Catalytic hydrolysis of PNPP by cobalt (II) and manganese (III) complexes with (15-Crown-5) salicylidenephenylimine

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Cobalt (II) and manganese (III) complexes with crowned salicylaldimine mono-Schiff base ligand have been employed as models to mimic hydrolase in catalytic hydrolysis of *p*-nitrophenyl picolinate(PNPP). The kinetics and the mechanism of PNPP hydrolysis have been investigated. The kinetic mathematical model of PNPP cleavage catalysed by these complexes has been proposed.

Keywords: crowned salicylaldimine mono-Schiff base, manganese (III) and cobalt (II) complexes, mimic hydrolase, catalytic kinetics

Introduction

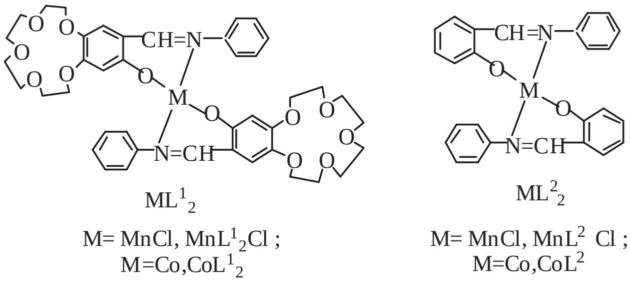
The hydrolysis of carboxyl esters and phosphoric esters is of paramount importance in biological and industrial processes.[1] Many researchers have been working hard to develop high efficiency and selectivity catalysts in order to achieve environmentally friendly and high-economy processes. A large number of studies on mimicing hydrolytic metalloenzymes have used metal ions Co (III),[2] Cu (II),[3] Zn (II),[4] Ni (II) [5] and La (III) [6] *etc.* as the active center of the mimic system, respectively, but Co (II) and Mn(III) complexes as the synthetic models of the metalloenzyme are infrequent.[7] Previously we have employed many kinds of bivalent transition metal complexes as catalyst of *p*-nitrophenyl picolinate(PNPP) hydrolysis in buffer solutions.[8-10] However, the effect of the structure of ligand-binding hydrophobic substitute groups on the catalytic hydrolysis of PNPP has been seldom reported. Recently, we reported that cobalt (II) Schiff base complexes containing crown ethers,[11] which can offer a hydrophobic micro-environment owing to the hydrophobicity of their outer ethylene groups, are good synthetic oxygen carriers and monooxygenase models.[12-13] But study of the catalytic performance of these complexes for PNPP hydrolysis has not been examined up to now. In this paper, the cobalt (II) (CoL^1_2) and manganese (III) (MnL^1_2Cl) complexes with (15-crown-5) salicylidenephenylimine (see Scheme 1) have been employed as models to mimic hydrolase, the catalytic performance, the kinetics and the mechanism of PNPP hydrolysis catalyzed by these complexes are reported. Compared with the crown-free analogous MnL^2_2Cl and CoL^2_2, the effect of crown ether ring on catalytic hydrolysis of PNPP is discussed.

Experimental

General methods and materials

Melting points were determined on a Yanaco MP-500 micro-melting point apparatus and are uncorrected. Infrared spectra were recorded on a Nicolet-1705X spectrometer (KBr, film). ^1H NMR. spectra were recorded on a Bruker AC-200MHz spectrometer using tetra-methylsilane as internal standard. Mass spectra were obtained on a Finnigan MAT 4510 spectrometer and Finnigan LCQ-DECA spectrometer. The metal ion content was measured by an IRIS-Advantage ICP emission spectrometer method. Other elementary analysis was performed on a Carlo Erba 1106 elemental analyser. Molar conductance was obtained on a DDS-11A conductivitimeter. Kinetic studies were carried out by UV-vis methods as detailed below.

All reagents, unless otherwise indicated, were of analytical grade and were used without further purification. Buffer solutions [tris(hydroxymethyl)aminomethane] (0.01M) were prepared by using distilled water and their pH values were adjusted by adding analytically pure nitric acid in all runs at 25°C. The ionic strength of the buffers was maintained at 0.1M KNO_3. PNPP stock solution for kinetics was prepared in acetonitrile. All aqueous solutions of complexes were prepared by using the desired pH value of the buffers solutions. The following compounds were prepared according to the literature: 4'-formyl-5'-hydroxybenzo-15-crown-5,[14] MnL^2_2Cl and CoL^2_2,[15] PNPP.[16]



Scheme 1 The structure of the complexes.

