International Journal of Chemistry Studies
www.chemistryjournal.in
ISSN: 2581-348X
Received: 19-05-2023, Accepted:04-06-2023, Published: 19-06-2023
Volume 7, Issue 1, 2023, Page No. 54-59

Biological activities and synthesis of metal-based heterocyclic compounds

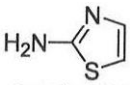
Shalini Sharma, D S Seth
Department of Chemistry, St. John's College, Agra, Uttar Pradesh, India

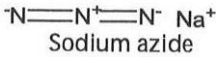
Abstract
The metal complexes of Cr(III) and Mn(II) with 2-aminothiazole (ATZ) and azide ion ligands have been prepared by reacting chromium and manganese nitrates with ligand 1:1 molar ratio for complex formation. The prepared complexes have been characterized by using elemental analysis (C, H, N, M), molar conductance, IR and UV-Vis spectral analysis. Also, their CV and TGA behaviors have been studied. All the complexes have an octahedral structure. The ligands coordinated to the metal ions through the nitrogen atoms of amino group of 2-aminothiazole and azide ion. The antimicrobial activities of the ligands and their metal complexes have been studied by using different bacterial species *viz., Escherichia coli, Lactobacillus brevis, Micrococcus luteus,* etc., and fungal species included *viz., C.albicans, Aspergillus niger* etc., Moreover, the prepared complexes have been evaluated for antioxidant activities and DNA binding properties. The complexes have larger antioxidant activity as compared to free ligand. The DNA-binding properties have been designed by fluorescence-emissions method. The obtained results advise that the complexes effectively bind to DNA because of metal complexes are well-known to speed up the drug action and the capability of healing agent which can repeatedly be enhanced leading coordination with a metal ion.

Keywords: metal complex, Cr(III), Mn(II), 2-aminothiazole, azide ion, biological activities

Introduction
Heterocyclic compounds containing sulphur and/or nitrogen atoms are of extensive use as building blocks in chemistry, wherever they are acknowledged as biologically active compounds with a wide range of action and textile dyes as well [1,3]. As a distinctive heterocyclic amine, 2-aminothiazole is the starting point for the synthesis of numerous compounds, together with sulfur drugs, biocides, fungicides dyes and chemical reaction accelerators and as intermediates in the synthesis of antibiotics, somewhere a huge number of 2-aminothiazoles have been substituted with different groups for pharmaceutical purposes [4,5] and are also second-hand in the syntheses of various types of dyes for synthetic fibers [6-10] next to their activity as corrosion inhibitors for mild steel protection[11] corrosion inhibitors for copper [12] and as an ionophore in the structure of a lutetium(III)-selective membrane sensor[13]. These derivatives keep on attracting the awareness of biologists because of their prevalent use in the behavior of the biological systems. Further more, thiazole is classify under five-membered heterocyclic class of compounds and is establish in a lot of natural and synthetic agents. Obviously, thiazole is accessible in a large number of terrestrial and marine compounds with different pharmacological activities. Thiazole is also present in the vitamin B1 (Thiamine). In synthetic substituted thiazole derivatives, 2-aminothiazoles [14] have shown a variety of biological behavior such as anti-HIV, antitubercular, antifungal, antibacterial, anti-inflammatory, anticonvulsant, antidiabetic, antihypertensive, antiprotozoal, anticancer, dopaminergic, plasminogen activator inhibitor-1, neuro protective and antioxidant. This broad spectrum of activities makes 2-aminothiazole as an beautiful moiety in therapeutic chemistry.[15] Literature search reveals that no work has been done on the mixed ligand complexes 2-aminothiazole and azide ion. In this research paper aims at the synthesis, characterization, thermal stability, antimicrobial, antioxidant and DNA- binding properties of Cr(III) and Mn(II) complexes containing 2-aminothiazole and azide ion as ligands.

Materials and methods
2-aminothiazole, NaN_3, $Cr(NO_3)_3.9H_2O$ and $Mn(NO_3)_4.3H_2O$ were purchased from Alfa Aaser Company and used as such. The organic solvents used were Di methylsulphoxide DMSO, $(CH_3)_2NC(O)H$, MeOH, EtOH which were of AR grade and used as such without additional purification.


