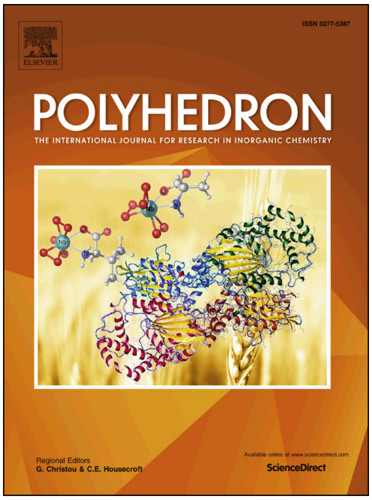

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Synthesis and Spectroscopic Characterization of a Photo-stable Tetrazinc(II)-Schiff base Cluster: A Rare Case of Ligand Centric Phenoxazinone Synthase Activity

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ABSTRACT

Herein, the synthesis and structural characterization and catalytic activity of a novel tetranuclear zinc(II)-Schiff base complex, $[Zn_4(\mathbf{L})_2(\mu_3\text{-}OCH_3)_2(CH_3OH)_2].2CH_3OH$ (**1**), [**L** = *N,N′*-bis(3-methoxysalicylidene)-1,3-diamino-2-propanol] was presented. Single crystal X-ray diffraction structural analysis revealed that the tetra-zinc(II) cluster crystallized in a monoclinic system with $P2_1/c$ space group. Interestingly, three different molecular bridges (methoxido-, alkoxido- and phenoxido-) simultaneously co-existed in assembling tetra-zinc(II) core, which was a very rare observation. To the best of our knowledge, this compound would be the first compound where a diverse coordination aspect was covered by a single solvent as terminal coordinator (CH_3OH), bridging(μ_3-CH_3OH) and solvent for crystallization in the existing scientific literature. The compound showed good photo-stability and excellent luminescence property with higher lifetime at transition state in ethanol. This zinc(II) complex revealed crucial role as an effective catalytic system towards oxidation of 2-aminophenol (2-AP) in ethanol. Additionally, the tetra-zinc(II) complex displayed potential phenoxazinone synthase like activity with momentous turn over number, $k_{cat}(h^{-1}) = 6.19\times10^2$ in ethanol under aerobic condition. ESI-MS and EPR spectral analysis of the reaction mixture between Zn(II) complex and 2-AP recommended that the course of catalysis proceeded through substrate-catalyst adduct formation and authenticated the radical mechanistic pathway in favour of oxidative coupling product. This tetranuclear zinc(II)-Schiff base complex would be considered as the first example that catalyzed the oxidative coupling of 2-aminophenol to aminophenoxazino compound under usual aerobic condition. As complementary, detailed quantum chemical computations, performed with density functional theory (DFT) were well corroborated with the experimental results. This was the first and rarest example where a tetrazinc(II)-Schiff base cluster exhibited catalytic oxidation of 2-AP through ligand centred radical activity.

Keywords: Zinc(II); Schiff base; X-ray structure; Bio-mimicking study; Phenoxazinone synthase activity.

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1. Introduction

Polymetallic cores containing atomic and/or molecular bridges based on first row transition metal ions (3d ions) with an organic backbone have drawn significant interest for their diverse applicability in modern science [1-3]. The investigation to design and construction of metallic clusters are growing on, not only for their stimulating structural aspects but also for their utility in developing the properties of electronic, optical, magnetic, and catalytic materials [4-6]. In all the transition metal ions, "zinc metal ion" remains one of the most fundamental contributors in the biological system and plays significant bio-functions in the living system. In this living world, nature controls elemental distribution in its own way and it is commonly observed that most of the fundamental basic processes are metal centric like Mg(II) in photosynthesis, Fe(II)/(III) in O_2 transport phenomenon, Fe-Mo metal ions in N_2 fixation etc. [7-9]. Therefore, designing of coordination compounds having the suitability to mimic the bio-functionalities of different metalloenzymes has become a serious challenge to the synthetic chemists. Bio-mimicking activities will not only provide lots of important basic information about their bio-functions but also lead to making a clarification of their mechanistic concern on such complex systems [10-12]. Zinc(II) Schiff base complexes are mostly treated as potential photo-active materials [13]. With a d^{10} electronic configuration, Zn(II) ions have a pronounced effect on their photo-physical properties. Unsaturation of coordination number at Zn(II) centre in several zinc complexes make it stronger Lewis acids. Other characteristic features of zinc(II) ions like Lewis acid activation, nucleophile origination, rapid ligand exchange, and stabilization of leaving group, make Zn(II) ion one of the most prevalent metal ion in material sciences [14,15].

Di-, tri-, and polynuclear zinc(II) complexes have created a huge appeal for their existence in the range of important metalloenzymes[16,17] like alkaline phosphatases, zinc-dependent aminopeptidases, and metallo-β-lactamases. After surveying the importance of oxidation reactions in laboratory and industry, it is of paramount interest to evaluate efficient catalysts that enable the course of catalysis in an effective and selective way [18]. In this regards, a (N,O)-donor polydentate Schiff base ligand, *N,N′*-bis(3-methoxysalicylidene)-1,3-diamino-2-propanol and its tetranuclear zinc(II)-Schiff base complex were synthesized. The compound showed good photo-luminescence properties in ethanol medium having higher lifetime span of the transition state. The structural features provided additional appeal to the synthetic chemists since a single solvent (methanol) covered diverse coordination aspects as terminal coordinator (CH_3OH), bridging(μ_3-CH_3OH) and solvent for crystallization and played a significant role in clustering this tetranuclear zinc(II) core. This complex was employed as an effective catalytic system towards 2-aminophenol oxidation in EtOH which revealed a significant turn over number, $k_{cat}(h^{-1}) = 6.19\times10^2$ in ethanol in favour of oxidative coupling of 2-aminophenol. ESI-Ms and EPR spectral analysis of the reaction mixture between Zn(II) complex and 2-AP authenticated that the course of catalysis proceeded through substrate-catalyst adduct formation in combination with the generation of radical in the catalytic cycles.

