

Experimental evidence on the sustainability of crystallographic and chiral symmetry of L-alanine under dynamic shocked conditions

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ABSTRACT

Over the years, crystallographic phase transitions of the polar and non-polar amino acids under static high-pressure-temperature conditions have been well documented, especially after the development of the diamond anvil cell techniques. In contrast, the crystallographic structural science of such primary amino acids under dynamic shocked conditions is still to be explored substantially as it is in a premature stage which requires being loaded with a few possible deep insights whereby exploring the structural kinetics could be a reality. In the present context, we have performed the acoustic shock wave recovery experiment on non-polar amino acid of L-Alanine powder samples such that X-ray diffraction (XRD) and circular dichroism (CD) spectroscopic techniques have been enabled to investigate the structural and chiral stability under shocked conditions. Based on the observed results, it is observed that the crystallographic structure with the $P2_12_12_1$ space group and the same chirality is maintained even at the 200-shocked condition with a transient pressure of 2.0 MPa. The observed dynamic shock wave loading structural stability outcome is identified to be well-matched with the previous static high-pressure compression results.

1. Introduction

In order to better understand the origin of life on Earth, extensive studies have been conducted on laboratory scale of high-pressure, high-temperature and ultraviolet irradiation on amino acids by conducting various experiments. It is proved that amino acids are the first building blocks of life on Earth which is one of the main areas of study for chemists and astrophysicists. There are still three unresolved questions about the origins of life in the universe and on Earth which are namely where, when, and how it happened [1–3]. On top of that, it is widely believed that “life cannot and never could exist without molecular dissymmetry” in light of the fact that absolute chiral homogeneity of molecules is a prerequisite for life to exist and that it would be impossible to have life-sustaining and self-replicating molecules without it.

Therefore, investigations involving amino acids at high temperatures and pressures are always essential for comprehending molecular dissymmetry. Numerous research teams have been working on high-pressure compression experiments with amino acid materials for the past 100 years with the goal of achieving various atomistic descriptions in the solid state and uncovering numerous phase transitions within them [4–6]. Such experiments can also give a new understanding regarding the origin of life in the cosmos and on Earth because they may introduce a few novel concepts related to the atomic theory of life.

L-alanine ($C_3H_7NO_2$) is one of the most significant non-polar amino acids among the 20 major amino acids. It has the orthorhombic structure and the $P2_12_12_1$ space group during crystallization. L-Alanine's fundamental crystal formation and functional characteristics are well understood [7–15], while its structural behavior under high pressure and

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temperature conditions is also understood to a certain extent [16–20]. For instance, Teixeira et al. conducted static pressure compression experiments in conjunction with Raman spectroscopy on L-alanine up to 4.3 GPa and observed a few new lattice Raman modes at 41 and 48 cm^{-1} at 2.3 GPa, where they hypothesized the formation of an unidentified new crystal structure [16]. Following this investigation, the same research team examined the same work again and found, by Raman scattering and energy dispersive X-ray powder diffraction measurements, an orthorhombic to tetragonal phase transition at 2.3 GPa and tetragonal to monoclinic phase transition occurring at 9 GPa [17]. The orthorhombic to tetragonal phase transitions, however, have not been observed later in powder and single crystal X-ray diffraction results up to 9 GPa, with only slight variations in the lattice parameters at the a and c axes (both are the same value at 9 GPa) [18,19]. In-situ infrared spectrometry study was carried out by Montgomery et al. up to 2.5 GPa and neither crystallographic phase transitions nor chiral alterations were discovered [20]. The maximum pressure compression value (15.46 GPa) was recently reported by Funnell et al. They also observed that the crystal symmetry ($P2_12_12_1$) is stable up to 13.6 GPa and transitioned from the crystalline to amorphous state at 15.46 GPa [21].

