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Simplified spreadsheet approach for investigating polyprotic acid equilibria

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Abstract

Background and objective: Understanding how polyprotic acids behave is critical in various fields, including chemistry, biology, and medicine. Elucidation of the behavior of polyprotic acids through utilization of alpha functions, which provide insightful graphical representations of species distribution with varying pH levels, has been considered. The current paper offers an in-depth exploration of alpha functions and their significance in portraying the proportions of ionized and non-ionized species, a crucial aspect in predicting acid behavior, buffering capacity, and identifying optimal pH conditions.

Materials and methods: The Microsoft Excel and its Solver tool to explore polyprotic acid equilibria have been utilized. Synthetic datasets were generated using Excel spreadsheets, while Solver facilitated non-linear curve fitting for parameter optimization.

Results and conclusion: A novel contribution of this paper lies in introducing Excel-based tools that facilitate the generation of alpha function plots. These tools simplify the process of visualizing species distribution and pave the way for more advanced analyses. Additionally, the paper showcases the utilization of Excel Solver for robust nonlinear curve fitting of dissociation constants. This advanced computational technique enhances the accuracy of dissociation constant determination, ensuring more reliable predictions. An intriguing aspect of the proposed method is its ability to exhibit robustness even when the total acid concentration is unknown. This characteristic sets the approach apart, overcoming a common challenge in acid-base studies and analytical chemistry. The ability to accurately determine dissociation constants in such circumstances further underscores the practicality and relevance of the method. The implications of this research extend beyond the realm of theoretical understanding. The practical applications of the findings are particularly noteworthy, with a significant impact on pharmaceutical industries. The insights gained from alpha function analysis can be instrumental in drug formulation processes and in optimizing the solubility of pharmaceutical compounds. These applications can potentially enhance drug efficacy, patient experience, and overall healthcare outcomes.

Keywords: Acid dissociation constants, Alpha functions, Nonlinear curve fitting, Polyprotic acids, Robustness, Species distribution plots

1. Introduction

The fundamental inquiry into acid dissociation constants lies within the intricate realm of chemistry, where the interplay of molecular entities orchestrates the ballet of matter. These constants, encapsulating the propensity of acids to relinquish protons and engage in transformative chemical reactions, constitute a cornerstone of understanding

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phenomena that span molecular dynamics, biological processes, and beyond. Their precise determination transcends the bounds of the laboratory, resonating across a panorama of scientific disciplines, catalyzing advancements in materials science, pharmaceuticals, biochemistry, and environmental studies [1,2].

Deciphering acid dissociation constants traces a trajectory punctuated by innovative methodologies and technological advances. From the foundational titration methods introduced by Arrhenius to the sophisticated potentiometric techniques embraced in contemporary science, researchers have pursued diverse avenues to unravel the equilibrium constants governing acid-base interactions. These constants underlie the structural integrity of biomolecules, the reactivity of organic and inorganic compounds, and the buffering capacity intrinsic to physiological systems [3,4].

However, amidst the ongoing technological evolution, the demand for accessible and streamlined methodologies has become increasingly pronounced. This is particularly evident when confronting the intricate conundrums posed by multiprotonic acids. These molecular entities, characterized by multiple sites of proton donation, defy simplistic classical models, necessitating novel strategies that bridge theoretical constructs with empirical realities.

In response to this pressing demand, the present research embarks on a transformative journey that seamlessly unites the domains of experimental chemistry and computational tools. At its heart lies an innovative strategy that capitalizes on the pervasive Microsoft Excel spreadsheet, an instrument synonymous with data manipulation, extending its reach into the domain of acid dissociation constants. Through an orchestrated interplay of linear signals- absorption and fluorescence- the approach metamorphoses pH-dependent measurements into a conduit for unveiling the enigmas concealed within multi-protonic acids [5].

Moreover, the research eschews conventional paths of arduous optimization and embraces the formidable capabilities of Excel's Solver feature. This mathematical powerhouse synergizes empirical data with computational finesse, making determining acid dissociation constants a remarkably approachable pursuit. This method circumvents the intricacies of manual optimization, charting a direct route to accurate parameter estimation.

As the exploration into acid dissociation constants unfolds through this novel methodology, this paper transcends a mere presentation of technique; it signifies a paradigm shift in perceiving and surmounting the intricacies of chemical equilibria. By elucidating the significance of this research within the broader tapestry of scientific progress, a bridge emerges between the specific inquiry at hand and the overarching narrative of scientific exploration. The audience is invited to embark on a transformative journey, where empirical observations and computational dexterity converge to unlock the concealed narratives within the delicate interplay of protons and molecular entities.

2. Materials and methods

An Excel-based approach was employed to study polyprotic acid equilibria and determine dissociation constants. Leveraging Microsoft Excel's spreadsheet capabilities, synthetic datasets were generated to simulate pH-dependent signal changes. The Solver tool within Excel facilitated parameter optimization and non-linear curve fitting by adjusting pKa values and molar absorptivity values. This user-friendly method is complemented by supplementary Excel spreadsheets, enhancing reproducibility and accessibility for researchers aiming to investigate complex acid equilibria.

