Synthesis, structural, mechanical, thermal, magnetic and antimicrobial properties of β-L -Glutamic acid

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Abstract:

β - L - Glutamic acid a semiorganic single crystal and grown by slow evaporation technique at room temperature. The lattice parameters of the grown single crystals were determined by using single crystal X – ray diffraction analysis and it reveals that the crystal lattice system is orthorhombic. Powder X-ray diffraction confirms the crystalline nature and the diffraction planes were indexed. The mechanical strength of the grown crystal was estimated using Vickers microhardness tester. The dielectric response of the grown crystal was analyzed at different temperatures in the frequency range from 50 Hz to 2 MHz. Thermal properties were investigated by thermogravimetric, differential thermal and differential scanning calorimetric analyses. Antibacterial and antifungal studies were carried out against ACDP declared harmful pathogens. The surface features of the grown crystal were analyzed using scanning electron microscopy analysis. The magnetic property was studied with the help of vibrating sample magnetometer.

Keywords
Growth from solution, X – ray diffraction, Thermal Properties, Antimicrobial activity and magnetic properties

1. Introduction

Nonlinear optical materials have a crucial role in the technology of photonics, laser technology, optical communication, data storage technology and frequency doubling process [1 – 3]. Amino acids have proved to be potentially interesting nonlinear materials for nonlinear applications and are more efficient at least one or more orders of magnitude than that of inorganic materials [4]. L - glutamic acid is an acidic amino acid with one amino and two carboxylic acid functional groups. These functional groups are capable of binding and expected to form metal organic hybrid structure by binding to the metal centre in different coordination modes. L-glutamic acid crystals have two polymorphs: granular α and plate-like β-forms [6]. The crystal structures of the two polymorphs were determined by X - ray [7 - 9] and neutron [10, 11] diffractions. The α -form is metastable and transforms to the stable β -form mediated by water [9, 10]. In the present investigation β-L glutamic acid (β-LGA) single crystals were grown using silver nitrate as an additive by slow evaporation solution growth technique. Grown crystals were characterized using single crystal and powder X – ray diffraction analysis and Scanning Electron Microscopy (SEM) analysis. Mechanical strength, Thermal properties, magnetic properties, Antibacterial and antifungal studies are reported.

2. Materials Preparation

Silver nitrate and L – glutamic acid were taken in 1:1 molar ratio and dissolved in double distilled water at room temperature. The solution was stirred for 3 hours to get homogeneity. The homogeneous solution was filtered and kept undisturbed for slow evaporation at room temperature. After a period of 7 days well developed β -LGA single crystal was harvested. The grown β -LGA single crystal of dimensions 11 mm × 3 mm × 3 mm are shown in Fig. 1.
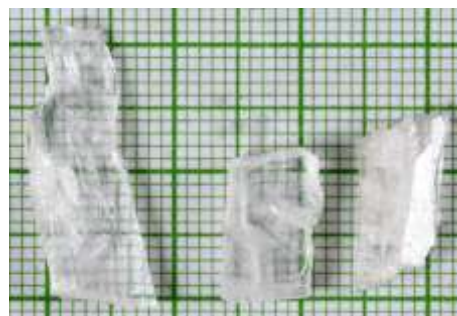
Fig. 1 As grown single crystals of β-LGA

3. Result and discussion
3.1 Single crystal X – ray diffraction

The unit cell dimensions were collected from a Bruker X8 Kappa APEXII single crystal X – ray diffractometer equipped with graphite monochromated MoKα radiation (λ= 0.7104Å). From the X - diffraction analysis, it has been found that the Ag is not incorporated in the compound but it favours the growth of β - L – Glutamic acid [10]. β - LGA crystallizes in orthorhombic system with noncentrosymmetric space group $P2_12_12_1$. Table 1 presents the unit cell parameters obtained and compared with reported [10].

