Some New Chromogenic Reagents for Copper(I) and Iron(II); Pyridyl-Substituted Pyrazine and Quinoxaline Compounds

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Summary: Fifteen new pyridyl-substituted pyrazine ligands have been synthesized and their IR and mass spectra are recorded. The ligands containing ethyl, methyl or phenyl groups adjacent to donor nitrogen atoms in aromatic pyridyl or pyrazine rings react only with copper(I), but the reagents 2,3-bis(2'-pyridyl)-5-phenyl-5,6-dihydropyrazine(III),2,3-bis(2'-pyridyl)-5-phenyl-6-methyl-5,6-dihydropyrazine(V), 2,5-diphenyl-3-(2'-pyridyl)-5,6-dihydropyrazine(IX) and 2,3-bis(2'-pyridyl)-5-phenylpyrazine(XI) react with copper(I) an iron(II) to for coloured compouns. Their reactions have been studied spectrophotometrically and the effects of methyl, ethyl and phenyl groups substitution on the reactions towards copper(I) and iron(II) have been investigated in terms of solution stability, molar absorptivity and wavelength of maximum absorbance.

Finally the reagent bis 2,3-(6-methylpyridyl)]-5,5,6,6-tetramethyl-5,6- dihydropyrazine(II) proved to be better chromogenic reagent for copper, have been applied for the spectrophotometric determination of copper in water samples and human head hairs.

Introduction

The pyridyl-substituted pyrazine and quinoxaline compounds containing ferroin and cuproin functionalities are of considerable interest because of their close resemblance to 1,10-phenanthroline and 2,2'-bipyridine. Goodwin and Lions [1], Jenson and Pflam [2], Pflam et al [3], Pfeiffer and Case [4], Schilt-and Hoyle [5], Stephen and his co-workers [6-9] and Khuhawar et al [10,11] have prepared a number of pyridyl substituted pyrazine ligands and have studied them spectrophotometrically. In the present work some more copper and iron selective reagents have been prepared and studied.

Experimental

Preparation of pyridyl-substituted dihydropyrazine and quinoxaline compounds (I to X)

Equimolar aqueous solution of 1-phenyl-1,2-diaminoethane hydrochloride, 1-phenyl-1,2-diaminopropane hydrochloride, 2,3-diaminopen- tane hydrochloride and 1,2-diamino-benzene hydrochloride was neutralized with ammonia and diamines were extracted with chloroform. The dried extract over anhydrous sodium sulphate was slowly added to the solution of 2,2'-pyridil, 6,6-dimethyl-2,2'- pyridil and 1-phenyl-2-(2'-pyridyl)-glyaxal in ethanol.

The mixture was refluxed for 30 min and most of the solvent was evaporated under vacuum desicator. The prodcut was dissolved in n-hexane repeatedly and the gummy mass solidified slowly and was finally recrystallised from n-hexane, petroleum spirit (40-60°C) or ethanol.

Similarly for the preparation of the reagents I, II, VIII, the refluxing solution of 2,2'-pyridil, 6,6-dimethyl-2,2'-pyridil and 1-phenyl-2-(2'-pyridyl) glyaxal in ethanol was added 2,3-dimethyl- 2,3-diaminobutane in ether. The mixture was refluxed for 30 min. was concentrated and cooled to -5°C. The compounds precipitated as crystalline materials and were recrystallised from petroleum benzene (60-80).

Diamine 2,3-dimethyl-2,3-diaminobutane was prepared by reported method [13] and 1-phenylethylenediamine hydrochloride, 1-phenyl-1,2-diaminopropane hydrochloride and 2,3-dia- minopentane hydrochloride were prepared by reduction of phenylglyoxime, methylphenylglyo- xime and ethylmethylglyoxime in ethanol with sodium metal following a general procedure [14].

All the spectrophotometric studies were carried out on Hitachi 220- Spectrophotometer. The elemental micro analysis was carried out by Elemental MicroAnalysis Ltd., U.K. Mass spectra were recorded at HEJ Research Institute of Chemistry, University of Karachi. Infrared spectra in the range of 4000-250 cm^{-1} were recorded on Hitachi 250-60 using KBr and in the range 3800-625 cm^{-1} on Unicam 1025 using nujol mull technique.