3. Results and discussion

3.1. Polyprotic dissociation equilibria

The concentrations of various species resulting from the dissociation of a polyprotic acid invariably adhere to equilibrium laws:

$H_nA <==> H^+ + H_{n-1}A^-$

$K_{a1} = [H_{n-1}A^-]\,[H^+] / [H_nA]$

$H_{n-1}A^- <==> H^+ + H_{n-2}A^{-2}$
$K_{a2} = [H_{n-2}A^{-2}]\,[H^+] / [H_{n-1}A^-]$
(1)
…
$HA^{-(n-1)} <==> H^+ + A^{-n}$
$K_{an} = [A^{-n}]\,[H^+] / [HA^{-(n-1)}]$

Different species of acid are present in a solution containing a polyprotic acid, wherein the fraction of the total acid concentration attributed to a specific species becomes a function of pH. In essence, at each pH, the various acid forms constitute a fraction of the overall concentration, their sum equaling unity:

$a_0 = [H_nA] / C_t$
$a_1 = [H_{n-1}A^{-1}] / C_t$
…
(2)
$a_{n-1} = [A^{-n}] / C_t$
where:
$C_t = [H_nA] + [H_{n-1}A^{-1}] + \ldots + [A^{-n}]$

By merging these equations, cohesive and structured functions emerge, allowing the distribution of diverse acid species as a function of pH to be examined and observed:

$a_0 = [H^+]^n / D$
$a_1 = K_{a1}[H^+]^{n-1} / D$
$a_2 = K_{a1}\,K_{a2}[H^+]^{n-2} / D$
…
(3)
$a_n = K_{a1}\,K_{a2}\ldots K_{an} / D$
and
$D = [H^+]^n + K_{a1}[H^+]^{n-1} + K_{a1}\,K_{a2}[H^+]^{n-2} + \ldots + K_{a1}\,K_{a2}\ldots K_{an}$ (4)

These equations elegantly reveal that for a specific acid possessing its distinct acid dissociation constants, the equilibrium distribution of species becomes solely a function of pH. Consequently, the recent equations aptly suit the task of plotting the distribution curves of species. Moreover, the equilibrium concentrations of all species can be computed at any given pH using these equations:

$[H_nA] = C_t\,a_0$
$[H_{n-1}A^{-1}] = C_t\,a_1$
….
(5)
$[A^{-n}] = C_t\,a_n$

A remarkably straightforward and concurrently practical procedure emerges one that harnesses software tools like *Excel* for determining acid dissociation constants of polyprotic acids based on the following equation:

$A_i = e_{HnA}\,[H_nA] + e_{Hn-1A}\,[H_{n-1}A^-] + \ldots + e_{A-n}\,[A^{-n}]$
(6)

In this equation, A_i represents the measured absorbance at the pH measurement, while $\mathbf{e_{HnA}}$, $\mathbf{e_{Hn-1A}}$, ..., and $\mathbf{e_{A-n}}$ are the molar absorption coefficients for each respective acid species. Through a simple procedure, when the absorbance of a solution with concentration $\mathbf{C_t}$ of a polyprotic acid H_nA is measured at varying pH values, absorbance values across the appropriate wavelength range at different pH values are obtained. Equilibrium concentrations in equation (6) conform to the equations mentioned earlier. Hence, fitting the absorbance changes relative to pH at a wavelength can lead to the determination of acid dissociation constants and the molar absorption coefficients of all species.

3.2. Alpha functions: exploring polyprotic acids

Alpha functions, also known as species distribution plots or distribution diagrams, are graphical representations that provide insights into the distribution of different chemical species of a polyprotic acid as a function of pH. These plots are especially useful for understanding how the relative concentrations of various ionized and non-ionized forms of the acid change with changes in pH. They play a vital role in elucidating the behavior of complex acid-base equilibria involving acids with multiple ionizable hydrogen ions [6].

In an alpha function plot, the x-axis represents the pH, while the y-axis represents each acid species' fraction (α) at a given pH. The sum of all fractions for a particular pH value is always equal to 1, reflecting the law of conservation of mass. Each curve on the alpha function plot corresponds to a different ionized acid form.