2. Experimental Section

2.1. Preparation of the Schiff base ligand and complex

(a) *Chemicals, solvents and starting materials*

High purity *o*-vanilin (Sigma, Germany), 2-hydroxy-1,3-diaminopropane (Aldrich, USA), zinc(II) sulphate hexahydrate (Merck, India), and all other solvents were purchased from the respective concerns and used as received. 2-aminophenol (2-AP) was procured from Sigma Aldrich Corporation (St. Louis, MO, USA). All other chemicals and solvents were of analytical grade and were used without further purification.

(b) *General Synthesis*

The Schiff base, **L** was prepared following a reported literature [19]. To prepare the Schiff base ligand, 2-hydroxy-1,3-diaminopropane (0.0890 g, 1 mmol) was refluxed with *o*-vanilin (0.3046 g, 2 mmol) in 20 ml dehydrated alcohol for 6 hours and after 7-10 days yellowish orange coloured compound was isolated from solution, which was dried and stored *in vaccuo* over $CaCl_2$ for subsequent use. Yield ~ 0.330 g (86%). Anal. Calc. for $C_{19}H_{22}N_2O_5$ (**L**): IR (KBr, cm^{-1}): 3365 (ν_{OH}), 1618 ($\nu_{C=N}$), 1333, 1245 (ν_{PhO}); UV-Vis (λ_{max}, nm): 255, 315, 403; ^1H NMR (δ ppm, 400 Mz, DMSO-d^6) δ = 13.55 (s, 1H), 9.92 (s, 1H), 9.92 (s, 1H), 7.26-6.90 (Ar-H, 6H), 5.94 (s, 1H), 3.93 (s, 3H) ppm. [**L**+H^+] m/z 359.16.

A methanolic solution (10 mL) of **L** (0.36 g, 1 mM) was added drop-wise to a methanolic solution (15 mL) of $ZnSO_4$ (0.53 g, 2 mM) and was kept the reaction solution on a magnetic stirrer for 30 minutes. The bright yellow coloured solution was filtered and the supernatant liquid was kept in air for slow evaporation. Yield for **1**: 0.37 g (~69% for **1** based on metal salt).
Anal. Calc. for $C_{44}H_{60}N_4O_{16}Zn_4$ (**1**): C, 45.46; H, 5.20; N, 4.82. Found: C, 45.51; H, 5.17; N, 4.89 %. IR (KBr, cm^{-1}): 3557 (ν_{OH}),1637,1616 ($\nu_{C=N}$); UV-Vis (λ_{max}, Abs, nm, 10^{-4} M, EtOH): 233 (0.83), 277 (1.10), 363 (0.132). [$C_{40}H_{44}N_4O_{12}Zn_4$+$H^+$], m/z 1035.13

2.2. Physical measurements

Infra-red spectrum (KBr) of the complex was recorded with a FTIR-8400S SHIMADZU spectrophotometer in the range 400-3600 cm^{-1}. ^1H NMR spectrum in DMSO-d^6 was obtained on a Bruker Avance 300 MHz spectrometer at 25°C and was recorded at 299.948 MHz. Steady-state absorption spcetral measurements were carried out with a JASCO model V-730 UV-Vis spectrophotometer. Elemental analyses were performed on a Perkin Elmer 2400 CHN microanalyser. Electrospray ionization (ESI) mass spectrum was recorded on a Q-TOF MicroTM Mass Spectrometer. Steady-state fluorescence measurements were carried out with a Hitachi F-7000 spectrofluorimeter. Molar conductivity measurement was operated using a Horiba LAQUA Benchtop DS70 conductivity meter. The X-band EPR spectra were recorded on a Magnettech GmbH MiniScope MS400 spectrometer (equipped with temperature controller TC H03), where the microwave frequency was measured with an FC400 frequency counter.

2.3. X-ray diffraction study

Single crystal X-ray diffraction data for **1** was collected using a Rigaku XtaLABmini (Fixed 2Theta and Distance) diffractometer equipped with Mercury375R (2x2 bin mode) CCD detector. The data were collected with graphite monochromated Mo-Kα radiation (λ=0.71075 Å) at 100 K for **1** using ω scans. The data were reduced using CrysAlisPro 1.171.38.46 (Reference

Rigaku Corporation: Tokyo,Japan) and the space group determination was done using Olex2. The structure was resolved by direct method and refined by full-matrix least-squares procedures using the SHELXL-97 software package using OLEX^2 suite [20,21].

2.4. Catalytic oxidation of 2-AP

In order to examine the phenoxazinone synthase activity, 1×10^{-4} M solution of **1** in EtOH was treated with 100 equiv. of 2-aminophenol (2-AP) under aerobic conditions at room temperature. Absorbance vs. wavelength (wavelength scans) of the solution was recorded at a regular time interval of 11 minutes for aminophenol oxidation in the wavelength range 300-800 nm. Kinetic experiments were performed spectrophotometrically [22,23] with Zn(II) complex, **1** in presence of 2-AP in EtOH at 25°C. 0.04 mL solution of the complex, with a constant concentration of 1×10^{-4} M, was added to 2 mL of 2-AP of a particular concentration (varying its concentration from 1×10^{-3} M to 1×10^{-2} M) to achieve the ultimate concentration of the complex as 1×10^{-4} M. The conversion of 2-AP to APX was monitored with time at wavelength of 434 nm (time scan) for **1** in EtOH. To determine the dependence of rate on substrate concentration, kinetic analyses were performed in triplicate. The phenoxazinone compound was separated in pure form by column chromatography using neutral alumina as column support and benzene-ethyl acetate as an eluant mixture. The compound was isolated in high yield (85% for **1**) by slow evaporation of the eluant. The product was principally confirmed and identified by ^{1}H NMR spectroscopy. ^{1}H NMR data for 2-amino-3H-phenoxazine-3-one (APX), ($CDCl_3$, 400 MHz,) δ_H: 7.61 (m, 1H), 7.46 (m, 3H), 6.48 (s, 1H), 6.39 (s, 1H), 6.27 (s, 1H).

