

Synthesis, Characterization and Biological Activity of Pure and Metal Ions Doped L-Proline Amino Acid

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Abstract- Alkali, Alkaline earth and transition metal ions doped L-proline single crystals were grown by solution growth technique. Due to variation in the nature of dopant, there is a modification in the growth habit, nonlinear optical property and mechanical hardness of the doped crystals. Fourier transform infrared (FTIR) spectra reveal strong absorption bands occur due to the presence of L-proline. UV-visible spectra show an improvement in the optical transmittance while doping on L-proline. The unit cell parameters and cell volume are obtained by powder X-ray diffractometer. Effect of dopant on the crystal defect on the grown crystals was characterized by Optical Microscopy. Anti microbial study of the grown crystals were analyzed using gram positive and gram negative micro organisms.

Index Terms- Solution growth technique, Dopant, Optical Microscopy, Powder X-ray diffractometer.

I. INTRODUCTION

The rapid development in the field of science and technology necessitates the search for newer and efficient nonlinear optical materials. In recent years, much attention has been paid to the research of novel nonlinear optical (NLO) materials, especially semiorganic crystals. Semiorganic compounds illustrate the following features:(1)dipolar structure composed of an electron donating and electron accepting group; (2) the contribution from the delocalized electrons belonging to organic ligand results in high nonlinear optic and electro-optic coefficients in the semi organic crystals also; (3) the organic ligand is ionically bonded to metal ion to impart improved mechanical and thermal properties; (4) exhibit wider transparency range, chemical stabilities and bulk crystal morphologies.

Nonlinear optics is one of the most attractive fields of current research in view of the applications in the areas such as optical modulation, optical switching, optical logic, frequency shifting and optical data storage and for developing technologies in telecommunication and signal processing[1,2]. Investigations were initially focused on purely inorganic materials, which were the first to demonstrate second-order nonlinear optical properties [3]. Attention was later directed towards organic materials due to their low cost, fast and large nonlinear response over a broad frequency range and inherent synthetic flexibility of high optical damage threshold [4]. However, organic materials are poor in thermal stability and mechanical hardness. It is difficult to grow large size and high quality single crystals. Recently, several complexes of proline were reported such as L-proline cadmium

chloride monohydrate and L-proline lithium chloride monohydrate [5].

Organic nonlinear optical materials have large optical susceptibilities, inherent ultrafast response times, and high optical thresholds for laser power as compared with inorganic materials. Amino acids are interesting materials for NLO applications as they contain a proton donor carboxyl acid (-COOH) group and proton acceptor amino (-NH₂) group in them [6]. Amino acids, when added as impurities, have improved material properties [7]. Amino acid, L-proline has formed several complexes, which are promising materials for second harmonic generation [8, 9].

The present study concentrate on the crystal growth of a new NLO crystal of alkali metals, alkaline earth metals and transition metals doped L-Proline by slow evaporation technique and its characterization along with its optical properties is reported. The grown crystals were subjected to powder XRD study to understand the crystal system and space group.

The transparency range of the crystal was estimated from UV-VIS spectrum to find out its optical property. Through etching study the defects that exist due to dopant also found. The antimicrobial study of pure and metal ions doped L-proline was also tested.

II. EXPERIMENTAL

2.1. Solubility study of pure and metal ions doped L-proline single crystals

The solubility of pure and metal ions doped L-proline single crystals were studied for six different temperatures 30, 35, 40, 45, 50 and 60°C as shown in fig.1. Initially, supersaturated solution was prepared at 30°C in an air tight container of 100 ml inside the constant temperature bath maintained with an accuracy of $\pm 0.01^\circ\text{C}$. After achieving the super saturation, the solution was analyzed gravimetrically and the solubility of pure L-proline and metal ions doped L-proline in 100ml of solvent was determined. This procedure was adopted for various temperatures and the solubility curves were drawn. It is observed from the solubility curves that the solubility increases with increasing temperature. The solvent was able to accommodate fairly more solute between the temperature ranges of 45 to 50 °C. Therefore this temperature range was suggested for the growth by temperature lowering method. Equal proportions of L-proline and metal sulphates were taken and were dissolved separately in deionized water. Then the solution of L-proline was poured into the dissolved metal sulphates mixture. The solution thus arrived was filtered twice to remove dust particles and un dissolved materials.