2-aminothiazole


Sodium azide

Synthesis of Metal Complexes
Synthesis of Cr(III) complex
0.75 g (7.5 mmol) of ATZ in EtOH and 0.49 g (7.53 mmol) of sodium azide in EtOH were added to the $Cr(NO_3)_3.9H_2O$.00 g (2.5 mmol) in MeOH followed by microwave irradiation for few seconds after each addition by using IFB 25 BG-1S model microwave oven. The consequential precipitate was filtered off, washed with 1:1 ethanol: water mixture and desiccated under vacuum. The obtained complex is with 82.05% yield and green colored.

Synthesis of Mn(II) complex
The $Mn(NO_3)_4.3H_2O$ 1.00 g (3.64 mmol) in MeOH, 1.6 g (16 mmol) of ATZ in EtOH were added to 0.47 g (7.23 mmol) of sodium azide in EtOH the followed by microwave irradiation for few seconds after each addition by using IFB 25 BG-1S model microwave oven. The resulting impulsive was filtered off, washed with 1:1 ethanol: water mixture and dried under vacuum. The obtained complex is 80.50% yield and pale brown colored.

Instrumentations

C,H,N,S elemental analyses were performed using Thermo Finnegan make, Flash EA1112 Series CHNS(O) analyzer. The electrical conductivity measurements were conducted using 10-3M solutions of the metal complex in acetonitrile with Systronic Conductivity Bridge (model number-304) at 30°C. The electronic spectra (DRS method) of Cr(III) and Mn(II) complexes were recorded on Varian, Cary 5000 model UV Spectrophotometer. Infra red spectra for the complexes and the free ligands were recorded on a Perkin Elmer, Spectrum RX-I, FT-IR spectrometer in KBr discs at room temperature. The cyclic voltammograms of the complexes were taken in acetonitrile medium using Princeton make (MC-Tech, Applied Research) equipment. Tetrabutyl ammonium tetrafluoroborates was used as the supporting electrolyte. The thermogravimetric analyses of the complexes were carried out using Perkin Elmer Diamond TGA/DTA Instrument.

Biological Activities

Antibacterial activity

The Cr(III) and Mn(II) complexes and the free ligands were tested for in *vitro* antibacterial and antifungal activity by well diffusion method using agar nutrient as medium. The bacterial activities of the free ligands and their complexes were evaluated against the strains cultured on potato dextrose agar as medium. The stock solution (10-2M) was prepared by dissolving the compounds in Dimethylsulphoxide (DMSO) and the solutions were successively diluted with different concentration. According to the typical method a well was made on the agar medium inoculated with the microorganisms. The well was filled with the test solution using a micropipette and the plate was incubated for 24 hours for bacteria and 72 hours for fungi at 35^0C. At the end of the period, inhibition zones formed on the medium were evaluated in millimeter (mm) diameter [16].

Antioxidant activity

In the assessment of antioxidant activity, the stock solution was diluted to final concentrations of 10–500 µg/ml. Ethanolic and DPPH solutions (EtOH 1 ml, DPPH 0.3 mmol) were added to sample solutions in (DMSO) (3 ml) at different concentrations (10–500 µg/ml). The mixture was shaken vigorously and adequate to stand at room temperature for 30 min. The absorbance was then measured at 517 nm in a ultra-violet spectrophotometer. The lower absorbance of the reaction mixture indicates superior free radical scavenging activity. EtOH was used as the solvent and ascorbic acid as the standard. The DPPH radical scavenging activity was designed by the following equation: DPPH Scavenging effect (%) = Ao – A1/ Ao× 100 where A1 is the absorbance in the presence of the samples or standards and Ao is the absorbance of the control reaction [17].

