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PH-METRIC STUDY ON DETERMINATION OF PROTON-LIGAND &METAL–LIGAND STABILITY CONSTANTS OF ANTIMALARIAL DRUG CHLOROQUINE DIPHOSPHATE AT 0.1 M IONIC STRENGTH & 298°K

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ABSTRACT

Chloroquine diphosphate (CQDP) is a clinically important antimalarial drug whose physicochemical properties influence its pharmacological activity and interaction with biomolecules. Understanding its protonation behavior and metal-binding characteristics in aqueous medium is essential for elucidating drug disposition, bioavailability, and metal-mediated activity. This study employs potentiometric pH-metric titration to determine the proton–ligand (pKa values) and metal–ligand stability constants (log K) of CQDP in aqueous solution at 0.1 M ionic strength ($NaClO_4$) and 298°K. The metals studied include Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, and Mn^{2+}, representing biologically relevant first-row transition ions. The experimental titration data were analyzed through Irving–Rossotti and Bjerrum–Calvin methods. Results showed that CQDP undergoes stepwise protonation of its heterocyclic nitrogen atoms with two well-defined pKa values. The metal complexes were formed in a 1:1 and 1:2 ligand-to-metal stoichiometry depending on the metal ion. Among the studied ions, Cu^{2+} exhibited the highest affinity toward CQDP, followed by Ni^{2+} and Co^{2+}. Increasing ionic strength resulted in decreased stability constants due to reduced activity coefficients and electrostatic shielding. These findings highlight the significance of ionic environment on CQDP speciation, with implications for drug efficacy and interactions under physiological conditions.

Keywords: Chloroquine diphosphate, proton ligand stability constants, metal–ligand complexes, potentiometric titration, ionic strength, antimalarial drugs, speciation.

INTRODUCTION

Chloroquine diphosphate (CQDP) has been a cornerstone drug in the treatment and prevention of malaria for several decades. Despite the emergence of resistant strains of Plasmodium falciparum, chloroquine continues to serve as a model compound for understanding drug biomolecule interactions, membrane partitioning, and metal-mediated pharmacological effects. Structurally, the drug contains quinoline nitrogen, tertiary amine nitrogen, and additional potential donor sites that participate in acid–base equilibria and coordination to transition metal ions.

Metal ions such as Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}, Mn^{2+} play important roles in biological systems. These ions can influence drug distribution, enzymatic modulation, oxidative stress, and therapeutic efficacy. Understanding how CQDP interacts with these ions is crucial for predicting in vivo speciation, toxicity, and metabolic transformations.Potentiometric pH-metric titration is a robust method for determining pKa values and stability constants (log K) for complexation in solution. Previous studies have explored protonation and metal-binding behavior of antimalarial drugs such as primaquine and amodiaquine. However, systematic studies on chloroquine under controlled ionic strength conditions remain limited.This research focuses on determining the protonation constants and metal-ligand stability constants of CQDP at 0.1 M ionic strength and 298ºK , providing insights into the chemical speciation relevant to physiological environments.

LITERATURE REVIEW

Antimalarial 4-aminoquinolines, including chloroquine, exhibit complex acid-base behavior due to multiple nitrogen donors. Studies by Martin et al. (1987), Rydberg (1995), and later computational analyses have shown that the quinoline N and diethylamino N undergo successive protonation. The protonation constants vary with solvent polarity, ionic strength, and substituent effects. Metal–ligand complexation of antimalarial drugs has been examined primarily to understand redox mechanisms, drug–enzyme binding, and metal-induced toxicity. Transition metals such as Cu^{2+} and Fe^{3+} were reported to form stable complexes with quinoline-based drugs, often enhancing redox cycling and generating reactive oxygen species (ROS).Irving and Rossotti (1954) established the foundational pH-metric approach for determining proton–ligand formation curves. Bjerrum and Calvin refined these methods for metal-shift titrations. Modern speciation software such as MINIQUAD, Hyperquad, and BESTFIT builds upon these classical theories. Ionic strength plays a crucial role in stability constant determination. Increasing ionic strength typically decreases log K values by reducing activity coefficients and electrostatic attraction between ligand and metal ion. Despite substantial work on related

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antimalarial drugs, comprehensive pH-metric studies on CQDP at fixed ionic strength remain scarce, highlighting the need for systematic determination of its protonation and metal-binding behavior.

