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**SUPPLEMENTARY MATERIAL**

# EXPERIMENTAL BIOLOGICAL STUDIES OF NOVEL TETRADENTATE HYDRAZONE Cu(II) COMPLEXES FOR POTENTIAL APPLICATIONS IN MEDICINAL CHEMISTRY

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## DNA binding studies

The complex's DNA binding ability was examined using UV-Vis and flourescence spectroscopy, with minor alterations to the previously published literature [1-4]. At room temperature, titrations were carried out with a buffer containing 10 mM Tris-HCI and 50 mM NaCl (pH 7.6). The complicated stock solution was made using 1% (V/V) DMF and 99% (V/V) Tris-HCI buffer. CT-DNA (lyophilized) was dissolved in Tris-HCI buffer overnight at 4 °C and utilized within 2 days as a DNA stock solution.A solution of CT-DNA gave a ratio of UV absorbance at 260 and 280 nm of ca. 1.8–1.9:1, indicating that the DNA was sufficiently free of protein [5]. The DNA concentration was determined from the absorbance at 260 nm using the molar absorption coefficient (6600 M−1 cm−1) at 260 nm [6].

## UV-Visible absorbtion studies

By adding increasing quantities of CT-DNA (0-100 μM) to a constant concentration of complex (100μM), absorption titrations were performed. The reaction mixture was allowed to incubate for 5 minutes before the absorption spectrum was recorded. After each addition, the intrinsic binding constant Kb of complex was calculated by using Wolfe-Shimmer equation (Eq. 1)[7].

$$[DNA]/(\varepsilon_a-\varepsilon_f)=[DNA]/(\varepsilon_b-\varepsilon_f)+ 1/K_b(\varepsilon_b-\varepsilon_f) \qquad \text{(Eq. 1)}$$

where [DNA] is the concentration of DNA in base pairs,$\varepsilon_a$, $\varepsilon_f$, and $\varepsilon_b$ correspond to $A_{obs}$/[compound], the extinction coefficient for the free complex, and the extinction coefficient for the complex fully bound with DNA. In plot of [DNA] / ($\varepsilon_a - \varepsilon_f$) versus [DNA], the intrinsic binding constant $K_b$ is given by the ratio of the slope to y-intercept.

## Viscosity measurements

Viscosity experiments were carried out using an Ubbelodhe viscometer at room temperature. The viscosity of CT-DNA solution (25 μM) was measured in the absence and presence of increasing

amounts of the compound (6.25-50 μM) in tris-HCl buffer (10 mM tris–HCl-NaCl; pH=7.6) containing %5 DMF solution. Flow time was measured three times with a digital stopwatch. Viscosity values were presented as (η/η0) 1/3 versus concentrations of [complex]/[DNA] where η was the viscosity value for DNA in presence of the compounds and η0 was the viscosity value of CT-DNA alone [2].

**DNA cleavage studies**

Plasmid DNA (pBR322) cleavage study of the complex was performed by agarose gel electrophoresis [8]. Stock solution of complex was prepared in DMF and diluted with Tris-HCI buffer to desired concentrations. Supercoiled (SC) DNA (pBR322) (25 ng/μL, 5 μL) in Tris–HCl (100 mM, pH 7.6) was treated with 100 μM of the complex in a total volume of 30 μL, and then incubated at 37 °C for 1-6 h with 1 h intervals in the presence and absence of $H_2O_2$ (5 mM) to obtain the optimal cleavage incubation time. The optimal complex concentrations for cleavage were also studied using the same methodology but with different complex concentrations (12.5, 25, 50, 100, and 200 M). After incubation, the reaction was quenched with 4 liters of loading buffer (0.25 percent bromophenol blue, 30 percent glycerol, 0.25 percent xylene cyanol, and 10 mM EDTA) and loaded on a 1 percent agarose gel with 1 g/mL ethidium bromide. In TBE buffer (40 mM Tris/borate and 1 mM EDTA, pH 8.0), electrophoresis was performed at 80 V for 1.5 hours. UV light was used to photograph and visualize the bands.

