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RESEARCH

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Binary and ternary inclusion complexation of lapatinib ditosylate with β-cyclodextrin: preparation, evaluation and in vitro anticancer activity

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Abstract

Background: Lapatinib ditosylate, an efficient tyrosine kinase inhibitor for breast cancer, poses pharmacokinetic issues, hence developing its oral delivery system is troublesome. The poor aqueous solubility of this medicament is a key impediment in developing its successful formulation. So, the current study aims to improve water *solubility of Lapatinib ditosylate by* using complexation technique with β-cyclodextrin and a suitable ternary agent.

Results: Binary and ternary complexes of *Lapatinib ditosylate* were synthesized by means of kneading and lyophilization using β-cyclodextrin and PVP K30. As a ternary agent, various hydrophilic polymers, as well as organic acids, were assessed, and PVP K30 was chosen for the final formulation based on its stability constant and complexation efficiency. When compared to pure *Lapatinib ditosylate*, both inclusion complexes demonstrated improved solubility, and drug dissolution. Differential scanning calorimetry (DSC), powder X-ray diffractometry (PXRD), Fourier transform infrared (FTIR), and scanning electron microscopic (SEM) techniques, all validated the complex formation. Docking studies picturized the geometry of Lapatinib ditosylate in β-cyclodextrin cavity. Using MCF-7 cell lines, investigation of anticancer activity of the pure drug and its synthesized complexes was carried out and the results revealed that the complexes had stronger anticancer activity than Lapatinib ditosylate alone.

Conclusions: Overall, it can be concluded that *Lapatinib ditosylate* complexation increased its aqueous solubility, resulting in its increased dissolution and in vitro anticancer activity in a breast cancer cell line.

Keywords: Lapatinib ditosylate, β-cyclodextrin, Polymer, Organic acid, Complexation, Ternary complex

1 Background

Even in the year 2022, breast cancer is still one of the leading causes of death in females and is yet affecting a large number of women every year [1]. Though, a breakthrough therapy is not found yet, dynamic research is being carried out in the concerned area. Among the five different types of breast cancer, human epidermal growth factor receptor 2 positive (HER2 +) breast cancer accounts for about 20–25% of cases. So, HER2-targeted and HER2-associated therapies were evolved and were found to be effective in controlling the neoplasm [2].

Lapatinib, a tiny molecule and a dual epidermal growth factor receptor (EGFR) as well as tyrosine kinase (TK) inhibitor, is found to be effective for HER2 + breast cancer and is approved by USFDA for metastatic settings [3]. It is available in the market as film-coated tablet for oral use. The tablet contains 405 mg lapatinib ditosylate monohydrate (LD), corresponding to 250 mg of lapatinib free base. Though the marketed preparation contains salt form of Lapatinib, its aqueous solubility is inadequate

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which leads to its variable bioavailability [4]. Therefore, a number of methods are being utilized on this active for its solubility enhancement purpose. Drug inclusion complexation is a well-known, well-studied, and widely utilized method for increasing solubility. Among the different cyclodextrins, β-cyclodextrin (β-CD) is the most commonly used cyclodextrin in marketed formulations due to its higher solubilization efficiency as well as its listing as GRAS excipient. The current research is carried out in an attempt to increase solubility of Lapatinib ditosylate with the aid of β-cyclodextrin [5].

Few research studies previously reported a range of natural, as well as synthetic, cyclodextrins, namely α, β and γ-CD, as well as randomly methylated β-cyclodextrin, hydroxypropyl β-cyclodextrin and sulfobutyl ether β-cyclodextrin (SBE-β-CD) for solubility enhancement of LD. Their phase solubility study concluded maximum solubility enhancement of LD with SBE-β-CD due to additional electrostatic interactions involved at molecular level [6]. Yet the current research utilized only β-CD due to its satisfactory suitability and the need to perform additional investigations with ternary co-complexing agents.

Multicomponent complex of β-cyclodextrin further enhances solubility of guest drugs, so this research targeted further solubility enhancement of Lapatinib ditosylate by using ternary agents like hydrophilic polymers or organic acids. Effect of pH on solubility of LD in presence of co-complexing agent was also studied [7]. Final ternary agent was selected based on preliminary phase solubility data, and both binary and ternary complexes of LD were prepared by kneading and freeze drying method. Solid-state evaluation of complexes was performed by techniques of FTIR, DSC, PXRD and SEM. The complexes were also assessed for their solubility and dissolution improvement as compared to free drug. Anticancer activity of the prepared complexes was carried out using MCF-7 cell line to assess their inhibitory effect.

2 Methods

2.1 Materials

Lapatinib ditosylate was purchased from Sumar Biotech LLP, Mehsana, Gujrat, India. β-cyclodextrin, PEG-4000, PVP K-30, HPMC E5, citric acid and tartaric acid were procured from Himedia Laboratories Pvt. Ltd., Mumbai, India. Deionized water was used for all the experiments.

2.2 Phase solubility analysis

A preliminary phase solubility analysis was performed based on Higuchi and Connors method in order to establish stability constant and complexation efficiency of LD with β-cyclodextrin. Excess of LD was added in 5 ml aqueous β-CD solution (0–20 mM) in 10 ml vials. The formed suspension was vortexed for a minute and then was placed in shaker bath at 25 °C for 48 h. Later, this suspension was withdrawn and filtered through 0.22 µm pore size filter. Analysis of this filtrate was carried out using UV–Visible spectroscopy (Shimadzu, UV-1800) at 261 nm for LD estimation. Phase solubility diagram was then constructed using β-CD concentration versus LD concentration. From the slope of obtained curve, the values of stability constant (*K*s) and complexation efficiency (CE) were estimated using Eqs. (1) and (2). In these equations, So represents intrinsic aqueous solubility of LD (mM) [8].

$$K_s = \frac{\text{Slope}}{S0(1 - \text{slope})} \tag{1}$$

$$\text{CE} = \frac{\text{Slope}}{1 - \text{slope}} \tag{2}$$

2.3 Effect of hydrophilic polymers on complexation

Polyvinyl Pyrrolidone (PVP K-30), Hydroxypropyl Methyl Cellulose (HPMC E-5) and Polyethylene Glycol—4000 (PEG—4000) were screened as ternary agents for this study. These hydrophilic polymers were found to significantly enhance the solubility of guest molecules with β-CD in a number of reports. In order to study their effect, excess of LD was added in 5 ml aqueous β-CD solution (1–20 mM) containing 0.5% w/v of each polymer. The suspensions were vortexed for a minute followed by their filtration through a filters of pore size 0.22 µm [5]. Analysis of filtrate for LD estimation was carried out by UV–Visible spectroscopy at 261 nm.

