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In Silico Modelling of Ionization and Solubility Profiles in Antihypertensive Agents: Validation Approaches Using Potentiometric and Shake Flask Techniques

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Abstract

The prediction of ionization and solubility profiles is a critical aspect of pharmaceutical development, particularly for antihypertensive agents where physicochemical properties significantly influence drug absor0ption and bioavailability. This review provides a comprehensive analysis of in silico modelling approaches used for predicting ionization constants (pKa) and aqueous solubility, with a specific focus on their validation using experimental techniques such as potentiometric titration and the shake-flask method. Various computational methodologies, including quantitative structure–property relationship models, quantum chemical approaches, and machine learning algorithms, are discussed in relation to their predictive performance and limitations. The review highlights the importance of integrating computational predictions with experimental validation to ensure reliability and applicability in drug development. Potentiometric and shake-flask methods are examined as complementary techniques that provide accurate determination of ionization and solubility parameters. Correlation studies demonstrate that incorporating ionization data significantly improves solubility prediction, while discrepancies between predicted and experimental values underscore the need for hybrid modelling approaches. Furthermore, the review addresses current challenges in physicochemical prediction and outlines future perspectives, including the application of artificial intelligence and physiologically based pharmacokinetic modelling. Overall, the integration of in silico and experimental methodologies offers a robust framework for optimizing antihypertensive drug development and improving therapeutic efficacy.

Keywords

In silico modelling; pKa prediction; solubility prediction; antihypertensive drugs; potentiometric titration; shake-flask method; drug development; ADME.

1. Introduction

Hypertension remains one of the most prevalent chronic cardiovascular disorders worldwide and is a major contributor to morbidity and mortality associated with stroke, myocardial infarction, and renal dysfunction. The pharmacological management of hypertension relies on multiple classes of drugs, including angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), beta-blockers, calcium channel blockers, and diuretics, each exhibiting distinct physicochemical and pharmacokinetic profiles. Despite significant advances in antihypertensive therapy, variability in drug absorption, bioavailability, and therapeutic response continues to present major challenges in clinical outcomes. These limitations are largely governed by intrinsic physicochemical properties such as

ionization behaviour and aqueous solubility, which directly influence drug dissolution, permeability, and systemic exposure (Avdeef, 2001).

Ionization, commonly expressed as the dissociation constant (pKa), plays a central role in determining the charge state of drug molecules across physiological pH environments. The extent of ionization affects membrane permeability, protein binding, and distribution characteristics. For instance, weakly basic antihypertensive drugs such as amlodipine and atenolol exhibit pH-dependent ionization that influences their intestinal absorption and bioavailability. Similarly, solubility remains a critical determinant of oral drug performance, particularly for compounds classified under Biopharmaceutics Classification System (BCS) classes II and IV, where dissolution is the rate-limiting step. The interplay between pKa and solubility governs the pH–solubility profile, ultimately dictating drug absorption across gastrointestinal segments (Steele & Austin, 2016).

In recent years, the integration of computational approaches into drug development has revolutionized the prediction of physicochemical and pharmacokinetic properties. In silico modelling techniques, including quantitative structure–property relationship (QSPR) models, molecular descriptors, and machine learning algorithms, have been widely employed to estimate pKa and solubility with increasing accuracy. Early works by Cruciani et al. (2009) and Lee et al. (2007) demonstrated the feasibility of predicting ionization constants using computational frameworks, while subsequent advancements incorporated structural, electronic, and thermodynamic parameters into predictive models. Similarly, solubility prediction has evolved through empirical models such as the General Solubility Equation and more sophisticated approaches integrating crystal packing and ionization effects (Johnson et al., 2007; Hewitt et al., 2009).

The application of in silico tools has been particularly significant in the development and optimization of antihypertensive agents. Computational studies have enabled the identification of novel ACE inhibitors, renin inhibitors, and vasorelaxant compounds through molecular docking and ADME profiling (Abdelhedi et al., 2017; Pavlos et al., 2025). Furthermore, predictive modelling has been utilized to assess pharmacokinetic behaviour, including absorption, distribution, and metabolic stability of antihypertensive drugs (Singh et al., 2017). However, despite the rapid advancements in computational methodologies, the reliability of predicted pKa and solubility values remains dependent on experimental validation.

Experimental techniques such as potentiometric titration and the shake-flask method continue to serve as gold standards for determining ionization constants and equilibrium solubility, respectively. Potentiometric methods enable precise characterization of ionization equilibria, even for poorly soluble compounds, by measuring pH changes during titration. In contrast, the shake-flask method provides direct measurement of thermodynamic solubility under equilibrium conditions and is widely regarded as a benchmark technique in pharmaceutical profiling. Comparative studies have demonstrated that these experimental approaches complement computational predictions and are essential for validating in silico models (Glomme et al., 2005; Lucero-Borja et al., 2020).

Despite the availability of both computational and experimental techniques, there exists a significant gap in the integrated analysis of in silico predictions and their experimental validation specifically for antihypertensive agents. Most studies focus either on predictive modelling or experimental determination, with limited efforts directed toward correlating and critically evaluating both approaches. This lack of integration hinders the development of robust predictive frameworks that can reliably guide drug design and optimization.

Therefore, the present review aims to provide a comprehensive and critical analysis of in silico modelling approaches for predicting ionization and solubility profiles of antihypertensive drugs, with a particular emphasis on their validation using potentiometric and shake-flask techniques. By synthesizing available literature, this review seeks to bridge the gap between computational predictions and experimental observations, thereby offering insights into improving the accuracy and applicability of physicochemical modelling in pharmaceutical development.

