Journal of Molecular Structure 977 (2010) 189–196

Contents lists available at ScienceDirect

Journal of Molecular Structure

journal homepage: www.elsevier.com/locate/molstruc

Synthesis, spectral investigations, antimicrobial activity and DNA-binding studies of novel charge transfer complex of 1,10-phenanthroline as an electron donor with π-acceptor p-Nitrophenol

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ARTICLE INFO

Article history:
Received 30 March 2010
Received in revised form 22 May 2010
Accepted 24 May 2010
Available online 1 June 2010

Keywords:
Charge transfer
DNA-binding
Pharmacology
UV-vis
Fluorescence spectroscopy
^{1}H NMR

ABSTRACT

Proton or charge transfer (CT) complex of donor, 1,10-phenanthroline (Phen) with π-acceptor, p-Nitrophenol (PNP) has been studied spectrophotometrically in methanol at room temperature. The binding of the CT complex with calf thymus (ct) DNA has been investigated by fluorescence spectrum, to establish the ability of the CT complex of its interaction with DNA. Stern–Volmer quenching constant (*K*sv) has also been calculated. The formation constant (K_{CT}), molar extinction coefficient (ε_{CT}), free energy (ΔG^o) and stoichiometric ratio of the CT complex have been determined by Benesi–Hildebrand equation. The stoichiometry was found to be 1:1. The CT complex was screened for its pharmacology as antibacterial and antifungal activity against various bacterial and fungal strains, showing excellent antibacterial and antifungal activity. The newly synthesized CT complex has been characterized by FTIR spectra, elemental analysis, ^{1}H NMR, electronic absorption spectra. TGA–DTA studies were also carried out to check the stability of CT complex.

1. Introduction

Charge transfer interaction within a molecular complex formed from an electron donor, D and electron acceptor, A, involves resonance with a transfer of charge from D to A [1,2]. Therefore, the excited state of the complex is easily achieved by direct excitation in the charge transfer band of the complex [3]. In general, the charge transfer complexation occurs as an ionic bond in simple low-radical pair interaction. The charge transfer complexes play the important roles in many biological systems such as DNA-binding, antibacterial, antifungal, insecticides and ion transfer through lipophilic membranes [4–10]. Besides, CT complexes act as intermediates in a wide variety of reactions involving nucleophiles and electron deficient molecules [11]. They can also be used in the study of drug acceptor binding mechanism [10]. The formation of the charge transfer complexes was utilized in the development of simple, rapid and accurate spectrophotometric methods for the analysis of ganciclovir in pure form as well as in its pharmaceutical formulation [12,13]. The fluorescence spectroscopy method has been used to investigate the interaction of selected antibiotic compounds with DNA [14]. The redox properties of CT complexes have been found to promote DNA-binding. The bidentate nitrogen ligands Phen and its derivatives have been the subject of research interests in recent years [15–18] due to the potential application as DNA cleaving agents and nonradioactive nuclei acid probes. Many priority phenols are known for their toxicity and carcinogenetic character [19,20].

$\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ molecular complexes of Phen derivatives with chloranil, picric acid and chloranilic acid were investigated spectrophotometrically [21]. The spectral data of the complexes obtained on the reaction of iodine with twin site donors such as 1,10-phenanthroline and its methyl and chloro derivatives, 1,7- and 4,7-phenanthroline and 2,2′-bipyridine were also obtained [22]. Charge transfer interactions of π-acceptor p-Nitrophenol (PNP) and other phenols with N,N′-bis [2-hydroxyethyl]-1,4,6,8-napthalenediimide (BHENDI) were investigated spectrophotometrically and spectroscopically to obtain their important properties [23]. The interest to study these particular organic compounds was to have an idea of them pharmacology. These compounds are used as parent material in pharmaceutical insecticides and the excess of thiocarbamides can be determined as charge transfer complexes. The Phen and its derivatives show high catalytic activity [24–26]. The importance and applications of Phen and PNP, prompted us to synthesize and carry out spectrophotometric studies of CT complex forward from Phen and PNP. The studies were also detected towards DNA-binding and antimicrobial activities.

This paper is in continuation of our studies on charge transfer interactions [27–29]. Herein, the synthesis and behavior of CT com-

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doi:10.1016/j.molstruc.2010.05.031

plexation between Phen with PNP as π-acceptor are reported. The stoichiometry and other physical parameters of the charge transfer complex under investigation were determined using Benesi–Hildebrand methods [30]. Studying the CT complex interaction with DNA is one of the most important aspects in biological investigations aimed at discovering and developing new type of antiproliferative agents [31]. DNA-binding studies of synthesized CT complex were done by fluorescence spectroscopy. The CT complex was also screened for its antifungal and antibacterial activity against various strains. The CT complex was characterized by instrumental techniques such as FTIR, ^{1}H NMR, TGA–DTA, and electronic absorption.

2. Experimental

2.1. Materials and reagents

1,10-Phenanthroline (Merck), p-Nitrophenol (Thomas Baker), Tetracycline, Nystatin and agar (Hi-Media, Mumbai) were used as supplied. Analytical grade (AR) methanol (Merck), DMSO (Merck) and HCl (Merck) were used without any further purification. Calf thymus DNA (ct-DNA) was purchased from Sigma (USA).

2.2. Analyses

The electronic absorption spectra of the donor (Phen), acceptor (PNP) and the resulting CT complex in methanol were recorded at room temperature in the region of 700–200 nm using a Shimadzu UV–vis Spectrophotometer model UV 1700 Pharma Spec with a 1 cm quartz cell paths length and DNA-binding studies of synthesized CT complex were carried out by RF-5301 fluorescence Spectrometer. FTIR spectra of reactants and the CT complex were recorded using KBr discs on the FTIR Spectrometer model Spectrospec 2020, ^{1}H NMR spectra of the CT complex, donor and acceptor were recorded in DMSO using Bruker advance II 400 NMR Spectrometer and elemental analysis were done by Elementar Vario EL III Carlo Erba 1108. The thermal analyses (TGA and DTA) was carried out under nitrogen atmosphere with a heating rate of 20 °C/min for thermogravimetric analysis (TGA) and DTA using Shimadzu model DTG-60H Thermal Analyzers.

2.3. Procedures

2.3.1. Preparation of standard stock solutions

A standard solution of 1,10-phenanthroline (donor) at a concentration of 1×10^{-2} M was prepared in different volumetric flask by dissolving 0.099 g of pure Phen in 50 ml of methanol and diluting with the same solvents and a standard solution of PNP 10^{-2} M (acceptor) was prepared by dissolving 0.069 g of pure PNP in a 50 ml volumetric flask using methanol.

