Proc. Indian Acad. Sci. (*Chem. Sci.*), Vol. 115, No. 4, June 2003, pp 175–183

Equilibrium and kinetic studies on ligand substitution reactions of chloromethyl(aquo)cobaloxime with aromatic and aliphatic N-donor ligands

D SUDARSHAN REDDY and S SATYANARAYANA*
Department of Chemistry, Osmania University, Hyderabad 500 007, India
e-mail: ssnsirasani@yahoo.com

MS received 21 February 2003; revised 13 May 2003

Abstract. Equilibria and kinetics of the reactions of chloromethyl(aquo)cobaloxime with histamine, histidine, glycine and ethyl glycine ester were studied as a function of pH at 25°C, 1·0 M ionic strength (KCl) by spectrophotometric techniques. Comparison of equilibrium constants and rate constants tells that the order is $K_{Hisdn} > K_{Hiamn} > K_{Gly} > K_{EtGlyest}$. The rate of substitution of H_2O varies with the p*Ka* of the incoming ligand and nucleophilic participation of the ligand in the transition state. The rate constants and equilibrium constants are correlated to the hardness and softness of the ligands and the Co(III) of cobaloxime.

Keywords. Alkylcobaloximes; ligand substitution reactions; hard and soft ligands.

1. Introduction

B_{12}-based enzymes are among the few cofactors known till now contain a metal–carbon bond. Axial ligation reactions of metalloporphyrin and cobaloximes in aqueous solution are dependent upon the particular metal ion,[1–3] equatorial ligands[4] and the axial ligands.[5–7] The study of simple models of the B_{12} coenzyme, such as the cobaloximes, $RCo(DH)_2L$, where L = neutral ligand and R = alkyl group, has furnished significant amounts of data[8,9] that have provided a foundation for understanding the behaviour of cobalamins.[10] These have also been the subject of extensive kinetic and mechanistic studies.[11] Ligand substitution reactions of coordinated H_2O in aquocobalamin ($VitB_{12}$) by OCN^-, SCN^-, CN^- and N_3^- were reported by Marques and Knapton.[12]

Compared to cobalamins and other model systems, cobaloximes have stronger Co–C bonds[13] and shorter Co–L (L = pyridine or substituted pyridines) bonds.[9] Eldik *et al*[14,15] studied the ligand substitution reactions of *trans*-$[Co(en)_2Me(H_2O)]^{2+}$ a simple model for coenzyme B_{12}, with cyanide and imidazole as entering ligands, and found that these ligands displace the coordinated water molecule *trans* to the methyl group and form the six-coordinate complex. There is a need to study ligand substitution reactions *trans* to the axial alkyl ligand in coenzyme B_{12} and various model complexes. It is known that methyl cobaloximes and coenzyme B_{12} undergo substitution of their axial benzimidazole ligand with protein histidine residues during complexation to the enzymes methionine synthase and methyl malonyl coenzyme A mutase respectively.[16,17]

*For correspondence

Since cobaloximes with amino acids and histamine are more closely related to the structural and bonding characteristics of corrin systems involved in biological mechanisms, we decided to explore the kinetics and equilibria of the axial ligation of chloromethyl(aquo)cobaloximes with ambidentate aromatic ligands (histamine, histidine) and unidentate aliphatic ligands (glycine, ethyl glycine ester).

2. Materials and methods

Histamine (histamine dihydrochloride), histidine (histidine monohydrochloride), glycine, ethyl glycine ester were obtained from Sigma and were used without further purification. KCl, HPLC grade methanol, acetic acid, HCl, phosphoric acid, formic acid were obtained from Fluka. Dipotassium hydrogen phosphate, potassium dihydrogen phosphate, potassium phosphate, *tris*(hydroxymethyl)aminomethane (Tris), sodium acetate, potassium hydroxide were obtained from Acros. Double distilled, deionized water was used throughout.

To maintain appropriate pH 0·2 M buffers of HCl (0–1·5 pH), KH_2PO_4 and H_3PO_4 (2·0 pH), HCOOH and KOH (2·5–3·0 pH), CH_3COOH and CH_3COONa (3·5–5·5 pH), K_2HPO_4 and KH_2PO_4 (6·0–8·0 pH), Tris and HCl (8·5–9·0 pH), K_2HPO_4 and K_3PO_4 (9·5–11·5 pH) are used.

