



# SYNTHESIS AND CHARACTERIZATION OF TRANSITION METAL COMPLEXES WITH AZO LIGAND DERIVED FROM 4-HYDROXY-6-METHYL-2-PYRANONE

Mahmoud Najim Al-Jibouri<sup>[a]</sup>

**Keywords:** Azo dyes derived from 4-hydroxy-6-methyl-2-pyranone; transition metal complexes; synthesis.

A series of metal (Cr(III), Fe(III), Mn(II), Co(II), Ni(II), Cu(II) and Zn(II)) complexes with 4-[(Z-(4-hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)-1-methyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one)] were prepared by azo coupling of diazotised 4-aminoantipyrine with 4-hydroxy-6-methyl-2-pyranone. The free ligand and its metal complexes were fully characterized on the basis of elemental analyses and <sup>1</sup>H NMR, FTIR and UV-Visible spectroscopy. The keto-enol tautomer azo ligand [HL] was a mixture of E and Z isomers as suggested by <sup>1</sup>H NMR and FT-IR spectroscopy. The chelating properties of the new azo ligand were studied towards Cr(III), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) ions, and the spectral data revealed that the nitrogen and oxygen atoms of –N=N–, C=O and –OH groups participated in bonding with the metal ions. The study of NMR, IR and electronic spectra indicated an octahedral structure for all metal complexes except zinc(II) complex, which has the tetrahedral structure.

Corresponding Authors

E-Mail: mahmoudnajim71@yahoo.com

[a] Chemistry department, College of Science-Al-Mustansiriya University

## Introduction

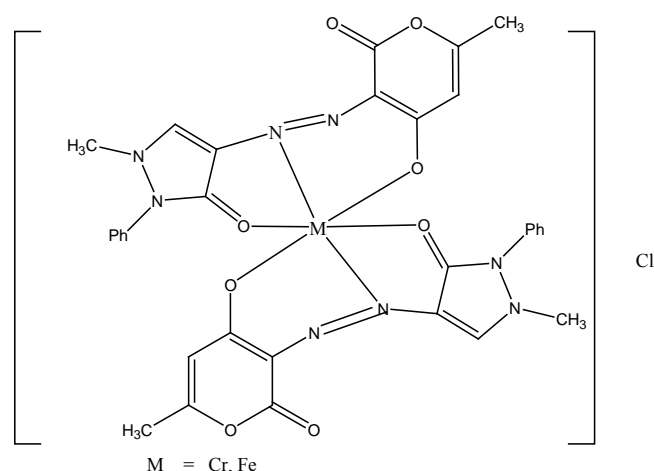
The study of azo dyes of pyrazoline-2-one and pyran-2-one derivatives has been of much interest in recent years due to their antibacterial, antiviral, anticancer, antifungal, antihelminthes and insecticidal activities.<sup>1</sup> In particular, the tautomerism of pyran-2-one ligands show a dramatic increase in the diversity of biological properties.<sup>2-4</sup> For example, some of these organic derivatives have shown hypoxia-selective cytotoxicity and they could be potentially useful for the treatment of solid tumours. Besides, some derivatives have presented excellent *M. tuberculosis* growth inhibition values, leading generally the lack of the two –N=N– and –C=O groups to the loss of the *antimycobacterial* activity.<sup>5-6</sup>

In an effort to improve bioavailability and pharmacological and toxicological properties of azo dye of 4-aminoantipyrine, many authors focused their research on the synthesis, characterization and biological evaluation of metal complexes of this family of organic compounds.<sup>7-9</sup> The spectral and magnetic studies of iron(III) complexes of hydroxyaryl azodyes, derived from antipyrine, prompted Rawther and Nair<sup>10</sup> to suggest from the IR data that the OH groups take part in co-ordination together with –N=N– nitrogen and C=O of pyrazolone ring.<sup>10</sup>

Antipyrine Schiff base derivatives can serve as antiparasitic agents and their complexes with platinum(II) and cobalt(II) ions have been shown to act as antitumor substance<sup>11</sup>. This prompted us to synthesize the Schiff base ligand, Fig. 1, containing the antipyrinyl moiety as well as the delocalized conjugated system followed by its reaction alone or mixed with 2-aminopyridine (2-ampy), 8-hydroxyquinoline (8-HOQu) or oxalic acid (Ox) with some di- and trivalent transition metal halides to gain insight into

the mode of coordination and geometry of the obtained complexes. These studies lead to several complexes with higher pharmacological activity than the free ligands, especially iron complexes bearing anti-*Mycobacterium tuberculosis* activity.

