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# Formulation development and characterization of lamotrigine-salicylic acid crystalline product: A strategy to improve oral release of drug for better management of epilepsy

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Lamotrigine, a FDA approved antiepileptic drug is widely used in the treatment of epilepsy and bipolar disorder. However, poor aqueous solubility and low dissolution rate limit its oral absorption leading to a delayed onset of action with reduced therapeutic effect. The present study aims for the development of formulation and characterization of lamotrigine-salicylic acid crystalline product for the improvement of oral release and absorption for better management of epilepsy. The crystalline product of LT are developed with SA at 1:1, 2:1, 3:1 molar ratios by solvent evaporation method. The experimental crystals have been characterized by different analytical techniques such as fourier transform infrared (FTIR) spectroscopy, differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD) etc. Presence of characteristic peaks for peptide in the experimental crystalline products in FTIR spectra indicates formation of strong covalent bond between LA and SA. In the DSC thermograms, melting endotherm of the formulations showed different melting points than pure LT. PXRD data depicted sharp peaks for the formulations, which further justified the successful formation of a new crystalline phase. Dissolution profile of the experimental crystal (L1S1 at 1:1 molar ratio) in simulated gastric fluid was higher than that of the pure drug and other formulations. The optimized crystalline product of LT may be used for the better oral treatment of epilepsy with early onset of action after successful *in vivo* studies.

**Keywords**: Crystal dislocation density, Crystal strain, Improved oral release, Lamotrigine, Salicylic acid

Poor aqueous solubility associated with low dissolution rate of many therapeutic agents remains as the major challenge in the drug development process[1]. Epilepsy can be characterized as abnormal electrical activities generated within the brain, which may affect a local area (partial seizure) or the entire brain (generalized seizure)[2]. The main goal for the management and control of epilepsy is the timely delivery of the drug with required therapeutic concentration to elicit early onset of action so as to reduce frequency and severity of seizures. However, Low solubility delays the onset of action, which directly limits the therapeutic efficacy of pharmaceutical agents to achieve desired clinical outcome. Among several techniques for the improvement of dissolution profiles of therapeutic agents such as solid dispersion, salt formation, complexation, nanocarrier system, etc., crystallization method has been employed as a suitable technique in pharmaceutical research[2,3]. A crystal is a type of molecular complex which contains two or more different molecules, bound together through covalent interactions in the same crystal lattice[4]. This modified formulation accepts pharmaceutically compatible guest molecules into its crystal lattice along with the active pharmaceutical ingredient (API) without interfering into their molecular arrangements[4]. Crystalline products are considered to be products of more rational design with higher stability as most of the crystallizing agents exist as stable solid forms at room temperature.

In formulation development, crystallization approach holds the uniqueness as it neither affects the pharmacological properties of incorporated drug molecule, nor changes its molecular structure, but alters several physicochemical characteristics of the entrapped drug molecule including melting point, solubility, permeability, tabletability, bioavailability etc[5,6]. Another advantage associated with crystals is their potentiality to be employed with all most all types of APIs including acidic, basic, and non-ionisable molecules[6].

Lamotrigine (LT), a Food and Drug Administration (FDA) approved anti-epileptic drug, is widely recommended for the treatment of epilepsy and

other bipolar disorders[7,8]. Chemically, LT is 6-(2,3-dichlorophenyl)- 1,2,4-triazine-3,5-diamine. Lamotrigine, a phenyl triazene class of anti-epilepsy drug is recommended for the treatment of primary and secondary tonic/clonic seizures, partial seizures etc[9]. LT significantly delays the incidence of repeat episodes with fewer side effects and is well tolerated in epileptic patients. The possible mechanism of action involves inhibition of voltage dependent $Na^+$ channels, which leads to sharp reduction in the release of some excitatory neurotransmitters like glutamate and aspartate-2 (Ref. 8)