Chloromethyl(aquo)cobaloximes were prepared by the procedure of Brown *et al*[18]. All manipulations were performed under minimal illuminations due to the photolability of carbon–cobalt bond. These alkyl(aquo)cobaloximes are photolabile, particularly in solution. They are soluble in alcohols and DMSO, less so in chloroform or water and virtually insoluble in ether and hydrocarbon solvents.

Measurements of pH were made with a Digisun digital pH-meter equipped with a combined glass electrode. The electrode was standardized at two pH values (pH = 4 and 9·2) with standard buffer solutions. UV and visible spectra were recorded at 435 nm on a Hitachi U-3410, the sample compartment of which is provided with a thermostat, and the concentration of chloromethyl(aquo)cobaloximes (0·00096M) was fixed. For axial ligation, single wavelength measurements were made on an Elico single beam spectrophotometer SL 171 model, the sample compartment of which was thermostated at $25 \pm 0{\cdot}1$°C.

3. Results and discussion

3.1 *Determination of equilibrium constants*

Apparent equilibrium constants (K_{app}) for the axial ligation of chloromethyl(aquo) cobaloximes were determined as below by spectrophotometric measurements at 435 nm (l_{max} of chloromethyl(aquo) cobaloxime). In 3 ml cuvettes, solutions containing $ClCH_2Co(DH)_2(OH_2)$, an appropriate buffer (0·2 M) to maintain pH, KCl to maintain ionic strength (1·0 M) and varying concentrations of ligand were taken and allowed to equilibrate in a thermostated holder at $25 \pm 0{\cdot}1$°C for 15 min prior to addition of cobaloxime.

$$K_{app} = \frac{[ClCH_2Co(DH)_2L]}{[ClCH_2Co(DH)_2H_2O][L]_{free}}. \quad (1)$$

Final absorbance readings were taken after equilibrium was established as indicated by the time independence of the readings. For such experimental setups, at a given pH, (2) is applied,

$$\Delta A = \Delta A_{max}\,[L]_f/(1/K_{app} + [L]_f), \tag{2}$$

where ΔA is the difference in absorbance between solutions containing cobaloxime and added ligand (L) and solutions containing only cobaloxime at the same concentration, ΔA_{max} is the maximum absorbance change thus obtained at high [L], and $[L]_f$ is the unbound ligand concentration. The data were analysed by a least-squares fit to the rearranged form of (2) to give

$$\Delta A = \Delta A_{max} - \{1/K_{app}(\Delta A/[L]_f)\}, \tag{3}$$

$$[L]_f = [L]_T - (C_T \Delta A/\Delta A_{max}). \tag{4}$$

$[L]_f$ is calculated from (4) using the measured value of ΔA_{max}, $[L]_T$ is the total concentration of added ligand and C_T is the total concentration of cobaloxime. Values of K_{app} were obtained from the least-squares fit of (3), i.e. the plot of ΔA vs $\Delta A/[L]_f$ and the slope is $-1/K_{app}$. Values for the equilibrium constants for axial ligation with respect to unprotonated ligand were calculated from the relation $K_{eq} = K_{app}/a_L$, where a_L is calculated from the relation $a_L = K_a/(K_a + [H^+])$.

3.2 *Determination of pseudo first-order rate constant* (k_{on})

For each ligand L, at various pH, first-order rate constants (k_{obs}) were determined from the absorbance measurements at the same wavelength used for K_{app} determinations under pseudo first-order condition with L in at least 10-fold excess over cobaloxime concentration (0·00096 M).

