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Original Research

Protolitic Equilibria and Stability Constants of Mn (II) and Ni (II) Complexes of 3-formylpyridine Thiosemicarbazone in Sodium Dodecyl Sulphate(SDS)-Water Mixture

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Abstract

Protonation constant of nicotinaldehyde thiosemicarbazone (NTSC) and its complexation with Mn (II) and Ni (II) were studied in SDS-water (1.15%w/v) mixture at 303K under nitrogen atmosphere and an ionic strength of 0.1 mol dm^{-3} pH-potentiometrically. The protonation constant of the ligand and stability constant of its Mn (II) and Ni (II) complexes were calculated by using MINIQUAD 75 computer program, and species distribution diagrams were produced using the HySS computer program. Selection of the best fit chemical models is based on statistical parameters. The predominant species detected for both metals for 1:1 and 1:2 ratios were ML, MLH; ML_2, ML_2H, and ML_2H_2 respectively. The appropriateness of experimental conditions is verified by introducing errors deliberately in the concentrations of ingredients of the solution. Distribution diagram and plausible equilibria for the formation and possible structure of the complex species are also presented.

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INTRODUCTION

Transition metal complexes of thiosemicarbazone have been receiving considerable attention largely of chemists and pharmacologists because of their wide range of biological and carcinostatic activities: antibacterial, antifungal, antiviral and anti-inflammatory agents (Beraldo and Gambino, 2004), antitumour, anticancer, and anti-HIV (Chandra and Kumar, 2007). They are also considered as excellent analytical reagents used in gravimetric and spectrophotometric determinations (Enyedy, 2010). They are interestingly promising in their use against cancer because of their selectivity. The main known effects related to their anticancer activity (Enyedy, 2011) are ribonucleotide reductase (RR) inhibition (Brockman *et al*., 1970), reactive oxygen species (ROS) production(Shao *et al*., 2006), topoisomerase II inhibition (Hall *et al*., 2000), mitochondria disruption (Yuan *et al*., 2004), and, more recently, a multidrug resistance protein (MDR1) inhibition (Ludwig *et al*., 2006 and Wu *et al*., 2007).

Although the free ligands of thiosemicarbazones showed interesting biological activity, transition metal complexes revealed greater biological activity (Pelosi, 2010; John *et al*., 2004; Belicchi-Ferrari *et al*., 2005; Gulea *et al*., 2004; Birca *et al*., 2004). And also studies made known that the metal complexes exert differential activity against the organisms' studied (Williams, 1972). This indicates that coordination to the metal activates the biologically active ligand and the metal serves to trigger the biologically active thiosemicarbazone ligand. Still the activity of the thiosemicarbazones is affected by the type and kind of substituent to the thiosemicarbazone moeity (Liberta and West, 1992). The coupling of thiosemicarbazones with metals, gives good biological activity due to the synergistic effect.

In solution thiosemicarbazones are known to exist in thiol form tautomerizing from the thione form. Complexation usually takes place via dissociation of the acidic proton, resulting in the formation of usually four-membered or depending on the alkyl/aryl group a five-membered chelate ring (Pal *et al*., 2002). Nicotinaldehyde thiosemicarbazone (NTSC) is used as anti tubercular drug combined with isoniazid (isonicotinic acid hydrazide, INH) (Grunberg and Leiwant, 1951). To understand the transport of thiosemicarbazone complexes in vivo it is essential to report the solution phase complexation of this ligand with some of the essential metal ions [Mn (II) and Ni (II)]. Thus, in this study the protonation constant of NTSC and its stability constant with Mn (II) and Ni (II) is reported and also the possible structures of the complexes are proposed.

MATERIALS AND METHODS

Reagent and Apparatus

Chlorides of Mn (II) and Ni (II), EDTA disodium dehydrate, sodium hydroxide pellets, potassium hydrogen phthalate (KHP) and oxalic acid were purchased from E.Merck.Pro.analysi. Nicotinaldehyde thiosemicarbazone (NTSC) was supplied by Fluka, AR and Sodium chloride was obtained from B.D.H.AnalaR. All reagents were analytical grade.