Table 1 Unit cell dimensions of β-L – Glutamic acid

Parameter	β -LGA (present study)	β form of LGA [10]
a	5.19 Å	5.17 Å
b	6.99 Å	6.95 Å
c	17.39 Å	17.34 Å
α, β and γ	90°	90°
Volume	630 A^3.	631 A^3.
Crystal System	Orthorhombic	Orthorhombic
Space group	$P2_12_12_1$	$P2_12_12_1$

3.2 Powder X – ray diffraction analysis

Powder X – ray diffraction pattern was recorded by using Bruker X – ray diffractometer with Cu Kα radiation (λ = 1.5406 Å) in the range of 10 to 80° in steps of 0.02°. Sharp powder XRD pattern is shown in Fig. 2 reflects the good crystallinity of the grown crystals. The obtained peaks were indexed using AUTOX 93 software package from the observed 2θ values.

Williamson and Hall [13] assume that size ($β_L$) and strain broadening($β_e$) are additive components for Bragg's peak broadening [14]. Size broadening can be related to Debye-Scherer formula $β_L=kλ/(Lcosθ)$. The strain induced broadening arising from crystal imperfection and distortion can be related as $β_e = Cε\tan θ$ where, $ε$ is either maximum tensile strain alone or maximum compressive strain which can be calculated from the observed broadening and C is the constant equals to 4 that depends on the assumptions made concerning the nature of the inhomogeneous strain. If both crystallite size and strain contributions present independently to each other, then their combined effects can be determined by convolution. The simplification of W–H is to assume the convolution is sum of $β_L$ and $β_e$. Using the former $β_{tot}$ of these then we get:

$$β_{tot} = β_L + β_e = Cε\tanθ + \frac{Kλ}{L\cosθ}$$

(1)

If we multiply this equation by cosθ we get

$$β_{tot}\cosθ = Cε\tanθ + \frac{Kλ}{L}$$

(2)

Plotting $β_{tot}\cosθ$ versus sinθ we obtain the strain component, from the slope (**Cε**), the size component and the intercept ($Kλ/L$). Williamson-Hall plot drawn for β -LGA from powder X – ray diffraction data and is illustrated in Fig. 3. The strain is assumed to be uniform in all crystallographic directions. The strain was calculated by Williamson – Hall equation. Microstrain of the β –LGA crystal is $1.38 × 10^{-4}$.
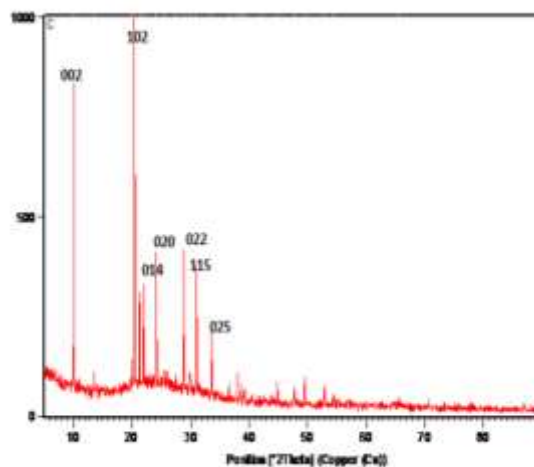
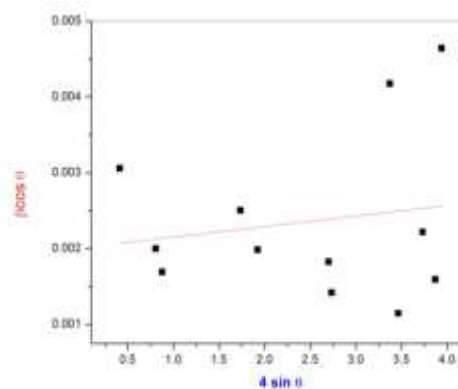

Fig. 2 Powder X – ray diffraction pattern

Fig. 3 W-H plot of β -LGA crystal.