Reaction progress was monitored by measurements of the change in the absorbance upon addition of chloromethyl(aquo)cobaloxime to a 3 ml cuvette, which contained KCl to maintained unit ionic strength, necessary buffer (0·2 M) to maintain pH and ligand in the thermostated (25 ± 0·1°C) cell compartment. First-order rate constants (k_{obs}) were obtained by least-squares fits of the data to (5) below,

$$\ln(A_t - A_\infty) = k_{obs}t, \tag{5}$$

where A_t is the absorbance at time t and A_∞ is the final absorbance. Second-order rate constants, k'_{on}, at a given pH for a given ligand were obtained from the slopes of least-squares fits of the data to (6)

$$k_{obs} = k'_{on}\,[L]_T + k_{off}, \tag{6}$$

where $[L]_T$ is the total concentration of L present. Values of k'_{on}, the pH independent second-order ligation rate constant, were calculated from $k_{on} = k'_{on}/a_L$, where a_L is as defined above.

3.3 *Determination of dissociation rate constant* (k_{off})

Ligand dissociation rate constants, k_{off} were measured spectrophotometrically by addition of a small volume of a solution containing preformed $ClCH_2Co(DH)_2L$ (usually 0·00096M $ClCH_2Co(DH)_2OH_2$ plus 0·00192 M ligand in 25% methanol) to cuvettes containing KCl and buffer (0·2 M) in the thermostated (25 ± 0·1°C) cell compartment of the spectrophotometer.

Absorbance was continuously monitored at the same wavelength (435 nm) used for K_{app} and k_{obs} measurements. Measurements were made in triplicate at each pH and first-order rate constants, k_{off}, were determined as above, (5). In all cases, the ligand dissociation proceeded to ≥ 99% completion. All plots of (5) were satisfactorily linear (correlation coefficients ≥ 0·998). All determinations were averaged to obtain a final value of k_{off} as

$$k_{off} = k\mathrm{I} + k\mathrm{II}\,[H^+]. \tag{7}$$

The values of the equilibrium constant K_{app} for the reaction of the glycine, ethyl glycine ester, histidine and histamine with chloromethyl(aquo)cobaloximes are given in table 1. Logarithmic plots of log K_{app} vs pH are shown in figure 1 which indicates that as the pH increases, the K_{app} increases and the affinity for ligands to neutral chloromethyl (aquo) cobaloxime increase in the order Glyest < Gly < Hiamn < Hisdn. If we compare the pH-dependent binding plots of glycine and ethyl glycine ester in both cases, K_{app} increases with increase in pH and after certain pH they become pH-independent, glycine shows pH dependence up to a value of 10 and later becomes pH-independent, whereas ethyl glycine ester binding is pH-dependent up to 8 pH and later becomes pH independent. The binding of histidine to chloromethyl(aquo)cobaloxime has been shown in figure 2.

Table 1. Formation constants for the axial ligation of $ClCH_2Co(DH)_2OH_2$ by L at 25°C.

	Log K_{app} at different pH values for ligand			
pH	Histidine	Histamine	Ethyl glycine ester	Glycine
4·5	2·44	2·10	–	–
5·0	2·92	2·59	–	–
5·5	3·38	3·06	–	–
6·0	3·75	3·46	–	–
6·5	3·99	3·76	1·91	–
7·0	4·11	3·92	2·47	–
7·5	4·16	3·98	2·82	1·27
8·0	4·17	4·01	3·04	1·76
8·5	4·18	4·01	3·13	1·25
9·0	4·18	4·02	3·17	2·70
9·5	–	–	3.18	3.07
10	–	–	3·19	3·32
10·5	–	–	3·19	3·44
11·0	–	–	–	3·49
K_{eq}	15180	10418	1550	3250

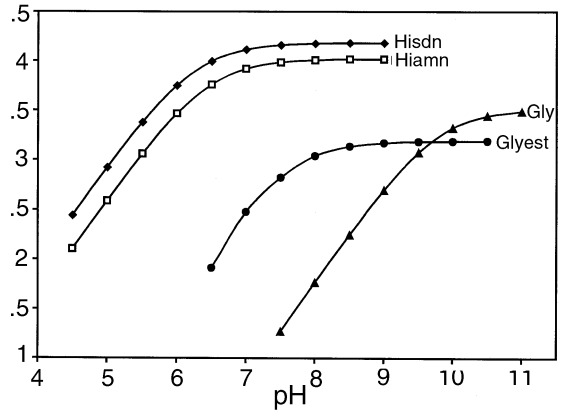

Figure 1. Dependence of log K_{app} on the pH for the axial ligation of chloromethyl (aquo)cobaloxime by different ligands at 25°C.