However, being a BCS class II drug, its absorption and bioavailability is dissolution rate limited. Thus, poor solubility linked with low dissolution rate delays its onset of action. Several formulation techniques such as nanoparticles[10], solid dispersion[11], salt forms[12], microemulsion[13], etc. have been reported over the past years for the improvement of dissolution rate and oral bioavailability of LT. Shinde *et al*., reported solid dispersion technique to enhance the solubility and dissolution characteristics of LT[11]. LT pellets were prepared with solid dispersions with polyvinylpyrrolidone K30 (PVP K30) and polyethyleneglycol 6000 as excipients by solvent evaporation method. The optimized formulation (PVP K30) at 1:5 drug:excipient molar ratio showed enhanced dissolution profile during the experimental study period. In another study, Chappa *et al*. investigated the structural and pharmaceutical properties of two novel cocrystals of LT formulated with maleic acid and malonic acid. The experimental cocrystals were evaluated by different *in-vitro* techniques[14]. The selected cocrystal formulations were subjected to *in-vitro* dissolution studies in 0.1 N HCl and compared with pure LT. Results showed a significantly higher dissolution rate of optimized LT-SA crystal than pure API.

However, crystallization technique of LT with salicylic acid for the enhancement of its solubility and release of drug in active form in simulated gastric fluid has not been reported yet. Thus, the present study aims to develop crystalline product of LT with salicylic acid and to evaluate their *in vitro* characteristics in order to establish an optimized formulation for improved release and oral absorption of LT.

## Experimental Section

### Materials

LT was obtained as a gift sample from Unichem Pvt. Ltd., Madhya Pradesh, India. Salicylic acid (SA) was procured from Sisco Research Laboratories, Maharashtra, India. Ethanol was purchased from Merck Specialties Pvt. Ltd., Maharashtra, India. All other chemicals used were of analytical grade.

### Method of crystal formation

The crystalline product of LT along with SA was prepared by conventional solvent evaporation technique with required modifications[15]. Briefly, an accurately weighed amount of LT was mixed with required quantity of SA. The mixture was then dissolved in ethanol (50% v/v). After that the resultant solution was dried at 40-50°C for 72 h. The organic solvent was slowly evaporated during the entire process to induce crystallization. Finally the crystallized products were recovered and used for further studies.

### Characterization

#### Fourier transform infrared (FTIR) spectroscopy

For the FTIR study, the pure drug (LT), excipient (SA) and different formulations (LT-SA crystals) were mixed properly with KBr (IR grade) in the ratio of 100:1 in separate vessels[16,17]. Corresponding pellets were prepared by applying a pressure of 5 tons for 5 min in a hydraulic press. The thin pellets were formed, which were then scanned with the help of a FTIR spectrophotometer (JASCO, FT/IR-4100) over a wave number range of 4000$cm^{-1}$ to 400 $cm^{-1}$ using spectra manager software version 2.0.

#### Differential scanning calorimetry (DSC)

DSC measurements for pure drug, SA and all the selected crystal formulations were carried out using a DSC-1 (Mettler Toledo DSC) using STAR$^e$ soft ware[18]. The temperature and heat flow of the instrument was calibrated using an Indium (m.p. 156.6°C) and zinc standards (m.p. 419.6°C). During the experiment, weighed amount of test samples (6 mg) were analyzed in crimped aluminum pans with pin-hole pierced lids. Throughout the experiment, $N_2$ atmosphere was maintained and a heating rate of 10°C $min^{-1}$ (within a temperature range of 30-300°C) was employed.

#### Powder X-ray diffraction (PXRD)

XRD study was carried out for the developed crystal products to get the information about the crystal structure, chemical composition along with physical changes in the materials. For the study, about 1 mg of dry powdered sample was placed on the glass slide and analyzed with a powder X-ray diffractometer (Ultima, IV, Japan)[19,20] During the experiment, an X-ray power of 40 kV/40 mA at a

detection angle (2θ), (2° - 75°) was employed for 120 seconds.

