



## Synthesis and Characterization of Transition Metal Complexes of Para-Aminosalicylic Acid With Evaluation of Their Antioxidant Activities

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### ABSTRACT

Complexing behavior of Para-aminosalicylic acid (PAS) towards Mn(II), Fe(II), Co(II), Ni(II), Cu(II), Zn(II), have been examined by Conductance and magnetic measurements and by UV-visible, IR and <sup>1</sup>H NMR. Thermal decomposition properties of the complexes are investigated by thermogravimetric analysis (TGA). The free ligand and its complexes were tested for antioxidant activity and evaluation of IC<sub>50</sub> values. It was found that PAS and its all complexes have low IC<sub>50</sub> value than standard ascorbic acid. All complexes have lower IC<sub>50</sub> value than PAS with Ni complex the least value. . Therefore, PAS and its complexes with these metal ions can act as antioxidants to reduce oxidative stresses.

**Keywords:** Para-aminosalicylic acid, metal complexes, thermal analysis, antioxidant activity, IC<sub>50</sub>

### INTRODUCTION

4-amino-2-hydroxy-benzoic acid or 4-aminosalicylic acid commonly known as para-amino salicylic acid (PAS), an amine derivative of salicylic acid, is an antibiotic and one among the World Health Organization's List of Essential Medicines. PAS is a well-known antibacterial drug used in combination with first line drugs like isoniazid, rifampicin, etc<sup>1</sup>. Recently, PAS has been found effective in treatment

of cancer and inflammatory bowel disease<sup>2</sup>. PAS also has modest activity against Influenza virus A<sup>3,4</sup>. PAS is one of the first anti-tuberculosis agents found to be effective in 1940s<sup>5</sup>. Today, PAS is used primarily as a second-line drug to treat multi drug resistant tuberculosis<sup>6</sup>. PAS, a bacteriostatic agent, is extremely helpful in controlling the growth of bacteria. It is also very useful in avoiding the development of the resistance of bacteria to the other anti-multi drug resistant -tuberculosis drugs<sup>7</sup>.

Various studies have been carried out to examine the effectiveness of anti-inflammatory drugs as scavengers of reactive oxygen species (ROS), viz. superoxide radical ( $2O^{\bullet-}$ ), hydrogen peroxide ( $H_2O_2$ ), hydroxyl ( $HO^{\bullet}$ ), and hypochlorous acid ( $HOCl$ )<sup>8</sup>. Halliwell and Gutteridge (1985) have reported that non-steroidal anti-inflammatory drugs (NSAIDs) are powerful free radical scavengers<sup>9</sup>. Further, it is reported that NSAID, including analogs of salicylates, can exert neuroprotective effect by blocking inflammatory processes in neurodegenerative diseases<sup>10</sup>. Therefore, PAS, being a salicylate analog, is expected to have the capacity of radical scavenging and hence can act as an antioxidant to reduce oxidative stress.

As a derivative of salicylic acid, PAS acts as a metal chelator<sup>11</sup>, is clinically effective drug for treatment of serious chronic manganese poisoning<sup>12, 13</sup>. This chelating tendency of PAS can be exploited for the formation of essential metal complexes which are useful for having better biological activities especially antioxidant activities. Further, these metal complexes are gaining attention because of their diverse applications in almost all fields of life. More specifically the medicinal properties of essential metal ion chelates are fascinating<sup>14</sup>. There is also a need to explore the role of trace elements, essential elements or micronutrients (in their native or chelate forms) both in plants and animals and to explain their specific and extended roles at various stages<sup>15</sup>.