Shock waves have been employed as an essential tool to address the aforementioned issues obtained through high-pressure experiments, and only a few articles have been published on this subject, particularly on the shock wave-assisted synthesis of amino acids [21–24]. The conformational phase change from phase-I (low-temperature $P2_1$) to phase-II (high-temperature $P2_1$) at the 50-shocked condition has recently been demonstrated by Sivakumar et al., and the high-temperature phase is observed by and large even at the 200-shocked condition [25]. L-Asparagine monohydrate undergoes significant lattice deformations [26], whereas L-Glutamic acid has a considerably greater degree of crystalline nature [27]. However, no phase transitions have been detected under shocked conditions in terms of structural stability. Similar shock wave recovery tests have been carried out on non-polar acid materials such as Glycine [28], Valine [29], and Isoleucine [30] and found a stable crystal structure when compared to polar-amino acid materials. Based on the current findings, the materials made of non-polar amino acids are currently being recognized as more shock-resistant than those made of polar amino acids. The shock wave recovery experiment on L-Alanine is not yet documented, to date, according to the literature.

In the current work, we intend to examine the structural and chiral stability of L-Alanine under shocked conditions as a continuation of our earlier articles on non-polar acid materials. The observed results are detailed in the following sections.

2. Experimental section

The Sigma Aldrich Company supplied the commercially available L-Alanine, and the fine powder samples were utilized for the current study. The necessary shock waves were produced using a semi-automatic Reddy tube, and its precise experimental information has been published elsewhere [31,32]. We produced a total of five samples of L- α -alanine, one of which was kept intact as a control sample. The other four samples were each dynamically loaded with 50, 100, 150, and 200 shock pulses. Typically, the sample in these experiments is placed in a sample holder that has been positioned 1 cm away from the shock tube's down-flight. The transient pressure and temperature of one shock pulse are 2.0 MPa and 864 K, respectively. In order to determine how shock waves affect the structural characteristics of crystals, the samples were then sent for XRD and CD spectral studies.

2.1. Analytical characterization details

The analytical instruments details are as follows; Powder X-ray diffraction (PXRD) [Rigaku - SmartLab X-Ray Diffractometer, Japan-CuK α_1 as the X-ray source ($\lambda = 1.5407 \text{ \AA}$), with the step precision of

$\pm 0.001^\circ$], have been utilized so as to understand the structural and magnetic structural stability of the test samples under shocked conditions. JASCO Corp J-715 CD spectrometer has been utilized to analyze the chirality in molecules through their optical activity. A scanning electron microscope (Tescan Vega 3) operating at the acceleration voltage of 10 kV was used to analyze the morphology of the title material.

3. Results and discussion

3.1. XRD results

The structural stability of the title amino acid has been assessed with conventional 1D powder X-ray diffractometry under shocked conditions. Before discussing the subject of this research, it is very important to have a thorough understanding of the crystal structure of the control sample. This knowledge is essential for highlighting shock wave impact changes in the test sample if any significant crystallographic changes took place under shocked conditions. According to the literature, non-polar L- α -Alanine crystallizes with the orthorhombic crystal symmetry and the space group is $P2_12_12_1$, wherein each unit cell contains four molecules. As per the literature, the unit cell dimensions are $a = 6.023$, $b = 12.343$ and $c = 5.784 \text{ \AA}$ [16–19]. All three hydrogen atoms in the ammonium group create hydrogen bonds with three separate neighbouring molecules when the molecule is in its zwitterion $\text{CH}_3\text{CHNH}_3^+\text{COO}^-$ state. The main intermolecular link in the crystal structure happens along the c-axis, where a chain of hydrogen bonds is visible and the other two hydrogen bonds connect these chains to form the three-dimensional network in the crystal lattice [16–19]. Fig. 1 displays the L-Alanine's single molecular unit as well as the crystal packing with the orthorhombic crystal symmetry.

In Fig. 2, the comparative XRD patterns of the control and simulated powder XRD patterns with the simulated XRD pattern are presented wherein CCDC CIF file 120203 has been used. The simulated powder XRD pattern is generated by Mercury software with the same diffraction angle step rate as the experimentally recorded control sample so as to make the most precise comparison of the XRD patterns. As seen in Fig. 2, the control and simulated XRD patterns of the L-Alanine are found to be well-matched with each other and hence it could be confirmed that the control sample has crystallized with the $P2_12_12_1$ crystal structure with the above-mentioned lattice dimensions.