Metal complexes of azo compounds could be divided into two categories, namely the ones in which the azo group is involved in bonding and the others in which it is not. The former are derived from azo compounds containing donor functions such as OH, NH<sub>2</sub>, COOH, SH, etc., in a congenial position so as to form six or five membered chelates.<sup>12</sup> In view of the importance of such azo dye bearing NO donor atoms of pyran-2-one and pyrazoline moieties, we describe here the coordination behaviour of organic ligand derived from azo ligand of 4-hydroxy-6-methyl-pyran-2-one (HL) towards some transition elements (Mn(II), Cr(III), Fe(III), Co(II), Ni(II), Cu(II) (Scheme 1) and Zn(II).



**Figure 1.** Proposed octahedral structure of Cr(III) and Fe(III) complexes of 4-[(Z-(4-hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)-1-methyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one)] (HL) ligand, (if M=Mn, Co, Ni, or Cu(II), there is no Cl in the outer sphere).

## Experimentals

UV-visible spectra were recorded on a Shimadzu UV-Vis spectrophotometer over 200-800 nm using  $10^{-3}$  M solutions of ligands and complexes in methanol, which was used as reference.

IR spectra were recorded on a JASCO FTIR spectrophotometer 4100 in the range  $400-4000\text{cm}^{-1}$ . Elemental analysis was carried out with a CHN Carlo-Erba 1106 elemental analyzer. The %Cl contents were measured via conductometric titrations against standard solution of silver nitrate after dissolving the Zn(II) and Cd(II) complexes in concentrated nitric acid then dilution with 5%v/v methanol.

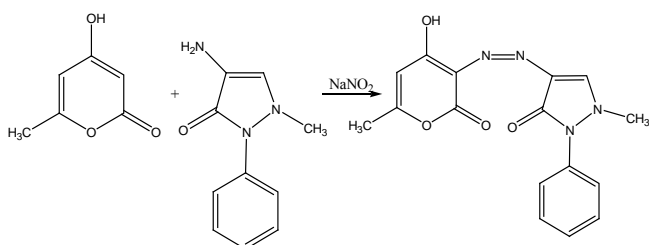
Magnetic susceptibility values were determined at room temperature on a Merck type instrument which was calibrated using  $\text{Hg}[\text{Co}(\text{NCS})_4]$  with Magnet Bruker magnetic susceptibility balance via Faradays method to deduce the magnetic moments of the solid metal complexes. The NMR spectra of free ligand was recorded in  $\text{DMSO-d}_6$  solvent on Bruker 300MHz spectrometer.

### Synthesis of diazonium salt of antipyrine

The reagents and solvents were of analytical grade and used without further purification. 4-Aminoantipyrine (0.01 mole) was diazotised by dissolving it in 1:1 HCl and adding  $\text{NaNO}_2$  (0.01 mol) solution keeping the temperature at  $0-5^\circ\text{C}$ . This product was dried over anhydrous  $\text{MgSO}_4$  and verified for purity (TLC).

### Synthesis of HL ligand

4-Aminoantipyrine (1.32 g, 0.01 mole) was diazotised and its diazonium salt was spontaneously added slowly dropwise to a well cooled alkaline solution ( $\text{pH}=8.5$ ) of 4-hydroxy-6-methyl-2-pyranone (1.26 g, 0.01 mole). The mixture was allowed to stand for 1 h. The pale brown product of HL ligand formed was filtered, washed successively with very dilute HCl and water, then recrystallised from boiling ethanol and stored over anhydrous  $\text{CaCl}_2$  in a desiccator (yield 80 %, m.p.  $120-122^\circ\text{C}$ ) (Scheme 1).