2. Physicochemical Basis of Ionization and Solubility

The physicochemical properties of drug molecules form the foundation for understanding their in vivo performance, particularly in relation to absorption, distribution, and overall bioavailability. Among these properties, ionization and aqueous solubility are critically interlinked parameters that govern the behaviour of antihypertensive agents in biological systems. A mechanistic understanding of these properties is essential not only for formulation development but also for the rational design of predictive computational models.

Ionization is defined by the acid–base dissociation constant (pKa), which represents the pH at which a drug exists in equal proportions of ionized and unionized forms. The ionization state of a molecule is highly dependent on the surrounding pH and directly influences its lipophilicity, permeability, and interaction with biological membranes. The Henderson–Hasselbalch relationship provides a quantitative framework to estimate the fraction of ionized and unionized species under varying physiological conditions. Weakly acidic drugs tend to remain unionized in acidic environments, while weak bases are predominantly unionized at higher pH values, thereby facilitating membrane permeation.

For antihypertensive drugs, ionization plays a decisive role in determining pharmacokinetic behaviour. For example, beta-blockers such as atenolol and bisoprolol possess basic functional groups that undergo protonation in the acidic gastric environment, leading to increased ionization and reduced permeability. Conversely, in the more alkaline intestinal environment, partial deionization enhances absorption. Computational studies have demonstrated that accurate prediction of ionization constants significantly improves the estimation of pharmacokinetic parameters, including absorption rate and distribution volume (Lee et al., 2007; Strope et al., 2018). Furthermore, advances in in silico prediction methods have incorporated molecular descriptors such as electron density, hydrogen bonding capacity, and atomic charges to refine pKa estimation (Cruciani et al., 2009).

Solubility, on the other hand, is defined as the maximum amount of a substance that can dissolve in a given solvent under equilibrium conditions. Intrinsic solubility refers to the solubility of the unionized form of a compound, whereas apparent solubility accounts for both ionized and unionized species and varies with pH. The pH–solubility profile is therefore a direct consequence of the ionization characteristics of the molecule. For ionizable drugs, solubility typically increases when the drug is in its ionized form due to enhanced interaction with the aqueous medium.

Antihypertensive drugs often exhibit complex solubility behaviour due to the presence of multiple ionizable groups and varying lipophilicity. Drugs such as losartan and valsartan, which belong to the ARB class, display poor aqueous solubility but improved solubility under specific pH conditions. This behaviour has significant implications for formulation strategies, particularly in the development of oral dosage forms where dissolution is a rate-limiting step. The relationship between solubility and lipophilicity is also critical, as highly lipophilic drugs tend to exhibit lower aqueous solubility but enhanced membrane permeability, leading to a delicate balance that must be optimized during drug development (Avdeef, 2001).

In silico prediction of solubility has evolved considerably, incorporating both empirical and mechanistic models. Early quantitative structure–property relationship (QSPR) models utilized simple descriptors such as molecular weight, logP, and hydrogen bond donors to estimate solubility (Ali et al., 2012). However, these models often lacked accuracy for structurally complex molecules. More advanced approaches have integrated crystal lattice energy, ionization effects, and solvent interactions to improve predictive performance. Johnson et al. (2007) developed a comprehensive model that accounts for intrinsic solubility, crystal packing, and ionization, demonstrating improved correlation with experimental data. Similarly, Hewitt et al. (2009) highlighted the challenges associated with solubility prediction, emphasizing the need for hybrid models that combine computational and experimental inputs.

The interplay between ionization and solubility is particularly important in the context of the Biopharmaceutics Classification System (BCS), where drugs are categorized based on their solubility and permeability characteristics. Many antihypertensive agents fall into BCS Class II, characterized by low solubility and high permeability, making solubility enhancement a critical factor in improving bioavailability. In such cases, accurate prediction of pKa and solubility profiles can significantly aid in the selection of appropriate formulation strategies, including salt formation, particle size reduction, and the use of solubilizing excipients.

In addition to formulation considerations, ionization and solubility also influence drug stability, protein binding, and tissue distribution. For instance, highly ionized drugs tend to exhibit lower plasma protein binding and limited tissue penetration, whereas unionized forms can readily cross biological membranes and accumulate in target tissues. These factors underscore the importance of accurately characterizing and predicting physicochemical properties during the early stages of drug development.

Overall, the physicochemical interplay between ionization and solubility forms the cornerstone of both experimental and computational approaches in pharmaceutical sciences. While in silico models offer rapid and cost-effective predictions, their reliability is inherently dependent on the accuracy of underlying physicochemical principles and their validation through experimental techniques. This interdependence highlights the need for an integrated framework that combines theoretical predictions with empirical data,

particularly in the development of antihypertensive agents where precise control over pharmacokinetic behaviour is essential.