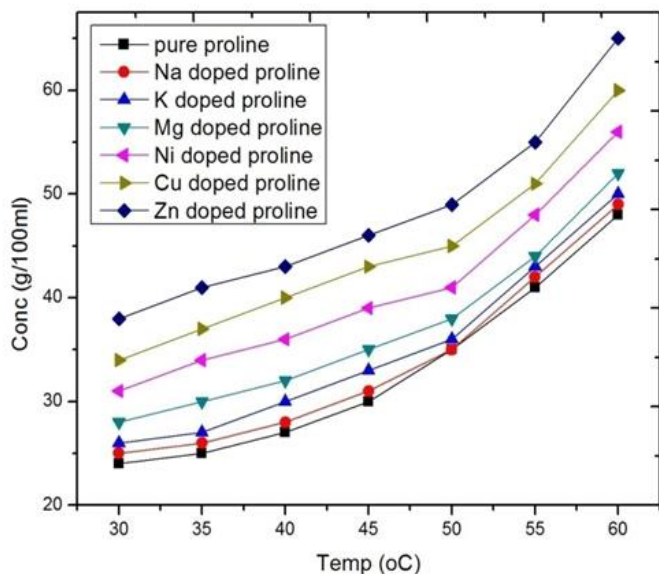


Fig.1 Solubility curve of pure and metal ions doped L-proline single crystals.

2.2 Synthesis of pure L-proline single crystal

The pure L-proline single crystals were grown by dissolving purified L-proline powders in appropriate amount of double distilled water and stirred well using magnetic stirrer for six hours to form a clear solution. Then the solution was kept in a constant temperature bath at 45°C. After a while the temperature had been set to slowdown by 1°C for every 1 hour till to reach 35°C. After reaching 35°C the temperature bath was kept constant at that temperature for about 3 hours. After that 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 after 35 days. The harvested crystals were washed several times with acetone and dried for further study.

2.3 Synthesis of metal ions doped L-proline single crystals

L-proline and metal sulphates (Na^{2+} , K^{2+} , Mg^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+}) in 1:1 stoichiometric ratio were mixed in deionised water. The reactants were stirred well using a temperature controlled magnetic stirrer for 6hrs to yield a homogeneous solution. Then the same procedure had been adopted as in the previous case. The finally formed solution was filtered using Whatmann filter paper. The filtered solution was then kept in crystal growth vessels followed by slow evaporation at room temperature. Good transparent metal ions doped L-proline crystals were obtained after 45 days. The harvested crystals were washed several times with acetone and dried for further study.

The photographs of the prepared pure and metal ions doped L-proline single crystals are shown in Fig.2

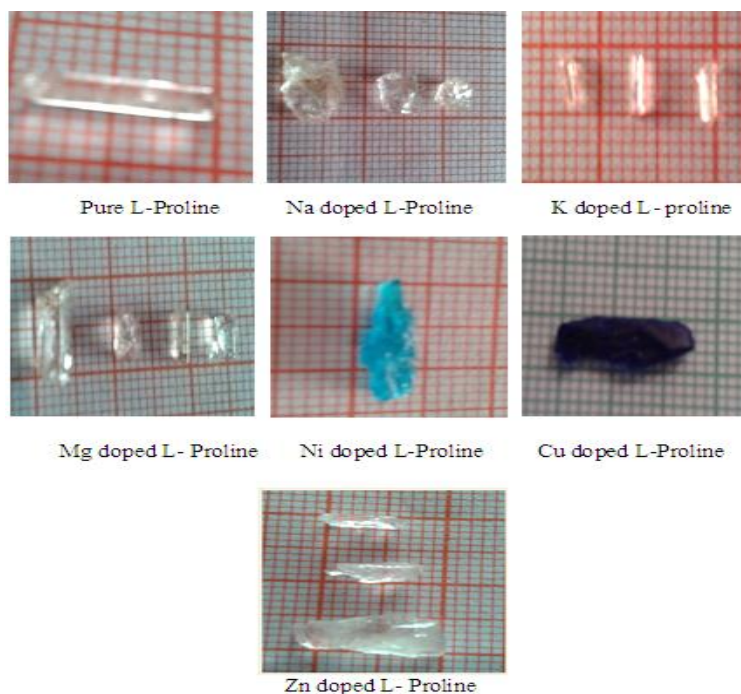


Fig.2 Photographs of grown good quality L-Proline single crystals.