2.4 Effect of organic acids on complexation

Citric acid and tartaric acid were also studied as ternary agents as they are reported to increase the solubility of entrapped drugs in β-CD. Therefore, excess of LD was added in 5 ml aqueous β-CD solution (1–20 mM) containing 0.5% w/v of organic acid. Further processing of these suspensions was carried out as discussed in Sect. 2.2. Final selection of co-complexing agent among hydrophilic polymers and organic acids for LD was based on the results of phase solubility data [9].

2.5 Effect of pH on complexation

Since LD is a weak basic compound with pKa of 7.2, its solubility is strongly influenced by pH variations. Therefore, the combined effect of pH, β-CD and PVP K-30 on their solubilizing efficiency was evaluated. A pH range of 3.2 to 11.2 was studied as an efficient pH range. To the buffered aqueous solutions containing 1 mM of β-CD alone or in combination with 0.5% w/v PVP K-30, excess

of LD was added. The vials were kept in shaker water bath for 48 h at 37 °C. Aliquots were withdrawn from each vial, filtered and the LD content was analyzed by UV–Visible spectroscopy [7, 10].

2.6 Preparation of solid inclusion complexes: Binary and Ternary

Binary complex of LD and β-CD was prepared in their equimolar ratio by kneading as well as freeze drying method. Similarly, ternary complex was prepared using equimolar ratio of LD and β-CD with 0.5% w/v of PVP K-30 added. The technique of lyophilization was used to prepare this complex [7, 11].

2.6.1 Preparation of physical binary and ternary mixtures

Physical mixtures (PM) of LD, and β-CD (LD-βCD-PM), as well as LD, β-CD, and PVP K-30 (LD-βCD-PVP-PM), were prepared in the same ratio as used for final complexation. The homogeneous mixing of all these sieved ingredients was carried out in a pestle mortar [11].

2.6.2 Kneaded binary complex

The kneaded complex (KD) of LD and β-CD was prepared in a 1:1 molar ratio. Both the ingredients were weighed accurately and mixed together uniformly in a mortar and pestle. Wetting of this mixture was carried out with the minimal quantity of ethanol–water mixture (60:40%v/v). The mix was kneaded thoroughly with a pestle for about an hour until a paste was obtained. This paste was allowed to equilibrate for about 3 h and dried under vacuum at 40 °C. This formed kneaded complex (KC) was coded as LD-βCD-KC and was stored in desiccator until further evaluation [12].

2.6.3 Preparation of Freeze-dried binary and ternary complexes

LD and β-CD were weighed in 1:1 mM ratio and added in water. The pH of this solution was adjusted to 3.2 using 0.1 N HCl. This solution was thoroughly mixed on a magnetic stirrer for 48 h at room temperature and was freeze dried using Martin Christ, Alpha 2–4 LSC freeze drier. Lyophilization was carried out at the vacuum of 0.3 mbar for 6 h. Similarly, ternary complexes were prepared utilizing 0.5 percent v/v PVP K-30, LD, and β-CD in a 1:1 mM ratio [11]. The lyophilized binary complexes (BC) and ternary complexes (TC) were coded as LD-βCD-BC, and LD-βCD-PVPK30-TC, respectively.

Furthermore, in order to study the effect of β-CD concentration on crystallinity of LD, binary complexes of LD with β-CD were prepared in 1:2, 1:3, and 1:4 ratio by freeze drying.

2.7 Differential scanning calorimetry (DSC)

Various techniques such as XRD, XRD, FTIR, and SEM were used to characterize the synthesized complexes. Differential scanning calorimeter, Mettler-Toledo GmbH, Switzerland, was used to record the thermograms of LD, β-CD, their PM, and lyophilized complexes. Indium was used to calibrate the system's temperature axis and cell constant. Then, in sealed pin holed aluminum pans, roughly 1–10 mg of material was weighed and analyzed at 10 °C/min heating rates over a temperature range of 100–300 °C with nitrogen purging at 50 ml/min. STAR SW 12.10 software was used to evaluate the results.

2.8 X-ray diffractometry

Bruker's D8 advance diffractometer was used to record PXRD patterns of LD, β-CD, PVP K30, and lyophoilized complexes using Cu Kα radiation (1.54 Aº) at 40 kV, 40 mA flowing through nickel filter. Over an angular range of 5° to 85° 2Θ, data were captured in a continuous scan mode with a step size of 0.01 and a step time of 0.1*s*. To eliminate the orientation effect, the recording was done with the sample holder rotated. DIFFRACplus EVA version 9.0 diffraction software was used to examine the diffractograms obtained.

2.9 Fourier transform infrared (FTIR) spectroscopy

On a Perkin-Elmer Sp.2 spectrometer, FTIR spectra of all of the above specimens were acquired using around 2 mg of each sample. The samples were scanned at IR wavelengths ranging from 450 to 4000 cm^{-1}. Spectrum 10 software was used to analyze the acquired spectra.

2.9.1 Molecular docking studies

Lapatinib ditosylate was molecularly docked with β-CD using Easy Dock Vina 2.2 and Autodock 4.2. The ligand structure of LD and its SMILES notation were prepared with Structure File Generator and online SMILES Translator, respectively. Open Babel 2.4.1. was used for geometry optimization of molecules under interaction study. The PDB co-crystal of β-amylase (PDB code:1BFN, resolution-2.07 Å) was used to mimic the crystal structure of β-CD and Easy Dock Vina 2.2 software was used to generate docking results of binary complex. These results were then represented on Autodock tools (ADT) version 1.5.6 [5].

2.9.2 Scanning electron microscopy

Using scanning electron microscopy, NovaNanoSEM, the surface morphology of binary and ternary LD complexes was investigated. Each sample was examined by sputter coating it with gold using an ion sputter under

0.000139 Pa vacuum and adhering its powder to a double-sided adhesive tape pasted over sample stubs. Under the scanning electron microscope, the materials were scanned with an electron beam of 10 kV acceleration potential, and pictures were acquired in secondary electron mode at various magnifications.

