

Antibacterial screening of Ln^{3+} metal complexes with isonicotinic acid hydrazide

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Abstract : In this paper, three Ln^{3+} metal ion complexes have been prepared with isonicotinic acid hydrazide as chelating agent by using simple condensation method. After the completion of the synthesis, the complexes have been characterized on the basis of elemental analysis, conductivity measurements, magnetic properties and spectral analysis. The eight coordinated complexes of Pr^{3+} ion and seven coordinated complexes of Nd^{3+} and Sm^{3+} ions have been confirmed by the chromatography and spectral methods say infrared spectroscopy, nuclear magnetic resonance and electronic spectroscopy. After the characterization of the structure of the metal complexes, the antibacterial screening of all the complexes have been completed against bacterium *Mycobacterium tuberculosis* by disc diffusion method.

(Keywords: Lanthanoids, Isonicotinic acid hydrazide, Chromatography and Spectroscopy, antibacterial Screening)

Introduction

The organometallic and coordination chemistry of lanthanides (Ln) is dominated by the trivalent state, according to their position in periodic table. The applications of the trivalent lanthanide complexes are innumerable, from industrial catalysis¹ to luminescence² to biomedical applications³.

Isonicotinic acid hydrazide, isonicotinyl hydrazide or isoniazid (INH)⁴ occurs as a nearly colorless crystalline solid that is very soluble in water. It is a well known [antibiotic](#), which is used to [cure blue tuberculosis](#)⁵. The research work on antibacterial screening of metal complexes of the first generation antitubercular drug isoniazid is although well known but not sufficient. A few

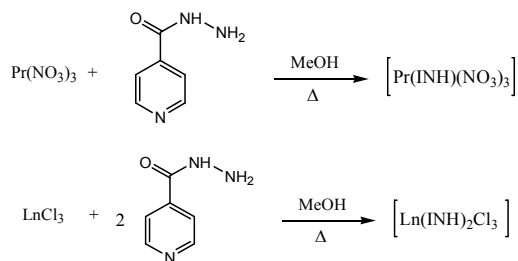
lanthanoid metal complexes of isoniazid are known⁶.

A mycobacterial catalase-peroxidase enzyme complex is required for the bioactivation of isoniazid^{7,8}. Drug efficiency depends upon linking behavior and its fastness to attack on acting site in body. Complexation of the drug may change its assay. This should be studied as it has become topic of much interest⁹.

In order to study the effect of complexation / chelation on efficiency of isonicotinic acid hydrazide, three lanthanoid metal cations say praseodymium (III), neodymium (III) and samarium (III) ions have been taken in this paper.

1. Experimental

All chemicals such as isonicotinic acid hydrazide, $\text{Pr}(\text{NO}_3)_3$, NdCl_3 , SmCl_3 , methyl alcohol, ethyl alcohol used in this study were of analytical grade. These chemicals were purchased from CDH or Merck and used as such. Schemes of the reactions are:



Where, Ln = Nd and Sm

Preparation of solution:

A 0.01 M isonicotinic acid hydrazid solution was prepared by dissolving the 0.07 g of isonicotinic acid hydrazid ligand in 50 ml methanol.

A 0.01 M solution of Ln (III) salts were prepared by dissolving 0.16 g of metal salts in 50 ml of methanol.

Preparation of complexes

The solution of Ln (III) salts and the ligand isonicotinic acid hydrazid in methanol were taken in euimolar ratio and mixed. The mixture was refluxed on a water bath for 2 h. On cooling in ice bath, the resulting solid complex was separated out. It was repeatedly washed with distilled water, diethyl ether and acetone several times and dried in a desicator over anhydrous calcium chloride, and then green solid complex was obtained.

The solubility tests of these complexes were performed. The complexes were found soluble in water and alcohol and insoluble in dimethyl sulphoxide (DMSO), dimethyl formamide (DMF), ether and acetone.

The thin layer chromatographies of these complexes were performed by using aluminium plates coated with silica gel-60GF254 as the stationary phase. The solvent system was consisted of chloroform:methanol:ammonia (4.3:4.3:1.5, V/V) as mobile phase. The R_f values were found 0.082 ± 0.04 .

1. Antibacterial Screening

All the synthesized complexes of metal ions with isoniazid have been screened against bacterium *Mycobacterium tuberculosis* by disc diffusion method.

Preparation of Meat Extract:

The 500 g of fat free minced meat was placed in 1000 ml of redistilled water for 24 hrs

in cold and stirred with sterile glass rod. The resulting solution was strained through a muslin cloth. The residue was discarded and the obtained filtrate had red colour. The surface fact was removed by skimming with a filter paper and it was boiled for 15 minutes till insoluble proteins became coagulated. The fluid was filtered through cheese cloth and its volume was increased by the addition of distilled water. Thus a clear yellow coloured meat extract obtained.

