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RESEARCH ARTICLE

STUDIES ON GROWTH, MORPHOLOGY, SPECTRAL AND ANTIMICROBIAL PROPERTIES OF ALKALI, ALKALINE EARTH AND TRANSITION METAL IONS DOPED L-ALANINE SINGLE CRYSTALS

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ABSTRACT

Single crystals of alkali, alkaline earth and transition metal sulphates doped alanine nonlinear optical materials have been grown from solution by slow evaporation method. The isoelectric point of the alanine is 6. So, the growth of crystals has been carried out at pH 6. The various functional groups present in the pure and doped crystals are elucidated from Fourier transform infrared spectroscopy study. UV-Visible transmittance is recorded to study the optical transmittance range for the grown crystals. The grown crystals have been subjected to powder X-ray diffraction studies to identify the crystalline nature. Surface morphology and defects due to dopant were studied by optical microscope. The antimicrobial studies of the grown crystals were studied using two gram positive and two gram negative bacteria.

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INTRODUCTION

L-alanine (LA) is the smallest, naturally occurring chiral amino acid with a non-reactive hydrophobic methyl group (-CH₃) as a side chain. LA has the zwitter ionic form (+NH₃-C₂H₄-COO⁻) both in crystal and in aqueous solution over a large range of pH. The crystalline state is well defined structurally and can be successfully used for a detailed examination of a broad range of molecular properties. L-alanine is an efficient organic NLO compound under the amino acid category. Attempts were made to grow bulk size single crystals of L-alanine with good transparency with different pH values. Thus L-arginine, L-histidine and L-alanine have been exploited for the formation of salts with different organic / inorganic acids. The compounds of metal ions doped L-alanine amino acid have been much less explored. The semi-organic crystals possess both the good qualities of host organic materials and additive inorganic materials (Ramajothi and Dhanuskodi 2007). These semi-organic NLO materials have been attracting great attention due to high non-linearity, chemical flexibility, high mechanical and thermal stability and good transmittance (Min - hua Jiang 1999). The amino acids exhibit specific features of interest such as (i) molecular chirality, which secures non-centrosymmetric crystallographic structure, (ii) absence of strongly conjugated bonds, which leads to wide transparency ranges in the visible and ultra-violet spectral regions and (iii) zwitter ionic nature of the molecule, which favors crystal hardness for applications in devices (Nicoud and Twieg 1987;

Parikh *et al.*, 2010). The α -carbon atom of L-alanine is bound with a methyl group making it one of the simplest α -amino acids with respect to molecular structure and also resulting in L-alanine being classified as an aliphatic amino-acid. The methyl group of L-alanine is non-reactive and is thus almost never directly involved in protein function (Prabha and Palaniswamy 2010). The presence of dopants in small amounts may greatly influence the crystallization kinetics of organic compounds from solution. Moreover, both organic amino acids and inorganic metals, with large second-order optical nonlinearities, as these materials have a significant impact on laser technology, optical communication and optical storage technology etc. Over the years many organic and inorganic materials have been developed (Singh *et al.*, 1993; Chemla and Zyss 1987; Badan *et al.*, 1993; Rajasekaran *et al.*, 2001) to cover the potential applications in ultra-violet, near-and far-infrared wavelength regions. Amino acid nonlinear optical materials are often formed by weak van der Waals and hydrogen bonds and hence possess high degree of delocalization. A dopant may affect the activity of the crystallizing solute in solution and interface with the crystal growth process through adsorption onto the growing surface. It has been reported that doping NLO crystals with organic impurities can alter various physical and chemical properties and doped NLO crystals can be used for various applications. It is identified that adding dopants changes the properties of the crystals (David Lechuga 1993). Hence the aim of this paper is to report the studies on growth morphology, spectral analysis and antimicrobial studies of some metal ions doped L-alanine crystals.