2.5. Detection of the presence of hydrogen peroxide in the catalytic oxidation of 2-AP

To detect the formation of hydrogen peroxide during the catalytic reaction, we followed a reported method [22b]. Reaction mixtures were prepared as in the kinetic experiments. During the course of the oxidation reaction, the solution was acidified with H_2SO_4 to pH = 2 to stop further oxidation after a certain time and an equal volume of water was added. The formed APX was extracted three times with dichloromethane. To the aqueous layer were added 1 ml of a 10% solution of KI and three drops of a 3% solution of ammonium molybdate. The formation of I_3^- could be monitored spectrophotometrically because of the development of the characteristic I_3^- band (λ_{max}= 353 nm).

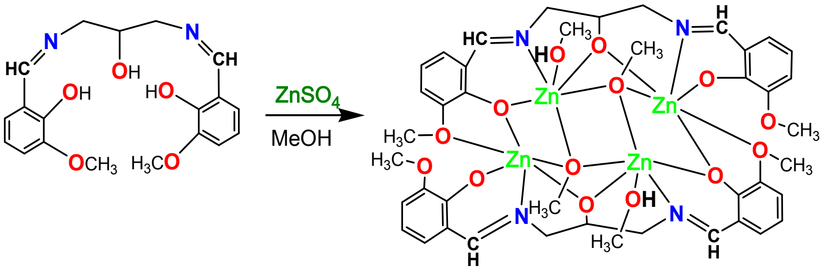
2.6. Theoretical details

To provide theoretical justification, detailed quantum chemical computations were operated using Gaussian 09W programme suite [24] without considering any symmetrical restrictions. The molecular structure of Zn(II)-Schiff base complex was optimized in vacuum utilizing B3LYP theoretical model and 6-311G basis set [25,26]. Then, the optimized structure of vacuum was again optimized in ethanol (ε= 24.852) considering Integral Equation Formalism Polarisable Continuum Model (IEFPCM) [27] as solvent model to corroborate the theoretical outcomes with the experimental results. To ensure the optimized structures to be true minima, stability calculations were performed and the global minima was further reconfirmed with no imaginary frequency. The ground state (S_0) and first singlet excited state (S_1) calculations were accompanied with density functional theory (DFT) and with time-dependent density functional theory (TD-DFT) respectively. The theoretical UV-Vis and fluorescence spectra were calculated with Multiwfn software [28]. The details of theoretical methods could be found somewhere else [29].

3. Results and discussion

3.1. Synthesis and formulation

The tetra-zinc(II) complex was prepared in the form of single crystals by mixing of zinc(II) sulphate to the polydentate Schiff base ligand in 2:1 mole ratio in methanol medium. The structural formulation of **1** was determined by different analytical techniques including single crystal X-ray diffraction study. The unique function of methanol solvent was most significant in this synthesis. Replacement of methanol by other solvents like ethanol, acetonitrile, dichloromethane did not produce the tetra metallic core. Therefore, the exclusive selectivity of the tetra metallic core towards methanol solvent developed the novelty of this complex.



Scheme 1. *Preparative procedure for zinc(II) complex.*

Controlled reactions exhibited that the tetra-zinc(II) Schiff base complex (**1**) could only be produced with zinc(II) sulphate with Schiff base ligand in methanol solvent. Other zinc(II) salts were not able to produce this compound in presence of Schiff base ligand.

3.2. Description of crystal structure

Single crystal X-ray structure analysis revealed that neutral tetrazinc(II)-Schiff base complex was crystallized in a monoclinic system with $P2_1/c$ space group. An ORTEP view of the asymmetric units of **1** was shown in Figure 1. The structural refinement parameters of **1** were listed in Table S1. Selected bond angles and bond distances of **1** were given in Table S2. The X-ray crystal structure of the zinc(II)-Schiff base complex (Figure 1) actually existed as a neutral tetra metallic core, $[Zn_4(\mu_3\text{-}OCH_3)_2(\mathbf{L})_2(CH_3OH)_2]$. Each of the Zn(II) centre in tetranuclear Zn(II)-Schiff base cluster exists in an octahedral geometry. The structural description is given by X. Liu *et al.* [30b]

The solvent methanol molecules acted a pivotal role in clustering four Zn(II) ions in association with two Schiff base ligands to a tetra metallic core through generation of methoxide ions (μ_3-O6) in the reaction medium. Also, another two methanol molecules coordinated with Zn(II) ions and fulfilled the coordination environments around each of the Zn(II) centres. So, phenoxido-O, alkoxido-O and methoxido-O were the driving forces in organizing four Zn(II) ions in an open dicubane geometry, having Zn···Zn distances varying from 3.059 to 3.137 Å (Zn1···Zn2, 3.137 Å to Zn2···Zn1, 3.059 Å) (Figure 1). Furthermore, two $[Zn_2(\mathbf{L})]$ units were interlinked through two methoxide ions, in which each Zn3 triangular unit was bridged by an oxygen atom (O6, O6) from a μ_3-OCH_3 group. An extensive investigation was executed to compare structural aspects with other reported tetrazinc(II)-Schiff base complexes [30], but in reality few tetra-zinc(II)-Schiff base complexes were available in scientific literature. In comparison with one reported tetra-zinc(II)-Schiff base complex, it was found that, Zn…Zn internuclear distances in **1** vary from 3.059 to 3.137 Å while Zn…Zn distances were found within 3.12−3.23 Å reported by Y.-X. Sun *et al.* [30a]. An identical tetrazinc structure is previously reported by X. Liu *et al.* [30b].