**Topoisomerase inhibition assays**

Relaxation tests were carried out with slight changes according to the manufacturer's (TopoGEN) specifications [9, 10]. In a final volume of 20 μL, the topoisomerase I (Topo I) experiment was done in reaction buffer containing 10 mM Tris-HCI (pH 7.9), 150 mM NaCI, 1 mM EDTA, 0.1% BSA, 0.1 mM spermidine, 5% gliserol, and 0.100 μg pHOT1 plasmid DNA. The complex was added to the reactions at different concentrations (5, 10, 25 M) while keeping the overall reaction volume constant (20 μL).3 U Topo I was added to start the reaction, which was then incubated for 30 minutes at 37 °C. Topo I activity was stopped with 2 μL of gel loading "stop" buffer (5% sarkosyl, 0.0025% bromophenol blue and 25% glycerol). Samples were placed directly onto a 1% agarose gel and electrophoresed in 1X TBE buffer (40 mM Tris/borate and 1 mM EDTA, pH 8.0) at 45 V for 3 hours. After 30 minutes of staining with EB (1 g/ml), the gel was destained in distilled water for 15 minutes.

The resulting gels were shot under UV light and visualized using a UV illuminator. Positive controls for Topo I was camptotechin (25 M).

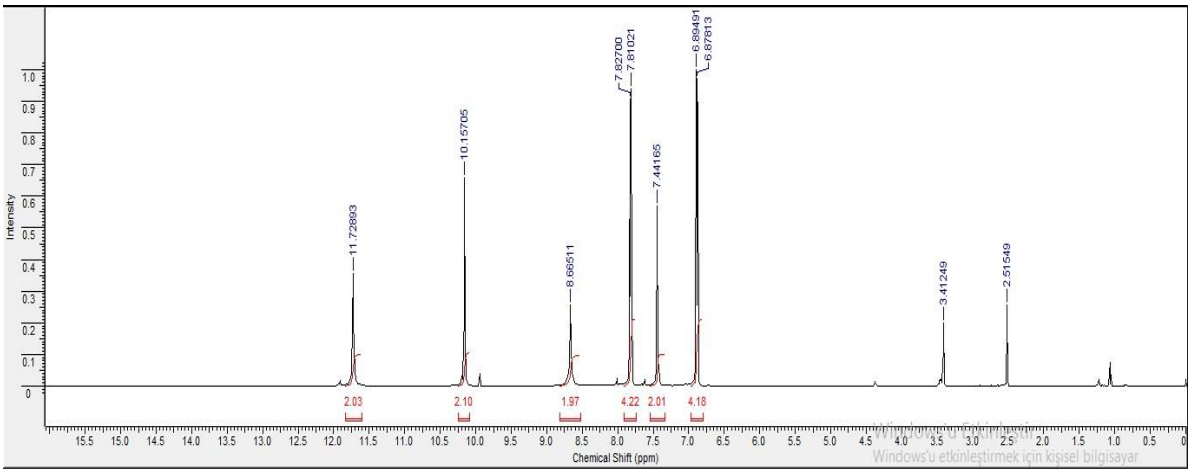


Figure S1:[1]H-NMR Spectrum of $H_2L^1$ Ligand.

