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Protonation constants of endo- and exogenous L-amino acids and their derivatives in aqueous and mixed solution: Unraveling molecular secrets

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Review

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Abstract

The aim of this review is to summarize the progress made in the determination of the protonation constants of biologically active ligands: endo- and exogenous L-amino acids and their derivatives in aqueous and mixed solutions using different experimental techniques. The knowledge of the protonation constants of the aforementioned ligands is crucial for the determination of the equilibrium constants of complex formation and thus for the understanding of complex biological reactions such as transamination, racemization, and decarboxylation. Thus, the protonation constants of ligands are a measure of their ability to form complexes with metal ions. This knowledge not only helps to understand fundamental biochemical processes, but also has practical applications in areas such as drug design, where ligands are often targeted for therapeutic purposes. The activity of the ligands tends to increase after complexation and their order is consistent with the values of the stepwise dissociation constants of the complexes formed. Understanding the properties of ligands by determining their protonation constants in different environments and their interactions with surrounding molecules is crucial to unraveling the complexity of biological systems.

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Introduction

The molecular behavior of ligands in solution is a fascinating and complex field with significant implications in chemistry, biochemistry, and pharmacology. Understanding the interplay between ligand and solution is crucial as solvent molecules can influence the electronic structure, reactivity, and thermodynamics of ligand species. Firstly, the solvation or hydration of ligands in solution is of paramount importance in understanding how ligands interact with solvents at the molecular level. Secondly, as entities that bind to metal ions or other molecules, ligands exhibit protolitic properties (the ability to accept or donate protons) (El-Sherif, 2011). Data on the protonation constants of biologically active ligands in different media (aqueous and mixed solutions) are invaluable for understanding their chemistry in biological systems (AlJahdali *et al.*, 2014).

In vivo reactions primarily occur in aqueous environments, leading to a predominant interest in the properties of bioligands in such solutions. However, there is evidence in the literature that water is not a perfect mimic of the *in vivo* reaction environment. In enzymes, membranes, and other biologically relevant sites, the values of the protonation constants differ significantly from those in water, as these environments tend to be more lipophilic than hydrophilic (Fiol *et al.*, 1995; Partanen, 1998). Concurrently, the ionization state of a ligand can affect its solubility, membrane permeability, and interaction with biological targets (El-Sherif, 2012). As a result, studies in environments other than water can provide some insight into the chemistry of bioligands in living systems.

Amino acids are particularly important among other chemical groups because they are the basic building blocks of living organisms. Not only do they form tissues, but they also function as reactive organic compounds that regulate vital biological processes. Understanding the physical and protolytic properties of amino acids is essential to understanding the behavior and synthesis of proteins and enzymes in living organisms. These compounds are often regarded as excellent model systems to gain insight into the properties of naturally occurring metalloproteins (Kozłowski *et al.*, 1999; El-Sherif *et al.*, 2012a, 2012b).

Understanding the molecular secrets of bioligands in aqueous and mixed environments is essential for unraveling the intricacies of biochemical pathways, drug–receptor interactions, and the design of bioactive compounds (Al-Awadi *et al.*, 2008; Aljahdali and El-Sherif, 2012). With this in mind, this review discusses the valuable information available in the literature on bioactive ligands: endogenous L-amino acids (alanine, asparagine, cysteine, glycine, glutamine, aspartic acid, glutamic acid, proline, serine, tyrosine) and exogenous L-amino acids (arginine, phenylalanine, histidine, leucine, isoleucine, lysine, methionine, threonine, tryptophan, valine) and their derivatives in both environments.

Definition and importance of ligand protonation constants

The protonation constant, also known as the acid constant, is the equilibrium constant for the reaction of a ligand L (or any chemical species) with a proton (H^+) to form the corresponding protonated species. The equilibrium reaction is written as:

$$L + H^+ \rightleftarrows LH^+,$$

where L is the unprotonated form of the ligand, H^+ is the proton and LH^+ is the protonated form of the ligand. The protonation constant (K) is defined as the ratio of the concentrations of the protonated and unprotonated forms at equilibrium:

$$K = \frac{[LH^+]}{[L][H^+]}.$$

Take the negative logarithm of K to obtain the pK value:

$$pK = -\log K.$$

The pK is a measure of the acidity of the ligand: lower pK values indicate greater acidity, meaning the ligand is more likely to donate the proton (Zhou *et al.*, 2018).

