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Spectroscopic characterizations of benzyl(ligand) Cobaloximes: DNA binding and antimicrobial activity

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ABSTRACT

Substitution reactions of benzyl(aquo)cobaloxime with O- donor ligands such as urea, acetamide, semicarbazide and formamide have been studied as incoming nucleophiles. These benzyl (Ligand) cobaloximes have been synthesized and characterized on the basis of IR, LC/MS, ^{1}H and ^{13}C NMR spectroscopy. The frequency changes in the IR spectra and shifts in the NMR were explained on the basis of back- donation of electrons from metal to the ligand and the basicity of the ligand. Further equilibrium constants for axial ligation of the benzyl(aquo) cobaloximes have been determined spectrophotometriclly as a function of pH at λ_{max} of 442 nm. Anti-microbial activity of the complexes have been screened in vitro against the microorganisms Escherichia coli. UV-visible scan of trans $PhCH_2Co(DH)_2H_2O$ with different concentrations of the ligand has been studied. The interaction of benzyl(ligand)cobaloxime with CT-DNA has been studied spectrophotometerically and binding constant has been calculated.

Key words: Benzyl(ligand)cobaloximes, stability constant, LC/MS, antimicrobial activity, DNA binding studies.

INTRODUCTION

The aim of this study is how the vitamin B_{12} models bind to the axial ligand and how the axial ligand stabilizes Co-C bond. Organocobaloximes, initially proposed as models of coenzyme B_{12} have now acquired an independent research field because of their rich coordination chemistry and potentional application in organic synthesis[1]. Organo bis(dimethyl glyoximato) cobalt(III) complexes (organocobaloximes, $CH_3Co(DH)_2L$), where L is a neutral ligand have been extensively studied, mainly for their interest as vitamin B_{12} models[2]. These model complexes

have shown that the ligand in the trans position to Co- C bond has a strong trans influence on the axial alkyl ligand and the nature of the alkyl group has an influence on the thermodynamic stability of the bond [3, 4]. There has been enormous study on the mono nuclear transition metal complexes in view of their interesting electrochemical, photophysical and photochemical studies [5]. 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 [6].

To provide further information concerning the ligand substitution reactions we have studied binding constant for a series of O-donor ligands such as urea, acetamide, semicarbazide and formamide. These were chosen because(i) they form stable complexes with cobaloximes; (ii) significant spectroscopic changes occur in their coordination so that reactions are readily followed spectroscopically; (iii) ligands with a wide range of pKa values; and (v) their coordination chemistry is expected to be simple[7]. Canpolat et al[8] reported that vic-dioxime complexes of cobalt(III) were the most active and may be promising for the development of new antibiotics. The cationic metal complexes possessing planar aromatic ligands may bind to DNA by intercalation which involves stacking of the planar ligand in between adjacent base pairs of the DNA duplex [9–12].

EXPERIMENTAL SECTION

Preparation of $PhCH_2Co(DH)_2L$

$PhCH_2Co(DH)_2H_2O$ was prepared using the procedure of Brown et al [13]. Eq(1-3)

$$Co(CH_3COO)_2.4H_2O + 2(DH)_2 \rightarrow \tfrac{1}{2}[Co^{11}(DH)_2(H_2O)]_2 + 2CH_3COOH + H_2O \quad (1)$$

$$\tfrac{1}{2}[Co^{11}(DH)_2(H_2O)]_2 + 1/2H^+ + 1/2\ H^- \rightarrow H[Co(DH)_2H_2O] \quad (2)$$

$$H[Co(DH)_2H_2O] + PhCH_2Br \rightarrow [PhCH_2Co(DH)_2(H_2O)] + HBr \quad (3)$$

All manipulations were performed under minimal illumination due to photolability of the organo cobalt bond and the solutions were covered with aluminum foil. $PhCH_2Co(DH)_2L$ complexes were isolated by mixing 1:1 of $PhCH_2Co(DH)_2H_2O$ and the base ligand in methanol . This mixture was heated at 40-50°C by constant stirring for 1-2 hours .Then the minimum amount of distilled water was added , the resulting precipitate of brown powder was filtered , washed with distilled water 95% methanol and ether and dried in vacuo (yields were 75-85 %).

Infrared spectra were determined on Perkin-Elmer FTIR-1600 spectrophotometer using KBr pellets. ^1H NMR spectra were recorded on a GE QE 300 NMR spectrometer at 25°C using a 5mm broad band probe. Samples were prepared by dissolving 25-50 mmoles in DMSO-d_6.