Nutrient Broth:

For the preparation of nutrient broth 1 g peptone, 500 mg sodium chloride and 100 ml meat extract were mixed and heated with continuous stirring till they dissolved. Now it was filtered through a filter paper and adjusted the pH at 7.4 by adding normal sodium hydroxide solution. The medium so obtained was poured into the sterile flask and plugged with sterile cotton plug which was then sterilized in an autoclave for 25 minutes.

Nutrient Agar:

Although agar is complex carbohydrate but it is not a source of nutrition for the bacteria but it used as a solidifying agent for the media. The 20 g of agar powder was placed in 100 ml of nutrient broth. The mixture was boiled in steam till a clear solution. It was then filtered through a glass-wool in hot medium, adjusting the pH by adding NaOH solution. Then the medium was kept in conical flask and heated in an autoclave for 15 minutes. Finally, it was transferred into the sterilized petridishes.

Inoculation of Test Media:

The four well isolated colonies having the same morphological type were selected from an agar plate culture with a loop of wire. The top of each colony was touched and transferred the growth to a tube containing 5 ml of suitable broth medium. The broth culture was incubated at 37°C for 8 hrs. The sterile cotton swab on woolen application was dipped into the inoculum. The excess of inoculum was removed from the swab

by several times rotating inside the wall of the test tube above the final level. The dried surface of a Miller-Hinto agar plate was inoculated by streaking the swab over the entire sterile agar surface. The streaking was repeated three times so as to ensure an even distribution of the inoculums.

Now within 10 minutes, antibiotic impregnated discs were applied on the surface of the inoculated plates with sterile forceps. Each disc was pressed on the medium to ensure complete contact. The discs have been arranged in such a way that they are not close from the edges of the plate and far enough apart to prevent overlapping of the zone of inhibition. Since some diffusion of test compound is almost instantaneous, a disc was not moved once it came in contact with agar surface.

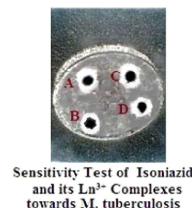
Incubation of Test Plate:

The individual plates were evenly dispersed on the incubator shelf at 37°C so as to make each plate reached approximately to incubator temperature for some time.

Interpretation:

After 24 hrs of incubation, the plates were examined and the diameter of the zones of complete inhibition was measured to the nearest whole millimeter with a sliding calipers.

For the sake of simplicity the petridishes containing discs of standard antibiotic ligands (as indicated by A) and complexes of metal ions Pr^{3+} , Nd^{3+} and Sm^{3+} (indicated as B, C and D respectively) only towards *M. tuberculosis* are shown in the following figure.



The observed screening data for the metal complexes and for the sake of comparison the data of standard drug isoniazid is given in following table:-I.

Table:-I												
	Isoniazid			$[\text{Pr}(\text{inz})(\text{NO}_2)_3]$			$[\text{Nd}(\text{inz})_2(\text{Cl})_3]$			$[\text{Sm}(\text{inz})_2(\text{Cl})_3]$		
Concn	10^{-3}	10^{-2}	10^{-1}	10^{-3}	10^{-2}	10^{-1}	10^{-3}	10^{-2}	10^{-1}	10^{-3}	10^{-2}	10^{-1}
<i>M. tuberculosis</i>	++	+++	++++	++	+++	++++	++	+++	++++	++	+++	++++

1. Results and Discussion

The complexes of isonicotinic acid hydrazid (isoniazid, INH), with lanthanide metal ions (Pr^{3+} , Nd^{3+} and Sm^{3+}) are anhydrous also supported from their analytical and spectral studies. All these complexes are quite stable at room temperature. The complexes are soluble in water, and alcohol. It was insoluble in dimethyl

sulphoxide (DMSO), dimethyl formamide (DMF), ether and acetone.

The elemental analyses of the complexes were carried out at Central Drug Research Institute (CDRI), Lucknow. The elemental analysis of the complex is given in table:-II.

Table:-II				
Complexes	% of metal Cal./obs.	% of C Cal./obs.	% of H Cal./obs.	% of N Cal./obs.
[Pr(INH)(NO ₃) ₃]	30.37/30.02	15.52/15.28	1.51/1.52	15.09/15.04
[Nd(INH) ₂ Cl ₃]	27.49/27.25	13.72/13.48	1.33/1.35	8.00/7.93
[Sm(INH) ₂ Cl ₃]	28.08/28.32	13.28/13.56	1.29/1.32	7.86/7.91

The melting points were determined by using open capillary tube in kjeldal flask filled with concentrated sulphuric acid.

