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Stabilisation of unusual simultaneous binding of four cytosine nucleobases to copper(II) by a novel network of bifurcated hydrogen bonding†

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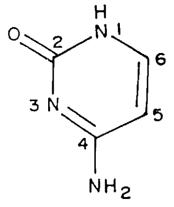
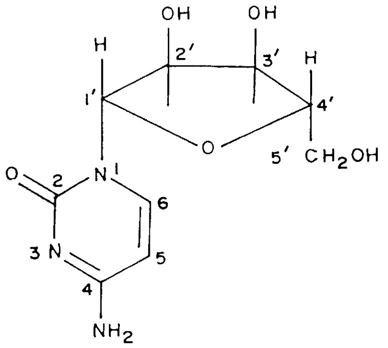
The crystal structure of tetrakis(cytosine)copper(II) perchlorate dihydrate has been determined. All the hydrogen atoms were obtained from Fourier-difference synthesis. The geometry around copper is a bicapped octahedron (4 + 2 + 2*). The adjacent cytosine rings are oriented head-to-tail with respect to each other and are roughly at right angles to the co-ordination plane. The exocyclic oxo groups form an interligand, intracomplex hydrogen-bonding network above and below the co-ordination plane with the exocyclic amino groups of alternate cytosine bases. The EPR and electronic spectra are consistent with the retention of the solid-state structure in solution. The steric effect of the C(2)=O group of cytosine is offset by the presence of the intracomplex hydrogen-bonding network. The trend in $E_{\frac{1}{2}}$ values of Cu^{II}–Cu^{I} couples for 1 : 4 complexes of cytosine, cytodine, pyridine, 2-methylpyridine and *N*-methylimidazole suggests that both steric effects and π-delocalization in imidazole and pyridine ligands and the steric effect of C(2)=O in pyrimidine ligands are important in stabilising Cu^{I} relative to Cu^{II}.

Bivalent transition-metal ions participate in nucleic acid–enzyme interactions during replication and transcription.[1,2] Their binding to bases of DNA is likely to be very important in inducing mutagenesis and metal carcinogenesis and in antineoplasticity of metalloantitumour drugs.[3] Like other metal ions, copper(II) destabilises the normal conformations of DNA and RNA double helices[4] by binding to bases and phosphate, but differs in that it helps in the regeneration of the thermally denatured double strand.[5] On interaction with DNA it binds to guanosine–cytidine pair rather than an adenosine–thymine one and selectively ruptures it. This preference seems to be related to the binding of Cu^{II} to N(3) of cytosine (Cyt) and in some way with the presence of secondary interaction between copper and the exocyclic carbonyl group of the Cyt.[6,7] As a convenient step toward the understanding of such a preferential interaction, copper(II) complexes[8–10] of Cyt have been isolated and investigated. The crystal structures of $[Cu(Cyt)_2Cl_2]$[8] and a few ternary Cyt[9,10] and cytidine[11] (Cyd) complexes of copper have shown the presence of a normal Cu–N(3) bond and provided information regarding the direct involvement in weak metal binding of the exocyclic O(2) group.

According to Martin and Sook-Huikim,[12] since the O(2) atom of the Cyt bases is bound at an average distance of 2.85 Å from copper, it could not be thought of as forming a true bond. They found that though the *ortho* oxo and amino groups in Cyt and Cyd are not as hindering for the binding of copper as are methyl and larger groups in *ortho*-substituted pyridines, the *o*-oxo group does not appear to strengthen the binding of copper by chelation. In an effort better to understand this effect we have investigated the 1 : 4 copper complexes of Cyt, Cyd and related heterocycles, *viz.* pyridine (py), 2-methylpyridine (mpy) and *N*-methylimidazole (mim). This report also deals with the successful isolation and novel crystal structure of the complex $[Cu(Cyt)_4][ClO_4]_2 \cdot 2H_2O$.‡



Cytosine



Cytidine

Experimental

Reagents were used as received from Sisco, India (cytosine), and Aldrich (cytidine). **CAUTION!** No incident occurred during handling of the perchlorate reported and as a solid it seems to be reasonably stable to shock and heat. In spite of these observations the unpredictable behaviour of perchlorate salts necessitates extreme care in their handling.

Synthesis of $[Cu(Cyt)_4][ClO_4]_2 \cdot 2H_2O$

To a solution of cytosine (0.44 g, 4 mmol) in water (10 cm^3) was added dropwise with stirring a solution of $Cu(ClO_4)_2 \cdot 6H_2O$ (0.37 g, 1 mmol) in methanol (2 cm^3). The resulting solution was filtered and kept aside for crystallisation by slow evaporation. The blue hexagonal crystals which separated were suitable for X-ray diffraction (yield, 0.58 g, 78%) {Found: C, 25.90; H, 3.25; Cu, 8.50; N, 22.55. Calc. for $[Cu(Cyt)_4][ClO_4]_2 \cdot 2H_2O$: C, 25.85; H, 3.25; Cu, 8.55; N, 22.65%}.

Attempts to isolate the corresponding Cyd complex were thwarted. The complex species $[Cu(Cyd)_4]^{2+}$, $[Cu(py)_4]^{2+}$, $[Cu(mpy)_4]^{2+}$ and $[Cu(mim)_4]^{2+}$ were generated in solution

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† *Non-SI unit employed:* G = 10^{-4} T.

‡ The crystal structure of $[Cu(Cyt)_4(ClO_4)_2] \cdot MeOH$ has been reported to be solved[13] but complete crystallographic data were not published.

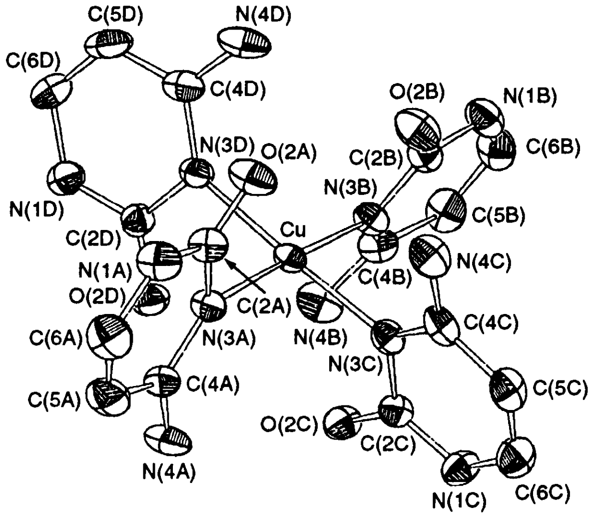

Fig. 1 An ORTEP drawing of the $[Cu(Cyt)_4]^{2+}$ cation showing the atom numbering and the thermal motion of the ellipsoids for non-hydrogen atoms

by adding the corresponding bases to copper(II) perchlorate in 4.1 : 1 molar ratio.