Determination of equilibrium constant

$$K_{app} = \frac{[PhCH_2Co(DH)_2L]}{[PhCH_2Co(DH)_2(OH_2)]\,[L]_{free}} \quad (4)$$

Apparent equilibrium constants, K_{app} Eq(4) for the axial ligation of benzyl(aquo) cobaloxime were determined by spectrophotometric measurements at 442nm(λ_{max} of benzyl (aquo) cobaloxime). In 3ml cuvette containing solutions of $PhCH_2Co(DH)_2(OH_2)$, an appropriate buffer (0.2 M) to maintain pH, KCl to maintain ionic strength (1.0M) and varying concentrations of ligand were taken in a cell maintained at 25ºC. Solutions were allowed to equilibrate in a thermostat cell holder for at least 15 minutes prior to addition of cobaloxime. $PhCH_2Co(DH)_2(OH_2)$. For a given pH, K_{app} is calculated from the experimental data as below,

$$\Delta A = [\Delta A_{max}[L]_f/[(1/ K_{app} +[L]_f], \qquad (5)$$

Where ΔA is the difference in absorbance between solutions containing only cobaloxime with and without ligand and, ΔA_{max} is the maximum absorbance change recorded at high ligand concentration. $[L]_T$ and $[L]_F$ is the total and unbound ligand concentration. The data were analyzed by a least square fit of Eq.(6) after rearrangement to give,

$$[L]_f = [L]_T-(C_T.\ \Delta A/ \Delta A_{max}) \qquad (6)$$
$$\Delta A = \Delta A_{max}-1/K_{app}\ \Delta A/[L]_F \qquad (7)$$

By using the measured value of ΔA and ΔA_{max}, $[L]_F$ can be calculated from Eq (6). Where $[L]_T$ is the total concentration of added ligand and C_T is the total concentration of $PhCH_2Co(DH)_2(OH_2)$. The pH independent of equilibrium constants were calculated from Eq.8.

$$K_{eq} = K_{app}/\alpha_L \qquad (8)$$

Where α_L , the fraction of ligand as free base, which calculated from Eq.6.

$$\alpha_L = K_a/(K_a+ [H^+]) \qquad (9)$$

RESULTS AND DISCUSSION

The formation of benzyl(aquo)cobaloxime is confirmed by ^1H, ^{13}C NMR and LCMS. Fig(1,2 ,3). The ligand substitution reactions of benzyl(aquo)cobaloxime $PhCH_2Co(DH)_2(OH_2)$ with urea, acetamide, semicabazide and formamide to form $PhCH_2Co(DH)_2L$ is shown as in Eq.10

$$PhCH_2Co(DH)_2H_2O + L = PhCH_2Co(DH)_2\ (L) + H_2O \qquad (10)$$

The photo-lability of alkylcobaloxime was first studied by Marzilli et al.[14]
The infrared spectra are presented in Table1.

Table 1. IR data for benzyl ligand cobaloximes

S No	Complex	CH_3	C=N	N-O	Co-N	(Co-N) #	N-H
1	$PhCH_2Co(DH)_2OH_2$	1375.3	1571.1	1223.2	510.0	-	3245.7
2	$PhCH_2Co(DH)_2U$	1374.6	1569.3	1229	509.9	450	3146.1
3	$PhCH_2Co(DH)_2AC$	1375.0	1567.9	1232.4	511.7	459.1	3188.0
4	$PhCH_2Co(DH)_2SC$	1374.5	1535.9	1231.1	512.6	462.0	3238.2
5	$PhCH_2Co(DH)_2FA$	1372.4	1540.5	1227.3	515.4	467.6	3334.3

Recorded as KBr discs and values in cm^{-1}, where U = urea, AC = acetamide, SC = semicarbazide, FA= formamide. ν(Co-N)# of ligand

The disappearance of the peak at 2362 cm^{-1} and appearance of a new peak at about 455cm^{-1} is assigned to the ν(Co-N) indicates the formation of benzyl(L)cobaloxime by replacement of H_2O molecule. The ν(Co-N)cm^{-1} is observed at 509.9-515.4cm^{-1}. Most of the bands appear as medium to strong sharp bands. The occurrence of well-defined sharp bands indicates that there is coordination between the metal and the lone pairs of electrons on the nitrogen. In all the complexes, the ν(O-H) band due to O..H-O hydrogen bridges in the ligand is assigned at 3400-3550cm^{-1} and all appear as very broad bands. The frequency band at around 1571 cm^{-1} is assignable to the ν(C=N) stretching frequency of dimethylgloxime. It is shifted to lower wave number when water is replaced by amides. Burger et al [15] reported on the basis of the frequency shifts of the C=N vibration that the lower the 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 [16], we could assign the peaks around 1232 cm^{-1} and 1066 cm^{-1} to the N-O stretching vibrations. 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 electronic spectra of benzyl(aquo)cobaloxime in methanol will show spin allowed $^1A_1g \rightarrow {}^1T_1g$ transition of an octahedral geometry in the region of 22624cm^{-1} due to $PhCH_2^-$ to Co(III) σ donation[17], which is drastically decreases in the formation of 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.[18]

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

The ^{1}H NMR spectra of $PhCH_2Co(DH)_2H_2O$ showed signal at 1.99 ppm that corresponding to the equatorial methyl groups, and the signal at 5.2 attributed to CH_2 of benzyl, aromatic CH appeared at (6.7-7.3 ppm). The dimethyl glyoxime methyl resonance of $PhCH_2Co(DH)_2L$ appear up field by about .05 ppm when compared with the values of the free dimethylglyoxime. The up field shift is due to the interaction of the benzyl group with equatorial dimethyl glyoxime methyl through space. 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[19]. Also these interactions cause the nonequivalence of the dioxime portions and the CH_2 protons become diastereotopic in 2-substituted benzylcobaloxime[20,21] or the anisotropy of cobalt atom alone has been invoked to explain the ^{1}H NMR shiftsTable.2 and the broadness of these resonances generally attributed to the quadrupolar relaxation by the ^{59}Co nucleus (I =7/2)[22].