Reagent solutions (0.2%) in ethanol or ethanol: water (1:1) were used. Solutions and analytical procedure were the same as reported earlier [8].

Analysis of water samples

Duplicate water samples (200 ml) from drinking water collected in steel tank and copper still collected in copper container were collected after running the water for 2-3 min. The water samples were transferred to a 500 ml separating funnel, followed by addition of 5 ml of 1% w/v ascorbic acid, 8 ml of acetic acid-acetate buffer pH 4.0, 10 ml of 0.2% w/v reagent solution (2,3-Bis-[2'-(6-methylpyridyl)]- 5,5,6,6-tetramethyl-5,6-dihydropyrazine), 5 g of solid $NaClO_4$ and 10 ml isoamyl alcohol. The contents were mixed and layers were allowed to separate. The organic layer was collected in 25 ml volumetric flask and the extraction was repeated with 8-10 ml of extracting solvent. Ethanol (1 ml) was added to each of the flask before adjusting the volume. The asborbance of the solution was measured against reagent blank prepared from deionized double distilled water. The average absorbance of atleast two samples was used to evaluate, the amount of copper from the calibration curve prepared from deionized double distilled water (200 ml) containing known amounts of copper.

The amount of copper in the water samples were also evaluated using bathocuproine as a complexing reagent.

Analysis of Copper in Human Head Hair Sample

Complete whole head hair sample (4 g approximate) was washed with acetone, followed by three rinses with water and again with acetone to remove the contaminants [15].

Exactly weighed hair sample in duplicate was digested with digestion mixture of nitric acid-sulphuric acid (5:1) [16]. The sample mixture was heated and additional digestion mixture was added and the process was repeated several times until the mixture became clear and was nearly dried. The residue was dissolved in deionized double distilled water and volume was adjusted to 50 ml. A reagent blank was also prepared.

Two solutions (10 ml) were taken from a sample, neutralized to pH 3-5 with 0.2 N sodium hydroxide. Five ml of acetic acid-acetate buffer (pH 4) was added, followed by 4 ml of freshly prepared 1% w/v ascorbic acid, 5 ml of 0.2% w/v organic reagent II (2,3-bis[2'-(6'-methylpyridyl)]-5,5,6,6-tetramethyl-5,6-dihydropyrazine), 2 g of sodium per -chlorate and 7-8 ml of the extracting solvent (isoamyl alcohol). The contents were mixed and the organic layer was collected in 25 ml volumetric flask and the extraction was repeated with 10 ml of solvent. Ethanol (1 ml) was added before adjusting the volume with solvent. The absorbance of the solutions was measured against reagent blank.

Table-1: Elemental Micro-Analysis of the Compounds (I-XV).

