Original Article

ENHANCEMENT OF ANTIBACTERIAL ACTIVITY BY THE ADDITION OF L-PHENYLALANINE ON THE GROWTH OF SULPHAMIC ACID SINGLE CRYSTALS

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Abstract

Pure sulphamic acid (SA) and L-phenylalanine added sulphamic acid (LPASA) were grown by slow evaporation technique. Structural analysis revealed that the orthorhombic crystal structure was retained for both the grown crystals and the crystallinity was improved upon the addition of L-phenylalanine. The vibrational analysis of both FTIR and Raman exhibited all the vibrational frequencies related to the stretching, deformation, and zwitterion nature of the sulphamic acid crystal. All the vibrational modes proved that the addition of L-phenylalanine enhanced crystalline nature. The elemental analysis confirmed the presence of all constituent elements and the surface morphological study affirmed the improvement in classification of crystal grains. The transmittance was raised to 92%. Moreover, the hardness of the crystal was enhanced. The thermal stability and hardness of the SA crystal was intensified with the addition of L-phenylalanine. The photoluminescence studies revealed that the blue emission intensity was increased on addition of L-phenylalanine. Therefore L-phenylalanine was considered as a good additive for sulphamic acid to improve the crystalline nature as well as the thermal and mechanical stability of the crystal. Addition of L-Phenylalanine with the pure SA enhanced the antibacterial activity was enhanced, which will be proficient in killing pathogenic bacteria. Moreover, the blue emission intensity was enhanced upon L-phenylalanine addition. Hence this material may be useful for application in blue emission LED devices.

Keywords: Crystal growth, single XRD, FTIR, optical studies, micro hardness, EDAX, SEM.

Introduction

Non - Linear Optical Materials (NLO) which shows second harmonic generation have become great importance owing to their technological improvement in optical communication, signal processing and instrumentation for the last few decades [1]. Normally the organic NLO crystals which possess huge NLO efficiency commonly have meager thermal and mechanical characteristics and are liable to damage while processing. Also, it is difficult to grow big size optical featured crystals for device implementation. Usually, organic crystals possess high nonlinear susceptibilities in comparison with inorganic crystals. But their poor optical transparencies, laser damage thresholds, low mechanical properties and restrictions its use in large scale [2]. The inorganic materials are widely used for device applications because of their high melting point, mechanical strength and chemical inertness [3].

Sulphamic acid (SA) is a traditional inorganic compound and a predominant commercial chemical which was produced in kilotons [4]. It is water soluble and stable at room temperature. Due to its thermal and mechanical stability JIS (Japanese Industrial Standard), BAMC (British Analytical Methods Committee) and IUPAC (International Union of Pure and Applied Chemistry) have proposed sulphamic acid as a standard compound for titrimetric analysis. Generally, amino acids have proton donar carboxyl acid (COOH) and proton acceptor amino (NH_2) group. Usually, amino acids are widely employed in the doping of crystals because the chiral carbon atoms present in them leads the crystallization process in noncentrosymmetric space group crystals as well as retain zwitterionic nature which improves the hardness of the crystal [5-7]. However, the work on amino acid addition in non-centrosymmetric crystals is scarce. Therefore in the present work we aim to understand the hardening effect and the crystallization progress of the inorganic sulphamic acid upon addition of the amino acid L-phenylalanine.