DNA binding properties

The DNA binding experiments involving interaction of the complexes with CT-DNA were conducted in Tris buffer containing HCl (0.01 M) adjusted to pH 7.2 with HCl acid. The CT-DNA was dissolved in Tris-HCl buffer and was dialyzed against the same buffer overnight. Solutions of CT-DNA gave the ratios of UV absorbance at 260 and 280 nm above 1.8, representing that the DNA was adequately free of protein. DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient 6600 dm3mol-1cm-1 at 260 nm. The stock solutions were stored at 4°C and used within 4 days[18]. For fluorescence-quenching experiments, DNA was pretreated EtBr for 30 minutes. The ligand/ complexes were then added to this mixture and their effect on the emission intensity was measured. Samples were excited at 450 nm and emission was observed between 500 nm and 800 nm.

Results and discussion

Elemental analysis and metal estimation

From the C,H,N analysis and the metal estimation, the empirical formulae for the prepared complexes were determined. It is well consonance with the hypothetical values. The analytical data are given in the Table-1. The experimental values are in good agreement with the theoretical values.

Molar conductance

Molar conductance measurements of the $[Cr(N_3)_3(ATZ)_3]$ and $[Mn(N_3)_2(ATZ)_4]$ complexes carried out using acetonitrile as the solvent at the concentration of 10-3 M, indicates non-electrolytic nature (1:0 type) [17] and the conductivity values were found to be 67.52-78.45 Ω-1cm^2 mol^{-1}. Thus the prepared complexes are non-electrolytic nature and there is no counter ion present in the out of the coordination sphere.

Magnetic moment and electronic spectra

In the diffused reflectance spectrum of $[Cr(N_3)_3(ATZ)_3]$ complex exhibits three absorption bands at 17623 cm-1, 23455 cm-1 and 28756 cm-1, which are assignable to the transitions $4A_2g \rightarrow 4T_2g$ ($\nu 1$), $4A_2g \rightarrow 4T_2g(F)$ (ν_2) and $4A_2g \rightarrow 4T_1g(P)$ (ν_3) respectively indicates octahedral geometry around the Cr(III) metal ion [19]. The magnetic moment at room temperature is 3.35 B.M which corresponds to the expected value for octahedral geometry of Cr(III) complex and d2sp3 hybridisation. The electronic spectrum of $[Mn_{(N3)}{}_2(ATZ)_4]$ complex displays the weak absorbtion bands at 16712, 18345, 25947 and 36455 cm^{-1} which are characteristic of octahedral geometry around Mn(II) complex and corresponding to the transitions, $6A_1g \rightarrow 4T_1g(G)$ (ν_1), $6A_1g \rightarrow 4Eg(D)$ (ν_2), $6A_1g \rightarrow 4T_1g(P)$ (ν_3) and $6A_1g \rightarrow 4Eg(G)$ (ν_4) respectively. These bands are both Laporte and spin-forbidden. However, due to instantaneous distortion of the octahedral structure around the metal cation, weak bands sometimes appear. The magnetic moment of the Mn(II) complex is 4.45 B.M indicates octahedral structure around the metal ion. The electronic spectra of Cr(III) and Mn(II) complexes shown in Figure-1.

FT-IR spectra

In order to determine the stretching mode of free ligands to the metal complex, the stretching frequencies of free ligands are compared with the metal complexes. The ligand 2-aminothiazole exhibited a strong band at 3332 cm-1 which could be assigned as $\nu(NH_2)$. The azide ion shows 645 cm-1 $\nu(N_3\text{-})$ frequency [20].The FT-IR spectra of the complexes were compared to those ligands 2-aminothiazole and the sodium azide. The frequency of the $\nu(NH_2)$ group was observed near 3320 cm-1 in complexes and they were shifted lower than those of resultant free ligand 2-aminothiazole. This shift refer to the coordination through a nitrogen atom of 2-aminothiazole and it is in good

agreement with earlier examples of reported complexes. After that, the (N_3-) group of the azide ion underwent higher frequency at after complexation, indicating the coordination of azide nitrogen to the metal atom. The low frequency region of the spectra revealed the presence of medium intensity bands in the region of 600-300 cm^{-1} due to ν(M-N) bond formation under investigation [21].

