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Macrocyclic Zn(II) Complexes: Synthesis, Structure and Hydrolysis of *p*-Nitrophenyl Acetate (pNPA)

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ABSTRACT

A new series of Zn(II) macrocyclic complexes has been synthesized by the reaction of Schiff bases (derived from dihydrazides and benzil/phthalaldehyde) in the presence of zinc(II) acetate dihydrate in ethanol. The complexes have been characterized by means of elemental and spectral (IR, ^{1}H and ^{13}C NMR) and SEM analysis. The kinetic studies were done to study the catalytic properties of these complexes towards the hydrolysis of *p*-nitrophenylacetate (pNPA) at 25°C in aqueous dimethylsulphoxide using phosphate buffer (pH = 7.0–8.5). During the reaction, absorbance of *p*-nitrophenolate increases with increase in time (i.e., increase of p-nitrophenolate concentration) which give information about the reaction progress i.e., ester hydrolysis. On the basis of these results, a plausible mechanism for the hydrolysis of pNPA is proposed. It is observed that coordinated water molecule might be serving as a good nucleophile that effectively catalyzes pNPA hydrolysis. Therefore, this type of complexes can be used as hydrolytic model enzymes.

Keywords: Macrocyclic ligand, zinc(II) complex, SEM, ester hydrolysis, kinetics

INTRODUCTION

Zinc, as a constituent of more than 300 enzymes, plays essential roles in biological systems. The active sites of these enzymes feature a zinc centre attached to the protein backbone by three or four amino acid residues, the nature of which influences the specific function of the enzyme (Bharathi *et al.* 2013). As one of the most prevalent transition metal cofactors in biological systems, it plays structural, signaling, and regulatory roles and is found in the most classes of the enzymes (most commonly hydrolases) (Andreini *et al.* 2006 and Sousa *et al.* 2009). The discovery of its presence as the catalytic centre in the hydrolytic metalloenzyme carbonic anhydrase (CA) (Keilin & Man 1939) was followed by its characterization in carboxypeptidase and soon after in enzymes of all classes (Vallee & Naurath 1954). In 1990, report analyzing the coordination spheres around Zn(II) in available protein crystal structures, including examples from different enzyme classes (Vallee & Auld 1990).

Most zinc enzymes are classified as hydrolyases because they catalyze the hydrolysis of condensed bonds present in many tissues which are most associated with proteins like esters (both phosphate and carboxylate), pyrophosphate and various types of peptides (Emsely 2001, Gani & Wilkie 1995, Lipscomb & Strater 1996, Wilcox 1996).

There are several reasons for the prevalence of Zn(II) as a catalyst in enzymes. First, it is earth abundant, with concentrations of 30 ppb in seawater and 75 ppm in the earth's crust (Hough *et al.* 1989). In order to understand why different zinc enzymes utilize different amino acid residues at the active site, it is necessary to understand how, and why, the chemistry of zinc is modulated by its coordination environment. Compared to other first-row transition metals, Zn(II) stands out because its filled *d*-orbital precludes it from participating in redox reactions and allows it to function solely as a Lewis acid. This particular property makes Zn(II) an ideal metal ion for reactions requiring a redox-stable cofactor to function as a Lewis acid catalyst (Baykal *et al.* 1999). As a d^{10} metal ion, Zn(II) has zero ligand field stabilization energy, so no geometry is electronically more stable than another (Gao *et al.* 2002*)*. This lack of an energetic barrier for Zn(II) may be important for its catalytic properties, allowing for changes in coordination number throughout the catalytic cycle (from four- to six-coordinate, for example, to accommodate the intermediate) and for alterations in the reactivity of the metal ion (Anbu *et. al*., 2013). Additionally, Zn(II) complexes can undergo rapid ligand exchange, enhancing the ability of Zn(II) to effect a catalytic cycle through efficient product release. A study of synthetic analogues of zinc enzymes (Mannich

et al. 2000, Kennedya *et al*. 2015 and Dalle *et al*. 2015) i.e., small molecules that resemble the structural and functional sites of the enzymes. Such studies are important because synthetic analogues are more amenable to structural, spectroscopic and mechanistic studies than are the enzymes themselves. Furthermore, several macrocyclic synthetic analogues (Rebilly *et al.* 2015) were studied as hydrolytic metalloenzymes.