III. RESULTS AND DISCUSSIONS

3.1 FT-IR Spectral analysis of pure and metal ions doped L-proline single crystals

The Fourier Transform infrared spectra were recorded for powdered samples of pure and metal ions doped L-proline crystals using Perkin- Elmer FTIR spectrometer by KBr pellet technique in the range of 400-4000 cm^{-1} . The FTIR spectra of pure and metal ions doped L-proline crystals are shown in fig.3. The effect of dopant on L-proline on the vibrational frequencies of the functional groups of pure L-proline crystal has been identified in the spectra. In the fig 3, the broad band around 3257 cm^{-1} is due to the O-H vibrations of water.

The peak obtained at 3419 cm^{-1} is due to stretching vibration of CH and the peak at 1600 cm^{-1} is due to the stretching vibration of C=O. The bands appeared at 793 cm^{-1} is assigned unambiguously to the wagging of NH_2 modes. The OH stretching vibrations is assigned in the range of 2565 cm^{-1} . The peak at 1398 cm^{-1} is due to the symmetric stretching of COO. These assignments are also supported in the literature.

An additional confirmation for the present assignment is the O-H in-plane-bend vibration at about 1112 cm^{-1} , which is typical for a non-hydrogen bonded carboxylic OH group. Similarly, the absorption maxima of the C=O stretch vibrations around 1780 cm^{-1} are almost identical for the L-proline amino acids.

Larger differences between the spectra are expected for the C-H and C-C stretch and bending vibrations, since among all the other amino acid L-proline is the only imino acid. The nitrogen atom present in the L-proline is secondary in nature. So the nitrogen in it interact with the metal ions change the stretching frequencies of C-H and C-C bonds. However, the spectra can be clearly distinguished in the C-H stretch and even better in the C-C stretch and C-H/N-H bending region. As usual, aromatic C-H

stretch vibrations occur above and aliphatic C–H stretch vibrations below 3000 cm^{-1} .

The splitting of the O–H in-plane-bend vibration of L-proline is due to harmonic couplings with the C–N stretch and C–H bend vibrations of the chiral carbon atom. Even the band shapes of the C–H stretch vibrations and of the C–C stretch and C–H/N–H bending vibrations between 1200 and 1600 cm^{-1} are mainly reproduced. Relative absorption intensities are also in good agreement with experiment.

From the spectra it is clear that, except for L-proline, the symmetric and asymmetric N–H stretch vibrations around at 3393 and 3088 cm^{-1} are barely visible in the simulated spectra. Indeed, we do not observe the N–H stretch vibrations in the present experiment. Only L-proline, where the nitrogen is incorporated in the five membered rings, is predicted to have larger infrared absorption intensity.

A remark to the $\nu(\text{C}=\text{O})$ backbone absorptions of L-proline is unusual compared to the amide absorption of other amino acids. It absorbs at 1618 and 1592 cm^{-1} . Assignments are according to Herlinger and Long (1970), νCN vibration which is assigned as 1409 cm^{-1} and it is sensitive to backbone conformations [10].

The broad band which absorbs at 3393 cm^{-1} corresponds to symmetric and asymmetric vibrations of NH_2^+ group. The band at 1618 cm^{-1} confirms the presence of COO^- group in a molecule. The presence of ring νCH and νCH_2 in the crystal conforms the absorption of band at 1362 cm^{-1} symmetric, 2985 cm^{-1} asymmetric and the bands at $3088, 1150$ and 1455 cm^{-1} both symmetric and asymmetric confirms the νCH_2 functional group present in a molecule. All these assignments show the formation of L-proline crystal in a perfect manner. The influence of dopant such as Na, K, Mg, Ni, Cu and Zn shifts the absorption of NH_2^+ and COO^- functional groups. The absorption bands at 1107 cm^{-1} confirms

the presence of both Na and K in L-proline. The bands at 1176 cm^{-1} as a broad peak and 873 cm^{-1} as a weak peak confirms the presence of Mg doped on L-proline. Again in the case of Ni and Cu the characteristic sharp peak occur at 1083 cm^{-1} and weak band at 1079 cm^{-1} confirms the presence of Ni and band at 1672 cm^{-1} confirms the presence of Cu dopant on L-proline. All the spectral assignments conforms the dopants present in L-proline.