Table.2. ^{1}H NMR data of benzyl ligand cobaloximes

S.No	complex	CH_3 of (DH)	C_6H_5	CH_2	NH_2	CH_3/OH of ligand
1	$PhCH_2Co(DH)_2OH_2$	1.99	6.7-7.3	5.2	-	-
2	$PhCH_2Co(DH)_2U$	1.98	6.7-7.3	5.2	7.5	OH = 11.7
3	$PhCH_2Co(DH)_2AC$	1.96	6.7-7.2	5.2	7.3	CH_3 = 1.74
4	$PhCH_2Co(DH)_2SC$	1.96	6.7-7.1	5.1	8.0	OH = 10.2
5	$PhCH_2Co(DH)_2FA$	1.97	6.6-7.4	4.7	7.9	OH = 11.3

Chemical shifts are in ppm

In the free ligand the position of O=C-NH_2 resonance occurs at about 5.7 ppm[23] which is shifted to down field in metal complexes due to the resonance of lone pair of electrons on NH_2

with –C=O. In the complexes of $PhCH_2Co(DH)_2U$, $PhCH_2Co(DH)_2FA$, $PhCH_2Co(DH)_2SC$ and $PhCH_2Co(DH)_2AC$ the signals at 7.5, 7.9, 8.0 and 7.3 ppm respectively corresponding to $–NH_2$ which are shifted to down field as compared to the free ligand. The OH groups of dimethylglyoximes absorb at about 11.7 ppm.Fig1.