Scheme 1. Synthesis of HL ligand.

### Synthesis of metal complexes

To a methanolic solution (10 ml) of HL (1 mmole, 0.345g) a metal salt solution (1 mmole,  $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ ,  $\text{MCl}_2 \cdot 6\text{H}_2\text{O}$  ( $\text{M}=\text{Co}$ ,  $\text{Ni}$ ),  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  and  $\text{ZnCl}_2$ ) in methanol was added dropwise under stirring.

The mixture was refluxed on a steam bath for about 2-3 hours. The metal to ligand ratio was kept at 1:2 for all metal ions except for zinc(II) where it was kept at 1:1. The deep colour complexes were filtered, washed with water, methanol and finally dried in an oven. The complexes were recrystallised from  $\text{CH}_3\text{CN}-\text{EtOH}$  (1:3) mixture and stored over anhydrous  $\text{CaCl}_2$  in a desiccators.  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO-d}_6$ ,  $\delta$ , ppm): 2.4 (s,  $J=8$  Hz, 3H,  $-\text{CH}_3$ -antipyrine), 3.4 (s, 3H,  $\text{CH}_3$ -pyranone), 4.5-4.76 (s, 1H,  $\text{CH}=\text{C}$ -antipyrine), 6.9-7.2 (m,  $J=12$ Hz, 5H, Ar-H), 8.4 (d,  $J=6$  Hz, 1H, OH), 8.9 (s, H,  $\text{H}-\text{C}=\text{C}$ -pyranone).

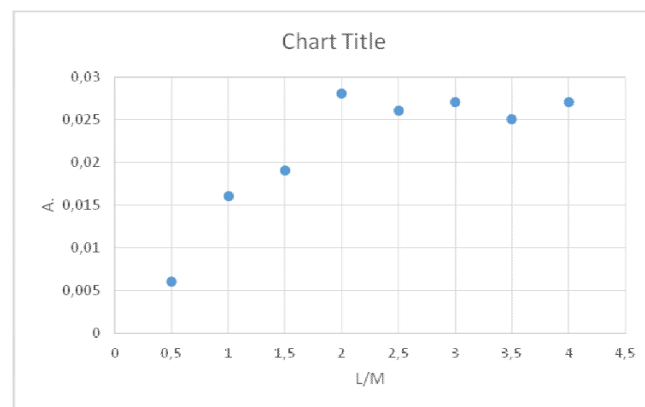
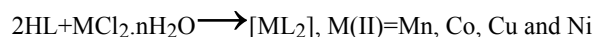


Figure 2. The absorbance-molar ratio relationship in the case of  $\text{Cu}(\text{II})-\text{L}^{1-}$  system ( $\lambda=560$  nm)

## Results and discussion

The following representative equations illustrate the formation of some of the complexes obtained



The synthesis route for the metal complexes is shown in Scheme 1. The complexes are stable solids in air, with varying shades of colouration and their structures were established from their elemental analyses, infrared and electronic and NMR spectra and magnetic moment values.

The results of the elemental analysis are in good agreement with the calculated values of 1:2 metal to ligand except for zinc(II) complexes in which 1:1 ratio was established. The complexes are completely soluble in DMF and DMSO, partially soluble in other polar solvents such as acetonitrile and methanol but are completely insoluble in non polar organic solvents. Low molar conductance values between  $27.2$  and  $38.3 \Omega^{-1} \text{cm}^2 \text{mol}^{-1}$  obtained for the complexes in DMF indicated that they are non-electrolytes and the nature of chlorine to metal bonds can be described as coordinative.<sup>13</sup>

However, the chromium and iron(III) complexes show molar conductance values in the range  $75-85 \Omega^{-1} \text{cm}^2 \text{mol}^{-1}$  confirming the electrolytic behavior in 1:1 ratio and support the presence of chloride ions in the outer sphere of the Cr(III) and Fe(III) complexes.<sup>14</sup> The analytical data and other physical properties of the complexes are recorded in Table 1.