#### *In-vitro* drug release study

The *in-vitro* drug release study for the selected crystalline product was performed according to US pharmacopoeia XXIII rotating paddle method in a dissolution apparatus (Dissolution tester (USP) TDT06L, Electrolab). For the experiment, weighed amount of the formulations placed in 900 ml of 0.1 N HCl at pH 1.2 in the dissolution vessel and set at 50 rpm rotation speed. The temperature during entire period of dissolution study was maintained at 37°C ± 0.5°C. At pre-determined time intervals (5, 10, 20, 30, 60 min), about 10 ml of samples were withdrawn from the dissolution chamber along with simultaneous replacement of fresh medium[21]. The samples were filtered and analyzed by UV-Visible spectrophotometer (JASCO V-630, Japan) at 267 nm against 0.1 N HCl as blank. All the studies were performed in triplicate.

## Results and Discussion

#### Formulation development

Crystallization technique is an emerging strategy to improve dissolution rate and oral bioavailability of poorly water soluble drugs. The technique is simple, devoid of any critical formulation steps, thus easy to standardize for industrial scale. In the present work, crystalline products of LT, a widely used antiepileptic drug, was prepared with SA as excipient. Various formulations were developed by varying molecular ratio of drug and excipient. In our work, the amount of SA was kept constant, whereas amount of LT was varied under identical experimental conditions. Among all the developed formulations, three formulations (L1S1, L2S1 and L3S1) with molar ratios such as 1:1, 2:1, 3:1 of LT with SA have been reported. The formulation composition of various crystal products was depicted in Table 1.

Table 1 — Formulation of lamotrigine-salicylic acid crystal products

| Formulation code | Lamotrigine (gm) | Salicylic acid (gm) | Molar Ratio | Solvent |
|---|---|---|---|---|
| L1S1 | 1.875 | 1 | 1:1 | 50% Ethanol |
| L2S1 | 3.750 | 1 | 2:1 | 50% Ethanol |
| L3S1 | 5.625 | 1 | 3:1 | 50% Ethanol |

The schematic representation of LT-SA crystal product has been illustrated in Fig. 1. It predicted formation of peptide bond in between the acidic group of SA with the amine group ($NH_2$) of LT with the removal of water. This type of bond is usually very strong covalent bonds which remain stable to hydrolysis, however, in presence of acidic pH (pH 2-3), it can be readily broken to liberate the entrapped drug molecule.

#### FTIR study

FTIR study was conducted to assess the incompatibility (if any) between various functional groups of API and the excipient and also to justify the formation of crystals. Data showed few changes in the characteristic peaks of experimental formulations as compared to free drug, which indicates that crystals were formed (Fig. 2). When the spectra of pure drug, and the selected crystal products were compared, the major functional groups of LT that is 3500 $cm^{-1}$ ($NH_2$ symmetric stretching), 850-550 $cm^{-1}$ (C-Cl stretching vibration), 3000-3250 $cm^{-1}$ (aromatic C-H stretching vibration) etc. were found in all the experimental crystal formulations (L1S1, L2S1, L3S1). However, shifting of some peaks was clearly observed in the FTIR data of crystal formulations. Particularly the