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Structure of L-alanine molecule

The α -carbon atom of L-alanine is bound with a methyl group ($-\text{CH}_3$), making it one of the simplest α -amino acids with respect to molecular structure and also resulting in L-alanine being classified as an aliphatic amino acid. The methyl group of L-alanine is non-reactive and is thus almost never directly involved in protein function. ($-\text{CH}_3$) is alkyl hydrophobic group which can't enter in hydrogen bond formation and is a non polar. The structure of L-alanine is shown in Fig-1.

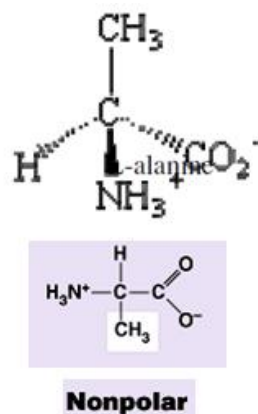


Fig. 1. Structure of L-Alanine

Experimental section

Synthesis of pure L-alanine single crystal

The pure L-alanine single crystals were grown by dissolving 10 mmol L-alanine powders in 20ml of double distilled water and stirred well using magnetic stirrer for six hours to form a clear solution. The solution was filtered and transferred to crystal growth vessels and crystallization was allowed to take place by slow evaporation under room temperature. Good quality single crystals were obtained within 10–15 days. The harvested crystals were washed several times with acetone and dried for studying further.

Synthesis of metal ions doped L-alanine single crystals

The metal ions doped L-alanine single crystals were grown by dissolving 10mmol of L-alanine and MSO_4 ($\text{M}=\text{Na}^{2+}$, K^{2+} , Mg^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+}) powders in 20ml of double distilled water and stirred continuously using magnetic stirrer for six hours at RT to form a clear solution. The solution was then filtered using the Whatmann filter papers and transferred to crystal growth vessels and crystallization was allowed to take place by slow evaporation under room temperature. Good quality single crystals were obtained within two weeks. The harvested crystals were washed several times with acetone and dried for further studies.

RESULTS AND DISCUSSION

Solubility Study

In order to assess quantitatively the effects of additives on the growth and nucleation, it was necessary to differentiate between changes in kinetics and changes in solubility since the

latter influences the super saturation for a given concentration of solute.

Solubility study of pure and metal ions doped L-alanine single crystals

The solubility study is generally carried out to know the amount of the material available for the growth and hence defines the size of the crystal that can be grown. The solubility curves for pure and metal ions-doped L-alanine samples in water at different temperatures ranging from 30 to 50°C are shown in Fig-2.

