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Research Article

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Coenzyme B_{12} model studies: Synthesis, characterization and axial ligation of benzyl (aquo) cobaloximes with N donor ligands and DNA binding studies

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ABSTRACT

Benzyl(aquo)cobaloxime has been prepared upon reacting this aquo complex with different N donor ligands (pyrazole, dimethyl pyrazole, alanine, alanine methyl ester and alanine ethyl ester) gives benzyl(ligand)cobaloxime [$PhCH_2Co(DH)_2L$]. Determination of equilibrium constant of the benzyl(aquo)cobaloxime with the above mentioned ligands as a function of pH at 25ºC, 1.0M ionic strength (KCl) by using spectrophotometric techniques. The rate of substitution of H_2O varies with the pka of the incoming ligand. The equatorial plane consists of four N atoms, two each from the dimethylglyoxime ligands, while the two axial positions are occupied by benzyl and neutral N donor base ligand The characteristic absorption band of ν(C=N) of dimethylglyoximato ligand in the benzylcobaloximes shifts to lower wave numbers as the donating power of the base ligand increases. The formation of benzyl ligand cobaloximes is confirmed on the basis of elemental analysis UV-Visible absorption spectroscopy, IR, ^1H, ^{13}C NMR spectra as well as LC/MS analysis. Binding of these complexes with calf thymus DNA (CT-DNA) has been investigated by absorption spectroscopy, steady-state emission spectroscopy and viscosity measurements. The experimental results indicate that the size and shape of the intercalating ligands have marked effect on the binding affinity of the complexes to CT-DNA. The complex [$PhCH_2Co(DH)_2PY$] binds with CT-DNA through an intercalative binding mode.

Key words: benzyl(ligand) Cobaloximes,equilibrium constant, ligand substituent reactions LC/MS.CT- DNA binding studies.

INTRODUCTION

The goal of this Perspective is to report the development of mechanistic insight over the past thirty years on the substitution behavior of bio-relevant Co(III) complexes that contain a single metal–carbon bond. Coenzyme B_{12} has long fascinated chemists and its unique property arises from the different catalytic activities of two different coenzymes. How the Co-C bond is activated toward homolysis or heterolysis is an enduring subject of research.[1,2] to highlight the influence of trans and cis ligands bound to cobalt(III) on the Co-C bond model complexes that contain Co(III) bound to the equatorial ligands and also bound to two trans axial ligands were synthesized and studied. Studies by using various equatorial ligands such as dimethylglyoxime, glyoxime, imine and amine have been studied.[3,4]

Co-C bond cleavage occurs considerably faster in the presence of enzyme, [5] there has been much speculation as to what is responsible for the rate enhancement and it now appears that a major contribution of Co-C bond homolysis arises from conformational changes in the enzyme which occurs upon substrate binding leading to sterically strained

adenosyl group and ultimately to cleavage of the Co-C bond.[6]

Model complexes for vitamin B_{12} have played an important role in understanding the behavior of ligand substitution reaction of vitamin B_{12} and the role of cobalt- carbon bond in coenzyme B_{12}.[7,8] The axial ligation reactions of metalloporphyrin ions in aqueous solution are dependent upon the particular metal ion.[9-14] spectroscopic studies on the model complexes help in establishing the basic relationship between structure and chemical properties. Kofod et al[15] characterized cobalt(III) compounds with classical ligands by spectroscopic techniques. Van Eldik et al [16,17] studied the ligand substitution reactions of trans $[Co(en)_2Me(H_2O)]^{2+}$ as a simple model for coenzyme B_{12} with cyanide and imidazole as incoming ligands where they found that these ligands displace the coordinated water molecule trans to the methyl group forming the six coordinated complex. Hence it is important to study ligand substitution reactions trans to the axial alkyl ligand in coenzyme B_{12} and related model complexes.

The interaction of transition metal complexes with nucleic acids is a major area of research due to the utility of these complexes in the design and development of synthetic restriction enzymes, chemotherapeutic agents, footprinting agents, spectroscopic probes, site-specific cleavers and molecular photoswitches.[18,19] The use of metal complexes as probes of DNA structure and information has been an active area of research.[20] DNA, as the hereditary substance of organism, carries and expresses the genetic information. It determines the types and functions of cells and plays a decisive role in life phenomena. In recent years, research of molecular device based on the interaction between ruthenium complexes and DNA have received extensive attention.[21]The effect of size, shape, hydrophobicity, and change on the binding of the complex to DNA has been analysed by changing the type of heteroaromatic ligand or metal center.[21] From these studies, it appears that the octahedral complexes at least partially intercalate one of their ligands between the base pairs of the nucleic acid double strand. Inorder for coordination compounds to intercalate in DNA, the intercalated ligand needs a flat, large surface area and a special geometry that permits overlapping between the aromatic ring of the intercalated ligand and the base pairs in DNA. In this paper, we have synthesized a series of benzyl ligand cobaloximes with Ndonor ligands and characterized by elemental analysis, LC/MS, IR and ^1H, ^{13}C NMR then extended our studies to the complex-DNA binding system using variety of physical methods.

EXPERIMENTAL SECTION

Pyrazole , 3,5-dimethyl pyrazole, alanine and alanine methyl ester were obtained from Sigma and used without further purification. KCl and HPLC grade of methanol were obtained from Fluka. Double distilled deionized water was used throughout the work to maintain appropriate buffers to maintain pH in the range of 0.5 to 12.5 as reported earlier.[22-24] $PhCH_2Co(DH)_2OH_2$ was prepared using a procedure of Brown et al.[25] All work with the benzyl(aquo)cobaloxime was performed in dim light (in dark room) and solutions were covered with aluminium foil due to photolability of organocobalt bond. pH measurements were made with a Digisum digital pH meter equipped with a combined glass electrode. The electrode was standardized at two pH values (pH = 4.0 and pH = 9.0) with standard buffer solutions. Spectra were recorded on a Hitachi U-3410, the sample compartment of which is provided with a thermostat of benzyl(aquo)cobaloxime (0.001M) at 442nm. For axial ligation single wavelength measurements were made on Elico single beam spectrophotometer SL 171 mode. The sample compartment of which was thermostated at 25± 0.1ºC.