Quantum mechanical calculations of the molecular structure of Zn(II)-Schiff base complex in the vacuum was also performed through density functional theory (DFT) method. The molecular structure for zinc(II) complex in vacuum was presented in Figure 2. The optimized structure displayed close resemblance with the crystal structure of the tetra zinc(II) compound. The structure in vacuum also showed similar bond connectivity and structural features for the Schiff base ligand towards zinc(II) ions. The molecular bridges and coordination environment for zinc(II) ions remained almost identical. The inter-nuclear distances among the Zn(II) ions and the inter-nuclear angles were also in well agreement with the X-ray structure. The theoretical bond distances and bond angles of the optimized structure of **1** in vacuum along with the experimental crystal structure data were also compared in Table S2.

3.3. Experimental and theoretical views on the solution properties of Zn(II) complex

The structural rigidity of the tetranuclear Zn(II) compound in solution state was investigated by different analytical methods like steady-state absorption and emission study, molar conductivity, ESI mass spectral analysis. The Zn(II) compound was soluble in most of the polar solvents like ethanol, dichloromethane and water.

The UV-Vis spectrum for the Zn(II) compound in ethanolic solution at room temperature showed characteristics absorption maxima's at 233, 277 and 363 nm (Table S3). High-intensity transitions at 233 and 277 nm were principally assignable to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ electronic transitions of –C=N- chromophore of Schiff base ligand in ethanolic solution [31]. These electronic transitions resembled very well with other Zn(II)-Schiff base based transitions [31]. A broad and low-intensity transition at 363 nm indicates ligand to metal centre charge transition (LMCT) in the zinc(II)-Schiff complex.

TD-DFT was also performed to determine the electronic properties of Zn(II) compound in solution. With an aim to get a better view on the nature of optical spectrum of Zn(II)-Schiff base compound, we have performed TD-DFT studies. The theoretical UV-Vis spectrum was also in well accordance with the experimental observations (Table S2). The theoretical absorption and fluorescence spectra were in accordance with the experimental results. The theoretical absorption (λ_{max}) maxima (278 nm) were very close to the experimental absorption maxima. In addition, the theoretical fluorescence spectra (λ_{em} = 480 nm) was also well corroborated the experimental findings ((λ_{em} = 476 nm). The electronic transitions in HOMO→LUMO/ HOMO+1→LUMO–1 (Figure 3, Table S3) provide better insights to make a statement about the electronic activity in solution. It is well known that lower the energy gap associated with HOMO & LUMO higher will be the activity and consequently lower will be the stability of a molecule.

The ESI-MS spectral analysis of **1** further confirms in favour of molecular integrity in solution for $[Zn_4(\mu_3\text{-}OCH_3)_2(\mathbf{L})_2(CH_3OH)_2]$ (**1**). ESI mass spectrum of **1** in ethanolic solution displays the base peak at m/z 517.35 for $[Zn_2(\mathbf{L})(OCH_3)+H^+]$ (Figure S3) while another very important peak at m/z 1035.13 indicates the molecular ion peak in EtOH medium. The ESI-Ms of ethanolic solution of Zn(II) compound confirms its structural integrity in favour of $[Zn_4(\mu_3\text{-}OCH_3)_2(\mathbf{L})_2]$ species in solution.

With an aim to investigate detail insights in solution phase we further recorded molar conductance values for the Zn(II) compound in EtOH at room temperature. In order to investigate the solution stability of the zinc compound in ethanol medium, we carried out the measurement of molar conductivity at room temperature under aerobic condition. For this, we have taken solution of 1.10×10^{-3} *M* concentration and recorded molar conductance value as 5.7 Scm2mol-1. Molar conductance value of this compound strongly suggests non-electrolytic nature in ethanolic solution.

3.4. Luminescence property with lifetime decay of the Zn(II) compound

The photoluminescence spectrum of the **1** (λ_{ex} 277nm) in ethanol at 298 K was presented in Figure S1. The emission wavelength for Zn(II) compound in ethanolic solution is 476 nm (Figure S2) upon excitation on 277 nm. The excited state mean lifetime of the tera-zinc(II) cluster was found to be 1.67 ns. Time dependent photoluminescence decay profile for this complex was given in Figure 4. It was seen that the compound was quite stable in solution and stable in its tetrameric form in solution. It could be assumed that the peak was probably derived from the $\pi^*\rightarrow$ n or $\pi^*\rightarrow\pi$ transitions. The nature of emission, in this case, indicated the possibility of ligand-to-metal charge transfer [32].

3.5. Phenoxazinone Synthase activity of the tetra-zinc(II) complex and its mechanistic inferences

The aminophenol oxidation activity by the tetra-zinc(II) cluster (**1**) was investigated using 2-aminophenol (2-AP) as a standard substrate in ethanol solvent under an aerobic condition at room temperature (25°C). To study this catalytic oxidation reaction, 1×10^{-4} M solution of Zn(II) complex was added to 1×10^{-2} M solution of 2-AP. The course of the catalytic reaction was monitored through UV–Vis spectrophotometer. After immediate mixing, a spectrophotometric scan was recorded with a time interval of 11 min for 3h. Common studies on 2-AP oxidation showed that 2-AP exhibits a high intensity single band at 267 nm [33] and we found same results. With the progress of time in the spectrophotometric titration, it was seen that characteristic signal at 267 nm for 2-AP gradually disappeared in solution and concomitantly an initial new band with incremental absorbance at 434 nm appeared (Figure 5). The appearance of the band at 434 nm for this tetrazinc-Sciff base compound was a definite sign for the production of phenoxazinone species in solution[33].