However, "perfect" synthetic analogues that mimic all aspects of zinc enzymes i.e., structures, functions and mechanism, are yet to be obtained. The construction of ligand design is very important in order to get perfect synthetic analogues and in continuation of our previous work a new type of Zinc(II) enzyme models of hydrolytic metalloenzymes have been synthesized.

MATERIALS AND METHODS

All chemicals and solvents used in this work were of GR grade. Melting points were obtained in open capillaries and are uncorrected. IR spectra were recorded in KBr with a Simadzu 8201 PC spectrophotometer. NMR spectra were recorded in DMSO-d6 with a Bruker DRX-300 (300 MHz) spectrometer using tetramethylsilane as an internal reference. Elemental analysis was performed with a Vario EL III Carlo Erba 1105 CHN analyser. Elemental (C, H, N) analysis indicated that calculated and observed values were within acceptable limits. The purity of compounds was checked by thin-layer chromatography using silica gel plate with ether and ethyl acetate as solvent system. An iodine chamber was used as developing chamber (Li *et al*. 2015). For scanning electron microscopy (SEM), gold sputter-coating was carried out on desired Zn(II) complex samples at pressures ranging between 1 and 0.1 Pa. A sample was loaded in the machine, which was operated at 10^{-3} to 10^{-2} Pa with an EHT of 15.00 kV and 300 V collector bias using a Leo microscope and SEM images were recorded. Absorption spectrometry was performed using a Shimadzu UV–visible spectrophotometer, model UV-1800. Kinetics of the reactions was studied in aqueous buffer solutions/ DMSO, following the increase in the absorption at 400 nm due to the release of *p*-nitrophenolate ion (Saki & Akkaya 2004). *p*-Nitrophenylacetate (pNPA) was prepared and purified using a method explained in the literature. The ligand design is very important in order to get perfect synthetic analogues and in continuation of our previous work (Pandey *et al*. 2014). The water used for kinetic measurement was obtained from a Millipore Elix 10 system. The solution of pNPA was prepared in aqueous DMSO. The synthetic route to the complexes is given in **Scheme 1.**

Schiff bases derived from dihydrazides and diketone/ aldehyde

Synthesis of esters

A mixture of appropriate acid (succinic acid /adipic acid / terephthalic acid) in (0.3 mole) absolute ethanol (105 cm^3) and concentrated sulphuric acid (50 cm^3) was refluxed in a flask for about 4 h on a steam bath. The solution was cooled and slowly poured with stirring in crushed ice. The resulting solution is neutralized with sodium bicarbonate. The ester, so formed was extracted with ether and dried over anhydrous magnesium sulphate.