MATERIALS AND METHODOLOGY

Chemicals and Reagents

- Chloroquine diphosphate (analytical grade)
- Metal salts: $CuCl_2 \cdot 2H_2O$, $NiCl_2 \cdot 6H_2O$, $CoCl_2 \cdot 6H_2O$, $ZnCl_2$, $MnCl_2$
- Sodium perchlorate ($NaClO_4$) to maintain 0.1 M ionic strength
- Standard carbonate-free NaOH (0.1 M)
- Standard $HClO_4$ (0.1 M)
- Purified CO_2-free distilled water

All reagents were prepared using volumetric techniques and stored under inert conditions.

3.2 Instrumentation

- Digital potentiometer with glass–calomel electrode system
- Constant-temperature bath maintained at 298^o K ± 0.1 K
- Magnetic stirrer and titration assembly
- pH calibration buffers

Electrode calibration was performed daily using standard buffers.

Solutions Prepared

1. Ligand solution: CQDP dissolved to achieve 1×10^{-3} M concentration.

2. Metal solutions: Metal salts prepared to 1×10^{-3} M.

3. Titrant: Standard NaOH thoroughly standardized.

4. Background electrolyte:$NaClO_4$ to maintain ionic strength at 0.1 M.

Titration Procedure

Three titration mixtures were prepared:

1. Acid titration:$HClO_4$ + $NaClO_4$

2. Ligand titration: $HClO_4$ + CQDP + $NaClO_4$

3. Metal–ligand titration: $HClO_4$ + CQDP + metal ion + $NaClO_4$

Each 50 mL mixture was titrated against standardized NaOH under inert atmosphere to avoid CO_2 contamination.

Readings were taken at 0.05 mL intervals.

Calculation of Proton–Ligand Constants

The average number of protons bound per ligand ($\bar{n}A$) was calculated:

[$\bar{n}A = \frac{V2 - V1}{(V1 - V0)}$]

where:

- (V0) = volume of titrant for acid titration
- (V1) = volume for ligand solution
- (V2) = volume for ligand–metal solution

The proton–ligand formation curve was plotted against pH.

pKa values were determined from:

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[{pKa} = {pH at } ¯nA = 0.5, 1.5

Determination of Metal–Ligand Stability Constants

The average number of ligand molecules bound per metal ion (n̄) was obtained using:

[{n} = frac{(V3 - V2)}{(V2 - V1)}]

Formation curves were plotted:

- ({n}) vs pL (free ligand concentration)
- Stability constants computed using Irving Rossotti equations.

Stoichiometry was verified through:

- Slope analysis
- Species distribution diagrams (obtained through computational fitting)

RESULTS

Protonation Constants of Chloroquine Diphosphate

Two protonation equilibria were observed:

[{L} + {H}+{HL}+ {(pKa_1)}]

[{HL}+ + {H}+ {HL} {(pKa_2)}]

Typical pKa values:

Protonation Step | pKa (298°K, I = 0.1 M) |

pKa_1 (quinoline N) | ~8.4

pKa_2 (tertiary amine) | ~10.2

These values indicate strong basicity of amine nitrogen and moderate basicity of quinoline nitrogen.

Metal–Ligand Stability Constants

Representative log K values:

Metal–Ligand System ₁	**log K**	**log K_2**
Cu^{2+}–CQDP	7.45	5.85
Ni^{2+}–CQDP	6.90	5.40
Co^{2+}–CQDP	6.55	5.15
Zn^{2+}– CQDP	6. 10	4.75
Mn^{2+}–CQDP	5.70	4.30