Considering these literature links, the complexation behavior of PAS with essential metal ions viz. Manganese (Mn), Iron (Fe), Cobalt (Co), Nickel (Ni), Copper (Cu) and Zinc (Zn) has been studied *in vitro*. The PAS and the prepared complexes were tested for antioxidant activities with 1,1-Diphenyl-2-picryl-hydrazyl (DPPH). Scavenging of DPPH radical is the basis of the popular DPPH antioxidant assay. Therefore, rate of reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction<sup>16</sup>. The antioxidant activities obtained in this manner were then compared with a positive control, Vitamin C/Ascorbic acid (AA). AA is regarded as the most important water-soluble micronutrient in human plasma and mammalian cells, required for multiple biological functions. In biological systems, AA is an

effective reducing agent and free radical scavenger, in addition to its antiscorbutic action<sup>17</sup>. The antioxidant activity was calculated on the basis of its radical scavenging activity (RSA) (percentage of inhibition) and  $IC_{50}$  (total antioxidant necessary to decrease the initial DPPH radical by 50%) values.

## MATERIALS AND METHODS

### Synthesis of metal complexes

All chemicals used were of analytical reagent grade (AR), and of highest purity available. Synthesis was carried out by the procedure as used by Soliman and Mohamed (2013) with minor alteration. The metal complexes were prepared by the addition of aqueous solution of the appropriate metal acetates to the methanolic solution of SA in 1: 2 ratio. The resulting mixture was stirred for 3h at room temperature and simultaneously checked for the reaction progress. The complexes were collected after slow evaporation of the solvent, then washed with methanol and air dried<sup>18</sup>.

### Instruments

Melting points were calculated on BUCHI melting point B-545 and molar conductance of solid chelates in methanol were measured using conductometer (Labtronics-Model LT-16). Infrared spectra were recorded on Shimadzu FT-IR type FTIR-8400S spectrometer. Electronic transitions, reaction progress and antioxidant activity were measured on Shimadzu UV Probe Version 2.33 spectrophotometer. The molar magnetic susceptibility was measured on powdered samples using the Faraday method. The diamagnetic corrections were made by Pascal's constant and  $Hg[Co(SCN)_4]$  was used as a calibrant. The  $^1H$  NMR spectra were recorded using Bruker Avance II 400 NMR spectrometer at SAIF PU Chandigarh. Thermo gravimetric analysis was carried out at STIC Cochin, Kochi at a heating rate of 20 °C/min.

### Antioxidant activity

The antioxidant activity in terms of free radical scavenging activity (RSA) of PAS and its complexes were measured by using 1, 1- diphenyl-2-picrylhydrazyl (DPPH) assay. 20, 40, 60, 80, 100 $\mu$ L of 100 ppm ethanolic solution of each compound were mixed with 4 mL of 0.1mM ethanolic solution of DPPH. The mixture was shaken vigorously and left to

stand for 30 minutes in the dark. The absorbance was then measured at 517nm against a blank. The control was prepared, as above, without any complex added and ethanol was used for the base line correction. AA was used as the reference (positive control) with the same concentrations as of PAS and its complexes. RSA was expressed as percentage inhibition and calculated as:

$$\text{RSA} = (\text{Absorption of control} - \text{Absorption of sample}) / \text{Absorption of control} \times 100^{19}$$

IC50 values were calculated from the regression of the plotted graph of RSC against the concentrations of the samples<sup>20</sup>.

## RESULTS AND DISCUSSION

The binary transition metal complex formation with PAS with the evaluation of radical scavenging activity in terms of IC<sub>50</sub> have been studied first time, and may be an area of interest because these complexes may affect the bio availability of PAS as these metal ions are present in relatively appreciable concentration in biological fluids<sup>21</sup>.

### Molar conductance measurements

Metal chelates are dissolved in methanol and the molar conductivity of 10<sup>-3</sup>M solutions is measured at room temperature. Table 1 shows the molar conductance values of the complexes which are located in the range of non-electrolytes<sup>22</sup> for all the complexes.

### Magnetic moment and Electronic spectral studies

The room temperature magnetic moments of the complexes are in agreement with the spin

free values (Table 1). The magnetic moment values for Mn(II), Fe(II), Co(II), Ni(II) and Cu(II) complexes indicate the high spin octahedral nature of these complexes.