2.9.3 Assay and solubility determination of complexes

For the assay procedure, the lyophilized complexes equivalent to 2 mg of LD were taken and dissolved in 5 ml of dichloromethane. The contents were vortexed until a clear solution was obtained. These solutions were filtered through a 0.22 µm porous filter, and LD content was determined at 261 nm using UV–Visible spectroscopy. To test the solubilizing potential of ß-CD and PVP K30, the excess of complexes was weighed and added in 10 ml of water in a 15 ml stoppered vial. The vials were stored in a shaker water bath (Lab Tech) at 37 °C for 48 h. The obtained suspensions were filtered, and LD content was determined by UV–Visible spectroscopy. The study was repeated thrice and average values were considered [13].

2.9.4 Dissolution study

Dissolution studies were conducted for LD, PM of LD and β-CD, LD-βCD-KC, LD-βCD-BC and LD-β-CD-PVP-TC using USP-II, paddle type dissolution test apparatus (Electrolab, India) as per guidelines of USFDA. The study was performed at 37 °C ±0.5 °C temperature with a paddle rotation speed of 55 rpm. The dissolution medium employed was 900 ml of 2% Polysorbate 80 in 0.1 N HCl. Aliquots from samples containing 250 mg of LD or its equivalent in PMs, binary and ternary complexes were withdrawn after 10. 15, 30 and 45 min, and the equivalent amount of fresh dissolution medium was added in order to maintain sink conditions. These aliquots were filtered (0.22 µm pores size), and LD content was determined by UV–Visible spectroscopy. The obtained data were plotted as a percentage of LD released vs. time. All these experiments were carried out in triplicate [14].

2.9.5 In vitro anticancer activity

The MCF-7 cell line was used to assess the effect of prepared complexes on cell toxicity. The effect of varying concentrations of samples on cell growth inhibition was also investigated. Beta cyclodextrin, Lapatinib ditosylate, and its prepared freeze-dried samples, LD-βCD-BC, and LD-βCD-PVPK30-TC were studied at the concentrations of 1, 2.5, 5, 10, 25, 50, 75, and 100 µg/ml. The culture medium was used to dilute the samples (DMEM with high glucose, FBS and Antimycotic 100X solution). A suspension of MCF-7 cells was seeded at a density of 1 X 10^4 cells per well in a 96-well plate. After then, the cells were exposed to all of the samples at various concentrations. Pure culture media was used as the control group. In a CO_2 incubator, all samples were incubated in triplicate for 24 h at 37 °C and 5% CO_2 (Thermo scientific BB150). After 24 h, the culture fluid was replaced with 20 µl of MTT (3-[4-dimethylthiazol-2-yl]-2, 5- diphenyltetrazolium bromide) and 80 µl of new media. The cells were then incubated for another 4 h. After that, 200 µL of DMSO was added to each well, which was incubated for further 10 min until the formazan crystal reaction occurred. Only living cells reduced the yellowish MTT to dark-colored formazan, thus each well was examined under a microscope to ensure cell survival. A microplate reader (Benesphera E21) was used to measure the absorbance of each sample at 550 nm. The obtained optical density and percent cell inhibition values were calculated [15].

3 Result

3.1 Phase solubility analysis

The purpose of this research was to establish the interaction strength between Lapatinib ditosylate and β-CD molecules, as well as to determine their stoichiometry. Figure 1 depicts phase solubility analysis results which clearly displays a linear increase in LD concentration with an increase in β-CD concentration, confirming the A_L type of solubility profile. According to Higuchi and Connors method, when the solubility of the substrate (drug) increases with an increase in ligand concentration (cyclodextrin), A-type phase solubility profiles are produced. When a linear rise in solubility of substrate is obtained with increased ligand concentration, then the profile is referred as "A_L type" [16]. A stochiometric complex of 1:1 ratio between the duo is confirmed as the slope of curve is smaller than unity. Intrinsic solubility of LD in water at 25 °C was found to be 0.0216 mM (So). The values of the stability constant and complexation efficiency were found to be 209.275 M^{-1} and 0.00452, respectively, implying the development of a stable complex. The presence of stable complexes is indicated by a Ks value greater than 100 M^{-1}. As a result, it can be concluded that β-CD is effective in increasing solubility of Lapatinib ditosylate [17].

3.2 Effect of hydrophilic polymers on complexation

The effect of polymers on solubility, stability constant, and complexation efficiency of β-CD with LD was studied using different hydrophilic polymers, namely Polyvinyl pyrrolidone (PVP K30), Polyethylene glycol (PEG-4000), and Hydroxypropyl methyl cellulose (HPMC E-5). As seen in Fig. 2, every polymer had a synergistic effect with β-CD. PVP K30 was found to be the most effective, followed by PEG-4000 and HPMC E5. The

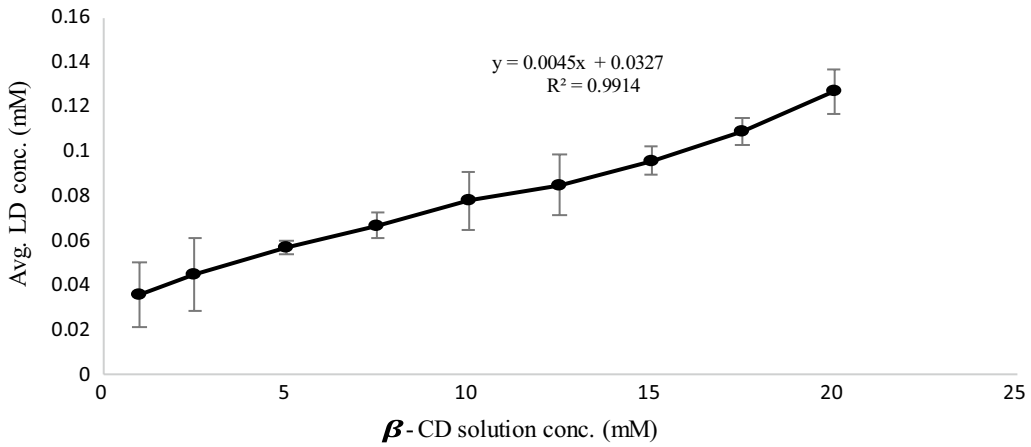


Fig. 1 Phase solubility study of LD in presence of different concentrations (1–20 mM) of β-CD