IR spectra were recorded in KBr discs on a Perkin-Elmer FTIR 1600 spectrometer , ^1H and ^{13}C NMR were measured on a varian XL-300 MHz spectrometer, with DMSO-d_6 solution as the solvent at room temperature by using tetramethylsilane (TMS) as the internal standard, Chemical shifts(δ) were given in ppm.

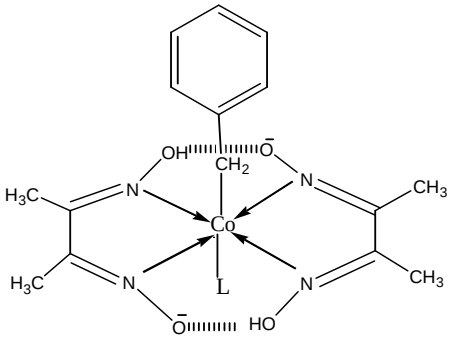
DNA binding

Concentrated stock solutions of metal complex was prepared by dissolving the complex in acetone: water (1:100) and suitably diluted with buffer to required concentrations for all experiments. All the experiments involving the interaction of the $PhCH_2Co(DH)_2H_2O$ complexes with DNA were conducted in pH 7.2. Tris buffer containing 5mM tris (hydroxymethyl)aminomethane (Tris), 50 mM NaCl in doubly distilled water. Solutions of CT DNA (calf-thymus DNA) showed a ratio of UV absorption at 260 and 280 nm, of about 1.91 indicating that DNA was sufficiently free of protein contamination.[26] DNA concentration per nucleotide was determined by ($\varepsilon = 6600M^{-1}cm$). at 260 nm.

RESULTS AND DISCUSSION

The formation of benzyl(aquo)cobaloxime is confirmed by electronic spectra, elemental analysis, IR ^{1}H NMR and LCMS. The complexes described in this paper were obtained by ligand substitution reactions of benzyl(aquo)cobaloxime $PhCH_2Co(DH)_2(OH_2)$ with 'L' where L is alanine, alanine methyl ester, pyrazole and dimethyl pyrazole to form $PhCH_2Co(DH)_2L$ which is shown as in Eq.(1) and structure.1.

$$PhCH_2Co(DH)_2H_2O + L = PhCH_2Co(DH)_2 (L) + H_2O \quad .....1$$



Structure .1: Benzyl(ligand)cobaloxime

The addition of equimolar or slightly excess of the (L) to 50 mg of the benzyl aquo cobaloxime $PhCH_2Co(DH)_2OH_2$ in methanol causes immediate color change from dark brown to yellow. This mixture was heated at 40-50°C by constant stirring for 1-2 hours, then minimum amount of distilled water was added, the resulting precipitate of yellow powder was filtered, washed with distilled water, 95% methanol and ether and dried in vacuo (yields were about 75%). The benzyl ligand cobaloximes $PhCH_2Co(DH)_2L$ which were actually isolated in quantitative yields were subjected for elemental analysis. Elemental analysis of these complexes exhibited that all the experimental values were in good agreement with the calculated values which confirming the formation of these complexes.Table(1).

All the complexes of benzyl ligand cobaloximes are light sensitive; Marzilli et al.[27] described the first application of near IR excited FT Raman spectroscopy to study the photo-lability of alkyl cobaloxime.

Table.1. Analytical data of $PhCH_2Co(DH)_2L$complexes

S.No	Complex $PhCH_2Co(DH)_2L$ Where L=	Formula (Mol.wt)	Found (cal) %		
			C	H	N
1	Water	$CoC_{15}H_{23}N_4O_5$	45.18	5.75	14.0
	OH_2	398	(45.22)	(5.77)	(14.7)
2	Alanine	$CoC_{18}H_{28}N_5O_6$	45.82	5.89	14.85
	CH_3CHNH_2COOH	469	(46.15)	(5.97)	(14.92)
3	Alanine methylester	$CoC_{20}H_{29}N_6O_4$	46.89	6.02	14.23
	$CH_3CHNH_2COOCH_3$	476	(47.20)	(6.21)	(14.49)
4	Pyrazole	$CoC_{18}H_{25}N_6O_4$	47.8	5.45	18.4
	$H_4N_2C_3$	448	(48.21)	(5.58)	(18.75)
5	Dimethylpyrazole	$CoC_{20}H_{29}N_6O_4$	50.36	5.94	16.87
	$H_8N_2C_5$	483	(50.42)	(6.09)	(17.64)

**Calculated values in parenthesis*

Electronic spectra:

Electronic spectra were recorded on an Elico BL 198 Biospectrophotometer, the sample compartment of which was provided with thermostat. The electronic spectra of benzyl(aquo)cobaloxime in methanolic solution will show spin allowed $^1A_1g \rightarrow {}^1T_1g$ transition in the region of 22624cm^{-1} due to $PhCH_2^-$ to Co(III) σ donation.[28]This band disappears or drastically decreases in benzyl(L)cobaloxime. The $^1A_1g \rightarrow {}^1T_2g$ band is masked by the intense charge- transfer bands. The charge –transfer spectra of the trans $PhCH_2Co(DH)_2L$ complexes show a band at 26315cm^{-1} due to intra-ligand π-π* transition of the coordinated dimethyl glyoxime.[29] The σ (DH) → σ* Co (LMCT) is masked by the intense short wavelength bands of alkyl (ligand) cobaloximes.