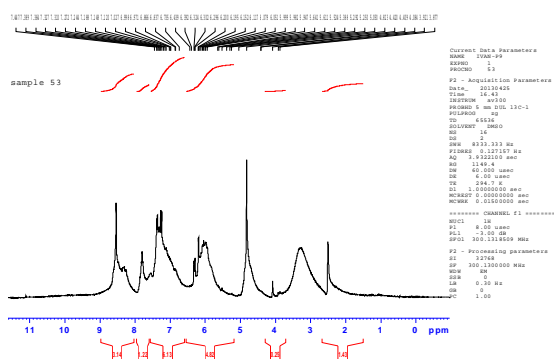
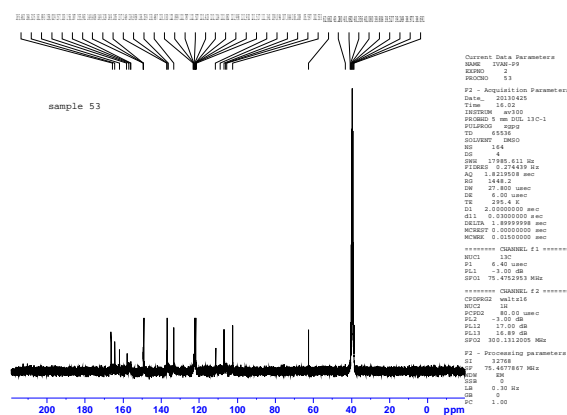
**Table 1.** Physical properties and elemental analysis of the prepared ligand HL and its metal complexes.

Compound	M.W. g mol <sup>-1</sup>	Colour	M.P. °C	C%	H%	N%	M%	Cl%
				Calc. (found)	Calc. (found)	Calc. (found)	Calc. (found)	Calc. (found)
HL	358	Pale brown	120-122	53.63(52.67)	3.92(3.21)	15.64(15.11)	-	-
[CrL <sub>2</sub> ]Cl	798	Dark green	250-252	53.045(52.17)	3.45(3.00)	15.08(15.71)	6.54(6.14)	4.44(4.33)
[FeL <sub>2</sub> ]Cl	803.5	Brown	290d	47.22(46.99)	3.30(2.72)	13.94(13.90)	6.98(5.82)	4.42(4.78)
[MnL <sub>2</sub> ]	766	Dark red	296d	50.40(49.62)	3.40(3.66)	14.66(14.77)	7.08(6.63)	-
[CoL <sub>2</sub> ]	768	Dark pink	306d	50.05(49.44)	3.91(3.44)	14.60(14.72)	7.29(6.94)	-
[NiL <sub>2</sub> ]	770	Orange	307d	49.37(49.00)	3.83(3.11)	14.40(15.11)	7.96(8.87)	-
[CuL <sub>2</sub> ]	777.6	Dark brown	290d	49.38(45.66)	3.50(2.65)	14.41(14.88)	8.20(8.09)	-
[ZnLCl]	452.5	Red	311d	24.69 (24.00)	2.80(2.13)	12.31(12.55)	13.26 (12.6)	7.84(8.33)

d=decomposition

**<sup>1</sup>H NMR spectra of the free azo ligand**

The <sup>1</sup>H NMR absorptions of the ligand in DMSO-d<sub>6</sub> is shown in Figure 3. The proton NMR spectrum of the ligand can be classified into three distinct classes, the methyl (N-CH<sub>3</sub>), (C=C-H) and hydroxyl (-OH) protons appear as singlet peaks and in the ranges 1.97-2.02, 8.9 ppm and 8.4 ppm respectively.<sup>15-18</sup> The broad singlet peaks found between 3.6-4.56 ppm are due to protons of -CH<sub>3</sub> related to pyran and N-CH<sub>3</sub> moiety respectively. However the multiple peak in the region 6.9-7.22 ppm could be attributed to resonance of aromatic and pyrazoline protons. More over, the Figure 4 shows the <sup>13</sup>C resonance of HL ligand, that records the absorptions at 155, 131, 121 to 25 ppm that are corresponded to C=O, C=N, C-N-C=C- and CH<sub>3</sub> groups respectively. The data obtained from <sup>13</sup>C NMR (Figure 4) with results of <sup>1</sup>H NMR together supports the the expected structure of azo ligand.