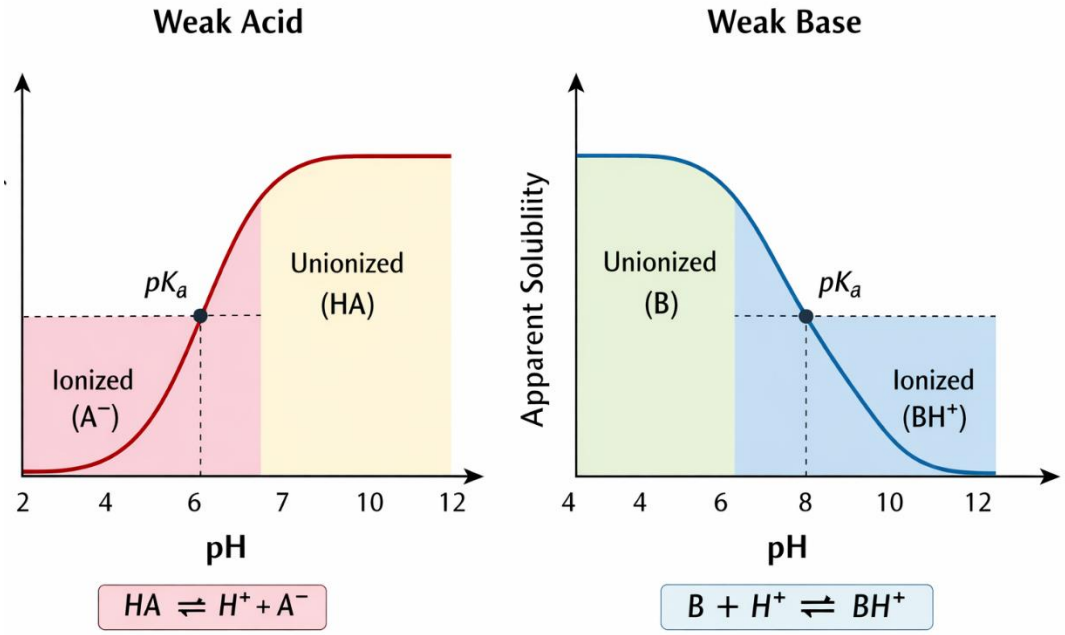

Figure 1. Graphical representation of pH–solubility and ionization relationship for weak acids and weak bases, illustrating the dependence of apparent solubility on pH.

3. Antihypertensive Drug Classes and Physicochemical Diversity

Antihypertensive therapy encompasses a broad spectrum of pharmacological classes, each characterized by distinct mechanisms of action and diverse physicochemical attributes. These variations in molecular structure directly influence ionization behaviour, solubility profiles, lipophilicity, and ultimately pharmacokinetic performance. Understanding the physicochemical diversity among antihypertensive agents is therefore essential for interpreting both in silico predictions and experimental validation outcomes.

Angiotensin-converting enzyme (ACE) inhibitors represent one of the most widely used classes in hypertension management. Structurally, these compounds typically contain carboxylic acid or thiol functional groups, which impart acidic characteristics and influence their ionization profiles. Drugs such as captopril and enalapril exhibit pKa values associated with acidic moieties, leading to increased ionization at physiological pH. This ionization behavior enhances aqueous solubility but may reduce membrane permeability, necessitating prodrug strategies in some cases to improve bioavailability. Recent physicochemical and computational investigations on captopril have highlighted the role of solvent interactions and ionic states in modulating its behaviour in aqueous systems (Sharma et al., 2026).

Angiotensin II receptor blockers (ARBs), including losartan and valsartan, display a markedly different physicochemical profile. These compounds are generally more lipophilic and possess multiple ionizable groups, resulting in complex pH-dependent solubility patterns. The presence of tetrazole rings in ARBs contributes to acidic ionization, while their bulky aromatic structures enhance lipophilicity. As a consequence, many ARBs are classified under BCS Class II, exhibiting low solubility but high permeability. Computational and experimental studies have demonstrated that accurate prediction of ionization states is crucial for modelling their absorption and distribution characteristics (Kritsi et al., 2013; Leon et al., 2023).

Beta-adrenergic blockers constitute another important class, characterized by the presence of secondary or tertiary amine groups that confer basic properties. Drugs such as atenolol and bisoprolol are predominantly ionized under physiological conditions, which contributes to their relatively high aqueous solubility but limited permeability across lipid membranes. This balance between solubility and permeability is a defining feature of BCS Class III drugs. In silico pharmacokinetic analyses have shown that ionization-dependent distribution plays a significant role in determining the therapeutic efficacy of beta-blockers (Singh et al., 2017; Oktaviani et al., 2023).

Calcium channel blockers (CCBs), such as amlodipine, exhibit amphoteric behaviour due to the presence of both acidic and basic functional groups. This dual ionization capability results in complex pH–solubility relationships, which can significantly influence dissolution and absorption kinetics.

Amlodipine, for instance, demonstrates moderate lipophilicity and pH-dependent solubility, making it sensitive to gastrointestinal conditions. Recent combined in vitro and in silico studies have further elucidated the interaction of amlodipine with biological systems, highlighting the importance of physicochemical profiling in predicting its pharmacological activity (Dańkowska et al., 2025).

Diuretics, including hydrochlorothiazide and related compounds, typically exhibit poor aqueous solubility and variable ionization characteristics. These drugs often contain sulfonamide or heterocyclic functional groups that influence their dissociation behaviour. The low solubility of many diuretics presents formulation challenges and necessitates careful optimization of dissolution properties. Regulatory frameworks, such as BCS-based biowaivers, emphasize the importance of understanding solubility and permeability characteristics in ensuring therapeutic equivalence (Van Wyk et al., 2024).

The structural diversity across antihypertensive classes underscores the complexity of predicting physicochemical properties using computational models. Variations in functional groups, molecular size, and electronic distribution contribute to differences in ionization and solubility behaviour, which must be accurately captured by in silico tools. Studies have demonstrated that incorporating structural descriptors and molecular interactions into predictive models significantly enhances their reliability (Lobell & Sivarajah, 2003; Wenlock & Barton, 2013).

Table 1. Representative Physicochemical Properties of Selected Antihypertensive Drugs

Drug	Class	Key Functional Groups	pKa	Log P	Solubility Profile	BCS Class
Captopril	ACE inhibitor	Thiol, carboxyl	~3.7	0.3	High	III
Losartan	ARB	Tetrazole, aromatic	~4.9	4.0	Low (pH-dependent)	II
Atenolol	β-blocker	Secondary amine	~9.6	0.2	High	III
Amlodipine	CCB	Amine, ester	~8.6	2.1	Moderate	II
Hydrochlorothiazide	Diuretic	Sulfonamide	~7.9	−0.1	Low	IV

The physicochemical heterogeneity observed among antihypertensive drugs highlights the necessity of integrating both computational and experimental approaches for accurate characterization. While in silico models provide valuable insights into structure–property relationships, their predictive accuracy is contingent upon robust experimental validation. This interplay becomes particularly critical when dealing with structurally complex molecules exhibiting multiple ionization sites and non-linear solubility behaviour.

