Green and Efficient Composition and Diagnosis of Pentdentate Schiff Base Donative Metal Complexes: Antimicrobial, Antifungal, Antioxidant Screening and DNA Binding

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Abstract

Background: Enforcement of sustainable and green chemistry protocols has seen colossal surge in recent times, the development of an effective, eco-friendly, simple and novel methodologies towards the synthesis of valuable synthetic scaffolds and drug intermediates. Recent advances in technology have now a more efficient means of heating reactions that made microwave energy. Efforts to synthesize novel heterocyclic molecules of biological importance are in continuation. Microwave irradiation is well known to promote the synthesis of a variety of organic and inorganic compounds. The aim of current study was to conceivea mild base mediated preparation of novel Schiff base of 2-Acetylpheno with trimethoprim drug (H_2TPBD) and its complexes with Cu(II),Co(II),Zn(II),Cd(II) and Ni(II).

Method: The products are likening with traditional processes for reaction time and their yield. (H_2TPBD) and the complexes were diagnosed by spectroscopic (Mass, NMR, UV–vis, IR spectral studies, analytical and magnetic data.

Results: All complexes were found to be six co-ordinate mono-hydrate as[M(TPBD)(H_2O)] [1:1(ligand:metal) ratio] type. The complexes exhibited biological activity against (B.subtilis, P.aeruginosa, C.albicansand Staphylococcus aureus) bacterial strains as compared to (H_2TPBD). The antibacterial efficiency showed the following trend: M(II)-complexes > (H_2TPBD) > parent drugs. Cu(II), Co(II), Zn(II), Cd(II)andNi(II) complexes had good antioxidant efficiencies than the free ligand (H_2TPBD). DNA binding study of complexes with (CT)-DNA utilizing binding nature of the complexes with CT DNA has moreover inveterate by viscometer and emission which then bespoken that complexes bound with CT DNA. The complexestook effective scavenging impact during the DPPH process.

Conclusion: [H_2TPBD] has been prepared by the condensation of trimethoprim drug and Acetylphenol and characterized by electronic absorption spectra, ^{1}H and ^{13}C-NMR and IR,mass UV-spectroscopies.

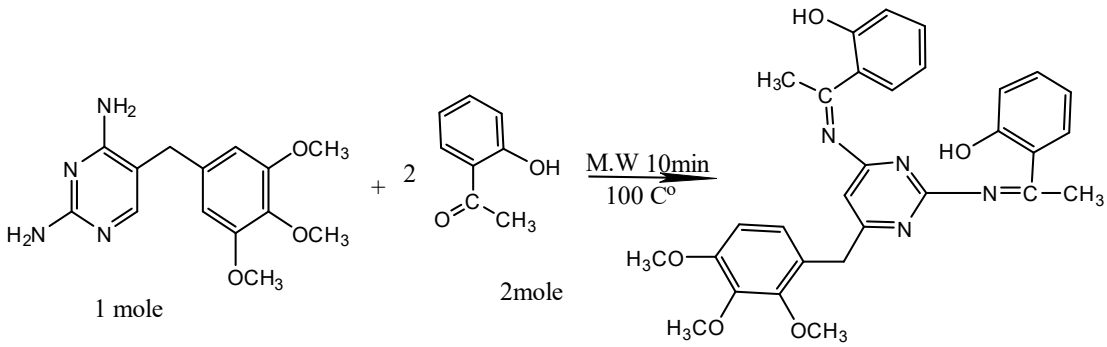
Keywords: *DNA cleavage; Microwave irradiation; Coordination compounds, Trimethoprim, Antioxidant.*

Introduction

Enforcement of sustainable and green chemistry protocols has seen colossal surge in recent times, the development of an effective[1], eco-friendly[2], simple and novel methodologies[3] towards the synthesis of valuable synthetic scaffolds and drug intermediates[4]. Recent advances in technology have now a more efficient means of heating reactions that made microwave energy[5]. Efforts to synthesize novel heterocyclic molecules of biological importanceare in continuation[6]. Microwave irradiation is well known to promote the synthesis of a variety of organic and inorganic compounds[7]. The development of the bioinorganic chemistry field has grew the attention in its complexeswere suggested for antimicrobial[8], anticancer[9], antibacterial[10], anticonvulsant[11], anti-inflammatory[12], anti fungal activities[13]. The essential metallic element and third most abundant is Copper that has antibacterial property and has a biological role in sustaining life[14]. A trace quantity of copper is required by all living organisms to

maintain their proper cellular functions[15]. We conceived a mild base mediated preparation of novel Schiff base of 2-Acetylpheno with trimethoprim drug (H_2TPBD) and its complexes with Cu(II),Co(II),Zn(II),Cd(II)andNi(II).