In Fig. 3, the control and shocked L-Alanine powder XRD patterns are shown for the diffraction angle range of 10 to 50 degrees. It is unable to detect any appreciable differences in the diffraction peak characteristics, such as the number of peaks over the specified diffraction angle and their shifts, after a preliminary analysis of the recorded XRD patterns of the control and the samples under shocked conditions. For instance, even at 50, 100, 150, and 200 shocked conditions, the control sample's diffraction lines (020), (110), (011), (120), (031), (040), (131), (002), (140), and (220) remain to be intact. The $P2_12_12_1$ space group symmetry is therefore evident from the initial screening of the XRD profiles, and the obtained results are in good agreement with the earlier static high-pressure X-ray diffraction observations [18,19].

On the other hand, under shocked conditions, only a small number of diffraction lines display mild modifications like diffraction peak splitting and shoulder peak creation. The zoomed-in illustrations of the control and shocked samples' XRD patterns are portrayed in Figs. 4 and 5 to provide detailed insights into the aforementioned alterations. The zoomed-in portion of the control and shocked L-Alanine samples are displayed in Fig. 4(a). Due to their high degree of crystalline nature, the control sample's diffraction lines (020), (110), and (011) have a significant amount of sharpness, as seen in Fig. 4(a). The (020) plane exhibits lower angle diffraction line shift in the case of the 50 shocked condition, which may have been caused by the expansion of the b-axis length. Additionally, under shocked conditions, the large axis length

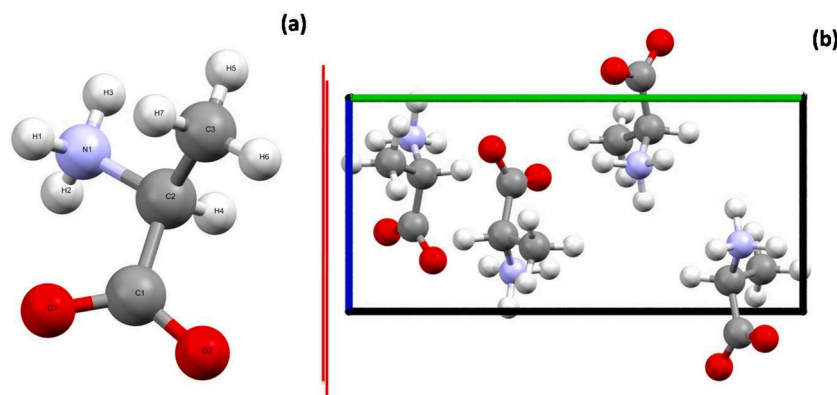


Fig. 1. (a) Single molecular unit of the L-Alanine and (b) crystal packing with the orthorhombic crystal symmetry (the figures are generated by mercury software using CCDC CIF file 120203).

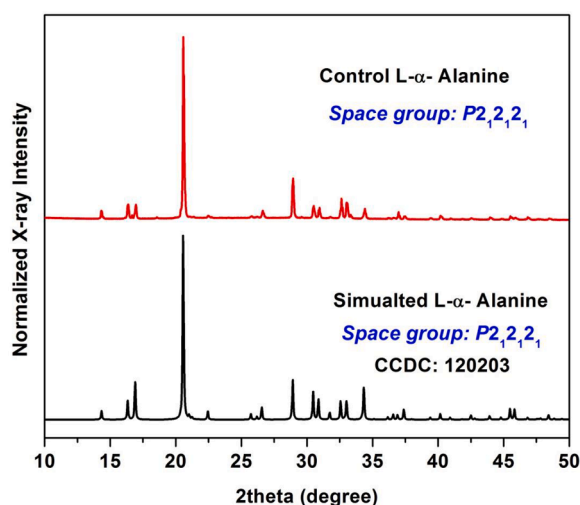


Fig. 2. Comparative XRD patterns of the control and simulated powder XRD pattern (CCDC CIF file 120203).