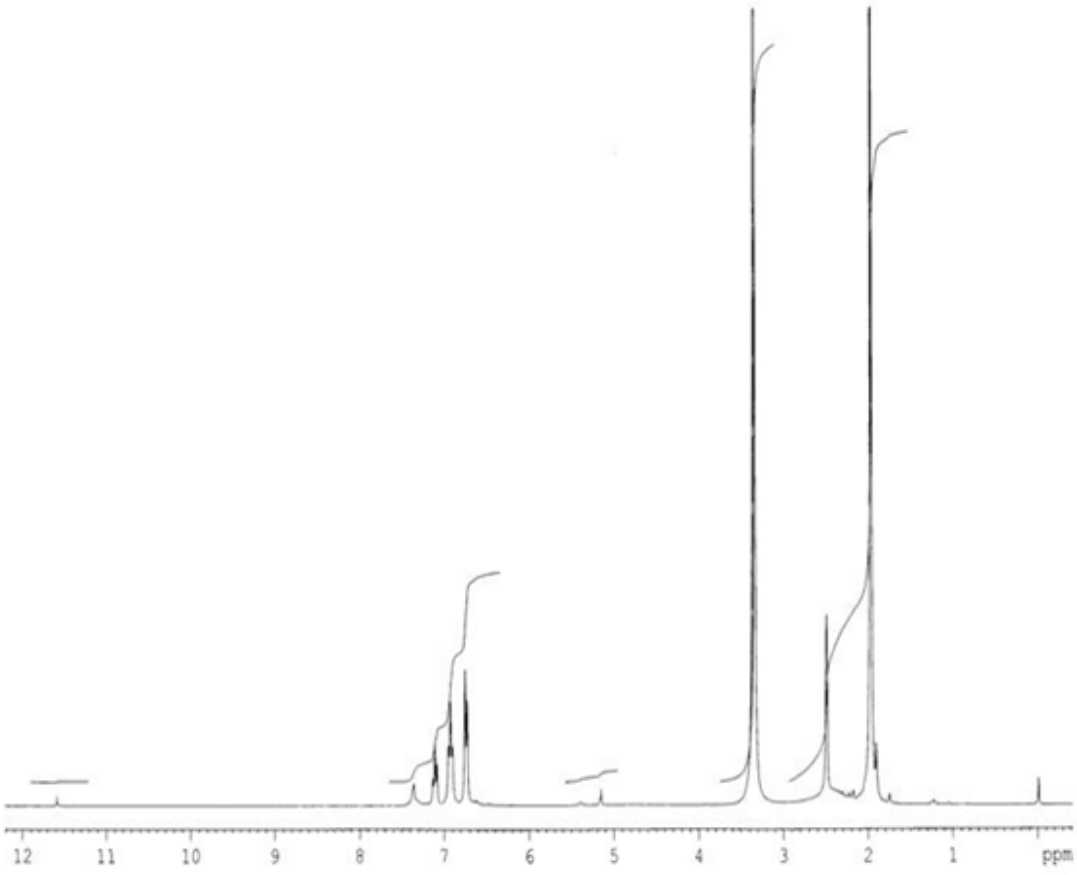

Fig.1. ^{1}H NMR Spectrum of $PhCH_2Co(DH)_2U$ complex in DMSO- d_6

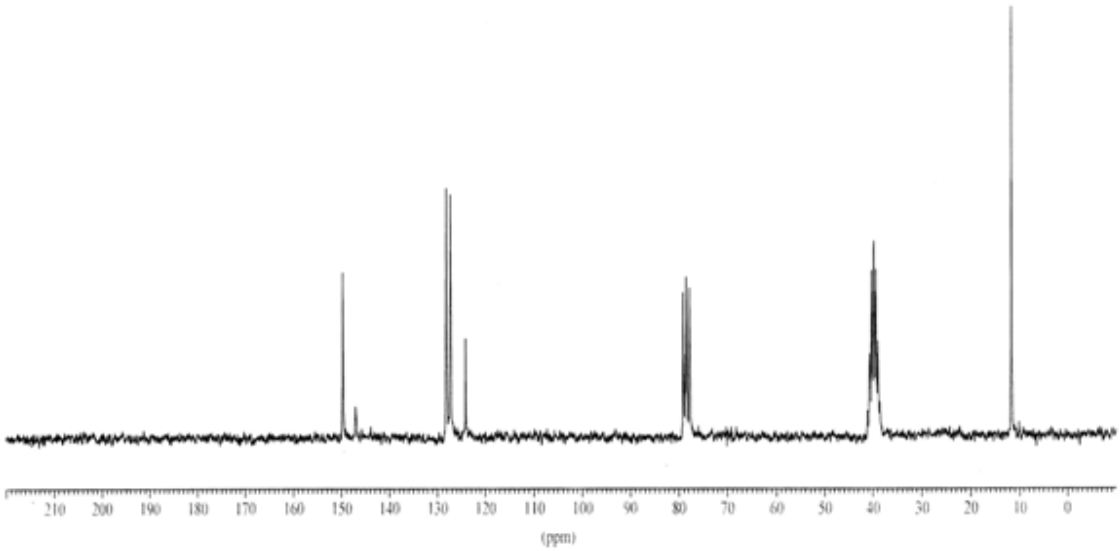

Fig. 2 : ^{13}C [^{1}H] NMR spectra of benzyl aquo cobaloxime in DMSO d_6 +$CDCl_3$

The ^{13}C NMR spectra of benzyl(aquo)cobaloxime peak at 11.73 ppm corresponding to four methyl groups and a peak at 149.7 ppm corresponding to four equatorial carbons of dioxime ν(CN), the remaining four peaks in the region of (124.1-128.1) corresponding to the aromatic carbons. Due to quadrupolar relaxation of ^{59}Co (I =7/2), benzyl carbon which is bonded to cobalt(III) did not observed..Fig.2.

In Mass spectra of formamide complex, the major peak is at 425 m/z which is corresponding to the exact average atomic mass of benzyl(formamide)cobaloxime suggesting the formation of this complex. Fig.3.

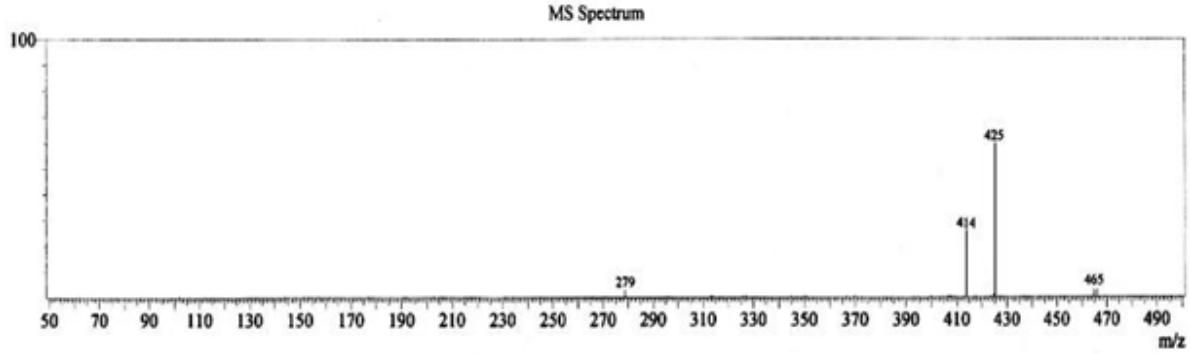

Fig. 3: Mass spectra of benzyl (formamide) cobaloxime

Elemental analysis:

In the elemental analysis of these complexes, it was exhibited that all the complexes are in good agreement with the calculated values which conforming the formation of benzyl(L)cobaloximes. Table.3.

Table.3. 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 H_2O	$CoC_{15}H_{23}N_4O_5$ 398	45.18 (45.22)	5.75 (5.77)	14 (14.7)
2	Urea NH_2CONH_2	$CoC_{16}H_{25}N_6O_5$ 440	43.43 (43.63)	5.65 (5.68)	18.91 (19.09)
3	Acetamide CH_3CONH_2	$CoC_{17}H_{26}N_5O_5$ 439	46.32 (46.46)	5.83 (5.92)	15.86 (15.94)
4	Semicarbazide $NH_2CONHNH_2$	$CoC_{16}H_{26}N_7O_5$ 455	41.90 (42.19)	5.66 (5.71)	21.68 (21.53)
5	Formamide $HCONH_2$	$CoC_{16}H_{24}N_5O_5$ 425	44.95 (45.17)	5.40 (5.64)	16.31 (16.47)

**Calculated values in parenthesis*

Determination of equilibrium constant:

The complex formation constants for the substitution of water molecule on $PhCH_2Co(DH)_2(OH_2)$ by urea, acteamide, semicabazide and formamide were determined by taking a fixed concentration of the complex and varying the ligand concentration presented by Eq(10).