Physical measurements

Elemental analyses were performed by College Science and Analytical Instrumentation Centre, VHNSN College, Virudhunagar, India. The copper content was determined by ethylenediaminetetraacetate titration after decomposing the complex with a mixture of concentrated HNO_3 and concentrated H_2SO_4 and extracting with concentrated HCl (2 cm^3). The infrared spectra were recorded as KBr discs on a Shimadzu 435 spectrophotometer, electronic spectra on aqueous solutions or Nujol mulls on filter-paper using a Hitachi U-3410 double-beam UV/VIS/NIR spectrophotometer. The EPR spectra were obtained on a Varian E-112 X-band spectrometer. The field was calibrated using diphenylpicrylhydrazyl (dpph). Frozen-solution spectra were measured for glasses of the complexes in methanol–water (1:4 v/v). The values of g_0 and A_0 were measured at ambient temperature and $g_{\parallel}$ and $A_{\parallel}$ at 77 K.

All voltammetric experiments were performed in a single-compartment cell with a three-electrode configuration on a EG&G PAR 273 potentiostat/galvanostat equipped with an IBM PS/2 computer and a HIPLOT DMP-40 series digital plotter. The working electrode was a glassy carbon disc and the reference a saturated calomel electrode (SCE). A platinum plate was used as the counter electrode. The supporting electrolyte was 0.1 mol dm^{-3} $NaClO_4$. Solutions were deoxygenated by purging with nitrogen gas for 15 min prior to measurements and during measurements a stream of N_2 was passed over the solution. All these experiments were carried out in aqueous solutions at 25 ± 0.2 °C maintained by a Haake D8-G water-circulating bath. The $E_{\frac{1}{2}}$ value observed under identical conditions for hydroxymethylferrocene in aqueous 0.1 mmol dm^{-3} $NaClO_4$ solution was 0.176 V *vs.* SCE and $\Delta E_p{}^0$ (ΔE_p at zero scan rate) was 73 mV.

Crystallographic data collection and structure analysis for $[Cu(Cyt)_4][ClO_4]_2\cdot 2H_2O$

The complex crystallised with eight faces, two hexagonal faces being at the ends. The crystal was cut along the hexagonal face and a portion mounted on a Enraf-Nonius CAD4 diffractometer for data collection. For unit-cell determination 25 randomly chosen reflections in the range 10 < 2θ < 15 were used. These were centred and the cell parameters refined by the least-squares method. The data collection was monitored with these at regular intervals of time for the orientation and intensity. Over the period of data collection the variation in intensity was much less than 10%. Finally, the intensity data were subjected to Lorentz polarisation[14] corrections but not absorption as the transmission range was < 1 (0.207). Details of the data collection and processing are summarised in Table 1.

Table 1 Crystallographic data for $[Cu(Cyt)_4][ClO_4]_2\cdot 2H_2O$

Formula	$C_{16}H_{24}Cl_2CuN_{12}O_{14}$
M	742.87
Crystal symmetry	Triclinic
Space group	$P\bar{1}$
Crystal dimensions/mm	0.2 × 0.2 × 0.375
Z	2
a/Å	11.663(2)
b/Å	12.601(1)
c/Å	11.410(2)
α/°	112.63(2)
β/°	112.75(2)
γ/°	69.07(1)
U/Å^3	1383.9
D_c/g cm^{-3}	1.78
D_m/g cm^{-3}	1.79
μ(Mo-Kα)/cm^{-1}	10.7
λ(Mo-Kα)/Å	0.7107
T/K	293
No. reflections measured	7279
No. unique reflections	6669 (R_{int} = 0.010)
No. observed reflections	5078 [$I_o \geqslant 3\sigma(I_o)$]
Scan type	ω–2θ
θ Range/°	1.0–28.0
Weighting scheme, w	$[\sigma^2(F) + 0.000\,215F^2]^{-1}$
R	0.051
R'	0.055

The structure was solved by the conventional Patterson method and refined by least squares. The copper position was determined from the Patterson vectors. Fourier-difference synthesis with phasing of this position revealed the positions of all the other non-hydrogen atoms. Subsequent Fourier-difference syntheses and refinement of these atoms revealed all the hydrogen atoms. One of the hydrogens, H(2D), which on refinement was closer to the metal was removed and fixed at a calculated position. The hydrogen atoms were refined isotropically and the non-hydrogen atoms anisotropically until the shift/e.s.d. was 10^{-3}. The refinement of this model using 5078 unique reflections with $I_o > 3\sigma(I_o)$ gave R = 0.0506.

The SHELX 76 system of programs[15] was used for structure analysis and further refinement was done with SHELX 400[16] and a DEC 1090 computer. Diagrams were drawn using the ORTEP II[17] programs.

Complete atomic coordinates, thermal parameters and bond lengths and angles have been deposited at the Cambridge Crystallographic Data Centre. See Instructions for Authors, *J. Chem. Soc., Dalton Trans.*, 1996, Issue 1.

Results and Discussion

Structure of $[Cu(Cyt)_4][ClO_4]_2\cdot 2H_2O$ and comparison with related structures

The complex $[Cu(Cyt)_4][ClO_4]_2\cdot 2H_2O$ exists in the crystal lattice as a bicapped octahedral cation (Fig. 1) with space group $P\bar{1}$, separated by perchlorate anions. Selected bond lengths and angles are given in Table 2. The four equatorial sites are occupied by N(3) atoms of four cytosine molecules. The nearly equivalent Cu–N(3A), Cu–N(3B), Cu–N(3C) and Cu–N(3D) distances [2.027(2), 2.032(2), 2.021(3) and 2.024(3) Å respectively] are similar to those reported[8–10,18] for other Cyt complexes. The adjacent bases are oriented head-to-tail with respect to each other. As a consequence the *trans* cytosine rings (A and B, C and D) have their O(2) atoms on the same side of

Table 2 Selected bond distances (Å) and angles (°) involving non-hydrogen atoms of $[Cu(Cyt)_4][ClO_4]_2 \cdot 2H_2O$ with estimated standard deviations (e.s.d.s) in parentheses

	Ring			
	A	B	C	D
Cu–N(3)	2.027(2)	2.032(2)	2.021(3)	2.024(3)
N(3)–C(2)	1.361(5)	1.366(6)	1.374(5)	1.369(6)
C(2)–N(1)	1.364(4)	1.357(4)	1.369(5)	1.369(5)
N(1)–C(6)	1.364(5)	1.359(6)	1.370(8)	1.362(5)
C(6)–C(5)	1.326(7)	1.331(8)	1.321(7)	1.324(8)
C(5)–C(4)	1.423(5)	1.421(5)	1.437(5)	1.438(6)
C(4)–N(3)	1.352(4)	1.345(5)	1.349(6)	1.344(4)
C(2)–O(2)	1.246(4)	1.242(5)	1.241(6)	1.227(4)
C(4)–N(4)	1.318(5)	1.324(6)	1.322(5)	1.329(6)
N(1)–H(1)	0.899(5)	0.799(6)	1.306(6)	1.051(7)
N(4)–H(2)	0.843(3)	1.099(4)	1.062(5)	1.080(3)
N(4)–H(3)	0.903(5)	0.801(5)	0.615(7)	0.827(5)
C(5)–H(4)	0.894(5)	0.833(4)	0.996(5)	1.022(3)
C(6)–H(5)	1.029(4)	0.791(4)	0.989(5)	0.817(6)
Cu · · · O(2)	2.775(2)	2.720(2)	2.717(2)	2.741(4)