Cyclic voltammetry

Cr(III) Complex

The Cr(III) complex exhibits one electron transfer quasi-reversible process with a reduction peak at Epc = 0.6V with the corresponding oxidation peak at Epa= 1.3V. The peak separation (ΔEp) of this couple is 0.7V. With increasing scan rates, the ΔEp value also increases giving more evidence of quasi-reversible Cr(III)/Cr(II) couple[22]. The difference between forward and backward peak potentials can provide a rough evaluation of the degree of the reversibility.

Mn(II) complex

The Mn(II) complex is exhibit two well defined quasi-reversible one-electron cyclic responses were observed, one at Epc = 0.6V with corresponding oxidation peak at Epa = 0.9V and the other at Epc = -0.9V with the corresponding oxidation peak at Epa= -0.5V respectively. The ΔEp values for the first redox couple, 0.3V is higher than the second redox couple 0.4V. ΔEp value is higher for the complex due to the difference between the original complex and the reduced species.

Thermo gravimetric analysis

The thermo gravimetric analysis (TGA) of Cr(III) and Mn(II) complexes (with ATZ: 2-aminothiazole, azide ion ligands) show two significant weight loss with common and specific stages, termed as two stage degradation. The weight loss at the range of 190-280ºC in TGA curves of complexes is termed the first stage of thermal degradation. In these case, the present weight loss is in the range of (19.14-29.11%), which may be attributed to the decomposition of azide ion. The onset of second step decomposition occurs in the range of 280-340ºC, which gives the loss of 2-aminothiazole (38.34 – 52.65%) bonding with the metal complexes. The experimental values are in full agreement with the percent weight calculated on the basis of stoichiometry proposed for the complexes [18].

Biological Activity

Antibacterial activity

The synthesized Cr(III) and Mn(II) complexes and the free ligand 2-aminothiazole are tested against the bacteria *viz*., *Escherichia* coli, *Lactobacillus brevis, Micrococcus luteus, Proteus vulgaris, Pseudomonas aeruginosa, Raoultella planticola, and Shigella flexneri* by agar-well diffusion method in vitro conditions as shown in Table-2. The complexes encompass prospective activity against the bacteria compared to free ligand 2-aminothiazole.

Antifungal activity

The antifungal activity of the free ligand 2-aminothiazole and the synthesized Cr(III) and Mn(II) complexes are tested against the fungi *viz., C.albicans, Aspergillus Niger, Aspergillus Flavus, Aspergillus oryzae and Aspergillus sojae* by agar -well diffusion method as shown in Table-3. The complexes have enhanced activity against the fungi compared to free ligand.

Antioxidant activity (Radical Scavenging Activity)

The DPPH (2,2″-diphenyl-1-picrylhydrazyl) radical assay provides an easy and rapid way to evaluate the antiradical activities of antioxidants. Fortitude of the reaction kinetic types DPPHH is a product of the reaction between DPPH• and an antioxidant.

(AH): DPPH• + AH → DPPHH + A

The reversibility of the reaction is evaluated by the addition of DPPHH at the end of the reaction. If there is an increase in the percentage of remaining DPPH• at the plateau, the reaction is reversible, otherwise it is a complete reaction. The 2,2″-diphenyl-1-picrylhydrazyl radical was used as stable free radical electron accepts or hydrogen radical to become a stable diamagnetic molecule[23]. It is a stable free radical containing an odd electron in its structure and usually used for detection of the radical scavenging activity in chemical analysis. The reduction capability of DPPH radical was determined by decrease in its absorbance at 517 nm induced by antioxidants. The graph was plotted with percentage scavenging effects on the y-axis and concentration (µg/mL.) on the x-axis. The scavenging ability of the ligand 2-aminothiazole, Cr(III) and Mn(II) complexes were compared with Vitamin C as a standard and shows enhanced activity, these results were in good agreement with previous metal complexes studies where the ligand has the antioxidant activity and it is expected that the metal moiety will increase its activity[24]. The Scavenging activity of free ligand and its complexes shows in Figure-2.