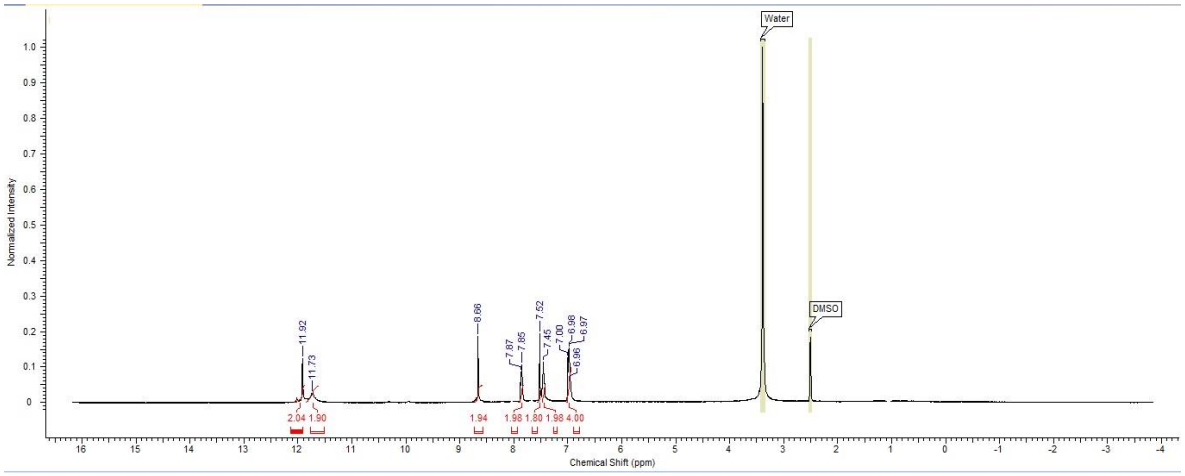


Figure S2:[1]H-NMR Spectrum of $H_2L^2$ Ligand.

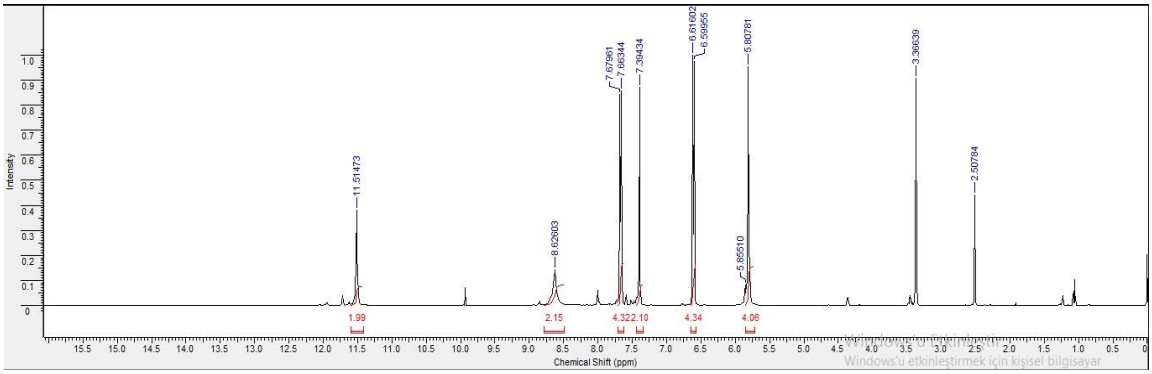


Figure S3:[1]H-NMR Spectrum of $H_2L^3$ Ligand.

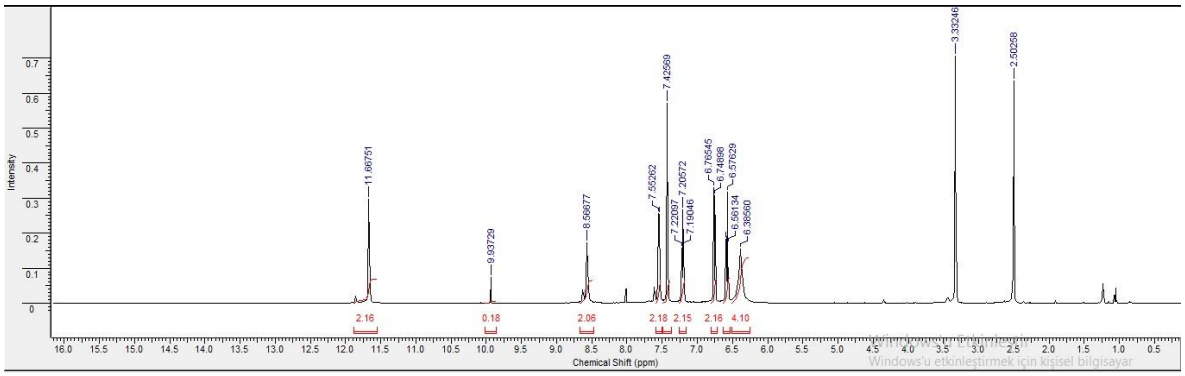


Figure S4:[1]H-NMR Spectrum of $H_2L^4$ Ligand.

