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GROWTH, CHARACTERISATION, ANTIMICROBIAL AND ANTICANCER ACTIVITIES OF L ALANINE ADDED NICKEL SULPHATE CRYSTALS

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ABSTRACT

Nickel Sulphate hexa hydrate (NSH) is hydrogen bonded crystal having a wide range of applications in various fields. An attempt was made to understand the effect of L alanine on NSH crystals. The crystals were grown by slow evaporation method in aqueous solution at room temperature. The grown crystals were characterized structurally, optically, thermally and biologically. X-ray diffraction studies proved the crystalline nature of the grown compound and it belongs to monoclinic structure. FTIR analysis confirmed the presence of various functional groups in the grown crystal. The results of UV-visible spectroscopy shows that the grown crystal has good optical transparency in the wavelength range 223-319 nm. Thermo gravimetric analysis proved that the crystal is stable up to 110ºC. Hence this crystal may be used in UV filters even at elevated temperatures. The grown crystal was evaluated for its biological efficacy and found to exhibit pharmacological properties such as antimicrobial and anticancer.

KEYWORDS: Ultraviolet filter, X-ray diffraction, FTIR and UV spectrum, Thermal stability, antibacterial, anticancer.

1. INTRODUCTION

Metal-organic compounds have attracted much more attention for their stable physico-chemical properties and better mechanical intension.[1] Hydrated metal (II) sulphates are widely used in different industrial processes.[2] $NiSO_4.6H_2O$ [NSH] is a chemical agent and is used in batteries, food and oil industry and in perfumery industry. In general majority of the

crystals show continual optical transmission from UV to near IR wavelength range. Very few selected crystals show discontinuity in the above range. For example NSH crystal which possess high transmission efficiency (>80%) in the narrow range 250 – 340 nm and moderate transmission at 450 – 600 nm and strong absorption over all other wavelengths are considered as UV light filter. They allow selected wavelength of light to pass through. Such filters are used in missile approach warning systems which locate and track sources of ultra-violet energy, enabling the system to distinguish the plume of an incoming missile from other UV sources that pose no threat. They are also able to estimate missile range. The success and efficiency of the system for helicopters or transport-type aircrafts depends on the UV sensors. Commercially available NSH crystals are widely used for these sensors. The biggest problem for these sensors arises due to low thermal stability of NSH crystals which is 72°C. Several other crystals such as Potassium Nickel Sulphate Hexahydrate (KNSH), Cesium Nickel Sulphate Hexa hydrate (CNSH), Iron Nickel Sulphate twelve hydrate (FNSH), Rubidium Nickel Sulphate Hexahydrate (RNSH), Ammonium Cobalt Nickel Sulphate Hexa hydrate (ACNSH) are reported as UV filter materials.[3-5] The SO_4 group in NSH may be considered as similar to the PO_4 group present in $NH_4H_2PO_4$ (ADP) and KH_2PO_4 (KDP). Considerable interest has been shown by several investigators in studying the effect of impurities (both inorganic and organic) on the nucleation, growth and physical properties of some hydrogen-bonded crystals like KDP and ADP and obtained several useful results.[6] Continuous demand for new anti-cancer drugs has stimulated chemotherapeutic research, based on the use of metals. The potential drugs which are developed in this way may be less toxic and more prone to exhibit anti-proliferative activity against tumors.[7] In general, metal based complexes have significant antibacterial, anticancer and antiviral effects. Moreover the presence of L alanine, an amino acid may also influence the biological activity of NSH.[8]

Aiming to discover new useful materials with better filter transmission property, higher thermal stability and efficient anti-microbial and anti-carcinogenic behaviour, in the present study, we have grown L alanine added nickel sulphate hexa hydrate [LANSH] single crystals by slow evaporation method and investigated the effect of L alanine as an impurity on the properties of NSH. The results obtained are reported herein and discussed.