IR Absorption spectra:

Infrared spectra were obtained on Perkin Elmer FT IR-1600 spectrometer using KBr pellets. Benzyl(aquo)cobaloxime shows the characteristic bands at 3086cm^{-1} due to H_2O molecule coordinated to Co(III) ion. The disappearance of the peak at 3086 cm^{-1} and the appearance of a new peak at about 455 cm^{-1} ν(Co-N) indicates the formation of benzyl(L)cobaloxime by replacement of H_2O molecule. The IR spectra of all the complexes investigated contains a weak broad band in the range of 1700- 1772 cm^{-1} this is attributed to intra-molecular hydrogen bridge (O..H–O).The frequency band at around 1571 cm^{-1} is assignable to the ν(C=N) stretching frequency of dimethyl gloxime. It is shifted to lower wave number when water is replaced by alanine, alanine methyl ester, pyrazole and dimethyl pyrazole. Burger et al[30] reported on the basis of the frequency shifts of the ν(C=N) vibration that the lower the ν(C=N) vibration frequency, the stronger the Co→N bond. Our results suggest that the increase in electron density on the cobalt causes the increase of back donation from Co(III) to nitrogen atoms of $(DH)_2$ ligands resulting in the increase in conjugation of the five membered chelate ring. From the experiments of Blinc and Hadzi[3], we could assign the peaks around 1232 cm^{-1} to the N-O stretching vibration and the peak at around 1066cm^{-1} is assignable to the unionized N–O..H of dimethyl glyoxime.These two bands are shifted to higher wave numbers when the fifth ligand changes from H_2O to amide which is in the approximate order of the strength of electron donating power. The band at around 512 cm^{-1} is assignable to Co-N stretching frequency between Co(III) and nitrogen atoms of dimethyl glyoximato ligands. This shift to higher wave numbers when the ligand changes from H_2O to amide derivatives which is the order of strength of the interaction of the base ligand with Co(III) complex. The bands at around 1445cm^{-1} and 1373cm^{-1} are due to the asymmetric and symmetric deformation vibrations respectively corresponding to methyl groups in dimethyl glyoxime. The bands at around 945cm^{-1} and 840cm^{-1} attributed to deformation vibration of OH on bis(dimethyl glyoximato)moiety and the band at around 752cm^{-1} to C=N-O deformation vibration. The characteristic absorption bands due to the axial ligands also observed.

The band at about 1030 cm^{-1} may be assigned to ν(N-N) of the pyrazole and dimethyl pyrazole ring.The IR spectra of free ligands were compared with the IR spectra of their corresponding cobaloxime complexes in order to identify the diagnostic bands. For the pyrazole and dimethyl pyrazole, monodentate coordination has been assigned through N-2 based on their IR spectra. An appreciable positive shifts of ν(C=N) (20-33 cm^{-1}) and ν(N=N) (18-27cm^{-1}) modes of pyrazole and dimethyl pyrazole ring in the IR spectra of the above cobalt(III) complexes compared with their corresponding free ligand band positions support the above pre-position that N-2 of pyrazole is the binding site.[31,32] Among the N donor ligands it is found that the ν(C=N) frequency shift order is H_2O > N donor (DMPY> PY &ALME > AL). It is found that the ν(C=N) is lowered more in benzyl (pyrazole)cobaloxime than in benzyl (dimethyl pyrazole)cobaloxime shows that pyrazole forms more stable complexes than dimethyl pyrazole and the ν(C=N) is lowered more in benzyl (alanine) cobaloxime than in benzyl (alanine methyl ester) cobaloxime shows that alanine complex forms more stable complexes than alanine methyl ester. As the donating power of the base ligand increases, the ν(C=N) at 1565 cm^{-1} shifts to lower wave number while ν(N-O) at 1232 cm^{-1} and 1066 cm^{-1}, ν(Co-N) at about 512 cm^{-1} shifts to the higher wave number region. These results can be explained as follows: the coordination of more electron donating base to the cobalt (III) ion causes the increase in the electron density on cobalt atom which facilitates the back donation from the cobalt(III) to the nitrogen atoms of dimethyl glyoximate equatorial ligands resulting in the increase in electron densities in C=N and N-O bonds. The increase in electron in N-O bonds causes the stronger hydrogen bridges of O−H…O and higher frequency shifts of N-O stretching vibrations. The facilitated back donation from the Co(III) to nitrogen atoms of the dimethyl glyoxime means the increased metal-donor π bond in the equatorial moiety of benzyl ligand cobaloxime which causes the stronger interaction of cobalt(III) with equatorial N atoms resulting in the frequency shifts of ν(Co-N) vibrations and causes more conjugation in the five membered chelating including cobalt(III) which effects the ν(C=N) vibrations to shifts to lower frequency. On contrary, the effects caused by the change of the sixth ligand (e.g., $PhCH_2$) were observed to be inverse. As the donating power of the sixth ligand decreases, ν(O..H–O) shifts to lower wave number while ν(N-O) shifts to higher.[33]

^{1}H and ^{13}C NMR spectra:

The ^{1}H NMR spectra were recorded on Varian Gemini 200 MHz NMR spectrometer. Samples were dissolved in DMSO-d_6. The ^{1}H NMR spectra of $PhCH_2Co(DH)_2H_2O$ showed signal at 1.98 ppm that corresponding to the equatorial methyl groups, and the signal at 5.2 attributed to CH_2 of benzyl, aromatic CH protons appeared at around (6.7-7.3 ppm). Fig.(1) contains well resolved absorptions corresponding to the ligand and the equatorial methyl groups of dimethyl glyoxime $(DH)_2$.The dimethyl glyoxime methyl resonance of $PhCH_2Co(DH)_2L$ appear up field by about 0.2 ppm when compared with the values of $PhCH_2Co(DH)_2H_2O$, the up field shift is due to the interaction

of the benzyl group with equatorial dimethyl glyoxime methyl groups through space.[34] Recently, the benzyl group was shown to have π-interaction with the equatorial dioxime and it is oriented over one of the dioxime wings and not over O–H..O.[35]Also, these interactions cause the nonequivalence of the dioxime portions and the CH_2 protons become diastereotopic in 2-substituted benzylcobaloxime,[36] or the anisotropy of cobalt atom alone has been invoked to explain the ^{1}H NMR shifts.