The significance and practical application of alpha functions or species distribution plots are as follows:

- *Visual Representation:* Alpha functions provide a clear and visual representation of how the proportions of different species change with pH. This visual insight helps researchers and chemists understand the dominant species in a solution at various pH values.
- *Predicting Behavior:* Alpha functions allow chemists to predict the behavior of the acid under different conditions. By observing the shifts in species distribution curves, chemists can anticipate how changes in pH might affect the acid's properties, reactivity, and interactions.
- *Buffering Capacity:* Alpha functions are essential for understanding the buffering capacity of solutions containing polyprotic acids. Buffer solutions are resistant to changes in pH, and alpha function plots help identify the pH range over which the buffer capacity is most effective.
- *Complex Equilibria*: For acids with more than two ionizable hydrogen ions, species distribution becomes more intricate. Alpha functions help unravel the complexity by showing how each additional proton dissociation impacts the species distribution.
- *Optimal Conditions:* Alpha functions aid in identifying the optimal pH conditions for specific chemical reactions or experimental procedures involving polyprotic acids. For instance, researchers can use these plots to determine the pH range where a specific acid form predominates.
- *Analytical Chemistry:* In analytical chemistry, alpha function plots are crucial for determining the optimal conditions for titrations, chromatography, and other techniques where pH plays a significant role in separation or analysis.
- *Teaching and Learning*: Alpha function plots are educational tools that help students understand acid-base equilibria and the concept of species distribution. They provide a practical and intuitive way to grasp complex chemical concepts.

In the pharmaceutical field, the impact of alpha functions is particularly pronounced:

- *Formulation Optimization*: Alpha function plots guide the optimization of drug formulations. They predict how pH variations impact species distribution, aiding in formulating drugs that maximize stability and bioavailability under specific physiological conditions.
- *Solubility and Dissolution*: Alpha functions assist in identifying the pH range where a drug's solubility is optimal, guiding the design of drug delivery systems that enhance therapeutic efficacy.
- *pH-Dependent Absorption*: Many drugs exhibit pH-dependent absorption. Alpha functions help understand how pH variations affect a drug's ionization state and absorption profile.
- *Buffering Effects*: Alpha functions help discern the pH range where buffering effects are prominent in drugs formulated with polyprotic acids.
- *Clinical Relevance*: Alpha functions help predict the physiological conditions under which drugs are effective, aiding in clinical decision-making.
- *Dosage Form Development*: Alpha functions guide the selection of pH conditions for oral dosage forms, ensuring desired drug release kinetics.
- *Pharmacokinetics and Pharmacodynamics*: Alpha functions predict drug behavior, optimizing dosing regimens and therapeutic outcomes.

Therefore, alpha functions or species distribution plots are indispensable for comprehending polyprotic acid behavior. Their applications extend to optimizing drug formulations, predicting drug behavior in physiological systems, and informing dosage form development. The intricate interplay of pH and species distribution, unveiled through alpha functions, empowers scientists to make informed decisions impacting drug stability, efficacy, and patient outcomes.

Considering the significance and informational content of distribution curves for polyprotic acids and the widespread availability of Excel software, this research aimed to initially design a simple and user-friendly spreadsheet for plotting these diagrams. The corresponding Excel file is freely available in the ***Supplementary Information*** of this paper. Figure 1 illustrates the extensive "Distribution" spreadsheet designed to draw distribution diagrams for uni-protic to hexa-protic acids. In cell **N1**, the user should input the capacity or, in other words, the number of dissociable protons of the acid. For instance, in the provided example, n=6 is considered.

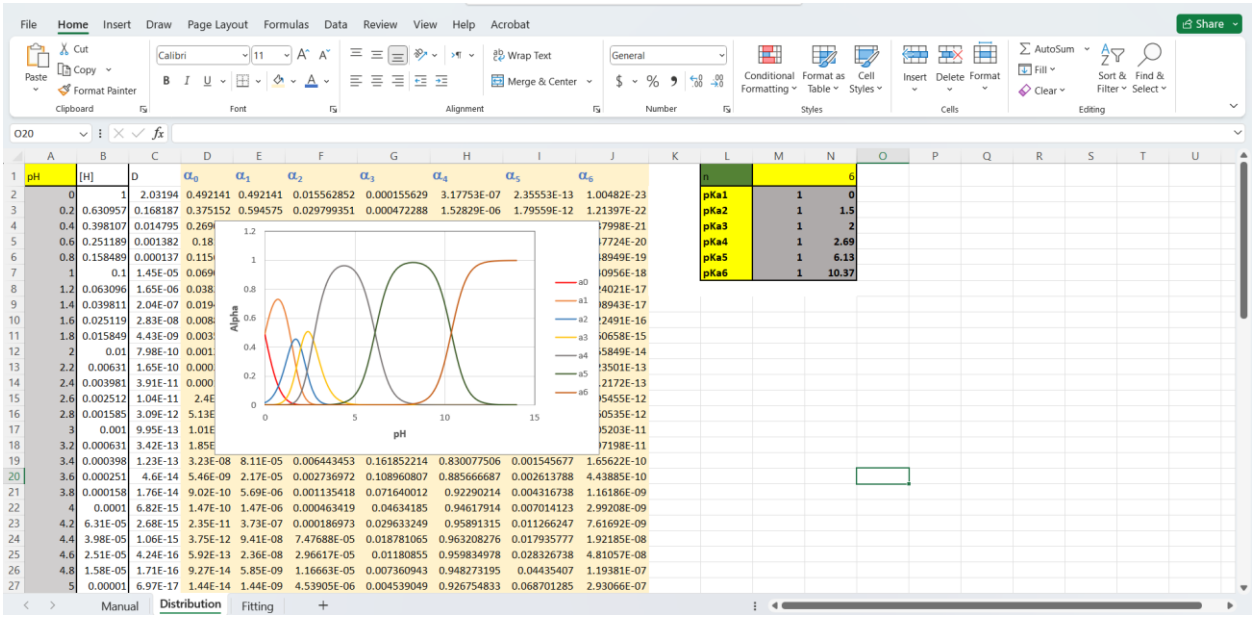

Figure 1- Designed “Distribution” spreadsheet for calculating and drawing the species distribution of EDTA as a hexa-protic acid

