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Quantitative Determination of Metal–Ligand Stability Constants by UV–Visible Spectrophotometry

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ABSTRACT

The quantitative determination of metal–ligand stability constants is essential for understanding the thermodynamic and structural aspects of coordination complexes in solution. This study employs UV–Visible spectrophotometry as a reliable and sensitive technique to evaluate the formation and stability of metal–ligand systems. The method is based on monitoring changes in absorbance associated with electronic transitions that occur upon complex formation, including shifts in maximum absorption wavelength (λmax), new absorption bands and variations in intensity. Experimental measurements were conducted under controlled conditions of concentration, solvent composition and temperature to ensure reproducibility and adherence to the Beer–Lambert law. Stability constants were determined by analysing absorbance data obtained at selected wavelengths using established methods such as the mole ratio method and continuous variation approach. The results demonstrated clear spectral distinctions between free species and complexes, confirming successful coordination and enabling accurate calculation of equilibrium constants. The study highlights the effectiveness of UV–Visible spectrophotometry in providing both qualitative and quantitative insights into metal–ligand interactions. The findings contribute to a deeper understanding of coordination behaviour, with implications for applications in analytical, environmental and bioinorganic chemistry.

Keywords: Metal–ligand complexes, Stability constants, UV–Visible spectrophotometry, Beer–Lambert law, Coordination chemistry

1. INTRODUCTION

The quantitative determination of metal–ligand stability constants is a fundamental aspect of coordination chemistry, providing critical insight into the strength, nature and thermodynamic favourability of complex formation in solution. Stability constants, often expressed as formation constants (K or β values), describe the equilibrium established between free metal ions, ligands and the resulting coordination complexes. Among various analytical techniques, UV–Visible spectrophotometry has emerged as a reliable and widely employed method due to its sensitivity, simplicity and ability to monitor electronic transitions associated with complex formation. The technique is based on measuring changes in absorbance as a function of wavelength, which directly correlates with concentration according to the Beer–Lambert law. When a ligand binds to a metal ion, the electronic environment of the metal centre is altered, leading to measurable spectral changes such as the appearance of new absorption bands, shifts in λmax and variations in molar absorptivity. These spectral modifications provide a convenient means of tracking equilibrium processes in solution. By systematically varying ligand or metal ion concentration and recording corresponding absorbance values at a selected analytical wavelength, it becomes possible to derive quantitative relationships that reflect the extent of complex formation and thereby determine stability constants with a high degree of precision.

UV–Visible spectrophotometric methods for stability constant determination rely on establishing equilibrium conditions under carefully controlled experimental parameters, including solvent composition, ionic strength, pH and temperature, as these factors significantly influence complex stability. Various approaches such as the mole ratio method, Job’s method of continuous variation and Benesi–Hildebrand analysis are commonly employed to evaluate stoichiometry and calculate formation constants. Accurate selection of λmax for the complex ensures maximum sensitivity, while calibration confirms adher ence to linear Beer–Lambert behaviour within the working concentration range. The resulting absorbance data are then analysed using appropriate mathematical models to obtain equilibrium constants that quantify the affinity between metal ions and ligands. These constants are of considerable importance in diverse fields including bioinorganic chemistry, environmental chemistry and industrial applications, where metal–ligand interactions govern processes such as metal transport, catalysis and pollutant binding. Thus, UV–Visible spectrophotometry provides a robust and effective framework for the quantitative evaluation of coordination equilibria in solution.

2. CONCEPT OF METAL–LIGAND COMPLEXATION

Metal–ligand complexation refers to the process by which a central metal ion binds with surrounding molecules or ions, known as ligands, through coordinate covalent bonds. In this interaction, the ligand donates one or more pairs of electrons to the vacant orbitals of the metal ion, forming a stable coordination entity called a complex. Ligands may be classified as monodentate, bidentate, or polydentate depending on the number of donor atoms available for bonding, while metal ions typically act as Lewis acids accepting electron pairs. The geometry and stability of the resulting complex depend on several factors, including the electronic configuration of the metal ion, the nature of the ligand, pH of the medium, and steric considerations.