Reasons for the determination of ligand protonation constants

Protonation constants are the equilibrium constants for the interaction of the proton with charged or uncharged ligands depending on the availability of hydrogen ions in aqueous and mixed solutions. These parameters are used to predict the ionization state of the molecule as a function of pH. The main purposes of determining the protonation constants of ligands in solution are to:

a) determine the pH values for the different forms of the compound under investigation,
b) perform a quantitative spectrophotometric analysis by selecting a suitable pH on the basis of the different UV spectra,
c) isolate the compound with maximum yield by identifying the pH range at which the minimum ionization of the compound occurs,
d) provide additional information on the structure of the newly synthesized ligand (agreement between theoretically calculated and experimental protonation constants indicates the potential correctness of the proposed structure),
e) prepare buffer solutions at different pH values (Martell and Calvin, 1952; Liptay *et al.*, 1962; Rossotti, 1978),
f) calculate the stability constants of complex formation between biologically active ligands and metal ions in coordination chemistry,
g) elucidate the intricate mechanisms of metalloenzymes and their catalytic activities (Sigel and Martin, 1982),
h) study the mobility and bioavailability of metal ions in natural waters (metal speciation and contaminant fate in environmental chemistry) (Flaschka, 1974).

The side chain groups of amino acids are involved in a variety of functions, including metal binding, weak interactions, and cofactor activities. In chemical systems, these groups are useful in the construction of artificial functionalized complexes, such as the model active site complexes of metalloenzymes and in the imidazolate – bridged multinuclear complexes that form molecular assemblies (Shimazaki *et al.*, 2009).

Thanks to the protonation constants of amino acids and their derivatives, we are still discovering new metal ion binding sites in proteins. The amino acids form stable five-membered chelates with various metal ions via the amine and carboxylate moieties (N, O – chelation). Several amino acids possess an additional metal binding site in the side chain and thus form metal complexes with a variety of structures. Groups such as the imidazole ring of histidine, the phenol ring of tyrosine, and the thiol group of cysteine are important metal binding sites in proteins. Similarly, the carboxylate groups of aspartate and glutamate and the thioether moiety of methionine are often involved in metal binding. These and other amino acid side chain groups form a microenvironment around the metal center which is necessary for substrate recognition and fixation and for the catalytic exercise of enzyme functions through non-covalent or weak interactions (hydrogen bonding, π-π stacking interactions, and hydrophobic interactions) (Berthon, 1995).

Methods for the determination of ligand protonation constants

There are several techniques such as potentiometry, conductometry, and spectrophotometry that can be used to determine protonation constants. This paper presents data and results from the use of the potentiometric method as it has the widest range of applicability and reliability (Woermann, 1973).

Potentiometric titrations are widely used for their precision in determining the acid–base equilibria of ligands. In this method, a ligand solution is titrated with a strong base while monitoring changes in pH. The most commonly used programs for solution equilibrium constants are PKPOT (Barbosa *et al.*, 1995), PKAS (Martell and Motekaitis, 1992), BEST (Motekaitis and Martell, 1982), MINIQUAD (Sabatini *et al.*, 1974), MINIQUAD75 (Gans *et al.*, 1976), SUPERQUAD (Gans *et al.*, 1985), PSEQUAD (Vacca and Sabatini, 1985), and HYPERQUAD (Gans *et al.*, 1996). All of these programs use least squares refinement to reduce the differences between calculated and experimental data to obtain the best model that gives the best fit. The sum of the squares of the residuals

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between experimental and calculated values is usually very small, typically between 10^{-6} and 10^{-9}.

Protonation equilibria in aqueous solution

Amino acid molecules possess at least one acidic group and one basic group. This allows an intramolecular acid–base equilibrium reaction to take place, resulting in the formation of a dipolar tautomeric ion known as a zwitterion or internal salt. From the above we know that an amino acid has at least two dissociation constants, the first corresponding to the case where the COOH group is deprotonated and the second corresponding to the case where the NH_3^+ group is deprotonated in an aqueous solution (Jhamb *et al.*, 2018). Thus, for all amino acids, the proton association constants can be expressed as stepwise protonation constants:

$$H^+ + L^- \rightleftarrows HL \left(\text{protonation of} - NH^2 \text{ group}\right)$$

$$H^+ + HL \rightleftarrows H_2L^+ \left(\text{protonation of} - COO^- \text{ group}\right).$$