Stock solution of (0.1mole dm^{-3}) chloride of Mn (II) and Ni (II) was prepared with slight acidification to repress the hydrolysis of the metal ion(Baes Jr. and Mesmer, 1976) in deionized water and standardized complexometerically with EDTA(Birk, J.P. 1970). A~0.05 mole dm^{-3} solution of NTSC was prepared freshly just before the titrations in 0.10 mol dm $^{-3}$ HCl. The solutions of sodium hydroxide, potassium hydrogen phthalate and sodium chloride were prepared as reported elsewhere. The NaOH solution was standardized with oxalic acid solution (0.05 mole dm^{-3}) and KHP (0.05 mole dm^{-3}) and the standard alkali solution was again used for the standardization of HCl. The strength of the prepared carbonate-free sodium hydroxide solution was determined by titrating it against hydrochloric acid solution using the Gran plot method(Gran, G 1988).

The pH-metric titrations were carried out with a Control Dynamics pH meter model APX 175 E/C in conjunction with a 0-14 pH range a combined glass calomel electrode. The pH meter was standardized before each titration with standard buffer solutions of pH 4.00, 7.00, and 9.00. A tip less double walled Pyrex glass vessel of 100 ml capacity fitted with a Perspex lid, through which the glass calomel electrode, gas inlet and out let tubes and burette tip were admitted, was used for carrying out the potentiometric titrations and the test solution was degasified with nitrogen gas. The temperature of the solution was maintained by passing water from a thermostat through the annular space between the walls of the titration cell.

Data Acquisition and analysis

Calvin-Wilson titration technique modified by Rossotti (Irving and Rossotti, 1953 and 1954) was employed for the study of protonation and complex equilibria of the ligand. Requisite volumes of hydrochloric acid (to give an overall concentration of 2.0 × 10^{-2} mole.dm^{-3}), sodium chloride (ionic strength was maintained at 0.1 mole.dm^{-3}), ligand solution in SDS-water(1.15% w/v) in the presence and absence of metal ions in a total volume of 50 cm^3 was titrated with ~0.2 mole.dm^{-3} sodium hydroxide at 303 K. The concentration of the ligand was 0.005 to 0.015 mol.dm^{-3}in different experiments. The same titrations were repeated in the presence of metal ion at 1:1, and 1:2 ratios of initial concentrations of metal to ligand. All the titrations are performed in triplicate by changing the concentrations of ligand to metal ratio in the total initial volume. After addition of each aliquot (0.1 ml) of sodium hydroxide, the pH - meter dial reading was recorded at regular intervals of time until two successive readings do not differ in more than 0.01 pH units. The titrations were performed up to a pH of ~12.0. In some of the titrations, the upper pH limit for rejecting data was determined by the appearance of opalescence leading to precipitation.

For a system containing a metal ion and a ligand forming N complexes, the formation of a complex can be represented as,

$$m.\mathrm{M}+l.\mathrm{L}+h\,\mathrm{H} \rightleftharpoons \mathrm{M}_m\mathrm{L}_l\mathrm{H}_h$$

and the overall formation constant is given by,

$$\beta_{mlh} = [\mathrm{M}_m\mathrm{L}_l\mathrm{H}_h]_i \,/\, [\mathrm{M}]_i^{m}.[\mathrm{L}]_i^{l}.[\mathrm{H}]_i^{h}$$

Where $[\mathrm{M}]_i$, $[\mathrm{L}]_i$ and $[\mathrm{H}]_i$ are the free concentrations of metal, ligand and hydrogen ion respectively at ith experimental point. Different species in solution possess different values of stoichiometric coefficients *m, l* and *h.* Positive value of h indicates protonated species and negative value indicates either deprotonated or hydroxylated species. Several computer programs have been proposed for the evaluation of both stoichiometric coefficients and formation constants for all the species present in solution. Most of these algorithms are based on the minimization of error square sum (U) between the experimentally determined quantities and the corresponding calculated ones using the estimated values of the formation constants. The values of the formation constants obtained by graphical procedures are taken as initial estimates. The computer based algorithms generate a vector of shifts to the formation constants and free concentrations. The modified constants after applying the shifts are then used as the initial estimates for the next cycle. If the chosen model is a true representation of the system and the initial estimates of formation constants are not far from the true values, the iteration will converge in a finite number of cycles. The procedure is repeated for different chemical models and the best-fit model is selected basing on the statistical parameters. The potentiometric titration data obtained in the present investigation was subjected to analysis by Miniquad-75 program (Gans *et al*., 1976). This program is robust and uses both constrained least-squares and Marquardt methods to achieve convergence even in extreme cases of non-convergence. Species distribution diagrams for all the systems under study were generated using HySS (Alderighi *et al*., 1999) program. The data from different experiments with different metal to ligand ratios were refined separately using Miniquad-75 program to yield species relevant to that particular composition. This way of refinement has been found to be better (Braibanti *et al*., 1982) compared to analysing the combined data from all the titrations at a time since the main part of the error in the stability constants derives from the variability from one titration to another. Therefore, the authors processed the data from different compositions separately using Miniquad-75 program. The best-fit models were selected on the basis of U (sum of the squares of residuals in mass balance equations), standard deviations in formation constants and other statistics like χ^2 test which tests the distribution of errors against a normal one.