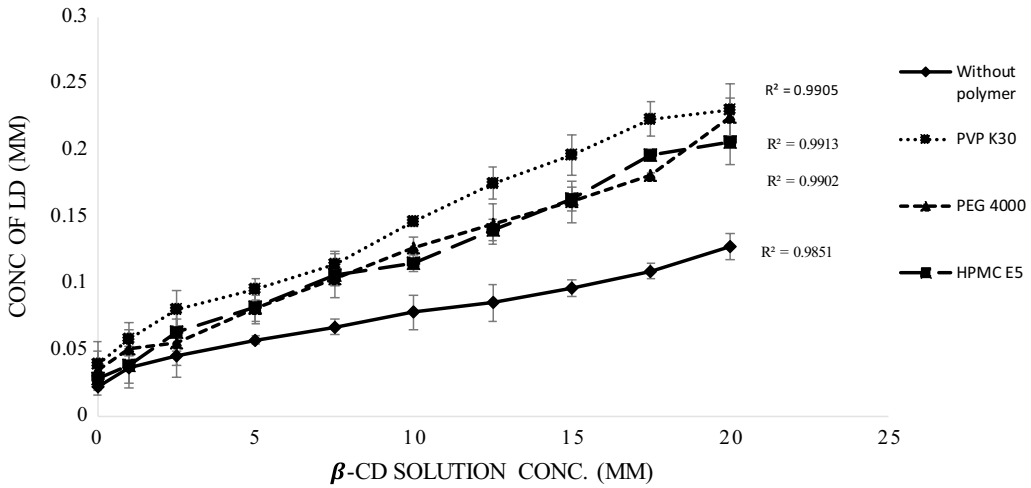


Fig. 2 The comparative solubility profile of LD in presence of hydrophilic polymer

solubility enhancement of LD was 5.87 times more with β-CD alone, at its 20 mM concentration. And, with 0.5 percent w/v of polymer added, the solubility of LD was improved by 9.53, 10.64, and 10.39 folds with HPMC E5, PVP K-30, and PEG-4000, respectively. The use of polymers enhanced the values of Ks and CE, implying that the medication, β-CD, and polymer formed a ternary complex. Table 1 summarizes these findings. PVP K30 was chosen for future research because it showed better solubilization enhancement potential, Ks, as well as CE.

Table 1 The complexation efficiency of β-CD in presence of 0.5%w/v of polymers and organic acids

Polymer	Ks (M^{-1})	CE
β-CD	209.27	0.00452
β-CD—PVP K30	444.03	0.009591
β-CD—PEG 4000	406.31	0.008776
β-CD—HPMC E5	406.31	0.008776
β-CD—Citric acid	378.06	0.008166
β-CD—Tartaric acid	363.94	0.007861

3.3 Effect of organic acids on complexation

Both the selected organic acids collaborated with β-CD in augmenting solubility of LD. 0.5% w/v of citric acid raised solubility of LD by 8.85 times, and similarly, tartaric acid elevated it by 8.65 times when combined with 20 mM β-CD solution. This effect can be seen from Fig. 3. These organic acids also boosted the values of Ks and CE, suggesting the creation of ternary complex of LD with β-CD. The values of Ks and CE in the presence of organic acids

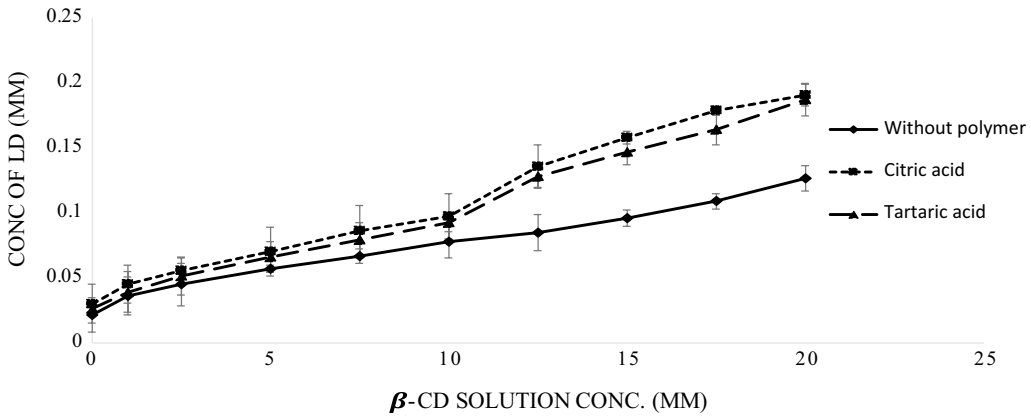


Fig. 3 The comparative solubility profile of LD in presence of organic acid

are depicted in Table 1. From this table, it can be inferred that the chosen hydrophilic polymers formed more stable complex with LD in association with β-CD. In comparison with organic acids, they also improved the solubility of LD to a bigger proportion. Hence, PVP K30 was selected for further complexation studies as it was found to be the most suitable ternary agent with β-CD.

3.4 Effect of pH on complexation

As LD is a weak basic compound with pK_a value of 7.2; its solubility is largely affected by the pH of the media. So, in this study, we studied the combined effect of pH, β-CD and polymer (PVP K-30) on LD solubility. pH of media governs the ionization status of a molecule and in turn affects complexation efficiency [18]. As per Henderson-Hasselbatch equation, at pH 11.2, LD exists in its unionized form and is expected to show maximum complexation efficiency with β-CD by entering in its cavity. However, maximum solubility enhancement was observed at pH value of 3.2 where LD undergoes maximum ionization. This could be attributed to the combined synergistic effect of ionized LD, β-CD and PVP K30. The addition of PVP K30 was particularly effective at this pH, as it in part counterbalanced the destabilizing effect due to increased drug ionization obtained with decreasing pH. Since, PVP K30 is a proton acceptor; it counterbalances this acidic pH of the complexation media without affecting the LD complexation with β-CD [19]. The effect of pH on solubility of LD in presence of β-CD and PVP K-30 is shown in Fig. 4.

