Synthesis, Characterization, DNA Binding and Cytotoxic Studies of Platinum(II) and Palladium(II) Complexes of the 2,2′-Bipyridine and an Anion of 1,1-Cyclobutanedicarboxylic Acid

Hassan MANSURI-TORSHIZI,* Saeid GHADIMY, and Niloofar AKBARZADEH

Department of Chemistry, Faculty of Science, University of Sistan & Baluchestan, Zahedan, Iran.
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Two neutral complexes of formula [M(bpy)(cbdca)] [where M is palladium(II) (Pd(II)) or platinum(II) (Pt(II)), bpy is 2,2′-bipyridine and cbdca is anion of 1,1-cyclobutanedicarboxylic acid] have been synthesized. These water soluble complexes have been characterized by chemical analysis and conductivity measurements as well as ^{1}H-NMR, ultraviolet–visible and infrared spectroscopy. In these complexes the ligand cbdca coordinates to Pt(II) or Pd(II) as bidentate with two oxygen atoms. They are nonelectrolyte in conductivity water. These complexes inhibit the growth of P_{388} lymphocytic leukemia cells and their targets are DNA. They invariably show ID_{50} values less than cisplatin. [Pt(bpy)(cbdca)] and [Pd(bpy)(cbdca)] have been interacted with calf thymus DNA and bind to DNA through coordinate covalent bond. In addition, the influence of binding of these complexes on the intensity of EtBr–DNA have been studied. They bind to DNA *via* a nonintercalating mode.

Key words platinium complex; palladium complex; DNA-binding study; 1,1-cyclobutanedicarboxylic acid

Since the discovery of the antineoplastic agent cisplatin (c-DDP) by Rosenberg *et al*.,[1] the drug has been in wide-spread use to date. However, because of its toxic side-effects, particularly renal damage, the search for safer platinum complexes and administration methods is still continuing. Several platinum and palladium containing compounds have subsequently been synthesized and tested.[2—4] One of these, carboplatin, was approved for treatment of ovarian cancer.[5] This has no kidney toxicity, there is less nausea, neurotoxicity, ototoxicity than cisplatin and the drug could be given without hydration treatment.[6] Recent work on the reaction mode of carboplatin focused on the intraction with DNA building blocks.[7] It was concluded that the reaction with carboplatin proceeds *via* a direct substitution mechanism, since the reaction with chloride was too slow to account for an aqua or chloro complex as the reactive species.[7—11]

Tobe and Kokhar[12] reported that change in the amine ligands have primary effect on antitumor properties of platinum(II)/palladium(II) [Pt(II)/Pd(II)] complexes. Recent kinetic work by van Eldik and his co-workers.[13] on [Pd(bpy)(cbdca)] [where bpy is 2,2′-bipyridine and cbdca is anion of 1,1-cyclobutanedicarboxylic acid] indicates that a decrease in reactivity was induced by increasing the steric hindrance on the amine ligands, and attributed to the axial sites becoming blocked for the incoming ligands. On substitution the two monodentate amine groups of carboplatin with bpy, the resulting complexes have a UV-absorption band in a region where DNA does not show absorption.[14] This enable us to carry out DNA-binding studies using A.M.Q. King method.[15] Thus, in this paper we report the synthesis, characterization DNA-binding and cytotoxic studies of bpy Pt(II) and Pd(II) complexes containing cbdca which are analogous to carboplatin.

Experimental

Commercially pure chemicals: K_2PtCl_4, $PdCl_2$, bpy, cbdca, highly polymerized calf thymus DNA sodium salt and Tris–HCl buffer were perchased from Aldrich U.S.A. and used as such. Solvents used were of laboratory reagent grade and they were purified before use by the standard methods.[16]

Electronic absorption spectra of the titled metal complexes were measured on a Shimadzu UV-265 recording spectrophotometer. Infrared spectra of the metals complexes were recorded on a Nicolet 5-DXB FT-IR spectrophotometer in the range of 4000—400 cm^{-1} in KBr pellets. Conductivity measurements were carried out on a Systronics conductivity bridge, model 305, with a cell (cell constant 0.59) using conductivity water as a solvent. Micro chemical analysis of carbon, hydrogen and nitrogen for the complexes were carried out on an elemental analysis of CHNO-Rapid Herause. ^{1}H-NMR spectra were recorded on a Brucker DRX-500 Avance spectrometer at 500 MHz in D_2O using sodium 3-trimethylpropionate as internal reference. Fluorescence measurements were carried out on a SPEX-Fluorolog spectrofluorimeter equipped with 1681, 0.22 m double spectrometer and DMIB spectroscopy laboratory coordination using a 1 cm^2 spectrofluorimeter quartz cuvette.