N(3C)–Cu–N(3D)	176.0(2)	N(3B)–Cu–O(2C)	81.4(1)	O(2A)–Cu–O(2D)	117.4(1)	N(3C)–Cu–O(2D)	129.8(1)
N(3B)–Cu–N(3C)	91.9(1)	N(3B)–Cu–O(2B)	54.3(1)	N(3C)–Cu–O(2C)	54.6(1)	O(2A)–Cu–O(2C)	139.8(1)
N(3A)–Cu–N(3C)	88.2(1)	O(2A)–Cu–N(3D)	80.7(1)	O(2A)–Cu–O(2B)	78.7(1)	O(2B)–Cu–N(3D)	96.1(1)
N(3B)–Cu–N(3D)	89.3(1)	O(2A)–Cu–N(3C)	95.6(1)	O(2B)–Cu–N(3C)	81.6(1)	O(2C)–Cu–N(3D)	129.4(1)
N(3A)–Cu–N(3D)	90.7(1)	O(2A)–Cu–N(3B)	130.7(1)	O(2C)–Cu–O(2D)	76.9(1)	Cu–N(3A)–C(2A)	109.1(2)
N(3A)–Cu–N(3B)	175.9(2)	N(3A)–Cu–O(2D)	83.6(1)	Cu–N(3A)–C(4A)	131.0(3)	Cu–N(3B)–C(2B)	107.2(2)
N(3D)–Cu–O(2D)	53.9(1)	N(3A)–Cu–O(2C)	95.4(1)	Cu–N(3B)–C(4B)	132.4(3)	Cu–N(3C)–C(2C)	107.2(2)
O(2B)–Cu–O(2D)	138.3(1)	N(3A)–Cu–O(2B)	129.7(1)	Cu–N(3C)–C(4C)	132.8(3)	Cu–N(3D)–C(2D)	131.7(3)
O(2B)–Cu–O(2C)	116.7(1)	N(3A)–Cu–O(2A)	53.3(1)	Cu–N(3D)–C(4D)	107.6(2)		

the equatorial plane. The two off-the-axis Cu · · · O(2) distances [Cu · · · O(2A) and Cu · · · O(2B)] of 2.775(2) and 2.720(2) Å above and the other two [Cu · · · O(2C) and Cu · · · O(2D) 2.717(2) and 2.741(4) Å] below the CuN_4 plane constitute the apices of a tetrahedron, extending qualitatively the co-ordination geometry to an unsymmetrical bicapped octahedron (4 + 2 + 2*). These Cu · · · O(2) distances are in agreement with those observed for other copper(II) complexes of Cyt.[8–10,18] The involvement of C(2)=O in axial binding is consistent with a slight decrease in the double-bond character [1.243(3) Å] compared to the free Cyt value[19] [1.237(2) Å]. It is probable that such secondary interactions, though weak, also play a role in the molecular conformation of the complex. The exocyclic O(2) is involved in bifurcated hydrogen bonding with exocyclic amine hydrogens of alternate Cyt [N(4D) · · · O(2B) 3.06(4), N(4C) · · · O(2B) 3.03(5), N(4D) · · · O(2A) 2.95(4), N(4C) · · · O(2A) 3.05(5), N(4A) · · · O(2C) 2.93(4), N(4A) · · · O(2D) 3.10(5), N(4B) · · · O(2C) 2.97(6), N(4B) · · · O(2D) 2.94(4) Å] and thus forms interligand, intracomplex hydrogen bonds (Fig. 2). Such hydrogen bonding may also contribute to the slight increase observed in the C(2)=O bond length.

The planes of the Cyt rings are roughly at right angles to the CuN_4 plane [dihedral angles 76.48(11), 100.20(11), 80.44(12) and 96.6(12)°], in order to minimise mutual repulsion. The six-atom framework of the four cytosine ligands is approximately planar. The out-of-plane deviation of O(2) is minimal, about 0.004–0.075 Å, evidencing exclusive intramolecular Cu · · · O(2) interaction. The involvement of both inter- and intra-molecular Cu · · · O(2) interactions in other compounds[8,11] would have resulted in higher out-of-plane deviations (0.19 Å). There are hydrogen-bonding interactions involving water and perchlorate oxygen atoms as acceptors and the exocyclic amino group at C(4) as well as N(1) of the bases as donors (Table 3).

There is considerable dissymmetry in the exocyclic bond angles at the heteroatom to which copper is bonded in the copper–pyrimidine base complexes where interligand hydrogen-bonding interactions or chelation takes place. The present structure shows Cu–N(3)–C(2) 107.2(2)° and Cu–N(3)–C(4) 132.4(3)° bond angles in all the four Cyt rings, implying definite attraction of O(2) atoms toward the copper(II) ion. This provides evidence for the Cu · · · O(2) interaction which overcomes steric repulsion arising out of the d_{xz} and d_{yz} orbitals of copper and the lone pair of oxygen when the neighbouring *cis* positions are unavailable for co-ordination.

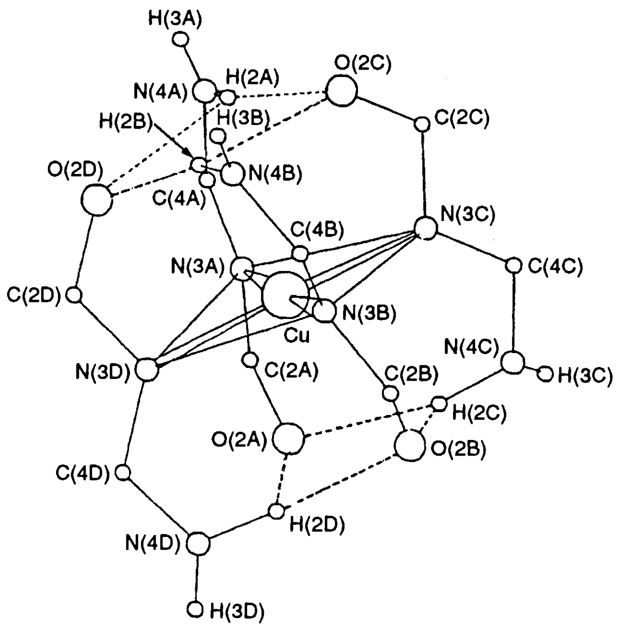

Fig. 2 Possible hydrogen-bonding interactions in the $[Cu(Cyt)_4]^{2+}$ cation

The perchlorate ions lie at a distance of 4 Å from the co-ordination plane, eliminating direct co-ordination to copper.[11] They are flanked by two cytosine groups [group Cl(10) between A and D and group Cl(20) between B and C]. The oxygen atoms of these groups are away from the flanking cytosine ring by a distance of 3.3 Å or more. Further, the ClO_4^- ions are not geometrically oriented for potential $Cu–OClO_3$ bonding which is also prohibited by the exocyclic groups of the four cytosine