As expressed in the set of equations (2), having acid dissociation constants is the only prerequisite for calculating alpha functions. Thus, in this spreadsheet (“Distribution”), the user needs to specify the presence (1) or absence (0) of the corresponding acid constants by selecting options 1 or 0 in cells **M2** to **M7**. In Figure 1, for an acid H_6A, all options in these cells are set to active (1).

Next, desired values for the acid pK_a's are entered in cells **N2** to **N7** for the active options. Particularly, Figure 1 showcases the utilization of the "Distribution" spreadsheet for the EDTA ligand.

Ethylenediaminetetraacetic acid (EDTA), recognized for its six ionizable hydrogen ions, presents a fascinating polyprotic acid with distinct characteristics. Its structure encompasses four carboxylates (COO^-) and two amines (NH_2) functional groups. EDTA's equilibrium distribution curve of acidic species, governed by these functional groups, showcases how its protonation and deprotonation events transform with changing pH. The carboxylate groups' deprotonation leads to negatively charged forms, influencing the curve's shape. The amine groups, able to accept protons, contribute to pH-dependent changes in species distribution [7].

Understanding EDTA's distribution curve is pivotal for harnessing its chelating properties. This knowledge guides its application in diverse fields, from analytical chemistry to pharmaceuticals. By aligning with the curve's trends, scientists can optimize EDTA's behavior based on pH, unlocking its potential across various scientific endeavors.

As another example, let's delve into the species distribution curve of Histidine. Histidine, recognized

as a three-protonic acid due to its three ionizable functional groups, presents intriguing polyprotic behavior. With both a carboxyl group (COOH) and two imidazole groups ($C_3H_4N_2$) in its side chain, Histidine undergoes sequential protonation and deprotonation reactions as the pH of its environment changes. This leads to the formation of distinct acidic species.

The distribution of these acidic species can be visualized through an acidic species distribution diagram. This diagram plots the proportions of Histidine's different forms as a function of pH. As the pH varies, the imidazole and carboxyl groups experience protonation or deprotonation, resulting in the prevalence of specific species at different pH levels [8,9]. At lower pH values, both imidazole and carboxyl groups are more likely to be protonated, leading to a higher concentration of species like "$HisH_3^+$" and "$HisH_2^+$." As the pH increases, the imidazole groups gradually lose protons, resulting in a shift towards the neutral "His" form, and the carboxyl group becomes deprotonated, contributing to the formation of "$HisHCOO^-$."