Synthesis of (H_2TPBD): The ratio (1:2) of ethanolic solution of (0.29g; 0.001mmol) trimethoprim drug and (0.281g; 0.002mmol) 2-Acetylphenol were mixed and irradiated in the microwave-oven insolution (3-4ml), then it was done in (1-2min) with good yield. [H_2TPBD] was isolated by crystallization after volume reduction by evaporation.



Scheme 1: Structure of (H_2TPBD) ligand

Synthesis of Complexes: The ethanolic-solution of the metal salt and ligand and were mixed in ratio of 1:1 and 0.1% ethanolic KOH was added to adjust pH(7-8) and was then irradiated insolution (4-5ml),then it was done in(3-6min). The resulting coloured products were then recrystallized with diethyl ether and ethanol.

MCl_2+H_2TPBD→[M(TPBD)(H_2O)]

Antimicrobial Evaluation: All the investigated (H_2TPBD) and its complexes were tested for their antibacterial activity (MIC) *in vitro* by broth dilution method with B.subtilis, P.aeruginosa, C.albicansand Staphylococcus aureus by disc diffusion technique taking Muller Hinton broth and byusing streptomycin as control and nutrient agar as medium.

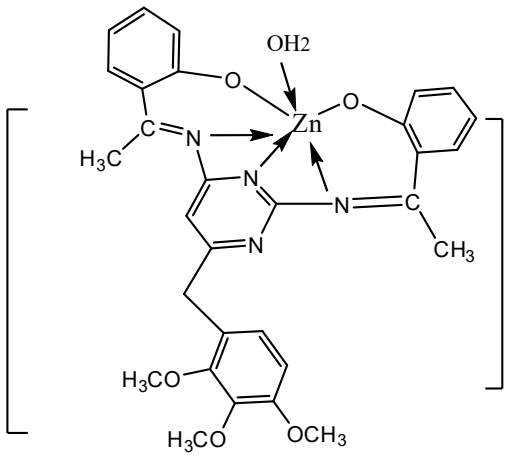
Antioxidant Studies: Antioxidant activities of compounds were investigated by using different free radicals (DPPH) assays.

The % inhibition was determined conforming to the following equation:

$$\%\ of\ radical\ scavenging\ activity = \frac{A_0 - A_1}{A_0} \quad 100$$

Where, A_1 is the absorbance of standard or sample and A_0 is the absorbance of control.

DNA Binding Studies: The experiments of the DNA binding were fulfilled in pH=7.5 (50mM Tris–HCl/1mM NaCl buffer),Tris–HCl/NaCl buffer utilizing (10%)from DMSO solution of the complexes. Experiments of absorption titration were made via employing concentrations of CT-DNA[40, 60 and 80lM]. Saving the concentration of the complexes constant, with suggested rectification for the absorbance of the CT-DNA itself. Specimens were equiponderated before recording each spectrum.



M= Co(II),Ni(II), Cu (II), Zn(II),and Cd(II)

Scheme 2: Suggested structure for complexes

Table (1): Some of physical properties and microanalysis of products

Compound	Empirical Formula	(Formula wt.)	Yield %	Colour	Elemental Analyses Found (Calc.) % (calculated)			
					C	H	N	M
[H_2TPBD]	$C_{30}H_{30}N_4O_5$	526.23	90	Pale Brown	64.24(64.69)	5.26(5.92)	13.07(13.72)	-
[Co(TPBD)(H_2O)]	$C_{30}H_{34}CoN_4O_8$	665.01	81	Olive	59.79 (58.65)	4.59 (4.39)	8.74 (9.24)	6.76 (7.78)
[Ni(TPBD)(H_2O)]	$C_{30}H_{32}N_4NiO_7$	664.77	89	Pale Green	57.40(58.67)	4.62(4.39)	8.57(9.25)	7.65(7.75)
[Cu(TPBD)(H_2O)]	$C_{30}H_{34}CuN_4O_8$	669.62	80	Greenth Brown	57.31(58.29)	3.43(4.36)	9.38(9.19)	8.53(8.34)
[Cd(TPBD)(H_2O)]	$C_{30}H_{34}CdO_5N_8$	671.46	83	Pale Yellow	58.02(58.15)	4.22(4.35)	8.11(9.16)	8.49(8.56)
[Zn(TPBD)(H_2O)]	$C_{30}H_{32}ZnO_7N_4$	718.44	81	Yellow	53.66(54.78)	4.44(4.10)	7.55(8.63)	12.83(13.86)