**Figure 3.** <sup>1</sup>H NMR spectrum of HL ligand in DMSO-d<sub>6</sub>**Figure 4.** <sup>13</sup>C NMR spectrum of HL ligand in DMSO-d<sub>6</sub>**IR spectra**

The important IR spectral bands and their assignment are given in Table 2. The free ligand in KBr disc shows broad band at 3470 cm<sup>-1</sup> which belongs to OH, thus it confirms the keto-enol tautomerism of the free azo ligand. The strong absorptions at 1450-1510 cm<sup>-1</sup> and 1730-1655 cm<sup>-1</sup> are typical for -N=N- and C=O(pyranone) moieties respectively.<sup>19</sup> The band due to C-O-C stretch of the pyranone ring was observed at 1115-1122 cm<sup>-1</sup>. In the IR spectra of the complexes, the band in the region ~ 1700-1625 cm<sup>-1</sup> gives a clear indication of chelated C=O group suggesting involvement of carbonyl oxygen of pyrazolone ring in the coordination with metal ions.

**Table 2.** FT-IR data of azo ligand and its metal complexes.

Symbol	$\nu$ C=O, $\nu$ OH	$\nu$ C=C, $\nu$ N=N-	$\nu$ M-N, $\nu$ M-O	Other bands, $\nu$ C-H, C-O
HL	1730, 1653, 3470	1630, 1440-1510(s)		3100(w), 2966, 1115-1122
CrL <sub>2</sub> Cl	1690(s)-1645	1590, 1433-1490	533(w), 420(m), 260-300	3033(w), 2972(m), 1110
MnL <sub>2</sub>	1678-1633(s)	1570, 1420-1500	530, 428	3023, 2982, 1200
FeL <sub>2</sub> Cl	1655-1642(s)	1541-1599(s), 1444	555(w), 470, 336	3030, 2882, 1122
CoL <sub>2</sub>	1674-1633(s)	1568-1606, 1433	555(w), 473(w)	3040, 2950, 1120
NiL <sub>2</sub>	1685-1625(s)	1585-1614(s), 1422	538, 430(w)	3094(br), 1111
CuL <sub>2</sub>	1700-1661(s)	1566-1606(s), 1418	560(m), 450(w),	3022(w), 1118
ZnLCl	1680-1633(s)	1544-1588(s), 1419	545, 450(m), 265(w)	2675(m), 1109(s)

s=strong, m=medium, br=broad, w=weak

As well as, the downshift in  $\text{-N=N-}$  vibration supports the participation of nitrogen atom in  $\beta$ -position in bonding with the metal ions. The weak to medium bands at  $560\text{-}530$  and  $420\text{-}477\text{ cm}^{-1}$  were due to vibrational modes of M-N and M-O bands, respectively.<sup>20-21</sup>