2.3.2. Synthesis of the solid CT complex

The solid CT [(Phen)(PNP)] product was synthesized by mixing the saturated solution of PNP (0.139 g, 1 mmol) in CH_3OH (10 ml) with a saturated solution of (0.198 g, 1 mmol) in CH_3OH (10 ml). The resulting solution was stirred for 15 min with heating at 50 °C and kept for 4 days at room temperature. The solid CT complex was separated as needle of off white CT complex which was filtered off and dried under vacuum over $CaCl_2$. The result of elemental analysis are: (theoretical values are shown in brackets): [(Phen)(PNP)], ($C_{18}H_{15}N_3O_4$) CT complex (M/w: 337.29 g): C, 63.99% (64.09%); H, 4.47% (4.48%); N, 12.40% (12.46%).

2.3.3. DNA-binding

Calf thymus DNA was dissolved in tris–HCl buffer (0.1 M, pH 7.4). The purity of the DNA solution was checked from the absorbance ratio A_{260}/A_{280} (≈1.8). Fluorescence measurements were made with a 150 W xenon lamp and a slit width of 5 nm. A fixed concentration of the compound (30 μM) was taken in a quartz cell and increasing amounts of ct-DNA solution was titrated using increments of 5 μM. The fluorescence spectra were recorded in the range of 300–450 nm upon excitation at 280 nm at 310 K.

2.3.4. Antibacterial activity

The antibacterial activity of newly synthesized CT complex was tested in vitro against two Gram-positive bacteria *Staphylococcus aureus* (MSSA 22) and *Bacillus subtilis* (ATCC 6051) and two Gram-negative bacteria *Escherichia Coli* (K 12) and *Pseudomonas aeruginosa* (MTCC 2488) strains using disc diffusion method [32,33]. The discs measuring 5 mm in diameter were prepared from Whatmann No. 1 filter paper sterilized by dry heat at 140 °C for 1 h. The sterile discs previously soaked in a concentration of the test complex were placed in a nutrient agar medium. The plates were invested and kept in an incubator for 24 h at 37 °C. The inhibition zone thus formed was measured (in mm) after 24 h. The screening was performed for 100 μg/ml concentration of test CT complex and antibiotic disc, Tetracycline (30 μg/disc, Hi-Media) was used as control.

The nutrient broth which, logarithmic serially twofold diluted amount of test CT complex and controls, was inoculated within the range 10^{-4}–10^{-5} cfu/ml. The cultures were incubated for 24 h at 37 °C and growth was monitored visually and spectrophotometrically like [34]. The lowest concentration (highest dilution) required to arrest the growth of bacteria is regarded as minimum inhibitory concentration (MBC). To obtain the diameter of zone, 0.1 ml volume was taken each and spread on agar plates. The number of colony forming units (cfu) was counted after 24 h of incubation at 35 °C.

2.3.5. Antifungal activity

The newly synthesized CT complex was also screened for its antifungal property against *Aspergillus niger* (Laboratory isolate), *Candida albicans* (IQA-109) and *Penicillium sp* (Laboratory isolate) in DMSO using standard agar disc diffusion method [35]. The synthesized CT complex was dissolved in DMSO. All cultures were routinely maintained on Sabouraud's dextrose agar (SDA, Hi-media, Mumbai) and incubated at 28 °C. Spore of fungal stain lawning was formed from 7 days old culture on sterile normal solution and diluted to obtain approximately 10^5 cfu/ml. The culture was centrifuged at 1000 rpm, pellets was resuspended and diluted in sterile NSS to obtain a viable count 10^5 cfu/ml. The inoculum of non-sporing fungi *C. albicans* was performed by growing the culture in SD broth at 37 °C overnight. With the help of spreader, 0.1 ml volume of the approximately diluted fungal culture suspension was taken and spread on agar plates. The fungal activity of CT complex was compared with Nystatin (30 μg/disc Hi-Media) as standard drug. The cultures were incubated for 48 h at 37 °C and the growth was monitored. Antifungal activity was determined by measuring the diameters of the zone (mm) in triplicates sets.

2.3.6. Determination of stoichiometry and formation constant

Stoichiometry is determined using the Benesi–Hildebrand method (straight line method) from stock solutions of acceptor and donor. On the other hand, the formation constant (K_{CT}) and molar extinction coefficient of the CT complex determined by Benesi–Hildebrand equation [30] valid under the condition $[A]_0 \gg [D]_0$ or $[D]_0 \gg [A]_0$, for 1:1 donor–acceptor complexes [36–38]. The concentration of donor was kept fixed at 4.5×10^{-5} M and that of the acceptor varied from 0.8×10^{-4} to 7.56×10^{-4} M. The electronic absorption spectra of the complex were measured in methanol at room temperature. The preparation of the molecular complex and the working procedure has been described elsewhere [39,40].

3. Results and discussion

3.1. Observation of CT bands and determination of formation constant

Taking into consideration the presence of an electron donating group in the electron donor system under investigation, on mixing the methanol solution of the donor and acceptor according to the condition $[A]_0 \gg [D]_0$ or $[D]_0 \gg [A]_0$ for 1:1 donor–acceptor complexes [36–38], different wavelength, λ_{CT}, of CT transition is observed relative to the wavelengths of donor and acceptor. This is further supported by the measurement of the absorption band of CT complex of Phen with π-acceptor PNP in methanol which produced CT characteristic broad bands in the UV region. In this region, both donor and acceptor are not absorbing.

The electronic absorption spectra of charge transfer complexes formed from different concentration and excess concentration of PNP with fixed concentration of Phen were recorded in methanol at room temperature and are shown in Fig. 1. The data for formation constant and molar extinction coefficient are reported in Table 1. The increase in absorption intensities for CT complex an addition of the acceptor part in the reaction mixture are reported in Table 1. The new absorption bands are observed at 316 nm and 233 nm which are neither of donor nor of acceptor and band for maximum absorbance have been consider as CT band [41] which is formed due to transfer of charge. It has shown to the transfer of phenolic proton from acceptor to donor by way of hydrogen bonding, assigned as N^+—H–O^-. The absorption intensities of the new bands increase with increases in the concentration of the acceptor in the mixture. The Phen is relatively electron rich and PNP is relatively electron poor molecules. When a solution contains both an electron rich and electron poor compound, they tend to associate with one another in loose interaction known as electron-donor–acceptor (EDA) complexes [42]. The new, low energy absorptions are observed in solutions containing both a donor and an acceptor