The absorption in the $2744\text{--}2456\text{ cm}^{-1}$ region is resulting from superimposed O–H and NH_2^+ stretching bands. The peak at 1661 cm^{-1} corresponds to the asymmetrical NH_2^+ stretching vibration. The absorption in the $1566\text{--}1457\text{ cm}^{-1}$ region is due to symmetrical NH_2^+ stretching vibration. Ratajczak et al [11] determined that the lack of any strong IR band at 1700 cm^{-1} clearly indicates the existence of the COO^- ion in Zwitter ionic form.

The peaks at 1414 cm^{-1} are assigned to symmetrical COO^- ion group stretching. The other peaks at 1376 and 1329 cm^{-1} are assigned to wagging of CH_2^+ group of the L-proline. The peaks at 1194 cm^{-1} are assigned to twisting NH_2^+ . The wagging vibration of COO^- is observed at 679 cm^{-1} . The peak at 780 cm^{-1} was attributed to the in-plane deformation of COO^- .

The broad band lies in the range $2710\text{--}3400\text{ cm}^{-1}$ in all the doped crystal corresponds to symmetric and asymmetric vibrations of NH_2 group. Very strapping absorption occur around 950 cm^{-1} is owing to sulphate ion. The FTIR spectra of doped crystals show a strong NH absorption peak at about 3200 cm^{-1} . When metal sulphates are doped with L-proline more NH stretch vibrations are pioneer due to doping and as a result the NH absorption peak becomes strapping. So FTIR spectra indirectly ascertain the presence of metal ions on L-proline crystal.

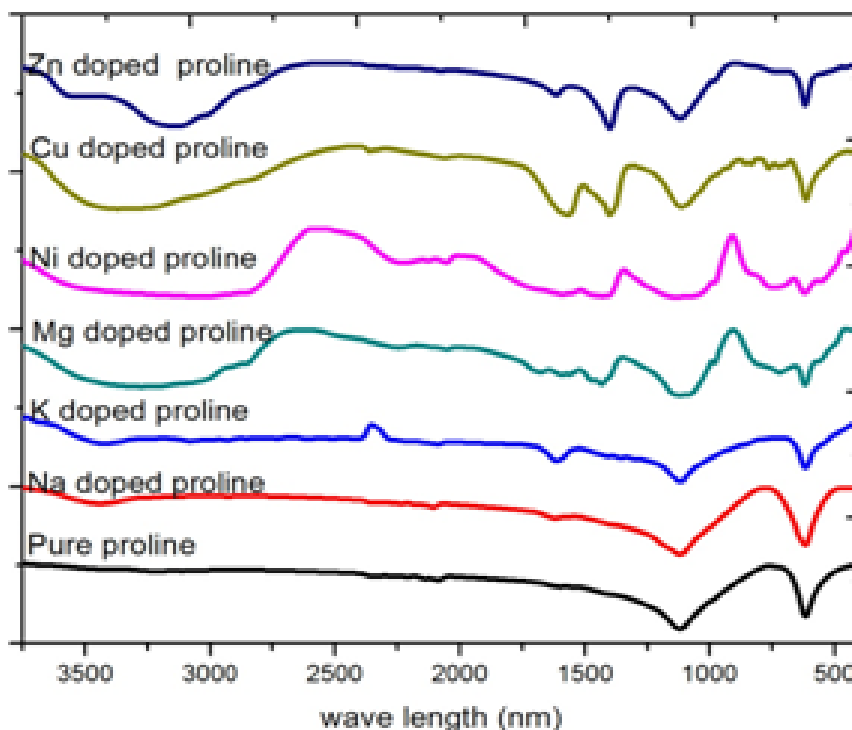


Fig.3 FT-IR Spectra of pure and metal ions doped L-Proline single crystals.

