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Ionization constants (pKa) in pharmaceutical chemistry: Experimental and computational determination in drug discovery and development

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Abstract

The ionization constant (pKa) is a major factor in the determination of the chemical and physical properties of drug molecules, which, in turn, affect their distribution, absorption and interaction with the biological targets as well as their pharmacokinetic fate. The present review is an attempt to shed light on the pKa significance in the contemporary drug discovery and medicinal chemistry, pointing its use in rational molecular design and lead optimization. This paper critiques both the experimental and computed methods of pKa determination. It focuses on the key analytical techniques, particularly, UV-visible spectroscopy and NMR spectroscopy. Furthermore, it reviews the recent advances in the in silico prediction platforms and software that are used for high-throughput pKa estimation in the modern screening pipeline. The exponentially increasing number of chemical libraries for screening has made the computational pKa prediction methods indispensable, especially in the early drug discovery phase, where they offer scalable and cost-efficient solutions. It is the accuracy of the experimental validation that still plays a significant role, particularly when it comes to structurally complex molecules. The NMR spectroscopy adds unique advantages to the resolution of individual ionization states in the multi-functional systems, as demonstrated by the case of aminoglycoside antibiotics. The pKa accurate determination and prediction is the basis for drug-like properties optimization and for the compounds' smooth transition throughout the stages of discovery and development. The combination of computational and experimental approaches results in the most reliable ionization profiles. The understanding of pKa behavior allows medicinal chemists to create more effective and less toxic drug candidates by the optimization of solubility, permeability, and target interactions. The use of advanced analytical and computational tools supports efficient compound triaging, accelerating the development of therapeutically relevant molecules.

Keywords: Drug development and discovery, Ionization constant (pKa), Pharmaceutical medicinal chemistry, pKa measurement techniques.

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1. General Introduction to Ionization Constants (pK_a)

Ionization constants (the pK_a of the conjugate acid) are among the most frequently studied physicochemical parameters and give considerable information about the physical and kinetic behaviour of chemical substances. pK_a is defined as the pH at which functional groups, such as amino groups and carboxylic groups, exist as 50% ionized and 50% un-ionized. The first concept of the dissociation constant comes from the law of mass action, provided by Ferner and Aronson [1]. The concept was further strengthened by the works of Reijenga, et al. [2]. pK_a is expressed as the following:

$$pK_a = -\log K_a$$

In this equation, K_a is the dissociation constant of the reaction. The dissociation of an acid in a solvent, such as water, is a reversible process proceeding in both forwards and backwards directions; the more dissociation, the stronger an acid is. The extent of the dissociation is measured in terms of K_a [2].

$$K_a = [H_3O^+]\,[A^-] / [HA]$$

K_a is the ratio of dissociated and un-dissociated acid in a solution and thus measures the strength of an acid in a solution. The higher K_a, the stronger the acid is because of the higher dissociation, in other words, the lower the pK_a, the stronger an acid, as shown in Figure 1 [3]. The main advantage of using pK_a instead of K_a is convenience. Due to the use of a logarithmic scale, a very large range of concentrations is covered in a convenient way. Use of this scale also has the advantage of converting very small values into easily tangible digits [2].