In $PhCH_2Co(DH)_2PY$ and $PhCH_2Co(DH)_2DMPY$ complexes, the electron donation from N→Co(III) leads to a pronounced deshielding of the proton adjacent to the N-2 nitrogen of pyrazole.[25] pyrazole H_4 is at 6.4 ppm and it is shifted to up field compared to the free ligand position, because of the loss in aromaticity due to the withdrawal of electron density from N-2 to Co(III) and as a result ring protons experience a higher shielding effect. When pyrazole coordinates to Co(III), the C-3 and C-5 signals separate and give two signal at 7.8 and 7.5 ppm respectively, Me_3 and Me_5 of dimethyl pyrazole appeared at 2.5 and 1.8 ppm respectively. In the case of free ligand of alanine and alanine methyl ester, the NH_2 is observed at 3.6 and 2.8 ppm respectively and upon coordination to metal these shifted to down field at 3.9 and 3.0 ppm due to the resonance of lone pair of electrons on NH_2 with –C=O. The peaks at 1.29 and 1.3 ppm are assignable to CH_3 groups and CH gives peak at about 2.6 ppm in each. The OH group of alanine gives very broad peak at 11.7 ppm.

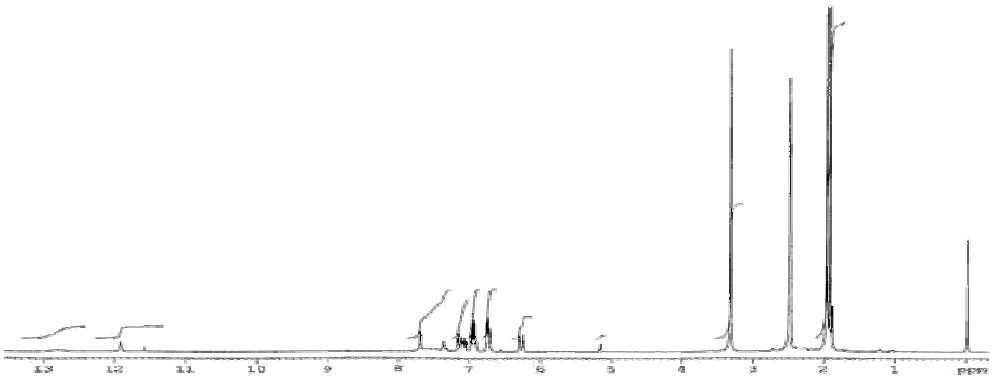


Fig.1.^{1}H NMR Spectrum of $PhCH_2Co(DH)_2PY$ complex in DMSO d_6

^{13}C spectroscopy has been recognized as one of the most promising tools for the study of the vitamin B_{12} model compounds.[37-39] The ^{13}C chemical shifts for oxime carbons appears at 165 ppm in free dimethylglyoxime, on coordination to the metal atom this signal shifts to the upfield at about 154 ppm, the broadness of ^{13}C resonance of CH_2–Co is generally attributed to the quadrupolar relaxation by the ^{59}Co nuclus (I = 7|2).[40] aromatic ^{13}C carbons appeared at around (127-130 ppm). Four equatorial methyl groups give one signal at about 12.5ppm. C_3, C_4 and C_5 of pyrazole and dimethyl pyrazole were absorbed at about 132, 110 and 129 ppm respectively. CH_3, CH and C=O of alanine and alanine methyl ester absorbed at about 18.2 and 57 and 175 ppm respectively.

LCMS analysis:

In the LCMS analysis of benzyl(aquo)cobaloxime, the only abundant peak is at 470 which is corresponding to the average atomic masses of benzyl(alanine)cobaloxime and in LC Chromatogram there is only one peak suggesting the formation of only one type of complex and the complex is Fig.(2).This result in a mass spectrum typically dominated by a single ion that corresponds to the molecular weight of the complex deprotonated in the positive ion mode $(M+H)^+$. For example, benzyl (alanine) cobaloxime with a molecular weight of 469 m/z in the positive ion mode will result in a spectrum with a base peak at 470m/z.Using LC/MS detector, it is possible to make structural predictions from the mass difference of the molecular ion and fragment ions seen in a mass spectrum.