3.3. Microhardness Studies

The Vickers microhardness test was carried out on a well – polished single crystal using Shimadzu Vickers microhardness tester fitted with a diamond pyramidal indenter. Different loads were applied on the selected plane. The loads ranging from 25 to 100g were applied for making indentations, keeping the time of indentation at 10s for all the cases. The diagonal length of the indentation marks were measured using micrometer eyepiece. The values of the Vickers hardness number Hv at different loads were calculated using the expression $Hv = 1.8544(P/d^2)$ kg/mm^2 where P and d are the applied load and average diagonal length of the indentation respectively. Fig. 4 shows the plot of hardness number versus applied load. It was observed that the hardness number increases with the increase of load, which is known as the reverse indentation size effect (RISE). The RISE signifies the plastic deformation nature of β -LGA crystal. At lower loads, dislocations propagate into in the material easily without experiencing mutual interaction. For an indentation load 100g cracks were initiated around the indenter. The work hardening coefficient of the β -LGA crystal was estimated using the Meyers relation $P = Kd^n$, where k is the material constant and n is the Meyers index. The work hardening coefficient was determined to be 3.6 by (Fig. 5) the least square curve fit method. Hence β -LGA belongs to softer material category.
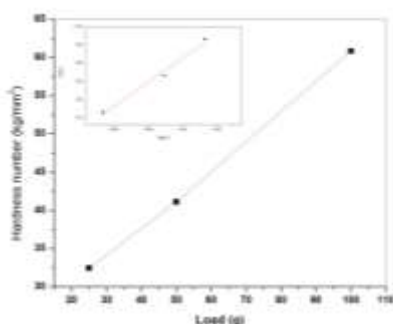

Fig. 4 Variation of Vickers hardness number with load for β-LGA and Meyers plot (inset).

3.4. SEM and Energy dispersive X – ray analysis

Scanning electron microscopy (SEM) was recorded using Quanta FEG 200 scanning electron microscope. Fig. 6(a) and (b) shows the SEM images of the grown crystal with resolution 1 µm and 5 µm respectively. From the image of 6(a), it is understood that the plate like morphology is more dominant than the rod shaped morphologies. The micro structure shown in Fig. 6(b) indicates different layers with aggregation of micro crystals of different sizes.
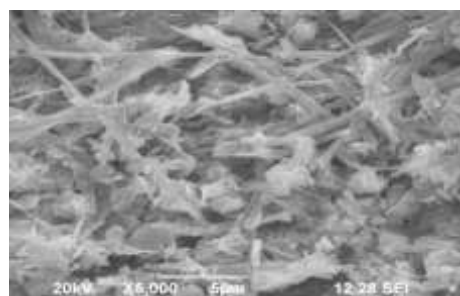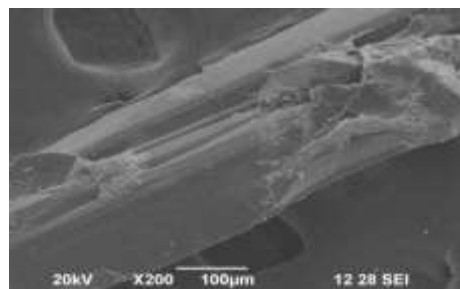

Fig. 6 (a) and (b) SEM images of β -LGA single crystal.

Elemental analysis was carried out for β LGA crystal by employing the energy dispersive X – ray (EDX) spectroscopy.EDS spectrum (Fig.7) confirm the composition of Carbon (C), Nitrogen (N) and Oxygen (O) in the grown crystal.
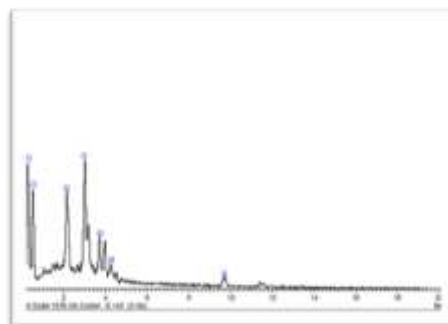

Fig. 7. EDX spectrum of β -LGA single crystal.