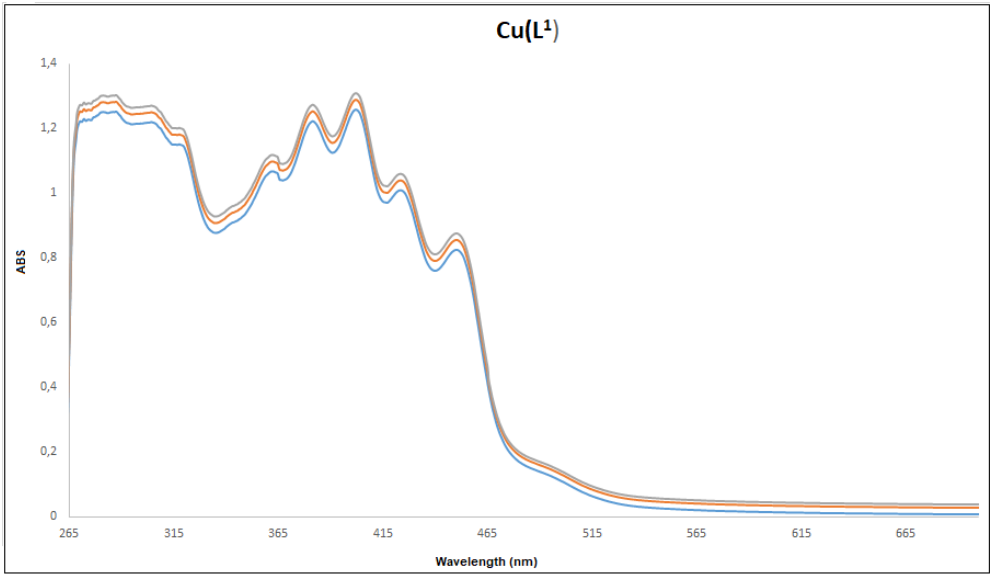


Figure S5: Stability spectrum of $Cu(L^1)$

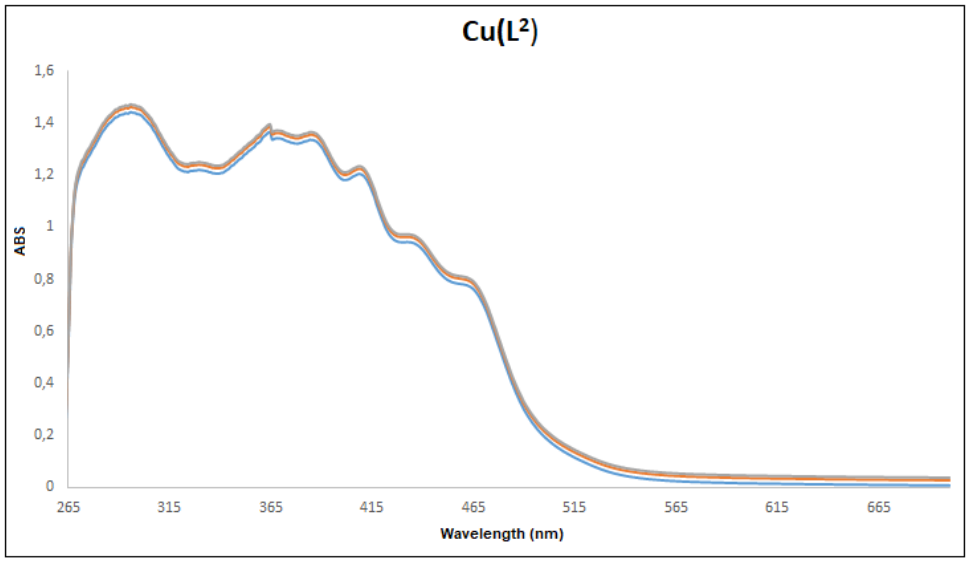


Figure S6: Stability spectrum of $Cu(L^2)$