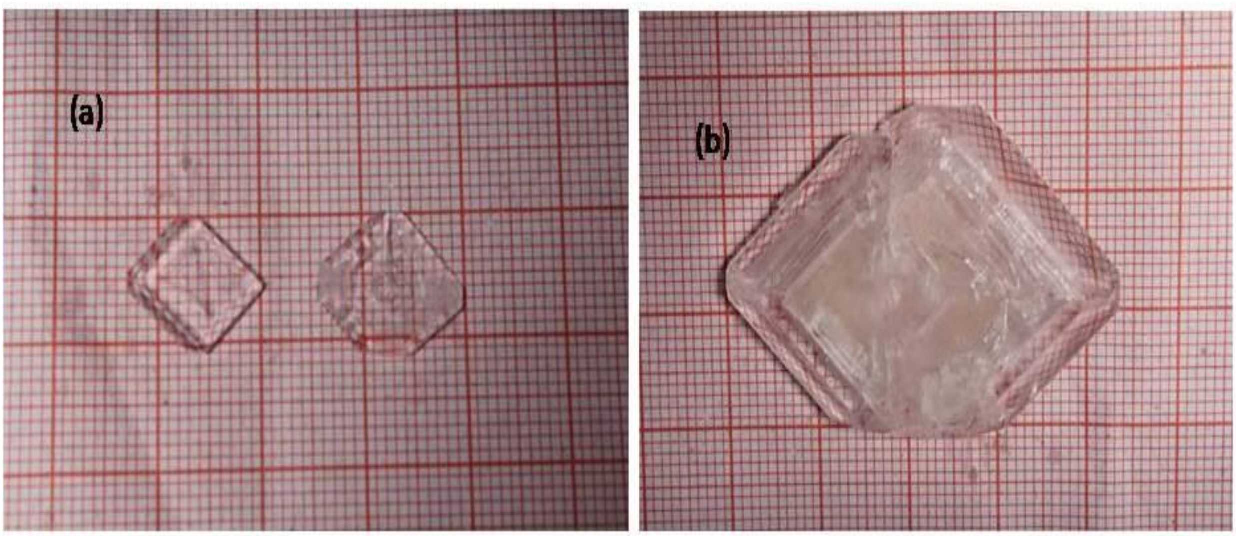
In this current work, L-phenylalanine added sulphamic acid (LPASA) was synthesized as crystals by slow evaporation growing technique. The structural, morphological, optical, thermal properties, as well as hardness of the crystal, were analyzed and investigated.

2. Experimental Methods

The L-phenylalanine added sulphamic acid crystals were grown using slow evaporation method. The starting materials used are high purity sulphamic acid and L-phenylalanine powder. Both the powders are taken in the ratio 3:1 and dissolved in double distilled water. The mixed solution was stirred using a magnetic stirrer at room temperature for several hours to get a clear transparent solution. The transparent saturated solution was filtered and transferred to the growth vessel and allowed to crystallize in a clean atmosphere at room temperature. After 20 days good transparent colourless crystals were harvested and used for characterization purpose. The photograph of grown crystals are shown in Fig. 1.

Powder X-ray diffraction (PXRD) analysis was carried out by irradiating the crystal with Cu K_α radiation (λ = 1.5406Å) using PANalytical XPERT-PRO diffractometer. The sample is scanned for a 2θ range of 10°- 80°. The Fourier transform infrared (FTIR) spectra was recorded between 4000 to 400 cm^{-1} by using Thermo Nicolet 380 FTIR spectrophotometer. Raman modes were examined using imaging spectrograph STR 530 mm Focal Length Laser Raman Spectrometer. The surface morphology of grown crystal was investigated by using Scanning Electron Microscope of VEGA3TESCAN at 228x and 347x magnification. The EDAX spectrum was recorded by Bruker Nano GmbH Berl in, Germany Esprit 1.9. UV-VIS analysis of the grown crystal is carried using UV-DRS spectrophotometer in the wavelength range of 190-1100 nm. The Vickers hardness of the samples was measured using the Shimadzu Model-HMV-2T. Thermogravimetry (TG) and differential thermal analysis (DTA) were measured at a heating rate 20°C/min between 35 and 1000°C in the nitrogen atmosphere using exstar/6300. The photoluminescence of the grown crystals were studied using Varian Cary Eclipse photo luminescence spectrophotometer.

Figure 1. Photographs of the grown crystals (a) SA (b) LPASA



3. Results and discussions

3.1. Single crystal X-ray diffraction

The BRUKER AXS KAPPA APEX 11 CCD Diffractometer was used to do single crystal X-ray diffraction. The single crystal XRD examination showed that the formed crystal belonged to an orthorhombic crystal system, and the measured lattice parameters for SA and LPSA were a = 8.10 Å, b = 8.17 Å, c = 9.29 Å and a = 8.09Å, b = 8.13Å, c = 9.31 Å respectively,with angles α=β=γ=90°.SA and LPASA have unit cell volumes of 614 A^3 and 615 A^3, respectively**,** and it's a, b, and c values differ from those of pure sulphamic acid. This shows that doping slightly alters the cell axes and cell volume**.**