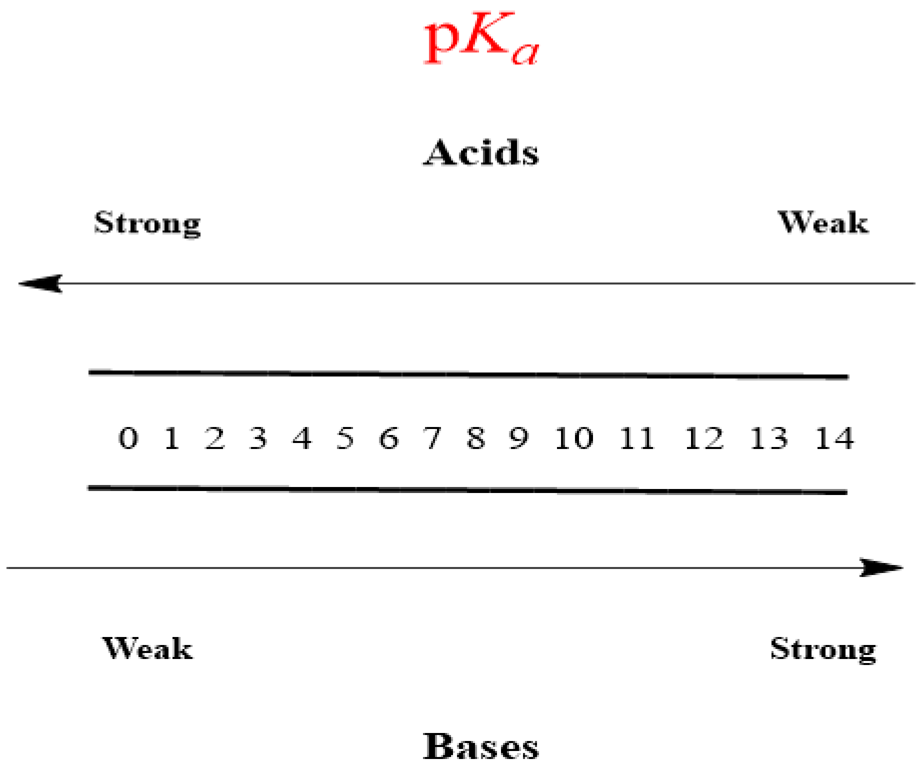

Figure 1.
The smaller the pK_a, value, the stronger an acid, the greater the pK_a, value the weaker an acid.

2. The Significant of pK_a in Medicinal Chemistry

2.1. From an Economic Perspective

The crucial role of pK_a in medicinal chemistry, particularly from an economic perspective, is evident in its influence on drug development, formulation, and efficacy. It is now abundantly evident that pharmaceutical companies are having a difficult time despite the fact that they have increased the amount of money they spend on research and development. Extensive pre-clinical profiling has, thankfully, contributed to a reduction in the number of drug failures that were caused by poor human pharmacokinetics. On the other hand, in more recent times, the reasons for medications being removed from clinical development have been based on commercial decisions, in addition to issues with formulation and toxicity [4]. Several additional variables, including increased regulatory scrutiny and a scarcity of simple targets that are also druggable, have also played a role in the industry's decreased productivity. A few observers have even questioned whether or not the pharmaceutical sector will be able to survive in the long run [5]. For the purpose of addressing the issue of high attrition rates and in accordance with the principles of good management practice, it has become vital for businesses to conduct an analysis of the entire drug discovery process in order to effectively compete in the current environment. In addition, medicinal chemists have been actively involved in the process of understanding therapeutic failures. They have participated in this process by analyzing and identifying the physicochemical features of compounds that are predictive of successful outcomes. These analyses are being conducted with the intention of enhancing the quality of substances that are going to be tested in clinical trials; nevertheless, there is some controversy regarding the degree to which these recommendations are being used [6]. Due to the fact that there is abundant information indicating that working with big and lipophilic compounds is associated with issues affecting promiscuity, metabolism, bioavailability, efflux, solubility, and plasma protein binding, property-based optimization is of the utmost relevance to the industry [7]. The acid-base character of pharmaceuticals is of critical significance to the way in which they behave since it influences the biopharmaceutical properties of the drugs as well as the formulation of the drugs [8, 9].