$PhCH_2Co(DH)_2H_2O + L = PhCH_2Co(DH)_2 (L) + H_2O$ …. (10)

The pH above and below the Pka values, the K_{app} values for the ligation of axial H_2O in $PhCH_2Co(DH)_2(OH_2)$ with the above ligands were determined as a function of pH by spectrophotometer Fig.4 and Table.3.

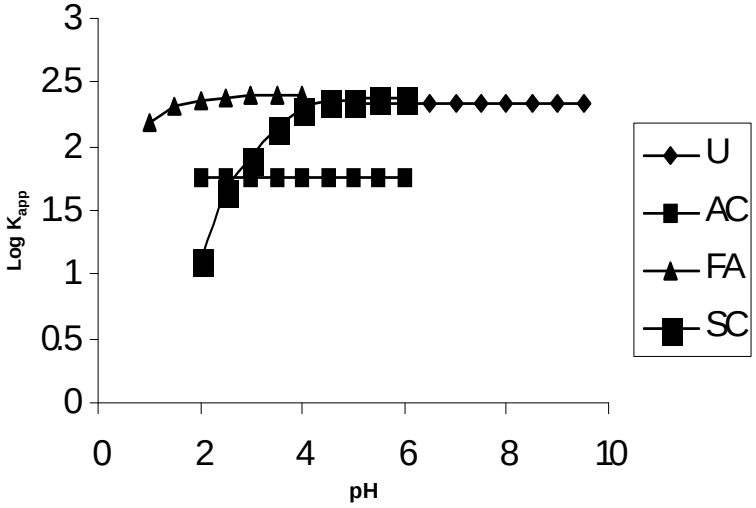

Fig.4: Dependence of apparent equilibrium constants ($logK_{app}$) on pH for the axial ligation of $PhCH_2Co(DH)_2H_2O$ at λ_{max} of 442nm by different ligands at 25°C

Table 4. Formation constants ($logK_{app}$) for the axial ligation of the benzyl(aquo)cobaloxime by different ligands at 25°C for different pH values

pH	U	AC	FA	SC
1			2.193	
1.5			2.307	
2		1.7581	2.363	1.11
2.5		1.7584	2.38	1.65
3		1.7589	2.39	1.9
3.5		1.7589	2.391	2.14
4		1.7589	2.391	2.3
4.5		1.7589		2.35
5	2.33	1.7589		2.36
5.5	2.332	1.7589		2.37
6	2.334	1.7589		2.37
6.5	2.337			
7	2.339			
7.5	2.339			
8	2.339			
8.5	2.339			
Keq	218.7	57.4	246.3	237.4

The values of equilibrium constant K_{app} for the axial ligation of $PhCH_2Co(DH)_2(OH_2)$ by various O-donor ligands given in Table (4). The pH dependent binding constants were measured from 1 to 4 for formamide, 2 to 6 for acetamide and semicarbazide and 5 to 8.5 for urea. They are

depending on the pKa of the ligands; as the pKa increases the K_{eq} increases , but above pKa, $logK_{app}$ is independent of pH.

It was observed that the K_{app} value below the pKa is very low due to the protonation of the ligand. As the pH increases the ligand is deprotonated and binds strongly to Co(III) complex, so that K_{app} is larger at the higher pH. The affinities of the ligands follow the order: <SC<U<FA as the basicity increases, the stability constant increases in these ligands. This stability order can be explained by considering the basicity of the ligands and σ-donation[24].

The UV/VIS scan of trans-$PhCH_2Co(DH)_2H_2O$ is given in Fig 5. Shows the variation of the absorption spectrum of the complex in the presence of different concentrations of the ligand urea depending on pKa values of the ligand as the concentration of the ligand increases, the absorbance decrease.