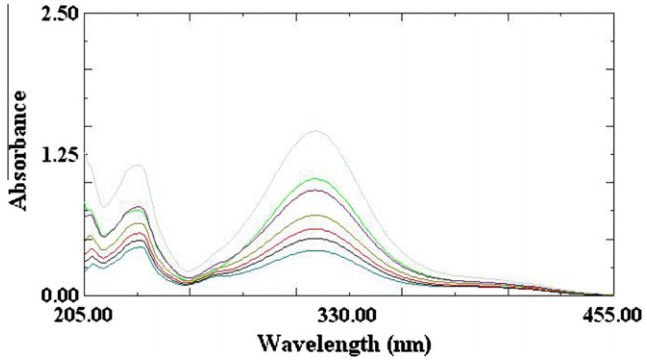


Fig. 1. Electronic absorption spectra of mixture of charge transfer complexes of; (1) Phen (4.5×10^{-5} M) + PNP (0.8×10^{-4} M); (2) Phen (4.5×10^{-5} M) + PNP (1.06×10^{-4} M); (3) Phen (4.5×10^{-5} M) + PNP (1.2×10^{-4} M); (4) Phen (4.5×10^{-5} M) + PNP (1.8×10^{-4} M); (5) Phen (4.5×10^{-5} M) + PNP (2×10^{-4} M); (6) Phen (4.5×10^{-5} M) + PNP (2.64×10^{-4} M) and (7) Phen. (4.5×10^{-5} M) + PNP (7.56×10^{-4} M) in methanol at room temperature, are shown with increasing concentration of acceptor from bottom to top.

Table 1
Absorption data for spectrophotometric determination of stoichiometry and formation constant (K_{CT}) and molar extinction coefficient (ε_{CT}) of the CT complex of Phen and PNP in methanol at room temperature.

Concentration of acceptor ($\times 10^{-4}$ M)	Concentration of donor (M)	Absorbance at λ_{CT} 316 nm	Formation constant (K_{CT}) l mol^{-1}	Molar extinction coefficient (ε_{CT}) l cm^{-1} mol^{-1}
0.80	4.5×10^{-5}	0.406	2.203×10^3	4.401×10^5
1.06		0.513		
1.20		0.588		
1.80		0.769		
2.00		0.935		
2.64		1.090		
7.56		4.118		

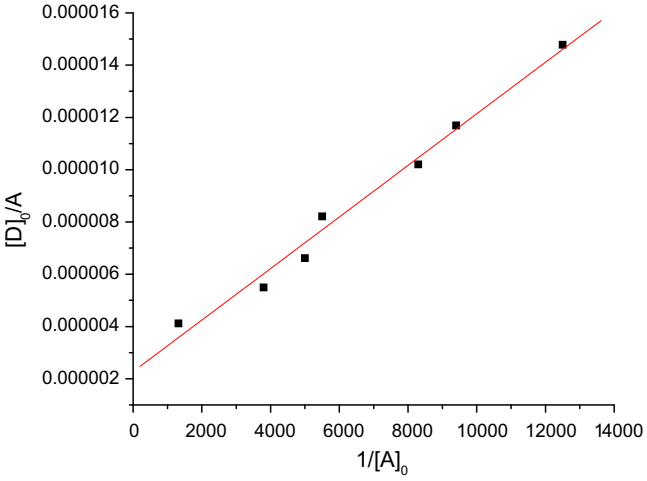


Fig. 2. Benesi–Hildebrand plot of the charge transfer complex of 1,10-phenanthroline with p-Nitrophenol, $[D]_o/A$ vs. $1/[A]_o$ in methanol at room temperature.

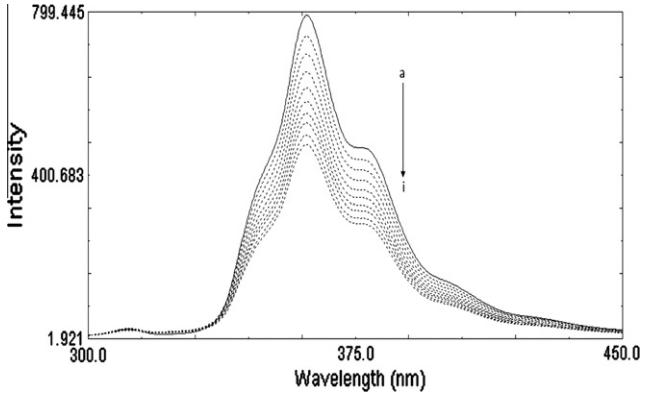


Fig. 3. Fluorescence emission spectra of CT complex of 1,10-phenanthroline and p-Nitrophenol in the absence (—) and presence of increasing amount of DNA from (a) to (i) (—).The arrow shows the intensity changes on increasing the CT complex concentration.

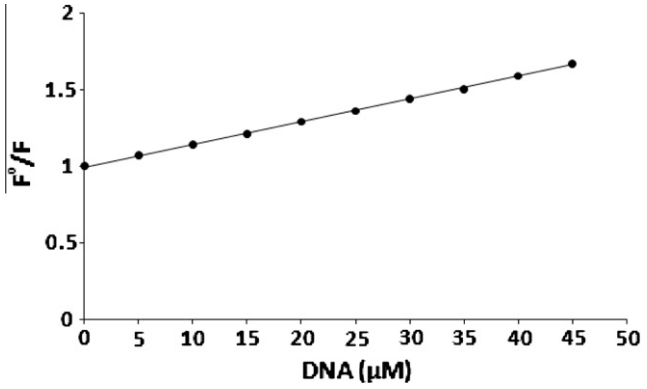


Fig. 4. Stern–Volmer plot for the binding of of CT complex of 1,10-phenanthroline and p-Nitrophenol with DNA.

Table 2
Antibacterial and antifungal activity of synthesized 100 μg/ml concentration of novel CT complex of Phen and PNP.

Diameter of zone of inhibition in mm at 100 μg/ml and DMSO as control					
Bacteria	CT complex	Tetracycline	Fungi	CT complex	Nystatin
Staphylococcus aureus	35.3 (±1.89)	20 (± 0.23)	*Aspergillus niger*	46 (±1.99)	24 (±0.24)
Bacillus subtilis	30.7 (±1.20)	21 (± 0.55)	*Candida albicans*	48 (±1.88)	23 (±0.58)
Escherichia Coli	26.0 (±0.82)	25 (± 0.88)	*Penicillium sp*	45 (±1.33)	25 (±0.88)
Pseudomonas aeruginosa	21.5 (±1.33)	24 (± 0.63)			