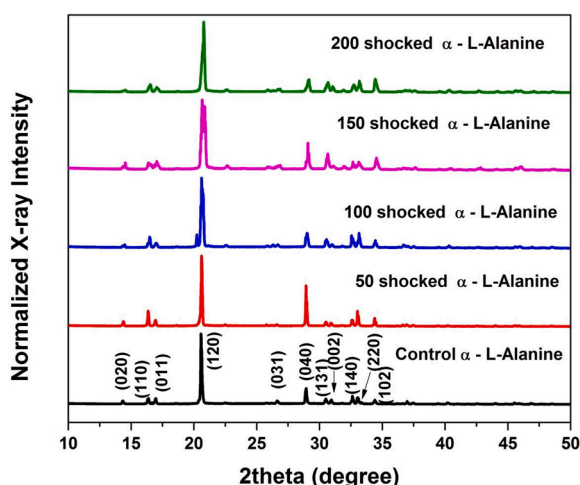


Fig. 3. Powder XRD patterns of the control and shocked L-Alanine powder samples.

plane has undergone significant modifications [28,33]. The (020) plane has undergone a significantly lower angle shift than the (011) and (110) planes because in the case of L-Alanine, the b-axis is nearly two times

higher than the a and c axes. Similar kinds of results have also been found for the non-polar amino acid material of α -Glycine under shocked conditions [28]. The normalized peak intensity with regard to the number of shock pulses is significantly decreased as the number of shock pulses is increased to 100, 150, and 200, respectively. However, due to the extended b-axis length, the (020), (110) and (011) planes have all undergone considerable modifications in terms of peak shift and peak intensity (Fig. 4(a)). The (020) plane intensity is essentially the same as the XRD pattern's baseline at the 200-shocked condition, while the (110) and (011) planes have retained about 50% of their intensity compared to the control sample.

Furthermore, the c and a axes are closely related to the three-dimensional network and hence these axes-dependent planes such as (110) and (011) have not undergone significant changes under shocked conditions [28,33]. Similar to this, the strongest diffraction line of the (120) plane features with respect to the number of shock pulses is displayed in Fig. 4(b). The diffraction line is somewhat displaced to the higher angle side at the 50-shocked condition, as seen in Fig. 4(b), but the intensity remains the same. The diffraction peak splitting is greatly increased at the 100-shocked condition and a new minute peak develops at 20.25 degrees which may be due to the re-organization of the lattice dimensions (Fig. 4(b)), whereas it vanishes at the 150-shocked condition. However, compared to the 100-shocked condition, the degree of peak splitting in (120) is literally increased at the 150-shocked condition which might have taken place because of the subtle changes occurring in the dynamics of the intermolecular hydrogen bonds which include proton shifts along the hydrogen bonds, rotations of selected hydrogen-bonded fragments, changes in their torsion barriers, different compressions of different types of the hydrogen bonds, etc. [18,19]. Peak splitting with similar results has been detected for the (020), (011), and (110) planes at the 150-shocked condition. Surprisingly, at the 200-shocked condition, the small diffraction peaks (shoulder peaks of the (120) plane, marked with blue aero marks) have vanished and transformed into a single peak, which could have been caused by the dynamic re-crystallization and re-organization of the intermolecular hydrogen bond lengths and positions. Similar outcomes have been observed for the (040) plane and the corresponding diffraction line profiles with respect to the number of shock pulses are displayed in Fig. 5(a), which is in accordance with the characteristics of the (020) plane while keeping the fact of (040) plane is a higher order plane of the (020). As can be observed in Fig. 5(a), the control sample's (040) plane has a moderate intensity compared to that of the (020), and under the conditions of the 200-shocked condition, the peak intensity is significantly lowered such that the degree of peak splitting is found to be increased. Peak splitting and modest peak shifts, as well as other similar modifications, also have happened in the (131) and (002) planes thereby the corresponding diffraction lines are depicted in Fig. 5(b). The overall