3.2. Powder X-ray diffraction

Figure. 2 shows the recorded powder XRD pattern of as grown pure sulphamic acid and L-phenylalanine added sulphamic acid crystals. The diffraction peaks in the XRD profile was matched to the standard JCPDS data. From the standard pattern, it was found that the observed XRD pattern of the grown crystals was well suited to the orthorhombic crystal system of sulphamicacid (JCPDS card no : 70-0060). In SA crystal the high intense peak was (221) and the other peaks also has sharp peaks. In L-phenylalanine added samples the crystallinity of the sample was increased and the most intense peak was along (211) direction. Also, the intensity of the strongest peak (211) was increased whereas the intensity of other peaks were decreased. This revealed that the addition of L-phenylalanine improved the crystalline nature of the sample and also the growth axis was enhanced along (211) direction. The change in the relative intensity of the various peaks was also noticed which proposed the successful incorporation of L-phenylalanine in the pure sulphamic acid. Moreover, no impurity peaks have appeared. Hence the grown crystals were phase pure. The unit cell parameters were calculated and tabularized. Using unit cell software package, the tabulated lattice parameters and structure were found to be in good accordance with the previous results of sulphamic acid [8-11]. From the unit cell values, it was noticed the addition of L-phenylalanine reduces the unit cell volume even though there was elongation in b and c-axis. The reduced cell volume was due to the contraction along a-axis. This may be accounted due to grain orientation along (211) direction.

Figure 2. Power X-ray diffraction patterns of (a) SA and (b) LPASA crystals

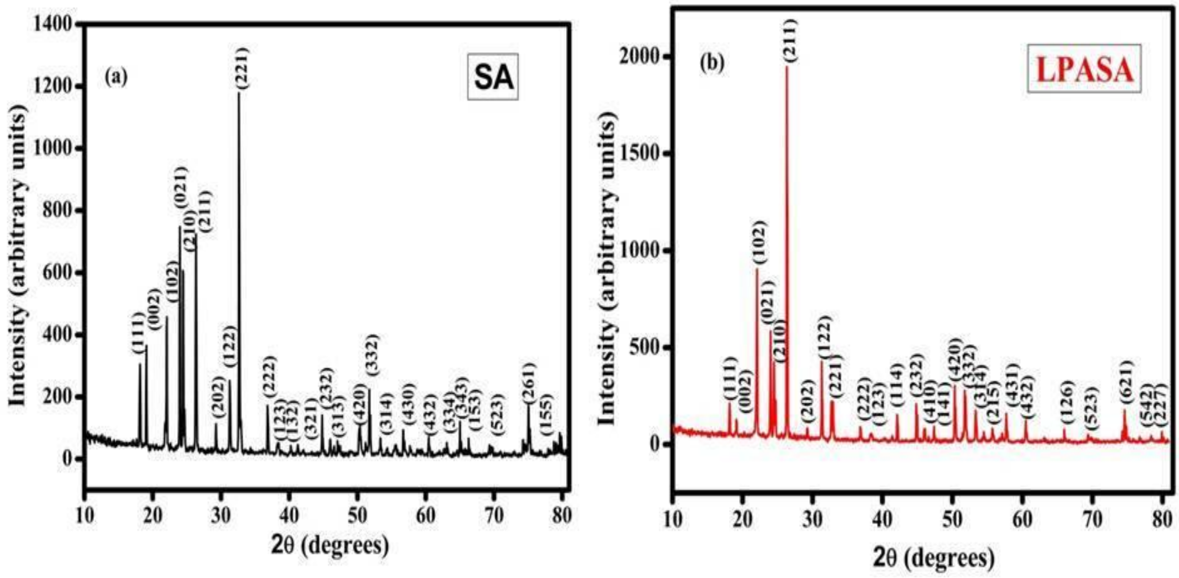


Table 1. Structure and lattice parameters of pure SA and LPASA single crystals.