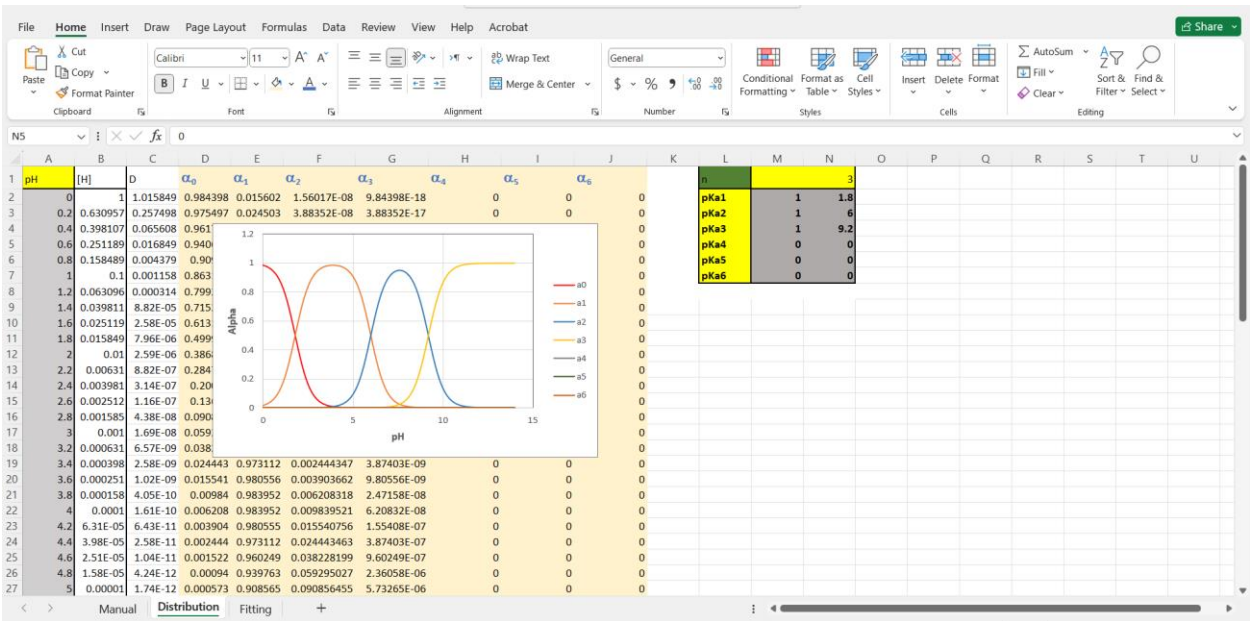

Figure 2- Designed “Distribution” spreadsheet for calculating and drawing the species distribution of Histidine as a tri-protic acid

Understanding the acidic species distribution diagram of Histidine provides insights into its behavior within biological systems and chemical reactions. In biochemistry, Histidine plays a pivotal role in enzyme catalysis, metal binding, and proton shuttle processes. The diagram allows scientists to predict how Histidine's various forms will dominate under different pH conditions, aiding interpreting its functional roles in biological and chemical contexts. In Figure 2, the "Distribution" spreadsheet has been utilized for Histidine as a three-protic acid. As observed, simple changes corresponding to the pK_a values of this amino acid result in the plotting of the species distribution curve.

3.3. Non-linear curve fitting for calculation the acidic dissociation equilibrium constants

Determining acidic dissociation equilibrium constants stands as a cornerstone in chemistry, underpinning our comprehension of molecular behavior and chemical interactions. These constants illuminate the intricate dance of protons, providing essential insights into the reactivity, solubility, and binding characteristics of molecules. A profound understanding of these cons-

tants is imperative across various domains, from elucidating the behavior of biomolecules to optimizing chemical processes in industries such as pharmaceuticals and materials science [5,10].

In pharmaceutics, the knowledge of pKa values holds remarkable significance. These values guide drug formulation, aiding in the design of pharmaceutical compounds with desirable solubility and bioavailability profiles. A drug's solubility, a critical factor affecting its absorption and effectiveness, is greatly influenced by its ionization state, which in turn is dictated by the pKa values of its functional groups. Accurate determination of pKa values enables formulation scientists to tailor drug molecules to optimal pH conditions, ensuring efficient absorption and targeted therapeutic effects. Furthermore, pKa values aid in predicting drug-drug interactions and understanding the behavior of drugs within various physiological environments.

Nonlinear curve fitting is a pivotal tool in determining pKa values, enabling the extraction of these constants from complex experimental data. By employing sophisticated mathematical models to fit the nonlinear protonation equilibria, scientists can unveil the characteristic acidic behavior of molecules and the corresponding shifts in ionization states with changing pH. This knowledge empowers pharmaceutical researchers to make informed decisions in drug development, formulation, and optimization, creating safer and more effective medications.

3.4. Excel solver: a versatile optimization tool

The procedure proposed for determining polyprotic acid's acid dissociation equilibrium constants is straightforward, practical, and easily adaptable using the designed "Fitting" spreadsheet. Samples of polyprotic acids with a total concentration Ct are prepared within a range of pH values, resulting in changes in the signal. For each sample with a specified pH, its signal is measured. The measured signal can be spectroscopic signals such as absorbance or fluorescence at a specific wavelength or any other instrumental signal that exhibits a linear relationship with the equilibrium concentration of at least one acid species. In this case, the absorbance signal is considered as an example. Therefore, two corresponding numeric sequences are obtained for each of the N samples: the pH numeric sequence in column A under the header "pH" and the measured absorption numeric sequence in column B under the header "A_{meas}" in the spreadsheet.

Solver, a robust tool within Excel, is widely acknowledged for its prowess in optimization and solving mathematical equations. This section delves into its specific application in nonlinear curve fitting. It highlights how Solver can adeptly ascertain optimal parameters for nonlinear models, particularly when modeling polyprotic acid dissociation equilibria [11].

The intricate realm of nonlinear curve fitting involves discerning the parameters of a mathematical model that effectively captures experimental observations. The polyprotic acid dissociation equilibria model is a combination of equations 5 and 6. Here, Solver takes center stage by iteratively refining model parameters (pKa values and molar absorptivity of the spectroscopically active species) to minimize the gap between model predictions and empirical data points. This iterative process hinges on minimizing a predefined objective function, often represented by the sum of squared differences between model predictions and actual measurements.