3.6. Magnetic properties

The magnetic property of the grown material was studied using a vibrating sample magnetometer at room temperature. The sample was taken in the form of fine powder to analyze this characterization with help of vibrating sample magnetometer. When β -LGA crystal sample was subjected to an external magnetic field, the dipoles within the atoms are subjected to a force tending to align them with the field. So the material will generate a cohesive field of its own. Some of the dipoles may rearrange their bonds within the material so that their new orientation is stable while others may stretch their bonds. If the magnetic field is increased, magnetic moment also increases gradually and gets magnetic saturation at 31.781×10^{-3} emu/g.

Then the material get demagnetization and moves in opposite direction, hence it forms hysteresis loop (Fig. 8). It is observed that β -LGA crystal has soft ferromagnetic nature. When the external field is removed they regain their original orientation. The relative strength of this residual magnetic field for β - LGA crystal was18.246 x10^{-3}emu/g and the coercivity was found that 496 x10^{-3} Tesla. The squareness ratio of the ideal hysteresis loop is 2 and for β -LGA crystal was 0.58.
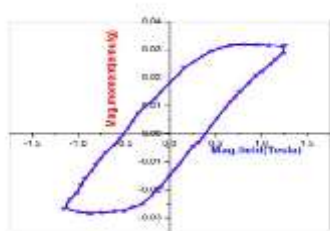

Fig. 8 Hysteresis loop for β -LGA crystal.

3.7 Thermal analysis

Thermogravimetric analysis of β-LGA crystals was carried out between 25 °C to 600 °C in nitrogen atmosphere using Perkin Elmer thermal analyzer at a heating rate of 10 °C /min. Initially 7.218 mg of the sample of β-LGA was taken for analysis. From the TGA trace, it is evident that the β-LGA is stable up to 199 °C and moisture free. The TGA curve shows two stages of weight loss pattern. The first weight loss (23%) starts at 199 °C ends at 280 °C. The second weight loss (57%) occurs between the temperature range 280 °C and 500°C. During these stages of decomposition of carboxyl and amine groups were eliminated. DTA curve shows two endothermic peaks at 219 °C and 303 °C which coincides with the weight loss steps observed in TG analysis. Simultaneous measurement of differential scanning colorimetric analysis was carried out and is shown in the Fig. 9. A small sharp peak was observed at 200°C indicates the decomposition of the material which the results of TGA and DTA.
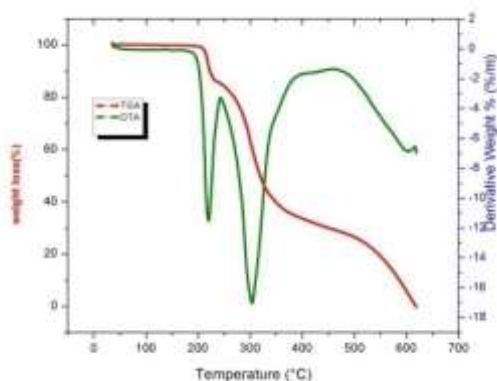

Fig. 9 TG – DTA analysis curve of β-LGA.

3.8. Etching

Etching study was carried out to characterize structural information, morphology, and dislocation of atomic structure of the grown crystal. A polished single crystal of β-LGA slice was etched in double distilled water. The crystal was dipped in the etchant for 5 sec. The sample was dried using tissue paper. Surface features were examined on the well developed plane. A polovision optical microscope fitted with motic camera was used with magnification of 100X. Etch patterns were photographed and are shown in Fig.10(a) & (b) respectively. When the crystal was etched in 5 sec. large number of etch pits are observed. On increasing the etch time to 10 sec linear etch pits are observed.

Fig. 10(a) Etch patterns for β-LGA (5 sec).

Fig. 10 (b) Etch patterns for β-LGA (10 sec).