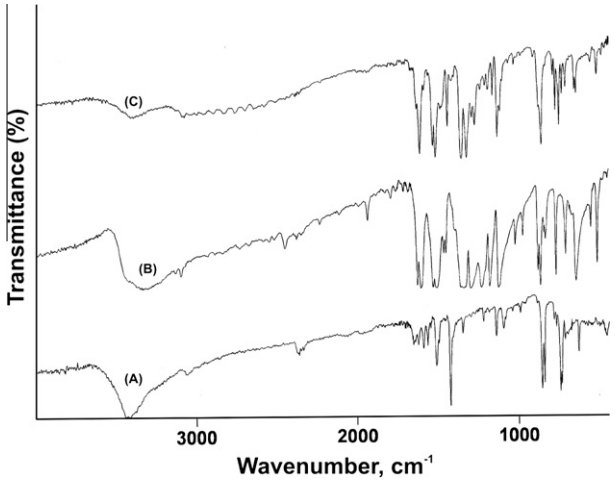


Fig. 5. FTIR spectra of (A) 1,10-phenanthroline; (B) p-Nitrophenol; (C) Charge transfer complex of p-Nitrophenol and 1,10-phenanthroline.

by Mulliken [43] as charge transfer interactions involving the excitation of an electron on the donor to empty orbital on the acceptor.

The formation constant (K_{CT}) for the CT complex of donor–acceptor, with molar extinction coefficient (ε_{CT}) were determined by the Benesi–Hildebrand [30] equation using the absorbance of the CT complex.

$$[D]_0/A = 1/K_{CT}\varepsilon_{CT} \cdot 1/[A]_0 + 1/\varepsilon_{CT} \quad (1)$$

where $[A]_0$ and $[D]_0$ are the initial concentrations of the acceptor and donor, respectively, A is the absorbance of the donor–acceptor mixture at λ_{CT} measured against the solvent as reference and ε_{CT} is the molar extinction coefficient. K_{CT} is the formation constant of the CT complex. In this case a typical linear plot is obtained and is shown in Fig. 2. The correlation coefficient for this plot was about 0.9. The values of ε_{CT} and K_{CT} are determined from the slop of such plot and are 4.401×10^5 l cm^{-1} mol^{-1} and 2.303×10^3 l mol^{-1}, respectively. The transition energy of CT complex is calculated to be 3.935 eV. The values of the formation constant (K_{CT}) are dependent on the nature and geometry of acceptors and donors. The stoichiometry of the CT complex was obtained as the straight line of Benesi–Hildebrand plots as shown in Fig. 2 and was found to be 1:1, similar to the stoichiometry of CT complexes of derivatives of 1,10-phenanthroline with aromatic nitrophenols [10,44].

3.2. Determination of free energy (ΔG^o)

The values of K_{CT} were used to calculate $-\Delta G^o$ values according to the equation [45,46].

$$\Delta G^o = -RT \ln K \quad (2)$$

The free energy, $-\Delta G^o$, is calculated to be 21.054 kJ mol^{-1}, indicating that the formation of the complex is exothermic and spontaneous.

Table 3
Infrared frequencies[a] (cm^{-1}) and band assignments for (A) Phen (B) PNP and (C) [(Phen) (PNP)] compound.

A	B	C	Assignments[b]
	3321 vs, br	3384 m, br	ν(O—H) of PNP; CTC ν($^+$NH)
3059 w	3084 w	3064 w	ν(C—H); aromatic
2960 w	2980 w	2955 w	ν(C=C); CH aromatic
	2948 w	2950 w	ν_s(C—H); CH_2 — CH_3
		2930 w	
		2878 w	Hydrogen bonding
		2811 w	
		2744 mw	
		2682 w	
		2625 w, br	
1690 w	1613 mw	1650 sh	ν(C=C); aromatic
	1589 vs	1592 vs	$\nu_{as}(NO_2)$; PNP
1587 m	1492 vs	1494 vs	δ(CH); CH def
1509			
1500		1463 sh	δ(CH); aromatic
1422 vs	1325 vs	1422 s	ν_{as}(CN)
1339 w		1334 s	
	1278 vs	1303 s	ν(C—O); C—OH
		1270 w	
	1218 vs	1214 w	$\nu_s(NO_2)$; PNP
	1169 s	1187 w	
	1106 vs		
1138 mw	1007 w	1102 vs	ν_s(CN)
1091		1094 sh	
1037 mw	963 w	1042 w	δ(CH); in-plane bend
988 w	850 vs	1009 w	
854 vs	819 w	889 w	
837 s		840 vs	
778 w	757 s	766 w	δ(CH); CH-rock
762 w		752 m	
740 s	627 vs	730 s	
694 w	694 s	711 w	δ(NH); NH def
622 m	628 vs	688 w	
637 mw		634 w	
		622 w	
525	536 mw	535 w	δ(CH); out-of-plan
447	496 s	493 w	
411	417 w	465 w	
		405 w	

[a] br, broad; m, medium; s, strong; sh, shoulder; w, weak; ν, stretching, δ, bending.
[b] Stretching and bending.

3.3. Pharmacology

The synthesized CT complex to be tested was dissolved in DMSO to obtain 100 μg/ml stock solutions. The diameter zones were measured which exhibited the growth of tested microorganism. The CT complex showed excellent microbial activities against various bacterial and fungal strains.

3.3.1. DNA-binding studies of CT complex using fluorescence spectroscopy

The fluorescence quenching technique is often used to monitor the molecular interactions because of its high sensitivity [47–49]. The interactions of CT complex of Phen and PNP, with calf thymus