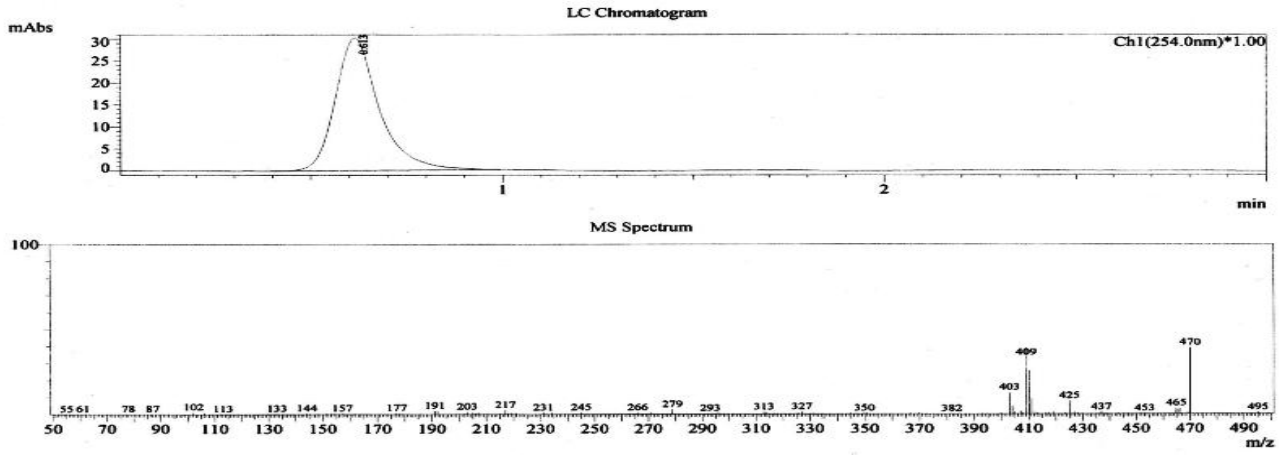


Fig.2. LC/MS Spectrum of [$PhCH_2Co(DH)_2AL$] complex

Determination of equilibrium constants

The formation constant, K_{app} for the substitution of added ligand L for water molecule in $C_6H_5CH_2Co(DH)_2(OH_2)$ by (alanine, alanine methyl ester, pyrazole and dimethyl pyrazole) was determined spectrophotometrically by measuring the absorbance changes of $C_6H_5CH_2Co(DH)_2(OH_2)$ upon addition of increasing concentration of ligand L.

$$C_6H_5CH_2Co(DH)_2H_2O + L \rightarrow C_6H_5CH_2Co(DH)_2\,(L) + H_2O \qquad \ldots\ldots 2$$

The elusive mechanism of B_{12} dependent enzymatic process, particularly those involving the homolytic Co-C bond breaking.[41] the Co-C bond homolysis is influenced by both equatorial and axial ligand. There are many factors which influence the above homolysis. These various factors are pH, basicity of the incoming ligand, concentration of the incoming ligand, nature of solvent, temperature etc. The above factors can be explained in the term of cis and trans labilising effect of the ligands (cis and trans) on the Co(III) center. In the present work, equilibrium constants were determined for the ligand substitution reaction of Benzyl (aquo)cobaloxime $C_6H_5CH_2Co(DH)_2(OH_2)$ with various bioactive N donor ligands, where the axial water has been substituted by the added ligand to form $C_6H_5CH_2Co(DH)_2(L)$. For this axial ligation, equation-4 has been followed for the equilibrium measurements. The complex formation was kept constant at $1.0X10^{-3}$ and different concentrations of the ligand L were added.

$$K_{app} = \frac{[\ C_6H_5CH_2Co(DH)_2L]}{[\ C_6H_5CH_2Co(DH)_2OH_2\ ][L]free} \qquad \ldots\ldots\ldots 3$$

The binding of ligand to $C_6H_5CH_2Co(DH)_2(OH_2)$ is dependent on pH. This is shown in UV- visible scan 1 in which the binding of the fixed concentration of L to benzyl(aquo)cobaloxime at different pH values is observed. In this scan, there is decrease in absorbance for the same concentration of ligand when the pH is increased. It tells clearly that the binding of ligand to benzyl(aquo)cobaloxime is pH dependent. For all the ligands studied, apparent equilibrium constants, K_{app} were obtained from the slopes of plots between ΔA vs $\Delta A/L_f$ (equation 4).

$$\Delta A = \Delta A_{max} - 1/K(\Delta A/[L]_f) \qquad \ldots 4$$

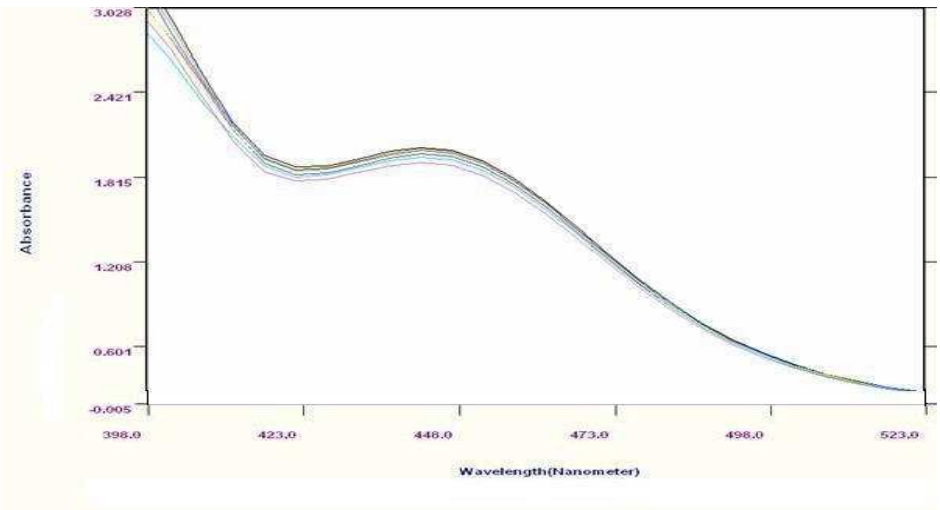
The anticipated pH dependence and pH independence of K_{app} was demonstrated for benzyl(aquo)cobaloxime as shown in Figs.(3). Values of K_{eq} were determined from each K_{app} measurement through Equation 5.

$$K_{eq} = K_{app}/\alpha_L \qquad \ldots 5$$

Where α_L is the fraction of ligand present as free base calculated from Equation 6 using pKa of the ligand (pKa = 2.53 for pyrazole at 25 ºC).

$$\alpha_L = K_a/(K_a + [H^+]) \qquad \ldots 6$$

Keeping the cobaloxime and ligand concentration constant spectra was recorded at different pH values. These spectra clearly show that as the pH is increased the binding of the alanine to $C_6H_5CH_2Co(DH)_2OH_2$ is increased (UV-visible scan1). Similarly the formation constants were determined for the other ligands studied. The K_{eq} for these ligands for benzyl ligand cobaloximes are given as follows: Al = 3133, ALME = 1177, PY = 549.8 and DMPY =490.90. The equilibrium constant (K_{eq}) was determined spectrophotometrically by the change in absorbance at λ_{max} of the respective benzyl aquo cobaloxime upon titrating the complex with varying concentration of ligand at a given pH, ionic strength 1.0M (KCl) at room temperature.