RESULTS AND DISCUSSION

Proton Ligand Equilibria of Nicotinaldehyde Thiosemicarbazone (NTSC)

A perusal of potentiometric titration data of NTSC indicates association of protons in the lower pH region and dissociation at higher pH. Figure 1 shows the protonation and deprotonation equilibria of NTSC . The best fit model obtained using Miniquad-75 program shown in Table 1 contained two stability constants β_{011} and β_{012} corresponding to the formation of LH^+ and H_2L^{2+}.

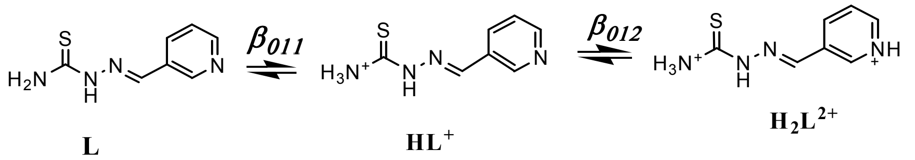


Figure 1: Proton-ligand equilibria of NTSC

Figure 2 shows the speciation of NTSC in its three forms from pH 2.4 to pH 11. At pH 11, about 24.2 % of the ligand was in LH form, and about 90.5 % was in L form. At pH's above pH 8.95, the most predominant species was L. Between pH 8.95 and pH 3.02, the most predominant species was LH. LH_2 was the most predominant species at pH's below pH 3.02. At pH 2.61, 98.6 % of the ligand is LH_2, the other only 0.4 was LH.

Table 1: Protonation constants of NTSC (nicotinaldehyde thiosemicarbazone) in 1.15 %(w/v) SDS-water mixture at 30°C and ionic strength 0.1 M (NaCl)

log β0lh (SD)		**Np**	**U_{corr}**	**χ^2**	**Skewness**	**kurtosis**	**R-Factor**	**pH-Range**
β 011	**β012**							
10.47 (1)	*14.90 (2)*	85	6.03	10.21	-0.22	3.5	0.01635	2.4-11.0

U_{corr}=Ux10^{-8}/NP-m; NP= number of points; m= number of protonation constants; SD=standard deviation

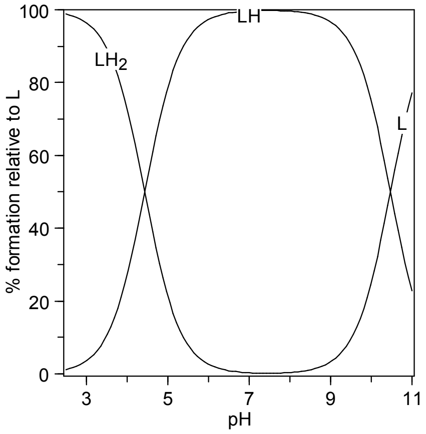
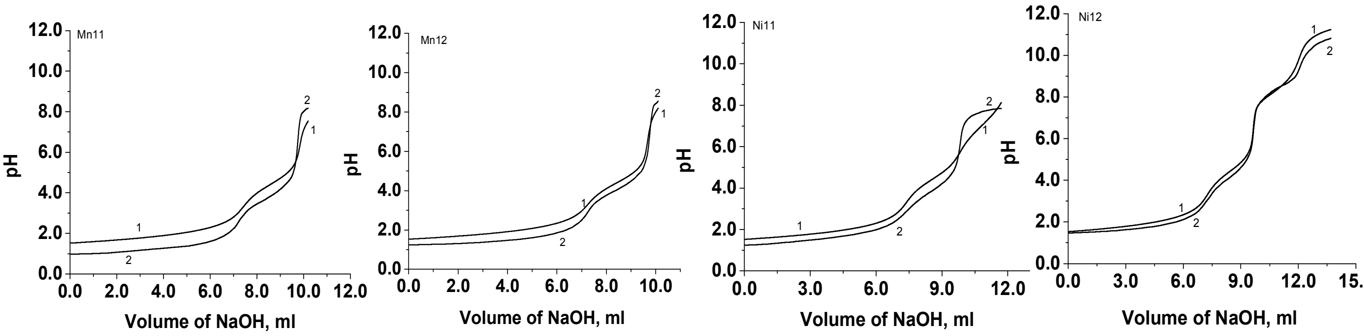