Characterization of Complexes: The complexes have been prepared by template process by treating [H_2TPBD]and MCl_2in ethanol. The complexes isolated in the current research along with melting point, colour, molar conductance and analytical values have been given in Table (1) [4].They are soluble in highly chelating solvents such as DMSO & DMF. An effort was taken up to crystallize the complexes in different solvent method under various experimental conditions. The melting of complexes with decomposition was in the temperature range(200–240°C).

IR Spectra of Complexes: The band at 3426 and 3365cm^{-1} was assigned to stretching vibration of O-H in [H_2TPBD]. This reside unaltered in the complexes spectra, signalizing that this group in not involved in chelation. The characteristic spectra of compounds possessed azomethine linkage also two (C=N) of trimethoprimdrug groups[9]. [H_2TPBD] hastridentated coordinate with metal ions. The IR spectrum of the ligand exhibited a peak at 1564cm^{-1} corresponded to vibration of (C=N) (trimethoprimdrug). In [H_2TPBD] spectra a new sharp band appeared at 1642 and 624cm^{-1} assigned to the azomethine linkage (C=N).Another frequency observed in the range 1200-1223cm^{-1} corresponded to C-O bond. In complexes, a new band appeared at 520–532cm^{-1} due to M–N indicating the coordination of metal ionswith nitrogen atom[10]. The appearance of a weak band at 463-497cm^{-1} assigned to weak(M–O) and confirmed the chalet ionwith oxygen atom[11].

Mass Spectral Studies: [H_2TPBD]spectrum showed the formation of a molecular ion peak at m/z = 526$[M]^+$ equivalent to its general molecular weight. In addition, to this, $[M]^+$ fragmented observed peak at m/z 333, 200, 168, 137, 134, 109, 93, 77, 76, 67, 56 and 32 equivalent to their molecular weights were due to the cleavage of $[C_{19}H_{17}N_4O_2]^+$, $[C_{11}H_{10}N_3O]^+$, $[C_9H_{12}O_3]^+$, $[C_8H_8NO]^+$, $[C_{10}H_{12}O_3]^+$, $[C_6H_5O]^+$, $[C_6H_6]^+$, $[C_5H_4]^+$, $[CH_4N_3]^+$, $[C_3H_3N_2]^+$, $[C_3H_6N]^+$ and $[CH_4O]$ groups, respectively[12].

UV-Vis Spectra and magnetic susceptibility: UV-VisSpectra of Cu(II), Co(II), Cd(II), Zn(II) and Ni(II) complexes were recorded at ca.10^{-3}M DMSO solution at room temperature. The band places of band maxima functions are listed in Table (2). [H_2TPBD] presented recognizable band at 37,037cm-^{1} would be due to π–π* for C=Ngroup and band at 28,490cm^{-1} is assigned for n–π* transition, respectively[13]. Co(II) complex showed three peaks which fall in the range 13,227-14,556cm^{-1} and23,752cm^{-1} attributed to $^4T_1g_{(F)}\rightarrow{}^4T_2g_{(F)}(\upsilon_1)$ and $^4T_1g_{(F)}\rightarrow{}^4T_1g_{(P)}(\upsilon_3)$ transitions, respectively, [14]. The ligand field parameters (Dq,B, β, β%) have also been calculated for Co(II) complex. The magnetic moment value of Co(II)complexes indicated the presence of three unpaired electrons. The magnetic moment value was found at 4.7BM, that is in the regarded region (4.3–5.2BM) for octahedral arrangement of the Ni(II) complexes[15]. The Ni(II) complex offered three absorption bands in the region 11792$cm^{-1}(\upsilon_3)$, 12345(υ_2),24509(υ_1) attributed to $^3A_2g_{(F)}\rightarrow{}^3T_1g_{(P)}(\upsilon_3)$,$^3A_2g_{(F)}\rightarrow{}^3T_2g_{(F)}(\upsilon_2)$ and $^3A_2g_{(F)}\rightarrow{}^3T_1g_{(F)}(\upsilon_1)$ transitions, respectively. The parameters (Dq,B, β, β%) were called the ligand field parameters that have been studied by using (Dq,B, β, β%). Ligand field parameters have been studied for

Ni(II) complexes.υ_2 was not noticed, but it might be studied by employing the relation $\upsilon_2=\upsilon_1+10Dq$. The data (B) equal(967$cm^{-1}$) that was further thandata of free ion, signalizing delocalization of d-electron and the orbital overlap on the ligand. The nephelauxetic ratio(β) was>1 that exposes the partial covalent nature of metal ligand bonds. The values 1174.8 and 1221cm^{-1} were (Dq) calculated data of crystal field schism energy. Thesevalues were well within the reignrecited for octahedral complexes[16]. The moment of magnetic data was found at2.86BM of Co(II)complexes.