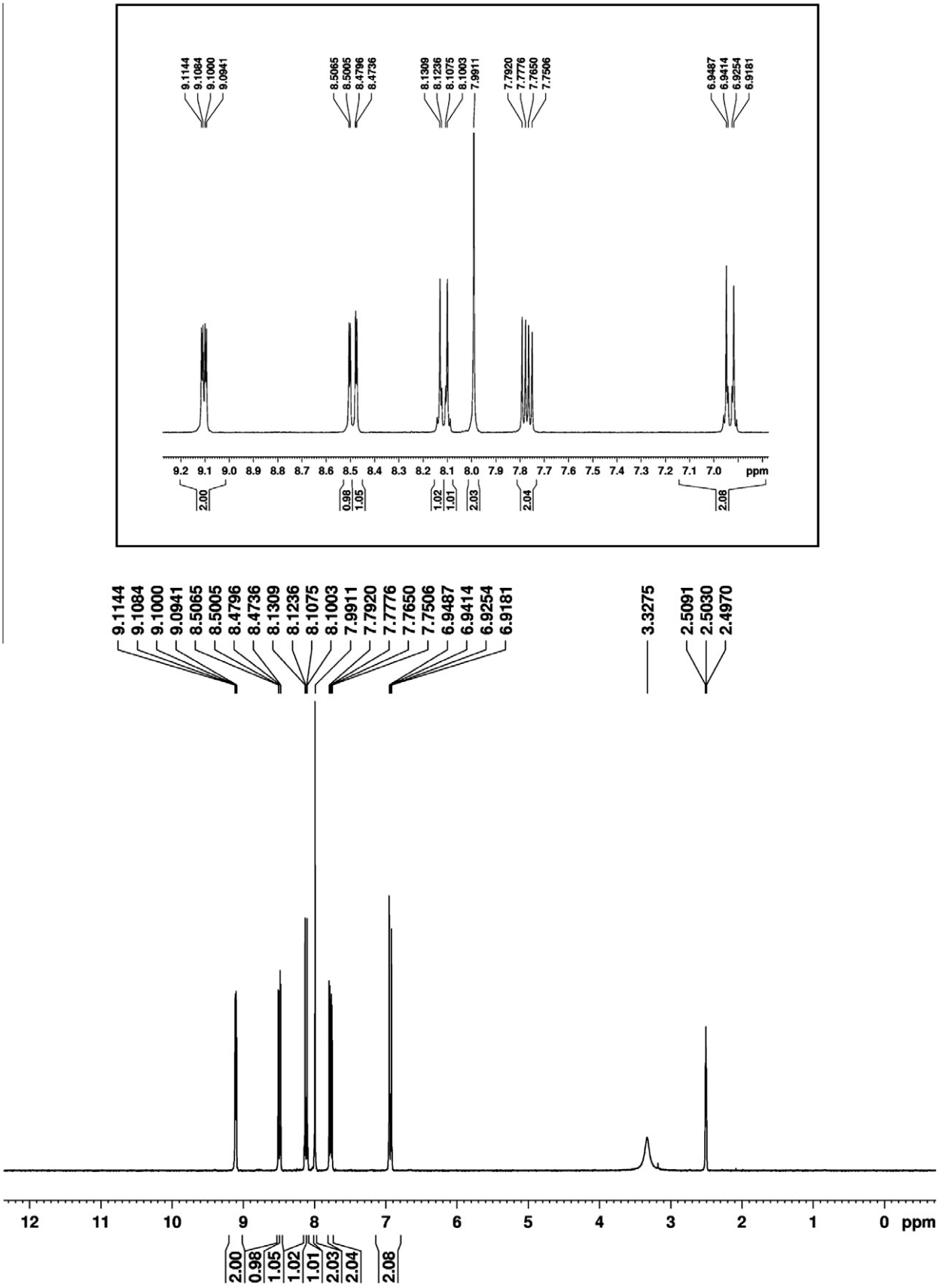
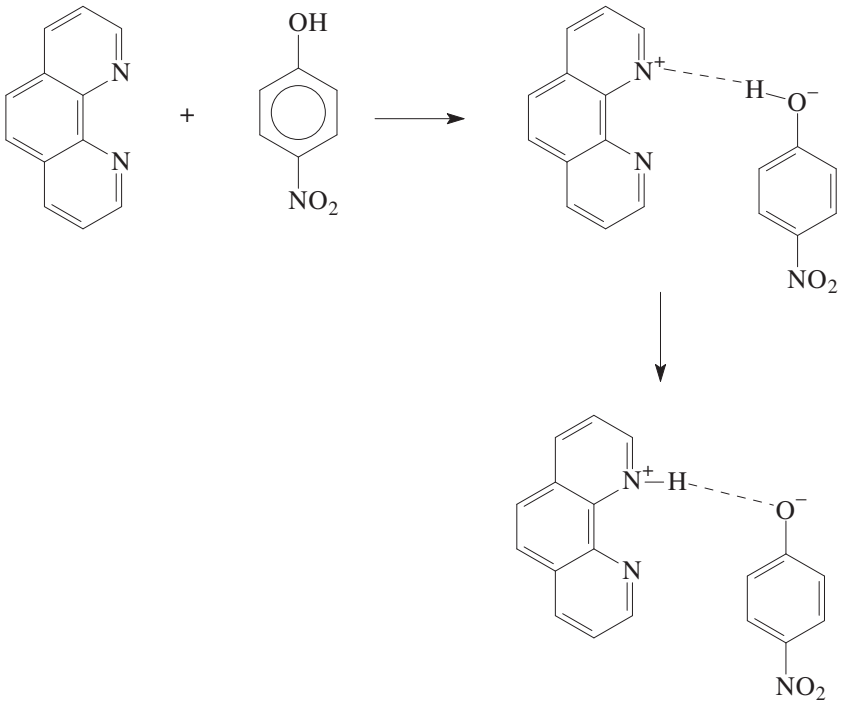


Fig. 6. ^{1}H NMR spectrum of charge transfer complex of 1,10-phenanthroline with p-Nitrophenol.

DNA was studied by fluorescence spectroscopy. Fluorescence quenching is usually classified as dynamic quenching and static quenching [50]. Dynamic quenching results from collision between fluorophore and quencher whereas static quenching is due to



Scheme 1. Proton transfer mechanism between 1,10-phenanthroline and p-Nitrophenol.

ground-state complex formation between fluorophore and quencher [51]. However the characteristic Stern–Volmer plot of combined quenching (both static and dynamic) is an upward curvature. The binding of CT complex with calf thymus DNA, was studied by monitoring the changes in the intrinsic fluorescence of CT complex at varying DNA concentrations. Fig. 3 gives the representative fluorescence emission spectra of the CT compound upon excitation at 280 nm. The addition of DNA caused a gradual decrease in the fluorescence emission intensity of the compound suggesting changes in the microenvironment of the fluorophore upon interaction with DNA. CT complex has effect on the fluorescence intensity of ct-DNA being quenched. To determine the DNA-binding ability of the compound, fluorescence intensity data were analyzed by the Stern–Volmer equation [52].

$$F^{o}F = 1 + Ksv[Q] \quad (3)$$

where F and F^0 are the fluorescence intensity with and without the quencher (ct-DNA), *Ksv* the Stern–Volmer quenching constant, and [Q] the concentration of the quencher. The *Ksv* for the CT complex was obtained from the slope of the Stern–Volmer graph as shown in Fig. 4 and was found to be $1.4 \times 10^4\,\mathrm{l\,M^{-1}}$ which depicts that the quenching may be static or dynamic.

3.3.2. Antibacterial activity studies

The biological activities of the synthesized CT complex have been studied for its antibacterial and antifungal activities using disc diffusion method [32,33] in vitro against two Gram-positive bacteria *Staphylococcus auras* (MSSA 22) and *B. subtilis* (ATCC 6051) and two Gram-negative bacteria *E. Coli* (K 12) and *P. aeruginosa* at concentration of 100 μg/ml. Tetracycline was used as standard drug for the comparison of bacterial results and screening data are given in table. The newly synthesized CT complex have exerted significant inhibitory activity against the growth of the tested bacterial strains and data reveal that CT complex have significant influence on the antibacterial profile of *S. aureus* and *B. subtilis*. The CT complex exhibited good inhibitory results against *E. coli* and *P. aeruginosa* as reported in Table 2.