DNA Binding – Emission study

The binding of free ligand and its complexes to CT-DNA can be studied by competitive binding experiments. EtBr is known to show fluorescence when bound to DNA, due to its strong intercalation between the adjacent DNA base pair. The fluorescent light is quenched by the addition of a second molecule [25]. The quenching extent of fluorescence of ethidium bromide binding to DNA is used to determine the extent of binding between the second molecule and DNA. The addition of the complex to DNA pretreated with ethidium bromide causes appreciable reduction in the emission intensity as shown in Figure-3 and Figure-4 indicating the replacement of the ethidium bromide fluorophore by the complex results in a decrease of the binding constant of the ethidium to the DNA.

According to the classical Stern-Volmer equation: Io/I = 1 + Ksvr, where Io and I are the fluorescence intensities in the absence and the presence of complex respectively. Ksv is a linear Stern–Volmer quenching constant, r is the ratio of the total concentration of complex to that of DNA. The quenching plots illustrate that the quenching of ethidium bromide bound to DNA by the complex are in good agreement with the linear Stern-Volmer equation, which also indicates that the complex binds to DNA. In the plot of I0/I versus CComplex/CDNA, K is given by the ratio of the slope to intercept. The data suggest that the interaction of complexes with DNA is strongest, which is consistent with the above absorption spectral results [26].

Table 1: Analytical data of Cr(III) and Mn(II) Complexes

S. No.	Complex	Elements found (Calc) %				Conductance (Ω−1cm2 mol−1)
		C	H	N	M	
1	$[Cr(N_3)_3(ATZ)_3]$	48.34 (51.07)	4.54 (4.90)	10.43 (11.05)	10.12(11.32)	78.45
2	$[Mn(N_3)_2(ATZ)_4]$	51.99 (52.67)	4.48 (5.89)	11.32 (12.05)	11.22 (12.31)	67.52

Table 2: Antibacterial activities free ligand 2-aminothiazole and its complexes

S. No.	Ligand/ Complexes	Conc. µg/ml	Zone of Inhibition in diameter (mm)						
			E.coli	*L. brevis*	*M.luteus*	*P.vulgaris*	*P.aeruginosa*	*R.planticola*	*S.flexneri*
1	ATZ	100	06	11	07	03	05	08	05
		200	14	09	15	06	11	25	23
		400	20	13	20	12	18	20	13
2	$[Cr(N_3)_3(ATZ)_3]$	100	35	28	20	25	23	33	28
		200	44	42	32	33	28	42	38
		400	20	13	44	42	38	38	52
3	$[Mn(N_3)_2(ATZ)_4]$	100	18	18	20	20	13	20	20
		200	23	32	28	35	28	28	35
		400	28	44	38	52	35	38	52
00-15 Resistant; 16-30 Moderate; 31-46 Highly activity; 46-60 Enhanced activity									

Table 3: Antifungal activities free ligand 2-aminothiazole and its complexes

S. No.	Ligands/ Complexes	Conc. µg/ml	Zone of Inhibition in diameter (mm)				
			C.albicans	*Aspergillus Niger*	*Aspergillus Flavus*	*Aspergillus oryzae*	*Aspergillus sojae*
1	2-aminothiazole	100	08	05	07	03	05
		200	14	09	15	06	11
		400	20	13	20	12	18
2	$[Cr(N_3)_3(ATZ)_3]$	100	33	28	20	25	23
		200	42	38	32	33	28
		400	20	13	44	42	38
3	$[Mn(N_3)_2(ATZ)_4]$	100	18	18	20	20	13
		200	23	32	28	35	28
		400	28	44	38	52	35
00-15 Resistant; 16-30 Moderate; 31-46 Highly activity; 46-60 Enhanced activity							