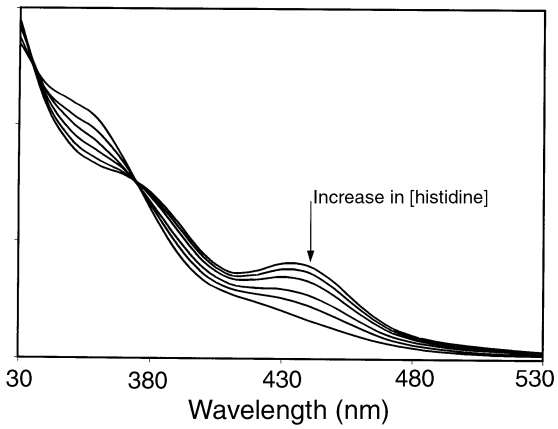

Figure 2. Binding of $ClCH_2Co(DH)_2H_2O$ with varying concentrations of histidine at pH = 6·5 and 25°C, isosbestic point = 376 nm.

Pseudo first-order rate constants are dependent on the pH. It is found that as pH is increased, rate constant also increases. In the case of histamine and histidine there is no increase in K_{app} at the pH above the p*Ka* of the ligand. This clearly indicates that in these ligands the binding is through the endocyclic nitrogen. If it binds through the NH_2 group, there should be an increase in K_{app} at higher pH, that is above the p*Ka* of the ligand. With histidine, the coordination is through the nitrogen of the imidazole ring, though there is a possibility of COO^- and NH_2 coordination, the NH_2 is mostly protonated below 8·0 pH. The plots of the pseudo first-order rate constants as a function of ligand concentration give straight lines with intercepts, the slope of which is k'_{on}. It is observed that as pH increases there is increase in k_{obs} and there is decrease in k_{off}. All the kinetic results are summarized in table 2.

Table 2. Kinetic data for the axial ligation of chloromethyl(aquo)cobaloxime by different ligands at 25°C.

k_{obs} (s^{-1})			k_{off} (s^{-1})			k_{obs} (s^{-1})		
pH	Histidine	Histamine	pH	Histidine	Histamine	C : L*	Histidine	Histamine
3·5	–	$1·5 \times 10^{-3}$	2·0	–	$8·0 \times 10^{-3}$	1 : 10	$1·20 \times 10^{-3}$	$1·80 \times 10^{-3}$
4·0	–	$1·8 \times 10^{-3}$	2·5	–	$4·9 \times 10^{-3}$	1 : 15	$2·00 \times 10^{-3}$	$1·86 \times 10^{-3}$
4·5	–	$2·5 \times 10^{-3}$	3·0	$2·5 \times 10^{-3}$	$2·5 \times 10^{-3}$	1 : 20	$2·80 \times 10^{-3}$	$1·90 \times 10^{-3}$
5·0	$1·2 \times 10^{-3}$	$4·0 \times 10^{-3}$	3·5	$1·9 \times 10^{-3}$	$1·6 \times 10^{-3}$	1 : 25	$3·40 \times 10^{-3}$	$1·96 \times 10^{-3}$
5·5	$2·4 \times 10^{-3}$	$4·4 \times 10^{-3}$	4·0	$1·5 \times 10^{-3}$		1 : 30	$4·00 \times 10^{-3}$	$2·00 \times 10^{-3}$
6·0	$5·1 \times 10^{-3}$		4·5	$1·4 \times 10^{-3}$		1 : 35	$4·80 \times 10^{-3}$	$2·04 \times 10^{-3}$
6·5	$6·0 \times 10^{-3}$					k_{on}'(dm^3 mol^{-1} s^{-1})	0·1464	0·01
7·0	$6·2 \times 10^{-3}$					*a*	$5·56 \times 10^{-2}$	$3·87 \times 10^{-3}$
						k_{on} (dm^3 mol^{-1} s^{-1})	2·63	2·58