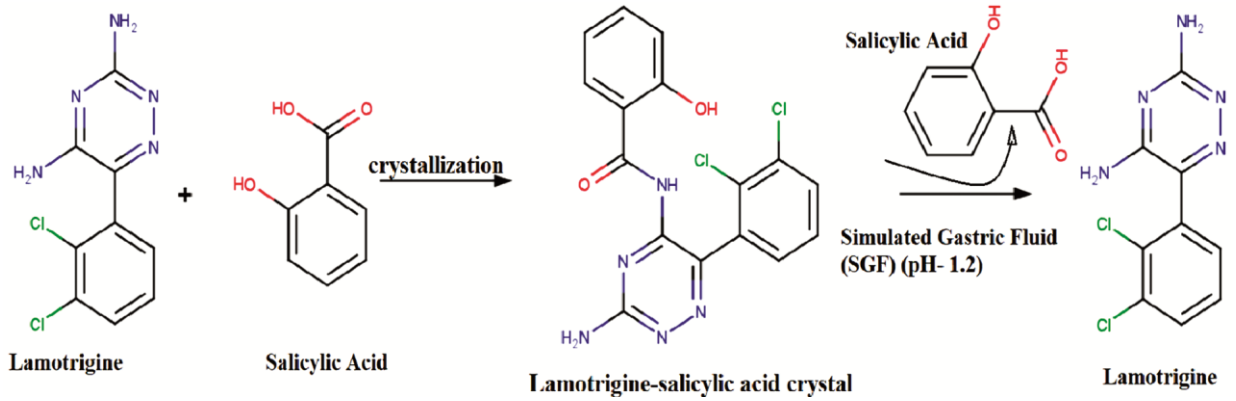


Fig. 1 — Schematic representation of LT-SA crystal formation and release of the drug under gastric acidic pH condition.

sharp peak observed for the drug at 3445.21 $cm^{-1}$ due to the presence of $NH_2$ group (symmetric stretching vibration) was found to be shifted to 3756.65 $cm^{-1}$ (L1S1), 3730.62 $cm^{-1}$ (L2S1), and 3832.83 $cm^{-1}$ (L3S1) in the tested formulations. A strong peak was observed at 1298.82 $cm^{-1}$ (L1S1, L3S1) and 1301.72 $cm^{-1}$ (L2S1) in the crystal products (C-N stretching vibration for aromatic amine), which was absent in the FTIR spectrum of the pure drug. This could be attributed to the formation of strong covalent bond (peptide bond) between the COOH group of SA and $NH_2$ group of LT during formation of crystal structure. Similarly the peak at 791.636 $cm^{-1}$ observed for LT (C-Cl stretching) was found to be shifted to 794.528 $cm^{-1}$ (L1S1), 708.712 $cm^{-1}$ (L2S1) and 795.493 $cm^{-1}$ (L3S1) respectively. In a nutshell, shifting in the characteristic peaks between the spectra of pure drug, excipient and formulation as well as appearance of new peaks in the crystal products justified the formation of new intermolecular bonds among the functional groups of LT and SA which might lead to the formation of novel crystal structure.

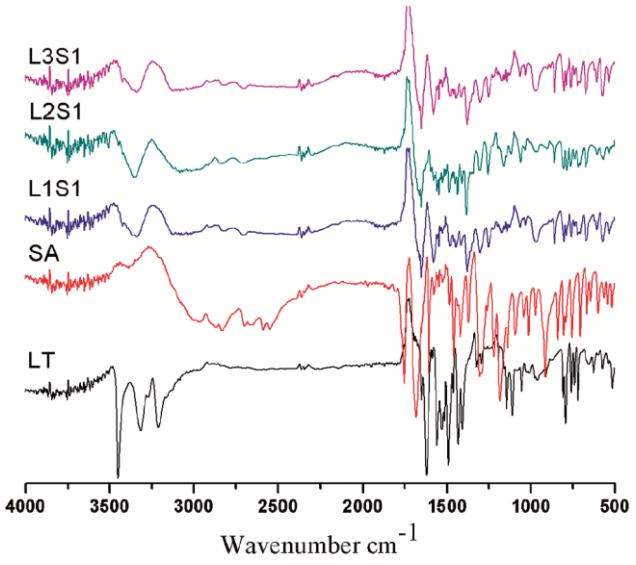


Fig. 2 — FTIR spectra of pure drug (LT), excipient (SA) and crystal products (L1S1, L2S1, L3S1)

**DSC study**

DSC experiment was carried out to depict the thermal behavior of the crystal form in relation to the individual components. DSC thermogram of LT and selected crystal products (L1S1, L2S1 and L3S1) were presented in Fig. 3. Data showed sharp endothermic peaks of LT at 215°C. Experimental