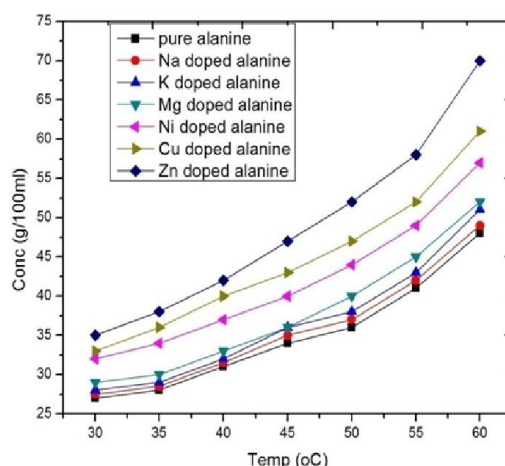


Fig. 2. Solubility curve of pure and metal ions doped L-Alanine single crystals

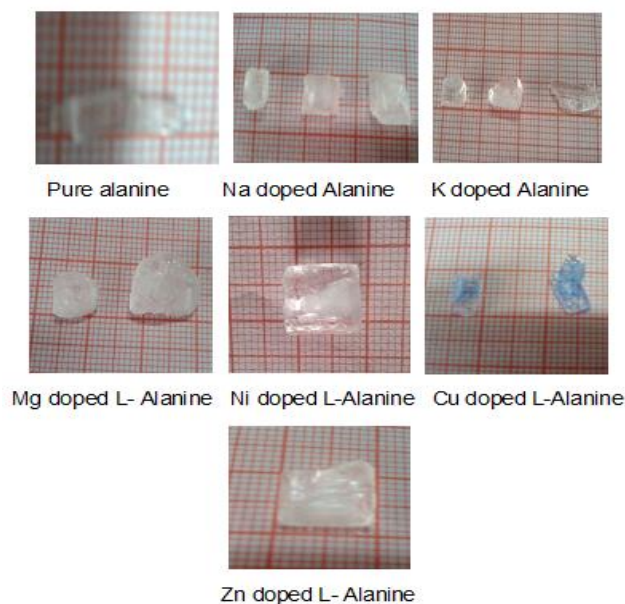


Fig. 3. Photographs of grown good quality L-alanine single crystals

Here, the solubility was the amount of solute in grams present in 100ml of saturated solution at a given temperature. From the graphs, it was observed that solubility increases with increase in temperature for both the compounds and it was found to be more for the transition metal ions-doped L-alanine. It is obvious that for the metal ions-doped sample, the solvent water

is capable of accommodating a slightly increased amount of solute for saturation at a particular temperature. Since the solubility of the compounds increases with increase in temperature, the samples of this study have positive temperature coefficient of solubility. Hence pure and metal ions doped L-alanine crystals can be grown from aqueous solution by slow solvent evaporation method. The photographs of the prepared pure and metal ions doped L-alanine single crystals are shown in Fig-3

FT-IR Spectral analysis of pure and metal ions doped L-alanine single crystals.

The Fourier Transform infrared spectra were recorded for powdered crystals of pure and metal ions doped all selected L-amino acids crystals using Perkin-Elmer FTIR spectrometer by KBr pellet technique in the range of 400-4000 cm^{-1} . For pure L-alanine crystals the absorption peak, as shown in Fig-4, at 3409 cm^{-1} is the indication of the presence of NH_3^+ group in the crystal. The peak at 2602 cm^{-1} is attributed to the C-H stretching mode of vibrations. The peak at 1575 cm^{-1} confirms the strong C=O stretching and at 1402 cm^{-1} shows COO^- symmetric stretching frequencies. Wave numbers 1305, 918 and 649 cm^{-1} show C-H bending. The O-CO bending mode at 768 cm^{-1} has been identified and assigned and at 846 cm^{-1} , a strong C-H wagging and twisting is identified. Thus, adding dopants into L-alanine crystal shows variations in the infrared spectra, especially by varying the nature of dopants like alkaline to alkaline earth and transition metal sulphates, they exhibit characteristic stretching frequency corresponds to that of particular metal. For example, in the case of alkali metal sulphates like sodium and potassium metal sulphates exhibit characteristic peak at 1107 cm^{-1} . Similarly in the case of alkaline earth metal like magnesium the stretching frequency falls at 1173 cm^{-1} and 873 cm^{-1} . For transition metals like Ni, Cu and Zn they exhibit stretching frequency around 1636 cm^{-1} , 1672 cm^{-1} and examinations of the peak position illustrates substantial shifts for the peaks at 3368, 1622, 1494, 1401, 1098, 711 and 473 cm^{-1} confirms the presence of Zn in L-alanine. All these assignments indicate the presence of metal ions on the amino acid L-alanine lattice in the crystal. The assignments for the absorption peaks of the FTIR spectra have been given in accordance with the data reported in the literature (Gross and Kalra 2002).

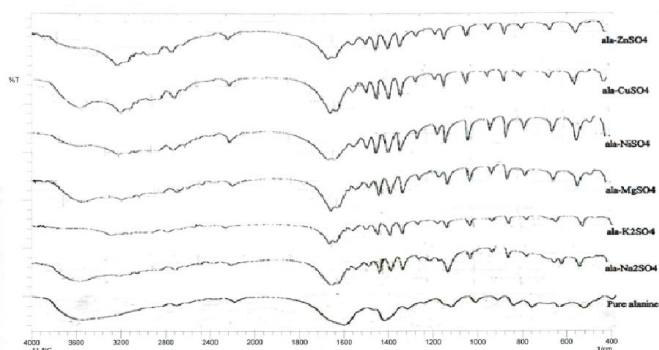


Fig. 4. FT-IR Results of pure and metal ions doped L-Alanine Single crystals

UV-Visible Spectra of pure and metal ions doped L-alanine single crystals

The UV-Visible-absorption spectra were recorded using Perkin-Elmer Lambda 35 UV-Visible Spectrometer in the range 190 nm to 800 nm for all the selected amino acid crystals. The transparent single crystalline samples were used and mounted in a standard manner so that equal areas of crystals were exposed to the radiation. The optical transmission range and the transparency cut-off-limits are important for NLO materials. The UV-Vis optical transmission spectra of pure and metal ions doped L-alanine crystals are shown in Fig-5.