Crystal	Structure	a(Å)	b(Å)	c(Å)	Volume(A^3)
SA	Orthorhombic	8.1266	8.0928	9.2298	607.0166
LPASA	Orthorhombic	7.9649	8.1087	9.2860	599.7360

3.3. Fourier Transform Infrared Analysis

The FT-IR spectra of the grown crystals SA and LPASA are shown in Fig. 3. From the spectra, it is clear that the band due to NH_3^+ mode of bonding was noticed at frequency of 3153cm^{-1} and 2873cm^{-1}. The band observed at 2458cm^{-1} and 2053cm^{-1}were due to tribands overtones/ combinations of hydrogen bonded OH bending modes. The band seen at 1806cm^{-1} and 1447cm^{-1}were due to deformation of NH_3^+ mode of vibration. The vibration bands observed at 1267cm^{-1}was due to degenerated SO_3^- stretching, whereas at 1068cm^{-1}was due to symmetric SO_3^- stretching. The rocking mode vibration NH_3^+ was noticed at 1002cm^{-1}which verified the formation of zwitterions in SA and LPASA crystals [12]. The N-S stretching vibration was observed at 690cm^{-1} and the band that occurred at 548cm^{-1}was due to degenerated SO_3^- deformation [13]. All the IR bands that observed in the grown crystals were in good agreement with earlier reports and were comparable with theoretically calculated bands [12, 14]. Table 2 shows the vibrational assignment of SA and LPASA crystals. However in L-phenylalanine added crystals almost all the modes were appeared. But the intensities were drastically decreased which revealed that there may be subtle change in the geometry of the sub lattice.

Figure 3. FTIR spectra of (a) SA and (b) LPASA crystals

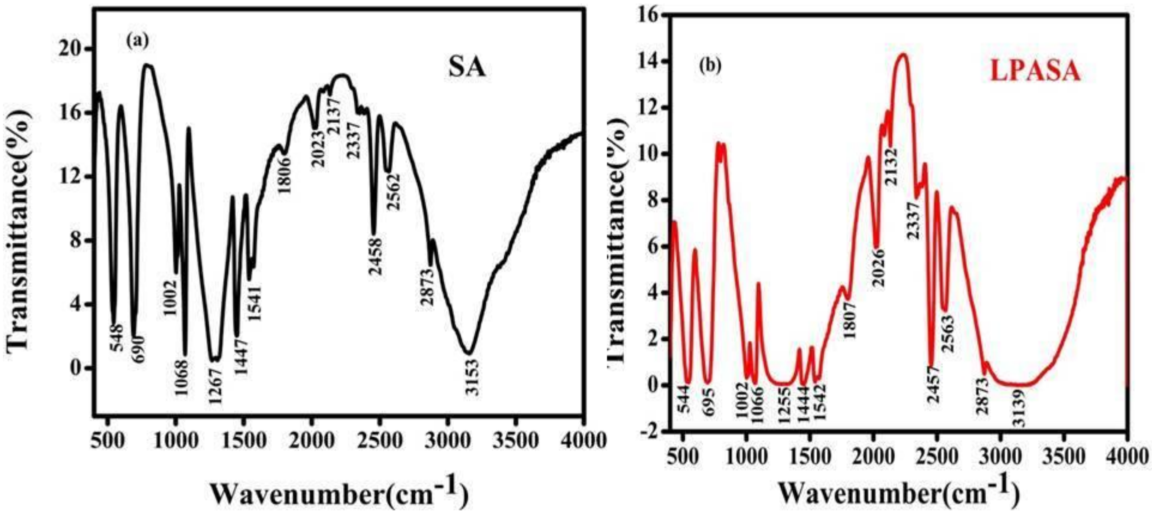


Table 2. Vibrational assignment of the pure and L-Phenylalanine added SA single crystal

Wave number (cm^{-1})		Assignment
Pure SA	LPASA	
548	544	Degen. SO_3^- deformation
690	695	N-S stretching
1002	1002	Rocking mode NH_3^+

1068	1066	Symmetric SO_3^- stretching
1281	1272	Degen. SO_3^- stretching
1447	1444	Sym. NH_3^+ deformation
1541	1542	Degen. NH_3^+ deformation
1806	1807	Symmetric NH_3^+ deformation
2023	2026	N-H Stretching
2458	2457	S-H Stretching
2873	2873	Symmetric NH_3^+Stretching
3153	3139	Degen.NH_3^+ Stretching