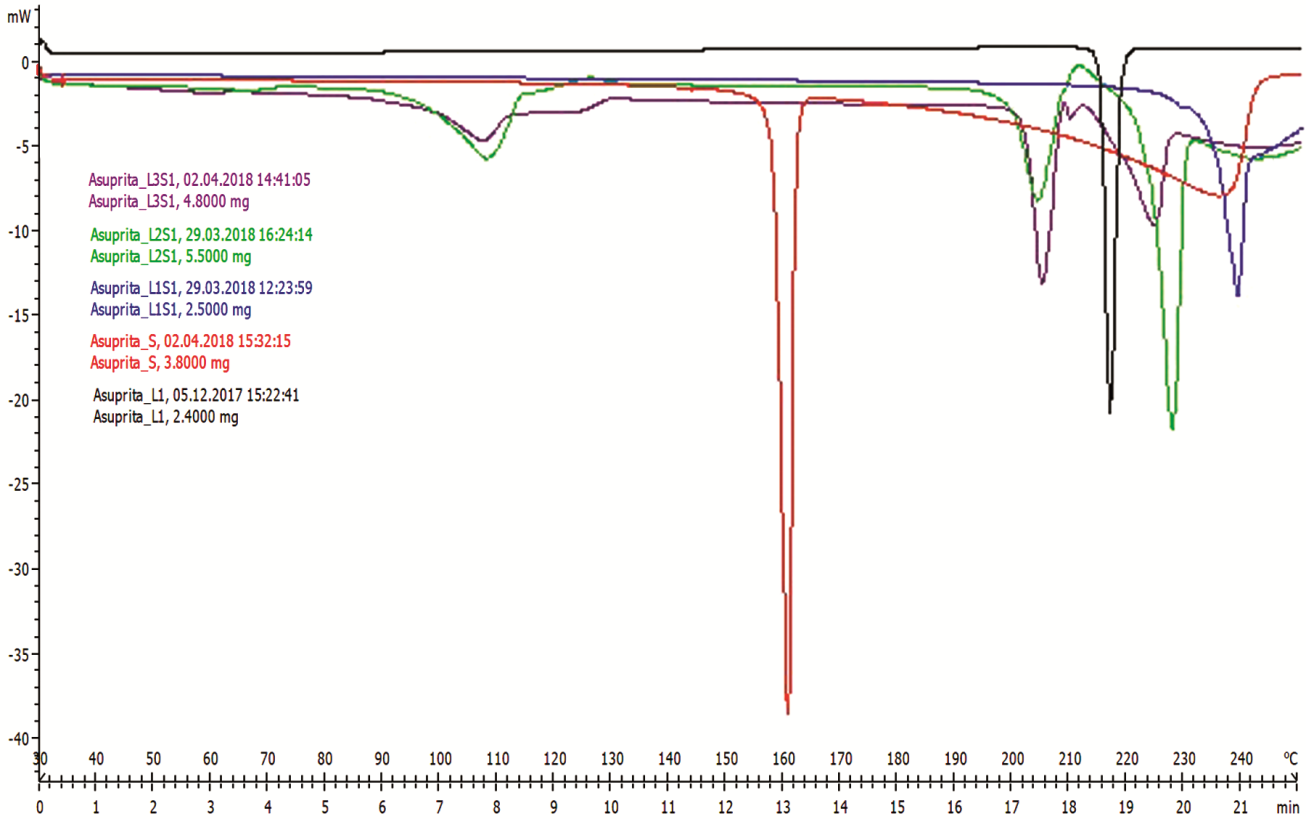


Fig. 3 — DSC thermogram of pure drug, excipient (SA) and the crystal products (L1S1, L2S1, L3S1)

crystals of LA-SA showed sharp endothermic peaks at 237.75, 226.01, and 201.35°C. The sharp endothermic values of crystal form and the individual component agreed with the melting range in the melting point determination. It was found that the thermal profile of crystalline products were distinct with different melting transitions than that of the indivisual components (LT, SA) which indicates formation of a novel crystal phase. The endothermic transitions further signified absence of any absorbed solvent or water molecules in the synthesized crystalline product and thus stable nature of the crystal phase.