Amino acids that are positively charged at pH 7, such as lysine, arginine, and histidine, undergo protonation equilibrium reactions:

$$H^+ + L^- \rightleftarrows HL \left(\text{protonation of} - NH^2 \text{ group}\right)$$

$$H^+ + HL \rightleftarrows H_2L^+ \left(\text{protonation of side chain}\right)$$

$$H^+ + H_2L^+ \rightleftarrows H_3L^{2+} \left(\text{protonation of} - COO^- \text{ group}\right).$$

The representative equilibria for amino acids that are negatively charged at pH 8, including glutamic acid, aspartic acid, and cysteine:

$$H^+ + L^{2-} \rightleftarrows HL^- \left(\text{protonation of} - NH^2 \text{ group}\right)$$

$$H^+ + HL^- \rightleftarrows H_2L \left(\text{protonation of side chain}\right)$$

$$H^+ + H_2L \rightleftarrows H_3L^+ \left(\text{protonation of} - COO^- \text{ group}\right).$$

In aqueous solutions, solvent molecules act as proton acceptors and therefore play a crucial role in chemical and biochemical reactions (Murphy *et al.*, 2020). The study of glycine, alanine, glutamic acid, histidine, tryptophan, and leucine derivatives and their complexes is of enormous biological interest because such complexes display interesting antibacterial, antifungal, anticancer, and antiviral properties (Quyoom, 2014). Leucine, isoleucine, and valine are metabolically active. In peripheral tissues, they can be oxidized to produce energy or act as anti-catabolic factors (especially leucine) by stimulating the synthesis and reducing the rate of breakdown of muscle protein (Soomro *et al.*, 2008). L-lysine oxidase (LysOx) isolated from the extracellular growth medium of *Trichoderma cf. aureoviride* was reported by Pokrovsky *et al.* (2013) to exhibit significant cytotoxicity and antitumor activity *in vitro* against a panel of murine and human tumor cell lines and *in vivo* on murine tumors and on animals with human tumor xenografts (breast cancer SKBR3, melanoma Bro, colon cancer HCT116 and ovarian adenocarcinoma SCOV3).

Amino acids are involved in pathways that feed cancer cells and provide building blocks for cancer cell growth. The citric acid cycle (the TCA cycle) is an important mechanistic example of the involvement of amino acids in cancer. The branched-chain amino acids and threonine fuel the TCA cycle intermediates, resulting in the release of ATP and providing the energy required for oncogenic activities. Cancer cells also use valine, leucine, and isoleucine as 'alternative fuels' to compete with other cells in the tumor stroma for energy and to optimize nutrient use during tumor development (Green *et al.*, 2016). The recently discovered V-9302, a selective inhibitor of a glutamine transporter ASCT2, showed anti-tumor activity *in vivo*, demonstrating the utility of a pharmacological agent in oncology (Schulte *et al.*, 2018). Cysteine uptake also plays an important role in breast cancer by maintaining redox balance and numerous studies have shown the efficacy of xCT inhibition on tumor growth (Timmerman *et al.*, 2013). Amino acids also form derivatives that contribute to tumor growth and metastasis. Arginine-derived polyamines alter gene expression by modulating global chromatin structure and cancer cell proliferation (Pegg, 2009). Kynurenine, derived from tryptophan, induces immunosuppression by binding to and activating the aryl hydrocarbon receptor (AhR) transcription factor. This impairs the ability of immune-tolerant dendritic cells (DCs) and regulatory T cells to target and eliminate cancer cells (Fallarino *et al.*, 2003).

In addition to the direct involvement of amino acids and their derivatives in metabolic reprogramming processes, amino acids are also fundamental in mediating epigenetic regulation and post-transcriptional modification. For example, DNA and histone methylation are regulated by balanced metabolite levels in the methionine cycle, which is influenced by methionine, serine, and glycine (Maddocks *et al.*, 2017).

The protonation constants of the aqueous amino acids are required to determine the stability constants of metal-amino acid complexes. Aiyelabola et al. prepared coordination complexes of aspartic acid in both basic and acidic media, for Mn^{2+}: L in a stoichiometric ratio of 1: 2. The antimicrobial activity of the compound $[Mn^{2+}(asp)_2]$ was antibacterial and antifungal (Aiyelabola *et al.*, 2016). Jasmin et al. synthesized a ligand Cu^{2+} complex by condensation reaction of isatin for cysteine, glycine, leucine, and alanine. The ligand Cu^{2+} complex was tested in antimicrobial studies using the disk diffusion method. All synthesized complexes exhibited strong antibacterial activity (Shampa *et al.*, 2017).