Synthesis of dihydrazides

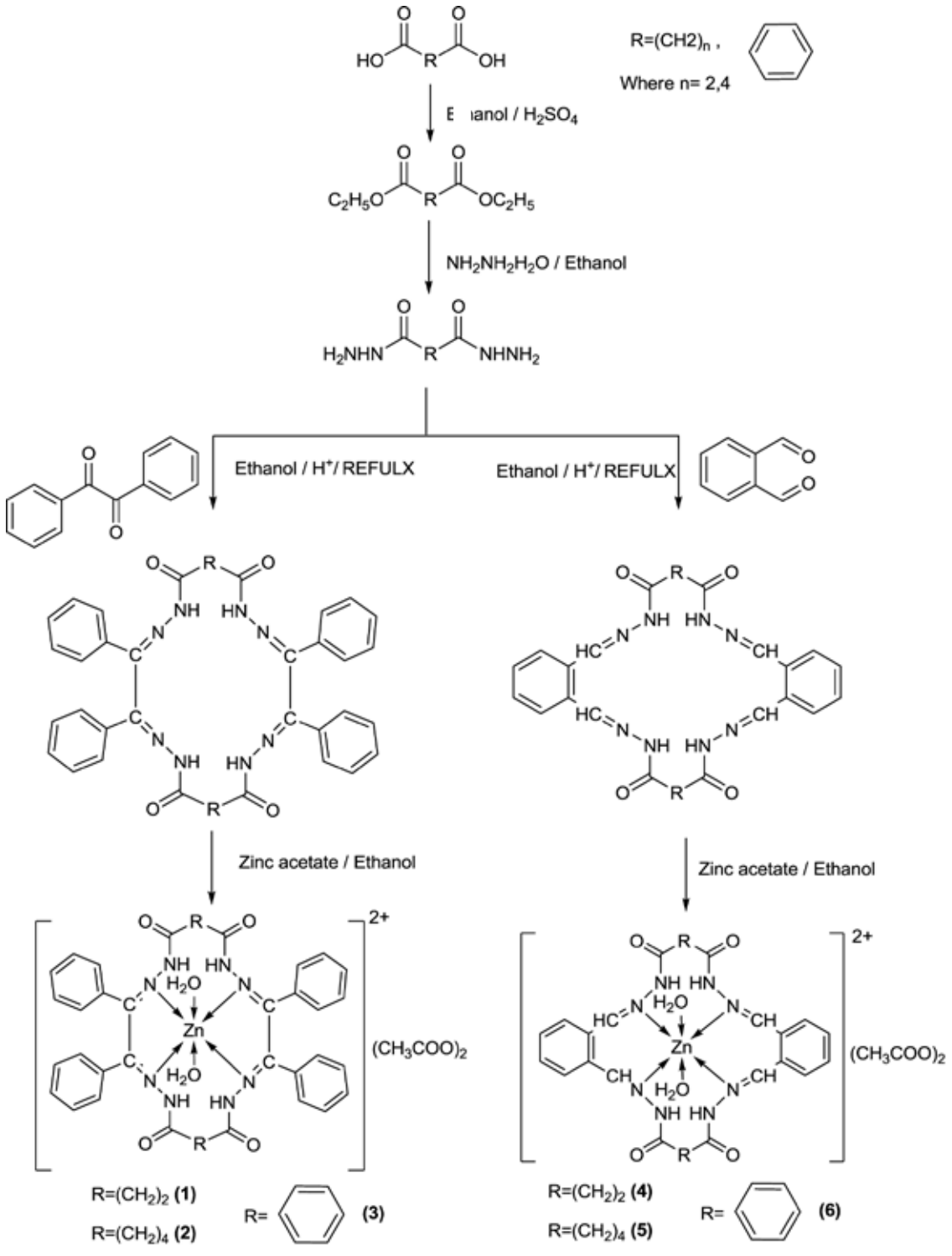
A mixture, ester of diacids and hydrazine hydrate in 1:2 molar ratios in ethanol was refluxed for *ca.* 4-5 h on water bath. The reaction mixture was cooled at room temperature poured into ice cold water. The compound precipitated from the clear solution. It was filtered off, washed and dried.

Synthesis of Schiff bases

A mixture of appropriate dihydrazide and benzil/ phthalaldehyde in 1:1 molar ratio was refluxed in alcohol (*ca.* 25 cm^3) containing few drops of conc. HCl for 5-6 h. The product thus separated was filtered, washed with ethanol and dried.