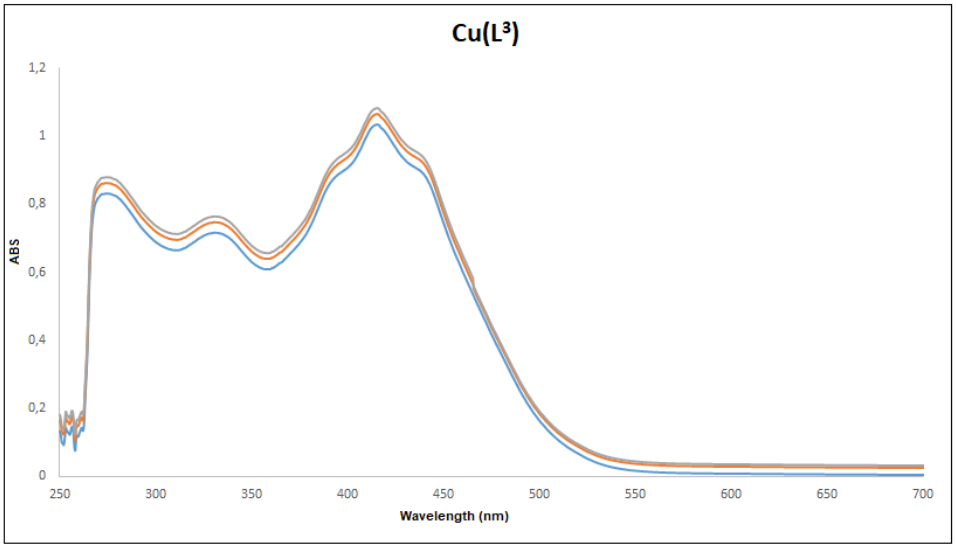


Figure S7: Stability spectrum of $Cu(L^3)$

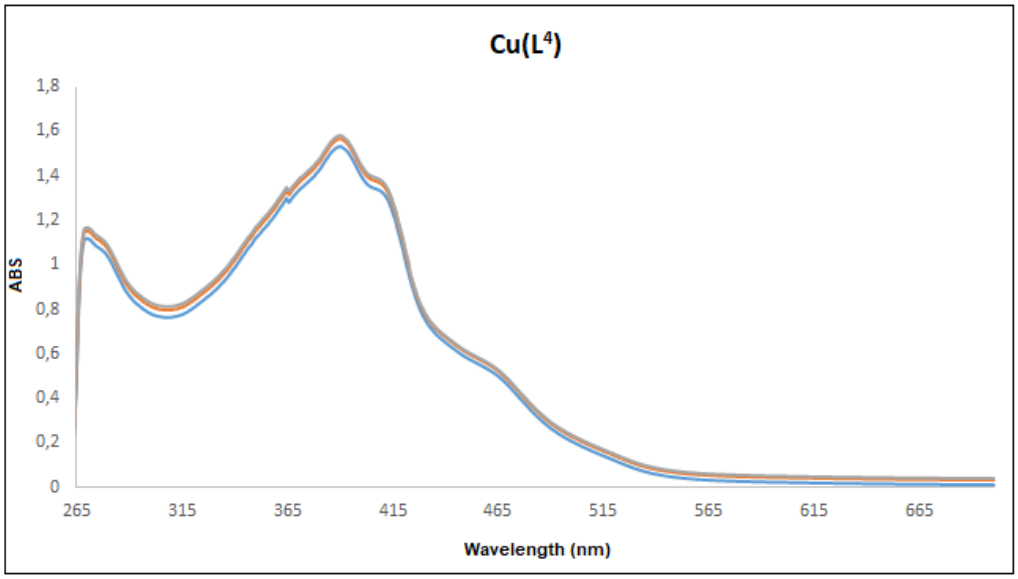


Figure S8: Stability spectrum of $Cu(L^4)$