**PXRD study**

PXRD is a very useful analytical technique, which is employed for the phase identification of a crystal structure and also reveals necessary clue on unit cell dimensions. It actually measures the diffraction pattern of crystalline material[22]. In XRD study, each API produces a specific pattern depending on the structure of its crystal lattice. Similarly, each polymorph, crystal or salt forms also have its own specific pattern. PXRD pattern of pure LT and selected crystal products were depicted in Fig. 4. For LT, the crystalline peaks were located at 12.39°, 17.35°, 25.44°, 26.68°, 27.78° and 28.33° (2Θ). Some new peaks were also observed in the prepared crystals. In our study, among the crystalline products, L1S1 showed a much sharper peak as compared to other formulations L2S1 and L3S1. The smaller peaks in L2S1 and L3S1 may be attributed to the phenomenon of amorphyzation. While comparing the graphs, each graph showed 100% relative intensity at different 2Θ angles, which are the clear indications of successful formation of crystal lattice.

Debye-Scherrer's formula was employed to find out other characteristic properties like particle size, strain and dislocation density which are tabulated in (Table 2).

Crystallite size was calculated using following equation:

$$D = 0.9\lambda/\beta\cos\theta \quad \ldots (1)$$

The particle strain in lattice was determined from the equation.

$$\varepsilon = \beta/4\tan\theta \quad \ldots (2)$$

Where, $\varepsilon$ = Strain, $\beta$ = Full Width Half maxima (FWHM)

D = Crystallite size, $\lambda$ = Wavelength

The dislocation density (δ), which represents the amount of defects in the sample is defined as the length of dislocation lines per unit volume of the crystal and it is calculated using Equation (3).

$$\delta = 1/D^2 \quad \ldots (3)$$

Particle size was found lowest in case of L1S1 as compare to pure LT as well as other formulations. The strain and dislocation value were found higher as compare to other formulations. The above mention changes may due to the binding of lamotrigine with salicylic acid.

***In vitro* drug release study**

*In vitro* drug release study is a key physicochemical parameter for all solid oral dosage forms that actually

Table 2 — Lamotrigine-salicylic acid crystal products characteristics from XRD analysis

| Formulation | Particle size (nm) | Strain | Dislocation density ($*10^4$) |
|---|---|---|---|
| LT | 77.31 | 0.337 | 1.67 |
| L1S1 | 54.75 | 0.398 | 3.34 |
| L2S1 | 78.76 | 0.371 | 1.61 |
| L3S1 | 76.23 | 0.058 | 1.72 |

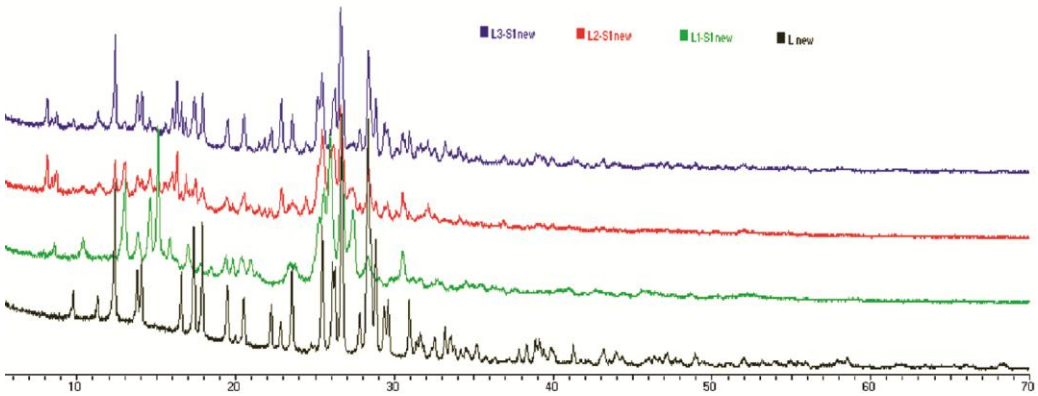


Fig. 4 — PXRD data of pure drug and the crystal formulations (L1S1, L2S1, L3S1)