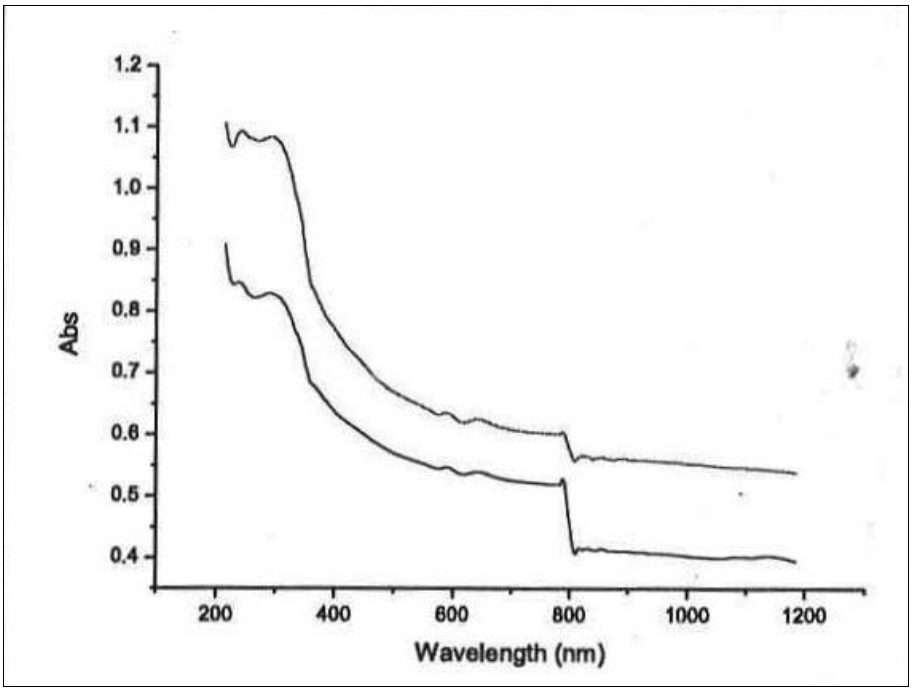


Fig 1: Electronic spectra of Cr(III) and Mn (II)