Utilizing solver for nonlinear curve fitting with polyprotic acid dissociation equilibria can be briefly explain as:

- *Model Formulation*: The foundation lies in defining a mathematical model that encapsulates the intricate behavior of polyprotic acid dissociation equilibria, harmonizing with empirical data patterns (equation 6, formulated in the corresponding cells of column C in “Fitting” spreadsheet).
- *Objective Function*: The crux is creating of an objective function that quantifies the deviation between model predictions and real data points. Typically, this function reflects the summation of squared residuals (formulated in cell G17).
- *Parameter Adjustment*: Distinct parameters of the

chosen model are identified for optimization using Solver (cells H2 to H7 the pKa values which are activated by selecting option 1 in cells G2 to G7, and also optional selected molar absorptivity values in the cells H8 to H14). These parameters undergo dynamic adjustment by Solver to minimize the designated objective function (cell G17).

- *Constraints (Optional)*: Where applicable, constraints on parameter values can be introduced to align with specific conditions. Applying the non-negativity constraint for fitted pKa and molar absorptivity values).

- *Applying Solver*: The journey commences as Solver is launched, with the objective function designated for minimization (cell G17). Parameters selected for adjustment are indicated, constraints defined, and Solver set into motion to optimize these parameters.

- *Iterative Solution*: Solver embarks on an iterative quest, navigating through an array of parameter values to minimize the objective function. Upon convergence, Solver furnishes the optimized parameter values that yield the most accurate data fit.

Solver's aptitude in nonlinear curve fitting, particularly concerning polyprotic acid dissociation equilibria, transcends mere computational utility. It empowers researchers to delineate the intricate equilibrium distributions of acidic species, shedding light on proton-donor tendencies. This tool bridges theoretical predictions rooted in the equilibrium constants and experimental observations marked by pH-dependent variations.

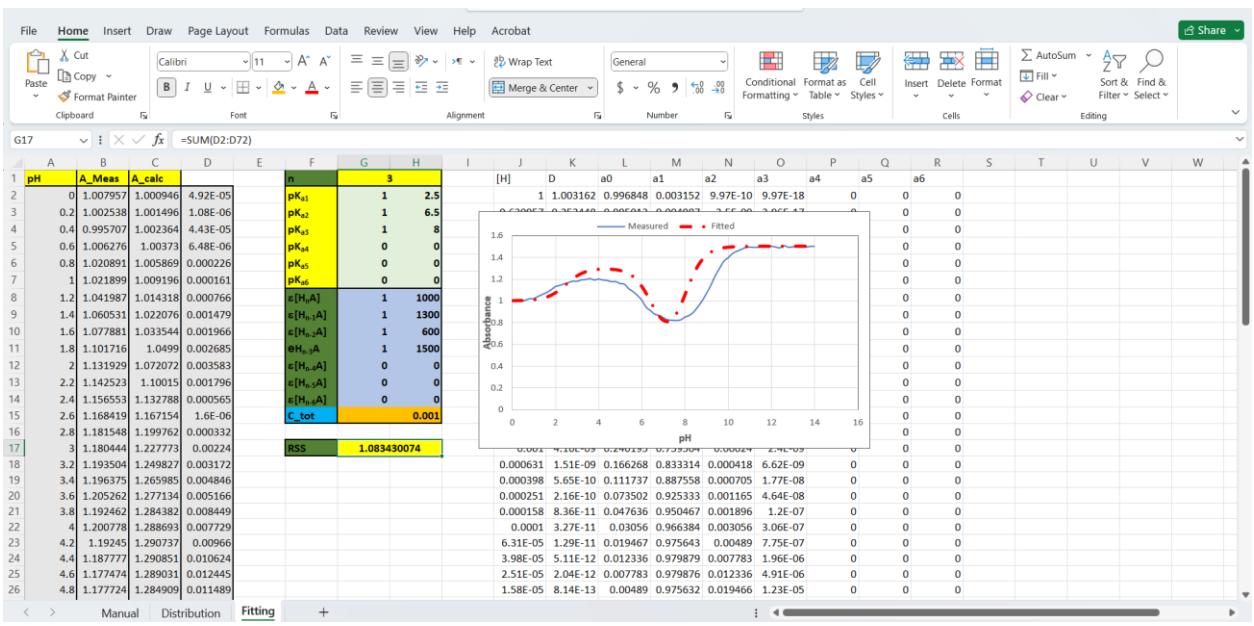

Figure 3- Designed “Fitting” spreadsheet for calculating the acid dissociation equilibrium constants of a triprotic acid; Initial estimates.

Figure 3 displays the designed "Fitting" spreadsheet for determining a three-protonic acid's acid dissociation equilibrium constants. Simulated data has been considered based on the acid dissociation constants of Histidine, with its species distribution curve shown in Figure 2. pH values and measured absorbances are entered into columns A and B, respectively. The values of pKa1, pKa2, and pKa3 need to be calculated. Initial estimates for these three constants should be entered in cells H2, H3, and H4. The closer these initial estimates are to the actual values, the faster the convergence of Solver optimization occurs. As seen in Figure 3, the initial estimates have been assumed as 2.5, 6.5, and 8. The

graph depicting the changes in absorbance with respect to pH provides preliminary insights into the spectroscopic active species, which contribute to the measured signal. From this absorbance curve about pH, it can be inferred that all four acid species under consideration exhibit absorbance at the selected wavelength. Given that in highly acidic and highly basic pH ranges, all the acid species exist in the forms of H3A and A-3, respectively, the molar absorption coefficients of these species can be calculated by knowing Ct and the measured absorbance. Therefore, only two molar absorptivity values need to be considered as additional fitting parameters, and their initial estimates should be entered in cells H9 and H10. The value of Ct is set to 0.001 M in cell H15. The observed difference between the calculated and measured absorbance curves at this stage indicates that the model parameters are not yet adjusted, a task managed by Excel's Solver. The Residual Sum of Squares (RSS), a measure of the difference between measured and calculated absorptions, is indicated in cell G17.