Synthesis of Schiff base ligand and their cobalt(II) and manganese (III) complexes 4-hydroxy-5-benzyliminemethylbenzo-15-crown-5(**HL^1**): A solution of aniline (5.0mmol) and 4'-formyl-5'-hydroxybenzo-15-crown-5 (5.0mmol) in EtOH (25cm^3) was stirred for 2h under a N_2 atmosphere at 80°C, then the mixture was cooled. The yellow precipitate was filtered and washed with ethanol. After recrystallisation from ethanol, yellow crystals were (1.45g, 75.0%) obtained. m.p. 101–103°C. ^1H NMR δ (ppm): 13.59 (s, 1H, OH), 8.44(s, 1H, CH=N), 7.42-7.19 (m, 5H, ArH), 6.86(s, 1H, ArH), 6.47(s, 1H, ArH), 4.17–4.07(m, 8H, $ArOCH_2$), 3.93–3.75(m, 8H, OCH_2CH_2O); IR (KBr, film) ν_{max}: 3227, 1628, 1225, 1140cm^{-1}; MS(*m/z*): 387(M^+); Anal. calcd for. $C_{21}H_{25}NO_6$: C 65.12, H 6.46, N 3.62; found: C 65.34, H 6.59, N 3.85

General methods for preparation of complexes: A solution of HL(1.0mmol) and $MnCl_2.4H_2O$ or $[Co(CH_3COO)_2]$ $4H_2O$(1.1mmol) in EtOH (15cm^3) was stirred for 2h under a N_2 atmosphere at 70°C, then the mixture was cooled and filtered, washed with MeOH to give the complexes, and the pure product was obtained after recrystallisation from EtOH.

CoL^1_2: dark brown. 55.1% yield. m.p: 171–174°C. IR (KBr, film) ν_{max}: 1616, 1224, 1134 cm^{-1}. Anal. calcd for $CoC_{42}H_{48}N_2O_{12}$: C 60.65, H 5.78, N 3.37; found C 60.79, H 5.86, N, 3.28. Λ_m($S.cm^2.mol^{-1}$): 12.6.

MnL^1_2Cl: purple, 59.2% yield, m.p. 192–194°C. IR.(KBr, cm^{-1}) ν_{max}: 1636, 1227, 1134; ESI-MS *m/z*: 863(M^+). Anal. cald for $MnC_{42}H_{48}N_2O_{12}Cl$.: C 58.40, H 5.56, N 3.24, Mn 6.37; found C 58.63, H 5.39, N 3.42, Mn 6.54. Λ_m($S.cm^2mol^{-1}$): 122.5.

Kinetics studies

Kinetic studies were carried out by UV-vis methods with a GBC 916 UV-vis spectrophotometer equipped with a thermostatic cell holder. Each kinetic run was initiated by injecting 30–70μl acetonitrile solution of the desired concentration (2.0×10^{-2}mol dm^{-3})PNPP into a 1cm cuvette containing 3ml of buffer solution containing the desired concentration of the complex. The pseudo-first-order rate constants for PNPP hydrolysis were determined by monitoring the release of *p*-nitrophenol at 400 nm under the conditions of more than 10-fold excess of substrate PNPP over catalyst concentration.

The pseudo-first-order rate constants (k_{ob}) of PNPP hydrolysis were obtained based on the initial rate method, *i.e.* according as the equations: $(rate)_0 = -(dC/dt)_0 = (dA/dt)_0/\varepsilon$ and $(rate)_0 = k_{ob}[ML]_0$ $(rate)_0$ was calculated initially, then the graph of $(rate)_0$ versus $[ML]_0$ was plotted; the k_{ob} was obtained from the slope of the straight line in the figure. Where $(rate)_0$ is the initial rate of PNPP hydrolysis, $[ML]_0$ is the initial concentration of the complex.