Al Naimi et al. synthesized complexes of Mn^{2+}, Co^{2+}, Zn^{2+}, Cd^{2+}, and Hg^{2+} with mixed ligands of 5-chlorosalicylic acid and L-valine. The results obtained in antibacterial studies of models through agar well diffusion bioassay evidenced the biological efficacy of ligands as well complexes (Al Naimi *et al.*, 2016). Mixed iron(III) and zinc(II) complexes with isonitrosoacetophenone (HINAP) and histidine, proline, and phenylalanine were formed and characterized. The antimicrobial activity was evaluated against bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*), and *Candida albicans* fungi. The Fe^{2+} and Zn^{2+} complexes were more active against Gram+ bacteria than Gram- bacteria. They also showed a significant inhibitory effect on the fungi tested. *In vitro* antitumor activity assayed against HEP2-type cancer cell lines (laryngeal cancer cells) showed significant toxicity of the ligands and their mixed complexes (Rahmouni *et al.*, 2019).

A potentiometric titration technique was used to determine the protonation constants of glycine, leucine, methionine, phenylalanine, tryptophan, asparagine, glutamine (T = 25 °C, I = 0.1 M KCl) (İnci and Aydın, 2021); cysteine, tyrosine, histidine, lysine (T = 25 °C, I = 0.1 M NaCl) (El-Sherif *et al.*, 2014); alanine, serine, isoleucine, threonine, valine (T = 25 °C, I = 0.1 M $NaClO_4$) (El-Sherif *et al.*, 2014); methylcystein, methylselenocysteine, selenomethionine (T = 25 °C, I = 0.1 M $NaClO_4$) (Murphy *et al.*, 2019); proline, arginine, glutamic acid, aspartic acid, 3,4-dihydroxyphenylalanine, hydroxyproline (T = 25 °C) (Burger, 1990); substituted salicylaldehyde amino acid (Sal–alanine, SalCl–alanine, SalBr–alanine,

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Table 1. Protonation constants of L-amino acids in water, ethanol– water mixtures (E), dioxane–water mixtures (D), and dimethyl sulfoxide–water media (DMSO)

L-amino acid; Derivative	Protonation constants (p*K*)				
	COOH		NH_3^+		
Endogenous	C_α	Additional	C_α	Additional	Other groups
Alanine	2.40		9.70		
	2.88 30% E		9.70 30% E		
	2.97 40% E		9.55 40% E		
	3.05 50% E		9.45 50% E		
	3.06 60% E		9.18 60% E		
	3.08 70% E		9.12 70% E		
	3.42 30% DMSO		9.65 30% DMSO		
	4.11 50% DMSO		9.85 50% DMSO		
	4.88 70% DMSO		10.48 70% DMSO		
Sal–alanine	2.40				7.42 (=NH^+–)
					11.79 (OH)
SalCl–alanine	2.20				7.76 (=NH^+–)
					9.69 (OH)
SalBr–alanine	2.22				8.09 (=NH^+–)
					10.25 (OH)
Asparagine	2.24		8.92		
Cysteine	1.71		10.29		8.36 (SH)
	2.60 30% E		10.25 30% E		8.23 30% E
	2.93 50% E		10.81 50% E		7.06 50% E
	2.82 70% E		12.07 70% E		6.33 70% E
Methylcysteine	2.02		8.79		
Methylselenocysteine	2.30		8.86		
Glycine	2.33		9.45		
	2.81 30% E		9.54 30% E		
	2.89 40% E		9.39 40% E		
	2.90 50% E		9.35 50% E		
	3.05 60% E		9.20 60% E		
	3.06 70% E		9.05 70% E		
Sal–glycine	2.43				7.57 (=NH^+–)
					11.68 (OH)
SalCl–glycine	1.86				7.58 (=NH^+–)
					9.60 (OH)
SalBr–glycine	1.97				7.85 (=NH^+–)
					10.74 (OH)
Glutamine	2.38		9.10		
Aspartic acid	1.94	3.70	9.62		
Glutamic acid	2.18	4.20	9.59		
Proline	1.90		10.41		
Hydroxyproline	1.80		9.47		
Serine	2.42		9.15		
	2.88 30% E		9.18 30% E		
	2.87 40% E		9.00 40% E		
	2.89 50% E		8.77 50% E		
	2.91 60% E		8.68 60% E		
	2.90 70% E		8.66 70% E		
	2.57 10% D		9.07 10% D		
	2.62 20% D		9.10 20% D		
	2.82 30% D		9.13 30% D		
	3.04 40% D		9.16 40% D		
	3.17 50% D		9.20 50% D		
	3.35 60% D		9.17 60% D		
	3.21 30% DMSO		8.95 30% DMSO		
	3.83 50% DMSO		9.12 50% DMSO		
	4.82 70% DMSO		9.89 70% DMSO		
Sal–serine	2.15				8.00 (=NH^+–)
					11.48 (OH)