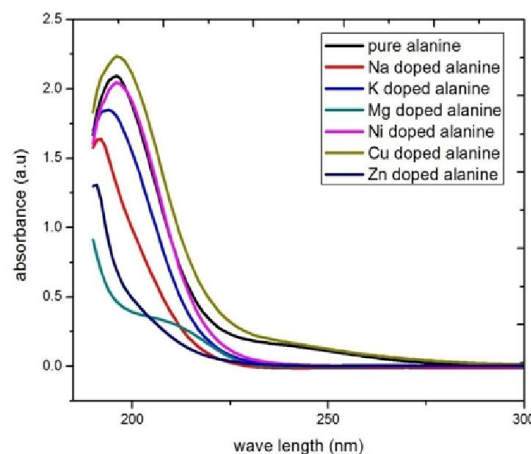


Fig. 5. UV Spectra of pure and metal ions doped L-Alanine single crystals

It is observed from the spectra, very low absorption takes place in the entire visible region and shows maximum absorption in the UV region. It is one of the most desirable properties of the crystals for the fabrication of optoelectronics devices. The UV cut off wave length was found to be 200 nm. The absence of absorption bands in the visible region and the wide band gap of the grown crystal attest to the suitability of the grown crystal for photonic and optical applications (Ramajothi and Dhanuskodi 2007). The smaller energy band gap shows that the defect concentration in the grown crystals is high; means though various metal ions are doped there is no change in the nature of absorption. The absorption intensity only varies without varying the nature of absorption. This shows if we dope metal ions on L-alanine their photonic and optical applications are enhanced in a remarkable manner. Altogether, the percentage of optical absorption increases on increasing the doping of metal ions on L-alanine. The increments are also in a linear manner when we doped alkali metal the absorption increasing slightly than the undoped one. While changing the nature of dopant like alkali, alkaline earth and transition metal ions on alanine, their absorbance also increased accordingly. The reason may be due to transition metals absorb UV radiation in a remarkable manner than its alkaline counterpart. So, transition metal ions doping increases the transparency of grown crystals of L-alanine. The lower cut-off at 200nm combined with the very good transparency window makes the material suitable for optoelectronics applications, the