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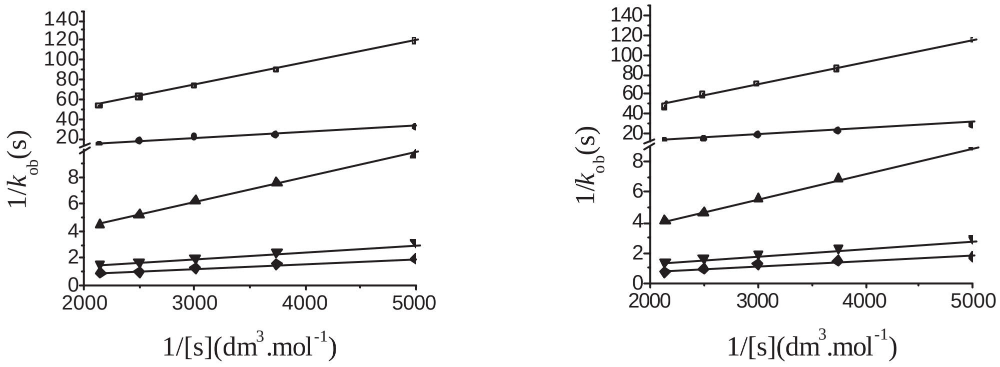
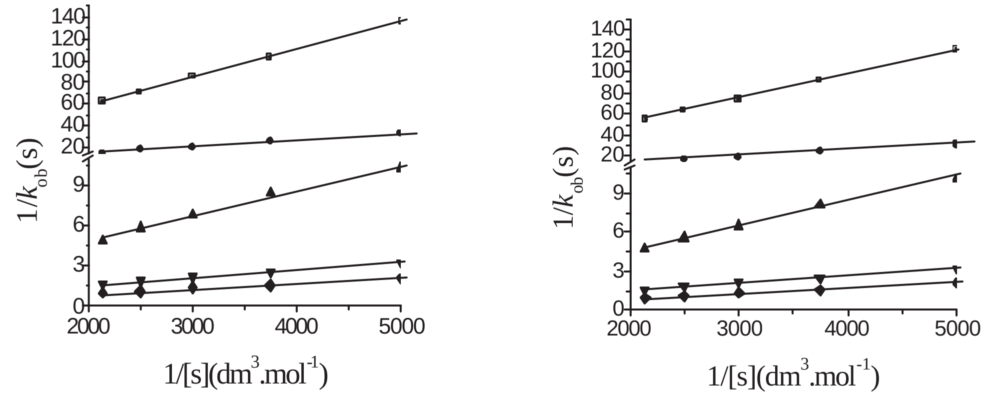


for $MnL^1{}_2Cl$ for $CoL^1{}_2$



for $MnL^2{}_2Cl$ for $CoL^2{}_2$

Fig. 1 Effect of substrate concentration on first-order-rate conatants for the catalytic hydrolysis of PNPP by complexes $MnL^1{}_2Cl$, $CoL^1{}_2$, $MnL^2{}_2Cl$ and $CoL^2{}_2$, in the buffer solution at 25°C. ■, pH7.00; ●, pH7.50; ▲, pH8.00; ▼, pH8.70; ◆, pH9.20.

Results and discussion

The pseudo-first-order rate constants for hydrolysis of PNPP at 25°C and pH 7.0

Table 1 lists the pseudo-first-order rate constants (k_{ob}) obtained at 25°C and pH 7.0. The pseudo-first-order rate constant (k_0) of PNPP hydrolysis in absence of catalyst is $0.78\times10^{-5}s^{-1}$ at 25°C, pH 7.00 and [PNPP] $=2.0\times10^{-4}$ mol dm^{-3}. Compared with k_0, the pseudo-first-order rate constants (k_{ob}) of the PNPP hydrolysis catalysed by the complexes (see Table 1) increases by a factor of ca. 1.08×10^3 times for $MnL^1{}_2Cl$, 1.12×10^3 times for $CoL^1{}_2$, 0.76×10^3 times for $MnL^2{}_2$**Cl** and 0.81×10^3 times for $CoL^2{}_2$**,** respectively, at 25°C, pH 7.00 and [PNPP] $=2.0\times10^{-4}$ mol dm^{-3}. But the pseudo-first-order rate constants (k_{ob}) of the PNPP hydrolysis catalysed by these two kinds of ligands are 4.32×10^{-4} s^{-1} for HL^1, 2.72×10^{-4} s^{-1} for HL^2, respectively, and the pseudo-first-order rate constants (k_{ob}) of the PNPP hydrolysis catalysed by these two kinds of metal salts are $1.50\times10^{-4}s^{-1}$ for $CoCl_2$, $1.19\times10^{-4}s^{-1}$ for $MnCl_2$, respectively, at 25°C, pH 7.00 and [PNPP] $=2.0\times10^{-4}$ mol dm^{-3}. The result of the comparisons of k_{ob} made between reaction rates in the presence of complex and in the presence of metal salts or uncomplexed ligands shows that the complexes are actually catalysis for PNPP hydrolysis.