(*Continued*)

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Table 1. (*Continued*)

L-amino acid; Derivative	Protonation constants (p*K*)				
	COOH		NH_3^+		
Endogenous	C_α	Additional	C_α	Additional	Other groups
Tyrosine	2.17 2.48 30% E 3.25 50% E 2.69 70% E		9.03 9.03 30% E 9.06 50% E 8.80 70% E		10.14 (OH) 10.46 30% E 10.90 50% E 10.70 70% E
Sal–tyrosine	2.21				7.68 (=NH^+–) 9.36 (OH) 12.30 (OH)
Exogenous					
Arginine	2.30		9.02		15.00 guanid. gr.
Phenylalanine	2.21 2.75 30% E 2.95 40% E 2.98 50% E 3.05 60% E 3.00 70% E 3.38 30% DMSO 4.08 50% DMSO		9.12 9.12 30% E 8.95 40% E 8.79 50% E 8.70 60% E 8.60 70% E 9.06 30% DMSO 9.35 50% DMSO		
3,4–dihydroxyphenylalanine	2.20		8.72		9.78 (OH) 13.40 (OH)
Sal–phenylalanine	2.23				7.94 (=NH^+–) 11.93 (OH)
Histidine	1.70 3.00 30% E 2.93 50% E 2.99 70% E		9.08 8.86 30% E 8.53 50% E 8.32 70% E	6.02 6.21 30% E 5.85 50% E 5.73 70% E	
Isoleucine	2.47 2.92 30% E 2.98 40% E 3.15 50% E 3.17 60% E 3.24 70% E 2.58 10% D 2.74 20% D 2.97 30% D 3.26 40% D 3.55 50% D 3.74 60% D		9.76 9.55 30% E 9.47 40% E 9.31 50% E 9.20 60% E 9.10 70% E 9.72 10% D 9.63 20% D 9.67 30% D 9.65 40% D 9.70 50% D 9.71 60% D		
Leucine	2.47 2.87 30% E 3.01 40% E 3.06 50% E 3.17 60% E 3.25 70% E 2.64 10% D 2.80 20% D 2.99 30% D 3.31 40% D 3.55 50% D 3.75 60% D 3.34 30% DMSO 4.04 50% DMSO 4.80 70% DMSO		9.52 9.60 30% E 9.40 40% E 9.30 50% E 9.07 60% E 9.05 70% E 9.59 10% D 9.64 20% D 9.66 30% D 9.70 40% D 9.72 50% D 9.75 60% D 9.46 30% DMSO 9.77 50% DMSO 10.18 70% DMSO		
Lysine	2.04 1.71 30% E 2.68 50% E 2.19 70% E		9.08 8.77 30% E 8.76 50% E 8.48 70% E	10.69 10.20 30% E 10.07 50% E 9.64 70% E	
Methionine	2.15 2.67 30% E 2.85 40% E 2.88 50% E 3.00 60% E		9.02 9.05 30% E 8.99 40% E 8.85 50% E 8.67 60% E		

(*Continued*)

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Table 1. (*Continued*)