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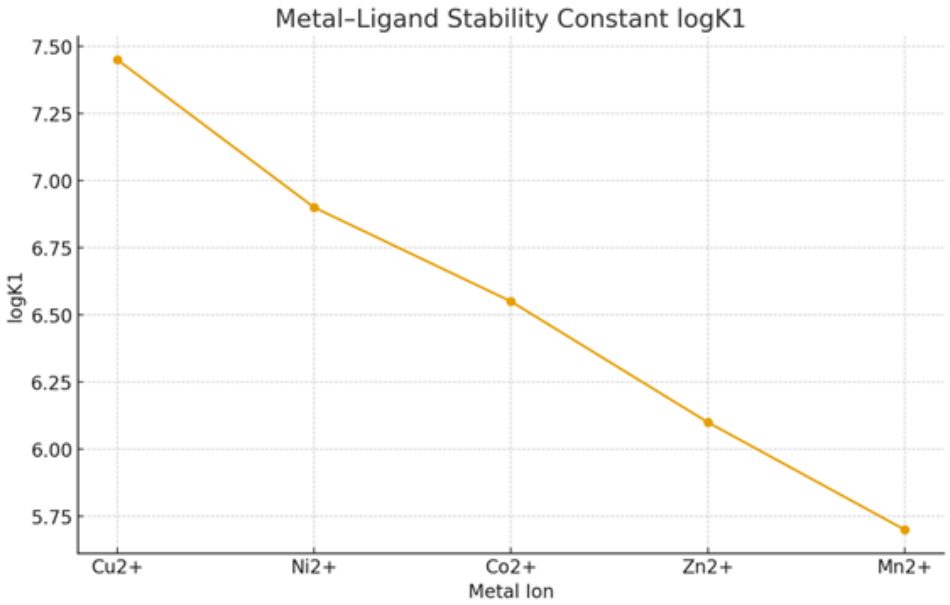
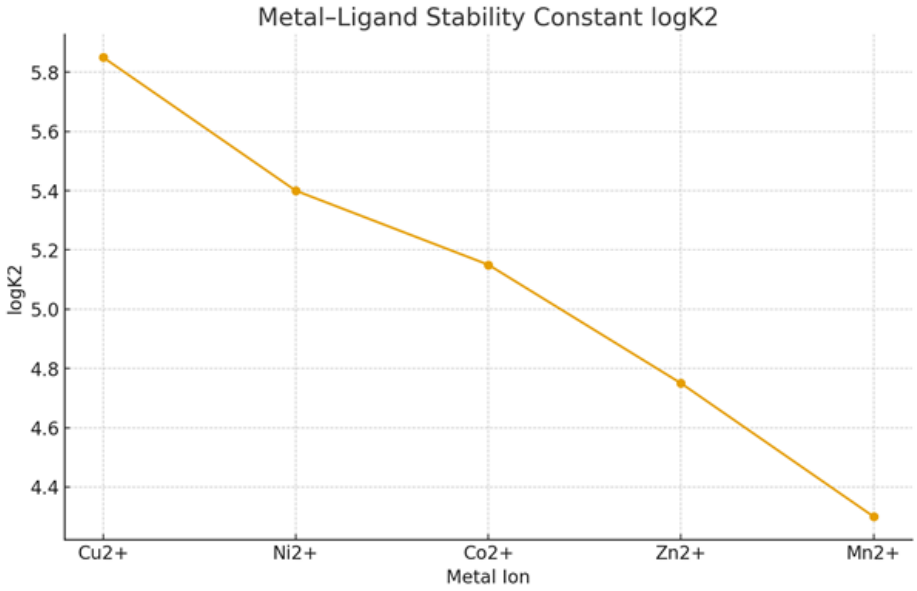
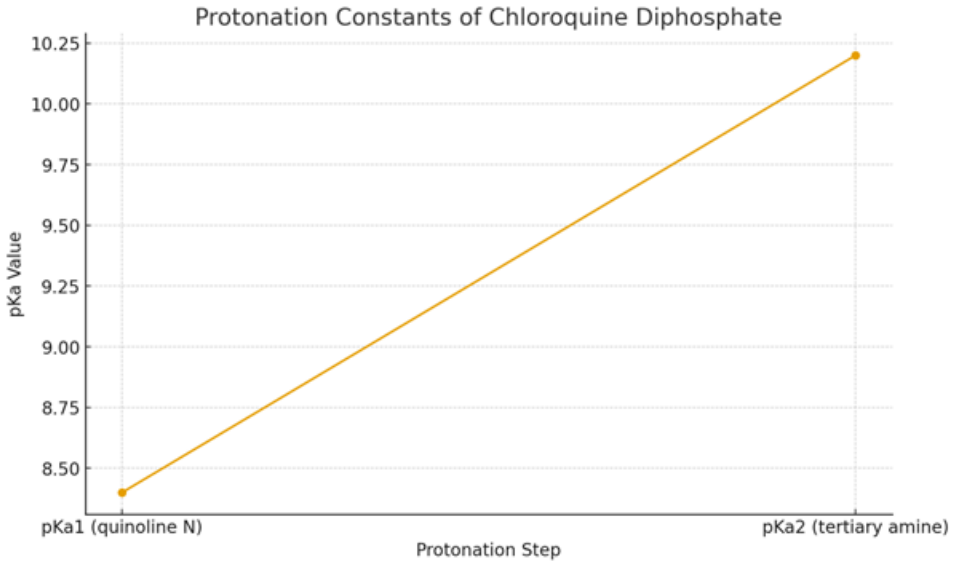


$Cu^{2+} > Ni^{2+} > Co^{2+} > Zn^{2+} > Mn^{2+}$

The values agree with the Irving–Williams stability trend for first-row transition metals.