Synthesis of Pd(II) and Pt(II) Compounds $[Pt(bpy)Cl_2]$ and $[Pd(bpy)Cl_2]$ were prepared by the procedures followed for making analogous bpy complexes.[17]

[Pt(bpy)(cbdca)]: $[Pt(bpy)Cl_2]$ (420 mg, 1 mmol) was suspended in 100 ml double distilled water and 19.7 ml of 0.10 M $AgNO_3$ (1.97 mmol) solution was added to it with constant stirring. This mixture was heated at 60 °C with stirring under dark for 6 h at 55 °C and then 16 h at room temparature (28 °C). The AgCl precipitate was removed by centrifugation. The clear solution was filtered through Whatman 42 filter paper and mixed with cbdca (144 mg, 1 mmol) and sodium bicarbonate (168 mg, 2 mmol). The reaction mixture was again stirred for 12 h at 50 °C and then filtered in hot. The clear solution was concentrated on water bath at 45 °C to 5 ml. The yellow precipitate were filtered, washed with small amount of chilled double distilled water and finally recrystallized from double distilled water and dried in vacuum desiccator over anhydrous calcium chloride. The yield was 70%. *Anal*. Calcd For [Pt(bpy)(cbdca)]: C, 38.95; H, 2.84; N, 5.68; Pt, 39.57. Found: C, 39.04; H, 2.93; N, 5.61; Pt, 39.49% . Absorption spectrum (H_2O): λ_{max} (ε_M), 318.6 (7630), 263.2 (7780), 232.5 (10820) nm.

[Pd(bpy)(cbdca)]: This complex was synthesized and recrystallized by following the preparative method of [Pt(bpy)(cbdca)] except $[Pd(bpy)Cl_2]$ was used in place of $[Pt(bpy)Cl_2]$. The yeild was 65%. *Anal*. Calcd for [Pd(bpy)(cbdca)]: C, 47.52; H, 3.47; N, 6.93; Pd, 26.33. Found: C, 47.46; H, 3.50; N, 6.93; Pd, 26.27%. Absorption spectrum (H_2O): λ_{max} (ε_M), 309.3 (11750), 245.7 (14880), 220.6 (31630).

Cytotoxic Studies The procedure followed was similar to that reported earlier[18] except that 2×10^6 P_{388} lymphocytic leukemia cells per ml were used in place of 1×10^6 P_{388} cells per ml in Tris–HCl buffer solution.

Binding Studies The difference UV absorption and fluorecence methods were used to determine the binding of metal complexes to DNA as described earlier[18,19] except that the metal complexes–DNA systems were incubated at 37 °C for 10 h before spectral measurements.

* To whom correspondence should be addressed. e-mail: torshizi@hamoon.usb.ac.ir

Results and Discussion

The mixed-ligand complexes of formula [M(bpy)(cbdca)] (where M is Pd(II) or Pt(II), have been prepared by interaction of α-diiminedinitro Pt(II)/Pd(II) complexes with cbdca. These complexes (1×10^{-3} M) show molar conductance values in the range of 5—6 $cm^{-1}\cdot ohm^{-1}\cdot mol^{-1}$ and these values are unchanged after 2 d. Thus, they can be considered as neutral complexes.[20)]

Spectral Studies The selective infrared spectral data of the Pt(II) and Pd(II) complexes in the range 4000 to 400 cm^{-1} are discussed. The infrared spectra of cbdca in KBr pellets showed a strong band at 1700 cm^{-1} which is assigned to the COOH stretching band of this ligand.[21)] In the Pt(II) and Pd(II) complexes the COO stretching band is observed at 1655 and 1642 cm^{-1}, respectively. This suggest that the carboxyl groups of the above dicarboxylic acid is coordinated as bidentate through two oxygen atoms[22)] as shown in Fig. 1.