2. MATERIALS AND METHODS

As per the estimated solubility data, saturated solutions of NSH(62.5 g/100 ml) and L alanine (16.72 g/100 ml) (99% purity) were prepared separately using doubly distilled water at room temperature with continuous stirring using magnetic stirrer (REMI 1MLH). The prepared

solutions were doubly filtered by Whatman No.1 filter paper and mixed in the ratio 3:1of pH 3 and stirred well for 5 hours to get good homogeneity. The beaker containing the mixture was closed with a pin holed aluminium foil and kept at room temperature in dust and vibration free environment for slow evaporation. A highly transparent, good quality LANSH single crystal of dimension 14x13x10 mm^3 was harvested on 61^{st} day and is shown in Fig.1. Recrystallization was carried out repeatedly to enhance the purity of the crystal.

Figure 1. Photograph of the grown crystal

3. CHRACTERISATION

Results of various studies carried out with the grown crystals are discussed hereunder.

3.1. Single X ray analysis (SXRD)

As grown crystal of LANSH has been subjected to single crystal XRD employing a Bruker AXS diffractometer using MoKα radiation (λ = 0.71073Å).The single crystal XRD reveals that the grown crystal belongs to monoclinic system with space group P2(1)/c. The lattice parameter values are given in Table 1, enabling a comparison. It shows that there is an observable change in the lattice parameters and decrease in cell volume of the grown LANSH crystals compared to that of NSH. This proves the incorporation of impurity molecules in the lattice of NSH crystals.[9]

TABLE 1. Cell parameters of the grown crystals

Crystal	a(A^0)	b(A^0)	c(A^0)	α= γ	β	Cell volume (A^{03})	Crystal system
NSH	9.878	7.214	24.065	90^0	98.37^0	1696.6	Monoclinic
LANSH	6.2377	12.4669	9.1824	90^0	106.928	683.35	Monoclinic

3.2 FTIR analysis

The FTIR spectrum of the grown crystals of LANSH had been recorded in the KBr phase in the frequency region 4000-400 cm^{-1} using Perkin Elmer spectrometer and is shown in Fig. 2.

Four normal modes are present in the infrared region for the sulphate anion (SO_4^{2-}): a non-degenerate symmetric bending ν_1, a doubly degenerate symmetric bending ν_2 and two triply degenerate symmetric stretching and bending ν_3 and ν_4 respectively.[10] Changes in protonation, metal complexation and solvation of SO_4^{2-} can modify S-O bond length and as a result, may change the symmetry of the anion. This leads to a shift in the vibrational bands to different wave numbers and causes the degenerate vibrations to become non-degenerate.

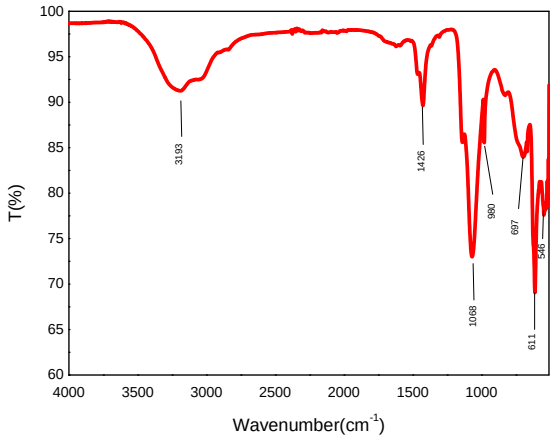


Figure 2. FTIR pattern of grown crystal

The broad envelope around 3178-2506 cm^{-1} indicates the presence of water and it belongs to free water symmetry stretch. The hydrogen bond O-H stretching frequency has been observed at 3193 cm^{-1}, i.e., in the region between 2000 and 3200 cm^{-1}.[9] The asymmetric stretch of sulphate (ν_3) appears at around 1236-915 cm^{-1}. The bending modes of sulphate (ν_4) are positioned at 980 and 697cm^{-1}. The presence of COO^- is confirmed through the peaks at 1426 and 546 cm^{-1}. The peaks at 1068 and 697 cm^{-1} represent the presence of CH_3 and C=O.[11,12] The spectra observed for the grown crystals are similar to that reported in the literature for $MgSO_4.7H_2O$[13] and $ZnSO_4.7H_2O$.[6]

3.3. Optical studies

The optical transmission spectra of the grown crystal was recorded using Shimadzu UV-Vis spectrophotometer between the wavelength ranges of 200 and 800 nm and is shown in Fig. 3.