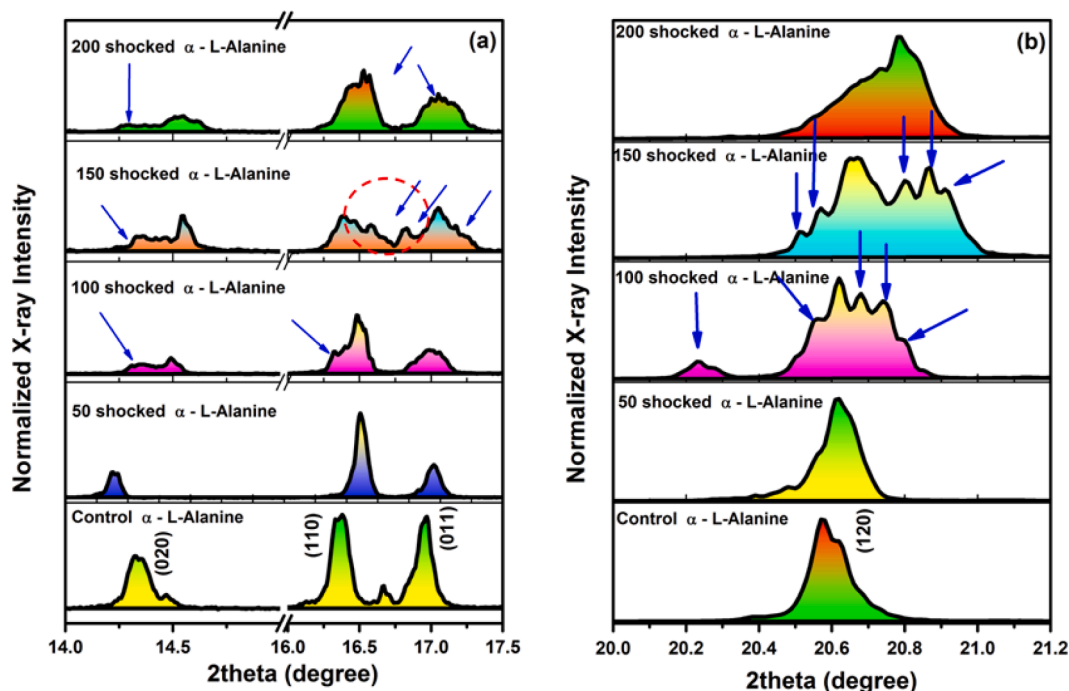


Fig. 4. Zoomed-in versions of the powder XRD patterns of the control and shocked L-Alanine powder samples (a) 14–17.5 degrees (b) 20–21.2 degrees

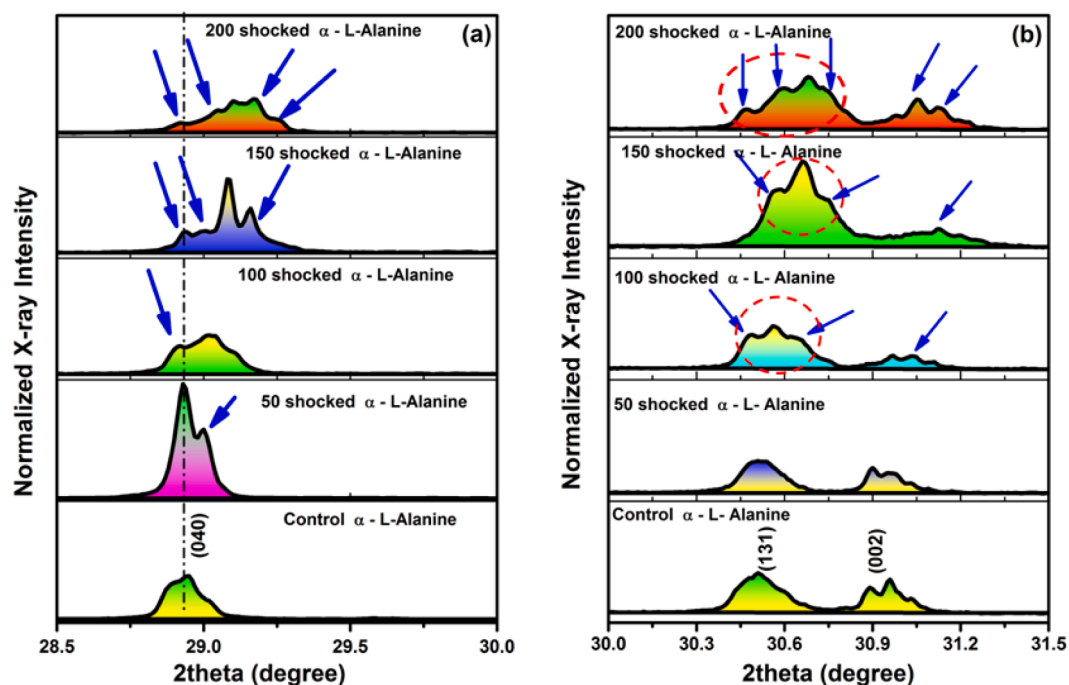


Fig. 5. Zoomed-in versions of the powder XRD patterns of the control and shocked L-Alanine powder samples (a) 28.5–30 degrees (b) 30–31.5 degrees

diffraction line characteristics clearly demonstrate that, similar to the non-polar amino acid materials such as Glycine [28], L-Valine [29], and L-Isoleucine [30], the title amino acid sample does not experience any structural phase transitions under shocked conditions.