### Electronic spectra and magnetic moments of metal complexes

The free azo ligand solution in methanol displays two distinct peaks at  $280$  and  $375\text{ nm}$  that are characterized of  $\text{-N=N-}$ ,  $\text{C=O}$  and other chromophore groups.<sup>22</sup> The magnetic moment value of Cr(III) complex was  $3.44\text{ B.M}$  suggested an octahedral geometry.<sup>22-23</sup> The conspicuous electronic spectral bands were at  $470\text{-}580\text{ nm}$  due to  ${}^4\text{A}_{2g}(\text{F}) \rightarrow {}^4\text{T}_{1g}(\text{F})$  and  ${}^4\text{A}_{2g}(\text{F}) \rightarrow {}^4\text{T}_{2g}(\text{F})$  transitions, respectively, that remarkably gave additional support to octahedral geometry of the Cr(III) complex. The brown solution of iron(III) complex in DMF showed spectral bands which were overlapped with CT bands. The bands observed at  $577\text{ nm}$  could very well be due to  ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{1g}$  transition. The magnetic moment value of  $5.64\text{ B.M}$  indicated the octahedral geometry.<sup>23</sup> The spectrum of Ni(II) complex was characteristic of an octahedral geometry with prominent bands at  $380\text{ nm}$  ( ${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}(\text{P})$ ) and  $670\text{ nm}$  ( ${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}(\text{F})$ ). The magnetic moment of  $2.78\text{ B.M.}$  also suggested an octahedral geometry. The Cu(II) complex was brown in colour due to strong C.T bands tailing off into the blue end of the visible region. The magnetic moment value ( $1.74\text{ B.M.}$ ) lies well within the expected range ( $1.7\text{-}2.2\text{ B.M.}$ ). The dark red solution of Zn(II) complex was diamagnetic and the spectral peaks were all similar to those of the ligand as d-d transitions were absent.<sup>24</sup> As the same d-d transitions for Cr(III), Fe(III), Mn(II) and Cu(II) complexes, the cobalt(II) and nickel(II) solutions in DMF exhibit spin-allowed transitions in the regions  $400\text{-}530$  and  $560\text{-}610\text{ nm}$  confirming the  ${}^4\text{T}_{1g} \rightarrow {}^4\text{T}_{2g}(\text{F})$ ,  ${}^4\text{T}_{1g} \rightarrow {}^4\text{A}_{2g}(\text{F})$  and metal to ligand charge transfer LMCT transitions, respectively, these agree well with the observed values of magnetic moments.<sup>25</sup> The red solution of zinc(II) complex in DMF showed high intensity peaks at  $225$  and  $367\text{ nm}$  due to electronic transitions of  $\text{-C=C}$ ,  $\text{C=O}$ , and  $\text{N=N-}$  chromophores and LMCT, respectively.<sup>25</sup>

**Table 3.** Electronic spectra, molar conductance and magnetic moments of the prepared metal complexes

Compound	$\lambda_{\text{max}}$ , nm	$\nu$ , $\text{cm}^{-1}$	$\Lambda$ , $\text{S cm}^2 \text{ mol}^{-1}$	$\mu$ , B.M.
HL	375 280	26666 35714	10	-
[CrL <sub>2</sub> ]Cl	344 570 580	29069 17543 17241	65	3.44
[FeL <sub>2</sub> ]Cl	300 577	33333 17331	58	5.64
[MnL <sub>2</sub> ]	341 433	29325 23094	40	5.5
[CoL <sub>2</sub> ]	400 533	25000 18761	23	3.44
[NiL <sub>2</sub> ]	288 566 610	34722 17667 16393	25	2.98
[CuL <sub>2</sub> ]	340 560	29411 17857	22	1.74
[ZnLCl]	367 225	27247 44444	28	0

$\Lambda$ =molar conductance in DMF solutions at  $0.001\text{ M}$  concentration.

### Conclusion

A series of novel chromium(III), iron(III), manganese(II), cobalt(II), nickel(II), copper(II) and zinc(II) complexes with a new azo ligand 4-[(Z-(4-hydroxy-6-methyl-2-oxo-2H-pyran-3-yl)-1-methyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one) were synthesized. The free ligand and the isolated solid metal complexes were fully characterized on the basis of C.H.N.M. elemental analyses,  ${}^1\text{H}$  and  ${}^{13}\text{C}$  NMR, FTIR and UV-Visible spectroscopic results. The formula of metal complexes were found to be  $[\text{ML}_2]\text{Cl}_n$ , for the  $\text{M}=\text{Cr}$ ,  $\text{Fe(III)}$  if  $n=1$  and  $\text{M}=\text{Mn}$ ,  $\text{Co}$ ,  $\text{Ni}$  and  $\text{Cu(II)}$  for  $n=0$ , while the zinc(II) complex has tetrahedral geometry with the formula  $[\text{ZnLCl}]$ . The infrared spectral data revealed that the new azo compound HL behaves as tridentate Lewis base via the  $\beta$ -nitrogen atom of  $\text{-N=N-}$  group and two oxygen atoms of  $\text{-deprotonated OH}$  and  $\text{-C=O}$  of 2-pyranone and pyrazoline moieties, respectively. According to the results obtained from elemental analyses, molar conductivity measurements in DMF solutions and magnetic susceptibility data, the octahedral structures for all metal(II) complexes except the zinc(II), which has tetrahedral structure, were proposed.