Kinetics on catalytic hydrolysis of PNPP

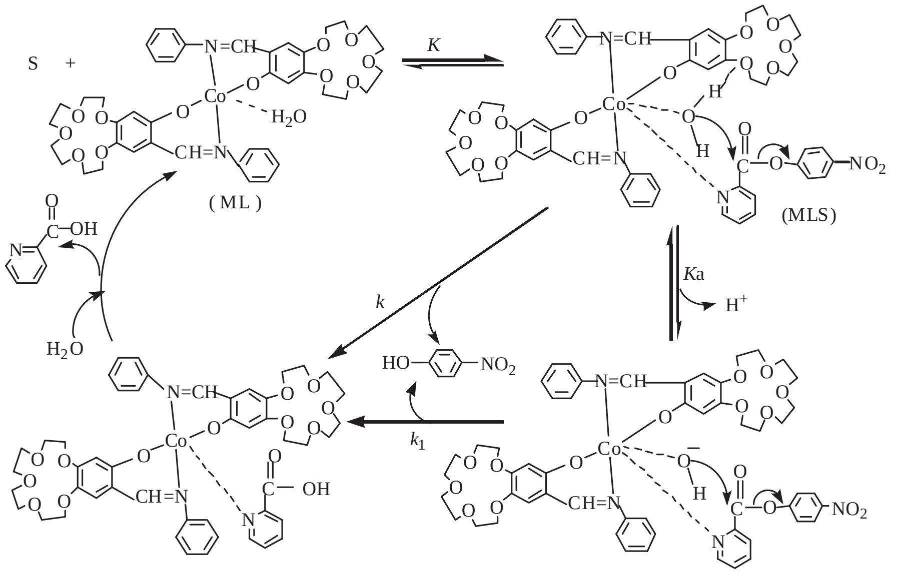
In aqueous solution, water could be coordinated to the central metal ion of the complex, and then would results in the hydrated complex. The hydrated complex may be the active species in the PNPP catalytic hydrolysis reaction.[17] Therefore, we assume that the mechanism of PNPP catalytic hydrolysis is similar to the hydrolysis reaction catalysed by the hydrolytic metalloenzyme (see Scheme 2).

Scheme 2 shows the course of PNPP hydrolysis catalysed by the complex $CoL^1{}_2$ as an example. Where S is the substrate PNPP. ML is the hydrated complex. MLS represents the intermediate made of ML and S. MLS^- is the anion intermediate. K is the association constant between the substrate and the hydrated complex ML. k is first-order-rate constant of the product formation, and is pH-dependent. K_a is the acidic dissociation constant of the intermediate MLS. k_1 is the first-order-rate constant, which is pH-independent.

Table 1 The pseudo–first-order rate constants of PNPP catalytic hydrolysis in buffer solutions

10^4 [PNPP]/mol/dm^{-3}	$0^2\ k_{ob}/1s^{-1}MnL^1{}_2Cl$	$10^2\ k_{ob}\ /s^{-1}CoL^1{}_2$	$10^2\ k_{ob}/s^{-1}MnL^2{}_2Cl$	$10^2\ k_{ob}\ /s^{-1}CoL^2{}_2$
2.00	0.84	0.87	0.59	0.63
2.67	1.12	1.18	0.76	0.89
3.33	1.37	1.41	0.87	1.11
4.00	1.66	1.74	1.01	1.33
4.67	1.91	2.12	1.23	1.59

Condition: 25±0.1°C, pH=7.00, I =0.1, [complex] = 1×10^{-5}mol/dm^{-3}.



Scheme 2 Proposed mechanism of PNPP hydrolysis catalysed by CoL^1_2.

Scheme 2 shows: (1) water-coordinating to Co(II) ion and forming a hydrogen bond is activated cooperatively by the central metal ion and crown ring, and the intramolecular hydroxide would be generated; (2) the N atom of the pyridine ring in PNPP molecule is coordinated to the central metal ion of the complex to form quickly the intermediate MLS with the association constant *K*; (3) the intramolecular Co(II) hydroxide as a nucleophile attacks the positive C atom on the carbonyl group of the PNPP to promote the departure of the *p*-nitrophenol with a first-order-rate constant *k*, this step is the rate-determining step of the total reaction and contains both acid ionisation equilibration of the step (II-1) with acidic dissociation constant K_a and the departure of *p*-nitrophenol of step (II-2) with the first-order-rate constant k_1; (4) picoline acid molecule-coordinating to the Co (II) ion is released and water is bonded to Co (II) ion again quickly.