The electronic absorption maxima of the Pt(II) and Pd(II) complexes in distilled water with their extinction coefficients are given in the experimental section. The band at 318 nm for Pt(II) and at 309 nm for Pd(II) complexes is tentatively assigned to charge-transfer transition from platinum or palladium *d*-orbital to π^* orbital of α-diimine. Other bands are assigned to first, second, and higher internal transitions of α-diimine.[23)]

In the ^{1}H-NMR spectrum of cbdca (pH*=7.00),[7)] the triplet at δ 2.34 is assigned to H-β and the quintets δ 1.82 to H-γ protons. These two resonances in the [Pd(bpy)(cbdca)] complex under neutral condition, are observed at δ 2.93 and 1.91 respectively. The H-β protons show downfield shift of 0.58 in [Pd(bpy)(cbdca)] as compared to free ligand which indicate bidentate coordination of cbdca ligand. The chemical shift of the bpy ligand in the [Pd(bpy)(cbdca)] and $[Pd(bpy)(H_2O)_2]^{2+}$ have been compared. The ^{1}H resonances of the bpy ligand in these two complexes, appear at δ 8.30 and 7.72 respectively. These small shift differences of bpy ligand, can be due to replacement of dicarboxylate by water which are both oxygen donor ligands.

The above NMR data are in good agreement with the results already obtained by van Eldik and his co-workers.[13)]

The ^{1}H-NMR spectrum of analogous Pt(II) complex shows similar splitting pattern. However, in this complex the H-β and H-α resonances are observed at δ 2.98 and 1.94 respectively.

Cytotoxic Studies The α-diimine Pd(II) and Pt(II) complexes with cbdca ligand were screened for their cytotoxicity against P_{388} lymphocytic leukemia cells.[24)] The ID_{50} values of the Pt(II) and Pd(II) complexes found to be 5 and 6 μmol/l, respectively. These values are much lower than cisplatin.[25)] These growth inhibition studies suggest that the target of these complexes is DNA of the cancer cells. Therefore, the interaction of these complexes with highly polymerized calf thymus DNA have been carried out as given below.

Binding Studies The interaction of [Pt(bpy)(cbdca)] and [Pd(bpy)(cbdca)] in 10 mM Tris–HCl buffer of pH 7.4 in the presence of 0.01 M sodium chloride with calf thymus DNA was studied using difference absorption spectroscopy.[19)] The hypochromic effect observed in the absorption spectra of [Pt(bpy)(cbdca)] and [Pd(bpy)(cbdca)] in presence of increasing concentrations of DNA is shown in Figs. 2a and b, respectively. Two isosbestic points are obtained at 323 and 310 nm for platinum complex (see Fig. 2a) and 318 and 300 nm for palladium complex (see Fig. 2b). This indicates that there is an equilibrium between bound and free forms of the metal complexes with DNA. The change in absorbance was measured at 319 nm for [Pt(bpy)(cbdca)]–DNA system and at 314 nm for [Pd(bpy)(cbdca)]–DNA system. The binding isotherms were constructed from these data using the Scatchard equation.[26)] The Scatchard plot obtained on titration of DNA (24 μM) with [Pt(bpy)(cbdca)] (4—48 μM) and the same plot obtained on titration of DNA (24 μM) with [Pd(bpy)(cbdca)] (4—48 μM) are given in Figs. 3a and b, respectively. Two linear Scatchard plots have been obtained. These linear plots indicate involvement of one binding process with independent binding sites on DNA. The association constant, *K*, has values of 2×10^4 and 1×10^4 and *n*, the number of binding sites per nucleotide, has values of 0.011 and 0.045 for [Pt(bpy)(cbdca)] and [Pd(bpy)(cbdca)], respectively. These binding parameters compare well with those of bpy–Pt(II) and Pd(II) complexes as reported earlier.[27,28)]

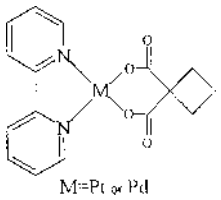

Fig. 1. Proposed Structures of [M(bpy)(cbdca)] Complexes
M is Pd(II) or Pt(II).