3.5 Assay and solubility determination of complexes

According to phase solubility experiments, LD generated a 1:1 complex with β-cyclodextrin. The inclusion of hydrophilic polymers improved the complexation propensity, and PVP K30 showed greatest improvement among the screened polymers. So, for the binary complex, 1 mM of each LD and β-CD were mixed, and for ternary complexes, 0.5% w/v of PVP K30 was added to it followed by pH adjustment to 3.2. The LD content of the complexes was determined after freeze drying. The assay of LD was 83.33% in the binary complex, LD-βCD-BC, and 87.85% in the ternary complex LD-βCD-PVPK30-TC. Saturation solubility tests found a considerable improvement in ternary complex solubility over binary complex solubility, as seen in Fig. 5. The solubility of LD in water was increased by 1.38 and 2.86 times, by lyophilized binary and ternary complexes, respectively. The kneaded binary complex of LD with β-CD increased solubility of API (Active pharmaceutical ingredient) by 1.25 times. These data clearly pointed enhanced solubility achievement of LD by means of lyophilization technique. Altogether, the complexation approach resulted in a substantial increase in aqueous solubility of LD.

3.6 Dissolution study

To comprehend the findings of dissolution experiments, the proportion of drug dissolved against time interval is used. Figure 6 shows the dissolution profiles of the LD and its carriers. The dissolution profile of LD in designated media revealed drug release of 16.12% at first time point; 10 min and only 32.43% at 45 min. The physical mixture released 20.35% LD after 10 min and 39.92% LD after 45 min demonstrating that β-CD had a wettability influence on drug release. The process of kneading and freeze drying resulted in complexation of LD in the cavity of β-CD, and therefore, enhanced drug release was seen with LD-βCD-KBC, LD-βCD-BC, and LD-βCD-PVPK30-TC formulations. In a binary complex formulation prepared by kneading method, a 23.16% drug release was achieved after 10 min and following 45 min it reached to 52.88%. Similarly, 33.04% LD release was seen

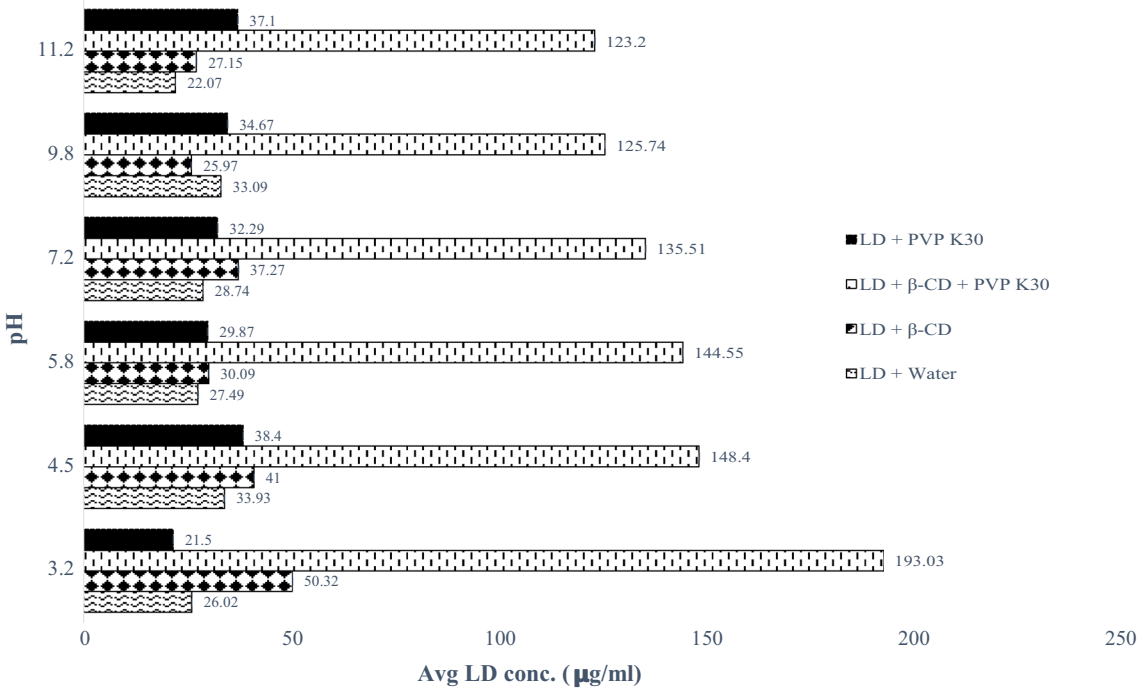


Fig. 4 Effect of pH on solubility of LD in presence/absence of polymer and co-polymer

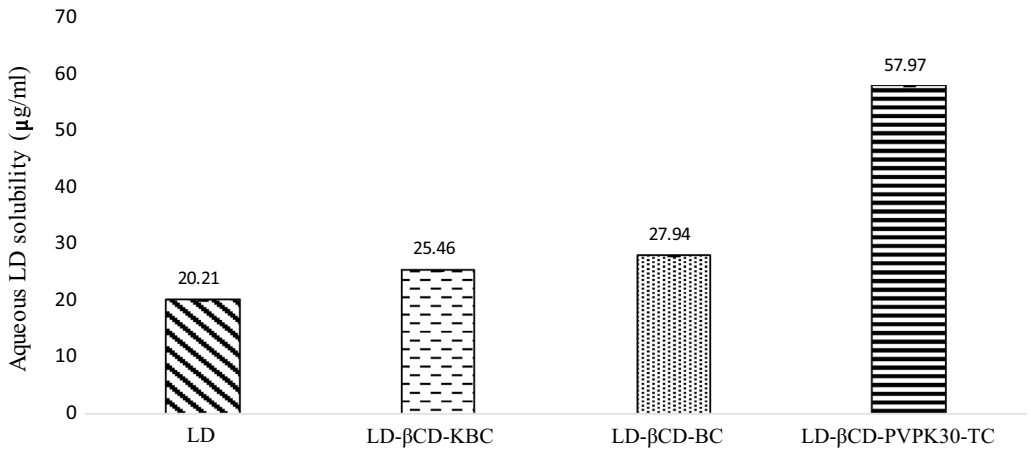


Fig. 5 The saturation solubility data of LD, kneaded binary complex (LD-βCD-KBC), freeze-dried binary complex (LD-βCD-BC) and freeze-dried ternary complex (LD-βCD-PVPK30-TC)

in first 10 min in case of LD-βCD-BC formulation which subsequently reached to 59.53% at the last time point; 45 min. The ternary formulation showcased maximum drug release to 65.36% at 45 min which is 2.01 times greater than pure LD.

The PVP K30 improved inclusion of LD in β-CD, resulting in greater drug release with the ternary complex. As a result, the produced LD-β-CD complexes had increased inclusion, complexation, solubility, and dissolution behaviors. With the addition of PVP K30, a hydrophilic agent, all of these metrics were improved even further.