The molar conductance values are too low to account for any dissociation. Thus

complexes are non electrolytes. The molar conductance of these complexes have been recorded in nitrobenzene by systonic digital conductivitymeter-304 and given in following table:-III.

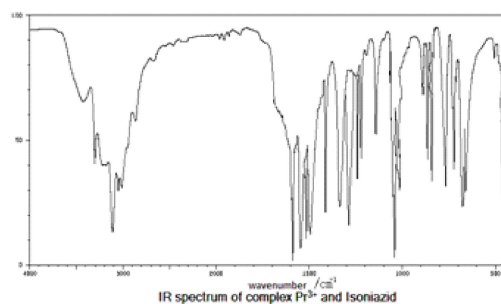
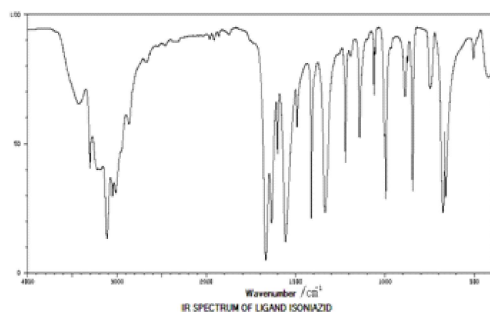
Table:-III					
Complexes	M.Pt.	Soluble	Conductance ($\Omega^{-1}\text{cm}^2\text{mol}^{-1}$)	μ_{eff}	Colour
[Pr(INH)(NO ₂) ₃]	215 ⁰ C	In water and alcohol	3.75	3.60	Green
[Nd(INH) ₂ Cl ₃]	235 ⁰ C	In water and alcohol	4.21	3.55	Lilac
[Sm(INH) ₂ Cl ₃]	240 ⁰ C	In water and alcohol	4.35	1.61	Pink

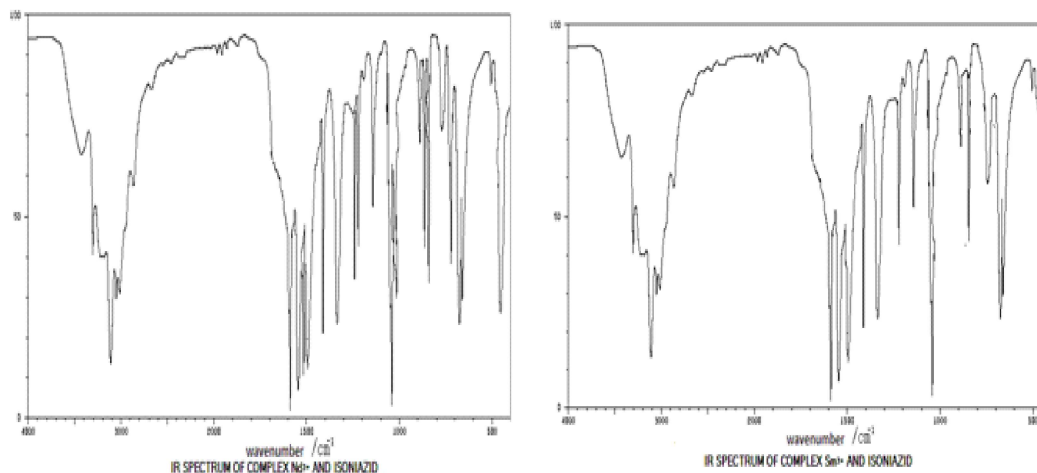
5. Spectral Analysis

The infrared, ¹H NMR and electronic spectra of these complexes were obtained from C.D.R.I., Lucknow.

IR

The infrared spectra of the solid Ln³⁺ complexes with ligand isoniazid are given below.





The IR spectral data and the assignment of the ligand isoniazid and its Ln^{3+} metal complexes are

given in following table:-IV.

Table:-IV						
Compounds	ν_{NH_2}	ν_{CO}	ν_{NH}	ν_{CN}	$\nu_{\text{C}=\text{C}}$	$\Delta\nu(\text{NO}_3)$
Isoniazid	3200	1660	3290	1080	1550	
$[\text{Pr}(\text{INH})(\text{NO}_3)_3]$	3185	1590	3292	1080	1545	210
$[\text{Nd}(\text{INH})_2\text{Cl}_3]$	3180	1580	3285	1075	1550	
$[\text{Sm}(\text{INH})_2\text{Cl}_3]$	3170	1595	3290	1080	1555	

The isoniazid expected to act as tridentate one, the possible coordination sites being pyridine-nitrogen, carbonyl of amide and NH_2 groups. The infrared frequencies in the present ligand associated with amide group (carbonyl-oxygen), NH_2 group and heterocyclic nitrogen are expected to lie influenced on complex formation with metal ions have been discussed.