The geometry of the metal complexes has been assumed from magnetic moment data and the electronic spectra of the complexes. The electronic spectra of the ligand, recorded by UV visible spectroscopy, show three bands at 208, 262 and 293nm (Table 1) which are attributed to the benzene ring  $\pi \rightarrow \pi^*$  transitions and to the chromophores  $n \rightarrow \pi^*$  transitions, respectively. These bands were shifted in the electronic spectra of the complexes to 214–239, 261–184 and 290–315 nm, respectively<sup>23</sup>.

The UV spectrum Ni(II) complex shows two bands at 590 and 560nm which may be assigned to  ${}^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$  and  ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$  transitions, thereby suggesting octahedral geometry of Ni(II) complex<sup>23, 22</sup>. UV spectrum of the Cu(II) complex shows a band at 718nm, suggesting the existence of  ${}^2E_g \rightarrow {}^2T_{1g}$  transition which is consistent with an octahedral (more specifically distorted octahedral) configuration<sup>24</sup>.

### IR Analysis

IR spectra is of great use in structural elucidation in absence of a powerful technique like single crystal XRD. The results of the IR measurements of PAS as well as metal complexes are tabulated in Table 2 (Fig. 1), wherein the assignments of the main bands (those affected by coordination) have been made. The sharp band at 3495 cm<sup>-1</sup> is assigned to the phenoxy O-H stretching vibration of PAS which disappears in metal complexes. Whereas, the sharp band at

**Table 1: Physical data with electronic spectra of PAS and its metal complexes**

Ligand/ Complex	Color	% Yield	Melting point (°C)	$\mu_{\text{eff}}$ (B.M.)	Molar conductance (S.m <sup>2</sup> .mol <sup>-1</sup> )	$n \rightarrow \pi^*$ (nm)	$\pi \rightarrow \pi^*$ (nm)	$d \rightarrow d$ (nm)
SA	Colorless		150 ± 2			293	262, 208	
Mn(II)	Colorless	80	225 ± 2	5.85	7.3491	310	262, 214	
Fe(II)	Reddish brown	75	> 285	5.05	6.90288	301	261	
Co(II)	Magenta	78	135 ± 2	5.01	9.28186	294	262, 239	
Ni(II)	Light green	80	> 285	2.90	4.86722	290	262, 238	560, 590
Cu(II)	Dark green	76	> 285	1.75	4.92852	315	284	718
Zn(II)	white	85	140 ± 2		8.9688	294	276	

3387 $\text{cm}^{-1}$  is assigned to the N-H stretching vibration of  $\text{NH}_2$  group, which is broadened in complexes. The broadening of this band in complexes may be because of the attachment of water as ligand which is also confirmed from thermogravimetric analysis. The broadness may also be because of the presence of aromatic protons which absorb in the range of 3110-3200  $\text{cm}^{-1}$ <sup>25</sup>. The characteristic absorption band at 1645 $\text{cm}^{-1}$  was observed which can be attributed to asymmetric  $\nu(\text{COO})$  stretching vibration<sup>26</sup>, which in complexes faced red shift (bathochromic shift) with hypochromic effect, suggesting of weakening of C=O bond and formation of M-O bond.

Complexation is also suggested on behalf of red shift (bathochromic shift) with hypochromic effect in C-O stretching vibrations of complexes to that of free ligand PAS (1228 $\text{cm}^{-1}$ )<sup>26</sup>. This suggests the weakening of C-O bond and formation of stronger M-O bond in complexes. In addition, bands in the region of 947-904 $\text{cm}^{-1}$  and 825 $\text{cm}^{-1}$  are assigned