The rate of PNPP spontaneous hydrolysis is much lower than that of PNPP catalytic hydrolysis, so the products of PNPP spontaneous hydrolysis can be neglected in kinetics calculation. Hence, Scheme 2 can lead to the rate equation:

$$\text{Rate} = k\,[\text{MLS}] \tag{1}$$

The association constants *K* can be expressed in terms of concentrations:

$$K = [\text{MLS}]/[\text{ML}][\text{S}] \tag{2}$$

Due to chemical balance principle, we have:

$$K_a = [H^+]\,[\text{MLS}^-]/[\text{MLS}] \tag{3}$$

According to the material balance, we have:

$$[\text{ML}]_T = [\text{ML}] + [\text{MLS}] \tag{4}$$

$$[\text{MLS}] = [\text{MLS}]_t + [\text{MLS}^-] \tag{5}$$

Combination of Eqns (2) and (4) leads to:

$$[\text{MLS}] = \frac{K[\text{S}][\text{ML}]_T}{1 + K[\text{S}]} \tag{6}$$

Combination of Eqns (3) and (5) leads to:

$$[\text{MLS}^-] = \frac{K_a[\text{MLS}]}{[H^+] + K_a} \tag{7}$$

Combination of Eqns (1) and (6) leads to:

$$\text{rate} = \frac{kK[\text{S}][\text{ML}]_T}{1 + K[\text{S}]} = k_{ob}[\text{ML}]_T \tag{8}$$

Rearrangement of Eqn (8) gives:

$$\frac{1}{k_{ob}} = \frac{1}{k} + \frac{1}{Kk[\text{S}]} \tag{9}$$

The rate Eqn in Scheme 2 can be expressed as:

$$k[\text{MLS}] = k_1\,[\text{MLS}^-] \tag{10}$$

Combination of Eqns (7) and (10) leads to:

$$k = \frac{K_a k_1}{[H^+] + K_a} \tag{11}$$

Rearrangement of Eqn (11) gives:

$$\frac{1}{k}\ \frac{1}{k_1} + \frac{1}{k_1K_a}\,[H^+] \tag{12}$$

In the above equations, [ML] and $[\text{ML}]_T$ are the free and the total concentration of the active species, respectively; [S] is the free substrate concentration and can be substituted by the initial concentration of the substrate based on the initial rate method; [MLS] is the concentration of the intermediate formed by the substrate and the hydrated complex in the buffer solution. $[\text{MLS}^-]$ is the dissociated concentration of the intermediate MLS, $[\text{MLS}]_t$ is the undissociated concentration of the intermediate MLS.

Based on Eqn (9), a linear plot, *i.e.*, $1/k_{ob}$ versus 1/[S] is obtained for MnL^1_2Cl, CoL^1_2, MnL^2_2Cl and CoL^2_2 complexes, by changing [S](see Fig. 1), and these plots allowed the evaluation of *k*, and the results of calculation are summarised in Table 2. From Table 2, it can be seen that the *k* values increase with increasing pH.

Effects of the acidity on the rate of PNPP catalytic hydrolysis

It is a known fact that the enzymatic activity is changed following the acidity of the reaction system. The ionic state in the hydrolase is interrelated to its catalytic activity and is controlled by the acidity of the reaction system because the spatial conformation of the enzyme and the stability of the intermediate forming from the substrate and the active species should be transformed following the changes of the acidity in the reaction system. Therefore, the acidity must be strictly controlled in the enzymatic catalytic reaction. The above Schiff base complexes as hydrolase mimics appear to show similar action to the natural enzyme in our experiment.

Figure 2 shows that the first-order-rate constant (*k*) of the PNPP catalytic hydrolysis is correlated to the acidity in the reaction system, namely, *k* increases with the increase of the pH value from 7.00 to 9.20. It implies that the reaction process can involve proton transfer at the rate-determining step in Scheme 2. In the process, the intermediate MLS was first ionised or generated proton transfer, and then the products were formed due to electronic transfer inside the intermediate MLS^-. The rate of PNPP catalytic hydrolysis depends on

Table 2 k (s^{-1})of the catalytic hydrolysis of PNPP by four complexes in buffer solution

pH	7.00	7.50	8.00	8.70	9.20
k(CoL^1_2complex)	0.522	1.231	2.832	3.971	10.17
k (MnL^1_2Cl complex)	0.113	0.267	0.552	0.894	1.122
k(CoL^2_2 complex)	0.109	0.341	0.925	2.564	7.143
k(MnL^2_2Cl complex)	0.050	0.124	0.248	0.418	0.617