Generally, all amides show two absorption bands¹⁰ at $<1640\text{ cm}^{-1}$ related to $\text{C}=\text{O}$, group known as amide-I band and 1600--

1500 cm^{-1} known as amide-II band. The amide-I band in INH appears at 1660 cm^{-1} . The complexes show a considerable negative shift from 1660 to $1595\text{--}1580$ in $\nu_{\text{C}=\text{O}}$ indicating a decrease in the stretching frequency due to the decrease in force constant of $\text{C}=\text{O}$ as a consequence of coordination through the carbonyl oxygen atom of the ligand isoniazid. The amide-II band appears at the normal position in the NH -deformation rather than C--N link. In the isoniazid, the absorption in $1565\text{--}1560\text{ cm}^{-1}$ reason has been assigned to amide-II absorption.

The NH stretching absorption in free ligand occurs¹¹ at 3290 and 3220 cm^{-1} which remains unaffected after complexation, it excludes the possibility of coordination through imine nitrogen. In spectra of all these complexes, the band occurs in the range of $<3200 \text{ cm}^{-1}$ attributed to NH_2 group (ν_{NH_2}), shifted to lower wave number and appears in 3185–3170 cm^{-1} region indicating the involvement of nitrogen atom of NH_2 group in coordination^{12,13}. The strong bands observed at 1575–1520 and 1080–1000 cm^{-1} are tentatively assigned^{14,15} to asymmetric and symmetric $\nu_{\text{C}=\text{C}} + \nu_{\text{C}=\text{N}}$ of pyridine ring and pyridine ring stretching and deformations remain practically unchanged in frequency and band intensities revealing non-involvement of pyridine-nitrogen and complexation through metal ions. Thus, on account of infrared spectral properties one can say that isoniazid acts as bidentate ligands supporting the previous work^{16–18}.

Another band appears at $<725 \text{ cm}^{-1}$ region in the complexes Nd^{3+} and Sm^{3+} with isoniazid indicating complexation of chloride ion through corresponding metal ions¹⁹. In Pr^{3+}

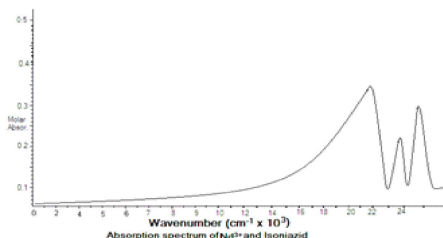
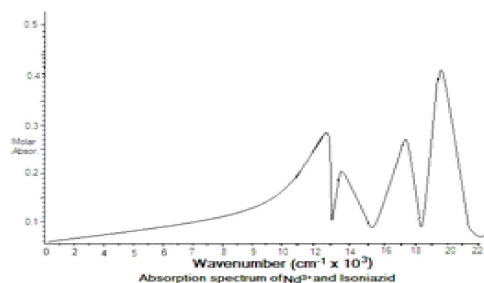
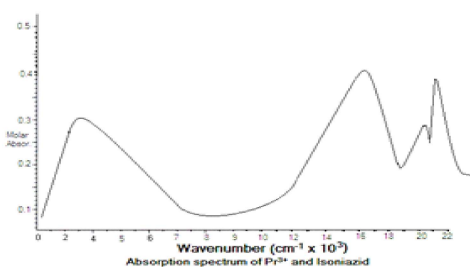
complex, a new band appears at 491 cm^{-1} . It may be due to $\text{Ln}-\text{O}$ stretching vibration¹⁰. Five absorption peaks at 1505 cm^{-1} (ν_1), 1025 cm^{-1} (ν_2), 817 cm^{-1} (ν_3), 740 cm^{-1} (ν_4) and 1295 cm^{-1} (ν_5), $\Delta\nu = \nu_1 - \nu_5$ i.e. 210 cm^{-1} in the spectra of Pr^{3+} complex are due to coordinated nitrates¹⁰ which behaves as bidentate ligand.

PNMR

An nmr triplet peak at $\delta = 5.90$ ppm indicating $-\text{CO}-\text{NH}-$ proton and a doublet peak at $\delta = 6.20$ ppm indicating $-\text{NH}_2$ proton shift to downfield at $\delta = 6.3$ ppm and 7.80 ppm respectively. It indicates that $-\text{NH}_2$ group involves in complexation they they decreases the electron density around the NH_2 group which shift the δ value to downfield. Further, with the involvement of carbonyl group in complexation the δ value of NH_2 group also shift downfield from 5.9 to 6.3 ppm.