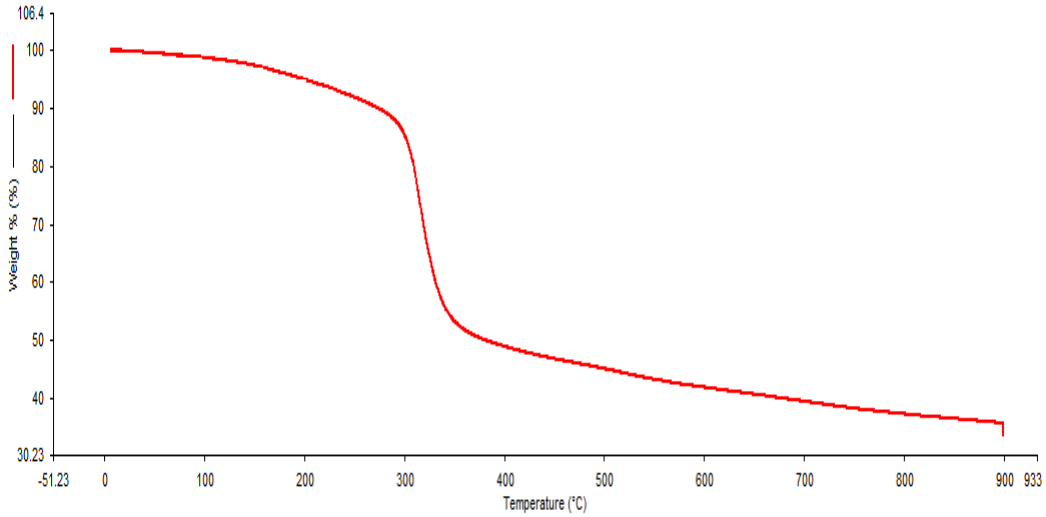


Figure S9: TGA diagram of Cu($L^1$)

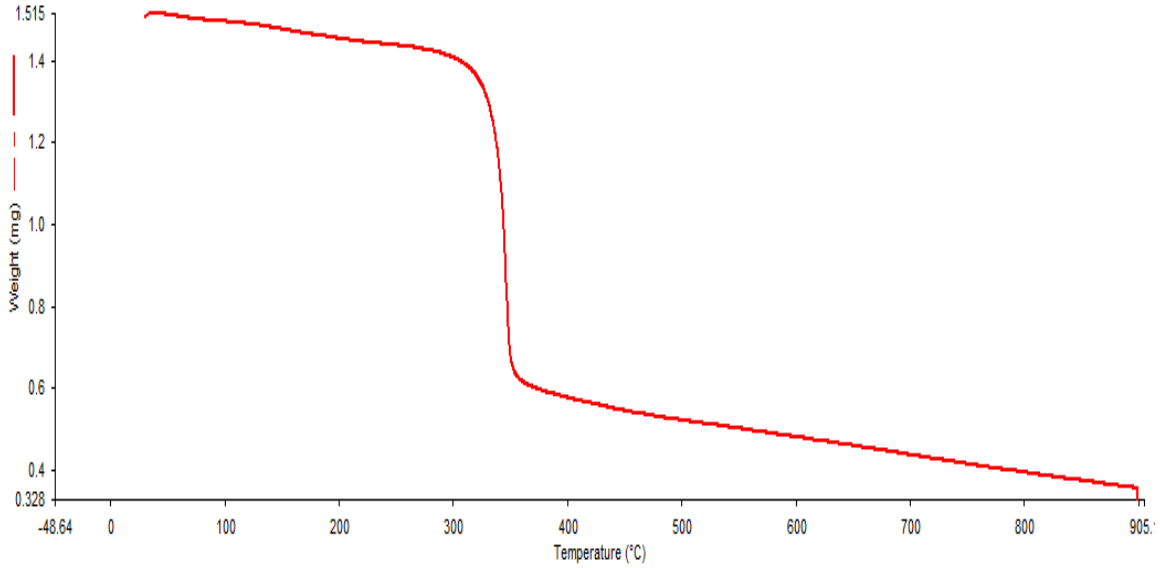


Figure S10: TGA diagram of Cu($L^2$)