In Fig. 6, the main diffraction line positions of the previously described non-polar amino acid materials, such as Glycine - (021) [28], L-Valine - (002) [29], and L-Isoleucine (002) [30] along with the L-Alanine (120) are shown which are provided in addition to the diffraction peak profile features. The Y-axis scale for all of the samples in comparison is fixed to be 5 degrees, and this approach is made only for

the purpose of improving technical comprehension of the structural stability under shocked conditions. All of the materials have an accurate fit to the linear equation based on the obtained line profiles of the non-polar amino acid materials stated above whereas, the data points of L-Alanine (120) plane are slightly increased with respect to the number of shock pulses compared to the other materials such as Glycine - (021) [28], L-Valine - (002) [29], and L-Isoleucine (002) [30]. It is obvious from the acquired profiles that L-Alanine is less structurally stable under shocked conditions than the aforementioned non-polar amino acid compounds.

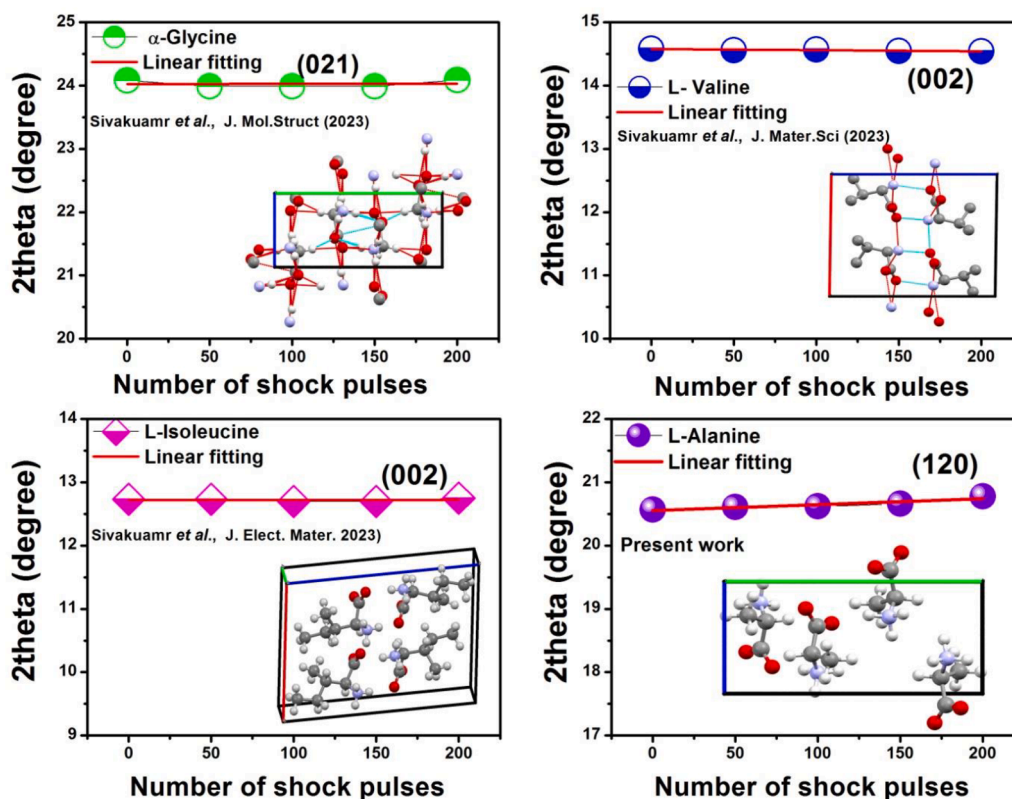


Fig. 6. Phase stability diagram of the non polar amino acid materials such as Glycine – (021) [28], L-Valine – (002) [29], and L-Isoleucine (002) [30] L-Alanine (120) [present work].