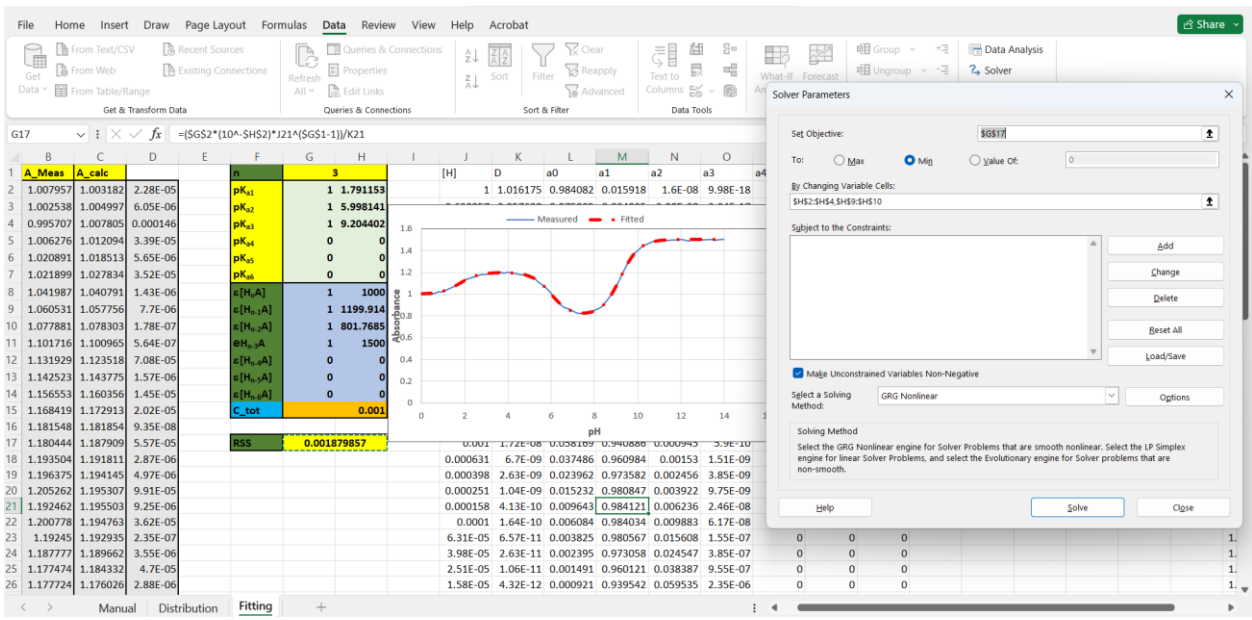

Figure 4- Designed "Fitting" spreadsheet for calculating the acid dissociation equilibrium constants of a triprotic acid; Adjustment of solver parameters and fitting.

Figure 4 demonstrates the execution of Excel Solver for fitting the data related to the proposed practical procedure, aimed at determining pK_{a1}, pK_{a2}, and pK_{a3}, as well as the molar absorptivity values of the species H_2A^- and HA^{-2}. The adjustment of these parameters is shown. The comparison between the final results of the Excel Solver's execution and the initial estimates, as depicted in Figure 3, illustrates a successful fitting process. The RSS has minimized and is indicated in cell G17. The absorbance curves relating to the changes in A with respect to pH, both calculated and measured, exhibit close agreement. In this scenario, the acid dissociation equilibrium constants and molar absorptivity values have been fitted and replaced in their respective cells by Solver optimization.

3.5. Robust determination of polyprotic acid dissociation constants

Various designs can be considered for studying equilibrium systems. The common feature of all these designs is that the equilibrium situation must be perturbed to extract relevant thermodynamic information from the system. A proper experimental procedure should cover the subspace of species

involved in equilibrium to exploit suitable thermodynamic information. Monitoring changes in the concentrations of equilibrium species as a function of pH in polyprotic acid systems can create a robust procedure for determining dissociation constants by covering the equilibrium space. The chemical model fitted in this procedure eliminates the need to know the polyprotic acid's total concentration. This feature can be highly significant in the thermodynamic study of polyprotic acids.