3.4. UV-VIS studies

It is very substantial to know the optical transparency of the grown crystals since they are used in several optoelectronic device applications. The Ultraviolet-Visible-near infrared spectroscopic measurements were done and the graph is shown in Fig. 6. It is clear from the figure that the grown crystals have transparency of 86% and 92% for pure SA and LPASA crystals respectively. The transparency of the LPASA crystals was found to increase due to increase in crystalline nature. The reduction of transmittance in SA crystals may be because of the scattering from the point and line defects as reported by M. Senthilpandian et al [15]. From Fig. 7 it was found that the lower UV-cut off wavelength for the SA crystal was 227nm, while in LPASA it was 234nm and percentage of absorption is high for L-Phenylalanine added sulphamic acid in comparison with pure sulphamic acid and it could be due to the influence of L-Phenylalanine [16]. The tauc's plot analysis was carried out to find the energy band gap values and shown in Fig. 8. The obtained optical band gap energies of pure and L-Phenylalanine added sulphamic acid were 3.7eV and 3.0eV respectively.

Figure 6. UV Transmission spectra of pure SA and GSA crystal.

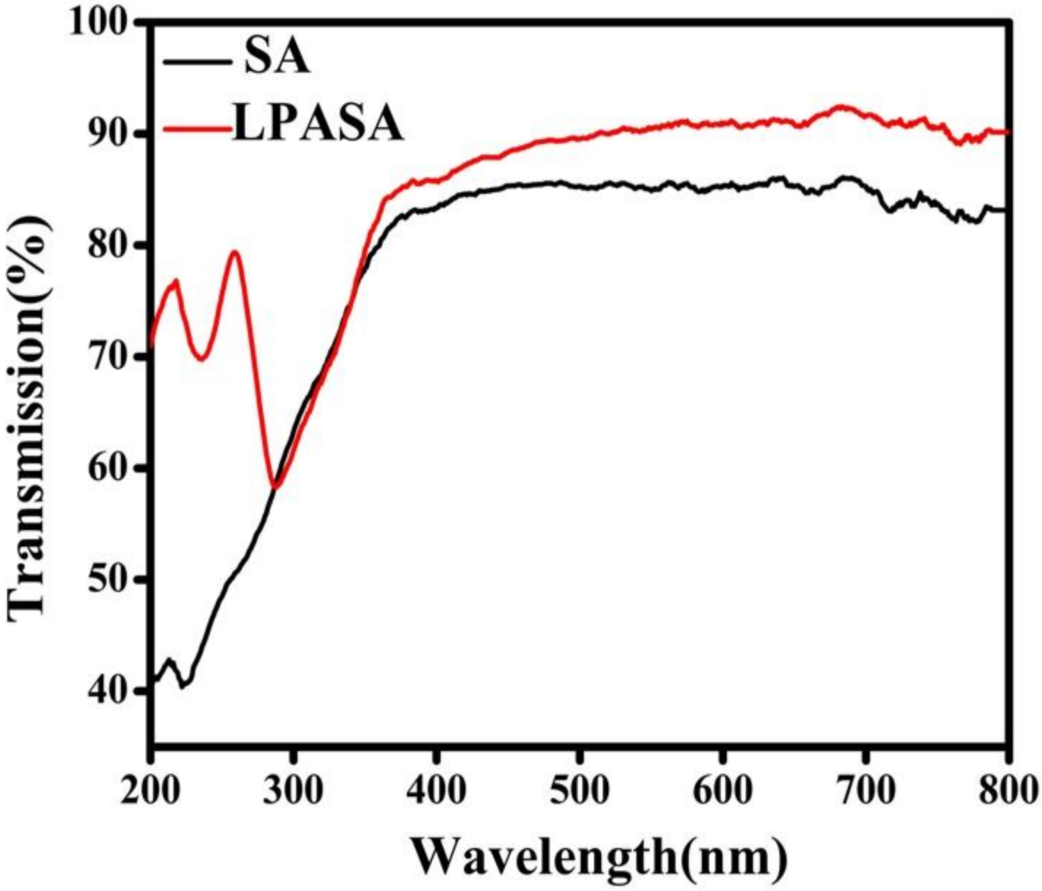


Figure 7. UV Absorption spectra of pure SA and LPASA crystal.