Electron Spectroscopy

The spectral data for the solution of Ln^{3+} complexes with ligand isoniazid investigated in acetonitrile as recorded in CDRI, Lucknow are as below;



The electronic spectral data and the assignment of Ln^{3+} metal complexes with ligand isoniazid are given in following table.

Table : Electronic spectral data of isoniazid with Ln³⁺ ions in cm⁻¹

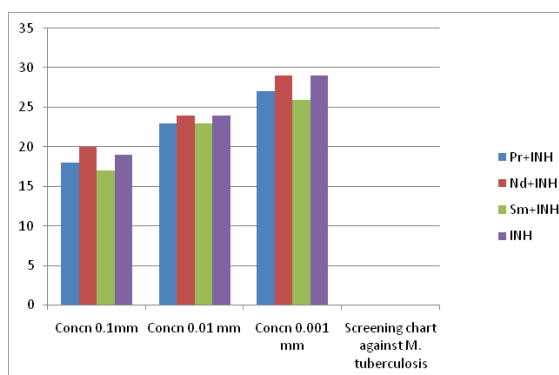
Table:-V		
complexes	Spectral Bands	Transitions
[Pr(INH)(NO ₃) ₃]	20620, 21230, 2230, and 16880	³ H ₄ ³ P ₀ , → ³ P ₁ , ³ P ₂ and ¹ D ₂
[Nd(INH) ₂ Cl ₃]	19480, 17260, 13630 and 12430	⁴ I _{9/2} ² G _{9/2} , → ⁴ G _{5/2} or ² G _{7/2} , ² S _{3/2} or ⁴ F _{7/2} and ⁴ F _{5/2} or ⁴ H _{9/2}
[Sm(INH) ₂ Cl ₃]	21520, 23835 and 24830	⁶ H _{5/2} ⁴ I _{13/2} , → ⁶ P _{5/2} and ⁴ F _{9/2}

The absorption bands of praseodymium (III), neodymium (III) and samarium (III) in the visible and UV-regions appear due to transitions from the ground levels ³H₄, ⁴I_{9/2}, and ⁶H_{5/2} to the excited J-levels of 4f-configuration²⁰, respectively. Some red shift or nephelauxetic effect is observed in CH₃CN solution of these complexes. This red shift is usually accepted as an evidence of higher degree of covalency²¹ comparatively.

Antibacterial Screening

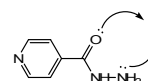
It is generally recognized²² that isoniazid is a pro-drug which is activated through an

oxidation catalyzed by an enzyme *KatG*. This enzyme exhibits catalyse peroxidase activity, converts isoniazid to a reactive species capable of acylation of an enzyme system found exclusively in the *Mycobacterium tuberculosis*. All the newly synthesized metal complexes of isoniazid were observed almost similar antibacterial activity. It may be due to the complexes were shown to be unstable in the gastrointestinal tract releasing the active isoniazid. Thus, it would appear that their activity resulted from the isoniazid and not the complexes, supporting the previous work^{23,24}.



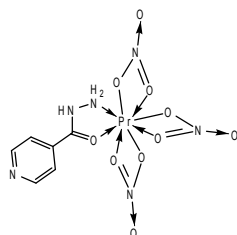
Conclusion

On the basis of IR analysis it is clear that the ligand isoniazid acts as a bidentate ligand coordinating through -NH₂ and C=O group. The ligands isoniazid also acts as neutral bidentate ligand coordinating through carbonyl group and NH₂ group.



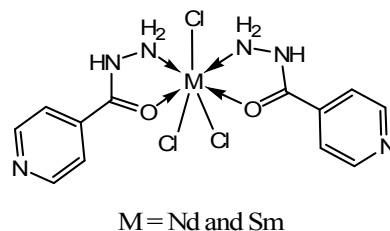
The complex of Pr (III) ion with ligand isoniazid has three nitrates which act as bidentate (confirmed by IR analysis) in addition to an

isoniazid ligand. So the resulting complex is eight coordinated confirmed as below;



On the other hand, complexes formed by Nd (III) and Sm (III) with isoniazid have three chloride coordinated in addition to two isoniazid. So the

seven coordinated structures of the complexes confirmed as:



All the complexes of Pr (III), Nd (III) and Sm (III) with isoniazid are almost similar effective towards bacterium *Mycobacterium tuberculosis*.

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