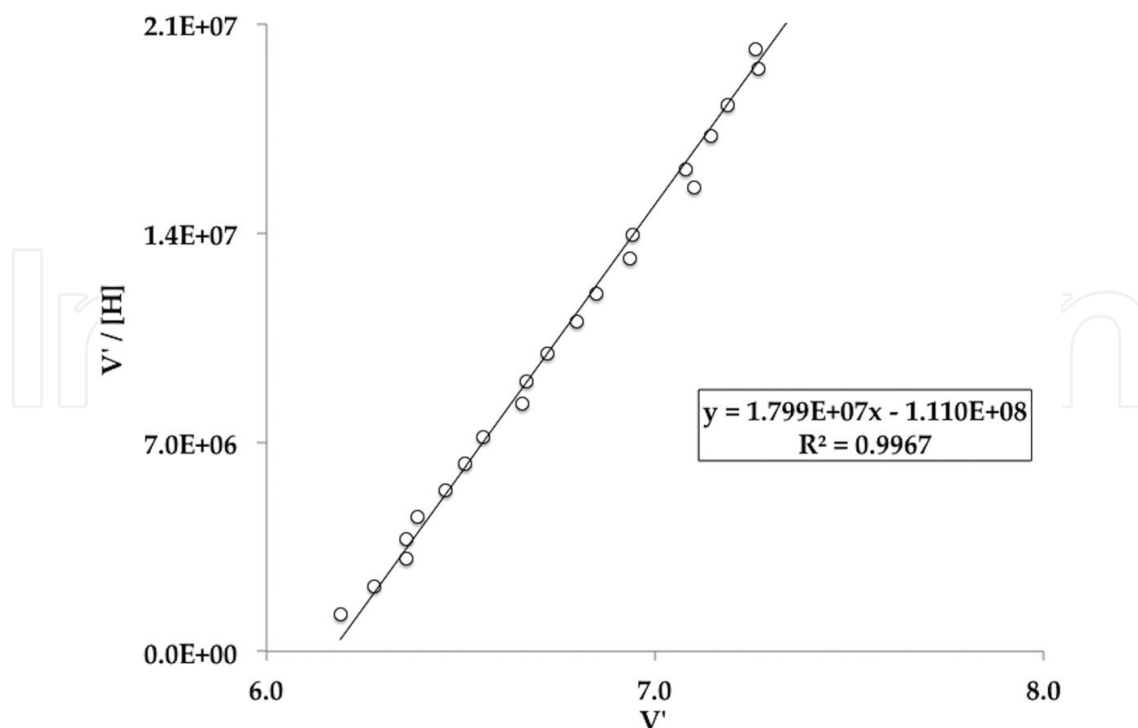


Figure 12. Proposed linearization method in the titration of ammonia in  $[H_3BO_3]=0.1941$  with HCl (0.077M).

## 11. Conclusions

The knowledge on the mathematical approach involved in the titration curves is very useful to check the validity of the procedure. A thoroughly theoretical study of the boric acid version of the titrimetric finish of the important Kjeldahl method has been carried out in this book chapter.

In order to locate the equivalence point of the ammonia-boric acid titration, an extension of the method of Schwartz has been devised. Though the method is non-approximate, it requires the knowledge of the acidity constant of the boric acid, which may be calculated by a trial and error procedure (minimization of the standard deviation of the regression line). Unlike the differential method, the proposed method makes use of all the experimental data, so no preliminary knowledge concerning the end point is needed. The method has proved very accurate when applied to synthetic titration curves and data, and in order to check its utility, it has been applied to the experimental data recently reported by Cruz [68]. In addition, titration data have been obtained in the laboratory and processed consequently, with good results.

The study of experimental ammonia-boric acid systems titrated with hydrochloric acid allows us to extract interesting conclusions. First, the behavior of experimental systems under study is far from the expected one on the basis of the theoretical model. This difference will be greater as the concentration of boric acid used as trapping agent for ammonia increases. Second, the proposed method works well so that it allows the straight line model fits properly the experimental data and leads to a reliable equivalence end point value by using a minimization criteria as indicated above. However, the parameter varied is empirical, without any physical

significance. Given the complexity of the systems with polynuclear borate species being present at high boric acid concentration, it tends to disappear by diluting the solution. It would be interesting on this respect to assay another weak acid other than boric acid as trapping agent, thus avoiding the concurrent equilibria of polyborate polymer species.

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