Figure 2: Distribution diagrams of protonated and deprotonated NTSC species in 1.15% w/v SDS-water mixture

Metal-ligand Equilibria

The solutions of ligand, and acid in the presence of metals were titrated with standard NaOH solution to determine the stability constants of complexes formed by Mn (II) and Ni (II) ions. The data obtained from metal ligand titrations was first subjected to analysis by classical procedures (Irving and Rossotti, 1953) to get the formation constants of simple mono-nuclear complexes ML and ML_2. Simulated titration curves were then generated using a computer program SOPHD developed in our laboratory to see whether these species satisfy the experimental data. The simulated titration curves thus obtained were plotted along with the experimental ones to identify the regions of pH where they differ. Titration curves for all the compositions are shown in Figure 3. The wide difference between the simulated and experimental curves reveals the presence of other major species in addition to simple mono-nuclear complexes. Different chemical models containing chemically plausible species depending on the nature of the ligand, metal and the pH region of difference in the curves were tested using the Miniquad-75 program. The required initial estimates of the formation constants were calculated basing on the formation constants of simple complexes and protonation/ deprotonation constants of the ligand.



a) (b)

Figure 3: Simulated and experimental titration curves for (a) Mn (II)-NTSC and (b) nickel (II)- NTSC systems for 1:1 and 1:2 (1.Simulated titration curve 2. Experimental titration curve) respectively

Various chemical models including all the plausible species ML, MLH, ML_2, ML_2H, ML_2H_2, MLH_{-1}, and MLH_{-2}, were tested using Miniquad-75 program. The best-fit models thus obtained for metal-NTSC is presented in Table 2. The investigation revealed that the best-fit models ML and MLH for 1:1 ratio ; ML_2, ML_2H, and ML_2H_2 for 1:2 ratio species between the ligand and Mn(II) and Ni(II) ions are formed depending on pH. The formation constants are in good agreement with Irving-Williams order(Irving and Rossotti, 1953;Irving and Williams, 1948) i.e. $Mn^{II} < Ni^{II}$.

The species distribution diagrams for metal-ligand systems (Fig.4) gives the concentration of formation of various species as a function of pH. Nicotinaldehyde thiosemicarbazone has two associable protons and one dissociable proton from tautomerization of thione. The different forms of NTSC are,H_2L^{2+}, LH^+ and L in the pH ranges below 2.5–6.0, 3.5-11.0 and above 9.0, respectively. The plausible binary metal-ligand complexes were predicted from the protonation data. The present investigation reveals the existence of ML, MLH, ML_2H, ML_2, and ML_2H_2, for both Mn(II) and Ni(II).Charges of complex compounds are omitted for simplicity unless otherwise.

The typical species distribution diagrams of metal ligand complexes of various systems are shown in Figure 4 which indicates the formation of NTSC complexes of Mn (II) and Ni (II) in the pH ranges 2.5–11.0, and 2.6–10.0 respectively. The protonated species MLH and ML_2H_2 are formed at low pH and ML_2H is formed at higher pH for both Mn (II) and Ni (II). ML species for both metals is formed at higher pH. The formations of various nicotinaldehyde thiosemicarbazone complex species are shown in the following equilibria:

$$M(II) + LH_2^+ \rightleftharpoons MLH^{2+} + H^+ \quad (1)$$

$$M(II) + 2LH_2^+ \rightleftharpoons ML_2H_2^{2+} + 2H^+ \quad (2)$$

$$M(II) + 2LH \rightleftharpoons ML_2H^+ + H^+ \quad (3)$$

$$MLH^{2+} \rightleftharpoons ML^+ + H^+ \quad (4)$$

$$M(II) + 2\,LH \rightleftharpoons ML_2 + 2\,H^+ \quad (5)$$

$$ML_2H_2^{2+} \rightleftharpoons ML_2H + H^+ \quad (6)$$

$$ML_2H^+ \rightleftharpoons ML_2 + H^+ \quad (7)$$

The typical distribution curves of species in binary mixtures are shown in Figure 4. The most important features of distribution diagrams are the pH limit of evolving and disappearance of compounds. At low pH the protonated species MLH, ML_2H and ML_2H_2 are prevalent and predominant from pH = 2.5 to pH = 8.0 for both 1:1 and 1:2 ratios. Beyond pH = 7.0 the non-protonated ML, and ML_2 complexes appear. The main metal species in both systems are ML, MLH, ML_2H, ML_2H_2. MLH is observed as a main species in acidic region which is gradually converted to ML, and ML_2 by increasing the solution pH.

