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## AN INVESTIGATION OF THE DNA BINDING PROPERTIES OF $Mn^{2+}$ , $Co^{2+}$ AND $Ni^{2+}$ COMPLEXES WITH 2-AMINOBENZONITRILE AND OCTANOATE ION AS LIGANDS

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### Keywords:

ABN, Octanoate ion, Complexes, DNA-binding property

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**ABSTRACT:** Three novel metal complexes of 2-aminobenzonitrile (ABN) and octanoate ion (OC) with  $Mn^{2+}$ ,  $Co^{2+}$ , and  $Ni^{2+}$  have been prepared by using microwave irradiation. The DNA-binding properties of the free ligand 2-aminobenzonitrile and its  $Mn^{2+}$ ,  $Co^{2+}$  and  $Ni^{2+}$  complexes have been investigated by fluorescence measurements. The results suggest that ABN,  $Mn^{2+}$ ,  $Co^{2+}$  and  $Ni^{2+}$  complexes both bind to DNA *via* an intercalative binding mode and the affinity for DNA is more strong in case of  $Mn^{2+}$ ,  $Co^{2+}$ , and  $Ni^{2+}$  complexes when compared with ABN. The intrinsic binding constants ( $K_b$ ) of the  $Mn^{2+}$ ,  $Co^{2+}$  and  $Ni^{2+}$  complexes and ligand with DNA were  $2.75 \times 10^4 M^{-1}$ ,  $3.08 \times 10^4 M^{-1}$ ,  $3.80 \times 10^4 M^{-1}$  and  $1.96 \times 10^4 M^{-1}$ , respectively.  $Ni^{2+}$  complex is strongly bound to DNA compared to other complexes and the ligand. Furthermore, the free radical scavenging activity of the free ligand and their complexes has been determined by measuring their interaction with the stable free radical DPPH. The complexes have larger antioxidant activity as compared to the ligands.

**INTRODUCTION:** In recent years, binding studies of transition metal complexes have become very important in the expansion of DNA molecule probes and chemotherapeutics <sup>1</sup>. DNA is the pharmacological target of many of the drugs that are at present in clinical use or advanced clinical trials. Targeting DNA to control cell functions by modulating transcription or by intrusive with imitation seems logical, instinctively appealing, and conceptually straightforward. Small ligand molecules bound to DNA artificially alter and/or inhibit the functioning of DNA.

These small ligand molecules act as a drug when alteration or inhibition of DNA function is required to cure or control a disease <sup>2</sup>. The study of interaction of drug with DNA is very thrilling and important not only for understanding the mechanism of interaction but also for the design of new drugs. The studies have shown that metal complexes can interact with DNA in different binding fashions and exhibit effective nuclease activities <sup>3,4</sup>.

On the other hand, among the aminobenzonitriles, 2-aminobenzonitrile (ABN) is used for the induction of nitrilase activity in arthrobacter, radioprotective agent and starting materials for the synthesis of biologically active compounds <sup>5-8</sup>. 2-aminobenzonitrile is one of the organic ligands in coordination chemistry which can coordinate to the metal ion through different modes *viz.*, monodentate, bidentate or bridging.

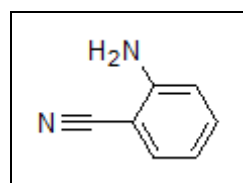
<p><b>QUICK RESPONSE CODE</b></p> 	<p><b>DOI:</b> 10.13040/IJPSR.0975-8232.10(12).5606-11</p> <hr/> <p>This article can be accessed online on <a href="http://www.ijpsr.com">www.ijpsr.com</a></p> <hr/> <p>DOI link: <a href="http://dx.doi.org/10.13040/IJPSR.0975-8232.10(12).5606-11">http://dx.doi.org/10.13040/IJPSR.0975-8232.10(12).5606-11</a></p>
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In general, the biological activities of the metal complexes differ from those of either the ligand or the metal ion itself and increased and/or decreased biological activities are reported for various metal complexes <sup>9</sup>.

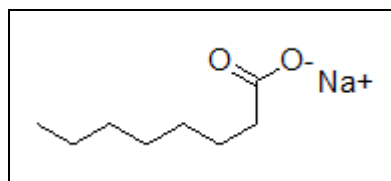
The present study aims at synthesis and DNA-binding investigation of  $Mn^{2+}$ ,  $Co^{2+}$ , and  $Ni^{2+}$  complexes with neutral bidentate 2-aminobenzonitrile and anionic monodentate octanoate ion ligands.