Controlled experiments using 2-AP in ethanol solvent upto 3h under aerobic condition (Figure S4) was also performed. The yield of phenoxazinone compound under controlled experiments was too small in comparison with the yield of tetra-zinc based catalytic oxidation and thus, neglected. Under this circumstance, it was recommended that auto oxidation of 2-AP was responsible for the conversion of 2-AP to phenoxazinone in the controlled experiment.

Kinetic studies of the catalytic oxidation of 2-AP were also performed to understand the catalytic efficacy for this tetrazinc-Sciff base compound. The kinetics of oxidative coupling of 2-AP under aerobic atmosphere were carried out following the method of initial rates. The growth of the phenoxazinone species in EtOH at 434 nm was monitored as a function of time (Figure S5) [34, 36]. The concentration of the substrate versus rate constants was plotted which showed rate saturation kinetics. The kinetics was analyzed based on Michaelis-Menten approach of enzymatic kinetics. The values of the kinetics parameters for **1** are found as V_{max} (Ms^{-1}) = 1.72×10^{-5} (Std. error=1.63×10^{-6}); K_M (M) = 3.23×10^{-3} (Std. error=5.98×10^{-4}); k_{cat} (h^{-1}) = 6.19×10^{2}. We didn't find a single tetra-zinc(II) complex in the scientific literature that exhibits aminophenol oxidation activity under aerobic condition. To the best of our knowledge, this tetranuclear zinc(II)-Schiff base complex would be the first and only example till date that catalyzed the oxidative coupling of 2-aminophenol to aminophenoxazino compound under normal atmosphere with a significant turnover number. For this cause, we couldn't present a comparison of phenoxazinone synthase activity between our zinc-Sciff base complex with the few reported tetranuclear Zn(II)-Schiff base complex. The catalytic efficiency of the zinc complex for aminophenol oxidation was found high, $k_{cat}/K_M = 1.91\text{x}10^5$ and indicative of very good catalytic efficacy for this oxidation reaction.

Large number of scientific literatures were reviewed to explore the mechanistic insights of catalytic phenoxazinone activity of this zinc(II) complex. One of the renowned scientists, P. Chaudhury *et al.*[36a] modeled a tetra-copper complex for the mimicking of catalytic oxidation of 2-aminophenol. He proposed that an "on-off" catalytic aspects of the radical in association with redox behaviour of the metal centers play most significant role during six-electron oxidative coupling of 2-AP in the catalytic oxidative coupling of 2-AP. Furthermore, another renowned scientist, T. P. Begley *etal*.[36b,c,d] recommended that catalytic function for the synthesis of 2-aminophenoxazinone proceeds through a sequential three consecutive 2-electron aminophenol oxidations.

It was quite unprecedented that zinc complexes containing Schiff-base ligands displayed similar functional activities like a copper(II) based metalloenzyme, phenoxazinone synthase. Generally, it was seen that zinc ions were involved in the redox process with the two-electron transfer. So in order to elucidate the reason favoring aminophenol oxidation activity exhibited by this Zn(II)-Schiff base complex, EPR study was carried out. The X−band EPR spectra for the solutions containing tetra-zinc(II) complex in presence and absence of 2-AP were separately recorded in CH_2Cl_2 at 298 K. The EPR spectra for the tetra-zinc cluster in presence of 2-AP are presented in Figure 6. Both of the EPR spectra for this Zn(II)-Schiff base complex and 2-AP under aerobic condition at 298 K remains silent and the compounds were considered as EPR inactive. Even mixing of different zinc(II) salts with 2-AP didn't produce any signal in the EPR spectrum (Figure S6). The EPR spectra were recorded promptly upon mixing of Zn^{II} complex with 2-AP in an inert atmosphere after 10 min, and 30 min time intervals. A nearly isotropic EPR signal at $g \approx 2.01$ was found in the solution mixture and shown in Figure 6. A control experiment, under identical experimental condition, was also performed and no characteristic signal for organic radical was detected from the mixture between different zinc(II) salts and 2-AP. Thus, EPR signal, which recommends the presence of radical species/species in solution, was a definite signature to confirm the generation of radical, only in presence of Zn(II)-Schiff base complex. Hence, the course of catalysis proceeds through the participation of ligand-centered radical species in solution. Therefore, generation of radical in the catalytic cycle was most likely responsible for that oxidative coupling of 2-aminophenol. To confirm this observation, the catalytic oxidation reaction was performed using a radical scavenger, TEMPO (TEMPO = 2,2,6,6-tetramethylpiperidinoxyl). Addition of two equivalent of TEMPO to the ethanolic solution of catalyst stopped the catalytic conversion from 2-AP to APX. This investigation provided further consolidated that catalytic oxidation for 2-AP substrate only proceeded through generation of ligand centric radical in solution, only in presence of this tetra-Zn^{II} complex. It was further observed that no catalytic oxidation reactions occurred in an inert atmosphere and no formation of APX was detected. However, exposure of the reaction mixture in aerobic atmosphere, formation of APX was noticed immediately. This particular observation helped us to predict that molecular oxygen remained one of the active reactants in the catalytic cycle. In the catalytic cycle, 2-AP was converted into the imino-benzoquinone radical in the first step of catalysis. In the next step, the release of APX was occurred with subsequent regeneration of the active catalyst.

Survey of scientific literatures on phenoxazinone synthase activity by different metal complexes showed that the oxidative coupling product of 2-AP, in general, proceeded through 6e oxidation or radical generation pathway. However, in reality, in two or three cases, scientists were able to trap the radicals in the course of catalysis. Radical detection in solution was very difficult and this might probably due to faster disproportionate of the radical in solution. However, not a single zinc complex was reported earlier that shows such catalytic oxidative coupling of 2-AP under normal condition through radical generation in solution. We were lucky enough in this regards to able to detect the radical in the course of catalysis.