2.2. In Drug Discovery

The recent rise in interest in ionization profiles is the result of a consistent analysis of a large number of physicochemical parameters having been carried out [10]. The characteristics that are associated with successful therapy outcomes will be outlined in this review, along with their correlation with acid/base properties [11, 12]. In this review, we will investigate the impact that charge state has on the interactions between drugs and receptors, as well as the pharmacokinetics of drug distribution within the body and the biopharmaceutical concerns that are relevant to possible medical applications. The identification and validation of a specific macromolecular target is often the first step in the process of discovering novel medications [13]. The performance of this action is carried out with the intention of improving the management of a certain sickness condition. It is possible to conduct tests on compounds in order to identify hits and leads that meet the criteria that have been established in advance for characteristics such as potency, functional activity, and physicochemical features [14]. Screen development makes managing this situation easier. Testing at this stage can be helpful in determining the quality of these ligands, which is necessary if oral bioavailability is required [15]. Through the ensuing process of changing a lead chemical into a pharmaceutical active compound, a multidimensional optimization of potency, selectivity, and biopharmaceutical properties is required [16]. These characteristics include absorption, distribution, metabolism, excretion, and toxicity [17-19]. With the aim of providing the most possible therapeutic benefit, this adjustment is carried out. Lipophilicity is a property of a medication that is determined by the ionization constants (pK_avalues) of important functional groups. The acid/base characteristics of a molecule have a direct impact on the lipophilicity of some medications [11]. Within the context of the interaction between the two parties, this is an essential component. Therefore, it is essential to do additional study and have a better understanding of acid/base profiles for research compounds, clinical prospects, and medications as a means to comprehend and monitor lipophilicity [20]. In general terms, there are four essential aspects of drug development that are affected by the acid/base equilibria of the particular molecule that is being considered. In addition to having a significant impact on a drug's pharmacokinetic and biopharmaceutical properties, a medication's acid/base characteristics will also have an effect on the drug's potency and selectivity as well as solubility [4, 5, 21-24].

3. Factors Affecting the pK_a Values

Contrary to the prevalent assumption that ionization constants are invariant, numerous factors indeed affect them. Additionally, the ionization constants are subject to change whenever there is a change in the length of the alkyl chain that is responsible for separating two functional groups. The inductive effect is one of the factors contributing to this change [25, 26]. At the same time as the inductive impact reduces when the distance between two functional groups rises, the contrary is also true when the distance is increased between the functional groups [27] (see Figure 2). Takeda, et al. [28] utilized ^{15}N nuclear magnetic resonance (NMR) spectroscopy in order to measure the pK_a values of spermidine [28]. This was done as a means to determine the pK_a values of the functional groups. The steric and resonance effects are likely to cause the pK_a values of the functional groups to drop. There are two examples that illustrate the ionization constants for each ionizable group. These examples include cadaverine (1,5-diaminopentane) and lysine, which is an amino acid that is chemically identical to cadaverine [29]. There are several other examples available. Lysine is composed of two amine groups that are arranged in a fashion that is analogous to that of cadaverine, in addition to three ionizable groups, one of which is carboxylic acid [30]. There are two ionizable groups in cadaverine, which are more specifically the two major amines (NH_2). The pK_a values for each ionizable group are shown in Figure 3, along with the balanced net ionic equations for both compounds. These equations are displayed with examples of compounds.

4. Measuring Ionization Constants (pKa)

Finding the ionization constants of pharmaceuticals is important in many areas, including food science, analytical chemistry, organic chemistry, medication development, and drug discovery [31-33]. An in-depth familiarity with the dissociation constants of pharmaceuticals and excipients is crucial for pharmaceutical systems since it provides information that is vital [34]. This knowledge is paramountcy necessary because a large number of medications are weak acids or bases [35]. Consequently, the chemical and biological reactivities depend on the degree of ionization of both the drug and the receptor [36]. Furthermore, the ionic characteristics of medications at varying physiological pH levels can be clarified by an understanding of ionization constants [37, 38]. When a chemical is released from an oral medication, it first comes into contact with gastric acid, the extremely acidic pH of the stomach [39, 40]. Finally, as the medicine moves through the intestines, its pH changes to something more alkaline [41, 42]. To pass the epithelial barrier in the gastrointestinal tract, drugs that become ions at the normal pH of the digestive system need to be delivered by specialized transporters [43]. One way that unionized medicines can be absorbed is through simple passive diffusion [44, 45]. In a similar line, the pH of 7.4—which is slightly alkaline or almost neutral—is what pharmacological substances face after they enter the bloodstream [46, 47]. This means that their ionization may render them less effective at penetrating the BBB [48-51]. An in-depth understanding of the dissociation constants might help medicinal chemists create new drug derivatives with improved efficacy or reduced toxicity [52]. One way to better understand the structure-activity relationships (SAR) of AGs, namely the order in which these similar functional groups acquire or give up protons, is to analyze the pK_a values of each amine present in these alkaloids [53, 54]. The sequence of target mRNA binding by major functional groups may be better understood as a result of these facts. Furthermore, the mechanisms of AG distribution and toxicity may be better understood by examining the selective interactions of different amines with AGs, which are controlled by steric effects and the ionization constants of these amines.