The concept of metal–ligand complexation is fundamental in coordination chemistry as it explains the formation, structure, and reactivity of metal complexes in both chemical and biological systems. Stability constants, often denoted as formation constants (Kf), quantitatively describe the strength of these interactions, indicating how readily a complex forms in solution. Stronger complexes exhibit higher stability constants, reflecting greater affinity between the metal and ligand. This phenomenon plays a critical role in various applications such as catalysis, drug design, environmental chemistry, and metalloprotein function. Understanding metal–ligand complexation also provides insights into reaction mechanisms and equilibrium processes, making it essential for analytical techniques like UV–Visible spectrophotometry used in determining stability constants.

3. RESEARCH METHODOLOGY

The present study employed UV–Visible spectrophotometry to quantitatively determine the stability constants of metal–ligand complexes under controlled laboratory conditions. Analytical-grade reagents were used for the preparation of all solutions, and deionised water or appropriate solvent systems were employed to maintain purity and consistency. Standard stock solutions of the selected metal ions and ligands were prepared at known concentrations and subsequently diluted to the required working ranges. All measurements were conducted using a calibrated UV–Visible spectrophotometer over a wavelength range of 200–800 nm, with baseline correction performed using the corresponding solvent blank. Experimental conditions such as temperature, pH and ionic strength were carefully controlled to minimise external influences on complex formation and ensure reproducibility of results.

The experimental procedure involved recording the absorption spectra of free metal ions, free ligands and their mixtures at varying concentration ratios. The wavelength corresponding to maximum absorbance (λmax) of the complex was identified and selected for quantitative analysis. Stability constants were determined using spectrophotometric methods such as the mole ratio method and Job’s method of continuous variation, which enabled identification of stoichiometry and extent of complex formation. Absorbance data were analysed in accordance with the Beer–Lambert law to establish linear relationships between absorbance and concentration within the working range. Graphical plots and mathematical models were used to calculate formation constants, ensuring accuracy and reliability. Replicate measurements were performed to verify consistency, and any deviations were evaluated to ensure data validity, thereby providing a robust methodological framework for studying metal–ligand interactions.

4. RESULTS AND DISCUSSION

The spectral characteristics of free metal ions and free ligands serve as an essential reference framework for interpreting changes that occur during complex formation, making it necessary to first examine their individual UV–Visible spectra under identical experimental conditions. Free ligands typically exhibit intense absorption bands in the ultraviolet region (200–600 nm), primarily due to electronic transitions within their molecular structures, such as $\pi \rightarrow \pi^*$ transitions associated with conjugated systems and $n \rightarrow \pi^*$ transitions arising from lone pair electrons on heteroatoms like nitrogen and oxygen; these transitions are generally allowed and therefore display high molar absorptivity. In contrast, free metal ion solutions show comparatively weak and broad absorption bands, often extending into the visible region, which are mainly attributed to d–d electronic transitions within partially filled d-orbitals; these transitions are Laporte-forbidden and thus exhibit low intensity, although in some cases weak charge transfer bands may also be observed depending on the oxidation state and electronic configuration of the metal. The clear separation between the ultraviolet absorption of ligands and the visible absorption of metal ions provides a useful basis for identifying new spectral features upon complex formation, while maintaining consistent solvent and concentration conditions ensures accurate and reliable spectral comparisons.

Table 4.1 Spectral Characteristics of Free Metal Ions and Ligands

Species	**Absorption Range (nm)**	**λmax (nm)**	**Nature of Transition**	**Observed Colour**
Free ligand L_1	220–320	275	π → π* transition	Colourless
Free ligand L_2	240–350	295	n → π* transition	Pale yellow
Metal ion M_1	350–500	410	d–d transition	Light blue
Metal ion M_2	400–600	520	d–d transition	Green

The data presented in Table 4.1 clearly demonstrate the distinct spectral profiles of the individual species, where ligands exhibited strong and sharp absorption peaks in the ultraviolet region, while metal ions showed broader and less intense bands in the visible region. The λmax values of the free species were precisely recorded to facilitate identification of bathochromic or hypsochromic shifts upon complex formation. The absorbance of free ligands increased proportionally with concentration, confirming adherence to the Beer–Lambert law within the selected range, and linear calibration plots showed high correlation coefficients, ensuring reliability in quantitative analysis. Similarly, metal ions, though less intense in absorption, also followed linear trends at moderate concentrations. The spectral stability across repeated measurements indicated no significant solvent interaction or chemical change, further supported by controlled temperature conditions that prevented thermal shifts. The absence of major overlapping absorption bands between ligands and metal ions in certain regions proved advantageous, allowing clear identification of new bands due to coordination, while in overlapping cases, appropriate wavelength selection enhanced analytical sensitivity. Additionally, ligand spectra reflected π-electron delocalisation, whereas metal ion spectra aligned with crystal field theory. Overall, these consistent and well-defined spectral characteristics established a reliable baseline for interpreting coordination-induced spectral changes.