	Compound	Mol Formula	M.P. °C	% Expected (% Found) C	H	N	MS m/z (% rel.intensity)
I	2,3-Bis(2'-pyridyl)-5,5 6,6-tetramethyl-5,6-dihydropyrazine	$C_{18}H_{20}N_4$	125	73.97 (73.49)	6.80 (7.49)	19.17 (19.29)	292(1.8), 277(100), 236 (14.9), 195 (21.9), 105 (54.3)
II	2,3-Bis[2'-(6-methyl-pyridyl)]-5,5-6,6-tetramethyl-5,6-dihydropyrazine	$C_{20}H_{24}N_4$	155	75.0 (75.05)	7.5 (8.05)	17.5 (17.93)	320(4.3), 305 (100), 264 (7.9), 223 (61.9), 119 (23.3).
III	2,3-Bis(2'-pyridyl)-5-phenyl-5,6-dihydro-pyrazine	$C_{20}H_{16}N_4$	165	76.92 (77.00)	5.12 (5.34)	17.95 (18.49)	312 (82), 311 (100), 284 (5.0), 235(71), 208 (33), 181 (53), 104 (56).
IV	2,3-Bis[2'-(6'-methyl-pyridyl)]-5-phenyl-5,6-dihydropyrazine	$C_{22}H_{20}N_4$	130	77.64 (77.71)	5.88 (5.67)	16.47 (16.97)	340(100), 263 (80), 236 (17.4) 209 (32.1), 118 (11.3) 92(33.9)
V	2,3-Bis(2'-pyridyl)-5-phenyl-6-methyl-5,6-dihydropyrazine	$C_{21}H_{18}N_4$	170	77.30 (76.56)	5.52 (5.74)	17.17 (17.11)	326 (20.1), 311(100), 284 (5.2), 208(22), 181 (29.8), 115 (13.2), 91 (15.8), 78(37.7)
VI	2,3-Bis[2'-(6'-methylpyridyl)]-5-phenyl-6-methyl 5,6-dihydropyrazine	$C_{23}H_{22}N_4$	130	77.97 (78.40)	6.21 (6.26)	15.84 (15.72)	354(24.5), 339(100), 312 (4.4), 277(30.7), 236 (18.4), 209(28), 105 (28.3).
VII	2,3-Bis[2'-(6'-methylpyridyl)-5-ethyl-6-methyl-5,6-dihydropyrazine	$C_{19}H_{22}N_4$	140	74.50 (74.57)	7.17 (7.53)	18.32 (18.21)	306(4.3), 291(28.9), 277(100), 262(12.2), 236 (4.4), 209 (7), 92 (21.9), 65 (14).
VIII	2-Phenyl-3-(2'-pyridyl)-5,5,6,6-tetramethyl-5,6-dihydropyrazine	$C_{19}H_{21}N_3$	132	78.35 (77.73)	7.21 (7.26)	14.43 (14.34)	291(6.1), 276 (100), 235 (24.5), 195 (22.8), 104(29.9).
IX	2,5-diphenyl-3-(2'-pyridyl)-5,6-dihydro-pyrazine	$C_{21}H_{17}N_3$	165	81.02 (81.05)	5.46 (5.07)	13.5 (13.57).	

Table-1: continued.

Compound	Mol Formula	M.P. °C	% Expected (% Found) C	H	N	MS m/z (% rel.intensity)
X 2-phenyl-3-(2'-pyridyl)-quinoxaline	$C_{19}H_{13}N_3$	120	80.56 (81.02)	4.59 (4.25)	14.84 (14.70)	283(28), 282(100), 255(4), 242(4), 179(10), 102(5.3)
XI 2,3-Bis(2'-pyridyl)-5-phenylpyrazine	$C_{20}H_{14}N_4$	160	77.41 (77.81)	4.51 (5.17)	18.06 (18.21)	310(42), 309 (100), 282 (44), 232 (2.6), 206 (3.5), 102 (21.0).
XII 2,3-Bis[2'-(6'-methylpyridyl)]-5-phenylpyrazine	$C_{22}H_{18}N_4$	175	78.10 (77.93)	5.32 (5.62)	16.56 (15.33)	338 (57.9), 337 (100), 323 (4.3), 296 (3.5),259(2.6), 208 (5.2), 102 (12.3).
XIII 2,3-Bis(2'-pyridyl)-5-phenyl-6-methylpyrazine	$C_{21}H_{16}N_4$	170	77.77 (77.34)	4.93 (4.86)	17.28 (17.05)	
XIV 2,3-Bis[2'-(6'-methylpyridyl)]-5-phenyl-6-methyl-pyrazine	$C_{23}H_{20}N_4$	130	78.42 (78.64)	5.67 (5.52)	15.89 (15.72)	352 (64.9), 351(100), 337 (3.5), 311 (3.6), 208(8.5), 115 (15).
XV 2,3-Bis[2'-(6'-methylpyridyl)]-5-ethyl-6-methyl-pyrazine	$C_{19}H_{20}N_4$	135	75.0 (74.73)	6.57 (7.23)	18.42 (18.31)	

The hair solutions were also analysed using spectrophotometric method with aid of bathocuproine as a complexing reagent.