to the stretching vibrations of M-OH<sub>2</sub> bond<sup>18</sup>. The medium band at 970 $\text{cm}^{-1}$  is assigned to out of plane bending  $\gamma(\text{OH})$ <sup>27</sup>, which was disappeared in complexes, indicates bonding through phenolic oxygen atom. Complexes also display weak bands in the region 424–455 $\text{cm}^{-1}$ . These bands are assigned to  $\nu(\text{M-O})$  stretching vibration. The weak bands of 970 and 968 $\text{cm}^{-1}$  in Ni(II) and Cu(II) complexes may be because of the out of plane  $\gamma(\text{CH})$  which becomes near degenerate with  $\gamma(\text{OH})$ , whose intensity is very low<sup>27</sup>. Therefore, from the IR spectra it is concluded that PAS behaves as bidentate ligand with OO coordination sites and binds to the metal ions through carboxylate O and phenolic O atoms.

### <sup>1</sup>H NMR Analysis

<sup>1</sup>H NMR spectra were carried out in DMSO-d<sub>6</sub> for the synthesized complex of Zn(II) and ligand PAS. Peak assignments are given in Table 3 for the corresponding protons and these are numbered in Fig. 2. Disappearance of the peak of phenolic

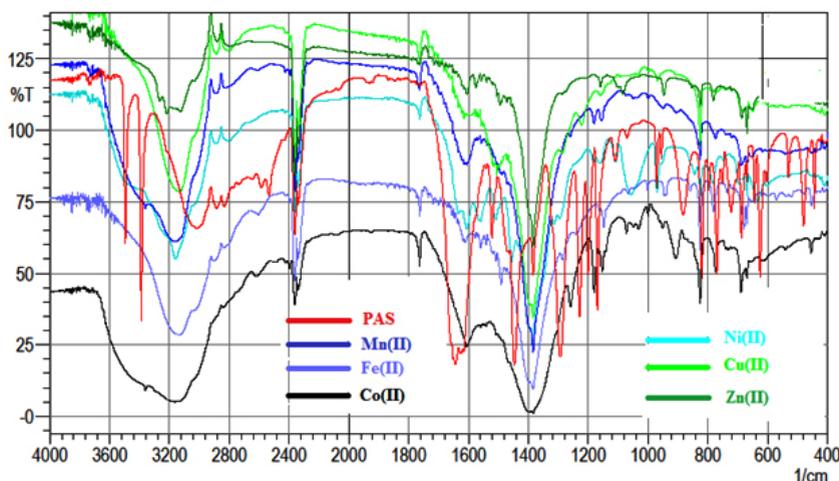
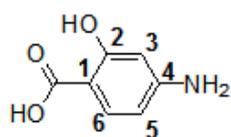


Fig. 1: IR plots of PAS and its metal complexes

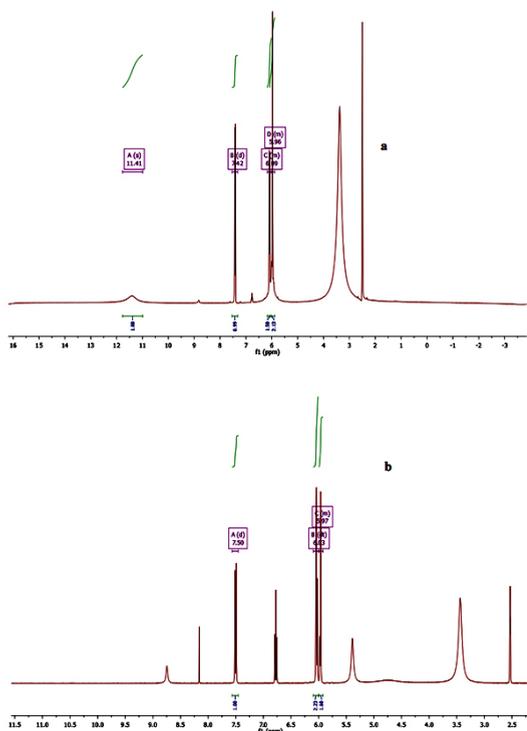
Table 2: IR spectra of PAS and its metal complexes