3.2 UV Spectra of pure and metal ions doped L-proline 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 are shown in Fig. 4. From the spectra, it is observed that both the pure and metal ions doped L-proline crystals show good absorption in the UV regions. The pure L-proline single crystal has absorption around 200nm. Thus the addition of metal ions on L-proline has increased the intensity of absorption than pure L-proline. The higher percentage of absorption for Cu doped L-proline when compared to pure and other metal ions doped L-proline suggests the presence of Cu enhance the optical activity of L-proline. The reason for this enhancement may be due to the hole formylation of Cu which when added to L-proline the lone pair electron on N of L-proline may facilitate the electronic transition in a wide manner than the other counterpart. Thus it has maximum absorption with highest intensity. The UV-VIS spectra give restricted information about

the structure of the molecule because the absorption of UV and visible light involves absorption or transmission of the electron from the ground state to higher energy states. From the spectra it is observed that the absorption percentage of metal ions doped L-proline is 15-18 % higher than that of the pure grown crystal. The doped crystal shows a superior absorption in the intact UV region. Good optical absorption and lower cut-off wavelengths are very important properties for NLO crystals. These properties were satisfied by the metal ions doped L-proline. Hence, these crystals may be used for the nonlinear optical applications in the above mentioned wavelength range. So these crystals can be used as NLO crystal properties.

From the spectra (Fig.4), it is evident that above crystal has UV cut off wavelength at 220 nm, which is sufficient for SHG laser radiation or other applications in the blue region. It further indicates that the crystal has wide transparency window between 200 nm and 800nm.

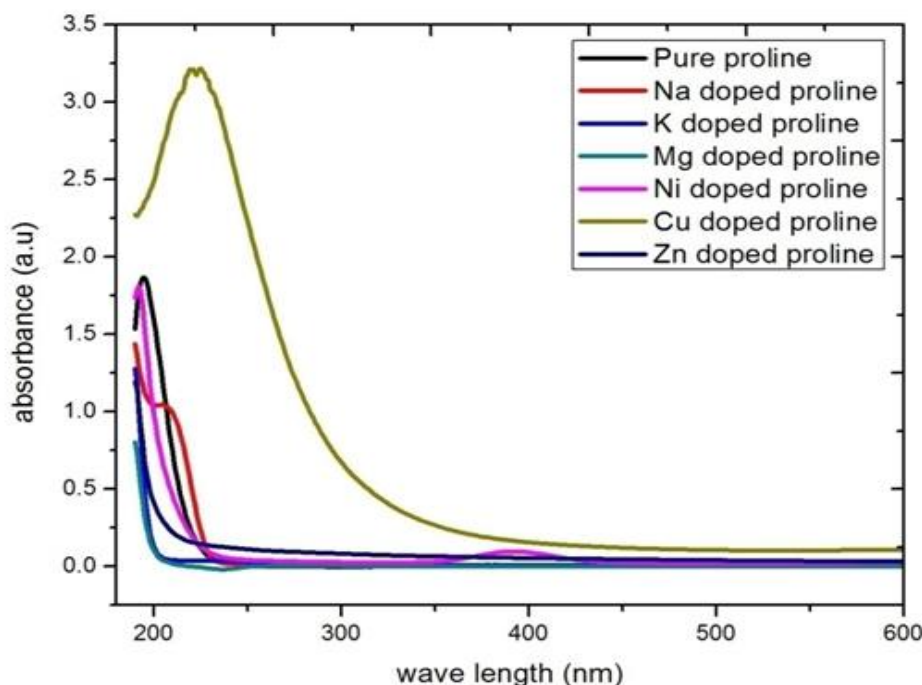


Fig 4 UV Spectra of pure and metal ions doped L-Proline single crystals

3.3 Powder XRD analysis of pure and metal ions doped L-proline single crystals

L-Proline exist in a monoclinic nature having unit cell dimensions of $a= 20.60$, $b= 6.315$ and $c= 5.186$. Using the tetragonal crystallographic equation the lattice parameter of the pure and metal ions doped L-proline crystals were calculated with in an accuracy of ± 0.002 . The crystals were identified by

comparing the interplanar spacing and intensities of the powder pattern with the JCPDS data of L-proline crystals. The variation in lattice parameters and d values for the doped samples can be attributed to the accommodation of impurity in the crystal lattice. The slight shift in the 2θ values of doped crystals suggests that crystal structure were slightly distorted compared to undoped L-proline. (fig-5 and data compiled in table-1).