Table 3 Possible hydrogen-bonding interactions (distances in Å, angles in °)

D	H	D–H	A	D···A	H···A	D–H···A
C(6A)	H(5A)	1.029(41)	$O(34^{I})$	3.203(6)	2.175(42)	177(4)
N(4A)	H(3A)	0.903(52)	$O(12^{II})$	3.020(5)	2.264(58)	141(5)
C(6B)	H(5B)	0.791(40)	$O(13^{III})$	3.291(7)	2.717(56)	131(5)
N(4B)	H(3B)	0.801(55)	$O(33^{IV})$	3.034(6)	2.295(59)	154(4)
N(4C)	H(3C)	0.615(76)	$O(11^{V})$	3.132(7)	2.624(73)	142(6)
N(4C)	H(3C)	0.615(76)	$O(14^{VI})$	3.057(7)	2.691(55)	121(6)
N(1D)	H(1D)	1.051(68)	$O(31^{VII})$	3.045(8)	2.038(74)	159(4)
N(1D)	H(1D)	1.051(68)	$O(32^{VII})$	3.187(7)	2.709(65)	108(4)
C(6D)	H(5D)	0.817(62)	$O(32^{VII})$	3.197(8)	2.664(71)	124(4)
N(4D)	H(2D)	0.827(56)	$O(10^{VIII})$	2.922(5)	2.145(49)	156(6)
N(4D)	H(2D)	0.827(56)	$O(34^{IX})$	3.241(8)	2.711(71)	123(5)

Symmetry relationships: I $x, y-1, z-1$; II $-x+1, -y, -z$; III $x, y+1, z+1$; IV $-x+1, -y+1, -z+1$; V $x, y, z+1$; VI $-x, -y, -z$; VII $x, y, z-1$; VIII $x-1, y, z$; IX $-x, -y+1, -z+1$.

molecules. They are held in lattice holes by Coulombic forces and exhibit no indication of disorder.

Infrared spectrum

The infrared spectrum of the $[Cu(Cyt)_4][ClO_4]_2$ exhibits a broad band around 3450 cm^{-1} due to the lattice water revealed in its crystal structure. Features at 3100 and 2900 cm^{-1}, appearing as a doublet, are assigned to ν(N–H) and coupled ν(N–H) + ν(C–H) vibrations respectively. The $\delta(NH_2)$ and δ[N(1)H] appearing around 1692 and 1520 cm^{-1} respectively are almost unchanged from those of free cytosine;[6,20,21] this is obviously because of the non-involvement of the $N(4)H_2$ group of cytosine in co-ordination and is consistent with N(3) as the site of complexation[21–23] as established by the crystal structure. The very strong ν(C=O) band observed at 1664 cm^{-1} in the spectrum of the free base[24] shifts to lower wavenumber (1645 cm^{-1}) with decreased intensity for the present complex, corresponding[25] to the established C(2)=O ··· Cu interaction. The strong band at around 1610 cm^{-1} is assigned to coupled ν(N–H) + ν(C=O) (amide I) vibrations.

The $\nu_3(ClO_4^-)$ is resolved into three bands at 1080, 1120 and 1140 cm^{-1}, consistent with the involvement of ClO_4^- in hydrogen bonding with NH of the Cyt ring, as revealed in the crystal structure; however, the $\nu_4(ClO_4^-)$ band at 620 cm^{-1} is unsplit. The IR-inactive ν_1 and ν_2 vibrations appear[22,26] at 470 and 920 cm^{-1} respectively, illustrating that the tetrahedral symmetry of the ClO_4^- ions is reduced on hydrogen bonding.

Electronic spectra

The solid-state (reflectance) spectrum of $[Cu(Cyt)_4][ClO_4]_2$ exhibits two ligand-field bands around 16 900 and 14 000 cm^{-1} (Fig. 3); this is consistent[27] with the tetragonally distorted octahedral geometry revealed by the crystal structure. On dissolution in water, however, only one band occurs at a low energy of 13 900 cm^{-1}; this shows that the solution structure does not parallel the solid-state one. Addition of an excess of Cyt ([Cyt]/[complex] = 0.1) to this solution does not alter the $\tilde{\nu}_{max}$ value; this rules out the possibility of any co-ordinative dissociation of Cyt or involvement of solvent water in co-ordination. It is suggested that the four Cyt bases are still held intact by the network of interligand hydrogen bonds. Thus the $\tilde{\nu}_{max}$ value is similar to those for CuN_4 complexes of selected monodentate heterocyclic bases, generated *in situ* (Table 4); the identity of the tetrakis complexes of py and Him (imidazole) in solution has been established[28] by EPR spectroscopy (see below). Further, as expected for the monodentate nature of the ligands, the ligand-field energies of all the present complexes are lower than those (17 000–19 000 cm^{-1}) for square-planar CuN_4 complexes of chelating ligands.[29–32]

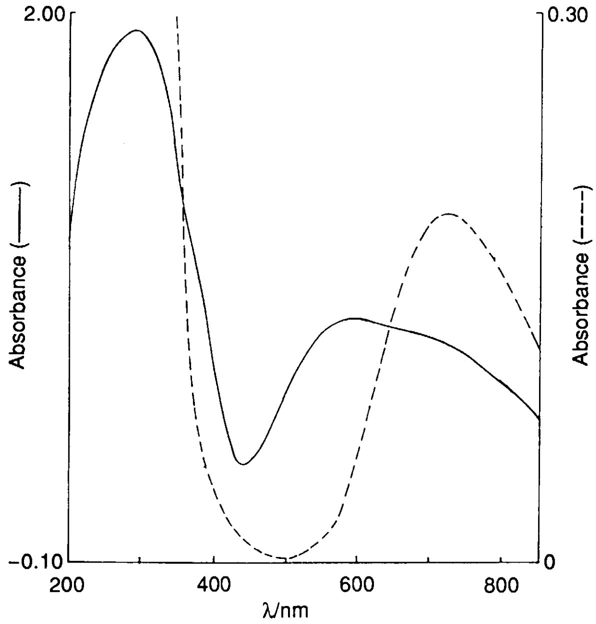

Fig. 3 Electronic spectra of $[Cu(Cyt)_4][ClO_4]_2 \cdot 2H_2O$ as reflectance (——) and in aqueous solution (- - -)