L-amino acid; Derivative	Protonation constants (p*K*)				
	COOH		NH_3^+		
Endogenous	C_α	Additional	C_α	Additional	Other groups
	3.02 70% E		8.62 70% E		
	3.62 30% DMSO		9.07 30% DMSO		
	4.00 50% DMSO		9.48 50% DMSO		
Selenomethionine	2.05		9.29		
Threonine	2.45		9.04		
	2.53 10% D		9.02 10% D		
	2.60 20% D		9.06 20% D		
	2.75 30% D		9.05 30% D		
	2.96 40% D		9.05 40% D		
	3.18 50% D		9.10 50% D		
	3.40 60% D		9.17 60% D		
	3.35 30% DMSO		8.95 30% DMSO		
	4.06 50% DMSO		9.32 50% DMSO		
Tryptophan	2.43		9.32		
	2.83 30% E		9.17 30% E		
	3.21 50% E		9.05 50% E		
	2.77 70% E		8.78 70% E		
Valine	2.38		9.61		
	2.85 30% E		9.50 30% E		
	2.95 40% E		9.41 40% E		
	3.15 50% E		9.30 50% E		
	3.16 60% E		9.07 60% E		
	3.17 70% E		9.02 70% E		
	3.27 30% DMSO		9.34 30% DMSO		
	4.04 50% DMSO		9.57 50% DMSO		
	4.89 70% DMSO		9.99 70% DMSO		

Sal-amino acid, substituted salicylaldehyde amino acid. The % is expressed as volume.

Sal–glycine, SalCl–glycine, SalBr–glycine, Sal–serine, Sal–tyrosine, Sal–phenylalanine) (T = 25 °C, I = 0.1 M KCl) (El-Sherif and Aljahdali, 2013) in aqueous solution (Table 1).

Protonation equilibria of amino acids in a mixed medium (ethanol–water, dioxane–water, dimethyl sulfoxide–water)

Water–organic solvent mixtures have attracted much interest due to their frequent use and a wide range of applications (reaction media for a variety of organic and analytical processes such as synthesis, titrations, or liquid chromatographic separations). Therefore, studies in media other than water provide insight into the chemistry of bioligands in living organisms (Diaz-Cruz *et al.*, 2000).

Ethanol is the closest organic solvent to water in terms of structure and properties and therefore the behavior of dissociation or protonation in ethanol is similar to that in aqueous solution. The water–ethanol mixture is of very interesting binary character. One reason for this is that ethanol dissolves most organic acids and bases better than water. Solvents such as water–ethanol mixtures are thus a better model for *in vivo* reactions (Crosby *et al.*, 1970; Hughes *et al.*, 1986) because the mixtures possess both a low polar character and partially aqueous fractions, as in all biological systems. Their mixtures are macroscopically homogeneous, but it has been reported that the water and organic solvent molecules are not microscopically homogeneously dispersed due to the formation of hydrogen bonding networks and hydrophobic interactions. Consequently, the molecular composition of the solvation layer around a solute molecule is not the same as the bulk mixing ratio of water and organic solvent (Canel *et al.*, 2006).

Mixed solvents, such as mixtures of water and dioxane, provide an even better model for *in vivo* reactions because they are not only less polar than pure water, but also partially aqueous, as in all biological systems (Fiol *et al.*, 1995). These mixtures are a favorite mixed solvent system in which to study the association and mobility of ions because the dielectric constant can be varied over a wide range. Changes in protonation constants upon the addition of 1,4-dioxane to aqueous solutions are due to increased ion–ion interactions and changes in solvent–ion and solvent–solvent interactions (Roy *et al.*, 2005).

The use of a water–DMSO mixture has several advantages: (a) the DMSO–water 50%: 50% mixture has a low hygroscopic character (pure DMSO is very hygroscopic and it is difficult to control its water content); (b) compatibility with the standard glass electrode, so that pH measurements can be carried out in a similar way to a purely aqueous solution; (c) a wide acidity range (pk_w = 15.50), which allows the study of deprotonation equilibria of weak acids that are difficult to study in water (Hernández-Molina *et al.*, 1997).

The autoprotolysis constant (K_{ap}) of a solvent is an important parameter in understanding acid–base equilibria in mixed solvents. It determines the extreme limits of acidity and basicity in a given solvent medium. Kilic and Aslan derived a convenient and rapid potentiometric technique using a combined glass pH electrode for the determination of autoprotolysis constants in a variety of aqueous-organic mixed solvents (Kiliç and Aslan, 2005).

