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Microwave synthesis and Antibacterial Activity of some Heterocyclic Schiff's base Metal Complexes of First Transition Series.

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ABSTRACT

A series of some Schiff base complexes of Cu(II), Ni(II), Co(II), Mn(II), Zn(II) with a tetra-dentate Schiff's base Diethyl 5-amino 4-methyl thiophene 3,5-dicarboxylate-2-acetyl 5-chloro thiophene(DAMTDACT) has been synthesized under microwave conditions. The process is economical, easy, environment friendly and provides better yields of metal complexes in a shorter period of time. All the synthesized metal complexes and ligand were characterized on the basis of elemental analysis, ^1H and ^{13}C NMR, IR and mass spectral data and study of antibacterial activity.

Keywords: Microwave synthesis, green approach, antibacterial activity, tetra-dentate Schiff's base.

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INTRODUCTION

Schiff bases and their bio-active complexes have been studied extensively over the past decade. Schiff bases provide potential sites for bio-chemically active compounds. Various transition metal complexes with tetradentate Schiff bases containing nitrogen and sulphur donor atoms play an important role in biological systems.

The antituberculous activity was ascribed to their ability to form more or less stable chelates with the transition metal ions [1-3]. Many drugs inhibit modified toxicological and pharmacological properties when they are in the form of metal complexes. The most widely studied metal in this respect is copper (II) which has proved to be beneficial in diseases such as tuberculosis, gastric ulcers, rheumatoid arthritis and cancers. Metal chelation therapy can emerge to answer the problems of multidrug resistance (MDR) as against various viruses, bacteria, fungi and other pathogens [4-7].

Microwave-assisted synthesis is a branch of green chemistry. The application of microwave-assisted synthesis in organic, organometallics and coordination chemistry continues to develop at an astonishing pace. Microwave irradiated reactions under solvent free or less solvent conditions are attractive offering reduced pollution, low cost and offer high yields together with simplicity in processing and handling. The salient features of microwave approach are shorter reaction times, simple reaction conditions and enhancements in yields [8-12]. Reports on the synthesis of metal complexes by microwave methods have been comparatively less. The present investigation aims at the conventional and microwave synthesis, characterization and bio-inorganic studies of Schiff bases involving Diethyl 5-amino 4-methyl thiophene 3,5-dicarboxylate-2-acetyl 5-chloro thiophene(DAMTDACT) and their metal chelates with Cu(II), Ni(II), Co(II), Mn (II), Zn(II).

Metal complexes of Schiff bases play a central role in the development of coordination chemistry. Keeping the above facts in the mind and in continuation of our research work on transition metal (II) complexes with Schiff bases, we report the synthesis and characterization of Co (II), Cu (II), Ni (II), Mn(II) and Zn (II) metal complexes of Schiff bases derived from the condensation of 2-acetyl-5-chloro thiophene and Diethyl 5-amino 4-methyl thiophene 3,5-dicarboxylate. Antifungal and antibacterial activity of Schiff base and its metal complexes have also been explored against different species of bacteria and Fungi. Thus the aim of this study is to observe the impact of chelation on the therapeutic value of the organic compounds.

It is well known that various organic ligands possess strong antibacterial, herbicidal, insecticidal and fungicidal properties [13]. It has also been reported that the activity of bio metals is very often altered through the formation of chelates with different biological relevant ligands [14-17]. It is suggested that the compounds having antimicrobial activity may act either by killing the microbe or blocking their active sites [18-20]. In addition to this the antimicrobial activity of the compounds also depends upon the nature of the microorganisms.

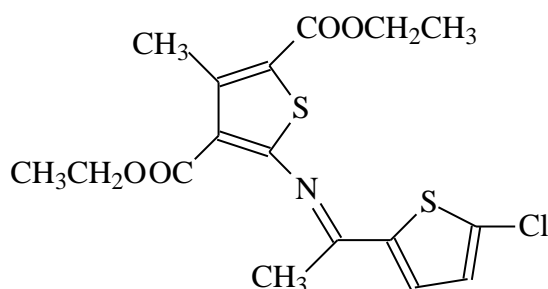


Figure 1: Structure of Ligand – (DAMTDACT)

MATERIALS AND METHODS

All the chemicals used were of AR grade and used without further purification. The infrared spectra were recorded in the range 4000-180 cm^{-1} with a Perkin Elmer 983 G spectrophotometer. The electronic spectra were recorded with Cary model 2390 spectrometer. The molar conductance of complexes in DMF ($\sim 10^{-3}$ M) was determined at 27 ± 20 C using a Systronic 303 direct reading conductivity bridge. The magnetic

susceptibility measurements were made using a vibrating sample magnetometer (VSM) operating at field strength of 5 KG. The ^1H NMR spectra was recorded on varian XL-300 MHz high resolution instrument in CDCl_3 solvent. The mass spectra were recorded using Fanning Mat 8230 Mass spectrometer.