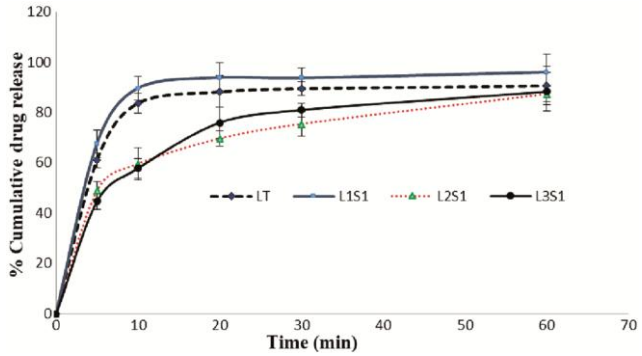


Fig. 5 — *In vitro* dissolution profile of pure drug (LT), and crystal formulations (L1S1, L2S1, L3S1) in simulated gastric fluid (0.1 N HCl, pH 1.2) for 60 min

characterizes how an API is extracted out of the solid dosage form. Appropriate selection of the *in vitro* experimental conditions that simulate the *in vivo* conditions leads to the generation of *in vitro-in vivo* correlation data[23]. Thus, to quantitatively elucidate the effect of solid-state modification on the dissolution characteristics as well as to select optimized product, *in vitro* dissolution studies were conducted for pure LT and LT-SA crystalline products (Fig. 5). The experiment was carried for 60 min in simulated gastric fluid (0.1 N HCl, *p*H 1.2) as the release medium. From the dissolution data, it was found that among the selected formulations, L1S1 (1:1 molar ratio) showed highest cumulative % drug release of 97.11 % within 10 min study period as compared to other formulations and pure drug. Among LT-SA crystals, L2S1 and L3S1 showed lesser amount of cumulative % drug release than pure LT. L1S1 thus could be selected as the optimized formulation, owing to its higher drug release property. The higher drug release property of L1S1 might be attributed to the higher compatibility between LT and SA with proper supramolecular arrangement of the subunits in the newly formed crystal lattice.

Formation of the peptide bond between the LT and SA was the uniqueness of this study, which was depicted schematically in Fig. 1. The same was also confirmed from the newly formed peaks observed in the FTIR spectra of crystal products. Though peptide bond is a type of strong covalent bond and thus seems difficult to split, but this can be easily broken under acidic *p*H condition (*p*H 3-4). In our case, simulated gastric fluid (*p*H 1.2) was used as the drug release medium, which might have helped to break the strong peptide bond easily to release the entrapped drug molecule. Thus the experimental crystal product would remain reasonably stable during the entire period of storage due to existence of strong covalent bond as well as it would also show satisfactory drug release property upon oral administration in acidic medium. These unique characteristics of the developed formulation would be certainly beneficial to improve their clinical applicability.

## Conclusion

The study reported a simple, industrially viable, standardized method for the development of stable crystalline product of LT using SA as the excipient to enhance dissolution rate and oral absorption of LT for improved management of epilepsy. FTIR confirmed successful formation of crystal of LT with SA (peptide bond formation) and DSC study further depicted absence of any chemical reaction between the drug and the excipient. Out of the selected formulations, L1S1 showed sharp peak as depicted from XRD study, which further justified successful formation of new crystal structure. The dissolution rate was higher for L1S1 as compared to other formulations and pure drug during the experimental time period. Owing to its satisfactory drug release profile in simulated gastric fluid (97.11 % within 10 min), L1S1 was reported as the optimized crystalline product. However, further *in vivo* studies are specifically warranted to establish the formulation for its future clinical applications in epilepsy after oral administration.

## Conflict of interests

The authors of the manuscript have no conflict of interest to declare.

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