3.9. Antimicrobial activities

Antibacterial activity of the compound was determined using well diffusion method [13]. Mueller Hinton agar medium was prepared and poured in the petri dishes. After solidification, wells were cut on the Mueller Hinton agar using cork borer. The test bacterial pathogens *E.coli*, *Pseudomonas aeruginosa* and *Shigella flesneri* were swabbed onto the surface of Mueller Hinton agar

plates. Stock solution was dissolving 10 mg β-LGA in 1 ml of dimethyl sulphoxide (DMSO). From this solution 25 µl of the test sample was pipetted out and impregnated into the wells. The plates were incubated for 30 min to allow the extract to diffuse into the medium. The plates were incubated at 37 ºC for 24 hours, and then the zone of inhibition was measured in millimetres. Each antibacterial assay was performed in triplicate and mean values were reported. The measured zone of inhibition data are presented in Table 2. Zone of inhibition is larger for *E.coli* than *Pseudomonas aeruginosa* and *Shigella flexneri.*

Table. 2. Antibacterial activity of the compounds treated against bacterial pathogens

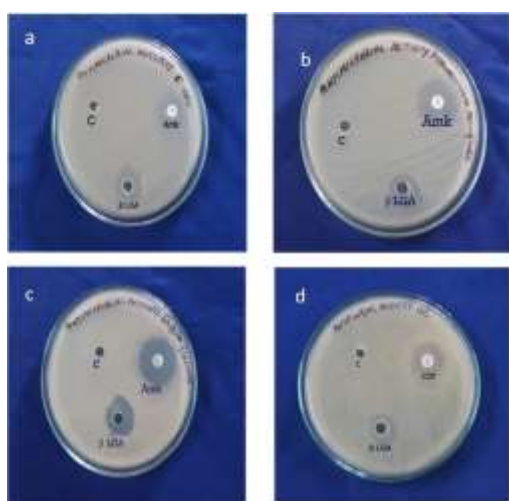| Test organisms	Zone of inhibition in millimeter (in diameter)		
	β-LGA	Solvent Control DMSO (Dimethyl Sulphoxide)	Standard Amickacin 30µg
E. coli	26	No Zone	25
Pseudomonas aeruginosa	18	No Zone	22
Shigella flexneri	19	No Zone	26

Fig. 11 Antibacterial activity of the β -LGA crystal treated against (a). *E .coli,* **(b).** *Pseudomonas aeruginosa* **, (c).** *Shigella flexneri and* **(d)** *Oidium caricae (antifungal)*

Antifungal activity of the compounds was determined using well diffusion method [14]. Mueller Hinton agar medium was prepared and poured in the petri dishes. After solidification, wells were cut on the Mueller Hinton agar using cork borer. The test fungal pathogens were swabbed onto the surface of Mueller Hinton agar plates. From the stock solution 25 µl was pipeted out and impregnated into the wells. The plates were incubated at 30 ºC for 72 hours, and then the zones of inhibition were measured in millimeters. Antifungal assay of *Oidium caricae* activities was performed in triplicate and mean values were reported. Co-trimoxazole was used as standard. The β-LGA sample shows less activity (ZOI:24mm) than the standard Co-trimoxazole(ZOI:27mm)

4. Conclusion

Single crystals of β - L – glutamic acid were grown by slow evaporation technique at room temperature with size $16 \times 3 \times 3$ mm^3. The single crystal X – ray diffraction reveals that the grown crystal belongs to orthorhombic system. The sharp well defined Bragg's peaks confirm the crystalline nature of the synthesized material. Vickers microhardness study showed a moderately good mechanical strength of the grown crystal. The SEM image shows that a few micro crystals were randomly distributed on the surface of the crystal. The thermogravimetric and DSC measurement established that the β-LGA crystal is thermally stable up to 200°C Antibacterial and antifungal studies are confirmed that the material possess antimicrobial activities. Etching revealed that dislocation and imperfection in the crystal lattice.

Conflict of interest: Nil

Ethical approval: Not required

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