4. In Silico Modelling Approaches for Ionization and Solubility Prediction (≈850 words)

The advancement of computational methodologies has significantly transformed the landscape of pharmaceutical research, particularly in the prediction of key physicochemical parameters such as ionization constants (pKa) and aqueous solubility. In silico modelling provides a rapid, cost-effective, and scalable approach for screening large libraries of compounds, thereby facilitating early-stage drug development and optimization. For antihypertensive agents, which often exhibit complex ionization behaviour and solubility challenges, these predictive tools have become indispensable.

4.1 In Silico Prediction of Ionization Constants (pKa)

The prediction of ionization constants has evolved from simple empirical correlations to sophisticated models incorporating quantum chemical calculations and machine learning techniques. Early computational approaches relied on fragment-based methods, where the pKa of a molecule was estimated based on contributions from individual functional groups. However, such models often failed to account for intramolecular interactions and electronic effects that significantly influence ionization behaviour.

More advanced methods incorporate quantum mechanics (QM) to calculate molecular electrostatic potentials, proton affinities, and solvation energies, thereby providing a more accurate estimation of pKa values. Cruciani et al. (2009) demonstrated that integrating molecular interaction fields with electronic descriptors enhances the predictive accuracy of pKa models. Similarly, Lee et al. (2007) developed computational frameworks that incorporate atomic charges and hydrogen bonding interactions, enabling reliable prediction of ionization constants across diverse chemical classes.

Recent developments have further improved pKa prediction through the application of machine learning algorithms trained on large datasets of experimentally determined values. These models leverage descriptors such as topological indices, molecular fingerprints, and physicochemical parameters to capture complex structure–property relationships. High-throughput in silico prediction systems have also

been developed to estimate ionization equilibria for pharmacokinetic modelling, significantly reducing the time required for compound screening (Strope et al., 2018).

For antihypertensive drugs, accurate pKa prediction is particularly critical due to the presence of multiple ionizable groups. For example, ARBs and ACE inhibitors often contain both acidic and basic moieties, resulting in amphoteric behaviour that complicates ionization profiles. Computational studies have shown that incorporating solvent effects and conformational flexibility into predictive models improves their applicability to such complex systems (Wenlock & Barton, 2013).

4.2 In Silico Prediction of Aqueous Solubility

Solubility prediction remains one of the most challenging aspects of computational modelling due to the multifactorial nature of solvation processes. Traditional approaches, such as the General Solubility Equation (GSE), provide a simplified framework based on melting point and lipophilicity. While useful for initial screening, these models often lack the precision required for structurally diverse drug molecules. Quantitative structure–property relationship (QSPR) models have been widely employed to predict aqueous solubility using molecular descriptors such as logP, molecular weight, polar surface area, and hydrogen bonding capacity. Ali et al. (2012) highlighted the importance of specific functional groups, such as phenolic moieties, in influencing solubility predictions. However, the accuracy of QSPR models is highly dependent on the quality and diversity of the training dataset.

More comprehensive models incorporate thermodynamic parameters, including crystal lattice energy, solvation free energy, and ionization effects. Johnson et al. (2007) developed a computational model that integrates intrinsic solubility, crystal packing, and ionization, demonstrating improved correlation with experimental data. Similarly, Hewitt et al. (2009) emphasized the need for hybrid approaches that combine empirical data with mechanistic insights to address the limitations of purely statistical models. Recent advancements in machine learning and artificial intelligence have further enhanced solubility prediction capabilities. Deep learning models trained on large chemical datasets can capture non-linear relationships between molecular structure and solubility, providing improved predictive performance. Schneckener et al. (2019) demonstrated the successful integration of in silico model outputs with machine learning algorithms to predict pharmacokinetic properties, including solubility and bioavailability.

4.3 Integrated ADME and Physicochemical Modelling

Modern in silico platforms increasingly adopt an integrated approach, combining pKa and solubility predictions with broader ADME (absorption, distribution, metabolism, and excretion) profiling. Such integration allows for a more comprehensive evaluation of drug candidates, particularly in terms of oral bioavailability and systemic exposure. Honorio et al. (2013) and El-Magboub (2017) highlighted the role of computational ADME modelling in predicting pharmacokinetic behaviour based on physicochemical properties. In the context of antihypertensive drug development, integrated modelling has been used to simulate dissolution profiles, absorption kinetics, and in vitro–in vivo correlations. Tools such as GastroPlus™ and DDDPlus™ have been employed to model drug absorption and establish relationships between physicochemical properties and pharmacokinetic outcomes (Duque, 2016). These approaches enable researchers to predict the impact of ionization and solubility on drug performance under physiological conditions.

4.4 Applications in Antihypertensive Drug Discovery

In silico modelling has been extensively applied in the discovery and optimization of antihypertensive agents. Molecular docking and simulation studies have facilitated the identification of novel ACE inhibitors and renin inhibitors, providing insights into drug–target interactions and binding affinities (Abdelhedi et al., 2017; Pavlos et al., 2025). Additionally, computational approaches have been used to evaluate the physicochemical and pharmacokinetic properties of candidate molecules, enabling the selection of compounds with optimal profiles (Singh et al., 2017; Kurmi et al., 2025). Recent studies have also explored the use of in silico tools for predicting the behaviour of antihypertensive drugs in complex biological environments. For instance, Guala et al. (2017) investigated the impact of antihypertensive drugs on myocardial oxygen balance using computational modelling, while Leon et al. (2023) employed in silico techniques to develop discriminative dissolution methods for combination therapies.