Results and Discussion

The reagents are readily prepared and their IR indicates one to three bands of weak to medium intensity above 1600 cm^{-1}, which could be assigned to C=N bands of dihydropyrazine and pyrazine rings. The reagents show a number of bands between 1600-1300 cm^{-1} due to C=C and C=N streching vibrations in pyridyl, dihydropyrazine and pyrazine rings. The reagents also show a characteristic doublet near 1600 cm^{-1} with peaks separated by 20 $\pm$ 5 cm^{-1}. The reagents containing the 2- pyridyl group show two bands at 800 $\pm$ 5 cm^{-1} and 760 $\pm$ 5 cm^{-1} and the 6-methyl-2-pyridyl group shows bands in the region 805 $\pm$ 5 cm^{-1} and 745 $\pm$ 5 cm^{-1}.

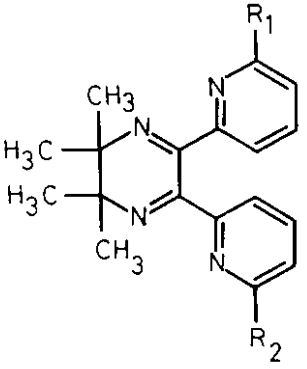
The mass spectra of the reagents I, II and VIII indicate molecular ion peak at m/z 292, 320 and 291, respectively with relative intensity in the range of 1.8-6.1%, and the base peak is obtained with the loss of -CH_3 group. All the three reagents successively lose m/z 41, 41 corresponding to C_2H_3N and C_3H_5 fragment from dihydropyrazine rings. The reagents I and VIII lose a main fragment corresponding to C_7H_6N (m/z 90) and II loss C_6H_4N(m/z 104) to give the peaks at m/e 105, 104 and 119, respectively.

M^+ peaks of reagents III and IV at m/z 312 and 340 lose -C_6H_5 followed successively by -CHN and -CHN from the dihydropyrazine ring to give peaks at m/z 181 and 209 respectively. The reagents III and IV subsequently lose corresponding to C_5H_3N and C_6H_5N respectively to give peaks at m/z 104 and 118. The reagents V and VI lose -CH_3 from the pyrazine ring to give base peaks at m/z 311 and 339 which subsequently lose the fragments corresponding to CHN, C_6H_4 and CHN to give the peaks at m/z 181 and 209 respectively.

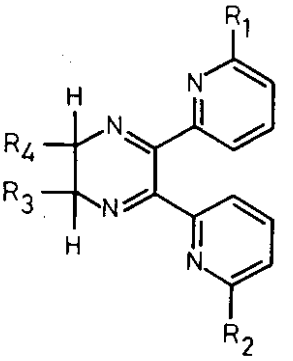
Mass spectra of the pyrazine compounds, X, XI, XII, XIV and XVI show molecular ion peaks at m/z 283, 210, 338, 352 and 309 respectively with relative intensity in range of 28-64%. The base peak is obtained with the loss of a hydrogen atom, followed by loss of fragment corresponding to C_6H_3CN from the reagents, X, XI and XVI to give peaks at m/z 179, 206 and 205 respectively. Reagent XIV loses fragement corresponding to -CH_3, followed by C = N and subsequently give peak at m/z 208. The base peak of the reagent XII lose the fragment corresponding to C_5H_4N to give peak at m/z 259 and finally join peak at m/z 208.

Spectrophotometric Studies

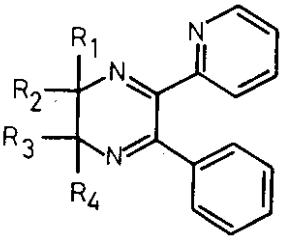
The pyridyl-substituted dihydropyrazine compounds I, II, IV, VI, VII, VIII react only with copper(I) and the reagents III, V, IX react with copper(I) and iron(II) to form coloured compounds, which are easily extractable in nonaqueous solvents as perchlorate ion association complexes. The absorptiometric properties of copper(I) reactions with dihydropyrazine compound (Table-2)