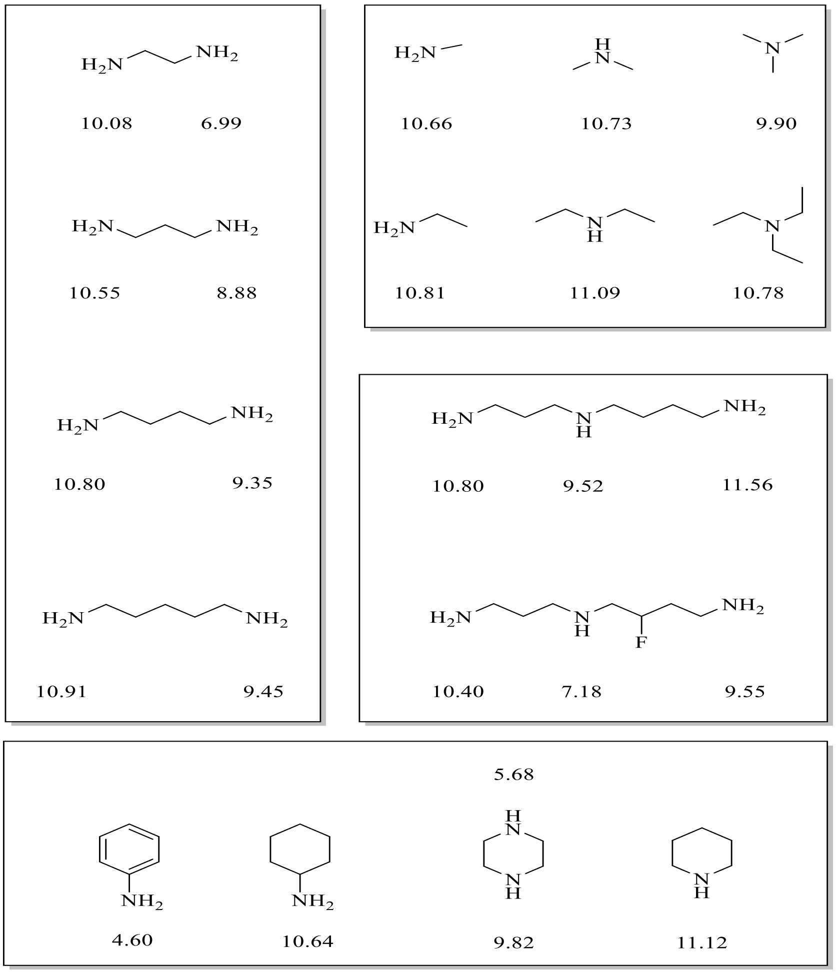

Figure 2.
The elongation of the alkyl chain leads to increased pK_a values of amines, on another hand, steric and resonance effects may reduce the pK_a values of amino groups in H_2O.
Source: Morgenthaler, et al. [55].