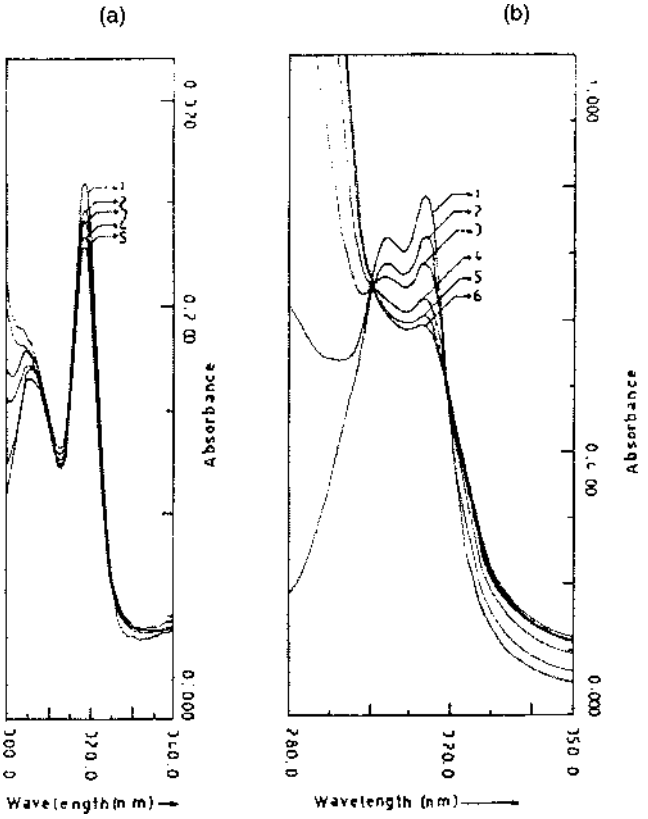

Fig. 2. Effect of Calf Thymus DNA on Absorption Spectrum of (a) [Pt(bpy)(cbdca)] (48 μM) and (b) [Pd(bpy)(cbdca)] (48 μM)
DNA concentrations were increasing in the order 0, 35, 57, 75, 84 and 100 μM for line 1 to 6 in (a) and 0, 62, 125, 153 and 187 μM for line 1 to 5 in (b).

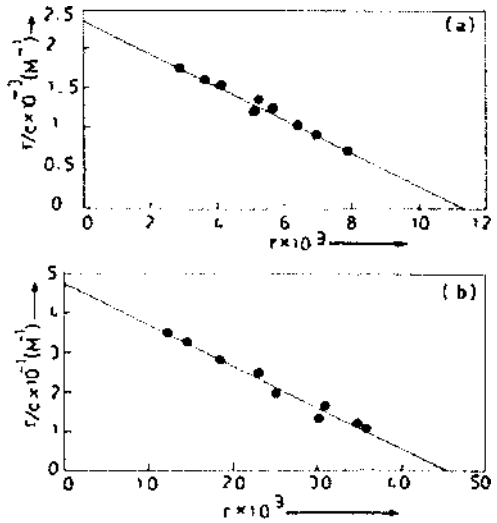

Fig. 3. Scatchard Plots for Binding of (a) [Pt(bpy)(cbdca)] (4—48 μM) to DNA (24 μM) and (b) [Pd(bpy)(cbdca)] (4—48 μM) to DNA (24 μM)

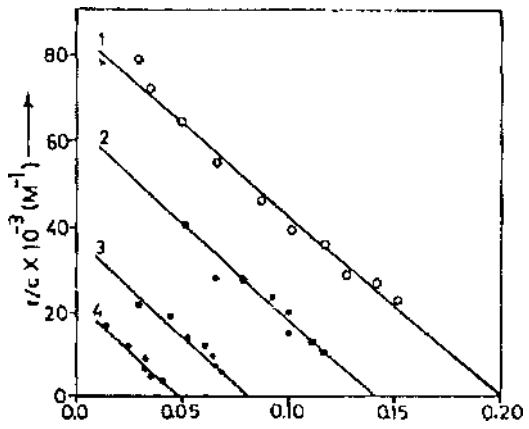

Fig. 4. Fluorescence Scatchard Plots for the Binding of EtBr (2.4—24 μM) to DNA (115 μM) in the Absence (○) and the Presence (●) of Increasing Concentrations of [Pt(bpy)(cbdca)]

r_f increases in the order of 0.0 (1), 0.108 (2), 0.216 (3) and 0.324 (4).

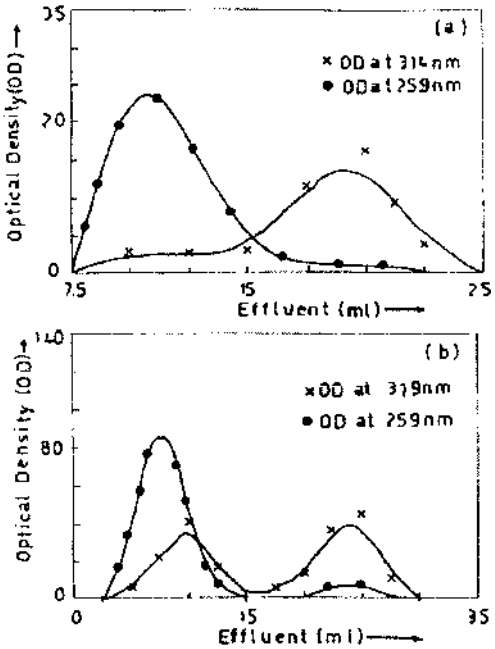

Fig. 5. Gel Chromatograms of (a) [Pt(bpy)(cbdca)]–DNA, (b) [Pd(bpy)(cbdca)]–DNA Complexes Obtained on Sephadex G-25 Column Equilibrated with 10 μM Tris–HCl Buffer of pH 7.4 in the Presence of 0.01 M Sodium Chloride

Each complex was 48 μM and DNA concentration was 120 μM.