Cu(II) complex spectrum presented absorption signals at 24271cm^{-1}, 14265cm^{-1} and 11641cm^{-1} which were referred to the transitions$^2B_1g\rightarrow{}^2A_1g$, $^2B_1g\rightarrow{}^2B_2g$ and $^2B_1g\rightarrow{}^2A_1g$ attributed to distorted octahedral structure. In addition substantiation was accomplished by magnetic moment 1.73BM which is compatible with submitted distorted octahedral structure for Cu(II) complex[17].

The electronic transition spectrum of the Zn(II) and Cd(II)complexes showed shoulder band at 421 and 409nm. This band can be attributed to the LMCT-transition. The electronic spectra of the Zn(II) and Cd(II) complexes did not show any (d→d)transition, which may be due to d^{10} electronic configuration. It has been reported that octahedral is the most favoured one.

Biological Effectiveness: In screening antibacterial efficacy of these compounds, we applied more than one screening organism to raise the prospect of exposing antibiotics essentials in examination materials[18; 19]. All of the tested compounds showed the following results: Bacteria: ligand was found to have no biological activity against all tested bacteria[20], but its complexes were found to have sensitivity for inhibition of Gram(+) more than Gram(-)bacteria. It was found in the order Co(II)>Cd(II)>Cu(II)>Zn(II)>Ni(II) for Gram-(+)and Ni(II)≈Zn(II)>Cd(II)>Co(II) for Gram-(-)bacteria, but Cu(II)complex did not show any antibacterial activity[20].

Antioxidants: These are materials or chemicals that give an electron to the free radical and transform it into harmless molecule. They may suppress radical positioning or reformdeterioration or decrease the energy of the free radical or cut-offseriesprevalence and reconstitute vellums.Free radicals ofligand mode is an antioxidant screening constructed on electrontransferthat manufactures a violet solution in ethanol[21;22] at room temperature is decreased in the existence of an antioxidant molecule, granting high to colourless solution of ethanol. The usage of the ligandchecksupplies arapid and easy path to estimate antioxidants by spectrophotometer and it may be beneficial to estimate different produces at a time. The proportion of antioxidant efficacy of each material was estimated by ligand free radical check[23; 24]. The determent of the ligand radical scavenging efficacy was completed depending to methodology recognized by radical scavenging efficacy of Brand-Williams and was evidenced as proportion repression of ligand radical and was determined by the following equation (Table 2).

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

Table 2: DPPH efficacy of compounds

Last Conc. of Complex × 10 M^{-5}	% Inhibition				
	[Co(TMAP)(H_2O)]	[Ni(TMAP)(H_2O)]	[Cu(TMAP)(H_2O)]	[Zn(TMAP)(H_2O)]	[Cd(TMAP)(H_2O)]
2.9411	45.94	40.43	35.43	33.24	30.64
6.0606	59.68	59.68	40.26	38.83	36.85
9.9032	64.05	64.05	45.68	42.98	40.83
12.6666	67.5	67.5	56.35	51.65	46.56
16.0606	72.98	72.98	61.08	57.54	50.54

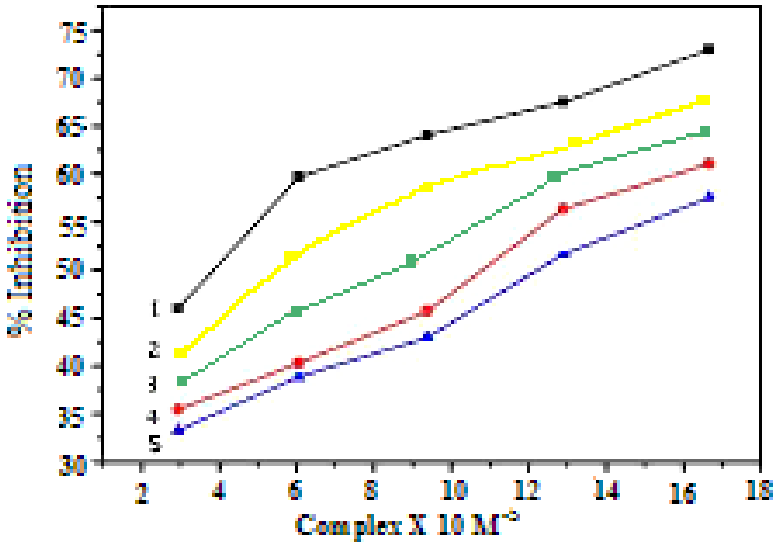


Figure 1: DPPH Activity.