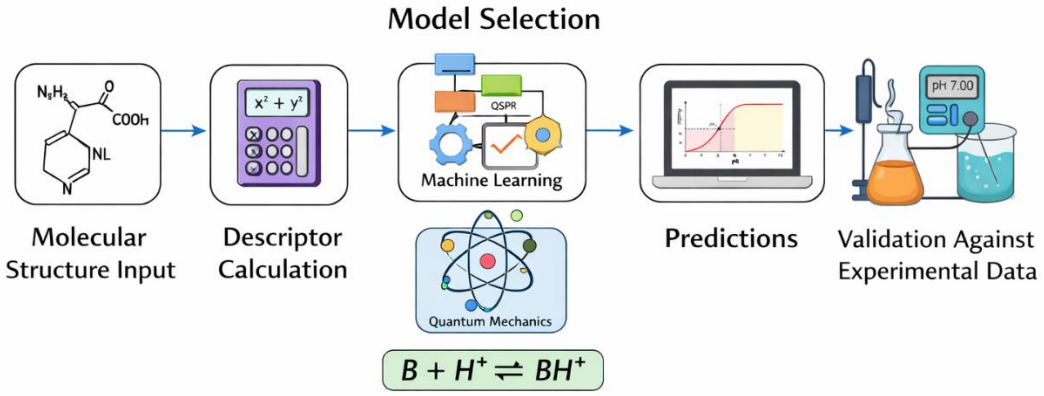

Figure 2. Workflow of in silico prediction of ionization and solubility, including molecular structure input, descriptor calculation, model selection (QSPR/ML/QM), and validation against experimental data.

4.5 Limitations of In Silico Models

Despite significant advancements, in silico models are not without limitations. Prediction accuracy can be affected by factors such as inadequate training datasets, inability to account for polymorphism, and limitations in modelling complex solvation phenomena. Furthermore, the presence of multiple ionizable groups and conformational flexibility in antihypertensive drugs can lead to discrepancies between predicted and experimental values. These limitations underscore the necessity of validating computational predictions using experimental techniques such as potentiometric titration and shake-flask methods. The integration of computational and experimental approaches is therefore essential for developing reliable and predictive models that can guide drug development processes.

5. Experimental Validation Techniques for Ionization and Solubility

While in silico models provide rapid and cost-effective predictions of physicochemical properties, their practical utility in drug development depends on rigorous experimental validation. Among the various analytical techniques available, potentiometric titration and the shake-flask method are widely recognized as gold standard approaches for determining ionization constants (pKa) and equilibrium solubility, respectively. These techniques play a crucial role in verifying computational predictions and ensuring the reliability of physicochemical profiling for antihypertensive drugs.

5.1 Potentiometric Titration for pKa Determination

Potentiometric titration is a well-established analytical technique used to determine the ionization constants of ionizable compounds by monitoring changes in pH during controlled addition of titrant. The method is based on the principle that the degree of ionization of a compound varies with pH, and this variation can be quantitatively analyzed to calculate pKa values. In pharmaceutical applications, potentiometric titration is particularly advantageous for compounds with multiple ionizable groups, as it allows for the resolution of individual dissociation constants. Advanced techniques such as the CheqSol method have further improved the applicability of potentiometric analysis, especially for poorly soluble drugs. Lucero-Borja et al. (2020) demonstrated that potentiometric CheqSol and standardized shake-flask methods serve as complementary tools in physicochemical profiling, providing reliable data for both ionization and solubility parameters.

The accuracy of potentiometric measurements is influenced by factors such as ionic strength, temperature, and the presence of co-solvents. Careful control of experimental conditions is therefore essential to ensure reproducibility. Additionally, the method requires precise calibration of pH electrodes and appropriate mathematical modelling to interpret titration curves. Mornar et al. (2006) highlighted the application of potentiometric titration in predicting bioavailability of phenolic compounds, emphasizing its relevance in pharmaceutical analysis.

One of the key advantages of potentiometric titration is its ability to analyze compounds with limited aqueous solubility by employing co-solvent systems or specialized techniques. Glomme et al. (2005) compared potentiometric titration with miniaturized shake-flask methods and demonstrated that potentiometric approaches can provide accurate pKa values even in high-throughput settings. Furthermore, this method is less time-consuming compared to traditional solubility assays and can be automated for large-scale screening. However, potentiometric titration also has certain limitations. The presence of multiple ionizable groups can complicate data interpretation, particularly when dissociation constants are closely spaced. Additionally, the requirement for specialized instrumentation and expertise

may limit its widespread application in routine laboratories. Despite these challenges, potentiometric titration remains an indispensable tool for validating in silico pKa predictions.

5.2 Shake-Flask Method for Solubility Determination

The shake-flask method is widely regarded as the reference technique for determining the equilibrium solubility of pharmaceutical compounds. This method involves equilibrating an excess amount of drug in a solvent under controlled conditions, followed by separation of the undissolved solid and quantification of the dissolved fraction using analytical techniques such as UV spectroscopy or high-performance liquid chromatography (HPLC). The simplicity and reliability of the shake-flask method make it the most commonly used approach for solubility determination. It provides direct measurement of thermodynamic solubility, which is essential for understanding drug dissolution and absorption characteristics. Saghaie and Mostafavi (2003) demonstrated the robustness of the shake-flask method in determining partition coefficients, while Port et al. (2018) highlighted its applicability across a wide range of drug classes, including neutral, acidic, and basic compounds.