Table 1. Binding Parameter for the Effect of [Pt(bpy)(cbdca)] on the Fluorescence of EtBr in the Presence of Calf Thymus DNA

Complex	r_f [a]	K [b]	n [c]
[Pt(bpy)(cbdca)]	0.00	4.26×10^5	0.200
	0.106	4.52×10^5	0.141
	0.210	4.77×10^5	0.081
	0.314	4.81×10^5	0.050

a) r_f is the formal ratio of metal complexes to nucleotide concentration. *b*) K is the association constant (M^{-1}). *c*) n is number of binding sites per nucleotide.

The fluorescence of ethidium bromide (EtBr) is greatly enhanced upon interaction of EtBr to DNA.[29,30] The influence of binding of some α-diimine Pt(II) and Pd(II) complexes of cbdca on the fluorescence intensity of EtBr–DNA complex has been studied. The number of EtBr molecules intercalated to DNA in the presence of [Pt(bpy)(cbdca)] and [Pd(bpy)-(cbdca)] were calculated using Scatchard analysis.[26] In these experiments the wavelength of excitation was set at 546 nm and the wavelength of emission at 605 nm. The saturation curves of fluorescence intensity for [Pt(bpy)(cbdca)]–DNA system at different r_f values (0.00—0.314) in the presence of increasing concentration of EtBr (2.4—24 μM) in 10 mM Tris–HCl buffer of pH 7.4 in the presence of 0.01 M sodium chloride were obtanined. The fluorescence Scatchard plots were obtained for binding of EtBr to DNA in the absence (○) and in the presence (●) of various concentrations of [Pt(bpy)(cbdca)] and are shown in Fig. 4. This Figure shows that the above complexes noncompetitively inhibit the EtBr binding to DNA (type-D behavior),[31] in which the slope that is K (association constant) remain constant while the intercept on the absissa that is n (the number of binding sites per nucleotide) decrease with increasing concentrations of metal complex. This suggests that the [Pt(bpy)(cbdca)] binds to DNA *via* nonintercalating mode. The values of K and n are listed in Table 1. Similar Scatchard plots have been observed for [Pd(bpy)(cbdca)].[27]

Other studies have also been carried out to determine the mode of the above interaction as followd.[27]

Each of the above metal complexes (48 mM) was incubated with calf thymus DNA (120 μM) for 10 h at 37 °C in Tris–HCl buffer pH 7.4. Each DNA–metal complex was passed through a Sephadex G.25 column equilibrated with same buffer. The elution of the column fraction of 2.5 ml was monitored at 319 and 259 nm for DNA–Pt(II) complex systems and 314 and 259 nm for DNA–Pd(II) complex systems. These results are given in Figs. 5a and b, respectively. These plots show that the peak obtained for the two wavelengths were not clearly resolved. This indicates that the DNA is not separated from the metal complexes and their binding to DNA is covalent and irreversible. The irreversible binding of metal complex to DNA is strictly true for kinetically inert Pt(II)–DNA complex and there is expected partial reversibility for kinetic labile Pd(II)–DNA complex as observed

above.

The precipitation of DNA from DNA–metal complex with absolute alcohol was also attempted. The presence of DNA–metal complex in the precipitate suggests the coordinate covalent bond which is responsible for stabilizing the above DNA–metal complexes.

Studies with heat denatured DNA show virtually no reversal in the binding of metal complex to DNA as compared to the native DNA. This suggests that the metal complex is strongly binding to denatured single stranded DNA.

Conclusions

Two water soluble complexes of formula [M(bpy)(cbdca)] which are analogous to carboplatin have been prepared by interacting appropriate α-diiminePt(II) and Pd(II) compounds with cbdca sodium salt. These complexes have been found to be better cytotoxic agents than cisplatin against P_{388} lymphocytic leukemia cells. [M(bpy)(cbdca)] complexes bind to calf thymus DNA through coordinate covalent bond as suggested earlier for the binding of cisplatin to DNA.