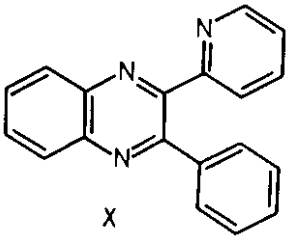


I R_1:R_2 = H
II R_1:R_2 = CH_3



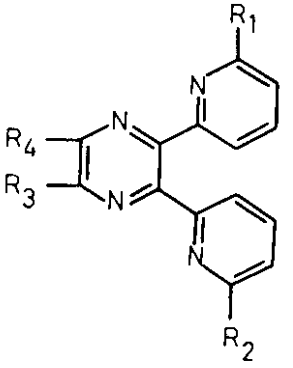
III R_1:R_2:R_3 = H:R_4 = C_6H_5
IV R_1:R_2 = CH_3:R_3 = H:R_4 = C_6H_5
V R_1:R_2 = H:R_3 = CH_3:R_4 = C_6H_5
VI R_1:R_2:R_3 = CH_3:R_4 = C_6H_5
VII R_1:R_2R_3 = CH_3:R_4 = C_2H_5





X

VIII R_1:R_2:R_3:R_4 = CH_3
IX R_1:R_2:R_3 = H:R_4 = C_6H_5



XI R_1:R_2:R_3 = H:R_4 = C_6H_5
XII R_1:R_2 = CH_3:R_3 = H:R_4 = C_6H_5
XIII R_1:R_2 = H:R_3 = CH_3:R_4 = C_6H_5
XIV R_1:R_2:R_3 = CH_3:R_4 = C_6H_5
XV R_1:R_2:R_3 = CH_3:R_4 = C_2H_5

Fig. 1: Structural Diagrams of the ligands.

Table-II: Quantitative Absorptiometric Data of the Reactions of Pyridyl-substituted pyrazine and quinoxaline compounds toward copper (I) and Iron(II).

Compound	Metal ion	Solvent	pH	λ−max nm	ε-mole^{-1} cm^{-1}	Chromophoric stability
I -		Ethanol	-	369	596	
				278	7349	
				206	11664	
	Cu(I)	Water	3-7	550	12500	6 hr
		Amyl alcohol	3-7	535	11300	24 hr
II	-	Ethanol	-	371	712 -	
				280	5547	
	Cu(I)	Water	2-7	555	12740	24 hr
		Amyl Alcohol	2-7	535	11790	24hr
III		Ethanol	-	272	7558	-
				220	1308	
	Cu(I)	Ethanol	3-8	545	7820	30 min
		Isoamyl alcohol	3-8	542	6190	2 hr
	Fe(II)	Ethanol	3-7	634	10280	2 hr
		Isoamyl alcohol	3-7	635	8410	2 hr
		Nitro-benzene	3-7	613	11480	24 hr
IV -		Ethanol	-	276	12220	-
				212	28900 -	
	Cu(I)	Ethanol	3-8	550	8940	1hr
		Isoamyl alcohol	3-8	545	9261	6 hr
V -		Ethanol	-	269	9270 -	
				211	23100 -	
	Cu(I)	Ethanol	3-7	530	8980	6 hr
		Amyl alcohol	3-7	545	7434	4 hr
	Fe(II)	Amyl alcohol	4-7	661	2390	3 hr
VI		Ethanol	-	281	6280 -	
				213	14380 -	
	Cu(I)	Ethanol	3-8	569	9040	1 hr
		Amyl alcohol	3-8	548	7863	6 hr

Table-2: continued.

Compound	Metal ion	Solvent	pH	λ−max nm	ε-mole^{-1} cm^{-1}	Chromophoric stability
VII	-	Ethanol	-	365	2740 -	
				282	14570 -	
				205	28650 -	
	Cu(I)	Ethanol	3-8	535	9740	1 hr
		Amyl alcohol	3-8	535	11310	3 hr
VIII	-	Ethanol	-	370	723 -	
				266	10930 -	
	Cu(I)	Ethanol	3-7	535	11360	24 hr
		Isoamyl alcohol	3-7	540	11240	24 hr
IX	-	Ethanol	-	318	23360	
				208	28570	
	Cu(I)	Ethanol	4-7	532	2720	30 min.
		Amyl alcohol	4-7	540	3510	30 min.
	Fe(II)	Ethanol	4-7	602	1100	30 min.
X	-	Ethanol	-	340	1970	
				265	3580	
				245	7080	
				209	4840	
	Cu(I)	Ethanol	3-7	520	5610	24 hr
		Amyl alcohol	3-7	520	6108	24 hr
XI	-	Ethanol	-	300	16310	
				265	14100	
				209	21000	
	Cu(I)	Ethanol	4-7	465	5150	2 hr
		Amyl alcohol	4-7	468	4890	8 hr
	Fe(II)	Nitro benzene	4-7	550	9090	24 hr
XII	-	Ethanol	-	325	8397	
				272	9559	
				208	39662	
	Cu(I)	Ethanol	3-8	475	6020	4 hr.