In order to gain further insight in this catalytic oxidation reaction in a solution state, ESI-MS spectra of a 1:100 mixture of complex **1** and 2-AP in ethanol solvent were also recorded. The spectrum (Figure S7) exhibited a base peak at m/z = 212.35 (100%) that was assignable to the quinine-sodium aggregate, $[2\text{-AP-H}^+]^+$. The peak at m/z = 1252.61 corroborated the formation of substrate-catalyst adduct, $[Zn_4(\mathbf{L})_2(\mu\text{-OCH}_3)_2(2\text{-AP})_2]$ species as an intermediate (Figure S7).

Another important structural aspect for this tetra-zinc(II) cluster was also investigated. To study photo-stability for the zinc(II) complex, molar conductance value was recorded for 1×10^{-4} M ethanolic solution keeping the solution in UV-Vis cuvette with passing of UV light for 120 mins. Under this condition, the molar conductance value was found to be 11 Scm^2mol^{-1} for **1**. The photo-stability of the complex in presence of the substrate (2-AP) was also studied. During the spectrophotometric titration for 120 mins, we have collected the ethanolic solution of the mixture (catalyst with the substrate) from UV-Vis cuvette and recorded the molar conductance values for the compound. The molar conductance value for the mixture was found to be 17 Scm^2mol^{-1} for **1**. All these conductance values corresponded to the non-electrolyte nature of the solution. The molar conductance values at room temperature helped us to predict that the pure tetra-zin(II)-Schiff base cluster were photo-stable in dry ethanol. We also tried to recycle this tetra-zinc(II) catalyst after the course of catalysis and were able to isolate the zinc catalyst in the form of tetra-metallic core. ESI-Ms and UV-Vis spectra (Figures S8 & S9) in ethanolic medium further attested the confirmation about the structural solidity of the tetra-zinc cluster in solution. So, the recycled catalyst was found in its original form except the loss of terminal coordinated methanol molecules.

From the EPR titration, *in situ* measurement of ESI-Ms spectrum for the zinc complex in presence of 2-AP substrate, now we were in a position to propose a mechanistic route through which the catalytic cycles were moving on. In order to detect the by product, we were able to detect I_3^{-} through spectrophotometrically and found the development of the characteristic I^{3-} band at 353 nm (Figure S10). Therefore, the course of catalysis indicated that substrate, at first, bound with catalyst (zinc-Schiff base complex) and formed a Catalyst-Substrate adduct. This adduct actually generated iminobenzoquinone radical employing imine chromophore of Schiff base ligand which consequently took part in oxidative coupling with another unit of 2-AP couple under aerobic atmosphere and produced phenoxazinone species in major amount with hydrogen peroxide.

4. Conclusion

In summary, a novel tetranuclear zinc(II)-Schiff base complex, $[Zn_4(\mathbf{L})_2(\mu_3\text{-}OCH_3)_2(CH_3OH)_2].2CH_3OH$ (**1**) in the form of single crystals was synthesized. Single crystal X-ray structural analysis revealed that **1** crystallized in monoclinic system with $P2_1$/c space group and exhibited stimulating structural features. This was the first zinc(II)-Schiff base compound where methanol solvent contributed significantly in assembling tetrazinc(II) core covering with diverse coordination aspects as terminal coordinator (CH_3OH), bridging(μ_3-CH_3OH) ligand and solvent for crystallization. This tetrazinc(II) structure was on the centre of inversion and three different molecular bridges (methoxido-, alkoxido- and phenoxido-) simultaneously coexist in the structure. The compound showed good photo-stability and excellent luminescence property with higher lifetime at transition state in ethanol. The compound showed good photo-luminescence property with higher lifetime (1.67 ns) at transition state in ethanol medium. This zinc(II) complex was also evaluated as an effective catalytic system towards oxidation of 2-aminophenol (2-AP) in ethanol. The tetrazinc(II) complex displayed good phenoxazinone synthase like activity with significant turn over, k_{cat}(h^{-1}) = 6.19×10^2 in ethanol under aerobic condition. ESI-Ms and EPR spectral analysis of the reaction mixture between Zn(II) complex and 2-AP recommended that the course of catalysis proceeded through substrate-catalyst adduct formation and generation of radical was the driving force for oxidative coupling of 2-AP. Most importantly, iminobenzoquinone radicals were very fast to disproportionate and extremely difficult to detect. In the true sense, this was the first and only example where a tetrazinc(II)-Schiff base cluster exhibited catalytic oxidation of 2-AP through ligand centred radical activity. Further, DFT outcomes results were in well agreement with the experimental observations.

Supplementary data

Supplementary crystallographic data are available free of charge from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-1223-336033; E-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk) upon request, quoting deposition number CCDC 1840633. Experimental information such IR, UV-Vis & photo-luminescence spectra, ESI mass spectra, rate vs. [substrate] plot, bond distance, & bond angle parameters are given here.