The ligand-field energy of the present complexes follows the order $[Cu(Cyd)_4]^{2+} < [Cu(Cyt)_4]^{2+} < [Cu(mpy)_4]^{2+} < [Cu(py)_4]^{2+} < [Cu(mim)_4]^{2+}$, while the absorption coefficients are in the reverse order. This is expected of the higher basicity of mim and the tetrahedral distortion induced by the sterically demanding *o*-methyl group in mpy. Similarly, the lower band energies observed for $[Cu(Cyt)_4]^{2+}$ and $[Cu(Cyd)_4]^{2+}$ may be consistent with enhanced axial interaction involving the four O(2) groups, revealed in the above crystal structure and/or tetrahedral distortion from planarity possibly achieved in solution.[33] Though the latter also accounts for the high absorption coefficient (ε 970 $dm^3\ mol^{-1}\ cm^{-1}$) of the visible band, it is ruled out on the grounds that it would involve breaking of the network of hydrogen bonds and then formation of hydrogen bonds with solvent water, which does not seem energetically possible. Further, on incremental addition of Cyt to an aqueous solution of $Cu(ClO_4)_2$ (Table 4) the ε value increases and reaches a maximum (923 $dm^{-3}\ mol^{-1}\ cm^{-1}$) on formation of the 1 : 4 complex; addition of an excess of Cyt ([Cyt]/[Cu] = 8 : 1) did not produce any significant change in the spectrum. It is obvious that the ligand-field band becomes intense only upon the formation of the network of hydrogen bonds below and above the CuN_4 plane, which probably confers a lower symmetry on copper. The complex $[Cu(Cyd)_4]^{2+}$ exhibits spectral features similar to those of $[Cu(Cyt)_4]^{2+}$ suggesting that the effect of the bulky sugar moiety far removed from the co-ordination site is negligible and

Table 4 Electronic spectral data ($\tilde{\nu}_{max}$/cm^{-1}, ε/dm^3 mol^{-1} cm^{-1} in parentheses) for the copper(II) complexes in aqueous solution *

Complex	Ligand field	Charge transfer
$[Cu(Cyt)_4][ClO_4]_2 \cdot 2H_2O$	13 900 (970)	37 600 (23 700)
1:0 Cu:Cyt	12 300 (21)	—
1:1 Cu:Cyt	12 900 (29)	—
1:2 Cu:Cyt	13 200 (59)	—
1:3 Cu:Cyt	13 600 (205)	
1:4 Cu:Cyt	13 900 (923)	—
1:4 Cu:Cyd	13 500 (832)	36 900 (28 200)
1:1:3 Cu:Cyt:Cyd	13 600 (492)	—
1:3:1 Cu:Cyt:Cyd	13 900 (657)	—
1:4 Cu:py	14 500 (164)	38 000 (13 200)
1:4 Cu:mpy	14 100 (247)	38 400 (15 300)
1:4 Cu:mim	14 900 (150)	—

* Concentration range 5 × 10^{-4}–1 × 10^{-2} mol dm^{-3}.

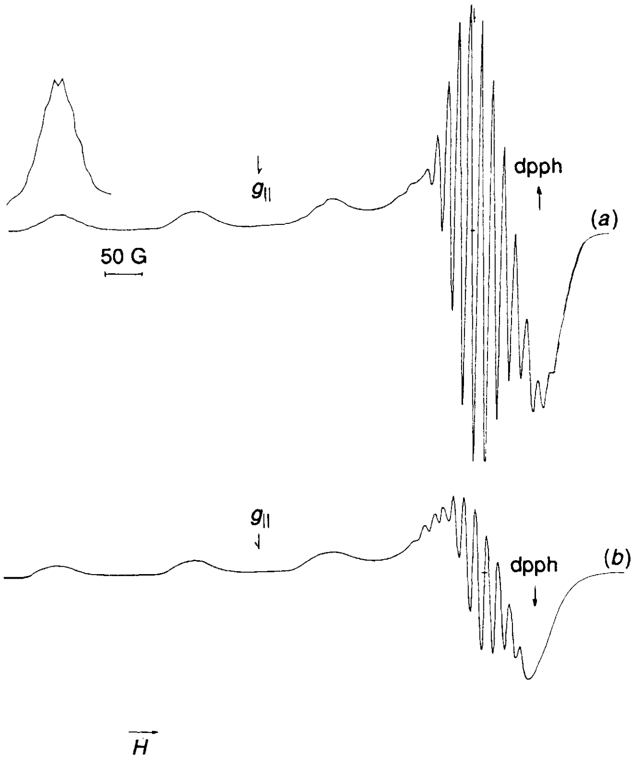

Fig. 4 The EPR spectra of (a) $[Cu(Cyt)_4][ClO_4]_2 \cdot 2H_2O$ and (b) 1:4 Cu:mim complexes in water–methanol (1:4) glasses at liquid-nitrogen temperature

that a similar network of hydrogen bonding is present. Thus the o-oxo group in Cyt and Cyd is not as hindering to binding of Cu^{II} as is the methyl group in mpy and in fact it does contribute measurably to strengthening the binding of Cu^{II} by semi-chelation. This is in contrast to the results of Martin and Sook-Huikim.[12]

The prominent UV band located around 37 700 cm^{-1} in all the present complexes is assigned[32,34,35] to ligand π→π* and overlapping N(σ)→Cu^{II} ligand-to-metal charge-transfer transitions.

EPR spectra

The powder spectrum of $[Cu(Cyt)_4][ClO_4]_2$ exhibits an isotropic spectrum at ambient and even at low temperatures suggesting that the molecules are not sufficiently magnetically dilute to cause resolution. The cryogenic solution spectrum, on the other hand, is axial (Fig. 4) with $g_{\parallel} > g_{\perp} > 2.03$, like those (Figs. 4 and 5) of the 1:4 complexes of Cyd, py, mpy and mim (Table 5) suggesting a $d_{x^2-y^2}$ ground state[36] in a distorted-tetragonal copper site. The $g_{\parallel}$ and $A_{\parallel}$ values (Table 5) of all these complexes lie on the $g_{\parallel}$ vs. $A_{\parallel}$ map[37] to the right of the aromatic N_4 delineator. The $g_{\parallel}$ values are higher, and $A_{\parallel}$ values lower, than those respectively of tetragonal CuN_4 chromophores[31,32] (2.19–2.21) consituted by chelating ligands, due to weak interaction of the monodentate ligands with copper rather than to any tetrahedral distortion,[33,38] as also revealed by electronic spectral data. The axial interaction[33,36] by the exocyclic C(2)=O group of four Cyt or Cyd ligands above and below the co-ordination plane, as discussed above, would be expected to enhance the $g_{\parallel}$ and decrease $A_{\parallel}$ values relative to other complexes but this was not observed.