Synthesis of Zinc(II) complexes

Zinc(II) acetate dihydrate (0.01 mol) dissolved in ethanol (30 cm^3) was added to a refluxing solution of appropriate Schiff base (0.01 mol) in ethanol (30 cm^3). The reaction mixture was refluxed for about 8-13 h. The colored complex was obtained. The complex was filtered off, washed thoroughly with ethanol and dried under *vacuo.*



Scheme 1: Reaction route for the synthesis of zinc(II) complexes with Schiff bases derived from dihydrazides and diketone/ aldehyde

Characterization of Complexes

Table 1. Infrared spectral bands (cm^{-1}) and analytical data of Zn(II) complexes of Schiff base macrocyclic ligands derived from dihydrazides and diketone/aldehyde

Complexes	Data (cm^{-1})	(%) Analysis Found (Calcd.)			M.P.(°C)
		C	N	H	
1	3370sb,3196m,1735s,1605m,1565m,1450w,1220s,1120s, 780w,730w,660w,610w,430m,385m,315w	55.81 (55.85)	4.88(4.92)	13.00 (13.03)	212-214
2	3425sb,3175m,2950w,1720s,1615m,1575m,1450w,1220 s,1125s,750w,700w,650w,615w,440m,400m,310w	57.61 (57.67)	5.45(5.50)	10.76 (10.83)	245-248
3	3402sb,3200m,1750s,1615m,1570m,1450w,1220s,1120s, 800w,710w,650w,610w,450m,405,310w	60.21 (60.29)	4.40(4.43)	10.63 (10.69)	255-259
4	3485sb,3185m,1740s,1610m,1560m,1450w,1225s,1120s, 910w,760w,715w,650w,610w,438m,395m,310w	47.46 (47.50)	4.78(4.84)	13.50 (13.56)	260-264
5	3449sb,3200m,1620m,1570m,1450w,1350w,1220s,1125s ,775w,720w,650w,610w,478m,445m,320w	50.22 (50.30)	5.51(5.54)	12.59 (12.70)	201-203
6	3440sb,3200m,1620m,1570m,1450w,1350w,1220s,1125s ,775w,720w,650w,610w,478m,445m,320w	53.73 (53.77)	4.21(4.26)	12.10 (12.15)	224-227

Table 2. ^{1}H-NMR spectral bands (δ, ppm) of of Zn(II) complexes of Schiffbase macrocyclic ligands derived from dihydrazides and diketone/aldehyde.

Complexes		δ, ppm					
	-CH_3CO	**-CH_2**	**-CH_2CO**	**H_2O**	**Aromatic ring**	**-NH-**	**=CH-**
1	1.78(s)	-	2.18(t)	5.48(s)	7.22-7.54(m)	8.12(s)	-
2	1.71(s)	1.51(quint)	2.19(t)	5.51(s)	7.21-7.50(m)	8.11(s)	-
3	1.73(s)	-	-	5.50(s)	7.19-7.41(m)	7.81(s)	-
4	1.81(s)	-	2.17(t)	5.53(s)	7.15-4.48(m)	8.10(s)	8.70(s)
5	1.70(s)	1.58(quint)	2.18(t)	5.52(s)	7.15-7.48(m)	7.90(s)	8.72(s)
6	1.75(s)	-	-	5.41(s)	7.24-7.51(m)	8.11(s)	8.75(s)

Table 3. ^{13}C NMR spectral bands (δ, ppm)) of Zn(II) complexes of Schiff base macrocyclic ligands derived from dihydrazides and diketone/aldehyde.

Complexes	δ, ppm					
	-COO	**-CH_3**	**-C=N**	**-CONH**	**CH_2**	**Aromatic ring**
1	182.4	23.5	156.3	176.3	23.2	135.4,132.2,131.2,130.6,129.4,128.7,127.4
2	182.7	23.6	156.9	176.9	23.5, 33.5	148.6,140.5,131.2,130.8,129.5,128.9, 128.4,128.3
3	182.8	23.4	157.0	177.0	-	135.4,131.1,130.8,130.2,129.3,128.7,128.3,127.4
4	182.6	23.8	156.6	176.6	23.5	137.4,133.4,130.7,130.5,129.7,129.4,128.9, 128.4,127.7
5	182.3	23.7	155.7	175.7	23.4, 33.1	152.7,138.8,132.5,131.1,129.4,128.7,121.7,115.4, 114.8
6	182.5	23.5	155.9	175.9	-	152.4,134.3,131.4,130.5,129.7,129.1,122.4,115.5, 114.6

Enzymatic studies

The newly synthesized compounds have been screened for their catalytic activity as a model for hydrolytic enzymes.