#### EXPERIMENTAL METHOD:

**Materials:** 2-aminobenzonitrile, sodium octanoate, and manganese nitrate, cobalt nitrate and nickel nitrate were purchased from Alfa Aaser Company and used as such. The organic solvents DMSO, DMF, methanol, ethanol were of AnalR grade and used as such without further purification.



2-aminobenzonitrile



Sodium octanoate

**Synthesis of Mn(II) complex:** 2-aminobenzonitrile 0.91g (7.38 mmol) in ~ 10 ml methanol and sodium octanoate 1.33g (8.01 mmol) in ~ 10 ml ethanol were added to the manganese nitrate 1.00 g (3.64mmol) in ~ 10 ml methanol followed by microwave irradiation for a 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. A pale yellow colored complex was obtained with 64.76% yield.

**Synthesis of Co(II) complex:** Sodium octanoate 1.14g (6.91 mmol) in ~ 10 ml ethanol and 2-aminobenzonitrile 0.83g (6.93 mmol) in ~ 10 ml methanol were added to the cobalt nitrate 1.00g (3.64 mmol) in ~ 10 ml methanol followed by microwave irradiation for a 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. A pink colored complex was obtained with a 63.77% yield.

**Synthesis of Ni(II) Complex:** 1.00 g (3.43 mmol) cobalt nitrate in ~ 10 ml methanol and 2-aminobenzonitrile 0.81g (6.88 mmol) in ~ 10 ml methanol were added to the Sodium octanoate 1.14g (6.90 mmol) in ~ 10 ml ethanol followed by microwave irradiation for a 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. A pale green colored complex was obtained with a 71.65% yield.

**DNA Binding Studies:** The DNA binding experiments involving the interaction of the Cr(III) complex and the ligands with calf thymus CT-DNA were conducted in Tris buffer containing HCl (0.01 M) adjusted to pH 7.2 with hydrochloric 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, demonstrating that the DNA was adequately free of protein. DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient  $6600 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$  at 260 nm. The stock solutions were stored at 4°C and used within 4 days. For fluorescence-quenching experiments, DNA was pre-treated with ethidium bromide (EtBr) for 30 min. The ABN,  $Mn^{2+}$ ,  $Co^{2+}$  and  $Ni^{2+}$  complexes then added to this mixture respectively 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 <sup>10</sup>.

**Antioxidant Activity:** Evaluation of antioxidant activity stock solution (1 mg/ml) was diluted to final concentrations of 10-500  $\mu\text{g/ml}$ . Ethanolic DPPH solution (1 ml, 0.3 mmol) was added to the sample solutions in DMSO (3 ml) at different concentrations (10-500  $\mu\text{g/ml}$ ) <sup>11</sup>. The mixture was shaken energetically and acceptable to stand at room temperature for 30 min. The absorbance was then measured at 517 nm in a UV-Vis spectrophotometer. The lower absorbance of the reaction mixture indicates higher free radical

scavenging activity. Ethanol was used as the solvent and ascorbic acid as the standard. The DPPH radical scavenging activity is designed by the following equation:

$$\text{DPPH Scavenging effect (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

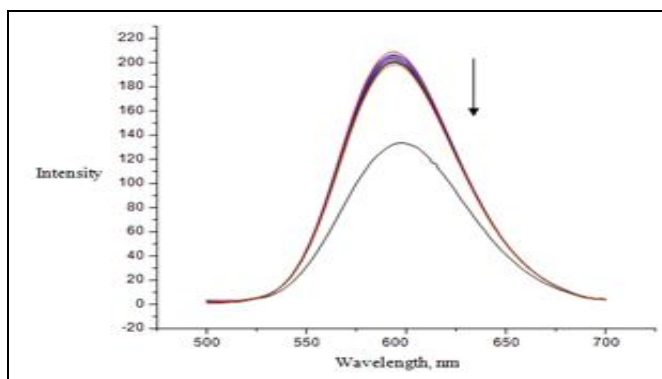
Where  $A_0$  is the absorbance of the control reaction and  $A_1$  is the absorbance in the presence of the samples or standard.