Microwave assisted synthesis were carried out in open glass vessel on a modified microwave oven model 2001 ETB with rotating tray and a power source 230V, microwave energy output 800W and microwave frequency 2450MHz. A thermocouple device was used to monitor the temperature inside the vessel of the microwave. The microwave reactions were performed using on/off cycling to control the temperature.

Conventional method for the synthesis of Schiff bases

The reaction mixture containing 2-acetyl-5-chlorothiophene(3g,0.0184 mol in 20ml of methanol) Diethyl 5-amino 4-methyl thiophene 3,5-dicarboxylate (4.74g, 0.0184mol in 20ml of methanol dissolved in hot condition) was taken in 250-ml round bottom flask and refluxed for 10h. On cooling the reaction mixture, On cooling the reaction mixture, light yellow colored product was formed. It was collected by filtration and washed with hot water and 50 % cold methanol. This compound was recrystallized from ethanol and dried in vacuo, yield 65% ; m. p. 257°C.(figure-2)

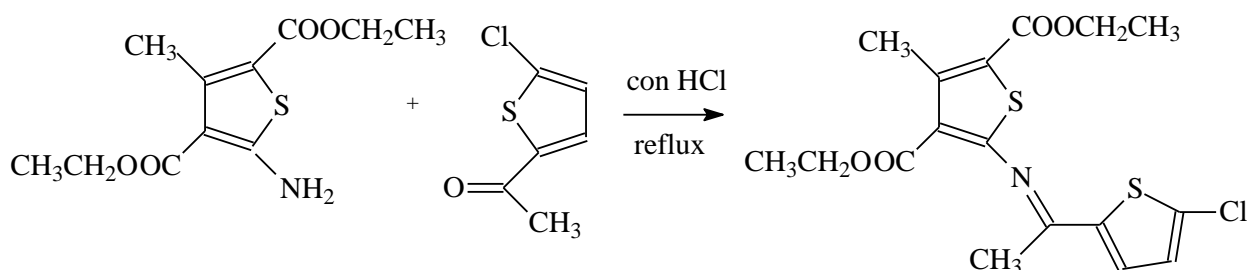


Figure 2: Synthetic route for the preparation of ligand- DAMTDACT

Microwave method for the synthesis of Schiff bases

The equimolar (1:1) ratio of methyl isobutyl ketone with 2-acetyl-5-chlorothiophene, and Diethyl 5-amino 4-methyl thiophene 3,5-dicarboxylate with isonicotinic acid hydrazide were mixed thoroughly in a grinder. The reaction mixture was then irradiated by the microwave oven by taking 3-4mL of dry ethanol as a solvent. The reaction was completed in a short time (4-5min) with higher (light yellow) yields. The resulting product was then recrystallized with ethanol, finally dried under reduced pressure over anhydrous CaCl_2 in a desiccator. The progress of the reaction, purity of the product was monitored by TLC using silica gel G (yield: 85%).

Conventional method for the synthesis of metal complexes

The metal complexes (Figs. 3) was prepared by the mixing of equal moles of metal salts dissolved in the methanol was added followed by 1 ml of 1M NaOAc was added, in 1:1 (metal: ligand) ratio. The resulting mixture was refluxed on water bath for 8- 10 h. A coloured product appeared on standing and cooling the above solution. The precipitated complex was, filtered washed with ether and recrystallized with ethanol several times and dried under the reduced pressure over anhydrous CaCl_2 in a desiccator. It was further dried in electric oven at 50-70°C (yield: 65-70%).

Microwave method for the synthesis of metal complexes

The ligand and the metal salts was mixed in 1:1 (metal: ligand) ratio in a grinder. The reaction mixture was then irradiated by the microwave oven by taking 3-4mL of dry ethanol as a solvent. The reaction was completed in a short time (5-9min) with higher yields. The resulting product was then recrystallized with ethanol and ether and finally dried under reduced pressure over anhydrous CaCl_2 in a desiccator. The progress of the reaction and purity of the product was monitored by TLC using silica gel G (yield: 80-85%).