3.3.3. Antifungal activity studies

The synthesized CT complex was also examined for its antifungal activity and Nystatin was used as standard drug for comparison of antifungal results. The test CT complex exhibited excellent inhibitory results for *A. niger* (Laboratory isolate), *C. albicans* (IQA-109) and *Penicillium sp* (Laboratory isolate) as given in Table 2. The data revealed that the CT complex has produced the marked enhancement in the potency as antifungal agent.

3.4. Comparative study of FTIR of CT complex and reactants

A careful investigation of the important characteristic peaks of the FTIR spectra of Phen, PNP and their 1:1 CT complex are recorded and shown in Fig. 5. Assignments of the characteristic infrared spectral bands of the free acceptor and donor as well as the formed CT complex are reported in Table 3. It is observed that the formation of CT complex is strongly supported by observing the main infrared bands of the reactant Phen and PNP in the product spectrum. It is observed that the positions of the most bands of donor and acceptor in the complex spectrum show shift in the frequency and changes in their band intensities compared with those of the free Phen and PNP. These changes strongly support the formation of CT complex and could be attributed to the expected symmetry and electronic structure modifications in both donor and acceptor units in the formed product relative to the free molecules. In general, spectra the CT complex show the characteristic bands due to various modes of vibration of the acceptor part, shift to lower wavenumber while those of the donor part acquire a counter shift. The shift of the nCH bands of the donor to higher wavenumber is considered to be a criterion for CT complex interaction [53] involving the transfer of an electron from HOMO of the donor to the LUMO of the acceptor or transfer of proton from acceptor to donor. These types of bands are due to the stretching

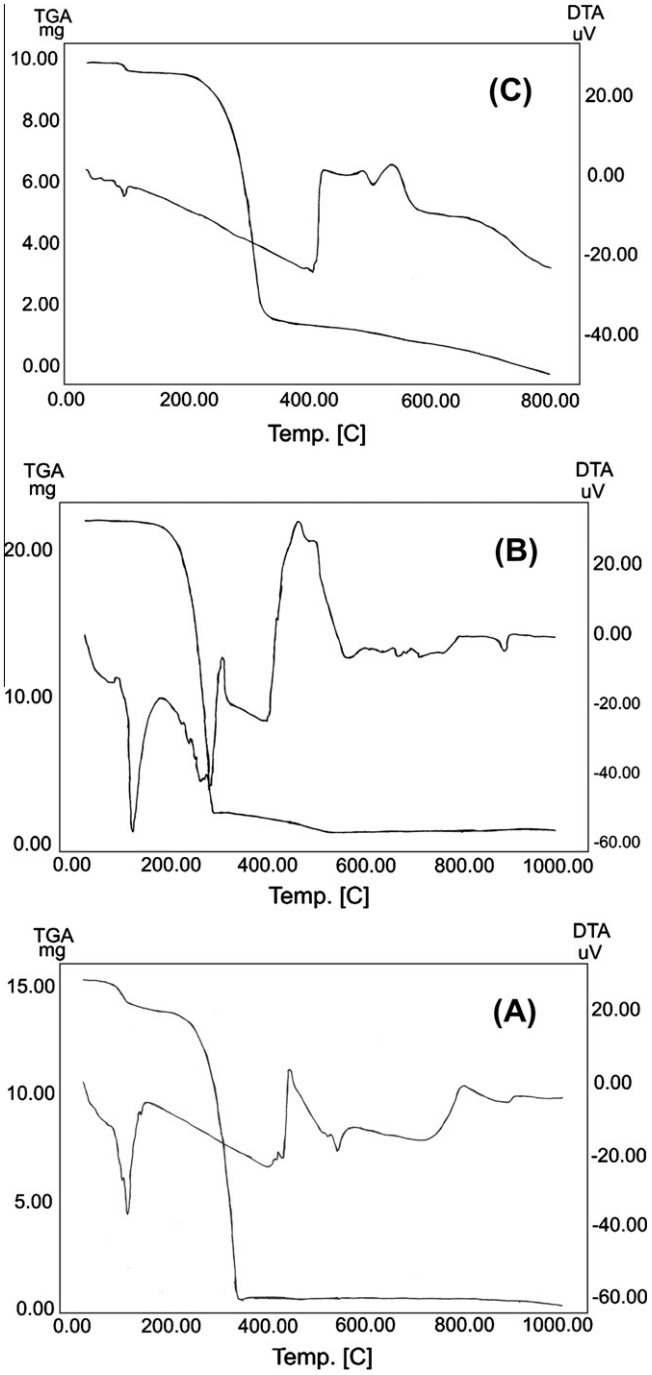


Fig. 7. TGA–DTA curves for (A) 1,10-phenanthroline; (B) p-Nitrophenol; (C) charge transfer complex of 1,10-phenanthroline and p-Nitrophenol.

mode of proton attached to a quaternary nitrogen atom [54].The stretching vibrations of the (C═N) and (C═C) peaks appear at 1422 cm^{-1} (very strong) and 1690 cm^{-1} (weak), in phenanthroline, respectively, and stretching vibration of —OH, —NO_2 and C═C peaks appear at 3321 cm^{-1} (very strong, broad), 1589 cm^{-1} (very strong) and 1613 cm^{-1} (medium, weak), respectively for PNP which is more acidic than alcohol. The infrared spectrum of CT complex shows a medium broad band for the N^+—H···O^- within the range 3224–3500 cm^{-1} and stretching vibrations of the C═N, C═C and NO_2 peaks appear at 1422 (very strong), 1650 cm^{-1} (sh) and 1592 cm^{-1} (very strong), respectively. It is also observed that some peaks for Phen and PNP do not appear in the IR spectra of CT complex of them. This behavior is in accordance with the charge migration from the donor to the acceptor [55,56], which give an additional evidence for interaction between Phen and PNP. Therefore, the FTIR data provided evidence for the existence of a new bands of medium weak intensity in the spectra of CTC prepared and indicated that the formation of quaternary amine species i.e., $^+$NH, from which a labile proton is expected. Therefore, these results reveal that the CT complex has just some changes in their band intensities and shift of some band frequency values.

3.5. ^{1}H NMR spectra

The nuclear magnetic resonance (^{1}H NMR) spectra of the CT complex, donor and acceptor were recorded in DMSO. The spectrum CT complex is shown in Fig. 6. The ^{1}H NMR spectrum of the CT complex was compared with the reactants and structure of donor, acceptor and proposed CT complexation are given in scheme 1.