Complex/ Ligand	$\nu(\text{phe})$	$\nu(\text{NH})$	$\nu(\text{COO})$ (asym)	$\nu(\text{C-O})$	$\gamma(\text{OH})$	$\nu(\text{M-O})$ ( $\text{H}_2\text{O}$ )	$\nu(\text{M-O})$
ASA	3495 s	3387 s	1645 s	1228 s	970 m		
Mn(II) complex		3360 w	1607 b	1180 w		904 w, 825 m	455 w
Fe(II) complex			1610 vs	1227 w		939 vs, 825 s	451 vs
Co(II) complex		3360 w	1607 vs	1180 m		906 m, 825 s	451 vs
Ni(II) complex			1634 w	1180 w	970 vs	825 m	453 w
Cu(II) complex			1614 w	1221 w	968 w	905 w, 825m	455 vs
Zn(II) complex		3265 w	1605 vs	1205 w		947 vs, 825 m	424 vs

proton in the complexes indicate the involvement of the phenolic oxygen atom in complexation (Fig 3). A shift in the values of ring protons of complexes in comparison to the proton of PAS also suggests the complexation. No shift in the peak of NH protons in complex nullifies the bonding through N atom of NH<sub>2</sub> group. So on the basis of analysis of the results obtained, it is concluded that the coordination of the PAS ligand to the metal ions occurs through the phenolic oxygen and carboxylic oxygen.



**Fig. 2: Numbering pattern of carbon containing protons for <sup>1</sup>H NMR peak assignments**



**Fig. 3: <sup>1</sup>H NMR of a) PAS and b) Zn(II) complex of PAS**

**Table 3: Peak assignments of protons for <sup>1</sup>H NMR of PAS and its Zn(II) complex**

Compound	$\delta$ (H3), ppm	$\delta$ (H5), ppm	$\delta$ (H6), ppm	$\delta$ (H <sub>NH</sub> )	$\delta$ (H <sub>OH</sub> )
PAS	6.09	6.09	7.42	5.96	11.41
Zn(II) complex	6.03	6.03	7.50	5.97	

### Thermal analysis

The complexes of PAS were also analyzed thermogravimetrically. The TG curves of complexes usually refers to three stages of mass losses within different temperature ranges. The first step of degradation corresponds to loss of coordinated water molecules which usually occurs in the range of 80-150°C. Therefore coordinated water molecules are confirmed from TGA analysis. Next degradation steps followed similar degradation trends as is shown in Figure 4 for Ni(II) complex of PAS.

On the basis of said facts, the proposed structures for the metal complexes are shown in Fig. 5. It is suggested that the ligand and metal combine in 2:1 ratio and metal is coordinated via O O atoms of the PAS to give octahedral geometry to the complexes.

### Antioxidant activity

Antioxidant activity of PAS and all its metal complexes were evaluated in terms of DPPH radical scavenging activity (RSA) at 5 concentrations of 0, 0.5, 1.0, 1.5 and 2 µg/mL of the compound and corresponding IC<sub>50</sub> values were obtained from their regression plot (Fig. 6). Ascorbic acid (AA) was used as a positive control. PAS and all its metal complexes show higher activity than the positive control, AA, with IC<sub>50</sub> values 2.75 µg/mL. The highest radical scavenging activity was observed for Ni(II) complex with IC<sub>50</sub> value 0.88µg/mL and for PAS it was lower than all the metal complexes with IC<sub>50</sub> value 2.13µg/mL.

From the data of IC<sub>50</sub> of AA, PAS and its metal complexes it is evident that all metal complexes are better antioxidants than PAS and standard AA. The higher antioxidant activity of metal complexes than PAS can be explained on some explanations cited in literature. It is reported by R Milaeva (2011)<sup>28</sup> that the antioxidant activity of sterically hindered phenolic antioxidants is increased by metal complexation because: i) Of the modification in the chemical structures of metal complexes by changing metal or ligand nature; ii) of the stabilization



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