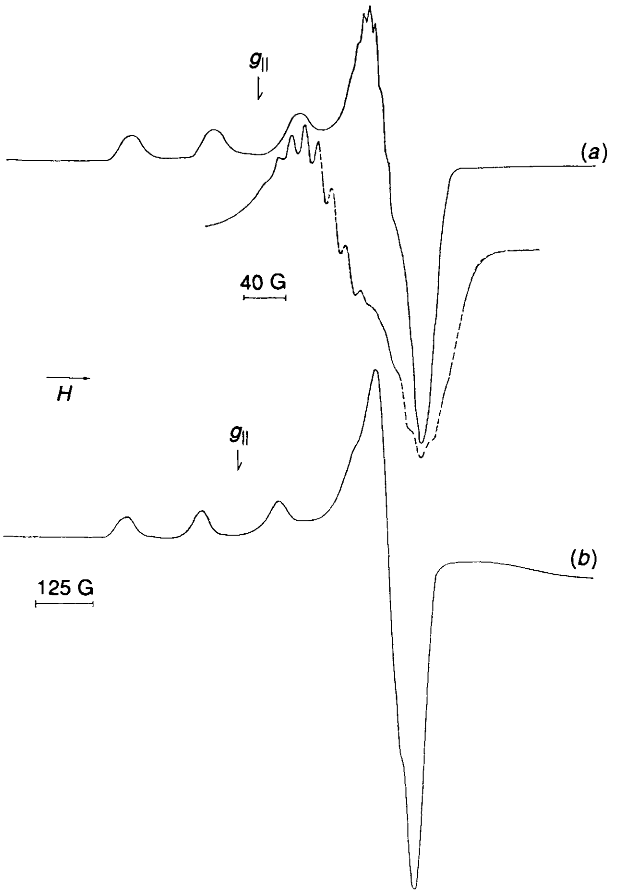

Fig. 5 The EPR spectra of 1:4 Cu:py (a) and Cu:mpy (b) complexes in water–methanol (1:4) glasses at liquid-nitrogen temperature

Further, the EPR spectral patterns and parameters observed for $[Cu(py)_4]^{2+}$ and $[Cu(mim)_4]^{2+}$ are consistent with those previously reported;[28,39] the ^{63}Cu EPR spectrum for $[Cu(py)_4]^{2+}$ shows[28] nitrogen superhyperfine lines on both the perpendicular and parallel components ($m_I = +\frac{3}{2}$) and the same at Q band is also axial with no high-field rhombic feature near the perpendicular region. For all the present complexes the $g_{\parallel}/A_{\parallel}$ quotient[37] ranges from 118 to 132 cm, evidence in support of the square-based geometry with no appreciable tetrahedral distortion. The observation of at least nine nitrogen superhyperfine lines on the perpendicular copper hyperfine component for $[Cu(Cyt)_4]^{2+}$, $[Cu(Cyd)_4]^{2+}$, $[Cu(mim)_4]^{2+}$ [Fig. 4(b)] and $[Cu(py)_4]^{2+}$ [Fig. 5(a)] also indicates the tetragonal geometry; the presence of at least nine such lines on the low-field parallel component also [Fig. 4(a)] confirms the retention of four ligands around Cu^{II} in a square-planar array as well as the identity of the tetrakis complex in solution. However, for $[Cu(mpy)_4]^{2+}$, the $g_{\parallel}/A_{\parallel}$ quotient is slightly higher than that for $[Cu(py)_4]^{2+}$ suggesting that the steric bulk of the o-methyl group induces a relatively higher tetrahedral distortion. This is consistent with the absence of nitrogen superhyperfine lines [Fig. 5(b)] and relatively high ε values for $[Cu(mpy)_4]^{2+}$. Our present results are in contrast to those from

Table 5 The EPR spectral data for binary copper(II) complexes in water–methanol (4 : 1 v/v) solutions

Complex	g_0	$A_0{}^a$	$g_\parallel$	$A_\parallel{}^a$	$g_\perp{}^b$	$A_\perp{}^b$	$g_\parallel/A_\parallel$	A(N)^a
$[Cu(Cyt)_4][ClO_4]_2\cdot 2H_2O^c$	2.114	86	2.253	189	2.045	35	119	14
$[Cu(Cyd)_4]^{2+}$	2.122	84	2.257	185	2.055	34^d	122	15
$[Cu(py)_4]^{2+}$	2.127	88	2.259	191	2.061	37	118	12
$[Cu(mpy)_4]^{2+}$	2.139	77	2.294	174	2.062	28	132	—
$[Cu(mim)_4]^{2+}$	2.117	85	2.258	186	2.047	34	121	16

^{a} A values in 10^{-4} cm^{-1}. ^{b} $g_\perp$ and $A_\perp$ computed as $\frac{1}{2}(3g_0 - g_\parallel)$ and $\frac{1}{2}(3A_0 - A_\parallel)$ respectively. ^{c} At room temperature the polycrystalline sample exhibited an isotropic signal with $g_{av} = 2.063$. ^{d} Computed as $\frac{1}{2}(3A_0 - A_\parallel)$.

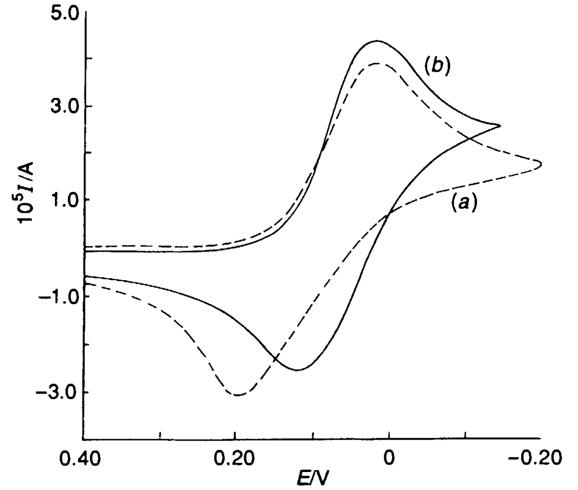

Fig. 6 Cyclic voltammograms in aqueous solution for (*a*) copper–cytosine (1 : 4) and (*b*) copper–cytidine (1 : 4) mixtures. [Cu] = 0.001 mol dm^{-3}. Supporting electrolyte, 0.01 mol dm^{-3} NaClO$_4$; scan rate 20 mV s^{-1}