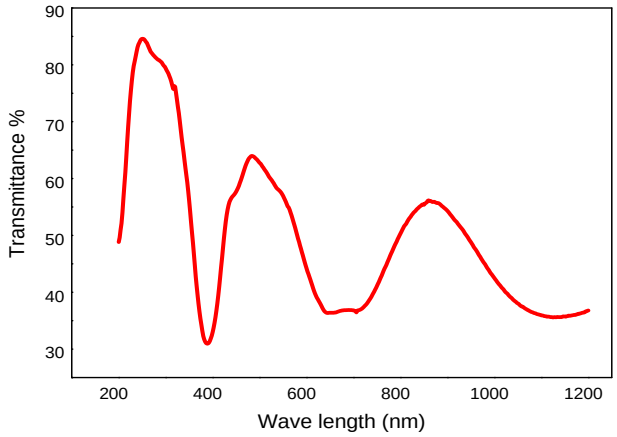


Figure 3. UV Transmission graph of grown crystal

The discontinuity found in the spectra is the characteristics of the absorption of hydrated transition metal ions $Ni(H_2O)_6$.[3, 6] From the point of view of application of the crystals understudy as filters in the mid-UV range, they should have maximum transmission in this wavelength range and minimum transmission in the visible spectral range.[14] It is found that the grown crystals have the best optical characteristics in the wave length range of 223–319 nm (mid range UV) and relatively low in the visible range. The transmission of LANSH crystal in the UV band is comparable with the other nickel compounds.[14]

3.4. Thermo gravimetric analysis (TGA)

TGA gives information regarding the distinctive aspects of phase transition, water of crystallization and thermal decomposition of the crystal and is of immense importance in fabrication technology as they provide information about the thermal stability of the material for fabrication.[11] In general, TGA gives the upper temperature limit of a material within which it can be used and it is an important factor for crystals that are to be used as Ultra violet light filter (UVLF). TGA was carried out between 25ºC and 700ºC at a heating rate of 20ºC / min using Perkin-Elmer thermal analyzer STA409PC in nitrogen atmosphere. The resulting thermo gram of LANSH is shown in Fig 4.