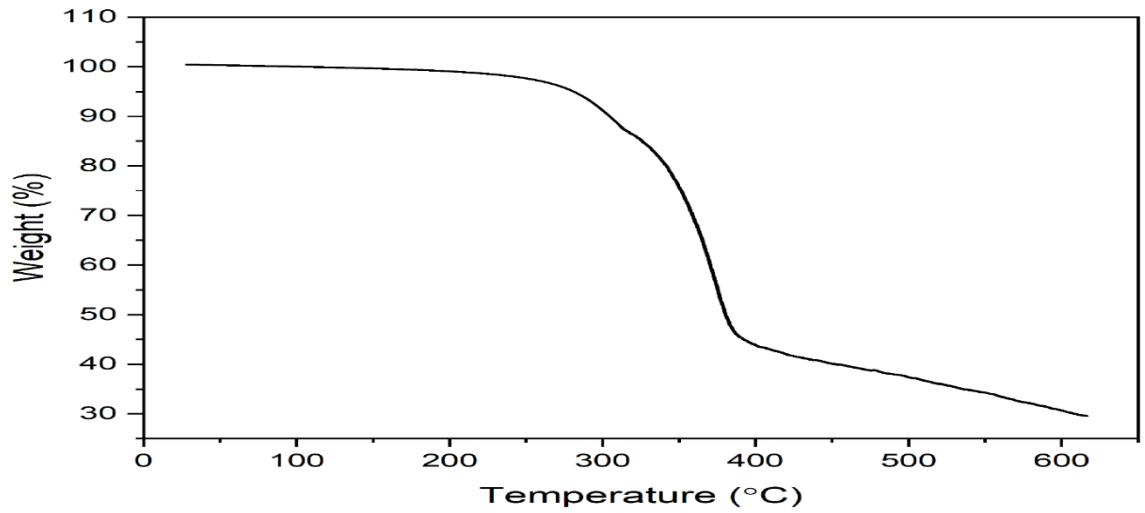


Figure S11: TGA diagram of Cu($L^3$)

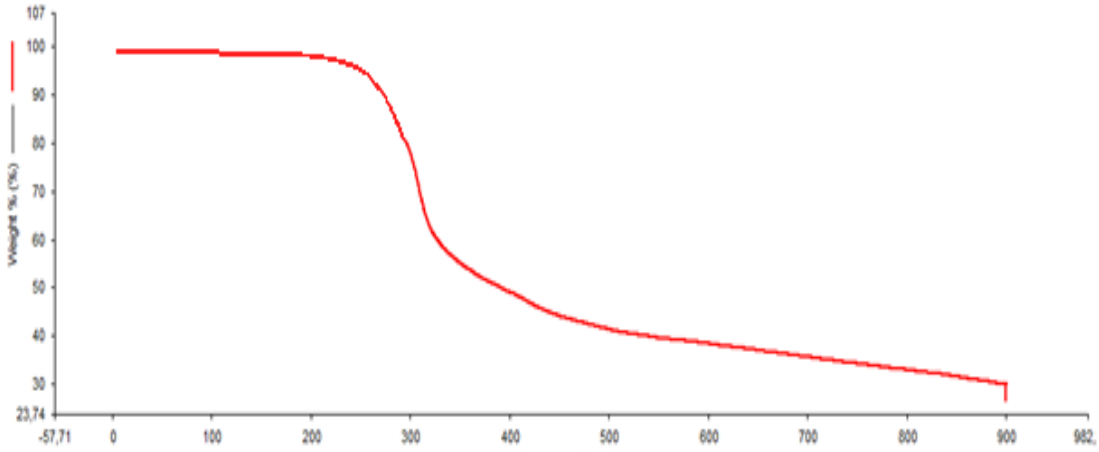


Figure S12: TGA diagram of Cu($L^4$)

## References

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