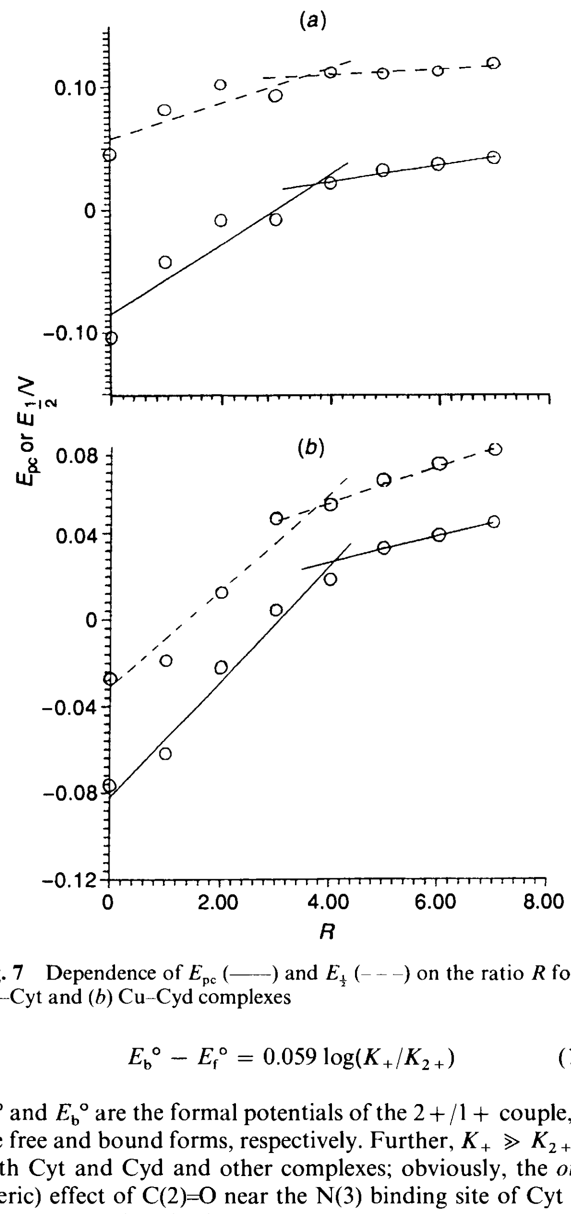

Fig. 7 Dependence of E_{pc} (——) and $E_{\frac{1}{2}}$ (– – –) on the ratio R for (*a*) Cu–Cyt and (*b*) Cu–Cyd complexes

previous EPR studies[40] on Cyd–copper(II) complexes, which show that nitrogen superhyperfine lines are observed only at a copper : Cyt/Cyd ratio of 1 : 26.

Electrochemical behaviour

The cyclic voltammogram of $[Cu(Cyt)_4][ClO_4]_2$ (Fig. 6) exhibits a well defined redox wave corresponding to the Cu^{II}–Cu^{I} couple. The diffusion coefficient, D (1.6 × 10^{-6} cm^2 s^{-1}), calculated [23,41,42] for the Cu^{II} ⟶ Cu^{I} process is typical of a one-electron redox ($n = 1$) process. Addition of an excess of Cyt (0.2 mmol) did not affect the redox parameters, supporting the inference from ligand-field spectral data that no co-ordinative dissociation of the complex occurs in solution. With the incremental addition of Cyd, py, mpy and mim to $Cu(ClO_4)_2$, the $E_{\frac{1}{2}}$ values of the Cu^{II}–Cu^{I} couple shift to more positive values, reach a maximum at $R = 4$ (R = [base]/[Cu]) and then remain constant indicating the formation of 1 : 4 complexes (Tables 6 and 7) similar to $[Cu(Cyt)_4][ClO_4]_2$. This is supported by plots of i_{pc}, E_{pa} and E_{pc} *vs.* R, which also show inflections around $R = 4$ (Fig. 7). The ΔE_p values of all the present complexes exceed the Nernstian requirement[43] of 59 mV in cyclic voltammetry (CV) and 90 mV full width at half-maximum (h.w.f.m.)[44] in differential pulse voltammetry (DPV), suggesting a quasi-reversible to irreversible Cu^{II}–Cu^{I} redox process. The perchlorate anion remains a spectator in this.

The $E_{\frac{1}{2}}$ value of the Cyd complex is lower than that for the Cyt complex; this illustrates that the electron-releasing CH (ribose) moiety at N(1) of Cyd confers enhanced stabilisation of Cu^{II}, compared to Cyt. Such a preference for Cu^{II} relative to Cu^{I} is elegantly illustrated by the ratio K_{2+}/K_{+} for the binding of Cu^{II} (K_{2+}) and Cu^{I} (K_{+}) ions to bases (Table 7). This ratio is estimated[45] from the net shift in $E_{\frac{1}{2}}$ using equation (1) where

$$E_b{}^\circ - E_f{}^\circ = 0.059 \log(K_{+}/K_{2+}) \qquad (1)$$

$E_f{}^\circ$ and $E_b{}^\circ$ are the formal potentials of the 2+/1+ couple, for the free and bound forms, respectively. Further, $K_{+} \gg K_{2+}$ for both Cyt and Cyd and other complexes; obviously, the *ortho* (steric) effect of C(2)=O near the N(3) binding site of Cyt and Cyd and π-delocalisation in other complexes are important in stabilising Cu^{I} over Cu^{II}. However, the C(2)=O groups contribute measurably to the stability of copper(II) complexes by semichelation or at least by forming a hydrogen-bonding network sandwiching the CuN$_4$ plane, as established by X-ray crystallographic and spectral studies.

The $E_{\frac{1}{2}}$ values for the mim, py and mpy complexes are typical of N$_4$ ligation in solution. The trend in these values obtained from CV agrees well with that in the $\tilde{\nu}_{max}$ values, illustrating the importance of basicity and the steric effect of the bases in stabilising Cu^{II} relative to Cu^{I}. These values differ from those calculated from DPV (Table 7, Figs. 8 and 9) for the same complex; this is probably because of complications like adsorption or rearrangement of the electrogenerated species.

Table 6 Redox data[a] for $Cu(ClO_4)_2$ in aqueous solution at a glassy carbon electrode with addition of cytosine, scan rate 20 mV s^{-1}

Added Cyt/mmol dm^{-3}	$E_{pc}(I)^b$	$E_{pc}(II)^c$	$E_{pa}(I)$	$E_{\frac{1}{2}}$	$E_{\frac{1}{2}}{}^d$
0.0	0.018	−0.730	0.194	0.106	0.067
0.2	0.010	−0.670	0.200	0.105	0.071
1.0	0.024	−0.656	0.212	0.118	0.077
2.0	0.038	−0.672	0.208	0.123	0.087
3.0	0.036	−0.680	0.214	0.125	0.091

[a] E in V *vs.*, SCE; add 0.244 V to convert into values *vs.* normal hydrogen electrode (NHE). [b] Appears as shoulder. [c] $Cu^I \longrightarrow Cu^0$ process. [d] DPV measurements. Supporting electrolyte, 0.1 mol dm^{-3} $NaClO_4$; $[Cu^{II}] = 0.001$, $[Cyt] = 0.2$ mol dm^{-3}.