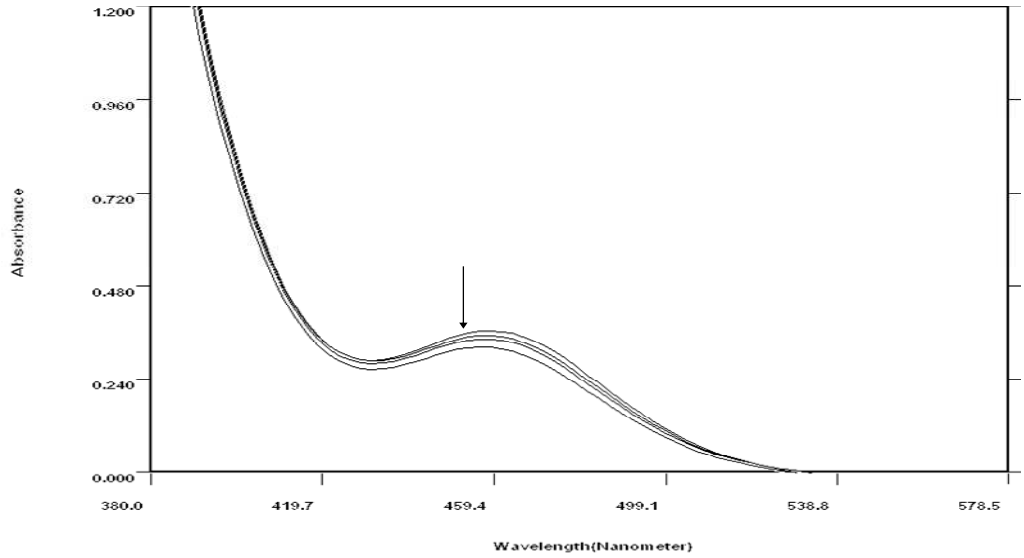

Fig .5:UV/VIS Scan: Binding of the benzyl(aquo)cobaloxime complex with different concentrations of urea at pH 6.5 and 25°C.

Antimicrobial activity

In this study, we have investigated that the antimicrobial activity of benzyl ligand cobaloximes against gram negative bacteria (E. coli) and gram positive bacteria (S.aureus). When the agar plates were added with these complexes and incubated for 24 hours at 37^0C in a humidified CO_2 atmosphere, it was observed that the studied complexes inhibit the growth of E. coli and S.aureus, the ranges of inhibited areas were 12-15 mm and the inhibited area for the standard compound is 16 mm (tetracycline). In general all these complexes were found to show antimicrobial activity and may be promising for progression of new drugs.

DNA Absorption Studies:

The application of electronic absorption spectroscopy in DNA binding studies is one of the most useful techniques [25-26]. 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.6 represents the absorption spectra of

$PhCH_2Co(DH)_2SC$ in the absence and presence of increasing amounts of DNA. In the UV region, the intense absorption bands observed for Co^{111} complexes attributed to intraligand $\pi\rightarrow\pi^*$ transition of the coordinated groups. Addition of increasing amounts of the complex results in hypochromism and bathochromic shift in the UV spectra of $PhCH_2Co(DH)_2SC$.