DNA binding studies: UV-Vis spectroscopy avails as the generality prevalent means to research the interactions between DNA-complexes. Bind complex-DNA through intercalation conducts in bathochromism and hypochromism, assign to intercalation method including a powerful accumulating interaction between the DNA, by pairs of base, and an aromatic chromophore[24]**.** The bands of MLCT-transition for complexes displayed bathochromism and hypochromism when concentration of DNA was increased that were related to bind between CT-DNA and the complexes by intercalation. Absorption of complexes in existence of DNA was displayed in Figure (2). The binding strength of the complexes, K_b constant of the intrinsic binding of DNA with the complexes, was calculated from the deterioration of the absorbance observed for complexes**.** K_b for CTDNA with the complexes was estimated from the Equation (1). K_b constants of Intrinsic binding of complexes gained were 1.41×10^{-4}, 1.38×10^{-4}, 1.37×10^{-4}, 1.35×10^{-4} and $1.32\times10^{-4}M^{-1}$. The constants of stability of metal complexes including Schiff base ligand were in the range 10^7–10^6M^1. For example, for Co(II), Cu(II), Ni(II), Cd(II) and Zn (II) were 1.17×10^{-5}, 1.7×10^{-4}, 2.35×10^{-5}, 3.12×10^{-4}, 2.675×10^{-5} and $2.98\times10^{-4}M^{-1}$. These data were lower than (EB) as binding constants were in the range 10^6–10^7 M^1[25].

Viscosity Calculations: For stabilization of the interactions DNA-complexes, viscosity calculations were completed. Physical probes of optical photo supply in dispensable, but not adequate proofs to back up a linking model**.** The calculations of hydrodynamic, which are critical to change of length, were considered like the mostcritical and least ambiguous screen of a linking insolation, in the non-attendance of crystallographic constitutional values. A conventional intercalation pattern demands that the helix of DNA lengthens like base pairs are isolated to harmonize the linking ligand, that due to the increase of DNA viscosity. EB, a recognized DNA intercalator, raises the relative viscosity greatly by lengthening the double helix of DNA through intercalation. Upon increasing the complexes concentrations, the relative viscosity of complexes rises comparable to the conduct of EB. The increased viscosity[28] follows the arrangement EB>1>2>3>4>5 (Figure 2).

Melting of DNA studies: The complexes intercalation into base pairs of DNA brings about stabilization of base accumulating in a way that promotes T for DNA. The DNA melting experience is beneficial in founding the range of intercalation[26]. The complexes were brooded with CT-DNA and their temperature (10-100°C) and the absorbance at 260nm was observed. Conductivity- and pH-determents were then executed previously and next warming the complexes(Figure 3).

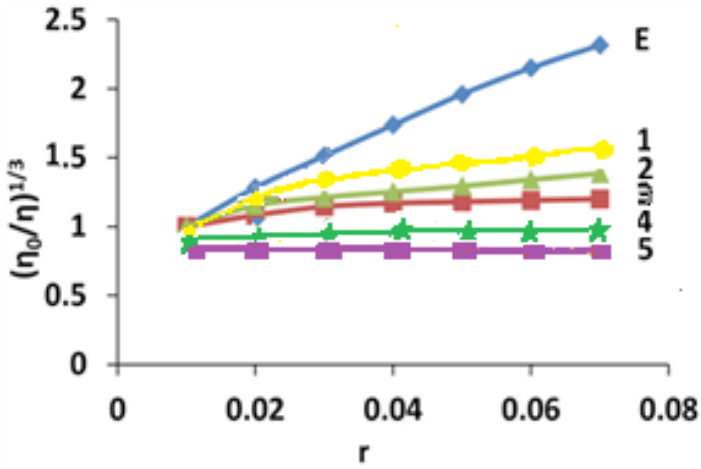


Figure 2: Effects of increasing amount of (EB). Complex(1): Co(II), complex(2): Ni(II), complex(3): Cu(II), complex(4): Zn(II)and complex(5): Cd(II)on ή of CTDNA at 29°C±0.1, [DNA]=15lM.