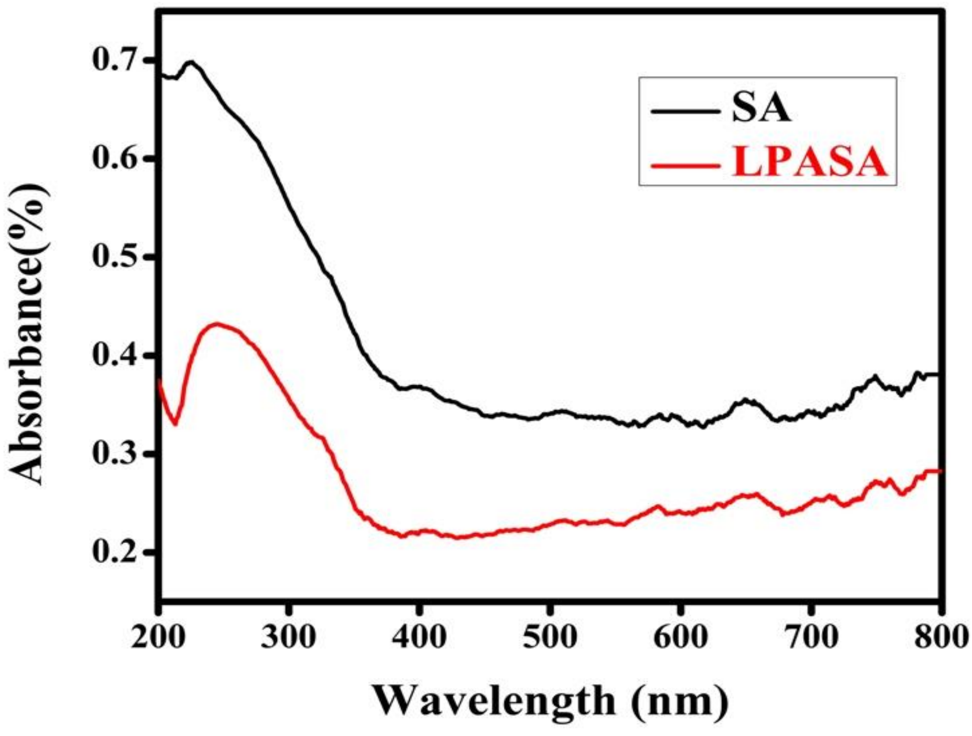
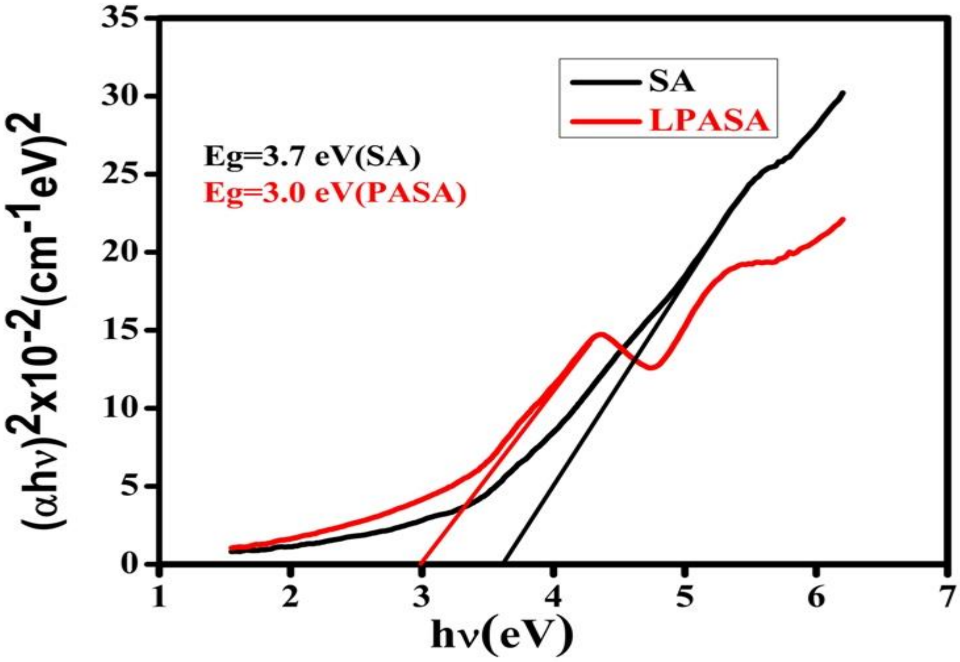


Fig.8.Tauc's plot for pure SA and LPASA crystal.