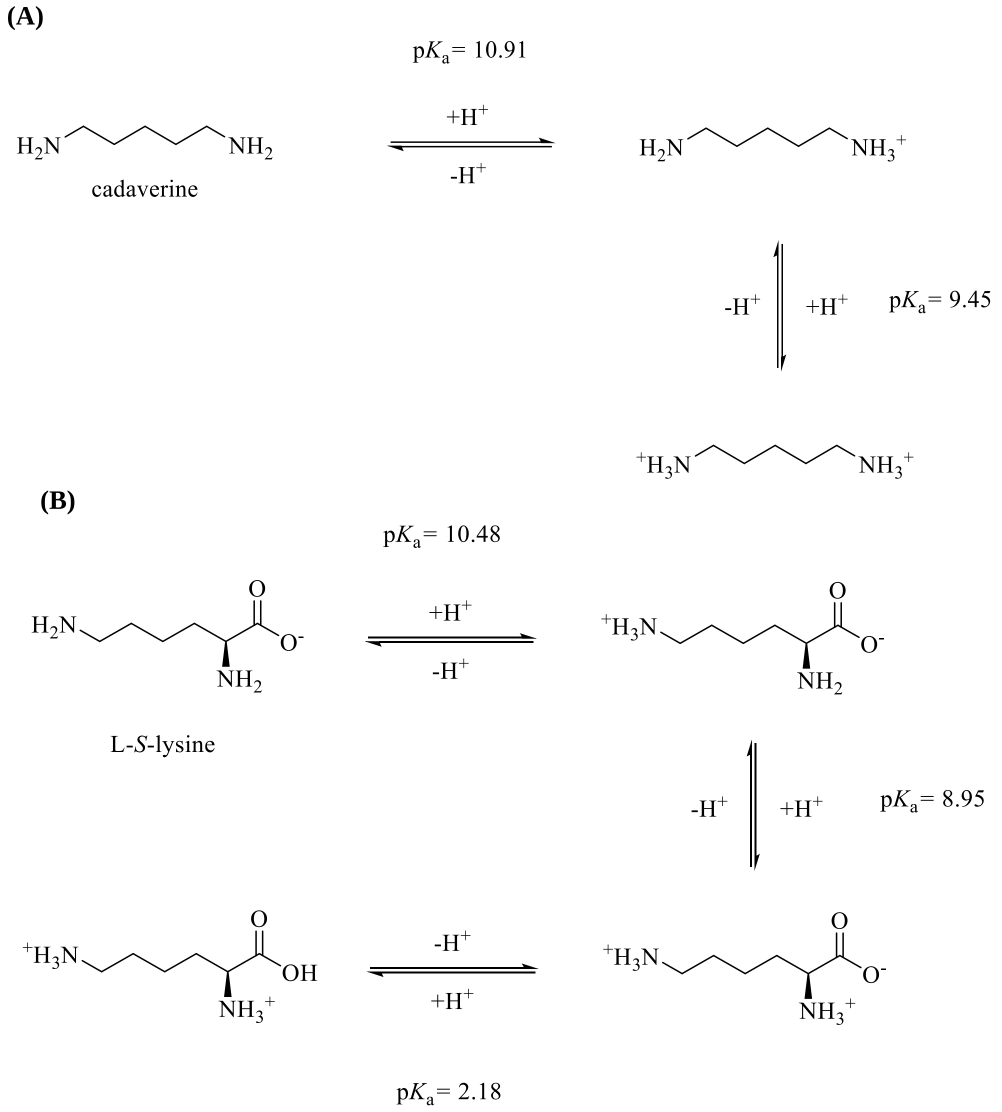

Figure 3.
For each pK_a (measured in H_2O), the balanced net ionic equations of cadaverine (A) and L-*S*-lysine (B) for the reaction are shown.
Source: Zhang and Vogel [56].