3.7 Differential scanning calorimetry (DSC)

Figure 7 depicts DSC patterns of LD, its physical mixture with β-CD, PVP K-30 and complexes prepared by freeze

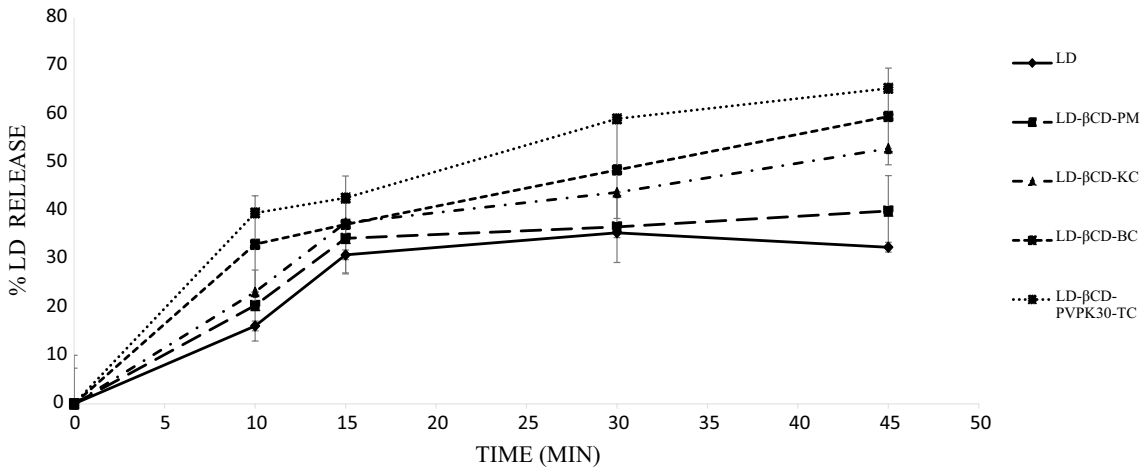


Fig. 6 The dissolution profiles of LD, PM's, binary and ternary complexes

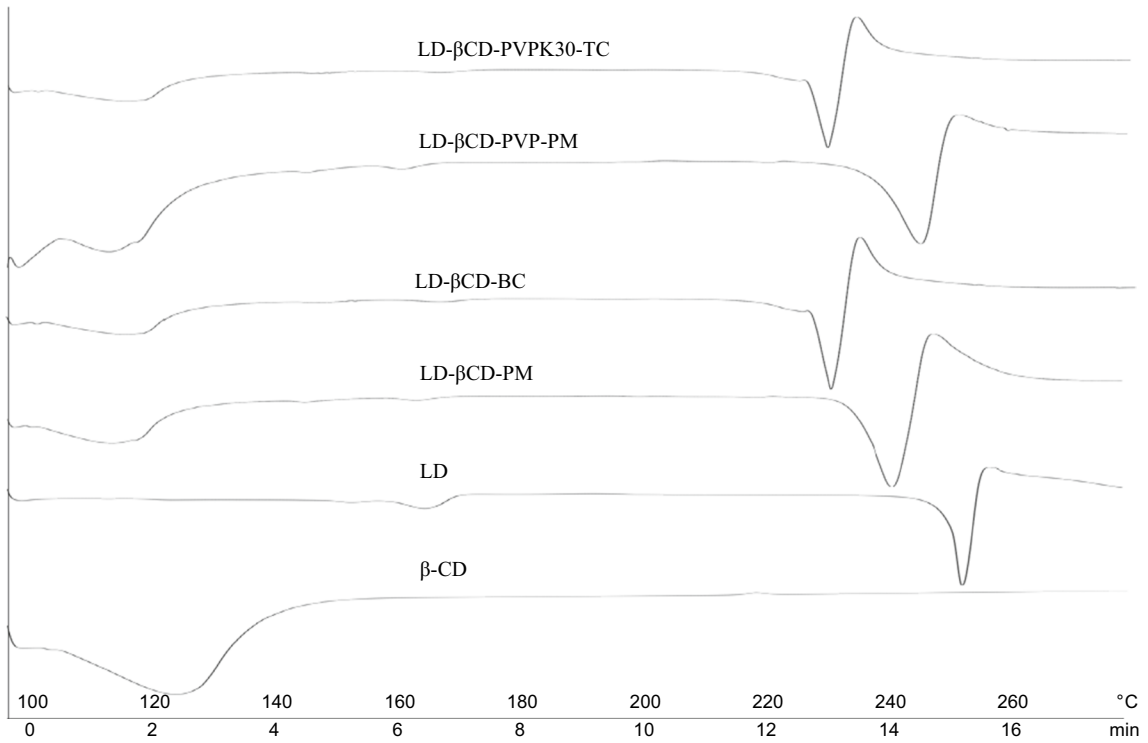


Fig. 7 DSC thermograms of β-CD, LD, physical mixture of LD-βCD, binary complex (LD-βCD-BC), physical mixture of LD-βCD-PVPK30 and ternary complex (LD-βCD-PVPK30-TC)

drying. Due to dehydration, β-CD exhibited a broad endotherm in the temperature range of 107–139 °C. LD displayed a sharp melting endotherm at 253 °C due to its crystalline nature. The enthalpy of this melting endotherm was 68.13 J/g. A physical mixture of LD with β-CD showed a broad endothermic event in the temperature range of 235–248 °C centering at 242 °C with enthalpy value of 125.50 J/g [20]. Presence of melting event in binary physical mixture clearly indicated retention of crystalline character of LD in it. However, enhancement of enthalpy (H_f) value designated possible interaction of β-CD with LD at solid-state level. This binary physical

mixture also depicted endotherm due to dehydration of β-CD from 107 to 139 °C. The binary lyophilized complex, LD-βCD-BC, revealed a sharp melting event at 230 °C indicating maintenance of crystalline nature of LD in the complex. However, this notable shift of M.P. of LD along with change in enthalpy ($H_f = 143.62$ J/g) confirmed successful synthesis of complex. A ternary physical mixture of LD with β-CD and PVP K-30, LDT-βCD-PVP-PM, denoted T_{peak} at 246 °C with enthalpy value of 102.09 J/g, implying profound interaction in the trio, although original crystalline nature of LD remained unaltered. The freeze-dried ternary complex, LD-βCD-PVPK30-TC, exhibited relocation of melting peak of LD from 253 °C to 232 °C indicating its partial engulfment in cavity of β-CD due to complexation. This peak shift was associated with the enthalpy of 110.02 J/g which differed from energy content of LD implying interaction of carriers with LD at molecular level. Altogether, thermograms of all the samples studied showed thermal events due to melting of LD; indicating absence of transition of crystalline structure to amorphous form, though the crystallinity has been reduced.