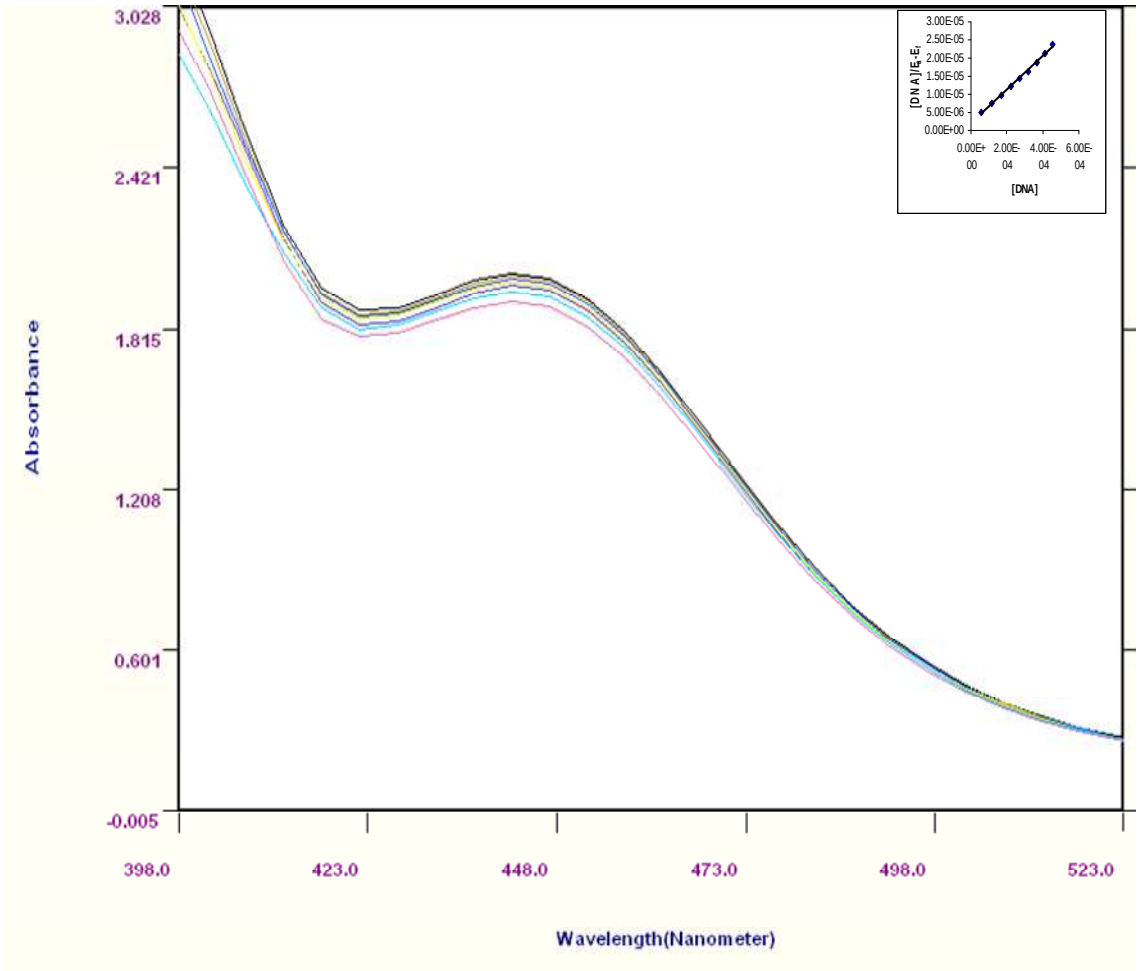

Fig.6: absorption spectra of $PhCH_2Co(DH)_2SC$) (top) in the absence of CT DNA, the absorbance changing upon increasing CT DNA concentrations. The arrow shows the intensity change upon increasing DNA concentration.

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.(11) to obtain the intrinsic binding constant, *K*.[27,28] Where $\sum_b$ refers to the extinction coefficient of the complex in the fully bound form.

$$[DNA]/(\sum_a - \sum_f) = [DNA]/(\sum_b - \sum_f) + 1/K(\sum_b - \sum_f). \quad (11)$$

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. $6.6\pm03.2X10^5M^{-1}$ 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 $PhCH_2Co(DH)_2SC$, 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 7.

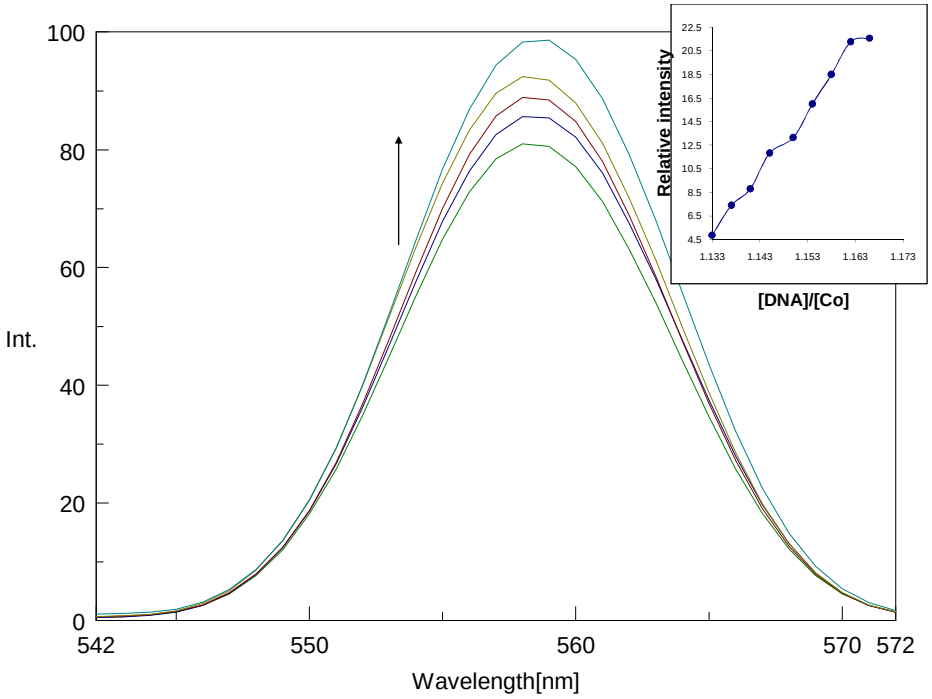

Fig.7: Fluorescence spectra of $PhCH_2Co(DH)_2(SC)$ (bottom) in the absence of CT DNA, the Fluorescence intensity increasing changing upon increasing CT DNA concentrations. The arrow shows the intensity change upon increasing DNA concentration

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

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.[29,30] 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 8.