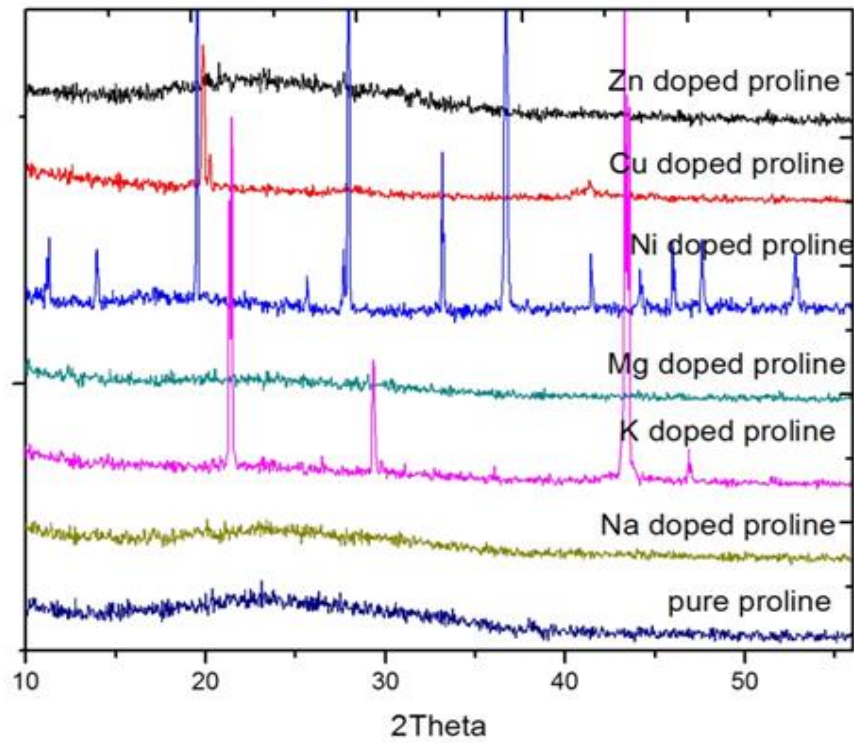


Fig 5 Powder XRD analysis of pure and metal ions doped L-proline single crystals

Table 1: Comparison of unit cell parameters of grown crystals along with pure L-proline

	Pure L-proline	Na doped L-proline	K doped L-proline	Mg doped L-proline	Ni doped L-proline	Cu doped L-proline	Zn doped L-proline
hkl	(310)(311) (-511)(421) (-312)(821)	(020)(201) (211)(231)	(211)(221) (820)(821) (442)(204) (214)(951)	(111)(220) (420)(022) (820)(313) (351)	(110)(-311) (710)(202) (-711)(330) (110)(731)	(111)(020) (731)(710) (910)	(111)(211) (121)(400) (040)(422) (342)
System	Monoclinic	Orthorhombic	Orthorhombic	Orthorhombic	Monoclinic	Monoclinic	Orthorhombic
Space Group	C ₂ (5)	P2 ₁ 2 ₁ 2 ₁ (19)	P2 ₁ 2 ₁ 2 ₁ (19)	P2 ₁ 2 ₁ 2 ₁ (19)	C ₂ (5)	C ₂ (5)	P2 ₁ 2 ₁ 2 ₁ (19)
Cell Parameters (Å)	a=20.60 b=6.315 c=5.186	a=11.64 b=9.037 c=5.620	a=19.59 b=7.839 c=5.619	a=19.59 b=7.839 c=5.619	a=20.60 b=6.315 c=5.186	a=20.60 b=6.315 c=5.186	a=11.64 b=9.037 c=5.620
Interfacial angles	$\alpha = \gamma = 90^\circ$ $\beta = 93.21^\circ$	$\alpha = \beta = \gamma = 90^\circ$	$\alpha = \beta = \gamma = 90^\circ$	$\alpha = \beta = \gamma = 90^\circ$	$\alpha = \gamma = 90^\circ$ $\beta = 93.21^\circ$	$\alpha = \gamma = 90^\circ$ $\beta = 93.21^\circ$	$\alpha = \beta = \gamma = 90^\circ$
JCPDS Card No.	32-1869	21-1805	51-2479	51-2479	32-1869	32-1869	21-1805

3.4 Etching studies of pure and metal ions doped L-proline single crystals

Chemical etching is a very simple and elegant technique to reveal the crystal defects and the crystal growth mechanism. Which is able to develop some features such as growth strains, etch spirals, rectangular etch pits, etc on the crystal surface, Here in the etching studies were carried out on the plane of the as grown single crystals of L-proline for 10-30s. Only some scattered etch pits and short strains were observed for the etching time of 10s. Increasing the etching time to 30s, the size of the etch pits enlarged and some growth strains spread along an axis. The observed etch pits, attributive to layer growth; confirm the

two-dimensional nucleation (2D) mechanism with less dislocations [12].