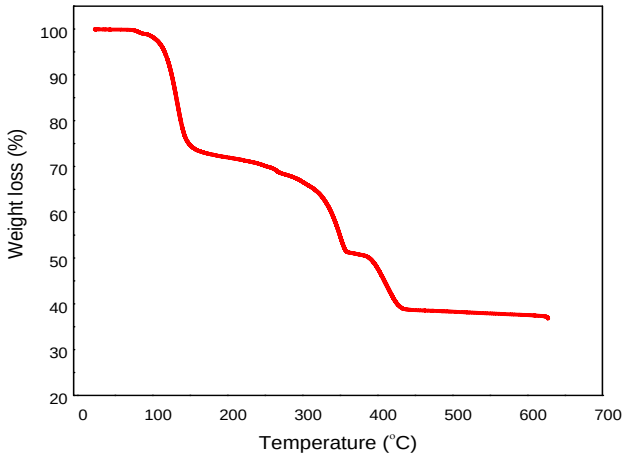


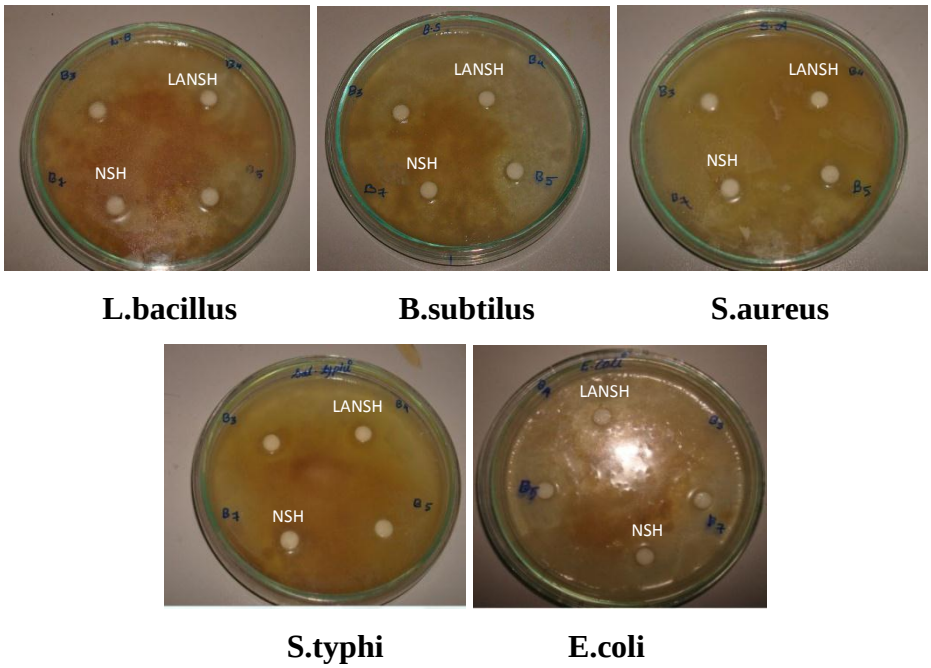
Figure 4. TGA spectra of grown crystal

The decomposition starts around 110°C and produces a sharp weight loss of 25% which may be due to physically adsorbed water and is followed by another gradual weight loss of 23% up to 360°C. So the compound has weight loss of about 48% due to the removal of six water molecules. In the temperature range of 388-430°C, 12 % of weight is lost. 40% of the remaining sample is stable up to 600°C in the form of NiO. It is observed from the figure that the decomposition temperature of the LANSH is increased by 38°C compared to NSH. It is to be noted that, the decomposing point of L alanine is 297°C. The presence of L alanine appears to increase the decomposition temperature of NSH, which led to networks of associated ions. It is observed from the literature that the presence of dopant with higher decomposing temperature increases the thermal stability of the compound.[5] Hence the grown crystal has better thermal stability compared to that of KNSH (97.2°C) and KCNSH (98°C).[3,15]

3.5. Antimicrobial analysis

Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern. The clinical efficacy of many existing antibiotics is being threatened by emergence of multi drug resistant pathogens. There is continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanism of action for new and re-emerging infectious diseases.[16] The biological screening is effective and the results show a significant antibacterial activity for the grown LANSH crystals over both types of bacterial strains. The grown crystals were screened for Gram positive (Lactobacillus, Bacillus subtilis and Staphylococcus aureus) and Gram

negative (Salmonella typhi and Escherichia coli) bacteria by agar well diffusion method by measuring the inhibition zone width as shown in Fig 5.



L.bacillus **B.subtilus** **S.aureus**

S.typhi **E.coli**

Figure 5. Photographs showing Zone of Inhibition (mm) against select bacteria

The inhibition zone diameters in the agar well diffusion assays for LANSH and NSH are given in Table 2 enabling a comparison. It is clear that the antibacterial activity of the grown LANSH crystals enhanced for L.bacillus, B.subtilus and S. typhi, remains same for S.aureus and decreases for E.coli.

TABLE 2. Zone of Inhibition against select bacteria (mm)

S. No	Sample	L.bacillus	B.subtilus	S.aureus	S.typhi	E.coli
1	LANSH	14 ± 0.2	13 ± 0.3	13 ± 0.1	11 ± 0.2	10 ± 0.6
2	NSH	12 ± 0.6	9 ± 0.3	13 ± 0.8	10 ± 0.1	12 ± 0.2

Among the five species, L.bacillus is more sensitive to the grown crystal action. The sensitivity for five bacterial species follows the order as Lactobacillus>Bacillus subtitlis>Staphylococcus aureus >Salmonella typhi>Escherichia coli. Hence the LANSH crystals may be considered for pharmacological applications.[17-19]

3.6 Anticancer studies

Cancer is among the most dreaded of human diseases. It is recognized as a major threat to health. Cervical cancer is the leading cancer which is associated with the death of women world- wide.[20] Experimental studies have shown that water-soluble nickel salts enhance the

mutagenicity of DNA damaging agents by inhibiting DNA repair processes. The inhibition of DNA repair may be one of the mechanism by which the water soluble nickel salts have a synergistic role in enhancing carcinogenesis after exposure to other nickel compounds. The mechanism for how nickel compounds cause carcinogenic effects is an area of continuing investigation.[21]

The human cervical cancer cell line (HeLa) was obtained from National Centre for Cell Science, Pune, India and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^0C, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly and the culture medium was changed twice a week.