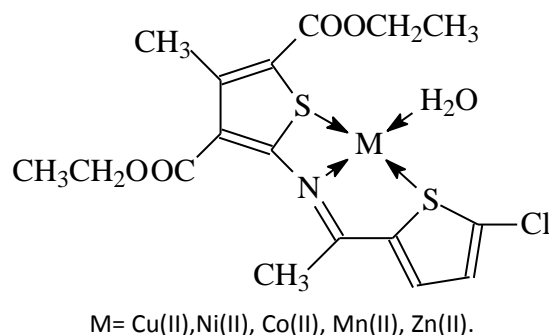


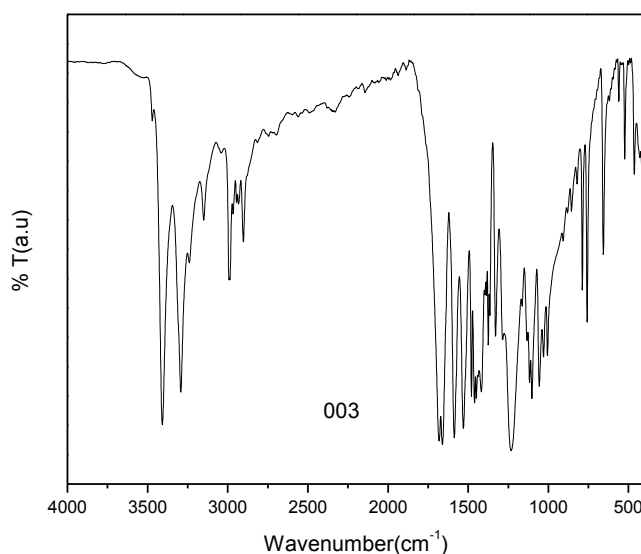
Figure 3: Proposed metal-ligand complexes

RESULTS AND DISCUSSION

The analytical data for all the complexes are given in Table- 1. The molar conductivity data of the complexes are consistent with the non-electrolytic nature [21,22] of the complexes. The ligand and complexes were characterized by elemental analysis to determine percentage of C, N, S and H. The observed and calculated percentages of the elements are in good agreement and support one ligand to a metal ion. The number of coordinated ligands to metal determined by Job's continuous method and Mole ratio method established 1:1 metal to ligand ratio.

IR and ^1H NMR Spectral Analysis

The reagents have been characterized by IR and ^1H NMR spectral data. The infrared spectra of DAMTDACT show bands at 1697 cm^{-1} for VC=N; 722 for VC=S; 1540 for VC=S; indicating the Schiff base formation. The lowering of VC=N of azomethine group to the extent of $30\text{-}50\text{ cm}^{-1}$ in all the complexes suggests the participation [23-25] of azomethine nitrogen in complexation. On coordination, this band is shifted to lower frequency suggests that the ligand is coordinated to metal ion via azomethine nitrogen in all complexes. This change in shift is due to the drift of the lone pair density of azomethine nitrogen towards metal atom [26]. In the far IR spectral region, additional medium to strong bands at $405\text{-}420$ and $325\text{-}355\text{ cm}^{-1}$ are assigned to VM-N and VM-S modes [27,28] respectively.



The magnetic moment (Table-4) value of Cu- DAMTDACT was 2.24 BM indicates one electron paramagnetism. This value is higher than the spin-only value of 1.73 BM for one unpaired electron. The higher value of the magnetic moment indicates that complexes are monomeric in nature and there is no metal-metal interaction along the axial position in the complex and have distorted octahedral environment [29-31]. The

magnetic moment of Co- DAMTDACT was found to lie in 2.05 BM. Monomeric cobalt complexes have lower magnetic moment values than would be expected for pure octahedral complexes suggesting flattening towards planar arrangement [32-36]. The magnetic moments of Ni (II) complex was observed at 3.73 BM. This value is in the range reported earlier for octahedral complexes [37] Zn(II) complex was observed at 2.65 BM.