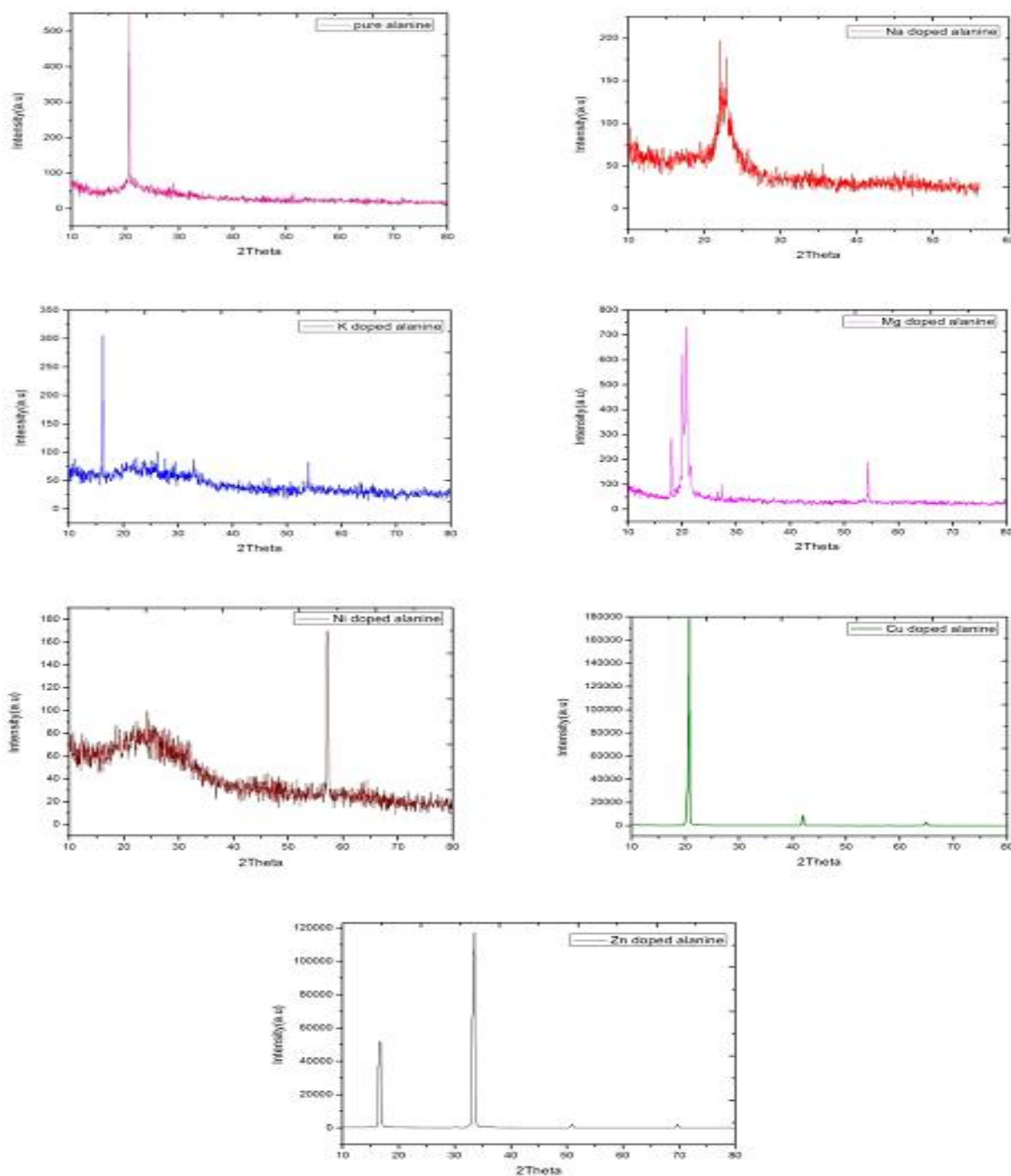


Fig. 6. powder XRD analysis of pure and metal ions doped L-Alanine

Table 1. Comparison of unit cell parameters of grown crystals along with pure L-Alanine

	Pure L-alanine	Na doped L-alanine	K doped L-alanine	Mg doped L-alanine	Ni doped L-alanine	Cu doped L-alanine	Zn doped L-alanine
Hkl	(120)	(211) (220)	(200) (103) (304)	(120)(021) (031)(340)	(013) (318)	(120)(318)	(110)(011) (220)(102)
System	Orthorhombic	Monoclinic	Monoclinic	Orthorhombic	Orthorhombic	Orthorhombic	Orthorhombic
Space Group	P2 ₁ 2 ₁ 2 ₁ (19)	P2 ₁ /c(14)	P2(3)	P2 ₁ 2 ₁ 2 ₁ (19)	PnC2 (30)	P2 ₁ 2 ₁ 2 ₁ (19)	P2 ₁ 2 ₁ 2 ₁ (19)
Cell Parameters	a=6.032 b=12.34 c=5.784	a=10.764 b=7.747 c=9.146	a=12.93 b=6.428 c=10.03	a=6.032 b=12.34 c=5.784	a=21.26 b=5.85 c=9.146	a=6.032 b=12.34 c=5.784	a=6.032 b=12.34 c=5.784
Interfacial angles	$\alpha=\beta=90^\circ$ $\gamma=90^\circ$	$\alpha=\gamma=90^\circ$ $\beta=117.24^\circ$	$\alpha=\gamma=90^\circ$ $\beta=99.2^\circ$	$\alpha=\beta=90^\circ$ $\gamma=90^\circ$	$\alpha=\beta=90^\circ$ $\gamma=90^\circ$	$\alpha=\beta=90^\circ$ $\gamma=90^\circ$	$\alpha=\beta=\gamma=90^\circ$
JCPDS Card No.	28-1508	37-1586	28-1751	22-1532	25-1885	22-1532	22-1532

generation of the second and third harmonics of the Nd: YAG.laser.