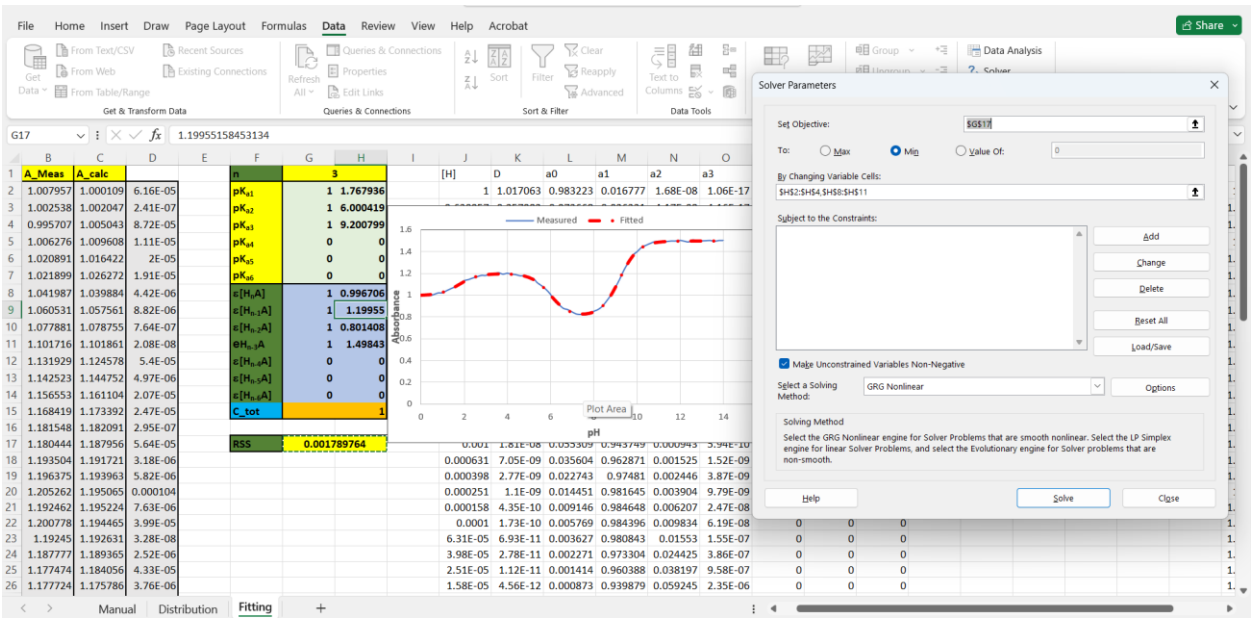

Figure 5- Designed "Fitting" spreadsheet for calculating the acid dissociation equilibrium constants of a tri-protic acid where the total concentration is arbitrary.

The "Fitting" spreadsheet can showcase this unique feature of the proposed method for determining dissociation constants of polyprotic acids. Let us assume that due to the investigated polyprotic acid's low solubility or impurity, it is impossible to accurately determine the actual value of Ct, or the determined value is inaccurate. In such cases, an incorrect value of Ct will be entered into the model. The interesting point is that the accuracy of the fitted acid constants is independent of the error in Ct. Therefore, applying the proposed method, makes it possible to consistently obtain accurate acid constants without having precise information about the total concentration. Repeating the fitting of the tri-protic acid shown in Figure 4 for an incorrect Ct value entered in Cell G15 (while the actual concentration is 0.001, a value of 1 has been entered, i.e., a thousand times the actual value) demonstrates that the accuracy of pKa values remains unaffected by it. The results of fitting by Excel solver have been shown in Figure 5.

The equilibrium constants remain accurate within the precision range of the method, even with a thousand-fold error in the total concentration value. However, as observed in the fitting results in Figure 5, the molar absorptivity values have been obtained in a perfectly linear proportion to the value of Ct, meaning that these coefficients are approximately one-thousandth of the calculated actual values. In cases where the total acid concentration is not available, the molar absorptivity values cannot be known, and all these coefficient values must be entered as unknown parameters into the fitting process.

4. Conclusion

In conclusion, providing simple, reliable, and robust

methods for studying acid dissociation equilibria in multi-proton acid systems holds paramount significance across various scientific disciplines, such as chemistry and pharmaceutics. In this study, we introduced an Excel-based approach for calculating and plotting distribution diagrams of various species in a polyprotic acid. Additionally, a straightforward procedure, utilizing Excel Solver, was presented alongside a spreadsheet for fitting experimentally measured data of a polyprotic acid under constant concentration and varying pH conditions. The proposed method allows for accurately determining acid dissociation constants even when the total acid concentration is not precisely known. The user-friendly design of the spreadsheets facilitates the generation of species distribution diagrams and data fitting for the polyprotic acid dissociation model. These spreadsheets are provided as supplementary information, enabling easy access and utilization. Integrating these methods into research and experimentation provides a robust toolkit for studying complex equilibrium systems and advances our understanding of acid dissociation phenomena in various scientific applications.

5. Conflict of interest

The author has no conflict of interest.

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