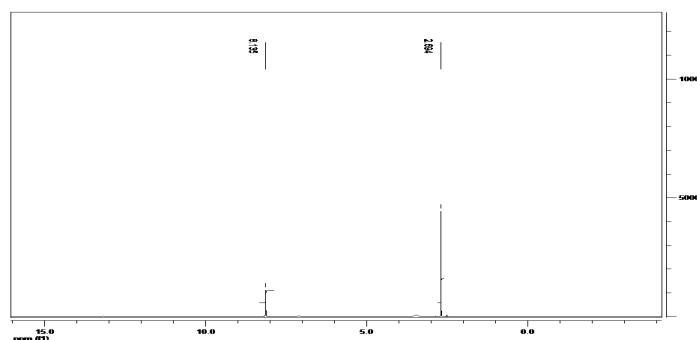
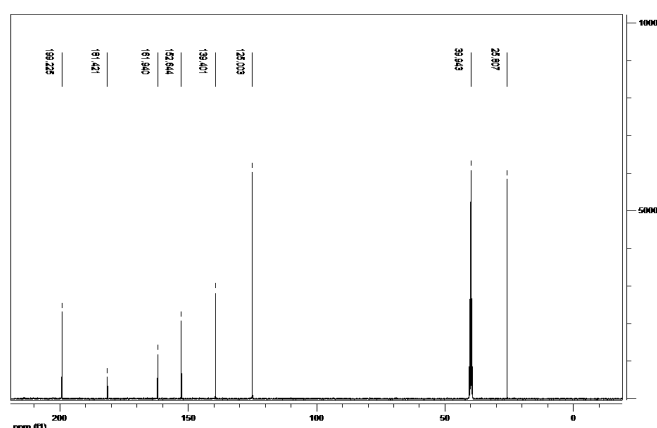


Figure 3: ^1H NMR Spectra of Ligand (DAMTDACT)



^{13}C - NMR spectroscopic analysis of the Schiff base (DAMTDACT)

^{13}C NMR showed that carbon (RCH_3) (a) of this compound appeared at 25.807 ppm, (R_3CH) (e) at 39.943 ppm, ($\text{N}=\text{C}$) azomethine (imines) group at 152.644 ppm, ($\text{N}=\text{C}-\text{R}$) at 139.401 ppm

The ^1H - NMR spectra of the Schiff bases were recorded in DMSO (Fig 3). For CH aromatic proton, the ligand shows singlet in the region 8.135 ppm. The ^1H -NMR signal at $\delta=2.694$ ppm sharp and singlet peak is due to $-\text{CH}_3$ proton [38, 39].

Table 1: The comparative results of conventional and microwave methods-Analytical Data of DAMTDACT and their metal complex

Compound / complex (colour)	M.Pt $^{\circ}\text{C}$	Reaction period		Yield %		Mol. Wt.	Elemental Analysis Found (calculated)						
		CMM(h)	MM (min)	CM	MM		C %	H%	N%	O%	S%	Cl%	M%
DAMTDACT (light-yellow colour)	82	10	5	68	84	401	50 (50.1)	4.9 (4.10)	3.4 (3.6)	15 (15.3)	15 (15.2)	8.7 (8.7)	-
DAMTDACT -Co(Light brown colour)	215	8	9	70	85	460	44.3 (44.4)	4.3 (4.3)	3.04 (3.05)	13.91 (13.92)	13.91 (13.9)	7.6 (7.6)	12.82 (12.84)
DAMTDACT -Cu(black colour)	262	10	8	55	84	464.5	43.9 (43.9)	4.3 (4.3)	3.02 (3.03)	13.7 (13.8)	13.7 (13.7)	7.5 (7.5)	13.7 (13.8)
DAMTDACT -Ni (light green colour)	280	8	5	61	85	459.7	44.3 (44.3)	4.35 (5.02)	3.04 (3.05)	13.9 (9.01)	13.9 (13.9)	7.61 (7.63)	12.7 (12.7)
DAMTDACT -Mn (yellowish green colour)	272	10	6	64	82	456	44.7 (44.6)	4.3 (4.3)	3.07 (3.09)	14.03 (14.04)	14.03 (14.04)	7.6 (7.6)	12.06 (12.09)
DAMTDACT -Zn (yellowish green colour)	300	8	6	64	82	466.38	43.74 (43.75)	4.28 (4.28)	3.0 (3.02)	13.7 (13.7)	13.7 (13.7)	7.5 (7.6)	14.01 (14.02)

Table 2: Selected IR bands (cm⁻¹) with tentative assignments

Compound	VC=N	VC-S	VC=S	VM-N	VM-S
DAMTDACT	1697	722	1540	-	-
Cu- DAMTDACT	1615	650	1560	420	355
Co- DAMTDACT	1608	708	1550	415	352
Ni- DAMTDACT	1610	707	1540	412	340
Mn- DAMTDACT	1623	712	1545	405	325
Zn- DAMTDACT	1620	720	1542	410	335