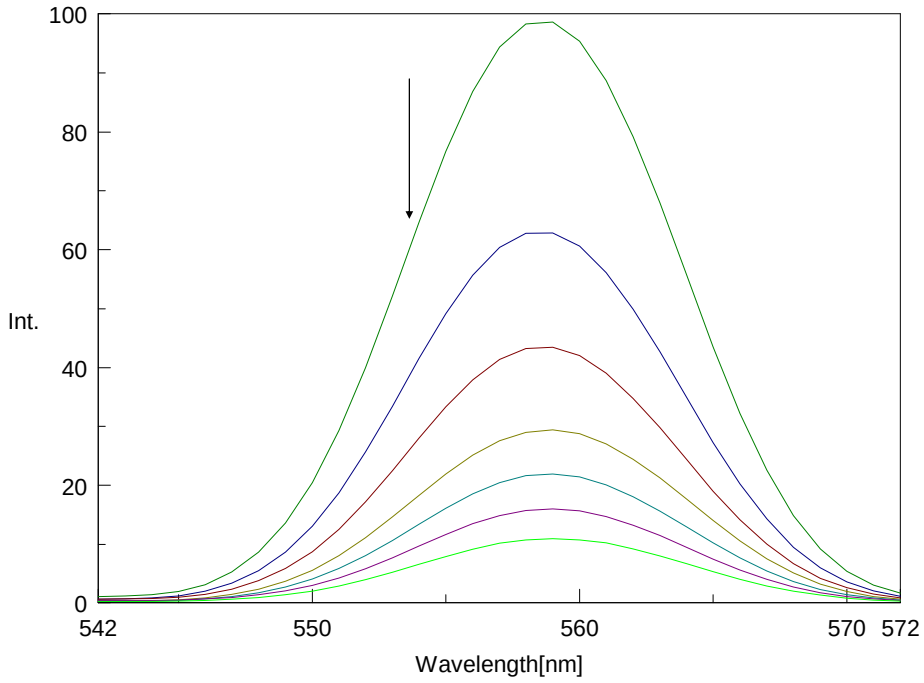

Fig .8: Fluorescence quenching spectra of $PhCH_2Co(DH)_2SC$ and complex+ DNA by ferrocyanide

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.9 shows the emission quenching curves of complex in absence of DNA (a) and presence of DNA(b).

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.[31,33] 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.10 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) $PhCH_2Co(DH)_2SC$ the relative specific viscosity of DNA increases steadily. The result suggests that the complex (b) $PhCH_2Co(DH)_2SC$ intercalates between the base pairs of DNA, which is consistent with our hypothesis.

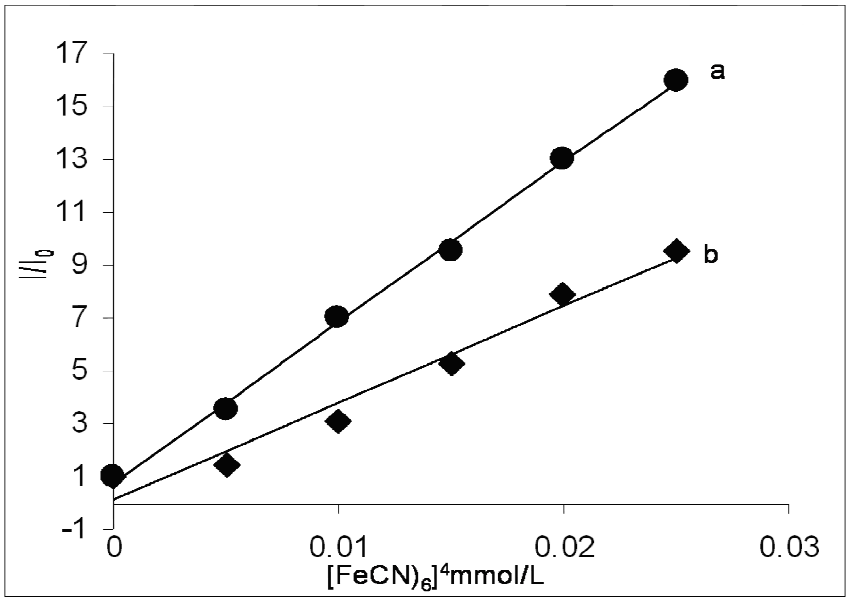

Fig .9: Emission quenching curves of $PhCH_2Co(DH)_2SC$ in absence of DNA (a) presence of DNA(b)

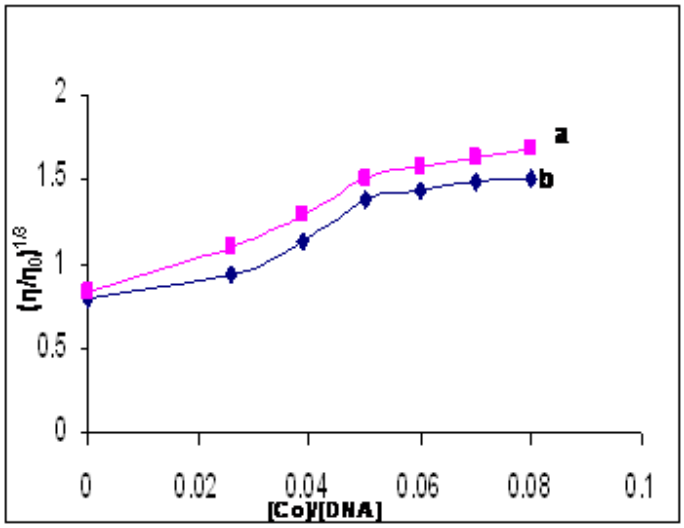

Fig10: Effect of increasing amounts of Ethedium bromide (a) and complex (b) $PhCH_2Co(DH)_2SC$ on the relative viscosity of CT DNA at 25±0.1 ºC.

CONCLUSION

Benzyl(aquo)cobaloxime reacts with urea, formamide, acetamide and semicabazide to form stable benzyl(ligand)cobaloxime whose stability has been explained on the basis of basicity of the ligand. The affinities of the ligands follow the order: AC<SC<U<FA as the basicitey of ligand increases, the stability constant increases. The binding constant suggest that the complex

$PhCH_2Co(DH)_2L$ binds with DNA. The result suggests that $PhCH_2Co(DH)_2L$ intercalates between the base pairs of DNA which is consistent with our hypothesis.

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