Table-2: continued.

Compound	Metal ion	Solvent	pH	λ-max nm	ϵ-mole^{-1} cm^{-1}	Chromophoric stability
		Amyl alcohol	3-7	475	5100	2 hr
XIII	-	Ethanol	-	298	18529	
				266	16100	
				207	26400	
	Cu(I)	Ethanol	4-7	466	3020	1 hr
		Amyl alcohol	4-7	465	4650	2 hr
XIV	-	Ethanol	-	300	1670	
				271	1720	
				205	3770	
	Cu(I)	Ethanol	3-7	495	5810	2hr
		amyl alcohol	3-7	487	4540	24 hr
XV	-	Ethanol	-	282	3553	
				247	2550	
				219	37202	
	Cu(I)	Ethanol	3-8	455	5830	1 hr
		Amyl alcohol	3-8	453	5770	6 hr.

(Fig. 2) indicate that compounds containing -N=C-C=N- grouping are able to react with copper(I) to form coloured complexes, but colour intensity and stability are enhanced by introducing the substituents near to the imine nitrogen atom. Thus the reagents I, II, IV, VI, VII are more sensitive chromogenic reagents for copper(I) with the molar absorptivities in the range of 0.9-1.2x10^4 l.mole^{-1} cm^{-1} with wavelength of maximum absorbance within 530-570 nm.

Similarly the pyridyl-substituted pyrazine and quinoxaline compounds, X, XII, XIII, XIV, XV also react only with copper and their reactions towards iron(II) are sterically hindered because of substituents adjacent to donor nitrogen atoms. The copper complexes absorb within 453-520 nm with molar absorptivities within the range 4.6-6.2x10^3 l.mole^{-1} cm^{-1}. The comparison of absorption spectra (Table-II) of copper(I) complexes in amyl alcohol as perchlorate ion association complexes again confirms that the ligands containing substituents adjacent to donor nitrogen atom proves favourable for copper(I).

Substitution of ethyl group instead of methyl in dihydropyrazine ring does not seem to have any favourable effect over methyl group and the reagent VII (ϵ = 1.13x10^4 at 535 nm) is slightly less sensitive than 2,3-bis[2'-(6-methylpyridyl)]-5,6-dimethyl-5,6-dihydropyrazine (ϵ = 1.15x10^4 at 539 nm) [8], because of ethyl group substitution instead of methyl in former ligand.

Similarly the substitution of phenyl group in dihydropyrazine ring does not also prove satisfac-

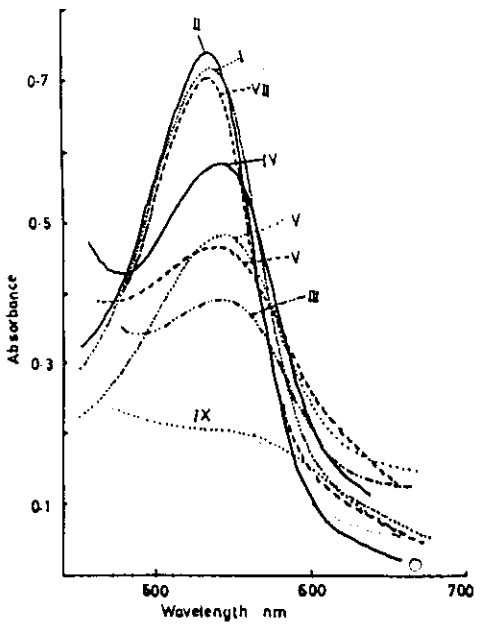


Fig. 2: Absorption spectra of copper(I) complexes of pyridyl- substituted dihydropyrazine compounds.