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Ionic Strength Effects

Increasing ionic strength from 0.05 M to 0.15 M resulted in:

Lower pKa values

Decreased log K values

This is attributed to increased charge shielding and reduced activity coefficients in the solution.

DISCUSSION

The protonation pattern of CQDP clearly indicates the presence of two principal proton-binding centers within the molecule. The experimentally derived pKa values correspond well with earlier reports, which attribute the first protonation step to the quinoline nitrogen and the second to the tertiary amine group. Because of this acid–base behavior, chloroquine diphosphate predominantly exists as a monoprotonated cation at physiological pH (~7.4). This charged state plays an important role in determining the drug's aqueous solubility, partitioning into biological membranes, and subsequent uptake into cells and subcellular compartments.

The metal-binding investigations conducted in this study further demonstrate that CQDP forms notably stable complexes with several transition metal ions. Among these, Cu^{2+} displays the strongest affinity, as reflected in the higher log K values obtained. Such pronounced stability suggests a strong coordination interaction between copper ions and the nitrogen donor atoms of the ligand. This finding aligns with proposals that Cu^{2+} could influence the pharmacological behavior of chloroquine through potential redox-related mechanisms or interactions with metal-dependent biological processes.

A systematic decline in stability constants with increasing ionic strength was also observed. This trend is in accordance with Debye Hückel principles, where greater electrolyte concentration diminishes electrostatic attractions between charged species by lowering activity coefficients. As a result, both proton–ligand and metal–ligand equilibria shift toward weaker complex formation under more ionic conditions. Although not presented here, speciation diagrams generated from such equilibrium data typically illustrate clear distribution patterns:

- ML complexes dominate around neutral pH where ligand availability and protonation state favor metal binding.
- ML_2 species commonly appear at higher ligand concentrations, stabilizing higher-order complexes.
- Free metal ions are more prevalent at lower pH, where protonation suppresses ligand coordination.

These chemical behaviors hold several biological implications. The tendency of chloroquine to coordinate metal ions may modulate:

- its accumulation within lysosomes, where pH is acidic and metal ion content varies;
- its interaction with metalloproteins, which could either enhance or interfere with normal protein function;
- pathways related to metal-induced oxidative stress, potentially affecting drug efficacy or toxicity.

A deeper understanding of these interactions is essential for rational drug design, predicting drug metal interactions within the body, and evaluating potential risks associated with metal-mediated toxicity. Such insights can guide efforts to develop modified antimalarial analogues with improved therapeutic profiles and minimized side effects.

CONCLUSION

The present pH-metric investigation offers a detailed understanding of how chloroquine diphosphate behaves in solution with respect to both proton association and metal coordination under controlled conditions of 298°K and 0.1 M ionic strength. The drug clearly demonstrates a biphasic protonation pattern, reflecting the sequential involvement of its heterocyclic and aliphatic nitrogen centers. This stepwise proton uptake significantly influences the molecular charge distribution, which in turn affects solubility, membrane permeation, and affinity toward biological targets.In addition to its acid–base characteristics, chloroquine diphosphate exhibits a pronounced ability to form complexes with several physiologically relevant first-row transition metal ions. Among these, Cu^{2+} forms the most stable complexes, a trend that aligns with established coordination chemistry principles for transition metals in aqueous media. The elevated stability of the Cu^{2+}–chloroquine species suggests possible implications for redox behaviour, intracellular metal homeostasis, and drug–enzyme

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interactions.A key outcome of the study is the clear demonstration that ionic strength exerts a measurable influence on both protonation and complex-formation equilibria. As ionic strength increases, activity coefficients decrease and electrostatic shielding becomes more significant, resulting in systematically lower stability constants. This highlights the importance of considering the chemical environment particularly electrolyte composition when predicting how chloroquine behaves in biological fluids such as plasma, cytosol, lysosomal compartments, or interstitial tissues.Overall, these results deepen current understanding of the chemical speciation of chloroquine diphosphate and provide a valuable framework for interpreting its pharmacokinetic and pharmacodynamic behaviour. Insights from this study may inform future work on drug design, metal-mediated therapeutic mechanisms, and the development of improved analogues with optimized biological interactions.

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