*Ratio of $[ClCH_2Co(DH)_2OH_2]$ $(9·6 \times 10^{-4})$M and [L]M

A soft or class b character has been assigned to Co(III) in cobaloximes[19] and is consistent with the observed greater ligand affinity of cyanide, imidazole,[20–23] histidine or histamine than that of the hard glycine or ethyl glycine ester. Furthermore, softness appears to be related to the ability of a cobalt complex to stabilize a Co–C bond. Co(III) to ligand *p* bonding is used to explain the reverse order for the dependence of ligation strength upon ligand basicity. The order of $ClCH_2Co(DH)_2L$ stability is attributed to the ability of imidazoles or histidine or histamine to accept electrons into higher energy unfilled *p** anti-bonding orbitals through $dp \rightarrow pp$ back bonding, whereas primary amines (glycine or ethyl glycine ester) cannot accept electrons in either fashion. The reverse order for the dependence of $ClCH_2Co(DH)_2L$ stability on ligand basicity among two series of ligands, aromatic (histamine and histidine) and aliphatic (glycine and ethyl glycine ester) is not unexpected based on the following reasons.

(1) An increase in basicity is associated with increased ability for *s* donation, for example glycine forms more stable complexes than ethyl glycine ester, since glycine is more basic (p*Ka* 9·74) than ethyl glycine ester (p*K*a 7·62).
(2) An increase in basicity is associated with decreased ability for the aromatic ligands to function as *p* acceptors. Hence, $K_{histidine} > K_{hiamn}$, though histamine is slightly more basic than histidine. Histidine and histamine bind to Co(III), via $N \rightarrow Co(III)$ as well as $Co(III) \rightarrow N$ *p* bond. Histidine is a better *p* acceptor than histamine, hence histidine forms more stable complexes than histamine.

Figure 3 shows the association of histidine with $ClCH_2Co(DH)_2OH_2$ (p*Ka* = 11·95[24]) at a fixed pH with time. The kinetics of substitution of the axial base in alkylcobaloximes and related cobalt complexes has been studied under a variety of conditions.[25,26] In none of the studies was the mechanism established conclusively although in all cases strong evidence was obtained that the intimate mechanism is dissociative (*Id* or *D*).

The small dependence of k_{on} upon ligand basicity within each series of ligands is clearly related to the fact that while the reacting complex is a soft acid, the ligand is hard. The rate constants are better correlated with the relative softness of the ligand.

The stability of pentacoordinate alkyl cobalt complexes and the evidence that both the dominant soft Co(III) complexes, $[Co(CN)_5H_2O]^{2-}$ and $[Co(NH_3)_5SO_3]^+$, undergo ligand substitution reaction as SN^1 mechanism,[27,28] clearly favour this mechanism for ligation reaction of $ClCH_2Co(DH)_2OH_2$. The coordination between the softness of a cobalt(III) complex and the stability of its pentacoordinate species permits an SN^1 mechanism for ligand substitution.[29]

Figure 4 shows the dissociation of histidine from $[ClCH_2Co(DH)_2$ Hisdn] at a fixed pH with time. The plot of pseudo first-order rate constant k_{obs} against histidine or histamine concentration is linear with a very small intercept, which may indicate that a small dissociation is accompanied by complex formation. This appears to be more likely at lower pH (i.e. much below the p*Ka* of histidine or histamine); this is probably due to the protonation of ligand. Kinetic studies could not be taken up at high pH by conventional

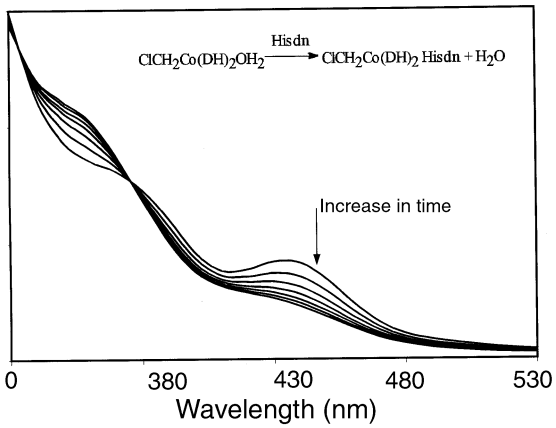

Figure 3. Association kinetics of $ClCH_2Co(DH)_2H_2O$ with Hisdn at pH = 5·5 and 25°C, isosbestic point = 376 nm.