The changes in the chemical shift values of donor and acceptor moieties in the ^{1}H NMR spectrum of CT complex have been observed relative to the free donor and acceptor same as the ^{1}H NMR spectra of CT complexes of 2,9-dimethyl-1,10-phenanthroline and 8-hydroxyquinoline and with picric acid and p-Nitrophenol, respectively [21,28]. The phenolic proton in the acceptor (PNP) usually appears at δ = 10.77 ppm [28] which is disappeared after complexation to make hydrogen bond with one nitrogen atom of Phen because H of —OH has more acidic character than other H atoms of PNP. Therefore, H of —OH became more easy to liberate. It is clearly obvious that new peak in spectrum of the CT complex at δ = 3.32 ppm (br s, 1H, $^+$N—H of CTC) is assigned to $^+$NH proton of CT complex due to proton transfer from acceptor donor which indicates the involvement of the phenolic —OH and one nitrogen atom of Phen. The doublets at δ = 6.91 ppm and δ = 8.10 ppm have been assigned to the one of each two protons of the same kind in PNP moiety in the CT complex whereas in free PNP they were observed at δ = 6.95 ppm and δ = 8.25 ppm respectively [28]. These upfield shifts in frequencies have been attributed to an increased electron density on PNP part of the CT complex due to the existence of the (N^+—H) charge transfer interaction between the donor and acceptor molecules. The triplet and singlet peaks at δ = 7.75 ppm and δ = 7.99 ppm are observed with small change in chemical shift and assigned to the one of each two protons in CTC. The two doublet at δ = 8.477 ppm and δ = 9.09 ppm are observed in the spectrum of the CT complex and each peak are assigned to the two proton.

3.6. Comparative study of thermograms for 1,10-phenanthroline, p-Nitrophenol and their CT complex

Thermal analysis was carried out for Phen, PNP and their CT complex. A heating rate of 20 °C/min within the temperature range

Table 4
Weight loss, enthalpy (ΔH), and degradation temperature (T), for CT complex of 1,10-phenanthroline and p-Nitrophenol.

Step	CT complex of Phen and PNP			1,10-Phenanthroline			p-Nitrophenol		
	Weight loss (%)	ΔH (J/g)	T (°C)	Weight loss (%)	ΔH (J/g)	T (°C)	Weight loss (%)	ΔH (J/g)	T (°C)
I	3.56	+21.55	142.37	9.37	+121.69	111.93	87.26	+64.34	254.83
II	81.56	+729.80	298.99	87.22	−117.10	280.67	6.04	+600.64	226.09
III	5.65	−127.36	499.77						

of 25–800 °C was used. The combined TGA and DTA thermograms for charge transfer complex along with acceptor and donor are presented in Fig. 7. It is clearly observed that [(Phen) (PNP)] exhibit three step degradation (Fig. 7C) which is typical thermal behavior of derivatives of PNP as reported elsewhere [23]. In first step, 3.558% of the compound is lost at around 142.2 °C which is thought to be a consequence of crystallization. This can also be reflected by the existence of corresponding endothermic peak (ΔH = 21.547 J/gm) observed on DTA thermograms. The second major weight loss (81.563%) is due to the decomposition of charge transfer complex into its constituents. The PNP is less aromatic (less carbon content) which is thus lost first. Therefore, this weight loss in CT complex is attributed to the loss of PNP prior to Phen, as clearly mirrored the first weight loss in TGA thermogram of PNP (Fig. 7). However, the large difference in the ΔH values, corresponding to loss of PNP which are estimated from DTA thermograms (Fig. 7B and C) is in perfect agreement with bonding between the hydrogen of PNP and the aromatic nitrogen of Phen in the complex, Table 4 presents the most important thermal analysis data obtained from the TGA–DTA thermograms. It is also interesting to note that the third degradation step exhibited by charge transfer complex at around 499 °C is the final loss of the carbon residue and azocyanine as well, resulting from the full decomposition of 1,10-phenanthroline same as derivates of 1,10-phenanthroline [24,57]. This step can also be observed in the TGA thermogram of donor (Fig. 7A) with slight difference in ΔH values of the second step (Fig. 7A) and the first step (Fig. 7C) corresponding to this weight loss which is due to charge transfer complex formation.

4. Conclusions

The electron donor 1,10-phenanthroline reacts with the π-acceptor p-Nitrophenol in methanol at room temperature to form the charge transfer complex. The forgoing discussion has shown that Phen forms with PNP 1:1 molecular complex in which phen was found to act as a n donor or a hydrogen acceptor. Further, the TGA–DTA thermograms of the solid state CT complex of phen and PNP supported for interaction between donor and acceptor by some change in enthalpy (ΔH), degradation temperature and weight loss for Phen, PNP and their CT complex. It is also observed that FTIR and ^{1}H NMR data provide evidence for the existence of new bands of CTC with some changes and indicate a charge transfer interaction associate with proton migration from acceptor to the donor followed by intermolecular hydrogen bonding which is assigned to N^+—H–O^- donor the formation of quaternary amine species ($^+$NH). The fluorescence spectrum studies carried out on the interaction of the CT complex with DNA show that the CT complex has well the ability of interaction with DNA, and CT complex shows excellent antibacterial and antifungal activity against various strains. Stern–Volmer quenching constant, Formation constant and other mentioned physical parameters are estimated.

Acknowledgments

Authors thank to the Chairmen Department of Chemistry for providing research facilities. One of the authors (I.M. Khan) gratefully acknowledges University Grant Commission, India for providing financial assistance.