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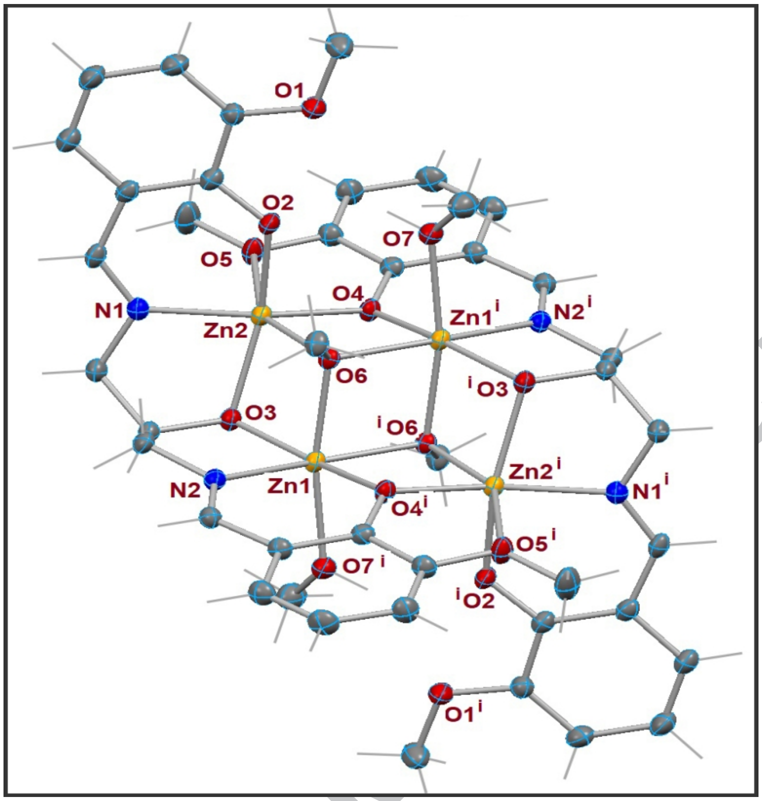

Fig.1. An ellipsoid plot (30% probability) of $[Zn_4(\mathbf{L})_2(\mu_3\text{-}OCH_3)_2(CH_3OH)_2].2CH_3OH$ (**1**) with atom numbering scheme.

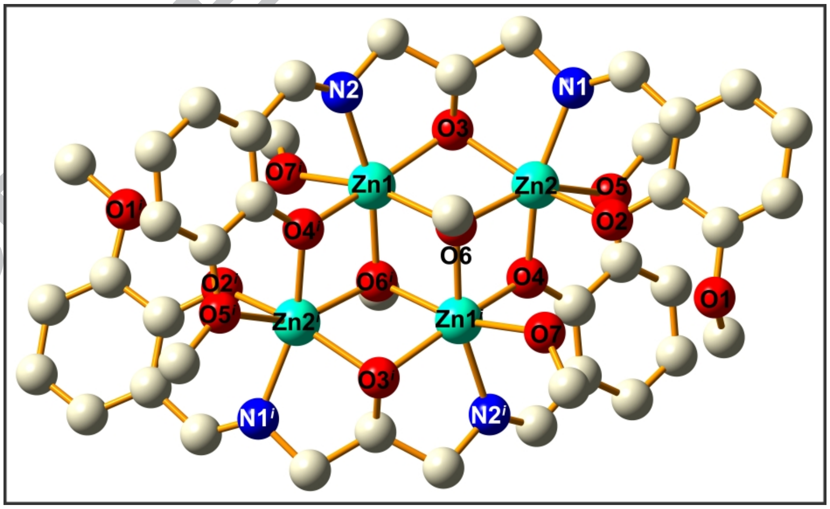

Fig.2. Optimized molecular structure of $[Zn_4(\mathbf{L})_2(\mu_3\text{-}OCH_3)_2(CH_3OH)_2]$ in vacuum using DFT/B3LYP/6-311G theoretical model.

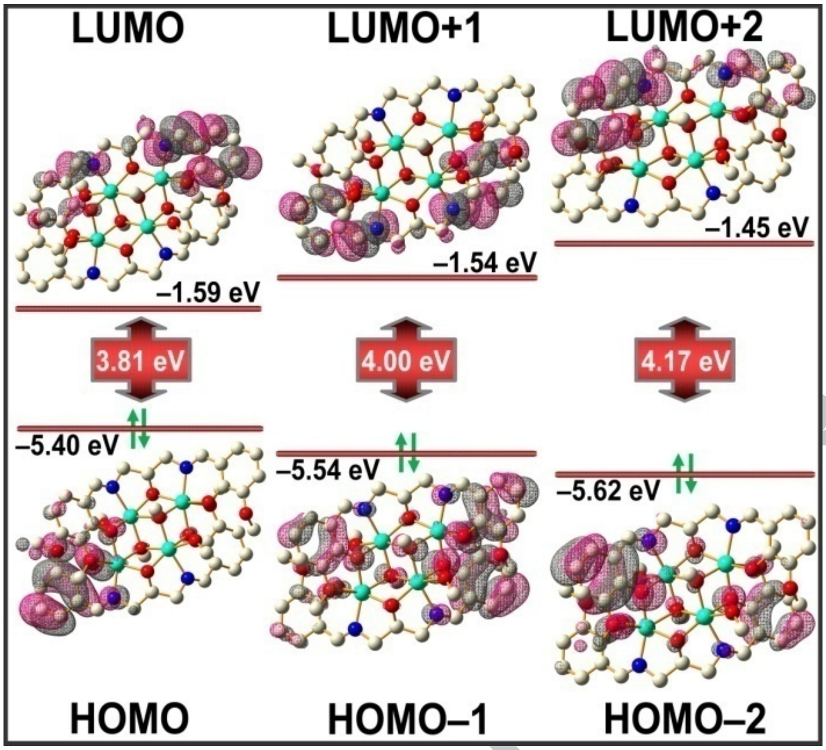

Fig.3. Images of the frontier molecular orbital's of **1** computed using B3LYP/6-311G theoretical method and IEFPCM/ethanol solvent model.