Powder XRD analysis of pure and metal ions doped L-alanine

The XRD data was collected using a X-PERT Phillips diffractometer with $\text{CuK}\alpha$ ($\lambda=1.540598 \text{ \AA}$), step of 0.05° and 2θ scanning between 10 and 80° . Finally, the samples were powders of crystals. Fig.6 shows the X-ray diffraction pattern of pure and metal ions doped L-alanine. The main peaks taken from the database gave information that the L-alanine crystal belongs to the orthorhombic system with a non-centrosymmetric space group $P2_12_12_1$. Using the powder X-ray diffraction study, the crystallinity of the grown crystals was confirmed and the diffraction peaks were indexed. Well defined Bragg peaks are obtained at specific 2θ angles indicating that the crystals are arranged in well ordered manner. The 'd' spacing and hkl values for prominent peaks in the spectrum were identified and compared with the JCPDS values. Using tetragonal crystallographic equation, lattice parameter values are calculated and listed in Table-1.

This data from the table suggests that the crystals retain almost the single phase structure and exhibit very slight variation in the unit cell parameters on doping of alkali metal ions on L-alanine. By doping alkali metal ions the crystal symmetry has changed as Monoclinic. The variation in the intensities of various diffraction patterns on changing the nature of dopant and their corresponding space groups, cell parameters, interfacial angles and the JCPDS card numbers are compiled in Table-1. This is in close agreement with the values obtained from single crystal X-ray diffraction analysis for L-alanine. Furthermore, the intensities appear in the same diffraction planes indicating that there is not much change in the orientation during the growth of the dopant on crystals. This is because, in general, metal ions seat on the site of lattice while doping.

Etching studies of pure and metal ions doped L-alanine

Etching is the selective dissolution of the crystal which reveals the crystal symmetry and lattice defects (Mukerji and Kar 1999). Patterns observed on surface like spirals, hillocks and step pattern, etc yield considerable information on the growth process and growth mechanism of the crystal. When a surface is etched, well defined etch patterns are produced at the dislocation sites. The chemical etching studies were carried out on the as grown crystals of pure L-alanine and metal ions doped L-alanine crystals to investigate the distribution of structural defects in the grown crystals. The surfaces of the samples were polished and then etched in the etching solution (ethanol) at room temperature for 10secs. Then soaked with a filter paper and examined under an optical microscope in reflection mode. Fig-7 illustrate the typical etch patterns observed on the (001) plane of the pure L-alanine and metal ions doped L-alanine crystals. When the crystals were etched in water, the square planar type etch pits were observed and the etch-pit density (EPD) for pure L-alanine crystal was found to be $2.5 \times 10^2 \text{ cm}^{-2}$ and for metal ions doped L-alanine crystal was around $14.28 \times 10^2 \text{ cm}^{-2}$. Usually, these etching pits are

considered as outcrop of dislocation lines. The shape of these etching pits is determined by the lattice structure and symmetry (Ochei and Kolhatkar 2000). This is also evident from the fact that the bottom of the crystal is flat. It means that the crystals are grown from the bottom of the beaker/container and not in the middle of the solution. The presence of dislocations strongly influences many of the properties of crystals. The generation of dislocations is strongly correlated with the formation of inclusions in the crystals. The formation of inclusions destroys the normal build of lattices, which results in stress and lattice mismatch in crystal. In order to relax the stress and adjust the lattice mismatch, lots of dislocations were produced. That's why so many dislocation etching pits are observed in doped L-alanine crystal.