A series of complex equilibria may be responsible for producing the ML_2H and MLH complexes. Simultaneous collision of M, and LH and decomposition of ML_2H_2 at pH about 6. whereas, the MLH is probably produced by combination of M and LH_2 followed by a fast deprotonation at pH below 6. This suggested that the formation pattern could be supported from the sharp descending of LH_2 concentration in the 2.5-6.0 pH region. In 1:1 ratio in the basic region ML was the only predominant species for both metal ions and a similar pattern is observed in 1:2 system wherein, the ML_2 species becomes the main species in basic region (Fig. 4 B&D). For both ratios in the physiological pH range the protonated species are the predominant ones.

Table 2: Parameters of best-fit chemical models of NTSC complexes of Mn (II) and Ni (II) in SDS-water mixtures (1.15% w/v) (Temperature = 303 K, ionic strength = $0.10 mol\ L^{-1}$)

Metal	*Log β_{mlh} (SD)*			Np	U_{corr}	χ^2	Skewness	Kurtosis	R-Factor	pH-Range
				1:1						
	110	**111**	------							
Mn(II)	4.32(5)	13.20(2)	------	28	1.27	30.48	0.13	4.40	0.00661	2.5-10.0
Ni(II)	5.63(4)	13.22(5)	-------	41	9.4	81.42	0.61	7.53	0.01886	2.5-9.0
				1:2						
	120	**121**	**122**							
Mn(II)	7.85(5)	16.88 (5)	24.14 (7)	25	0.76	28.57	-0.64	4.55	0.00575	2.5-11.0
Ni(II)	8.98(2)	17.25(5)	24.82(8)	46	0.38	43.10	-0.12	4.21	0.00438	2.8-10.0

U_{corr}=Ux10^{-8}/NP-m; NP= number of points; m= number of species; SD=standard deviation

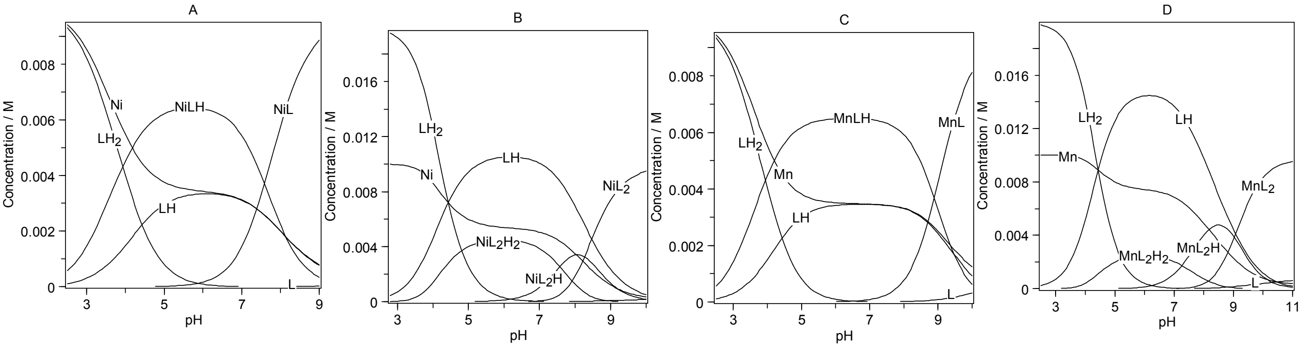


Figure 4: Distribution diagram of NTSC-Metal complexes in 1.15 %(w/v) SDS-water mixture. (A) & (B) are 1:1 &1:2 ratios for Ni(II) and (C) &(D) graphs are 1:1 &1:2 ratios for Mn(II) complexes.

Effect of Systematic Errors on Best Fit Model

Error in the concentrations of alkali, mineral acid, metal and ligand affects the magnitudes of stability constants. Such parameters are said to be influential parameters. Since there is no way to directly study the effect of systematic errors in the influential parameters on the magnitude of stability constant in MINIQUAD75, the best to do is provision of pessimistic errors in the concentrations of mineral acid, alkali, ligand, and metal.