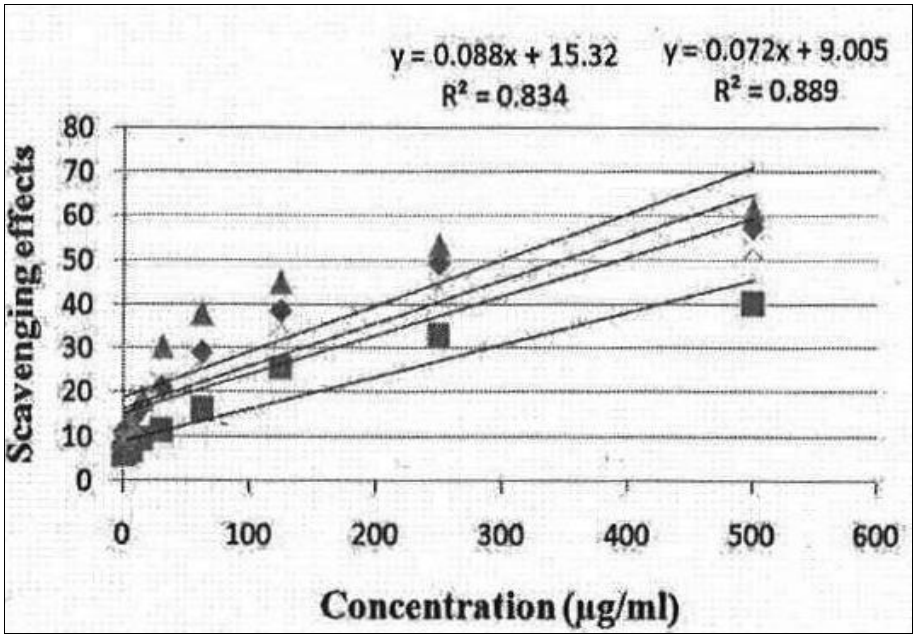


Fig 2: Scavenging activities of free ligands and their complexes

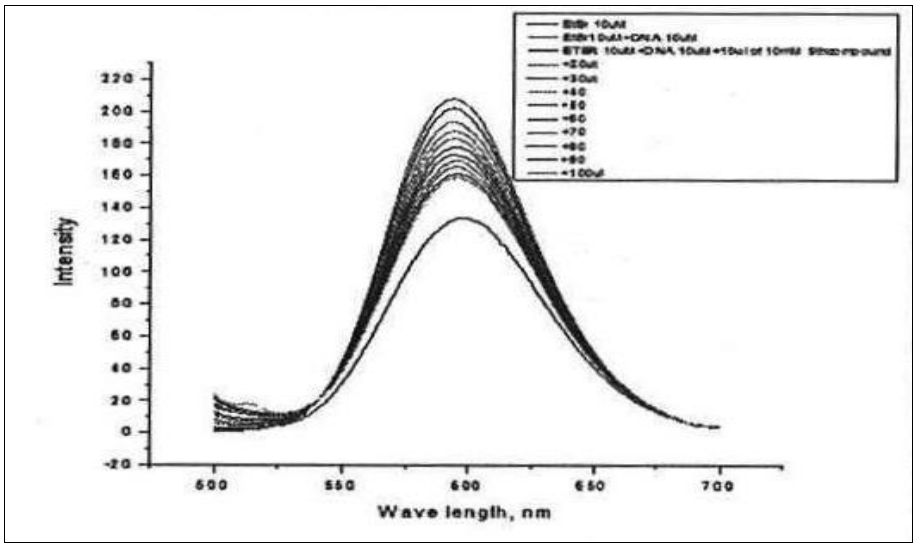


Fig 3: Emission spectrum of EtBr bound to DNA in the absence and presence of Cr (III) comples

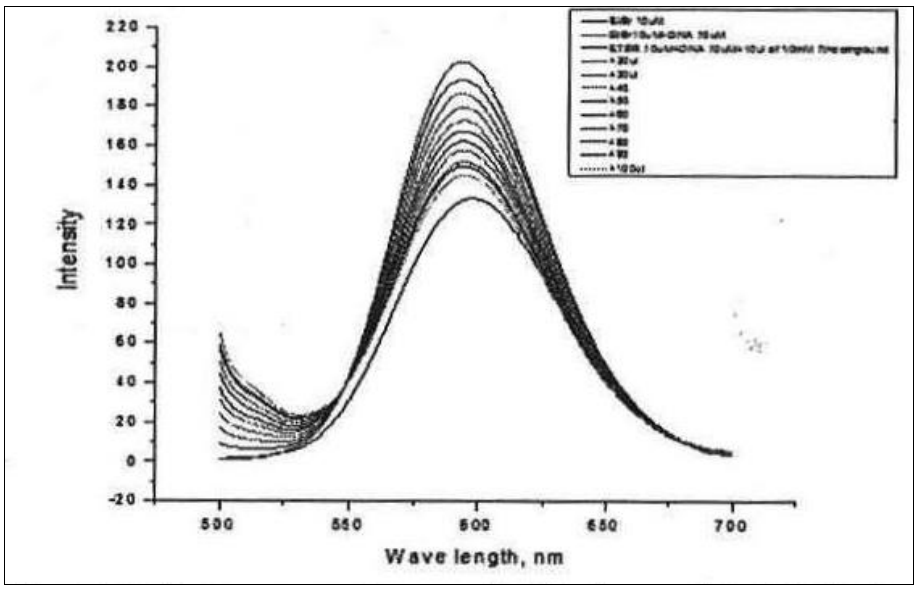


Fig 4: Emission spectrum of EtBr bound to DNA in the absence and presence of Mn (II) complex

Conclusion

In the current study, our work were to synthesize and characterize the new heterocyclic compounds of Cr(III) and Mn(II) complexes (ATZ: 2- aminothiazole; N_3- : Azide ion). The synthesized metal complexes were characterized by elemental analysis (C, H, N, M), molar conductance, IR and UV-Vis spectral analysis and also, their CV and TGA behaviors have been studied. Based on the analytical, molar conductance, spectral and magnetic moment, octahedral geometry have been suggested for the Cr(III) and Mn(II) complexes. The metal complexes have significant antimicrobial and antioxidant activities as compared to the free ligands. The effectiveness of the DNA binding of the complexes is being confirmed by means of change in intensity of emission in the case of emission spectral studies.