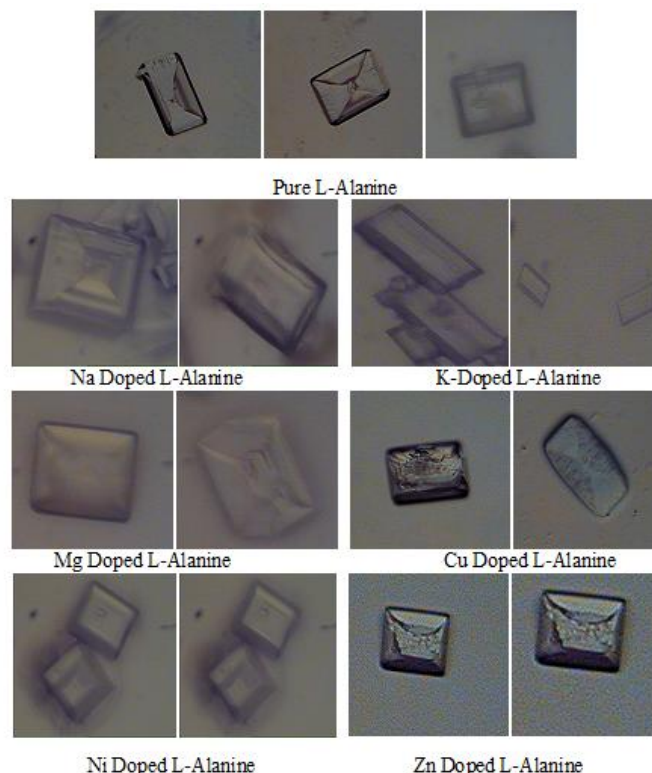


Fig. 7. Surface revealing of etched pure and metal ions doped L-alanine single crystals

Antimicrobial activity of pure and metal ions doped L-alanine single crystals.

Antibacterial activity was measured using a Well-diffusion method according to the National Committee for Clinical Laboratory Standard (1999). Petri plates containing 20 ml of Nutrient Agar medium were inoculated with a 24 hour culture of the bacterial strains. Wells (6mm diameter) were punched in the agar and filled with $30\mu\text{l}$ of L-alanine and metal ions doped L-alanine. The plates were incubated at 37°C for 24 hours. The antimicrobial activity was assessed by measuring the diameter of the area in which bacterial growth was inhibited around the well. Both gram positive and gram negative organisms like *Enterobacter*, *Proteus*, *Klebsiella* and *Streptococcus* were tested against the pure and metal ions doped L-alanine were tested and are shown in Fig-8.