4.1. Methods for Measuring Ionization Constants (pKa)

4.1.1. Potentiometry

The straightforwardness and affordability of potentiometric titration have rendered it one of the most prevalent techniques for pK_a determination [57-62]. In a potentiometric titration, a specified volume of reagent is incrementally introduced to a solution containing the analyte [63]. The alteration in potential during the reaction is thus quantified using two electrodes: an indication electrode and a reference electrode [64]. These are frequently incorporated into what is now referred to as a mixed pH electrode [65]. Graphing potential against volume produces a sigmoid curve, with the inflection point indicating the equilibrium potential. The potential can be linearly translated into pH, equivalent to pK_a, using standards with known pH [2].

The cumbersome hydrogen electrode was soon supplanted by the well-known glass electrode. The pH measurement apparatus is fully automated and self-regulating [66]. During its initial application, accurately estimating pH from the titration slope was challenging [67]. Nevertheless, numerous software applications have emerged throughout the years to mitigate the aforementioned problems [68]. Potentiometric titration necessitates a comparatively substantial sample volume in contrast to separation techniques like high-performance liquid chromatography (HPLC) [2].

4.1.2. High Performance Liquid Chromatography (HPLC)

Initial observation of the time of elution can be changed by adjusting the pH level was made by Fuguet, et al. [69] and Wiczling, et al. [70]. The performance of the HPLC was optimized by altering the pH, specifically in the context of ion-exchange chromatography. This method is effective as long as the analyte possesses a chromophore for detection, even in samples that are not quite pure, since HPLC is a separation technique [2, 71].

Following the development of reversed-phase HPLC in the late 1970s, sophisticated models were established correlating the capacity factor (k) to the degree of dissociation (α) [72]. Horváth provides a comprehensive overview of the principle, presenting a universally applicable equation for acidic components:

$$k = (1 - \alpha)\cdot k0 + \alpha\cdot k\text{-}1 \quad (14)$$

where k0 and k-1 represent the capacity factors for the non-ionic and ionic species, respectively, in reversed-phase HPLC [73, 74]. Graphing k against pH produces a sigmoid curve, with the inflection point at $\alpha = 0.5$ occurring at pH = pK_a [2].

4.1.3. The UV-Visible Spectroscopy

The UV-visible spectroscopic technique was initially established by Holmes and Snyder [75]. Then, Flexser further elucidated this in 1935 by establishing several ionization constants [76].

The method is advantageous because of its speed, accuracy, and potential for automation to minimize errors. However, the UV spectrophotometric pH titration method can't pinpoint the exact location of protonation in molecules that have a lot of similar functional groups. As a result, it is unsuitable for quantifying the ionization constants of certain functional groups, which are composed of many such functional group molecules [2, 77]. There is currently no published UV data readily available for measuring the ionization constants of aminoglycoside antibiotics [78].

4.1.4. Nuclear Magnetic Resonance (NMR) Spectroscopy

Nuclear magnetic resonance (NMR) spectroscopic methods have been developed for quantifying pK_a values of individual amines [79-84]. Furthermore, when a molecule possesses numerous functional groups of a comparable nature, potentiometric titration cannot definitively determine the site of protonation. Consequently, it is inadvisable to utilize it for quantifying the ionization constants of functional groups in intricate compounds, such as AGs [85]. NMR spectroscopy for pK_a measurement relies on the notion that certain nuclei possess both charge and spin. The NMR chemical shifts of a molecule are contingent upon its magnetic surroundings. Thus, a progressive variation in acidity or basicity results in modifications of their chemical shifts (δ), as illustrated in Figure 4 [80, 86, 87]. This phenomenon is occasionally employed to determine the ionization constants. The chemical shifts are graphed versus the solution's pH; the pK_a values can be derived from the inflection points of the sigmoidal curves [2]. The method was initially documented by Emwas [88] for determining the pK_a of amines in Emwas [88].