Hydrolysis of 4-nitrophenylacetate using Zn(II) complexes

Kinetic study of hydrolysis of *p*-nitrophenyl acetate was measured by the UV spectral method using a UV spectrophotometer equipped with a thermostatic cell. The hydrolysis rate of *p*-nitrophenylacetate was measured by an initial slope method by the increase in 400 nm absorption of the released *p*-nitrophenolate. The reaction solution was maintained at 298±0.1 K. The ionic strength was maintained to 0.1 with $NaNO_3$. Phosphate buffer (0.1 M, pH=7.0-8.5) was used to maintain the pH. For initial rate determination the following procedure was employed. *p*-Nitrophenyl acetate ($8.3x10^{-5}$M), complexes ($1.0x10^{-3}$M) and phosphate buffer (pH=7.0-8.5) were mixed. The UV absorption at 400nm was recorded immediately. The reference experiment did not contain any catalyst (complex). The increase in the concentration of *p*-nitrophenolate was measured. The initial slope (< 5% conversion) of a plot of the measured absorbance verses time was observed. The experiments were run in triplicate and data are represented in tabular form (Table 4) and the progress curves of absorbance for reference catalysts (complexes) are shown in Fig. 1

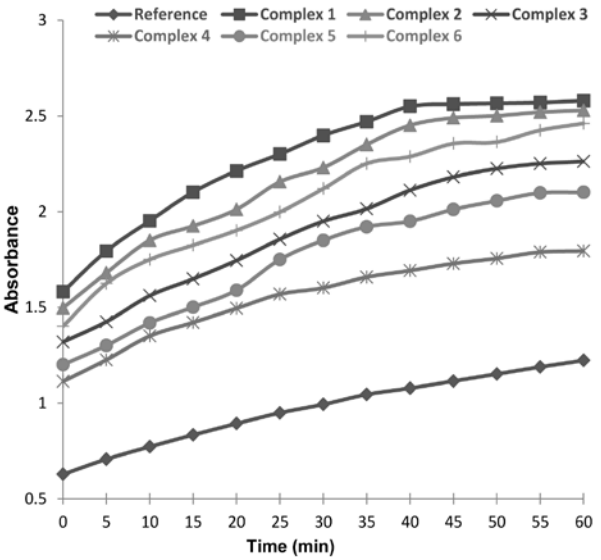


Fig. 1. Time course of absorption at 400nm (298 ± 0.1 K, pH = 7.0).

Table 4. Time-progress data showing the changes in absorbance at 405 nm associated the hydrolysis of *p*-nitrophenylacetate by a number of complexes (1-6).

Time (min)	Absorbance[(a)]						
	Reference	Complex 1	Complex 2	Complex 3	Complex 4	Complex 5	Complex 6
0	0.628	1.582	1.497	1.319	1.113	1.201	1.401
5	0.707	1.794	1.68	1.425	1.226	1.301	1.625
10	0.772	1.952	1.85	1.561	1.35	1.419	1.75
15	0.834	2.102	1.925	1.649	1.421	1.501	1.825
20	0.893	2.213	2.012	1.745	1.495	1.59	1.902
25	0.949	2.301	2.156	1.856	1.569	1.75	1.998
30	0.993	2.398	2.23	1.95	1.602	1.85	2.12
35	1.045	2.47	2.351	2.015	1.658	1.921	2.251
40	1.078	2.55	2.451	2.112	1.692	1.95	2.288
45	1.115	2.562	2.49	2.181	1.729	2.012	2.356
50	1.152	2.566	2.501	2.225	1.756	2.056	2.364
55	1.189	2.57	2.52	2.251	1.789	2.098	2.425
60	1.223	2.58	2.529	2.262	1.795	2.101	2.461