### References

- Chalaca, M. Z., Figueroa-Villar, J. D., Ellena, J. A. and Castellano, E. E., *Inorg. Chim. Acta*, **2002**, 328, 45.
- Bouchama, A., Bendaâs, A., Chiter, C., Beghidja A. and Djedouani, A., *Acta Cryst., Section E*, **2007**, 63(9), 1205-1207.
- Khalil, A., Hassan, M. A., Mohamed, M. M., Elsayed, A. M., *Dyes Pigments*, **2005**, 66, 241-245.
- Weisberger, A., Wiley R. H., Wiley, P., Eds. *The chemistry of heterocyclic compounds. Pyrazolinones, pyrazolidones and derivatives*, John Wiley, New York. **1964**
- Gupta, D. R. and Gupta, R. S., *J. Ind. Chem. Soc.*, **1966**, 43(5), 377-379.
- Gupta, D. R. and Ojha, A. C., *J. Ind. Chem. Soc.*, **1970**, 47(12), 1207-1208.
- Rawther, S. and Nair, M. R. G., *J. Ind. Chem. Soc.*, **1992**, 69, 157-161.
- Hassib, H. B., Abdel Latif, S. A., *Spectrochim. Acta, Part A*. **2003**, 59, 2425-2434.
- Kratzl, K., Fostel H. and Sobczak, R., *Monatsh. Chem.*, **1972**, 103(9), 677.
- Makedonski, P. B., Johannes, H. H., Wichem, J., Grahn, W., Kowalsky, W., *Dyes Pigments*, **2004**, 16, 109-119.
- Aysha, T., Lycka, A., Lunák, S. Jr., Machalický, O., Elsedik, M. and Hrdina, R., *Dyes Pigments*, **2013**, 98, 547-556.
- Gup, R., Kirkan, B., *Spectrochim. Acta*, **2005**, 62, 1188-1195.
- Geary, W. J., *Coord. Chem. Rev.*, **1971**, 7(1), 81-122.
- Kruger P. J. and Smith, D. W. *Can. J. Chem.*, **1967**, 45(14), 1611-1618.
- Porter, Q. N. and Balda, J., *Mass Spectrometry of Heterocyclic compounds* (Weissberger, A. and Taylor, E. C., eds) Wiley-Interscience, John. Wiley and Sons, Inc., USA, **1971**.
- Budzikiewicz, H., Djerassi, C. and Williams, D. H., *Mass spectrometry of Organic Compounds*, Holden-Day, **1967**, San Francisco.
- Dyer, J. R. *Application of absorption spectroscopy of organic compounds*, Prentice Hall, Eaglewood Cliffs, N. J. **1965**

- <sup>18</sup>Batterham, T. J., *NMR Spectra of Simple heterocycles* (Taylor, E. C. and Weissberger, A., eds. *General Heterocyclic Chemistry Series*, John Wiley and Sons, Inc. New York, USA. **1973**.
- <sup>19</sup>Silverstein, R. M., Webster, F. X., *Spectrometric Identification of Organic Compounds*, John Wiley and Sons, Inc., New York, **1998**.
- <sup>20</sup>Nakamoto, K., *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, Wiley, New York, **1986**.
- <sup>21</sup>Drago, R. S. *Physical Methods for Chemists*, 2<sup>nd</sup>. edition, Saunders Colleges Publishing, New York. **1977**.
- <sup>22</sup>Sutton, D., *Electronic Spectra of Transition Metal Complexes*, McGraw Hill, , London, **1968**.
- <sup>23</sup> Dutta, R. L. and Syamal, A., *Elements of Magnetochemistry*, **1992**
- <sup>24</sup>Cotton, F. A. and Wilkinson, G., *Advanced Inorganic Chemistry*, John Wiley, New York, **1980**.
- <sup>25</sup>Seth, S., Aravindakshan, K. K., *Spectrochim. Acta Part A Mol. Biomol. Spectr.*, **2013**, *2(1)*, 415-422.

Received: 10.03.2014.

Accepted: 06.04.2014.