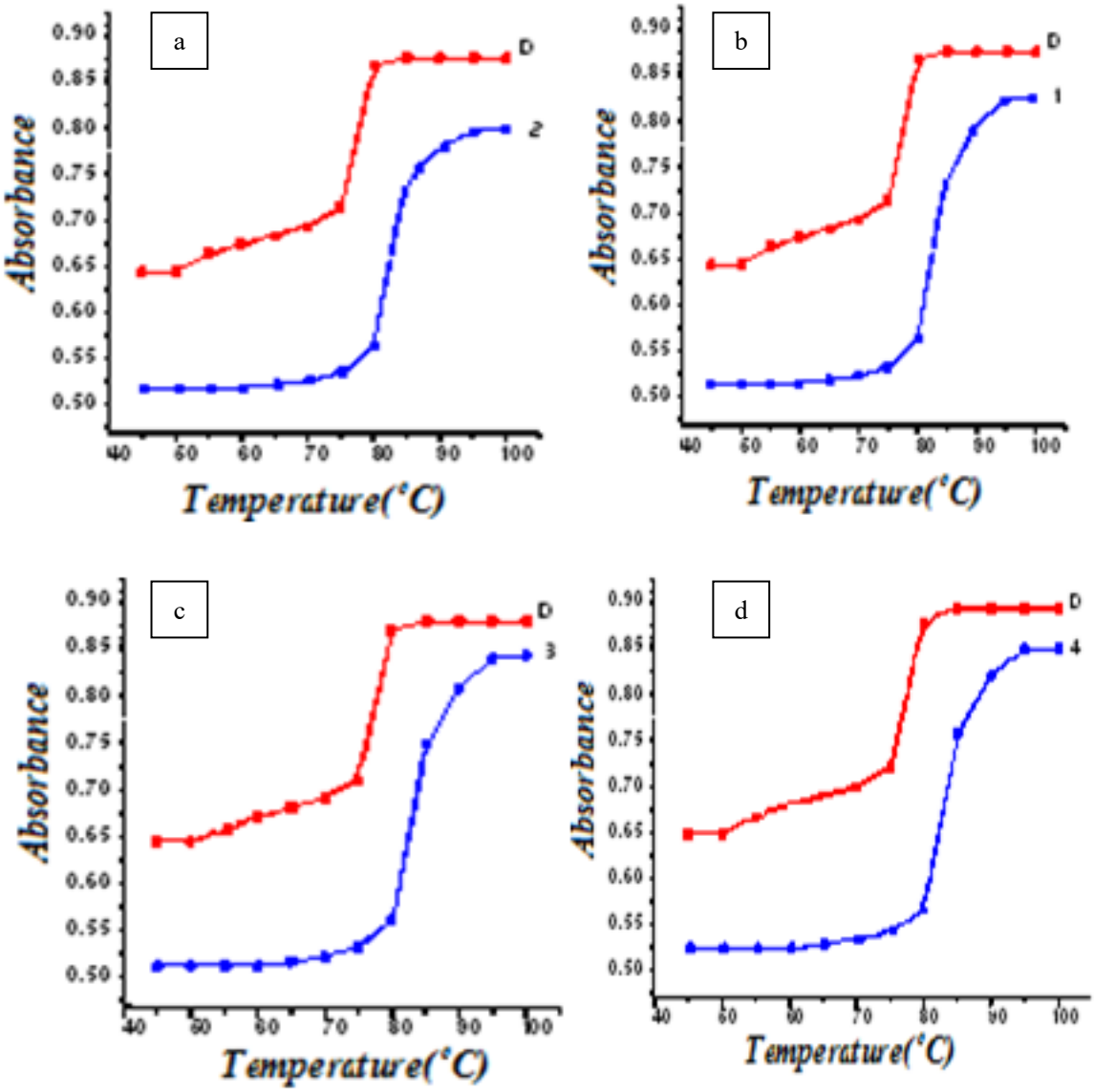


Figure 3 Plots of Abs versus T (C) for the melting of CT-DNA: (only DNA), DNA+ Co complex(a), DNA+ Nicomplex(b), DNA+ Cucomplex(C), DNA+ Zn complex (d) DNA+Cdcomplex(f).

Ethical Clearance: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding.

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