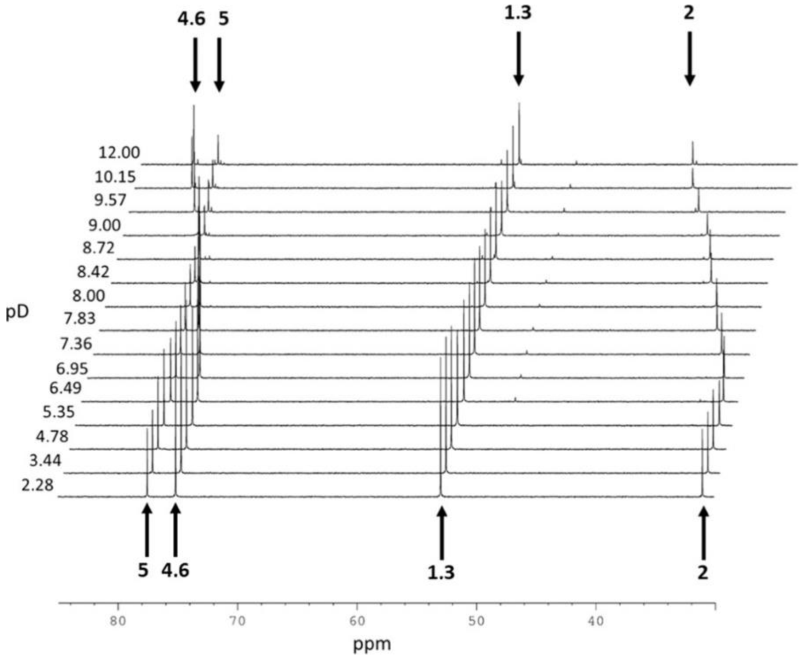

Figure 4.
Stack plot of the ^{13}C NMR spectra of 2-deoxystreptamine.
Source: Wallace, et al. [80] and Kogej and Muresan [89].

When a suitable chromophore is present, the primary advantage of NMR spectroscopy compared to potentiometry or UV spectroscopy is its efficacy in isolating and quantifying the distinct ionization constants of functional groups within complex compounds, such as AG antibiotics, which possess multiple ionizable groups [90-92]. Consequently, NMR spectroscopy was used to determine the pK_a values of amines in certain aminoglycosides. Clear assignments were established for each amine substituent on the clinically significant aminoglycosides tobramycin, kanamycin B, amikacin, sisomicin, and netilmicin through differences in the NMR spectroscopic chemical shift (δ) utilizing ^{1}H, ^{13}C, and ^{15}N HMBC, as illustrated in Figure 3 [89]. This data illustrates the study of individual amine basicity using NMR techniques, which is impossible with alternative analytical methods.

4.1.5. Computational Method, In Silico Prediction of pK_a values.

While the actual measurement of pK_a values is considerably convenient and straightforward nowadays, such assessment of millions of compounds within vast screening libraries has turned out to be costly and unfeasible, if not impossible, for virtual libraries during the early stages of drug discovery [93, 94]. This makes the in-silico prediction of pK_a values indispensable in contemporary drug discovery. Nowadays, the widely used standard methods of pK_a prediction for pharmaceutical substances [95] can be subdivided into two main groups: empirical and quantum chemical. Based on the detailed methodology employed, the empirical methods can be further divided into three sub categories: 1) linear free-energy relationships (LFER), techniques which make use of empirical relations of Hammett and Taft; 2) quantitative structure property relationships (QSPR), methods which correlate pre-calculated structural descriptors with pK_a values; and 3) database look up, i.e., those techniques which search for similar structures in a prebuilt database of molecules with known experimentally determined pK_a values [96, 97]. One of the strengths of the empirical methods is their high speed, which is useful when processing large databases of drug-like molecules [97]. Quantum chemical methods, an alternative to empirical methods, are supposed to have higher accuracy since they are based on, or closer to, first principles when calculating quantum mechanical descriptors [98]. These, however, are much more time-consuming compared to empirical methods [98].