To study the effect of β-CD concentration on crystallinity of complex, additional binary inclusion complexes of LD were prepared with β-CD using their 1:2, 1:3, and 1:4 ratio. Their recorded thermograms are overlayed in Fig. 8. The thermogram of binary complex prepared in 1:2 ratio shows thermal event in the temperature range of 226–237 ℃ with a T_{peak} at 232 °C. The enthalpy associated with this melting event was 152.47 J/g. This enhanced heat content confirms a significant interaction between LD, and β-CD in the complex. Also, heating curve of binary complex prepared in 1:3 ratio showed T_{peak} at 236 °C with the enthalpy of 160.36 J/g due to melting. Both these complexes presented higher enthalpy values with widening of melting curve, indicating enhanced entrapment of LD molecules in β-CD cavity. This higher entrapment could be associated with the use of increased number of β-CD molecules as the complexes prepared employed higher molar ratios of β-CD. The

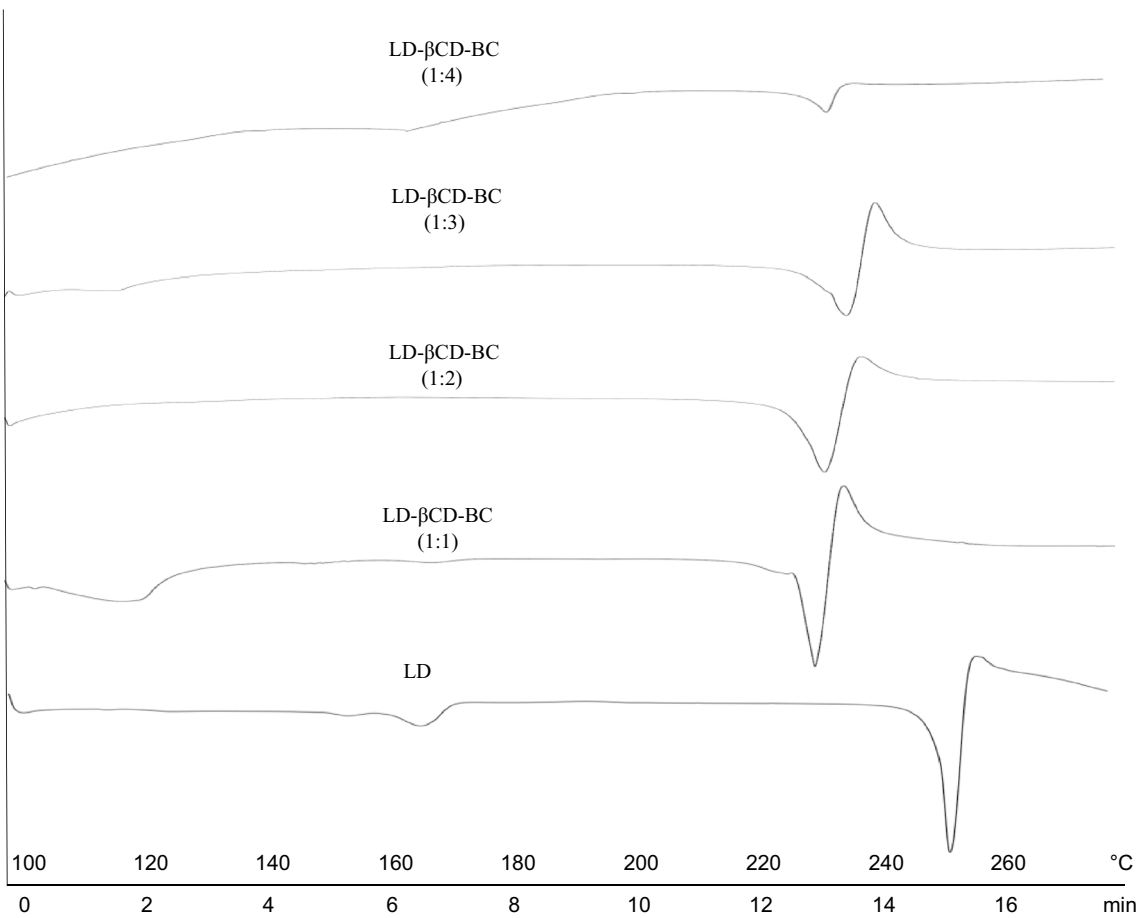


Fig. 8 DSC thermograms of LD, and its prepared freeze-dried, binary complexes of different ratios, LD-βCD-BC (1:1), LD-βCD-BC (1:2), LD-βCD-BC (1:3), and LD-βCD-BC (1:4)

binary complex of LD with β-CD prepared in 1:4 molar ratio showed a very small thermal event at 231 ℃ indicating retention of modest crystalline character of complex. Thus, it can be stated that increase in β-CD concentration has resulted in significantly reduced crystallinity of complexes due to enhanced entrapment of LD molecules. Further investigation of all these samples was carried out by PXRD to correlate and conclude the data.

3.8 Powder X-ray diffraction (PXRD)

Figure 9 shows the PXRD patterns of β-CD, PVP K30, LD and its lyophilized binary and ternary formulations. The cross-linked polysaccharide, β-CD, showcased few sharp peaks at 2Θ values of 5.1, 7.3, 17.6 and 23.2, showing its partial crystalline nature. Diffractogram of Lapatinib ditosylate flaunted its sharp characteristic peaks at 2Θ values of 18.2, 20.9, 24.5 and 26.9 owing to its crystallinity [20]. PVP K30 showed diffused X-ray diffraction pattern indicating its amorphous form. The lyophilized binary complex, LD-βCD-BC, in its X-ray diffractogram indicated only two peaks at 2Θ values of 8.4 and 14.3, which can be attributed to the reduced crystallinity of LD in carrier, β-CD. Similarly, the ternary lyophilized formulation, LD-βCD-PVPK30-TC, presented almost diffused diffraction pattern with a seemingly single peak at 24.5 2Θ value. These findings pointed further reduced crystallinity of ternary formulation as compared to the binary one. PXRD results are thus consistent with results of DSC, indicating that generated freeze-dried formulations have significantly reduced crystallinity as compared to pure active ingredient, LD. Also, the study confirmed that complete transformation to amorphous form of LD was not achieved even in ternary formulation though it was processed by freeze drying.