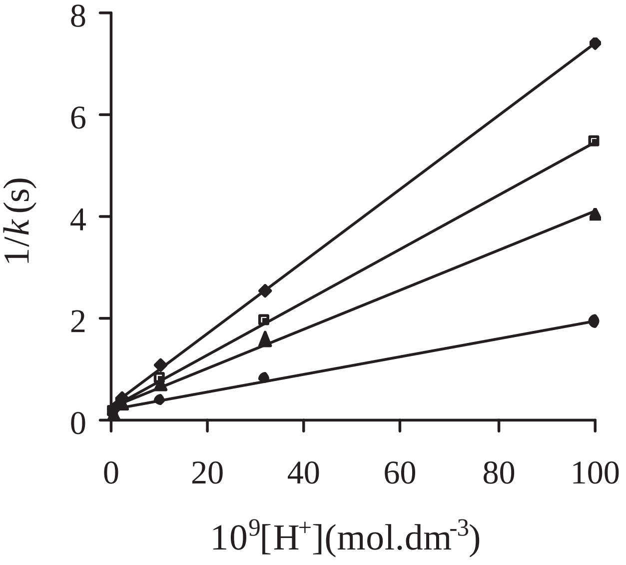


Fig. 2 pH-rate profile for the catalytic hydrolysis of PNPP by complexes, in the buffer solution at 25°C (●, CoL^1_2; ▲, MnL^1_2Cl; ■ CoL^2_2; ♦, MnL^2_2Cl).

the stabilisation of the intermediate MLS^-. According to the principles of the chemical equilibration, it is favorable for the intermediate MLS to form the intermediate MLS^- and generate the products in the alkaline solution. Hence, the first-order-rate constant (k) increases with the increase of pH in the reaction system. On the basis of Eqn (12), the k_1 and K_a values can be obtained from the slope and the intercept of the plot 1/k versus [H^+] (see Fig. 2.) The results show k_1 and pK_a values are 5.62 (s^{-1}) and 7.89 for CoL^1_2, 4.26 (s^{-1}) and 7.95 for MnL^1_2Cl, 4.09 (s^{-1}) and 8.11 for CoL^2_2, 3.77(s^{-1}) and 8.15 for MnL^2_2Cl, respectively.

Effects of the complex structure on the rate of PNPP catalytic hydrolysis
Generally, the enzymatic catalytic activity and selectivity are correlated with the enzymatic structure. The synthetic hydrolase used in this paper exhibits similar effects of structure. From Table 2, it can be seen that the catalytic activity of the complexes MnL^1_2Cl and CoL^1_2 is higher than that of complex MnL^2_2Cl and CoL^2_2 in the PNPP catalytic hydrolysis. The reasons may be: (1) the hydrophobic PNPP is more easily bonded to complexes MnL^1_2Cl and CoL^1_2 than bonded to complexes MnL^2_2Cl and CoL^2_2 due to the hydrophobic action between PNPP and the crown ring: (2) because of formation of the hydrogen bond between oxygen atom of the crown ring and hydrogen atom on water in the intermediate MLS, the water is activated cooperatively by both central metal ion and the crown ring. The above K_a values of MLS show that the forming rate of the intermediate MLS^- is more quickly in the system of MnL^1_2Cl or CoL^1_2 than in MnL^2_2Cl or CoL^2_2.

Conclusion

We have investigated the catalytic hydrolysis of carboxylic esters (PNPP) by cobalt (II) and manganese (III) complexes with crowned salicylaldimine. The results show that above the complexes exhibit high activity in the PNPP catalytic hydrolysis through comparing the hydrolyses in absence of the complexes and in the presence of metal salts or uncomplexed ligands; The pseudo-first-order rate constants (k_{ob}) of PNPP hydrolysis catalysed by Schiff base complexes containing crown ethers are about 1000 times higher than that of spontaneous hydrolysis of PNPP; the rate of the PNPP hydrolysis catalyzed by the complexes increases with the increase of pH value of the buffer solution. The studies also indicate that the catalytic activity of the MnL^1_2Cl and CoL^1_2 complexes-containing two crown rings is higher than that of crown-free analogous complexes CoL^2_2 and MnL^2_2Cl.

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