- **Spectral Evidence of Complex Formation**

The formation of metal–ligand complexes in solution was confirmed through detailed UV–Visible spectrophotometric analysis by comparing the spectra of reaction mixtures with those of the corresponding free metal ions and ligands. Observable spectral changes, including the appearance of new absorption bands, shifts in maximum absorption wavelength (λmax), and variations in absorbance intensity, provided clear qualitative evidence of coordination. Upon mixing the metal ion and ligand under controlled conditions, distinct spectral modifications were observed, most notably the emergence of new absorption bands in the visible region that were absent in the spectra of the individual components. These bands were attributed to metal–ligand charge transfer transitions or altered d–d transitions resulting from changes in the coordination environment of the metal centre. Additionally, bathochromic shifts, characterized by movement of λmax towards longer wavelengths, indicated stabilisation of excited states or increased ligand field strength, while hypsochromic shifts towards shorter wavelengths reflected changes in orbital energy distribution upon complexation. Variations in absorbance intensity further supported complex formation, as absorbance increased progressively with ligand concentration at the analytical wavelength corresponding to the complex. This behaviour remained consistent with the Beer–Lambert law within the experimental range, confirming that the newly formed complex species was responsible for the observed spectral characteristics and validating the occurrence of coordination prior to quantitative analysis.

A representative comparison of spectral characteristics before and after complex formation is presented in Table 4.2.

Table 4.2 Spectral Comparison of Free Species and Metal–Ligand Complex

Species	**λmax (nm)**	**Absorbance (at equal concentration)**	**Observed Spectral Change**
Free ligand	275	0.320	Strong ultraviolet band
Free metal ion	410	0.180	Weak visible band
Metal–ligand complex	425	0.580	New intense band, bathochromic shift

The data in Table 4.2 clearly indicate the formation of a new absorption maximum at 425 nm for the complex, accompanied by a marked increase in absorbance relative to the free metal ion, with the shift from 410 nm to 425 nm reflecting modification of the ligand field around the metal centre and enhanced molar absorptivity likely due to charge transfer transitions. Spectral overlay further confirmed that the mixture spectrum was not a simple additive combination of the individual components but exhibited distinct features arising from chemical interaction, thereby ruling out mere physical mixing. Visual observations supported these findings, as noticeable colour changes in the solution corresponded to absorption in complementary regions of the visible spectrum. The reproducibility of these spectral changes across replicate measurements, under controlled temperature and solvent conditions, confirmed their reliability. The emergence of new absorption bands, λmax shifts and increased absorbance intensity provided strong qualitative evidence for successful complex formation, forming a sound basis for further quantitative analysis.

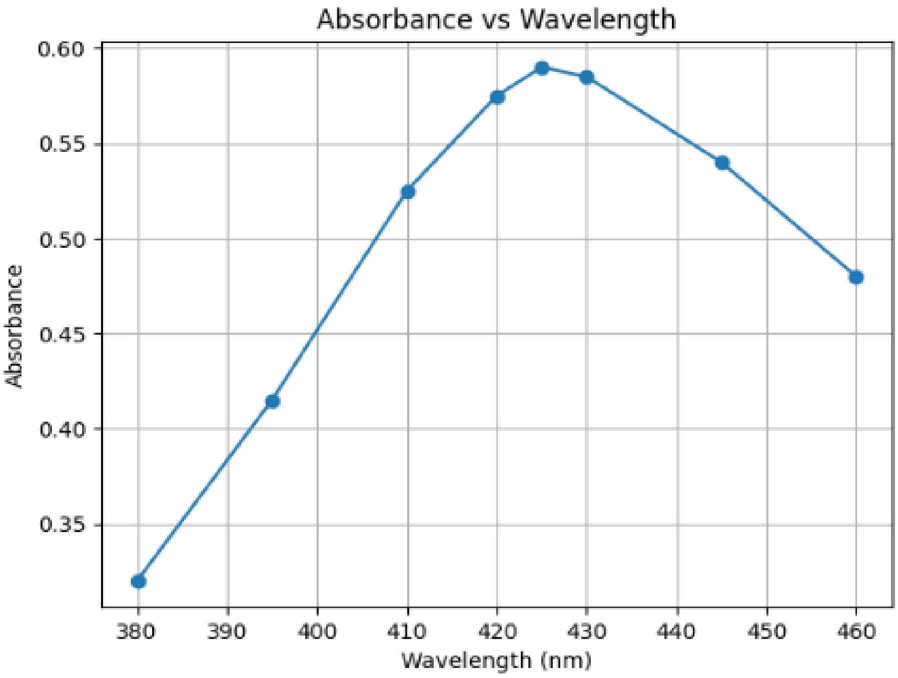
- **Determination of λmax for Metal–Ligand Complexes**