## RESULTS AND DISCUSSION:

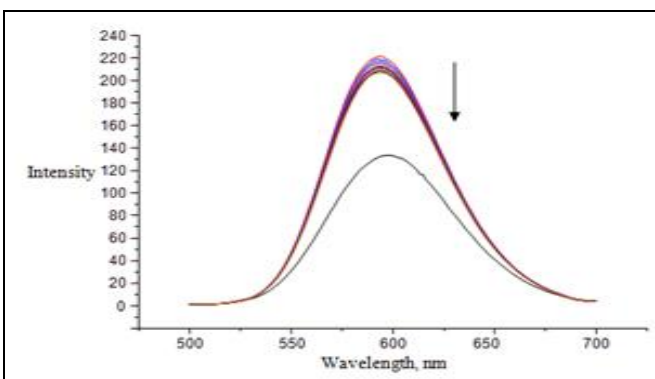
**DNA Binding Properties:** The binding of 2-aminobenzonitrile,  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$  and  $\text{Ni}^{2+}$  complexes to CT-DNA can be studied by competitive binding experiments. Ethidium bromide (EB) is known to show fluorescence when bound to DNA, due to its strong intercalation between the adjacent DNA base pair. The fluorescent light is quenched by the addition of a second molecule<sup>12, 13</sup>. 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 an appreciable reduction in the emission intensity, 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 **Fig. 1-4**.

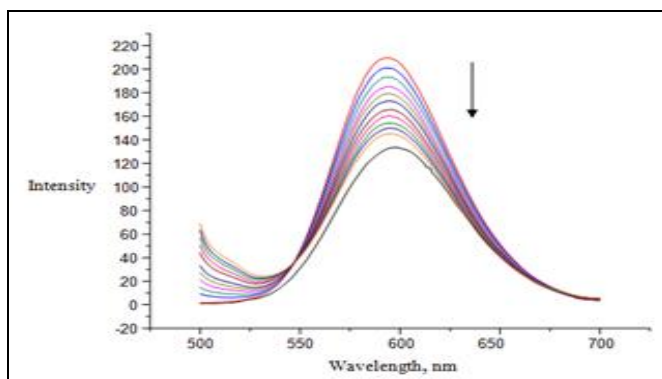
According to the classical Stern-Volmer equation:  $I_0/I = 1 + K_{sv}r$ , where  $I_0$  and  $I$  are the fluorescence intensities in the absence and the presence of complex respectively.  $K_{sv}$  is a linear Stern-Volmer quenching constant,  $r$  is the ratio of the total concentration of complex 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  $I_0/I$  versus  $C_{\text{Complex}}/C_{\text{DNA}}$ ,  $K$  is given by the ratio of the slope to intercept.



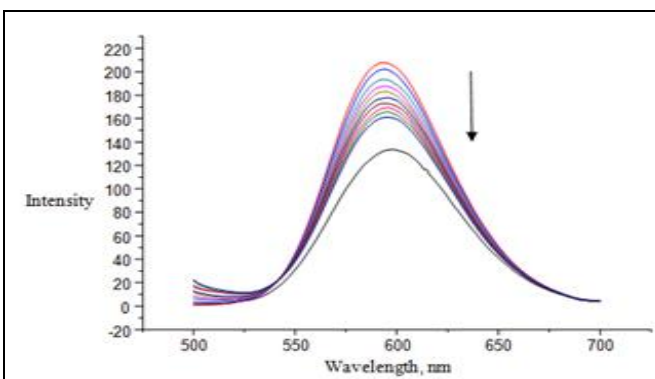
**FIG. 1: EMISSION SPECTRUM OF EtBr BOUND TO DNA IN THE PRESENCE OF 2-AMINO BENZONITRILE. ARROWS INDICATE THE INTENSITY CHANGES UPON INCREASING CONCENTRATION OF THE 2-AMINO BENZONITRILE**



**FIG. 2: EMISSION SPECTRUM OF EtBr BOUND TO DNA IN THE PRESENCE OF  $\text{Mn}^{2+}$  COMPLEX. ARROWS INDICATE THE INTENSITY CHANGES UPON INCREASING CONCENTRATION OF THE COMPLEX**