References

[1] R.S. Mulliken, J. Am. Chem. Soc. 74 (1952) 811.
[2] R.S. Mulliken, J. Phys. Chem. 56 (1952) 801.
[3] W. Jarzeba, J. Mol. Liq. 68 (1996) 1–56.
[4] R. Mandal, S.C. Lahiri, J. Indian Chem. Soc. 76 (1999) 347.
[5] F. Gutmann, C. Johnson, H. Keyzer, J. Molnar, Charge Transfer Complexes in Biochemistry Systems, Marcel Dekker Inc., 1992.
[6] D.J. Brown, S.F. Mason, The Pyrimidines, Interscience Publishers, John Wiley & Sons, New York, 1962.
[7] S.M. Sondhi, M. Johar, S. Rajvanshi, S.G. Datidar, R. Shukla, R. Raghubir, J.W. Lown, Aust. J. Chem. 54 (2001) 169.
[8] M. Kidwai, S. Saxena, S. Rastogi, R. Venkataramanan, Curr. Med. Chem. Anti-Infective Agents 2 (2004) 269.
[9] A. Dozal, H. Keyzer, H.K. Kim, W.W. Way, Int. J. Antimicrob. Agent 14 (2000) 261.
[10] J. Feng, H. Zhong, B.D. Xuebau, Zir. Kexu. 27 (6) (1991) 691.
[11] S.H. Bazzi, A. Mostafa, S.Y. AlQaradawi, E. Nour, J. Mol. Struct. 842 (2007) 1.
[12] A.A. Gouda, Talanta 80 (2009) 151.
[13] C.S.P. Sastry, T.E. Divakar, U.V. Prasad, Talanta 33 (1986) 164.
[14] X. Lu, L. Wang, H. Liu, R. Wang, J. Chen, Talanta 73 (2007) 444.
[15] L. Feng, X. Liu, H. Chao, L. Ji, J. Inorg. Biochem. 101 (2007) 56.
[16] Q. Zhang, F. Zhang, W. Wang, X. Wang, J. Inorg. Biochem. 100 (2006) 1344.
[17] Francesco P. Fanizzi, M. Lanfranchi, G. Natile, A. Tiripicchiot, Inorg. Chem. 33 (1994) 3331.
[18] N. Margiotta, P. Papadia, F. Lazzaro, M. Crucianelli, F. De Angelis, C. Pisano, L. Vesci, G. Natile, J. Med. Chem. 48 (2005) 7821.
[19] B. Nasr, G. Abdellatif, J. Electrochem. 152 (2005) 113.
[20] A. Bebeselea, F. Manea, G. Burtica, L. Nagy, G. Nagy, Talanta 80 (2010) 1068.
[21] A.S. Gaballa, C. Wagner, S.M. Telab, J. Mol. Struct. 876 (2008) 301.
[22] N.S. Rao, G.B. Rao, Spectrochima. Acta Part A 46 (1990) 1107.
[23] M.S. Refat, H.M.A. Killa, Ivo Grabeher, Spectrochem. Acta Part A 68 (2007) 123.
[24] G. Li, W. Mo, H. Lin, H. Xiong, Appl. Catal. A: General 333 (2007) 172.
[25] J.M. Kruse, W.W. Brandt, Anal. Chem. 24 (8) (1952) 1306.
[26] W.L. Mo, H. Xiong, T. Li, X.C. Guo, G.X. Li, J. Mol. Catal. A Chem. 247 (2006) 227.
[27] I.M. Khan, A. Ahmad, Spectrochim. Acta Part A 73 (2009) 966.
[28] I.M. Khan, A. Ahmad, Spectrochim. Acta Part A 76 (2010) 315.
[29] I.M. Khan, A. Ahmad, Mol. Cryst. Liq. Cryst. 515 (2009) 154.
[30] H.A. Benesi, J.H. Hildebrand, J. Am. Chem. Soc. 71 (1949) 2703.
[31] C. Zhou, J. Zhao, Y. Wu, C. Yin, P. Yang, J. Inorg. Biochem. 101 (2007) 10.
[32] R. Cruickshank, J.P. Duguid, B.P. Marmion, R.H.A. Awain, Medicinal Microbiology, 12th ed., vol. 11, Churchill Livinggstone, London, 1995, p. 196.
[33] A.H. Collins (Ed.), Microbiology Method, second ed., Butterworth, London, 1976.
[34] Z.A. Siddiqi, M. Khalid, S. Kumar, M. Shahid, S. Noor, Euro. J. Med. Chem. 45 (2010) 264.
[35] A.W. Bauer, W.M.M. Kirby, J.C. Sherris, M. Turck, Am. J. Clin. Path. 45 (1966) 493.
[36] H. Duymus, M. Arslan, M. Kucukislamoglu, M. Zangin, Spectrochim. Acta Part A 65 (2006) 1120.
[37] M.S. Refat, S.A. Sadeek, H.M. Khater, Spectrochim. Acta Part A 64 (2006) 778.
[38] S. Bhattacharya, K. Ghosh, S. Chattopadhyay, Spectrochim. Acta Part A 65 (2006) 659.
[39] Y.M. Issa, A.E. El-Kholy, A.M. Hindawey, R.M. Issa, J. Indian Chem. Soc. I, VII (1980) 216.
[40] M. Gaber, H.A. Desouki, E.H. Hossani, Spectrosc. Lett. 25 (4) (1992) 463.
[41] D.A. Skoog, Principle of International Analysis, third ed., Saunder College Publishing, New York, 1985 (Chapter 7).
[42] M.E. El-Zaria, Spectrochim. Acta Part A 69 (2008) 216.
[43] R.S. Mulliken, J. Chim. Phys. 61 (1964) 20.
[44] R. Abou-Ettah, A. El-Korashy, J. Phys. Chem. 76 (1972) 2405.
[45] E.H. El-Mossalamy, A.S. Amin, A.A. Khalil, Spectrochim. Acta Part A 58 (2002) 67.
[46] E.H. El-Mossalamy, J. Mol. Liq. 123 (2006) 118.
[47] Hu Y. Liu, L.X. Zhang, R.M. Zhao, S.S. Qu, J. Mol. Struct. 750 (2005) 174.
[48] L.F. Tan, Z.J. Chen, J.L. Shen, X.L. Liang, J. Chem. Sci 121 (2009) 397.
[49] N. Wang, L. Ye, B.Q. Zhao, J.X. Yu, J. Med. Biol. Res. 41 (2008) 589.
[50] Y.J. Hu, Y. Liu, X.S. Shen, X.Y. Fang, S.S. Qu, J. Mol. Struct. 738 (2005) 143.
[51] A. Papadopoulou, R.J. Green, R.A. Frazier, J. Agric. Food Chem. 53 (2005) 158.
[52] A.M. Pyle, J.P. Rehmann, R. Meshoyrer, C.V. Kumar, N.J. Turro, J.K. Barton, J. Am. Chem. Soc. 111 (1989) 3051.
[53] R.D. Kross, V.A. Fassel, J. Am. Chem. Soc. 79 (1957) 38.
[54] L.J. Ballamy, The Infrared Spectra of Complex Molecules, Chapman and Hall, London, 1975. p. 290.
[55] H.M.A. Salman, U.M. Rabie, E.M. Abd-Alla, Can. J. Anal. Spectrosc. 49 (2004) 1.
[56] R. Foster, Organic Charge-Transfer Complexes, Academic Press, London, 1969.
[57] R. Santi, A.M. Romano, R. Garrone, L. Abbondanza, M. Scalabrini, G. Bacchilega, Macromol. Chem. Phys. 200 (1999) 25.