Table 3: Effect of errors in influential parameters on Mn (II)-NTSC complex stability constants in SDS-water mixture (1.15% w/v)

Ingredient	% Error	log β_{mlh} (SD)	
		110	111
	0	4.32(5)	13.20(2)
Acid	-5	5.15 (8)	14.05 (3)
	-2	rejected	13.83 (3)
	+2	3.64(49)	13.49 (3)
	+5	rejected	13.16(8)
Alkali	-5	rejected	11.12 (88)
	-2	rejected	13.37 (7)
	+2	5.06 (7)	14.02 (3)
	+5	5.67(13)	14.31(8)
Ligand	-5	4.34 (6)	13.63 (1)
	-2	4.38(6)	13.69 (1)
	+2	4.42 (7)	13.77 (3)
	+5	4.46(7)	13.83 (2)
Metal	-5	4.42 (7)	13.75 (2)
	-2	4.41(7)	13.73 (2)
	+2	4.36 (6)	13.70 (1)
	+5	4.34 (6)	13.68 (1)

This may help to critically evaluate the best fit model because the data acquisition was done under varied experimental conditions with different accuracies. The results of a typical system given in Table 3 emphasize that the order of the ingredients that influence the magnitudes of stability constants due to incorporation of errors is alkali > acid > ligand > metal. As seen from the results in the table species are even rejected when errors are introduced in the concentrations of the alkali and acid. The rejection of species and increased standard deviations in the stability constants on introduction of errors confirm the appropriateness of the experimental conditions (like concentrations) and the choice of the best fit models.

Structures of Complexes

As depicted from structural studies, most thiosemicarbazone ligands coordinate through oxygen, nitrogen and sulfur donor atoms in their (N, S) bidentate form or (N, N, S or O, N, S) tridentate form, to form metallic complexes of varied molecular geometry (Pal *et al*., 2002; Bakkar *et al*., 2003; El-Shazly *et al*., 2006; Rios and Valcarcel, 1985; Hans Stiinzi, 1981). Thus, Nicotinaldehyde thiosemicarbazone is expected to coordinate as (N, S) bidentate form to produce metallic complexes with transition metal ions with distinct molecular geometry. Mono-and bis- NTSC complexes have been identified for M (II)-NTSC systems of the transition metal ions. Even though NTSC has five potential donor sites, the structural studies show thiosemicarbazones coordinate through the azomethine nitrogen and sulfur of thiol. Therefore, in the complexes of Mn(II) and Ni(II)-NTSC for protonated complexes the protons can likely be ascribed to the non-coordinating hydrazinic nitrogen atom and pyridine moiety and coordination occurs through azomethine nitrogen and thiolate sulfur donor set (N, S^-). The simple species ML_2 assumedly contain ligands coordinated to Ni (II) and Mn (II) through azomethine nitrogen and thiolate sulfur donor atoms as illustrated in Figure 5. Hence, octahedral structures are proposed for the complexes of Ni (II) and Mn (II) with NTSC.

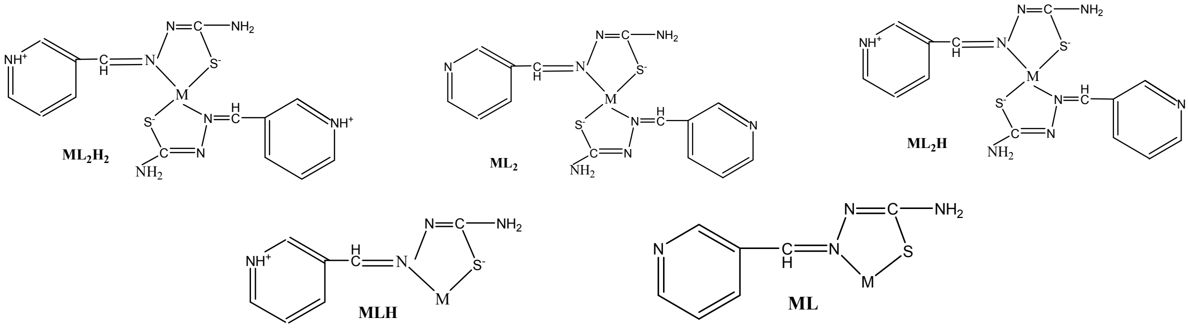


Figure 4: Proposed Structures of M (II) - NTSC complexes of Ni (II) and Mn (II)