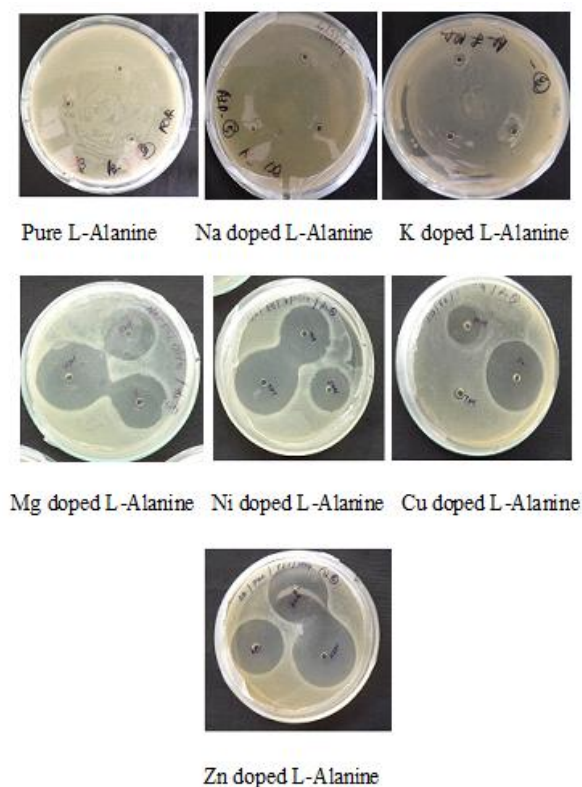


Fig. 8. Antimicrobial activity of pure and metal ions L-Alanine single crystals

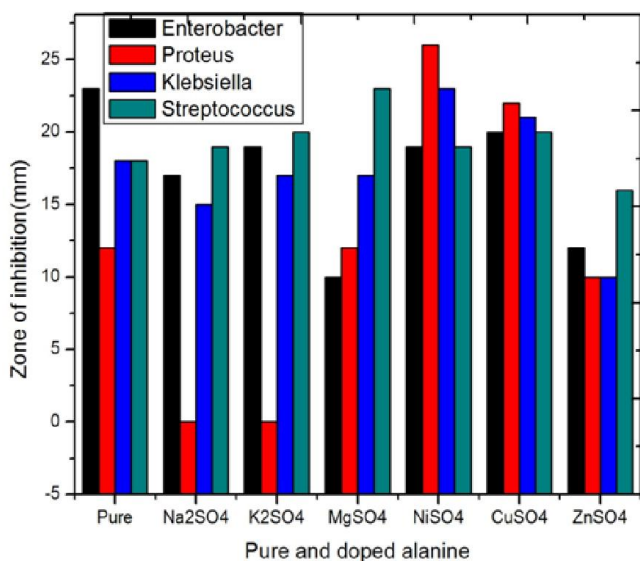


Fig. 9. Zone inhibition areas of pure and metal ions doped L-Alanine single crystals

Due to the aliphatic nature of L-alanine the zone of inhibition is very very low for pure L-alanine, because there is no chance of electron delocalization. But in the case of metal ions doped L-alanine there is considerable amount of zone of inhibition. The reason may be due to the influence of metal ions that enhance the electron delocalization in a reasonable manner. In the case of transition metal ions the zone of inhibition is higher compared to alkali and alkaline earth metals. Again in the case of transition metal ions Cu^{2+} exhibit maximum zone of

inhibition than the other transition metal ions counterpart like Ni and Zn, and this may be due to hole formylation nature of Cu^{2+} which has maximum electronic transition that may prevent the growth of micro organism on the crystal medium. The sensitivity of the microorganisms against the pure and metal ions doped L-Alanine are represented as bar diagram and is shown in Fig.9.

Conclusion

The present study deals with growth of L-Alanine (non-essential), amino acid single crystals and alkali, alkaline earth and transition metal ions doped above said amino acid by using conventional slow evaporations solution growth method. The functional groups present in the grown crystals were identified by recording FTIR spectra. All the recorded FTIR spectra exhibited characteristic peaks corresponding to the nature of the dopant metal ions and other functional groups like amino and carboxylic acid present in amino acids. The optical absorbance of the grown crystals confirms that L-alanine shows maximum absorption in the UV region. So, crystals of L-alanine are used for the fabrication of optoelectronics devices. The absence of absorption bands in the visible region and the wide band gap of the grown crystal and the smaller energy band gap in various metal ions doped L-alanine exhibited their photonic and optical applications are enhanced in a remarkable manner. From the XRD, we found that L-alanine crystal belong to the orthorhombic system with a non-centrosymmetric space group $P2_12_12_1$. By doping alkali metal ions the crystal symmetry has changed as monoclinic. The chemical etching studies were carried out on the as grown crystals and typical etch patterns observed on the (001) plane of the pure L-alanine and metal ions doped L-alanine crystals. When the crystals were etched in water, the square planar type etch pits were observed. These etching pits are considered as outcrop of dislocation lines. The shape of these etching pits is determined by the lattice structure and symmetry. All the grown crystals were evaluated in vitro for antibacterial activity by using filter paper disc method against different strains of bacteria, viz, *Enterobacter*, *Proteus*, *Klebsiella* and *Streptococcus*. All the crystals along with standard antibacterial Streptomycin were used at 50 and 100 ppm concentrations. Due to the aliphatic nature of L-alanine the zone of inhibition is very very low for pure L-alanine and metal ions doped L-alanine, because there is no chance of electron delocalization in it.

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