Figure 6 shows the surface of the L-proline single crystal using water as an etchant at room temperature for 5 and 7s. First, the crystal sample was completely immersed in the etchant and then etched sample was cleaned using a tissue paper and the etch patterns were observed using an optical microscope in the reflection mode. When the crystal was etched for 5 and 7s, the round hole etch were observed. Well defined etch pits were observed while increasing the etching time. The shape of the etch pits may be changed by varying the concentration of the solvent (Sangwal 1992) [13]. Moreover, factors such as temperature of etching, stirring, and adsorption of impurities or reaction

producers, which alters the absolute value of these rates, also lead to the change in the geometry of etch pits (Gilman et al 1957)[14]. The resulting etch patterns are the characteristic distribution of defects and nature of defects in the sample and also confirmed the two-dimensional nucleation growth mechanism. The etch pits produced by etchants are composed from solvents in which a crystal is highly or poorly soluble.

The diamond shaped etches pits obtained for pure L-proline revealed that L-proline has no defects on its own and it confirms the monoclinic nature with perfect crystallinity. The further change of etch pits while doping on L-proline confirms the change in morphology of doped L-proline compared to undoped one.

The beautiful hexagonal forms shown in Mg doped L-proline are those produced by some distortion in the crystal lattice. These etch pits are definitely limited by hexagonal pyramidal faces characteristically symmetrical to six planes of symmetry which accords with all experimental results obtained in this type of doped crystal. The nature of the solvent, then, seems not to affect the final results. Solution phenomena, therefore, are of primary importance when considering the symmetry content and classifications of crystals.



Fig.6 Surface revealing of etched pure and metal ions doped L-proline single crystals

3.5 Antimicrobial activity of pure and metal ions doped L-proline single crystals

The antibacterial activity results of the pure L-proline and their metal ions doped L-proline were given in Fig.7. Antimicrobial results showed that all the synthesized compounds possess biological activity. The activity generally increases with increasing the concentration of the compounds.

The data revealed that the activity of the metal ions doped L-proline enhanced than the undoped L-proline. This enhancement in the activity is rationalized on the basis of the structures of metal ions by possessing an additional electron delocalization of the presence of these metal ions than the pure L-proline. This enhances the trans amination and resamination reactions in biological system. It has also known that the metal ions with nitrogen and oxygen donor systems of L-proline might inhibit enzyme production, since the enzymes which require these groups for their activity appear to be especially more susceptible to deactivation by the metal ions upon bonding with L-proline[15]. On bonding the polarity of the metal ion will be reduced to a greater extent due to overlap of the L-proline nitrogen orbital and partial sharing of positive charges of metal ion with donor groups like L-proline. Further it increases the delocalization of pi electrons over the whole L-proline enhances the lipophilicity of the formed crystal. This again enhances the penetration of metal ions doped L-proline into lipid membrane and blocking the metal bonding sites on enzymes of microorganisms, hence increases the biological activity [16].

Here we noticed that both in bar diagram and in measuring the diameter of zone of inhibition of Cu^{2+} doped L-proline, we found that Cu^{2+} exhibited higher zone of inhabitation than all the other crystals. The reason may be due to greater chance of delocalization of Cu^{2+} (d^9 system) with secondary nitrogen of L-proline. Because of this greater and unique nature of this Cu^{2+} ions exhibit greater inhibition zone than all other counter parts.