3.2. Circular dichroism spectroscopic results

Circular dichroism (CD) spectroscopy is an optical spectroscopic method that exploits the differential absorption of left- (L-form) and right- (D-form) circularly polarized light by such chromophores, which can be harnessed to derive structural information about protein conformations [15]. Montgomery et al., have performed vibrational optical rotatory dispersion spectral analysis of L-alanine with the static pressure compression technique and found that there are no changes in the chirality with the application of pressure (2.5 GPa) which might be because of the unchanged asymmetric bonds arranged around the carbon atoms [20]. In the present work, the circular dichroism spectroscopic technique has been implemented to identify the chirality changes under shocked conditions over the wavelength region from 180 to 400 nm. For this measurement, the control and 200-shocked L-alanine samples have been considered such that the obtained CD spectra are presented in Fig. 7. If there are any chirality changes, the observed signal must be in the opposite direction (like left to right).

But, in the present case, there is no such kind of directional changes observed in the 200-shocked sample which provides possible evidence for the stability of its own chirality {L(+)} and the schematic diagram of the unchanged chirality is displayed in Fig. 8.

3.3. Scanning electron microscopic images

The scanning Electron Microscopic (SEM) technique was used to visualize the surface morphological changes of the test sample under shocked conditions. Note that significant modifications have been detected in the surface morphologies of amino acid samples under shock pressure and temperature in the previously published papers. The SEM images of the control and shocked samples are presented in Fig. 9 and based on the observed SEM micrographs, there is no strong evidence for significant shock wave-induced changes that have occurred on the test

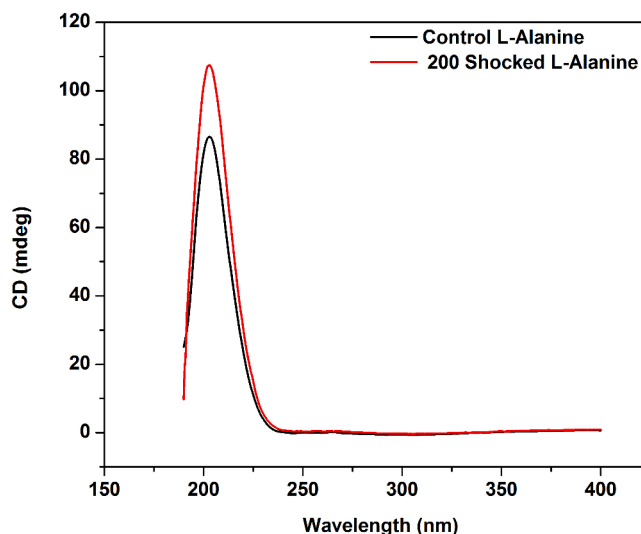


Fig. 7. CD spectra of the control and 200 shocked L-alanine sample.

samples similar to that of L- α -Leucine and L-Asparagine monohydrate. In the instance of L- α -Leucine, it is necessary to observe that the phase transition from L- α -Leucine –I to –II enables the irregularly shaped thin platy crystal morphology to transform to a well-defined rectangular plate-like morphology with a highly smooth surface at the 50-shocked condition [25]. Whereas, in the case of L-Asparagine monohydrate, the control sample has a very rough surface which turns into a highly smooth surface at the 50-shocked condition because of the occurrence of the dynamic recrystallization that is induced by the shock waves [26]. In the case of the α -Glycine sample, subtle morphological changes are

Informed consent

All people involved in or responsible for the research are informed and consent.

CRediT authorship contribution statement

Sivakumar Aswathappa: Conceptualization, Data curation, Methodology, Writing – original draft. **Lidong Dai:** Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Writing – review & editing. **Sahaya Jude Dhas Sathiyadhas:** Validation, Writing – review & editing. **Martin Britto Dhas Sathiyadhas Amalapushpam:** Validation, Writing – review & editing. **Vasanthi Thangavel:** Formal analysis, Validation. **V.N. Vijayakumar:** . **Raju Suresh Kumar:** Formal analysis, Validation. **Abdulrahman I. Almansour:** Formal analysis, Resources, Validation.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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