Table 7 Voltammetric behaviour[a] of copper(II) complexes in aqueous solution at scan rate 20 mV s^{-1}

Complex	E_{pc}	E_{pa}	$E_{\frac{1}{2}}$	$E_{\frac{1}{2}}{}^b$	$E_b - E_f$	K_{2+}/K_+	$10^6\ D/cm^2\ s^{-1}$
$Cu(ClO_4)_2$	−0.072	0.102	0.015	−0.035	—	—	6.4
Cu:Cyt (1:4)	0.018	0.198	0.108	0.107	0.142	0.004	1.6
Cu:Cyd (1:4)	0.018	0.122	0.070	0.052	0.087	0.034	1.4
Cu:py (1:4)	−0.062	0.216	0.077	0.062	0.097	0.023	8.7
Cu:mpy (1:4)	−0.124	0.294	0.085	0.045	0.080	0.044	6.4
Cu:mim (1:4)	−0.002	0.130	0.066	0.078	0.113	0.012	1.4

[a] E in V *vs.* SCE; add 0.244 V to convert into values *vs.* NHE. [b] DPV measurements. Supporting electrolyte 0.1 mol dm^{-3} $NaClO_4$. $[Cu^{II}] = 0.001$, [base] = 0.2 mol dm^{-3}.

Thus a difference of as large as 50 mV has been observed for $[Cu(mpy)_4]^{2+}$ which exhibits irreversible redox behaviour (very high ΔE_p value).

Conclusion

The present study leads to the conclusion that copper(II) can bind to as many as four cytosine or cytidine molecules simultaneously. Such simultaneous binding is apparently stabilised by semichelation involving the C(2)=O and N(3) sites, and supported by the unique bifurcated hydrogen-bonding network, in spite of the *ortho* effect (steric crowding) expected of a C(2)=O group. This behaviour is similar to that of $[Pd(mcyt)_4][NO_3]_2{\cdot}2H_2O$ (mcyt = 1-methylcytosine).[46] The retention of four co-ordinated cytosines even in solution is evidenced by electronic and EPR spectral studies. So it is clear that the C(2)=O group is not as hindering for copper(II) binding as the *o*-methyl group in mpy. The steric demand and axial interaction by C(2)=O, however, tend to destabilise Cu^{II}, as inferred from spectral and electrochemical data. Thus the effect of co-ordination of N(3) is not simple. This is not surprising as it is frequently the case in studies of metal-ion complexation by nucleic acid constituents that single-site specificity is not encountered and chelate formation results. The constancy of the Cu–N(3) and Cu ··· O(2) bond distances and also of the Cu ··· O(2)–C(2) bond angle in the present and other cytosine complexes[8,11,18] suggests that this type of interaction may be specific for Cu–Cyt co-ordination in square-planar complexes[23] and may play a role in the recognition of cytosine residues in nucleic acids by Cu^{II}.

The important observation that copper can be involved in simultaneous binding of four cytosines is relevant to the reversible Cu^{2+}-induced unwinding of DNA. Models proposed[47] for Cu^{II}–DNA interaction include cross-link formation at guanosine-cytidine pairs involving N(7) of guanine and N(3) of cytosine, resulting in breaking of hydrogen bonds. Further, it is not unreasonable to expect that copper can bring four single strands together through interaction with cytosine, which is preferred due to the concomitant hydrogen-bonding network formed; however, it is admittedly a large step from a nucleic acid base to single-stranded DNA. Nevertheless, such unusual structures or interactions may be relevant to cross-linking observed in melted DNA and polynucleotides.

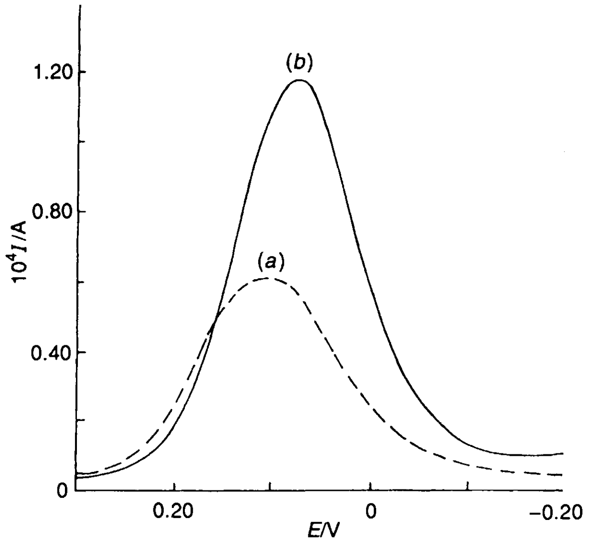

Fig. 8 Differential pulse voltammograms of 0.001 mmol dm^{-3} $[Cu(Cyt)_4]^{2+}$ (*a*) and $[Cu(Cyd)_4]^{2+}$ (*b*). Supporting electrolyte 0.01 mol dm^{-3} $NaClO_4$. Sweep rate 1 mV s^{-1}. Pulse amplitude 50 mV

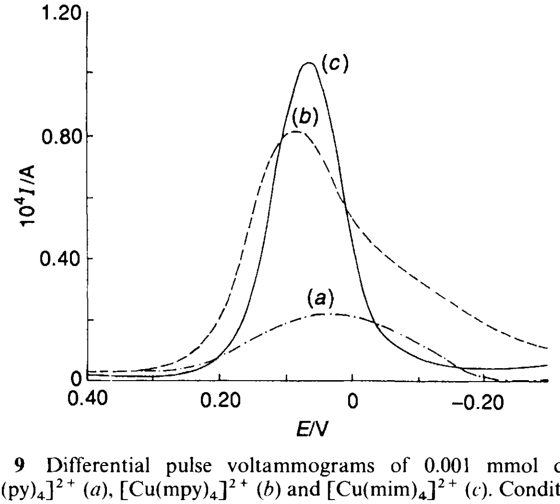

Fig. 9 Differential pulse voltammograms of 0.001 mmol dm^{-3} $[Cu(py)_4]^{2+}$ (*a*), $[Cu(mpy)_4]^{2+}$ (*b*) and $[Cu(mim)_4]^{2+}$ (*c*). Conditions as in Fig. 8

Acknowledgements

M. P. and I. S. thank the University Grants Commission, New Delhi, India [F. 12–52/86(SR-III)] for financial support. I. S. thanks the Council of Industrial and Scientific Research, for a fellowship. We thank Regional Sophisticated Instrumentation Centre, Indian Institute of Technology, Madras for EPR spectra.

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Received 5th June 1995; *Paper* 5/03560K