CONCLUSIONS

In the present work, pH-potentiometric speciation study was performed to determine protonation constant of ligand and stability constant of complex to assess binary species for NTSC with Mn(II) and Ni (II) metal ions in 1.15%(w/v) SDS-water mixture. The common species formed are ML, MLH for 1:1 ratio and ML_2H, ML_2H_2 and ML_2 for 1:2 ratio for both metals. Compared to Mn (II), Ni (II) forms similar but more stable complexes as expected. At the physiological pH range the ML_2H complex is charge neutral which enables an easier passage across the cell membrane. It was also concluded that the formation of protonated complexes is favorable with both metals in the physiological pH range. The order of ingredients influencing the magnitudes of stability constants due to intentional incorporation of errors in their concentrations is alkali> acid> ligand > metal.

Conflict of Interest

Conflict of interest none declared.

REFERENCES

Alderighi, L., Gans, P., Ienco, A., Peters, D., Sabatini, A., Vacca, A. (1999). Hyperquad simulation and speciation (HySS): a utility program for the investigation of equilibria involving soluble and partially soluble species. *Coordination Chemistry Review* 184: 311-318.

Baes Jr. C.F. and Mesmer, R.E. (1976) The hydrolysis of cations. Oak Ridge National Laboratory.

Bakkar, M.S., Siddiqi, M.Y. and Monshi, M.S. (2003). Preparation and investigation of the bonding mode in the complexes of Pt(II) with thiosemicarbazone ligands. *Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry* 33(7): 1157-1169.

Belicchi-Ferrari, M., Bisceglie, F., Casoli, C., Durot, S., Morgenstern-Badarau I., Pelosi,G., Pilotti, E., Pinelli, S., Tarasconi, P. (2005). Copper(II) and Cobalt(III) Pyridoxal Thiosemicarbazone Complexes with Nitroprusside as Counterion: Syntheses, Electronic Properties, and Antileukemic Activity. *Journal of Medicinal Chemistry 48*(5): 1671-1675.

Beraldo, H. and Gambino, D. (2004). The wide pharmacological versatility of semicarbazones, thiosemicarbazones and their metal complexes. *Mini-Reviews in Medicinal Chemistry* 4: 31-39.

Birca, M., Tapcov,V., Prisacari, V., Gulea, A., Buraciova, S. (2004). Synthesis and antimicrobial properties of Cu complexes with thiosemicarbazones of substituted salicylic aldehydes. *COFrRoCA 2004, Actes du Colloque Franco-Roumain de Chimie Applique, 3^{rd} Bacau, Romania, sept. 22-26,* 44-45 (Fr). Rome. ISBN: 973-8392-36-5.

Birk, J.P. (1970). Mechanism of the Reduction of Vanadium(V) by Hexacyanoferrate(II) in Acidic Aqueous Solution. *Inorganic Chemistry* 9: 125.

Braibanti, A., Dallawali, F., Mori, G. and Vigonl, B. (1982). Analysis of variance applied to determinations of equilibrium constants *Talanta* 29: 725-731.

Brockman, R.W., Sidwell, R.W., Arnett, G., and Shaddix, S. (1970). Heterocyclic thiosemicarbazones: correlation between structure, inhibition of ribonucleotide reductase, and inhibition of DNA viruses. *Proceedings of the Society for Experimental Biology and Medicine* 133:609-614.

Chandra, S. and Kumar, A. (2007). Spectral, IR and magnetic studies of Mn(II), Co(II), Ni(II) and Cu(II) complexes with pyrrole-2-carboxyaldehyde thiosemicarbazone (L). *Spectrochimica Acta Part A* 68: 469-473

El-Shazly, R.M., Al-Hazmi, G.A.A., Ghazy, S.E., El-Shahawi, M.S. and El-Asmy, A.A. (2006). Synthesis and spectroscopic characterization of cobalt(II) thiosem icarbazone complexes. *Journal of Coordination Chemistry* 59(8): 845-859.

Enyedy, E.A.., Nagy, N.V., Zsigó, É., Kowol, C.R. , Arion,V. B., Keppler, B.K., and Kiss, T. (2010). Comparative Solution Equilibrium Study of the Interactions of Copper(II), Iron(II) and Zinc(II) with Triapine (3-Aminopyridine-2-carbaldehyde Thiosemicarbazone) and Related Ligands. *European Journal of Inorganic Chemistry* 1717-1728.

Enyedy, E.A., Primik, M.F., Kowol,C.R., Arion,V.B., Kiss, T. and Keppler, B.K. (2011). Interaction of Triapine and related thiosemicarbazones with iron(III)/(II) andgallium (III): a comparative solution equilibrium study. *Dalton Transaction* 40: 5895.