tory in improving the sensitivity of copper(I) complex. In fact the reagent IV ($\varepsilon = 8.9 \times 10^3$ at 555 nm in ethanol) is less sensitive than 2,3-bis[2'-(6'methylpyridyl)]-5,6-dihydropyrazine ($\varepsilon 1.09 \times 10^4$ at 538 nm in ethanol) [6] due to a phenyl group substitution in dihydropyrazine ring. Probably the phenyl group substitution is disturbing the coplanarity of rings, which results in decrease rather than an increase in the molar absorptivity of copper(I)-complex. It may be noted that a bathochromic shift of about 17 nm has occurred in the λ-max of copper(I) complex due to greater resonance contribution, because of phenyl substitution in dihydropyrazine ring.

The substitution of the phenyl group in dihydropyrazine ring to form the ligand III indicates its colour reactions with iron(II), with a bathochromatic shift of 13-40 nm, with an increase in chromophoric stability as compared to unsubstituted ligand 2,3-bis(2'-pyridyl)-5,6-dihydropyrazine [6], but a decrease in molar absorptivity is again observed. The introduction of phenyl groups is probably disturbing the coplanarity of the rings here also, which decreases rather than an expected increase in molar absorptivity.

The reagent XI reacts with copper(I) and iron(II), but its iron(II) - complex when extracted in chloroform or amyl alcohol as the perchlorate ion association complex, readily decomposes; however, when extracted in nitrobenzene, it is highly stable and shows no sign of change in absorbance up to one week.

Quantitative Studies

The reagent II having maximum number of methyl groups substituted adjacent to donor nitrogen shows highest sensitivity and reasonable chromophoric stability among the series towards copper(I) with molar absorptivity of 1.27×10^4 at 555 nm in aqueous solution, with no sign in change in absorbance upto 24 hours. The copper(I) complex is easily extractable in amyl alcohol, 1,2-dichloroethane, chloroform and nitrobenzene as perchlorate ion association complex.

The effect of pH on extract ion of the copper as copper(I) - complex in amyl alcohol was investigated. The results indicate that the colour of the complex reaches and remain maximum between pH 2-6.

It is interesting to note that the reagent shows its characteristic colour reactions with copper(I) even in 1M hydrochloric acid, but at this high, aciditty the reagent blank develops a yellow colour. Hence a pH 3.5-4.5 was used through out the study. The copper(I) complex when extracted in amyl alcohol obeys the Beer's law over the range 0.2-7 μg/ml of copper and gave a straight line calibration curve.

In order to check the validity of the calibration curve, the test solutions of copper were analysed and relative percentage error was found within $\pm$ 0-5%.

The effect of diverse ions on the extraction of copper(I) in amyl alcohol was also checked. The cations Al(III), Bi(II), Cd(II), Ca(II), Co(II), Fe(II), Pb(II), Mg(III), Hg(II), Ni(II), Ru(II), Ag(I), Sn(II), U(VI), Zn(II) and anions F^-, Cl^-, Br^-, SO_4^{-2}, PO_4^{-3} citrate, tartrate when added in proportion ten times the concentration of copper(I), only iron(II) interferred. This was probably due to the

formation of a yellow bis-iron(II) complex of low colour intensity capable of extraction in an organic phase. However, the interfering effect of iron(II) was successfully removed by adding tartrate to mask the iron before complexation. Moreover iron(II) when present at concentration comparable to copper(I) did not interfere.

Finally drinking water sampels distilled water sample from copper still, collected in copper tank and human head hair samples were analysed for copper contents with reagent II and the results were 14.3 $\pm$ 0.6 μg/l; 455 $\pm$ μg/l & 2.3 $\pm$ 0.12 μg/g respectively, as compared to 15.6 $\pm$ 0.6 μg/l, 480 $\pm$ 2.5 μg/l & 3.0 $\pm$ 0.12 μg/g, respectively when the samples were analysed using bathocuproine as complexing reagent. The results agree quite well, but the results obtained with the reagent II are slightly lower than obtained using bathocuproine.

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