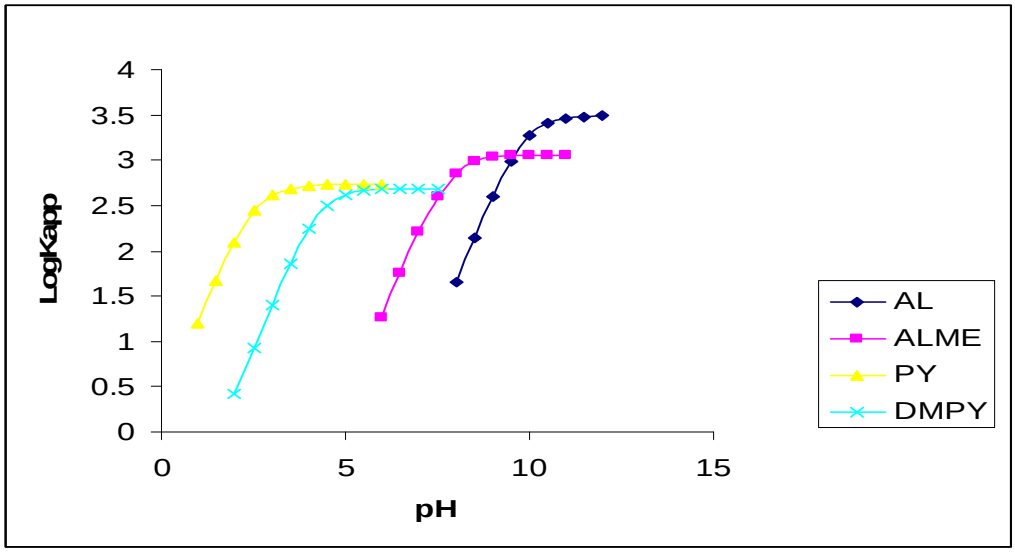
SCAN-1:Dependance of binding of benzyl (aquo)cobaloxime with increase in [pyrazole] at pH = 3.0 25^0 C

It is found that as the pH increases the apparent equilibrium constant (K_{app}) increases then it become stable, this is attributed to the competition of H^+ with Co(III) to bind with ligand. As the pH increases, the ligand becomes completely deprotonated. Moreover Co(III) is assigned as soft acid character[42] recently Sudershan Reddy et al [43] studied the axial ligation reactions of urea and thiourea with benzyl(aquo) cobaloxime to show that soft S donor thiourea forms more stable complex than hard donor urea with soft cobalt(III) center. The order of stability of cobaloxime with respect to amides and thio amides is that the S donor ligand has greater stability than the corresponding O donor ligand based on HSAB principle. Among N donor series the order of stability of complex with respect to the ligands is DMPY< PY< ALME< ALA. Steric crowding on the ligand also plays an important role in the stability of the complex. Jensen and Kiskih[44] proved that $p(n\text{-}But)_3$ would react faster due to its basicity and back bonding ability but pyridine forms more stable complexes than $p(n\text{-}But)_3$ due to steric hindrance. Therefore, the more basic dimethyl pyrazole forms less stable complex than pyrazole due to steric hindrance.The equilibrium constant for the axial ligation of benzyl(aquo)cobaloxime $PhCH_2Co(DH)_2OH_2$ with alanine and alanine methyl ester is dependent on the basicity (pKa) of the incoming ligand. The increase in the equilibrium constant with an increase in the basicity is well explained by the σ donor ability of the ligand, alanine and alanine methyl ester, the stability order with benzyl cobaloxime studied is $K_{AL} > K_{ALME}$, where DMPY (pKa = 4.26) and PY (pKa = 2.53) do not follow basicity order i.e., $K_{PY} > K_{DMPY.}$

DNA Binding Studies:

The interaction of the complex with DNA was investigated using absorption spectra. The absorption spectra of complex in the absence and presence of DNA (at a constant concentration of the complex) was studied. Fig.4 represents the absorption spectra of $PhCH_2Co(DH)_2PY$ in the absence and presence of increasing amounts of DNA. In the UV region, the intense absorption bands observed for Co^{III} complexes attributed to intraligand $\pi\text{-}\pi^*$ transition of the coordinated groups. Addition of increasing amounts of the complex results in hypochromism and bathochromic shift in the UV spectra of $PhCH_2Co(DH)_2PY$. The absorbance (*A*) of the most red-shifted band of each investigated complex was recorded after successive additions of CT DNA. The intrinsic binding constant *K*, was determined from the plot of [DNA]/($\sum_a - \sum_f$) vs [DNA], where [DNA] is the concentration of DNA in base pairs, the apparent extinction coefficient is obtained by calculating A_{obs}/[complex] and $\sum_f$ corresponds to the extinction coefficient of the complex in its free form. For the complex the observed data were then fit in to Eq.(7) to

obtain the intrinsic binding constant, *K*.[45] where $\sum_b$ refers to the extinction coefficient of the complex in the fully bound form.



AL = Alanine, ALME = Alanine methyl ester, PY = pyrazole, DMPY =Dimethyl pyrazole

Fig.3: Dependance of formation constants ($LogK_{app}$) on the pH of the axial ligation of $C_6H_5CH_2Co(DH)_2OH_2$ by different N donor ligands (L) at 25 ^{0}C in aqueous solution, ionic strength 1.M(KCl).