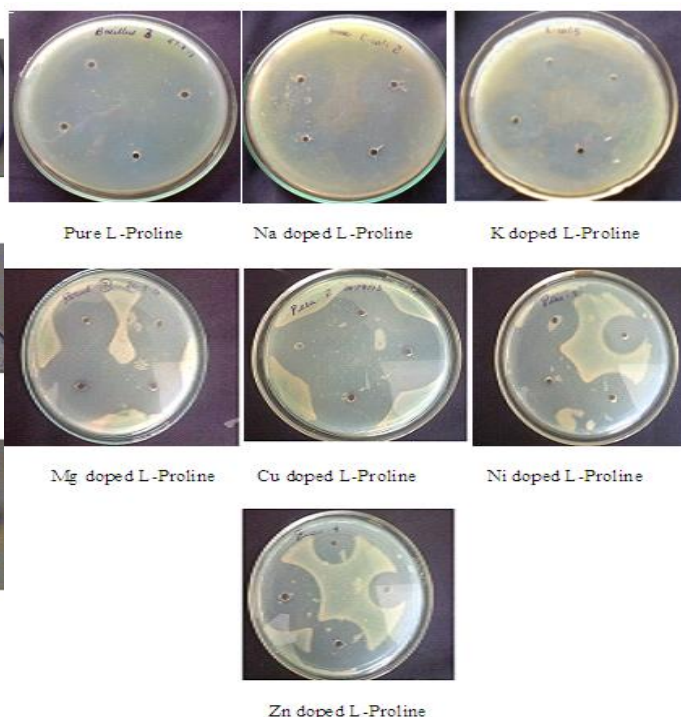


Fig 7 Antimicrobial activity of pure and metal ions L-Proline single crystals

The sensitivity of the microorganisms against the pure and metal ions doped L-proline are represented as bar diagram is shown in Fig 8

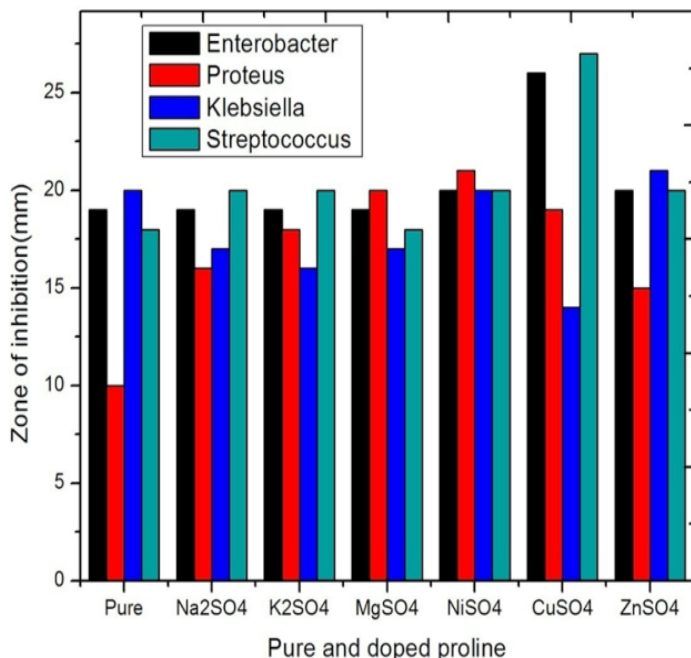


Fig.8 Zone inhibition areas of pure and metal ions doped L-Proline single crystals.

IV. CONCLUSION

The present study deals with growth of L-Proline(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 UV Visible absorption of pure L-proline single crystal has absorption around 200 nm and the addition of metal ions on L-proline has increased the intensity of absorption than pure L-proline. The higher percentage of absorption for Cu doped L-proline when compared to pure and other metal ions doped L-proline suggests the presence of Cu enhance the optical activity of L-proline. The reason for this enhancement may be due to the hole formylation of Cu which when added to L-proline the lone pair electron on N of L-proline may facilitate the electronic transition in a wide manner than the other counterpart.

L-Proline exist in a monoclinic nature having unit cell dimensions similar to metal ions doped L-proline. The slight shift in the 2θ values of doped crystals suggests that crystal structure were slightly distorted compared to un doped L-proline.

The diamond shaped etches pits obtained for pure L-proline revealed that L-proline has no defects on its own and it confirms the monoclinic nature with perfect cristanality. The further change of etch pits while doping on L-proline confirms the

change in morphology of doped L-proline compared to undoped one.

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.

The antimicrobial activity of L-proline and the metal ions doped L-proline revealed that the activity of metal ions doped L-proline is higher than the undoped L-proline. This enhancement in the activity is rationalized on the basis of the structures of metal ions by possessing an additional electron delocalization of the presence of these metal ions than the pure L-proline. This enhances the transamination and resamination reactions in biological system. It has also known that the metal ions with nitrogen and oxygen donor systems of L-proline might inhibit enzyme production, since the enzymes which require these groups for their activity appear to be especially more susceptible to deactivation by the metal ions upon bonding with L-proline.

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