The monolayer cells were detached with trypsin-ethylene diamine tetra acetic acid to make single cell suspensions and viable cells were counted using a hemo cytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/ml. One hundred micro litres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dispersed in phosphate buffered saline and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95% air and 100% relative humidity. The control and triplicate was maintained for all concentrations.[22, 23] The % of cell inhibition was determined using the following formula.

% of Cell Inhibition = 100- Abs (sample)/Abs (control) x100.

The results obtained are shown in figure 6 and is consolidated in table 3.

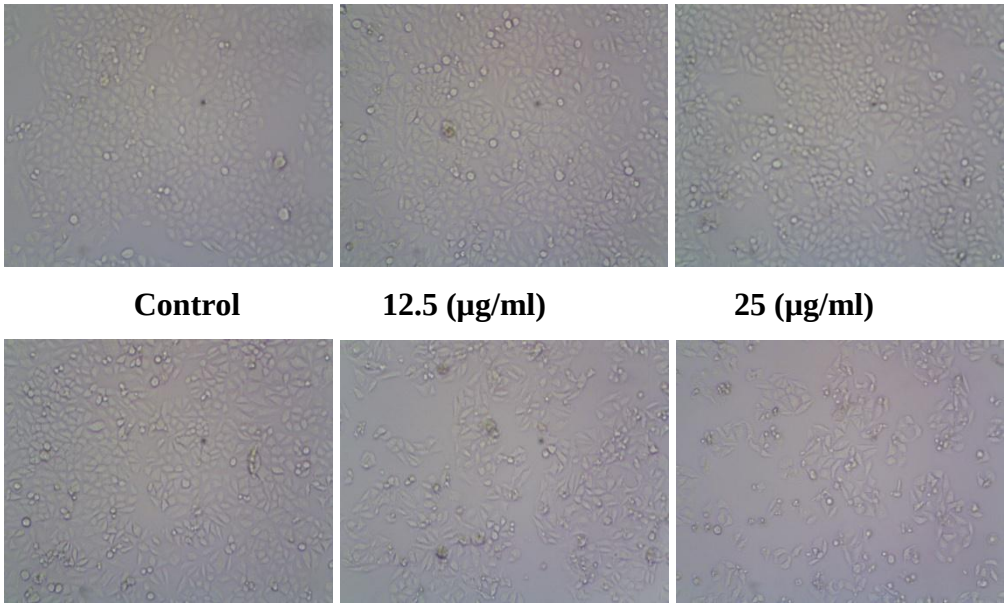


Figure 6 Photographs showing cancer cell inhibition for different concentrations

TABLE 3. Percentage of cancer cell inhibition

Sample Concentration (µg/ml)	Cell Inhibition %
12.5	0.638468
25	1.516361
50	10.69433
100	47.00718
200	57.62171

It is clear from the above result that at higher concentrations the LANSH crystal exhibit good anticarcinogenesis activity.

4. CONCLUSION

Single crystals of LANSH have been grown by slow evaporation method at room temperature. The cell parameters of LANSH crystals prove the monoclinic structure with the space group of $P2_1/c$. The FTIR study confirms the presence of SO_4 and H_2O molecules. The Optical transmission spectrum shows that the grown crystal is highly transparent in mid UV region. From TGA, it is observed that the decomposition temperature of LANSH crystal is 110^oC which is much greater than that of pure NSH crystals. As grown LANSH crystal is transparent in mid UV region and thermally stable, it is well suited for UV light filters even at high temperatures. Hence LANSH crystals may be recommended for UV light filters as sensors in spaceships especially in the area of defence services. The product was assayed for their antimicrobial and anticancer activity and the results obtained are encouraging. Hence

the grown crystal may also be considered for pharmacological applications in designing better and more active drugs.

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