Several papers have reported the performances of various pK_a prediction software packages on drug discovery projects. New chemical entities were tested using five tools, the results from which showed average absolute errors of 0.86-1.28, leaving room for improvement [99]. Additionally, ACD and Marvin were also applied as tool-of-choice medicinal chemistry tools, while obstacles with some chemical moieties were discussed [100]. Nine substances were compared, and the ranking of performances including the average performance, was ADME Boxes, ACD/ pK_a DB, and SPARC according to accuracy and coverage [95]. Recently, the prediction of pK_a and protonation state by using machine learning was reported to be applied to Epik version 7. This new version reaches median absolute errors of 0.42 pK_a units across test sets and recovers 95% of the most populated protonation states, thus enabling fast and accurate predictions for drug-like molecules [101]. Support Vector Machines predicted pK_a for carboxylic acids using Quantum Chemical Topology descriptors with high accuracy [102]. Besides, a new methodology was presented using Grid Molecular Interaction Fields, which showed very good predictive power for acidic nitrogen compounds and *N*-heterocyclic bases [94]. In a subsequent study, utilizing an improved MoKa prediction tool with high-throughput pK_a measurements enhanced the prediction accuracy for diverse chemical groups [94]. These studies highlight that improvement in pK_a prediction accuracy is continuous, and it is important to expand training datasets with new experimental measurements.

5. Conclusions

In drug development, the pK_a values are important due to their great influence on physicochemical properties and biological activities. The pK_a gives a measure of the degree of ionization that a compound will undergo at any pH and, by consequence, its solubility, permeability, and overall pharmacokinetic profile in a pharmaceutical context. Understanding the pK_a of drugs is very important for several reasons. Most importantly, it helps in predicting the ionization state of the drug at various physiological conditions, such as in the acidic environment of the stomach and the neutral to slightly basic conditions of the blood and intracellular compartments. Such an understanding is crucial for the optimization of drug absorption and bioavailability. As such, drugs that undergo GI ionization might require transporters to be absorbed, whereas drugs in nonionized states can easily be absorbed through passive diffusion. Moreover, there is an important impact on the pK_a value with regard to drug formulation and stability. The ionization state is one of the major factors affecting drug chemical stability in media, its solubility, and interaction with pharmaceutical formulation excipients. Therefore, precise determination of pK_a values plays a decisive role in the design of both stable and efficient pharmaceutical formulations.

In drug design, the knowledge of the pK_a values is important to guide the process of optimization in chemical structure refinement for maximum efficacy with limited toxic liability. Knowledge of the dissociation constants for different functional groups gives medicinal chemists the capability to develop superior pharmacological derivatives. By appropriate manipulation of pK_a in a given functional group, the ability of the drug to permeate the biological barrier for instance, the blood-brain barrier could be improved, enhancing its therapeutic potency. Among the different techniques for pK_a determination, NMR spectroscopy has some special merits, especially in cases where complex molecules possess more than one ionizable group. NMR spectroscopy allows precise pK_a determination based on the changes in chemical properties of nuclei as a function of pH. This provides a big advantage over classical techniques, especially for compounds like the aminoglycoside antibiotics that contain a number of similar functional groups, allowing a distinction between the pK_a values of individual amines in such complex molecules to be very informative about their SAR and mode of action. Determination of ionization constants has basic importance in research and development within the pharmaceutical field. Advanced techniques, such as NMR spectroscopy, have considerably enhanced our capabilities for precise measurement of these constants, hence assisting in the development of stronger, safer drugs. Recently, the pK_a prediction software has become an increasingly critical component of modern drug discovery, especially when dealing with large libraries of compounds. Currently, ACD/ pK_a, Marvin, and Epik represent some of the in silico pK_a prediction tools that have successfully estimated pK_a values rapidly and accurately, even for complex molecules. Empirical and quantum chemical methods are being implemented by these tools, enabling the screening of millions of compounds and greatly reducing the time and cost associated with experimental pK_a determination. The utilization of advanced experimental techniques such as NMR spectroscopy and pK_a prediction software will remain paramount in drug discovery and development.

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