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References

1) Rosenberg B., Van Camp L., Trosko J. E., Mansour V. H., *Nature* (London), **222**, 385 (1969).
2) Amo-Ochoa P., Gonzalez V. M., Perez J. M., Masaguer J. R., Alonso C., Ranninger C. N., *J. Inorg. Biochem*., **64**, 287 (1996).
3) Ratbindra N., Soumitra B., Gbosb K., Moghaddas S., *J. Inorg. Biochem*., **65**, 199 (1997).
4) Philip K. W., Kharatishvili M., Qu Y., Farrel N., *J. Inorg. Biochem*., **63**, 9 (1996).
5) Barnard C. F. J., Cleare M. J., Hydes P. C., *Chem. Brit*., **22**, 1001 (1986).
6) Hacker M. P., Douple E. B., Krakoff I. H. (ed.), "Platinum Coordination Complexes in Cancer Chemotherapy," Martinus, Boston, 1984.
7) Frey U., Ranford J. D., Sadler P. J., *Inorg. Chem*., **32**, 1333 (1993).
8) Barnham K. J., Djuran M. I., del S. Murdoch P., Ranford J. D., Sadler P. J., *Inorg. Chem*., **35**, 1065 (1996).
9) Guo Z., Hambley T. W., del S. Murdoch P., Sadler P. J., Frey U., *J. Chem. Soc., Dalton Trans*, **1997**, 469.
10) Allsopp M. A., Sewell G. J., Rowland C. G., Riley C. M., Schowen R. L., *Int. J. Pharmaceut*., **69**, 197 (1991).
11) Canovese L., Callalini L., Chess G., Tobe M. L., *J. Chem. Soc*., *Dalton Trans*., **1988**, 2135.
12) Tobe M. L., Kokhar A. R., *J. Clin. Hematology and Oncology*, **7**, 120 (1977).
13) Shouky A., Rau T., Shoukry M., Van Eldik R., *J. Chem. Soc*., *Dalton Trans*., **1998**, 3105.
14) Mansuri-Torshizi H., Srivastava T. S., Chavan S. J., Chitnis M. P., *J. Inorg. Biochem*., **48**, 63 (1992).
15) King A. M. Q., Nicholson B. H., *Biochem. J*., **114**, 679 (1969).
16) Furniss B. S., Hannaford A. J., Rogers V., Smith P. W. G., Tacheil A. R., "Vogel's Textbook of Practical Organic Chemistry," Longman, London, 1978, p. 204.
17) Palocsay F. A., Rund J. V., *Inorg. Chem.*, **8**, 524 (1969).
18) Mansuri-Torshizi H., Srivastava T. S., Parekh H. K., Chitnis M. P., *J. Inorg. Biochem*., **45**, 135 (1992).
19) Mital R., Srivastava T. S., *J. Inorg. Biochem*., **40**, 111 (1990).
20) Angelici R. J., "Synthesis and Technique in Inorganic Chemistry," Saunders, Philadelphia, 1969, p. 17.
21) Williams D. N., Fleming I. (ed.), "Spectroscopic Methods in Organic Chemistry," 4th ed., McGraw-Hill, London, 1987.
22) Nakamoto K. (ed.), "Infrared and Raman Spectra of Inorganic and Coordination Compounds," 4th ed., Willey, New York, 1986.
23) Mital R., Jain N., Srivastava T. S., *Inorg. Chem. Acta*, **166**, 135 (1989).
24) Puthraya K. H., Srivastava T. S., Ambonkar A. J., Adwankar M. K., Chitnis M. P., *J. Inorg. Biochem*., **25**. 207 (1985).
25) Mital R., Srivastava T. S., Parekh H. K., Chitnis M. P., *J. Inorg. Biochem*., **41**, 93 (1991).
26) Scatchard G., *Ann. N.Y. Acad. Sci*., **51**, 660 (1949).
27) Milal R., Ray K. S., Srivastava T. S., Battacharya R. K., *J. Inorg Biochem*., **27**, 133 (1986).
28) Blake A., Peacocke A. B., *Biopolymer*, **6**, 57 (1967).
29) Butour J. L., Macquet J. P., *Eur. J. Biochem*., **78**, 455 (1977).
30) LePecq J. B., Paoletti C., *J. Mol. Biol*., **27**, 87 (1967).
31) Howe-Grant M., Wu K. C., Bauer W. R., Lippard S. J., *Biochemistry*, **15**, 4339 (1976).