One of the major strengths of the shake-flask method is its ability to accommodate a variety of experimental conditions, including different pH environments, temperatures, and solvent systems. This flexibility allows researchers to construct detailed pH–solubility profiles, which are critical for predicting drug behaviour in physiological conditions. Additionally, the method can be adapted for high-throughput screening by miniaturization and automation, as demonstrated in comparative studies with potentiometric techniques (Glomme et al., 2005). Despite its widespread use, the shake-flask method is not without limitations. The technique is inherently time-consuming, often requiring several hours to days to achieve equilibrium. Furthermore, issues such as supersaturation, polymorphic transitions, and adsorption of drug onto container surfaces can affect the accuracy of solubility measurements. Careful experimental design and validation are therefore essential to ensure reliable results. Recent advancements have focused on improving the efficiency and reproducibility of the shake-flask method through automation and integration with analytical techniques. High-throughput platforms have been developed to enable rapid screening of multiple compounds, thereby enhancing the applicability of this method in early-stage drug development (Faller et al., 2006).

5.3 Complementarity of Potentiometric and Shake-Flask Methods

The combined use of potentiometric titration and shake-flask methods provides a comprehensive framework for physicochemical profiling. While potentiometric techniques offer precise determination of ionization constants, shake-flask methods provide direct measurement of solubility under equilibrium conditions. Lucero-Borja et al. (2020) emphasized that these methods are complementary rather than competitive, and their integration can significantly enhance the reliability of physicochemical data. For antihypertensive drugs, which often exhibit complex ionization and solubility behaviour, the use of both techniques is particularly important. The interplay between pKa and solubility necessitates a combined approach to accurately characterize pH-dependent solubility profiles. Furthermore, validation of in silico predictions using these experimental methods ensures that computational models are grounded in empirical data, thereby improving their predictive performance.

Table 2. Comparison of Potentiometric and Shake-Flask Methods

Parameter	Potentiometric Method	Shake-Flask Method
Primary Output	pKa (Ionization constant)	Equilibrium solubility
Principle	pH change during titration	Saturation and equilibrium
Accuracy	High	Very high (gold standard)
Time Requirement	Moderate	High
Applicability	Ionizable compounds	All compounds
Limitations	Complex data interpretation	Time-consuming, equilibrium issues

In summary, experimental validation techniques remain indispensable for confirming the accuracy of in silico predictions. The integration of potentiometric and shake-flask methods provides a robust and reliable approach for characterizing the physicochemical properties of antihypertensive drugs, thereby bridging the gap between computational modelling and real-world pharmaceutical applications.

6. Correlation Between *In Silico* Predictions and Experimental Data

The reliability of in silico models for predicting ionization (pKa) and solubility is ultimately determined by their agreement with experimentally obtained values. Establishing a robust correlation between predicted and measured data is therefore essential for validating computational tools and ensuring their

applicability in drug development. In the context of antihypertensive agents, which often exhibit complex ionization equilibria and solubility behaviour, such validation becomes particularly critical.

6.1 Statistical Approaches for Model Validation

Quantitative comparison between predicted and experimental values is typically assessed using statistical parameters such as the coefficient of determination (R^2), root mean square error (RMSE), and mean absolute error (MAE). A high R^2 value indicates strong agreement between predicted and observed data, while low RMSE and MAE values reflect minimal deviation. These statistical metrics provide a standardized framework for evaluating the performance of computational models across different datasets. Early studies demonstrated that incorporating ionization effects into solubility prediction models significantly improves correlation with experimental data. Johnson et al. (2007) reported that models accounting for intrinsic solubility and ionization achieved superior predictive accuracy compared to those based solely on lipophilicity. Similarly, Raevsky et al. (2015) conducted a comparative analysis of local and global predictive models, highlighting that hybrid approaches integrating multiple descriptors yielded improved performance in solubility prediction. In the case of pKa prediction, the inclusion of electronic and structural descriptors has been shown to enhance model reliability. Cruciani et al. (2009) and Lee et al. (2007) demonstrated that computational models incorporating molecular interaction fields and atomic charges achieve strong correlation with experimental potentiometric data. High-throughput prediction systems have further improved validation efficiency, enabling rapid comparison of predicted ionization constants with experimental values across large compound libraries (Strope et al., 2018).

6.2 Correlation in Antihypertensive Drug Studies

Several studies have specifically evaluated the correlation between in silico predictions and experimental measurements for antihypertensive agents. Sharma et al. (2026) investigated the physicochemical behavior of captopril in aqueous systems using both computational and experimental approaches, demonstrating good agreement between predicted and observed ionization characteristics. Similarly, Leon et al. (2023) employed in silico tools to develop dissolution methods for hydrochlorothiazide and valsartan, achieving reliable prediction of drug behaviour under physiological conditions. Studies focusing on angiotensin receptor blockers and beta-blockers have also highlighted the importance of accurate ionization modelling. Kritsi et al. (2013) used in silico techniques to investigate the interaction of telmisartan with its target receptor, emphasizing the role of ionization in drug binding and pharmacological activity. Likewise, Oktaviani et al. (2023) demonstrated that computational predictions of physicochemical properties can effectively guide the design of novel antihypertensive derivatives. In addition to small-molecule drugs, peptide-based antihypertensive agents have also been studied using combined in silico and experimental approaches. Abdelhedi et al. (2017) and Zheng et al. (2019) reported that molecular docking and computational analysis of ACE-inhibitory peptides showed strong correlation with experimentally observed antihypertensive activity. These findings underscore the broader applicability of in silico modeling across different classes of therapeutic agents.

6.3 Integration of Solubility and Ionization Data

The correlation between predicted and experimental solubility is inherently linked to the accuracy of pKa prediction, as solubility is strongly influenced by the ionization state of the drug. Studies have shown that incorporating pKa values into solubility models significantly enhances predictive performance. Lobell and Sivarajah (2003) demonstrated that combining calculated pKa and lipophilicity values improves the prediction of aqueous solubility and pharmacokinetic parameters. Comparative studies between experimental techniques have further validated computational predictions. Glomme et al. (2005) reported good agreement between miniaturized shake-flask methods, potentiometric titration, and calculated solubilities, highlighting the reliability of integrated approaches. Similarly, Lucero-Borja et al. (2020) emphasized that combining potentiometric and shake-flask data provides a comprehensive validation framework for in silico models.