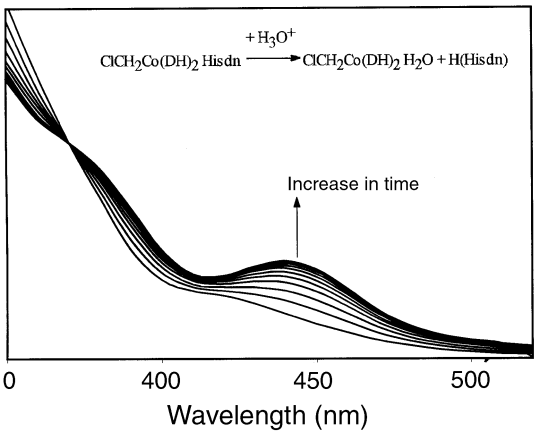

Figure 4. Dissociation kinetics of $ClCH_2Co(DH)_2$Hisdn into $ClCH_2Co(DH)_2H_2O$ at pH = 1·0 and 25°C, isosbestic point = 376 nm.

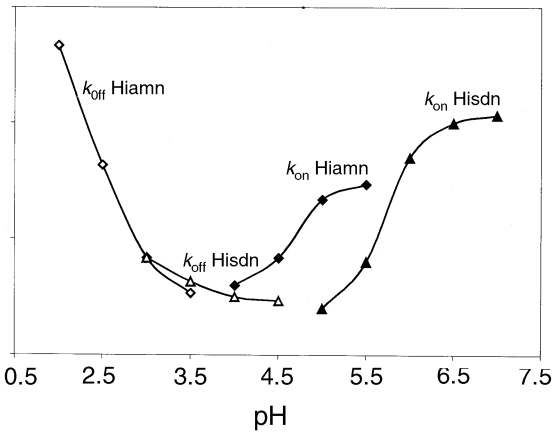

Figure 5. Dependence of k_{obs} on the pH for the axial ligation of chloromethyl (aquo) cobaloxime at 25°C.

methods due to fast reactions. This is supported by the observed high binding constant values at high pH. In case of histidine and histamine, as the pH is increased the rate of formation of complex increases. In case of histamine there is not much change in the k_{obs} even the pH is increased above 7·0. In both the cases, as the pH is decreased from a value of 4·0 the rate of dissociation is initially constant but on reaching 2·5 there is a sudden increase in the dissociation rate constant. That means the bound histamine or histidine comes out from the complex at lower pH easily. This supports the very low binding constant at lower pH and high binding constant at higher pH. The rate of dissociation (k_{off}) increases with decreasing pH (figure 5).

To compare the rate constants of the various ligands for the formation of complex with $[ClCH_2Co(DH)_2OH_2]$, we have calculated second-order rate constant, k'_{on}, from the slopes of the pseudo first-order rate constants as a function of concentration of the ligand. Since this is also pH-dependent, for better comparison we have calculated k_{on}, the pH independent second-order rate constant. The order of k_{on} is as follows CN^- >> 1-Meimd[11] > Imd > Histidine > Histamine > Gly > Etglyest. This is in accordance with the order of basicity and *p* back-bonding ability of the ligands. Though the basicities of glycine and ethyl glycine ester are larger than that of imidazole, histidine, or histamine the k_{on} of glycine and ethylglycine ester are much smaller. But among glycine and ethyl glycine ester again, they follow the basicity order, k_{on} of glycine > ethyl glycine ester. This can be explained based on *p* back-bonding and HSAB principle. Since imidazole and CN^- are less basic than glycine, these react much faster due to the ability of *p* back-bonding in addition to *s* bond and soft–soft interaction between Co(III) and CN^- or imidazole.

4. Conclusions

In the ligation reaction of $ClCH_2Co(DH)_2OH_2$ the *p*-accepting ligands (cyanide, imidazole, histidine or histamine) react more rapidly than the purely *s* donor (glycine or ethyl glycine ester). The greater reactivity of the histidine and histamine compared to

glycine or ethyl glycine ester stands in contrast to the opposite reactivity order for these nucleophiles with various unsaturated carbon electrophiles.