RESULTS AND DISCUSSION

SEM Analysis

The morphology of the Zn(II) complexes were investigated using SEM. Fig. 2 depicts the SEM images of the synthesized macrocyclic Zn(II) complexes. We observed that there are well-arranged structures of the synthesized complexes in the micrographs. Rod-like, granular, flake-like micro shaped and well arranged micro rectangular type structures are observed for Zn(II) complexes.

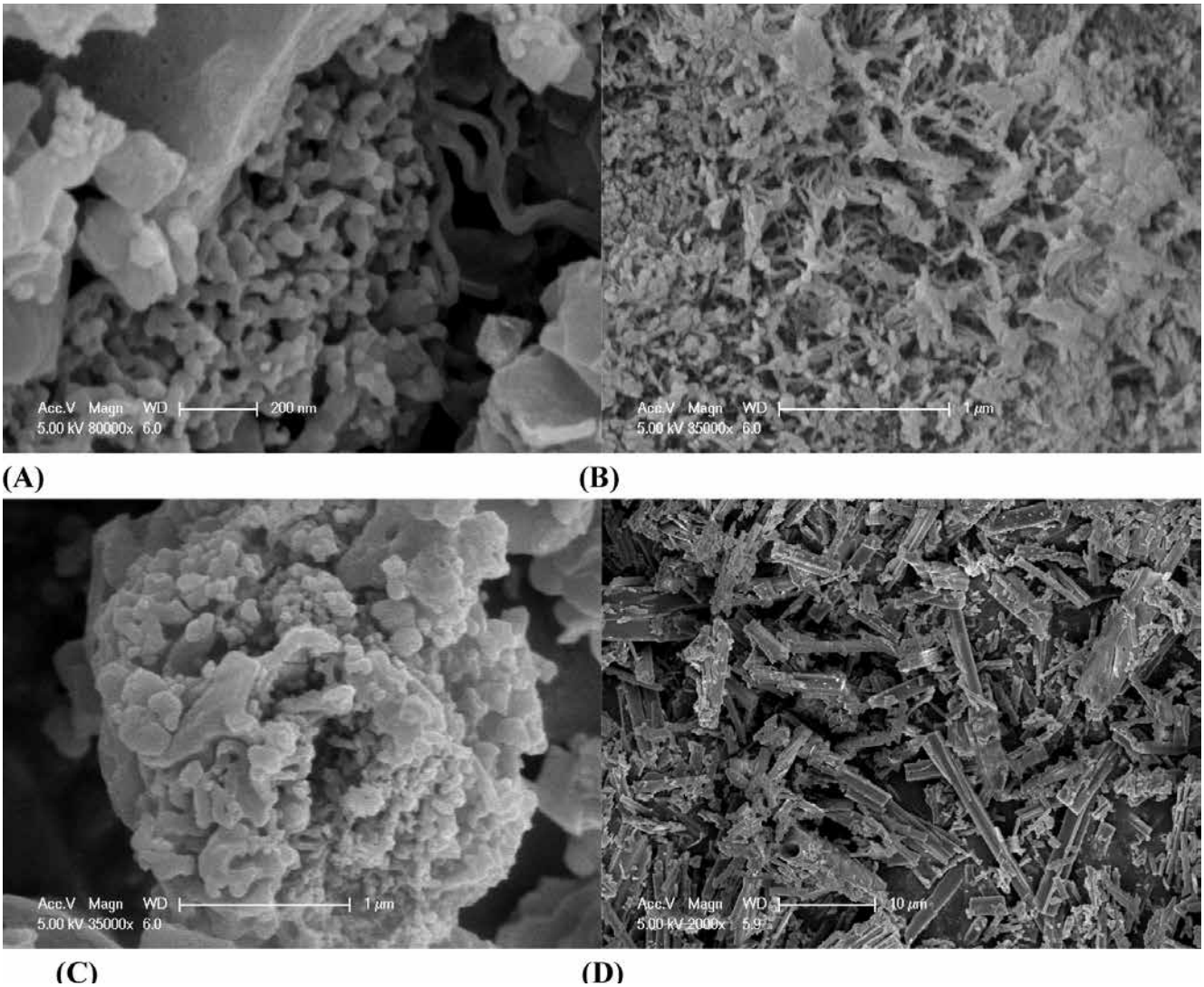


Fig. 2. SEM images (A) complex 1, (B) complex 3, (C) complex 5,(D) complex 6

Kinetic Study

Kinetic studies of the hydrolysis reactions were done using pNPA as a model substrate in buffered solution at different pH values. In the macrocyclic complexes, each Zn(II) ion is associated with two water molecules. It has been found that in many examples of metalloenzyme models, the metal-bounded water molecule appears as a nucleophile at pH=7.0 to react with electrophilic substrates (carboxyesters, phosphates, amides, etc.), wherein the prior activation of the nucleophile is essential. For example, in carbonic anhydrase the Zn(II)-bound water at the active centre deprotonates to yield the good nucleophile Zn(II)-OH which attacks the electrophilic centre of the substrate. During the experiment an absorption band typical of the *p*-nitrophenolate ion is observed at 400 nm, the intensity of which increases with time. The time course of the absorption at 400 nm at 298 ± 0.1 K (pH = 7.0) is shown in Fig. 1. It is observed that the hydrolysis of the complexes takes about 60 min. The liberated *p*-nitrophenolate is identified as one of the products by comparing