6.4 Sources of Deviation Between Predicted and Experimental Data

Despite generally strong correlations, discrepancies between predicted and experimental values are frequently observed. These deviations can arise from several factors, including limitations in computational models, experimental variability, and the intrinsic complexity of drug molecules. One of the primary challenges in solubility prediction is the inability of many models to accurately account for crystal lattice energy and polymorphism. Differences in solid-state forms can significantly influence solubility but are often not considered in computational models. Hewitt et al. (2009) highlighted that solubility prediction remains a challenging task due to the multifactorial nature of solvation processes. Similarly, the presence of multiple ionizable groups and conformational flexibility can complicate pKa

prediction. Wenlock and Barton (2013) noted that structural complexity and intramolecular interactions can lead to deviations between predicted and experimental ionization constants. Additionally, environmental factors such as temperature, ionic strength, and solvent composition can affect experimental measurements, further contributing to discrepancies.

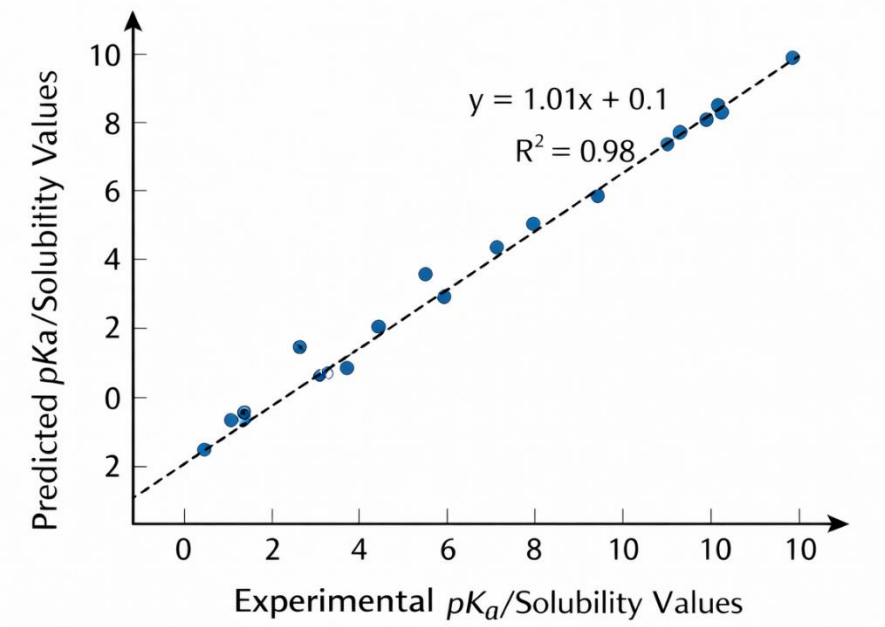

Figure 3. Correlation plot showing predicted vs experimental pKa/solubility values for representative antihypertensive drugs, illustrating linear relationship with high R^2.

6.5 Implications for Drug Development

The integration of in silico and experimental data provides a powerful approach for optimizing drug candidates and reducing development time. Accurate prediction of ionization and solubility enables the identification of potential formulation challenges at an early stage, thereby facilitating rational design strategies. Furthermore, validated computational models can be used to simulate drug behaviour under various physiological conditions, supporting decision-making in formulation and clinical development.

Table 3. Summary of Selected Studies Correlating In Silico and Experimental Data

Study	Drug/Compound	Methodology	Key Outcome
Sharma et al., 2026	Captopril	In silico + experimental	Strong agreement in ionization behaviour
Leon et al., 2023	Valsartan/HCTZ	In silico dissolution modelling	Accurate prediction of dissolution
Abdelhedi et al., 2017	ACE peptides	Docking + in vitro	High correlation with activity
Glomme et al., 2005	Multiple drugs	Shake-flask vs potentiometric	Good agreement across methods
Lobell & Sivarajah, 2003	Various compounds	pKa + logP modelling	Improved solubility prediction

In conclusion, the correlation between in silico predictions and experimental data is a critical determinant of model reliability. While significant progress has been made in improving predictive accuracy, the integration of computational and experimental approaches remains essential for addressing the complexities of antihypertensive drug development. A balanced and validated framework that combines both methodologies offers the most effective strategy for accurate physicochemical profiling.

7. Challenges and Limitations

Despite significant advancements in both in silico modelling and experimental methodologies, several challenges continue to limit the accuracy and applicability of predicting ionization and solubility profiles of antihypertensive drugs. These limitations arise from the inherent complexity of molecular systems, constraints in computational algorithms, and variability in experimental conditions. One of the primary challenges in in silico prediction lies in the accurate modelling of structurally complex molecules. Many antihypertensive agents possess multiple ionizable groups, tautomeric forms, and conformational flexibility, all of which complicate the prediction of pKa values. Computational models often rely on simplified assumptions and may fail to fully account for intramolecular hydrogen bonding, electronic

delocalization, and solvent interactions. As a result, discrepancies between predicted and experimental ionization constants are frequently observed, particularly for amphoteric compounds (Wenlock & Barton, 2013).