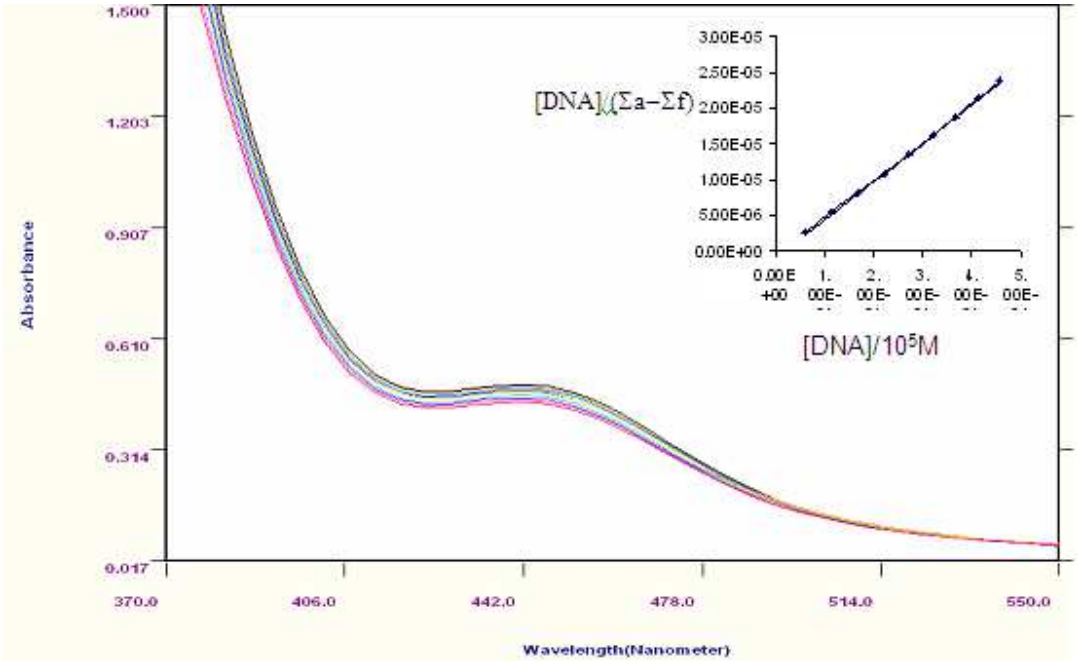


Fig.4: absorption spectra of $PhCH_2Co(DH)_2PY$) (top) in the absence of CT DNA, the absorbance decreasging upon increasing CT-DNA concentrations.

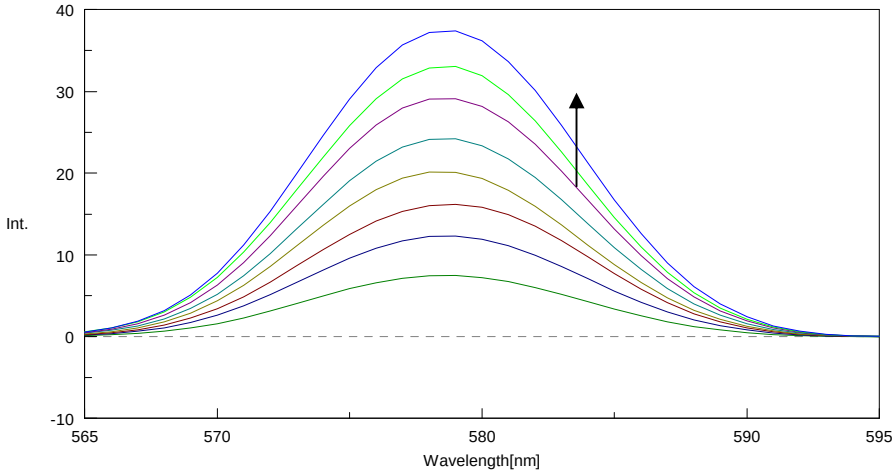


Fig.5: Fluorescence spectra of $PhCH_2Co(DH)_2PY$ complex in aqueous buffer 7.2 in absence and presence of CT DNA, the Fluorescence changing upon increasing CT DNA concentrations. The arrow shows the intensity change upon increasing DNA concentration

$$[DNA]/(\sum_a - \sum_f) = [DNA]/(\sum_b - \sum_f) + 1/K(\sum_b - \sum_f). \qquad (7)$$

Each set of data, when fitted to the above equation, gave a straight line with a slope of $1/(\sum_b - \sum_f)$ and a *y*-intercept of $1/K_b(\sum_b - \sum_f)$. K_b was determined from the ratio of the slope to intercept. Intrinsic binding constant K of ca. $5.4\pm02.1X10^5M^{-1}$ was obtained from the decay of the absorbance. The binding constant indicates that the complex binds strongly to the DNA.

Fluorescence Studies:

The complex of $PhCH_2Co(DH)_2PY$, exhibits luminescence in Tris buffer at pH 7.0 at ambient temperature with a maximum at 560 nm. Binding of the complex to DNA was found to increase the fluorescence intensity. The emission spectra of the complex in the absence and presence of CT DNA and the plot of the relative intensity versus the ratio of [DNA]/[Co] are shown in Fig 5.

Upon addition of CT DNA, the emission intensity increases steadily. This implies that complexes can strongly interact with DNA.