the UV spectra at the end of the reaction. This compound shows the maximum absorption at pH = 7.0 which is greater as compared to the reaction rate of the unanalyzed hydrolysis of pNPA. During the experiment, it is observed that the reaction rate decreases on going to more alkaline pH. On the basis of the above results a plausible mechanism of catalytic hydrolysis is shown in Fig. 3. pNPA interacts with the coordinated OH group, *p*-nitrophenolate dissociates and the nucleophile binds with the acetate. The latter can easily decompose into acetate and water-coordinated Zn(II) complex in aqueous solution and the catalytic cycle completed.

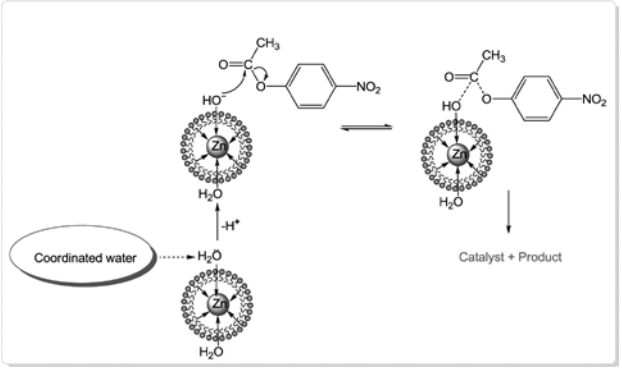


Fig. 3. Plausible mechanism of catalytic hydrolysis.

CONCLUSION

New macrocyclic Zn(II) complexes have been synthesized and characterized. The catalytic activity of these complexes have been investigated towards the ester hydrolysis and found that metal bounded water might be critically active species. The catalytic mechanism for the hydrolysis of pNPA possibly involved in this type of reaction. The much higher catalytic activity of the zinc(II) complex toward the hydrolysis of pNPA was found at pH 7.0 i.e., the most suitable condition for the hydrolysis of ester. It seems interesting to observe that on the basis of results obtained; these complexes may serve as model compounds to hydrolytic enzyme. Work is also under progress to investigate the catalytic activity of more complexes towards the hydrolysis of phosphate esters at different pH, temperatures and concentrations.

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