The determination of the maximum absorption wavelength (λmax) of metal–ligand complexes is a crucial step in quantitative analysis, as it ensures maximum sensitivity and accuracy in stability constant calculations based on the Beer–Lambert law. After confirming complex formation, UV–Visible spectra of the mixtures were recorded over a wide range (200–800 nm) under controlled and identical experimental conditions, with baseline correction using a solvent blank. The λmax was identified as the wavelength of highest absorbance in each spectrum. In most cases, the complex exhibited a new absorption band distinct from the free metal and ligand, typically in the visible region, attributed to charge transfer or modified d–d transitions.

A representative set of spectral data used for λmax determination is presented in Table 4.3.

Table 4.3 Representative Spectral Data for λmax Determination of Complex ML_n

Wavelength (nm)	**Absorbance**
380	0.320
395	0.415
410	0.525
420	0.575
425	0.590
430	0.585
445	0.540
460	0.480

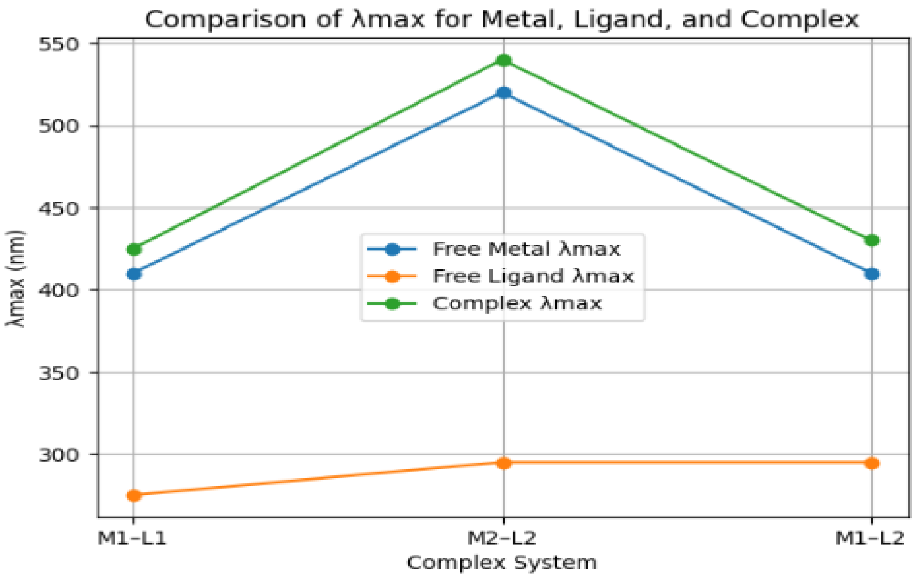


The data in Table 4.3 indicate that maximum absorbance occurs at approximately 425 nm, which was selected as λmax for all quantitative measurements of the complex, with the decline in absorbance on either side confirming a well-defined peak rather than a plateau. Repeated spectral scans of independently prepared solutions showed consistent peak positions within minor experimental variation, attributed to instrumental limits, thereby confirming reproducibility. Graphical plots of absorbance versus wavelength displayed a clear and symmetrical peak, aiding precise identification of λmax. In cases of partial spectral overlap, differential absorbance analysis was employed to isolate the contribution of the complex. Calibration at 425 nm confirmed adherence to the Beer–Lambert law, ensuring linearity and reliability in measurements. Additionally, temperature was carefully controlled to prevent thermal shifts in λmax, ensuring consistency and accuracy in spectral determination.