The DNA double helix, containing a stacked array of base pairs in its core, represents a unique and efficient medium for long-range electron transport.[46] This observation is supported by the emission quenching experiments with $[Fe(CN)_6]^{4-}$ as quencher. The ion $[Fe(CN)_6]^{4-}$ has been shown to be able to distinguish differentially bound Co^{3+} species and positively charged free complex ions should be readily quenched by $[Fe(CN)_6]^{4-}$. The complexes bound to DNA can be protected from the quencher, because highly negatively charged $[Fe(CN)_6]^{4-}$ would be repelled by the negative DNA phosphate backbone, hindering quenching of the emission of the bound complex. The method essentially consists of titrating a given amount of DNA-metal complexes with increasing the concentration of $[Fe(CN)_6]^{4-}$. The ferro-cyanide quenching curves for these complexes in the presence and absence of CT DNA are shown in Figure 6. The absorption and fluorescence spectroscopy studies determine the binding of complexes with DNA. The emission intensities of the complexes in presence of DNA increase as compared to the intensity of complexes alone. Fig.6. shows the emission quenching curves of complex in absence of DNA (a) presence of DNA(b).a

$[FeCN)_6]^4$mmol/L

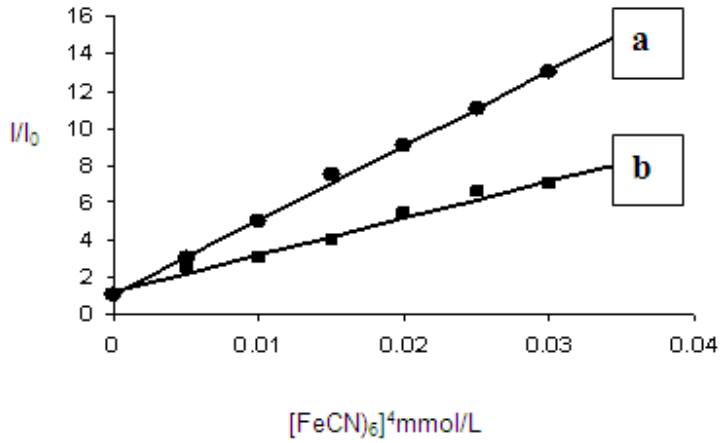


Fig .6: Emission quenching curves of complex in absence of DNA (a) presence of DNA(b)

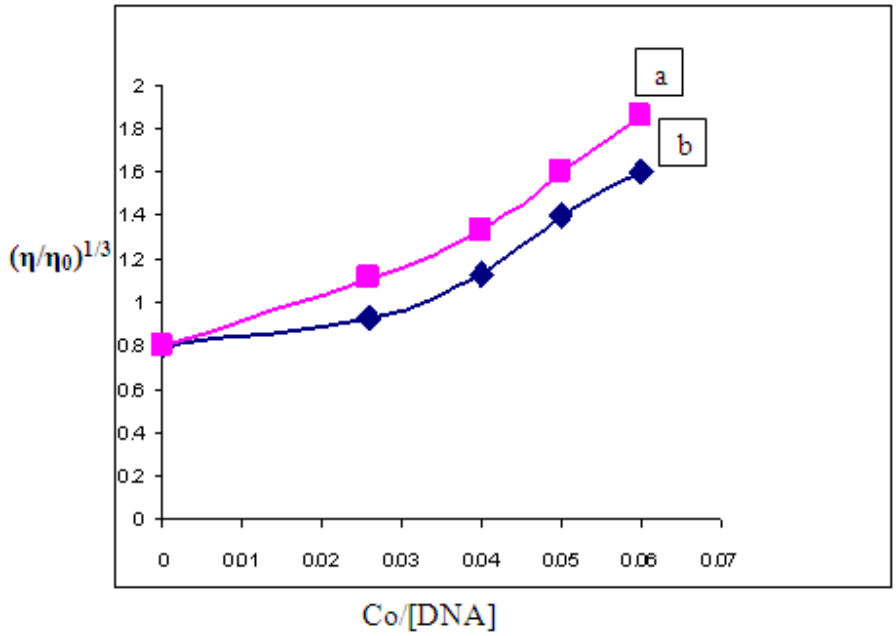


Fig.7:Effect of increasing amounts of Ethedium bromide (a) and complex (b) $PhCH_2Co(DH)_2PY$ on the relative viscosity of CT DNA at 25±0.1 °C.

Viscosity measurements:

To further clarify the interaction between the complexes and DNA, viscosity measurements were performed. Optical photochemical probes provide necessary but not sufficient clues to support a binding model. Hydrodynamic measurements that are sensitive to the length change (i.e. viscosity and sedimentation) are regarded as the least ambiguous and the most critical test of a binding model in solution in the absence of crystallographic structural data.[47,48] A classical intercalation model requires that the DNA helix lengths are separated to accommodate the binding ligand leading to an increase of DNA viscosity. A classical intercalation model demands that the DNA helix must lengthen as base pairs are separated to accommodate the binding ligand, leading to the increase of DNA viscosity. Fig.7 shows the changes in viscosity upon addition of the complex (b) as well as the known DNA intercalator ethidium bromide. On increasing the amounts of (b) $PhCH_2Co(DH)_2PY$ the relative specific viscosity of DNA increases steadily. The result suggests that the complex (b) $PhCH_2Co(DH)_2PY$ intercalates between the base pairs of DNA, which is consistent with our hypothesis.

CONCULSION

Cobalt(III) complexes of equatorial dimethylglyoxime, known as cobaloximes, have been accepted as model compounds for vitamin-B_{12}.The equilibrium constant for the axial ligation of benzyl(aquo)cobaloxime $PhCH_2Co(DH)_2OH_2$ with alanine, alanine methyl ester, pyrazole and dimethyl pyrazole were dependent on the basicity (pKa) of the incoming ligand whereas pyrazole forms more stable complexes than dimethyl pyrazole because of steric hindrance of the substitutents at C_3 and C_5 of DMPY. We have shown that $PhCH_2Co(DH)_2PY$ binds to DNA by intercalation of the benzyl ligand between base pairs of DNA. Evidence for intercalation is provided by the moderate hypochromism in the spin allowed $^1A_1g \rightarrow {}^1T_1g$ transition in the region of 22624cm^{-1} due to $PhCH_2^-$ to Co(III) σ donation.

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