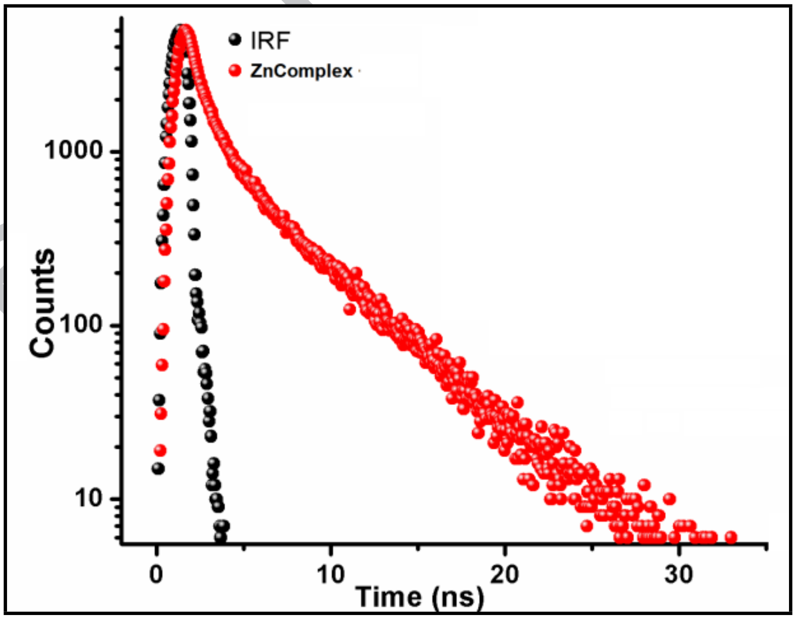

Fig.4. Time dependent photoluminescence decay profile for zinc(II) complex in EtOH

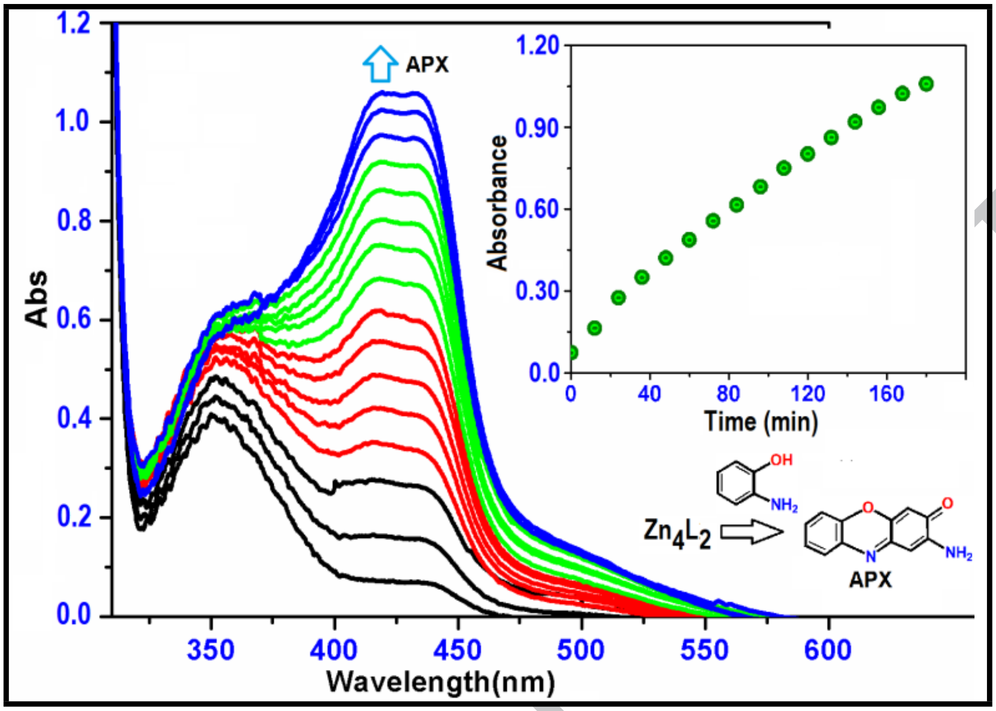

Fig.5. Growth of phenoxazinone species at 434 nm upon addition of 10^{-4} M zinc complex (**1**) to 100 equivalents of 2-AP under aerobic condition in ethanol at 25 ^{0}C. The spectra were recorded after every 11 min. Inset: Time vs Absorbance plot.

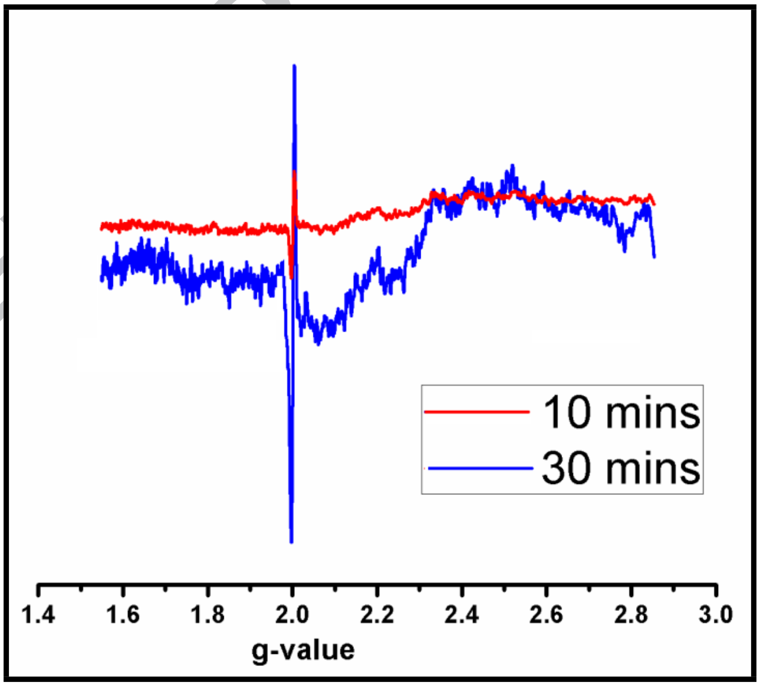

Fig.6. X-band EPR spectra of Zn-Schiff base complex in mixing with 2-AP at 10 min & 30 min in CH_2Cl_2 solution at 298 K.