Gans, P., Sabatini, A. and Vacca, A. (1976). An Improved Computer Program for the Computation of Formation Constants from Potentiometric Data. *Inorganica Chimica Acta* 18: 237-239.

Gran, G. (1988). Equivalence volumes in potentiometric titrations. *Analytica Chimica Acta* 206: 111-123.

Grunberg, E. and Leiwant, B. (1951). Anti-Tubercular Activity *in vivo* of Nicotinaldehyde Thiosemicarbazone and Its Isomers. *Proceedings of the Society for Experimental Biology and Medicine* 77:47-50.

Gulea, A., Poirier, D., Roy, J., Spinu, S., Coziri, N., Birca, M., Tapcov, V. (2004). Anticancer effect of sulfanilamide complexes of Cu(II) with thiosemicarbazones of substituted salicylic aldehydes. *COF-rRoCA 2004, Actes du Colloque Franco-Roumain de Chimie Applique, 3rd Bacau, Romania, Sept.22-26,* 68(Fr). Rom. ISBN: 973-8392-36-5.

Hall, I.H., Lackey, C.B., Kistler, T.D. (2000). Cytotoxicity of copper and cobalt complexes of furfural semicarbazone and thiosemicarbazone derivatives in murine and human tumor cell lines. *Pharmazie* 55: 937-941.

Irving, H.M., Rossotti, H.S. (1953). Methods for Computing Successive Stability Constants from Experimental Formation Curves. *Journal of the Chemical Society* 3397-3405.

Irving, H.M., Rossotti, H.S. (1954). The Calculation of Formation Curves of Metal Complexes from pH-Titration Curves in Mixed Solvents. *Journal of the Chemical Society* 2904–2910

Irving, H.M. and Williams, R.J.P. (1948). Order of Stability of Metal Complexes. *Nature (London)* 162: 746-7.

John, R.P., Sreekanth, A., Rajakannan, V., Ajith, T.A., Kurup, M.R.P. (2004). New copper (II) complexes thiosemicarbazones and polypyridyl co-ligands: structural, electrochemical of 2-hydroxyacetophenone *N* (4)-substituted and antimicrobial studies. *Polyhedron* 23: 2549-2559.

Liberta, A. E., West, D. X. (1992). Antifungal and antitumor activity of heterocyclic thiosemicarbazones and their metal complexes: current status. *BioMetals* 5(2):121-126.

Ludwig, J.A., Szakács, G., Martin, S.E. (2006). Selective toxicity of NSC73306 in MDR1-positive cells as a new strategy to circumvent multi drug resistance in cancer. *Cancer Research* 66: 4808-15.

Pal, I., Basuli, F., and Bhattacharya, S. (2002). Thiosemicarbazone complexes of the platinum metals. A story of variable coordination modes. *Proceedings of the Indian Academy of Sciences: Chemical Sciences*114(4):255-68.

Pelosi, G. (2010). Thiosemicarbazone Metal Complexes: From Structure to Activity. *Open Crystallography Journal* 3: 16-28.

Rios, A. and Valcarcel, M. (1985). Simultaneous kinetic determination of copper, cobalt and nickel by means of group interchange reactions.*Talanta* 32(9): 851-858.

Shao, J., Zhou, B., Di Bilio, A.J. (2006). A Ferrous-Triapine complex mediates formation of reactive oxygen species that inactivate human ribonucleotide reductase. *Molecular Cancer Therapy* 5: 586-592.

Stiinzi, H. (1981). Copper Complexation by Isatin β-Thiosemicarbazones in Aqueous Solution. *Australian Journal of Chemistry* 34: 2549-2561.

Williams, D.R.(1972). Metals, ligands and cancer. *Chemical Review* 72: 203-213.

Wu, C., Shukla, S., Calcagno, A.M., Hall, M.D., Gottesman, M.M. and Ambudkar, S.V. (2007). Evidence for dual mode of action of a thiosemicarbazone, NSC73306: a potent substrate of the multidrug resistance linked ABCG2 transporter. *Molecular Cancer Therapy* 6: 3287-3289.

Yuan, J., Lovejoy, D.B. and Richardson, D.R., (2004). Novel di-2-pyridyl-derivediron chelators with marked and selective antitumor activity: *in vitro* and *in vivo* assessment. *Blood* 104: 1450-1458.