References

1. Ibatullin UG, Petrushina TF, Leitis LY, Minibaev IZ, Logvin BO. Chemistry of Heterocyclic Compounds,1993:*29*(5):612-615.
2. Das D, Sikdar P, Bairagi M. Journal of Medicinal Chemistry,2016:109:89-98.
3. Singh K, Singh S, Taylor JA. Dyes and Pigments,2002:54(3):189-200.
4. Geronikaki A, Vicini P, Dabarakis N, Lagunin A, Poroikov V, Dearden J, *et al.* European Journal of Medicinal Chemistry,2009:44(2):473-481.
5. Kreutzberger A, Tantawy A, Archiv der Pharmazie,1981:314(11):968-969.
6. Khalifa ME, Gobouri AA. Polish Journal of Chemical Technology,2017:19(4):28-35.
7. Khalifa ME, Metwally MA, Abdel-Latif E, Amer FA. Int. J. Text. Sci.,2012:1(6):62-68.
8. Metwally M, Khalifa M, Attia E, Amer F. Polish Journal of Chemical Technology,2010:12(1):1-6.
9. Metwally MA, Abdel-Latif E, Amer FA, Kaupp G. Dyes and Pigments,2004:60(3):249-264.
10. Khalifa ME, Abdel-Latif E, Gobouri AA. Journal of Heterocyclic Chemistry,2015:52(3):674-680.
11. Yüce AO, Mert BD, Kardaş G, Yazıcı B. Corrosion Science,2014:83:310-316.
12. Al-Hajjar FH, Al-Kharafi FM. Corrosion science,1988:28(2):163-171.
13. Pourjavid MR, Razavi T. Chinese Chemical Letters,2012:23(3):343-346.
14. Joshi KC, Pathak VN, Arya P. Agricultural and Biological Chemistry,1979:43(2):199-201.
15. Rajapandiyan K, Shanthi S, Vidya S. Chemical & Biological Sciences,2013:3(3):68-70.
16. Govindharaju R, Durairaj P, Maruthavanan T, Marlin Risana M, Ramachandramoorthy T. Int. J. Pharm. Sci. Drug Res,2019:11(5):174-180.
17. Govindharaju R, Balasubramaniyan S, Rajasekar K, Ramachandramoorthy T. International Journal of Pharma Research & Review,2014:3(10):8-13.
18. Govindharaju R, Balasubramaniyan S, Palanivelan L, Marlin Risana M, Mukil Meenakshi, V. Int J Pharm Sci & Res,2019:10(11):5137-5145.
19. Govindharaju R, Durairaj P, Maruthavanan T, Marlin Risana M, Ramachandramoorthy T. Int. J. Pharm. Sci. Drug Res,2019:11(5):174-180.
20. Palanivelan L, Balasubramaniyan S, Govindharaju RR amachandramoorthy T. International Journal of Advanced Scientific Research and Management,2018:3(12):66-70.
21. Kulkarni AD, Patil SA, Badami PS. International Journal of Electrochemical Science,2009:4(5):717-729.
22. Donzello M, Dini D, Arcangelo G, Ercolani C, Zhan R, Ou Z. *et al. J. Am. Che. Soc*.,2003:125:14190-14194.
23. Govindharaju R, Muruganantham N, Balasubramaniyan S, Palanivelan L, Jayalakshmi B, Rajalakshmi K, Ramachandramoorthy T. Int. J. Pharm. Investigation,2019:9(4):158-163.
24. Gao E, Zhu M, Yin H, Liu L, Wu Q, Sun Y, Journal of Inorganic Biochemistry,2008:102(10):1958-1964.
25. Muruganantham N, Govindharaju R, Anitha P. Int J Pharm Sci & Res,2019:10(12):5606-5611.
26. Govindharaju R, Muruganantham N, Balasubramaniyan S, Palanivelan L, Jayalakshmi B, Rajalakshmi K, *et al.* Int. J. Pharm. Investigation,2019:9(4):158-163.