Table 3: Molar conductance data of metal complexes of DAMTDACT

DAMTDACT – Complex	Conductance(Ohm ⁻¹ Cm ² mol ⁻¹)
Cu- DAMTDACT	26
Co- DAMTDACT	24
Ni- DAMTDACT	22
Mn- DAMTDACT	36
Zn-DAMTDACT	25

Table 4: Magnetic moment data of metal complexes of DAMTDACT

DAMTDACT - Complex	Magnetic Momentum(B.M)
Cu- DAMTDACT	2.24
Co- DAMTDACT	2.05
Ni- DAMTDACT	3.73
Mn- DAMTDACT	2.12
Zn-DAMTDACT	2.65

Antimicrobial activities

The *in-vitro* Antimicrobial activity of the synthesized Schiff base ligands and their corresponding metal complexes on selected bacteria *E. coli* and *S. aureus* and two fungi *A. niger* and *C. albicans* was carried out. All of the tested compounds showed good biological activity against microorganism. On comparing the biological activity of the Schiff base and its metal complexes with the standard bactericide and fungicide, it is shown that some metal complexes have good activity as compared to the standard but all the complexes are more active than their respective ligands. The higher inhibition zone of metal complexes than those of the ligands can be explained on the basis of Overtone's concept and Chelation theory. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalization of π -electrons over the whole chelating ring and enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms. There are other factors which also increase the activity are solubility, conductivity and bond length between the metal and ligand⁴⁰⁻⁴³. The bactericidal and fungicidal investigation data of the compounds are summarized in Tables 5 and 6. The results of the investigations account for the anti-pathogenic behavior of the compounds and this efficacy is positively modified on complexation.

$$\% \text{ Activity index} = \frac{\text{zone of inhibition by test compound (diameter)}}{\text{zone of inhibition by standard (diameter)}} + 100$$

Table 5: Antibacterial screening data for the ligands and their complexes

Compound	<i>E. coli</i>						<i>S. aureus</i>					
	Diameter of inhibition zone(mm)			% Activity index			Diameter of inhibition zone(mm)			% Activity index		
	25	50	100	25	50	100	25	50	100	25	50	100
DAMTDACT	10	15	18	45	60	62	12	16	19	63	64	76
Cu-DAMTDACT	13	16	20	59	64	68	13	15	20	68	71	80
Co- DAMTDACT	14	17	21	63	68	72	12	14	18	63	66	72
Ni- DAMTDACT	16	19	23	72	76	79	11	15	18	57	71	72
Mn-DAMTDACT	17	20	24	77	80	82	10	16	20	52	76	80
Zn- DAMTDACT												
Streptomycin	22	25	29	100	100	100	19	21	25	100	100	100

(Standard)												
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Table 6: Antifungal screening data for the ligands and their complexes

Compound	Diameter of inhibition zone (mm); Concentration in ppm					
	<i>A. nizer</i>			<i>C. albicans</i>		
	25	50	100	25	50	100
DAMTDACT	12	15	21	13	16	20
Cu- DAMTDACT	14	18	23	14	19	24
Co- DAMTDACT	15	20	24	15	18	22
Ni- DAMTDACT	16	19	23	14	17	21
Mn-DAMTDACT	15	21	25	18	20	25
Zn-DAMTDACT						
Miconazole (Standard)	22	25	32	24	26	30

CONCLUSION

In the present research studies, our successful efforts are synthesis of some newly compounds from the conventional as well as microwave methods. These synthesized compounds have been characterized by various physicochemical, VSM and spectral analyses. In the result of microwave-assisted synthesis, it has been observed that the reaction time decreased from hours to minutes and availability of the product within better yields compared to the classical method. Electrical conductivity data suggest that all the complexes fall in the semiconducting range. The antimicrobial data show that the metal complexes to be more biological active compared to those parent Schiff base ligand against all pathogenic species. The compounds also inhibit the growth of fungi and bacteria to a greater extent as the concentration is increased. The Schiff base ligands were found to be biologically active and their metal complexes displayed enhanced antimicrobial activity against one or two strains. Chelation tends to make the ligand act as more powerful and potent bactericidal agent. Further chelation can help in MDR problems.

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