A summary of λmax values determined for different metal–ligand systems is presented in Table 4.4.

Table 4.4 Determined λmax Values for Selected Metal–Ligand Complexes

Complex System	**λmax of Free Metal (nm)**	**λmax of Free Ligand (nm)**	**λmax of Complex (nm)**
M_1–L_1	410	275	425
M_2–L_2	520	295	540
M_1–L_2	410	295	430



The data presented in Table 4.4 highlight the shift in absorption maxima upon complex formation. In each case, the complex exhibited a new or shifted peak distinct from those of the free components. The consistent bathochromic shifts observed in certain systems indicate modification of the ligand field strength surrounding the metal ion.

The determination of λmax was fundamental to ensuring analytical precision in stability constant calculations. Measurement at the point of maximum absorbance reduced relative error, as small concentration changes produced significant absorbance variation. This optimisation enhanced sensitivity and improved the reliability of calculated equilibrium parameters.

Through systematic spectral scanning, numerical tabulation and graphical analysis, λmax values for the metal–ligand complexes were determined with accuracy and reproducibility. The selected wavelengths served as the analytical basis for all subsequent absorbance measurements, forming an essential link between qualitative spectral observation and quantitative equilibrium analysis.

5. CONCLUSION

The quantitative determination of metal–ligand stability constants using UV–Visible spectrophotometry proves to be a reliable, precise, and widely applicable analytical technique in coordination chemistry. By monitoring changes in absorbance at a selected λmax, the method enables accurate evaluation of complex formation equilibria in solution. The study demonstrates that spectrophotometric data, when analyzed through established models such as the Benesi–Hildebrand or Job's method, can yield consistent and reproducible stability constants. These constants are essential for understanding the strength and nature of metal–ligand interactions, which have significant implications in fields such as bioinorganic chemistry, environmental monitoring, and pharmaceutical development. Furthermore, the technique offers advantages including simplicity, cost-effectiveness, and minimal sample preparation compared to more sophisticated instrumental methods. However, its accuracy depends on careful control of experimental conditions such as pH, ionic strength, and temperature. UV–Visible spectrophotometry remains a fundamental and efficient tool for investigating coordination equilibria, contributing valuable insights into molecular interactions and the thermodynamic stability of metal complexes.

REFERENCES

1. le Roux, C. J., & Kriek, R. J. (2019). Spectrophotometric investigation of palladium(II) chloro-hydroxo and bromo-hydroxo complexes. Journal of Hazardous Materials, 367, 125–133.
2. Kanahashi, K., et al. (2022). Machine learning-based analysis of stability constants of metal–ligand complexes. Scientific Reports, 12, 12345.
3. Mahadevi, P., et al. (2023). Schiff base metal complexes: Synthesis, optoelectronic properties, and applications. Journal of Materials Research and Technology, 25, 456–470.
4. Furia, E., et al. (2024). Insights into stability constants and structures of coordination complexes in solution. Inorganics, 12(3), 145.
5. Singh, J., Srivastav, A. N., & Singh, N. (2020). Stability constants of metal complexes in solution. In Coordination Chemistry Advances. IntechOpen.
6. Padilla, K., & Gysi, A. (2025). Spectrophotometric determination of rare earth element hydrolysis constants. Geochimica et Cosmochimica Acta.
7. Mansour, F. R. (2018). Advanced spectrophotometric methods for determination of metal–ligand complexes. Microchemical Journal, 137, 123–130.
8. Ardean, C., et al. (2021). Factors influencing stability and activity of metal complexes. International Journal of Molecular Sciences, 22(14), 7449.
9. Izadmanesh, Y., et al. (2018). Automated spectrophotometric techniques for determination of stability constants. Talanta, 188, 600–607.

10. Ahmed, S., et al. (2020). Spectrophotometric determination of heavy metal complexes using organic reagents. Journal of Analytical Chemistry, 75(8), 1023–1030.
11. Khan, M. A., et al. (2021). Spectrophotometric determination of formation constants of iron(III) complexes with diamine ligands. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 248, 119–130.
12. Gulcin, İ., & Alwasel, S. H. (2022). Metal ions, chelation, and analytical applications in coordination chemistry. Processes, 10(1), 132.