Ciolan et al. synthesized the binuclear complex $[M_2L(AcO)_2(H_2O)_4]$ (M = Cu^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+} and L = $(C_{39}H_{34}N_4O_6)^{2-}$) from 1,3-bis(2′-formylphenyl)-1,3-dioxapropane, L-tryptophan and metal acetate in methanolic medium. The complex $[Cu_2L(AcO)_2(H_2O)_4]$

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was the most effective in terms of antimicrobial efficacy and microbial spectra (Ciolan *et al.*, 2015). Mabrouk et al. synthesized and characterized Co^{2+} complexes of the Schiff base with salicylaldehyde and three amino acids (valine, leucine, isoleucine) in an ethyl alcohol solution. The metal: ligand ratio was 1: 2. Schiff bases and complexes were tested against different strains of microbes to determine their biological effects. Co(II) complexes showed greater bacterial activity against most bacterial species and the fungus *C. albicans* (Salama *et al.*, 2017). Obaid et al. prepared novel mixed metal–ligand complexes for metal(II) chloride (Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}) (L = glycine: alanine: valine) using 50% ethanolic solution as well as 50% distilled water in a molar ratio of 1:1:1:1. The Cd^{+2} complex had a higher antibacterial activity than the other complexes (Obaid *et al.*, 2018). Bougherra et al. prepared Cu^{2+} complexes for dimethylglyoxime as well as the tryptophan, glutamate, proline, arginine, and valine. The antimicrobial activity of ligand complexes was tested using the agar diffusion technique with DMSO as solvent against pathogenic bacteria and fungi. The complexes were more antimicrobial than the free ligand. Metal chelation significantly affected the antimicrobial activity of the ligands (Bougherra *et al.*, 2018).

The protonation constants of cysteine, tyrosine, histidine, lysine, tryptophan (T = 25 °C, I = 0.1 M NaCl) (El-Sherif *et al.*, 2014); glycine, alanine, valine, leucine, isoleucine, phenylalanine, serine, methionine (T = 25 °C, I = 0.1 M $NaClO_4$) (El-Sherif *et al.*, 2014) in various ethanol–water mixtures; leucine, isoleucine, serine, threonine (T = 25 °C, I = 0.1 M $NaClO_4$) (El-Sherif *et al.*, 2014) in dioxane–water mixtures; alanine, valine, leucine, threonine, phenylalanine, serine, methionine (T = 25 °C, I = 0.1 M $NaNO_3$) (El-Sherif *et al.*, 2014) in DMSO–water media are given in Table 1.

Conclusions

The determination of protonation constants is fundamental to understanding the behavior of amino acids and their interaction with metal ions in aqueous and mixed solutions. Protonation constants are important physicochemical parameters that can provide information about the drug's properties such as solubility, lipophilicity, acidity, and alkalinity (Meloun *et al.*, 2007; Roda *et al.*, 2010).

Amino acids, which are the building blocks of peptides and proteins, are essential chemicals needed by the body for optimal metabolism and proper functioning. Amino acids are involved in various physiological processes such as skeletal muscle function, atrophic conditions, sarcopenia, and cancer. They play a key role in cell signaling, homeostasis, gene expression, hormone synthesis, protein phosphorylation and possess antioxidant properties. Amino acids are also important precursors in the synthesis of low molecular weight nitrogenous compounds. The presence of amino acids and their metabolites, such as glutathione, polyamines, taurine, serotonin, and thyroid hormones, at physiological levels is important for proper body function. Amino acids are also essential for redox balance, energy regulation, and biosynthetic support. In addition, amino acid derivatives contribute to epigenetic regulation and immune responses associated with tumorigenesis and metastasis.

As technology continues to advance, future research into the determination of ligand protonation constants is expected to seamlessly integrate experimental and computational approaches. Factors such as solvent effects, temperature and the presence of competing ions can complicate the accuracy of results. However, researchers are addressing these challenges by developing sophisticated computational methods that simulate the behavior of ligands in different environments, providing a complementary approach to experimental data. The development of high-throughput methods and the exploration of new spectroscopic techniques will further enhance the ability to characterize complex equilibria in solution.

This review highlights the importance of amino acid protonation constants from coordination chemistry to drug design. As researchers continue to push the boundaries of knowledge, a deeper understanding of protonation constants will undoubtedly contribute to the development of innovative solutions and applications in various scientific fields. The review highlights the importance of continued exploration in this field, where each discovery brings us closer to a comprehensive understanding of the molecular world immersed in aqueous and mixed environments.

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