3.5 Antibacterial activity

Mueller-Hinton Agar medium is a reagent for the Disk Diffusion Test. E. coli had a 22 mm half-life for known conventional antibiotics while absorbing the zone of inhibition; however it was longer in the LPASA sample. The absorbed zone of inhibition expanded in comparison to the usual antibiotic zone of inhibition for klebsiella, bacillus, staphylococcus aureus, and serattia. The antibacterial activity of SA and LPASA is shown in Table.3. We deduced from this that the produced crystals are more efficient than conventional antibiotics. It was observed that the addition of LPA with SA further increased the inhibition. It shows the enhancement of anti bacterial activity with the addition of LPA with SA. When compared to other antibacterial drugs, gram negative bacteria serattia have a higher zone of inhibition.

Table 3 Antibacterial effect of pure S A and LPSA crystal.

Bacteria name	SA Zone of inhibition[mm]	LPSA Zone of inhibition[mm]	Standard antibiotic AMIKACIN Zone of inhibition[mm]
E.COLI	28	29	22
KLEBSIELLA	30	31	19
BACILLUS	29	30	20
STAPHYLOCOCCUS AUREUS	26	27	22
SERATTIA	33	33	19

3.6 Z-scan measurements:

The Z-scan method was used to examine the LPASA crystal's third-order NLO characteristics. Z-scan is a very good technology for analyzing the nonlinear absorption coefficient (β) and third order nonlinear refractive index (n_2). Figures 9 and 10 demonstrate the graphs obtained using the LPASA's open aperture and closed aperture methods, respectively. Here, β was determined using an open aperture Z-scan approach. For the purpose of getting any optical limiting applications, this property is crucial. 5.17 x 10^{-9} cm^2/W is the computed value of the third order nonlinear refractive index (n_2). Hence 3.08 x 10^{-4}cm/W is the nonlinear absorption coefficient. The self-focusing nature was shown by the nonlinear refraction's positive value. 4.93 x 10^{-6}esu is the third order susceptibility ($\chi^{(3)}$) of LPASA.