**FIG. 3: EMISSION SPECTRUM OF EtBr BOUND TO DNA IN THE PRESENCE OF  $\text{Co}^{2+}$  COMPLEX. ARROWS INDICATE THE INTENSITY CHANGES UPON INCREASING CONCENTRATION OF THE COMPLEX**



**FIG. 4: EMISSION SPECTRUM OF EtBr BOUND TO DNA IN THE PRESENCE OF  $\text{Ni}^{2+}$  COMPLEX. ARROWS INDICATE THE INTENSITY CHANGES UPON INCREASING CONCENTRATION OF THE COMPLEX**

The Kb values for  $Mn^{2+}$ ,  $Co^{2+}$  and  $Ni^{2+}$  complexes and ligand with DNA were  $2.75 \times 10^4 M^{-1}$ ,  $3.08 \times 10^4 M^{-1}$ ,  $3.80 \times 10^4 M^{-1}$  and  $1.96 \times 10^4 M^{-1}$ , respectively. Furthermore,  $Ni^{2+}$  complex is complex strongly binds to DNA compared to other complexes and the ligand. The binding constant (Kb) values indicate that interaction of the complexes with DNA is intercalative mode<sup>14</sup>.

**TABLE 1: DNA-BINDING CONSTANT (Kb) OF LIGAND AND ITS COMPLEXES**

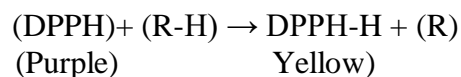
S. no.	Ligands/ Complexes	Binding constant (Kb)
1	2-aminobenzonitrile	$1.96 \times 10^4 M^{-1}$
2	$Mn^{2+}$ complex	$2.75 \times 10^4 M^{-1}$
3	$Co^{2+}$ complex	$3.08 \times 10^4 M^{-1}$
4	$Ni^{2+}$ complex	$3.80 \times 10^4 M^{-1}$

### Anti-oxidant Activity:

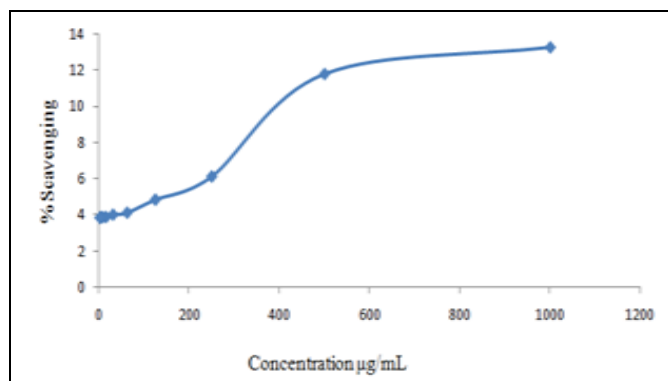
**DPPH Radical Scavenging Assay:** The scavenging activity of a chemical/or compound on the DPPH radical as a fast and reliable parameter to measure the *in-vitro* antioxidant activity of such sample has been used by diverse researchers<sup>15</sup>. This assay is based on the measurement of the decrease in the molar absorptivity of DPPH at 517 nm after reaction with the test compound. The effect of antioxidants on DPPH radical scavenging

is due to the hydrogen donating ability or radical scavenging activity of the samples<sup>16</sup>.

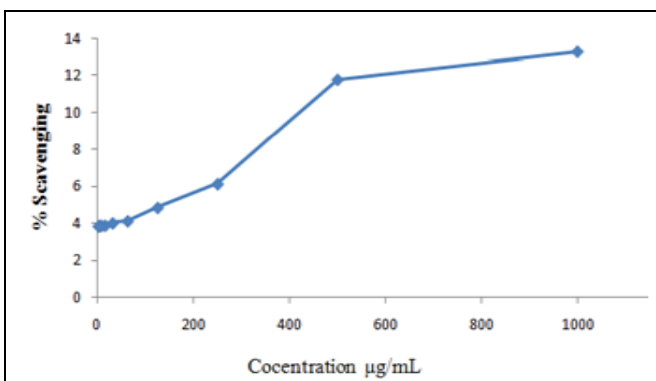
The scavenging reaction between (DPPH) and an antioxidant (R-H) can be written as:



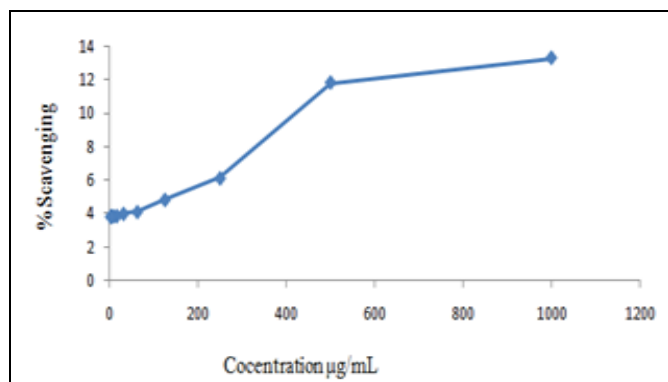
Antioxidants react with DPPH, a stable free radical that is reduced and as a result, the absorbance decreases due to the formation of the DPPH-H from the DPPH radical. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or samples in terms of hydrogen donating ability<sup>17</sup>. The graph was plotted with the percentage of scavenging effects on the y-axis and concentration ( $\mu\text{g/ml}$ ) on the x-axis. The scavenging ability of the 2-aminobenzonitrile (ABN) with  $Mn^{2+}$ ,  $Co^{2+}$ , and  $Ni^{2+}$  complexes were compared with ascorbic acid as standard. The complexes showed enhance activity as a radical scavenger compared with ascorbic acid, 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<sup>18-20</sup>.



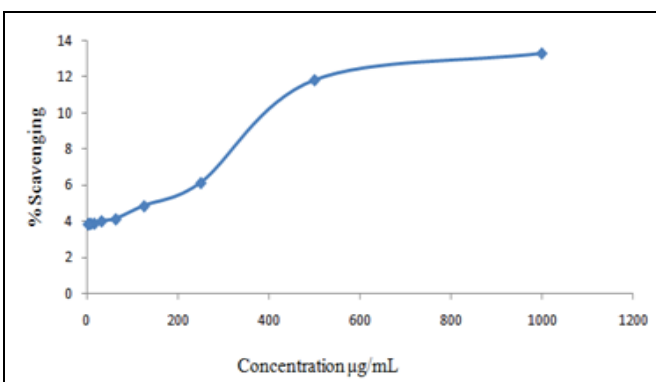
**FIG. 5: ANTIOXIDANTAL ACTIVITY OF 2-AMINO BENZONITRILE**



**FIG. 6: ANTIOXIDANTAL ACTIVITY OF  $Mn^{2+}$  COMPLEX**



**FIG. 7: ANTIOXIDANTAL ACTIVITY OF  $Co^{2+}$  COMPLEX**



**FIG. 8: ANTIOXIDANTAL ACTIVITY OF  $Ni^{2+}$  COMPLEX**



**TABLE 2: ANTIOXIDANT ACTIVITY OF LIGANDS AND THEIR COMPLEXES**

S. no.	Ligands/ Complexes	IC <sub>50</sub> values (µg/ml)
1	2-aminobenzonitrile	>1000
2	Mn <sup>2+</sup> complex	348.64
3	Co <sup>2+</sup> complex	572.46
4	Ni <sup>2+</sup> complex	270.94

The Mn<sup>2+</sup>, Co<sup>2+</sup>, and Ni<sup>2+</sup> complexes were found to have better antioxidant activity than the free ligand. At the lowest concentration (125 µg/ml) the antioxidant activity of the free ligand was found to be 10% but, upon complexation, it increased significantly to the range of 25.02% - 54.88% in all the complexes **Fig. 5-8**.

**CONCLUSION:** In the present study, our efforts were to synthesize the Mn<sup>2+</sup>, Co<sup>2+</sup> and Ni<sup>2+</sup> metal complexes with 2-aminobenzonitrile and octanoate ion as ligands. The new complexes were synthesized using microwave irradiation. The synthesized metal complexes were characterized by DNA binding properties and antioxidant activities.

The metal complexes have significant antioxidant activity as compared to the free ligands. The effectiveness of the DNA binding property of the complexes is being confirmed by means of change in the intensity of emission in the case of emission spectral studies.

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**CONFLICTS OF INTEREST:** Nil

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