Solubility prediction presents an even greater challenge due to its dependence on multiple interrelated factors, including crystal lattice energy, polymorphism, particle size, and solvent effects. Many computational models do not adequately incorporate solid-state properties, leading to significant deviations from experimentally measured solubility values. Hewitt et al. (2009) emphasized that solubility prediction remains one of the most difficult tasks in computational chemistry due to the complex thermodynamics of dissolution. Another critical limitation is the quality and diversity of datasets used to train predictive models. Machine learning approaches, while powerful, are highly dependent on the availability of high-quality experimental data. Inadequate or biased datasets can result in overfitting and poor generalizability, limiting the applicability of models to new chemical entities (Raevsky et al., 2015). Additionally, many models are developed using datasets that do not adequately represent the structural diversity of antihypertensive drugs, further reducing predictive reliability.

Experimental techniques, although considered gold standards, are also subject to limitations. Potentiometric titration can be affected by factors such as electrode calibration, ionic strength, and the presence of co-solvents, which may introduce variability in pKa determination. Similarly, the shake-flask method is time-intensive and susceptible to issues such as supersaturation, polymorphic transitions, and adsorption of drug molecules onto container surfaces. These factors can lead to variability in solubility measurements and complicate the validation of computational predictions (Glomme et al., 2005; Lucero-Borja et al., 2020). Furthermore, the lack of standardized protocols across laboratories can hinder reproducibility and comparability of results. Differences in experimental conditions, analytical techniques, and data interpretation methods can contribute to inconsistencies in reported values, thereby affecting the validation of in silico models. Overall, while both computational and experimental approaches have significantly advanced the field of physicochemical profiling, their limitations highlight the need for integrated methodologies. Combining robust computational models with standardized experimental validation can help overcome these challenges and improve the reliability of predictions in antihypertensive drug development.

8. Future Perspectives

The field of physicochemical prediction in drug development is undergoing rapid transformation, driven by advances in computational science, data analytics, and experimental automation. Future developments in in silico modelling of ionization and solubility are expected to focus on improving predictive accuracy, expanding applicability to structurally complex molecules, and enhancing integration with experimental validation techniques. One of the most promising directions is the application of artificial intelligence (AI) and deep learning in physicochemical property prediction. Unlike traditional QSPR models, which rely on predefined descriptors, deep learning algorithms can automatically extract relevant features from molecular structures and capture complex non-linear relationships. Recent studies have demonstrated that integrating machine learning with in silico modelling significantly improves the prediction of pharmacokinetic parameters, including solubility and bioavailability (Schneckener et al., 2019). As larger and more diverse datasets become available, these models are expected to achieve higher levels of accuracy and generalizability. Another emerging trend is the integration of physicochemical predictions with physiologically based pharmacokinetic (PBPK) modelling. PBPK models simulate drug absorption, distribution, metabolism, and excretion using physiological parameters, enabling a more comprehensive understanding of drug behaviour in vivo. By incorporating predicted pKa and solubility data into PBPK frameworks, researchers can better predict drug performance under various physiological conditions, including disease states such as hypertension. This integrated approach has the potential to bridge the gap between in vitro, in silico, and in vivo studies, thereby improving translational outcomes (Duque, 2016).

Advancements in experimental techniques are also expected to complement computational developments. High-throughput screening platforms and automated analytical systems are being developed to generate large volumes of high-quality physicochemical data. These data can be used to train and validate computational models, thereby enhancing their predictive reliability. Faller et al. (2006) highlighted the importance of high-throughput in vitro profiling in supporting drug discovery, and similar approaches are likely to play a crucial role in future research. In addition, there is growing interest in developing hybrid models that combine computational predictions with experimental data in a unified framework. Such models can leverage the strengths of both approaches, providing more accurate and reliable predictions. For instance, integrating potentiometric and shake-flask data with in silico

predictions can improve the characterization of pH-dependent solubility profiles, particularly for drugs with multiple ionizable groups.

From a regulatory perspective, the increasing acceptance of computational modelling in drug development is expected to drive further innovation. Regulatory agencies are increasingly recognizing the value of in silico tools in supporting decision-making processes, particularly in the context of Quality by Design (QbD) and risk assessment. This trend is likely to encourage the adoption of integrated modelling approaches in both academic and industrial settings. Overall, the future of in silico modelling of ionization and solubility lies in the development of integrated, data-driven frameworks that combine computational and experimental methodologies. Such approaches have the potential to significantly enhance the efficiency and reliability of drug development, particularly for complex therapeutic classes such as antihypertensive agents.

9. Conclusion

The accurate prediction and characterization of ionization and solubility profiles remain fundamental to the successful development of antihypertensive drugs. These physicochemical properties directly influence drug absorption, bioavailability, and therapeutic performance, making them critical determinants in formulation design and pharmacokinetic optimization. The emergence of in silico modelling approaches has significantly enhanced the ability to predict pKa and solubility, enabling rapid screening and rational selection of drug candidates during early-stage development. Computational methods, including QSPR models, quantum chemical calculations, and machine learning algorithms, have demonstrated considerable progress in predicting physicochemical parameters. However, their reliability is inherently dependent on validation through experimental techniques. Potentiometric titration and shake-flask methods continue to serve as gold standards for determining ionization constants and equilibrium solubility, respectively. The integration of these experimental approaches with computational predictions provides a robust framework for physicochemical profiling. In the context of antihypertensive agents, the structural diversity and presence of multiple ionizable groups present unique challenges that necessitate a combined modelling–experimental strategy. Studies have consistently shown that incorporating ionization data into solubility models improves predictive accuracy, while experimental validation ensures the reliability of computational outputs. Despite existing limitations, the convergence of in silico and experimental methodologies offers a powerful approach for optimizing drug design and development. Future advancements in artificial intelligence, high-throughput experimentation, and integrated modelling frameworks are expected to further improve the accuracy and applicability of physicochemical predictions. Overall, a synergistic approach that combines computational efficiency with experimental rigor represents the most effective strategy for advancing antihypertensive drug development and ensuring improved therapeutic outcomes.

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