Fig. 9 Z-scan open aperture of LPASA crystal

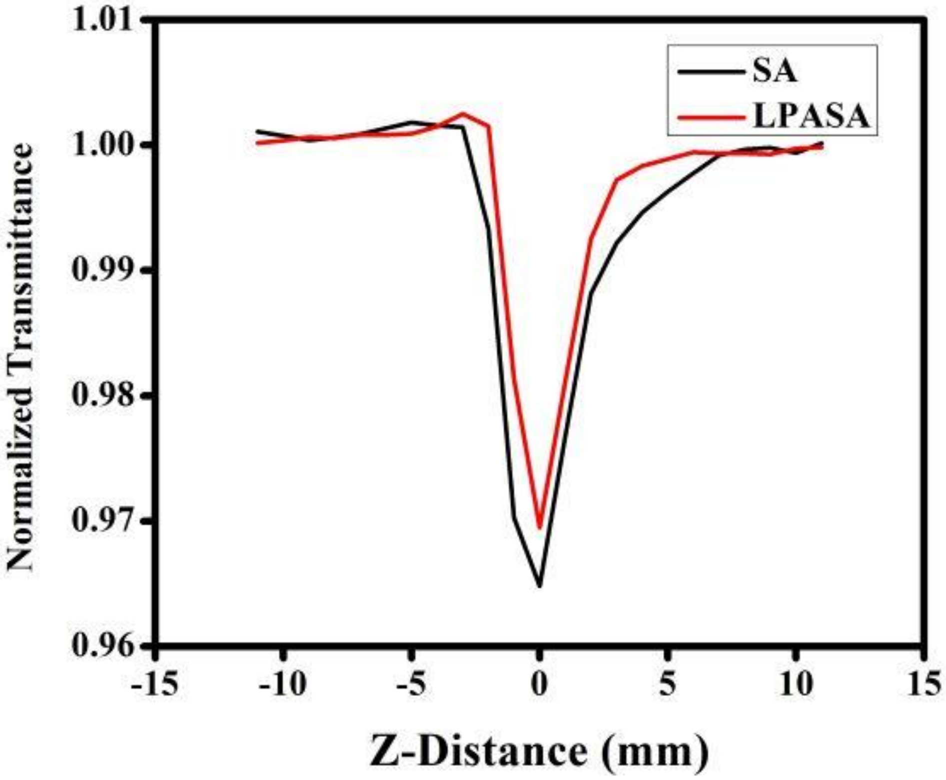
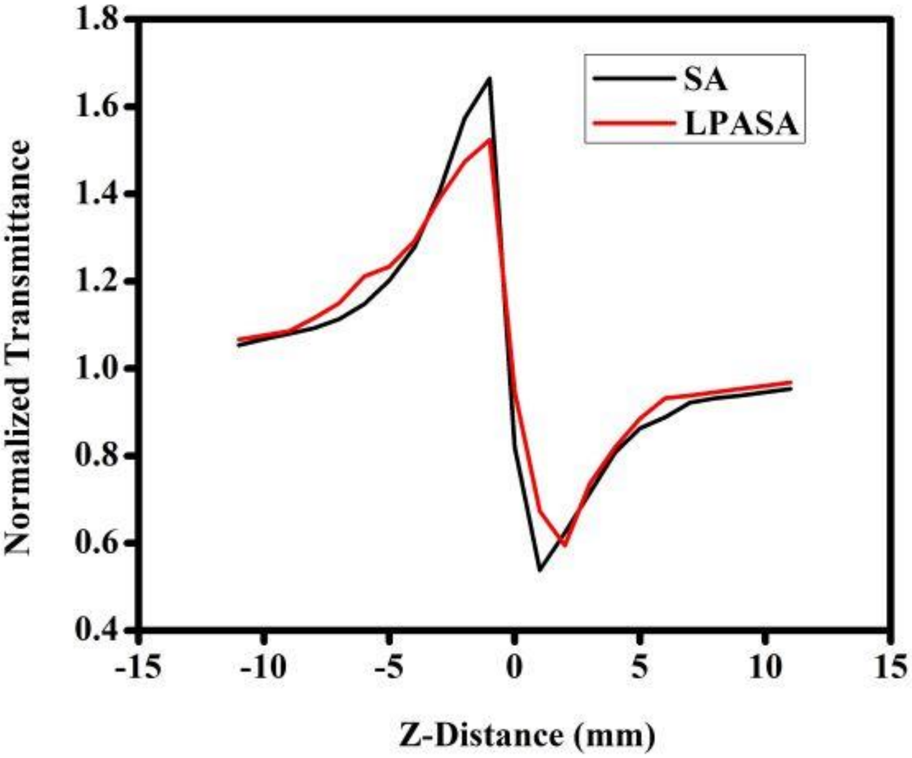


Fig. 10 Z-scan closed aperture LPASA crystal



4. Conclusion

The growth of pure and L-phenylalanine added sulphamic acid crystals have been attained by slow evaporation technique at room temperature. Single X-ray diffraction studies verify the crystal structure and lattice parameters. A small change in the lattice parameters and volume of the crystals was noticed when L-phenylalanine is added to sulphamic acid. The FTIR and analysis verified that there is no phase change was noticed when L-phenylalanine is added to sulphamic acid and all the vibrational modes are present with slight deviation in the peak position. The optical study shows that the crystalline perfection is excellent as the transparency is very high. The value of band gap was found to decrease between 3 and 4 with the addition of L-phenylalanine. The antibacterial activity showed that upon addition of L-Phenylalanine in pure SA the antibacterial activity was enhanced, which will be helpful for killing pathogenic bacteria. The third order nonlinear refractive index, absorption coefficient and optical susceptibility were calculated by the Z-scan technique and it revealed that the SA and L-Phenylalanine added SA crystal possessed self focussing and two-photon absorption process.

5. Acknowledgement

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6. References

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