Acknowledgements

We gratefully acknowledge the University Grants Commission, New Delhi for financial support.

References

1. Ashley K R and Leipoldt G J 1981 *Inorg. Chem.* **20** 2326
2. Leipoldt G J, Van Eldik R and Kelm H 1983 *Inorg. Chem.* **22** 4146
3. Ashley K R, Shyu S and Leipoldt G J 1980 *Inorg. Chem.* **19** 1613
4. Choo P L, Mulichak A M, Jones Jr. R W, Bacon J W and Pett V B 1990 *Inorg. Chim. Acta* **171** 183
5. Brown K L and Satyanarayana S 1992 *Inorg. Chim. Acta* **201** 113
6. Hirota S, Kosugi E, Marzilli L G and Yamauchi O 1998 *Inorg. Chim. Acta* **275** 90
7. Rajeshwar Rao A, Sridhar V and Satyanarayana S 1999 *Proc. Indian Natl. Acad. Sci.* **A69** 1
8. Randaccio L, Bresciani-Pahor N, Zangardo E and Marzilli L G 1989 *Chem. Soc. Rev.* **16** 229
9. Bresciani-Pahor N, Forcolin M, Marzilli L G, Randaccio L, Summers M F and Toscano P J 1985 *Coord. Chem. Rev.* **63** 1
10. Kim S H, Chen H L, Feilchenfeild N and Halpern J 1988 *J. Am. Chem. Soc*. **110** 3120
11. Sudarshan Reddy D, Rajeswar Rao A and Satyanarayana S 2002 *Indian J. Chem.* **A41** 2475
12. Marques H M, Knapton L, Xiang Zou and Brown K L 2002 *J. Chem. Soc., Dalton Trans.* 3195
13. Halpern J 1983 *J. Pure Appl. Chem.* **55** 3677
14. Hamza M S A, Ducker-Benfer C and Van Eldik R 2000 *Inorg. Chem.* **39** 3777
15. Alzoobi B M, Hamza M S A, Ducker-Benfer C and Van Eldik R 2002 *Eur. J. Chem.* 968
16. Drennan C L, Huang S, Drummond J T, Mathews R G and Ludwig M C 1994 *Science* **266** 2669
17. Manica F, Keep N H, Nakagawa A, Leadlay P F, McSwerney S, Ramussen B, Bosake P, Diat O and Evans P R 1996 *Structure* **4** 229
18. Brown K L 1986 *Organometallic syntheses* (eds) R B King and J J Eisch (Amsterdam: Elsevier) **3** 186
19. Hague D N and Halpern J 1967 *J. Inorg. Chem.* **6** 2059
20. Sridhar V and Satyanarayana S 2000 *Proc. Indian Acad. Sci.* (*Chem. Sci.*) **112** 579
21. Sridhar V and Satyanarayana S 2001 *Indian J. Chem.* **A40** 165
22. Sridhar V, Sudarshan Reddy D, Ravikumar Reddy N and Satyanarayana S 2002 *Proc. Indian Acad. Sci.* (*Chem. Sci.*) **114** 1
23. Sridhar V, Sudarshan Reddy D, Ravikumar Reddy N and Satyanarayana S 2002 *Proc. Indian Acad. Sci.* (*Chem. Sci.*) **114** 11
24. Garlatti R D, Taugher G and Costa G 1983 *Inorganic. Chim. Acta* **71** 9
25. Poon C K 1973 *Coord. Chem. Rev.* **10** 1
26. Herlinger A W and Brown T L 1972 *J. Am. Chem. Soc*. **94** 388
27. Haim A J, Grassi R J and Wilmarth W K 1965 *Advan. Chem.*, No. 49
28. Halpern J, Palmer R A and Blakley L M 1966 *J. Am. Chem. Soc.* **88** 2877
29. Firth R A, Hill H A O, Pratt J M, Thorp R G and Williams R J 1969 *J. Chem. Soc*. *A* 381