The X-ray diffractograms of prepared additional binary inclusion complexes of LD with β-CD in the ratio of 1:2, 1:3, and 1:4 ratio are presented in Fig. 10. The binary complex prepared in 1:2 molar ratio showed very small intensity diffraction peaks at 2Θ values of 8.4 and 14.3. This phenomenon clearly indicated reduced crystalline nature of 1:2 complex as compared to the 1:1 binary complex prepared using same method. The amount of β-CD was further enhanced when the binary complex was prepared in 1:3 molar ratio. PXRD pattern of this complex did not show even a weak diffraction peak nor it displayed a hollow pattern, representing very weak crystal nature of the complex. The PXRD spectra of lyophilized 1:4 complex showed apparently diffused diffraction pattern indicating a rough attainment of amorphous nature in the complex while retaining a tiny amount of the ordered state. All these PXRD results are in agreement with the DSC data, confirming the hypothesis of severely reduced crystallinity of binary LD complex with increasing β-CD concentration.

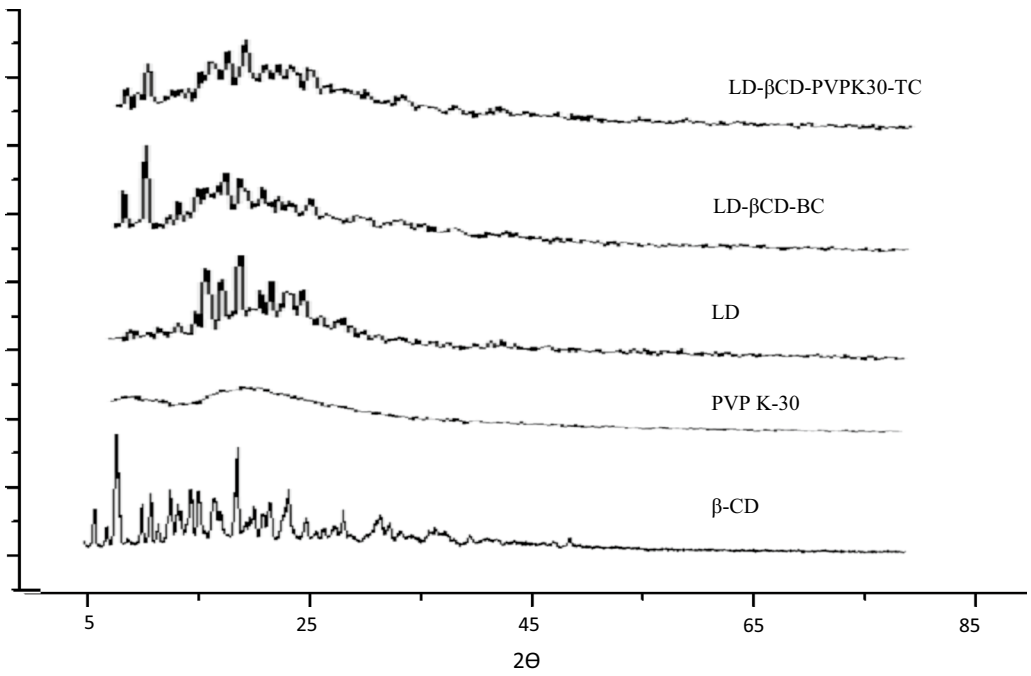


Fig. 9 The X-ray diffractograms of β-CD, PVP K30, binary complex (LD-βCD-BC) and ternary complex (LD-βCD-PVPK30-TC)

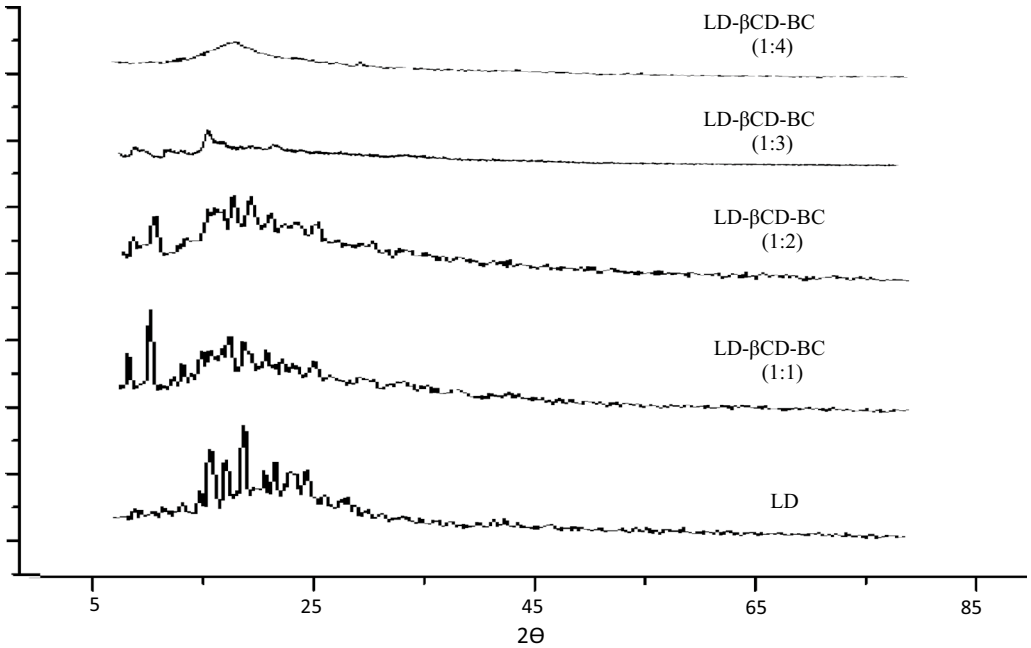


Fig. 10 The X-ray diffractograms of LD, and its prepared freeze-dried, binary complexes of different ratios, LD-βCD-BC (1:1), LD-βCD-BC (1:2), LD-βCD-BC (1:3), and LD-βCD-BC (1:4)

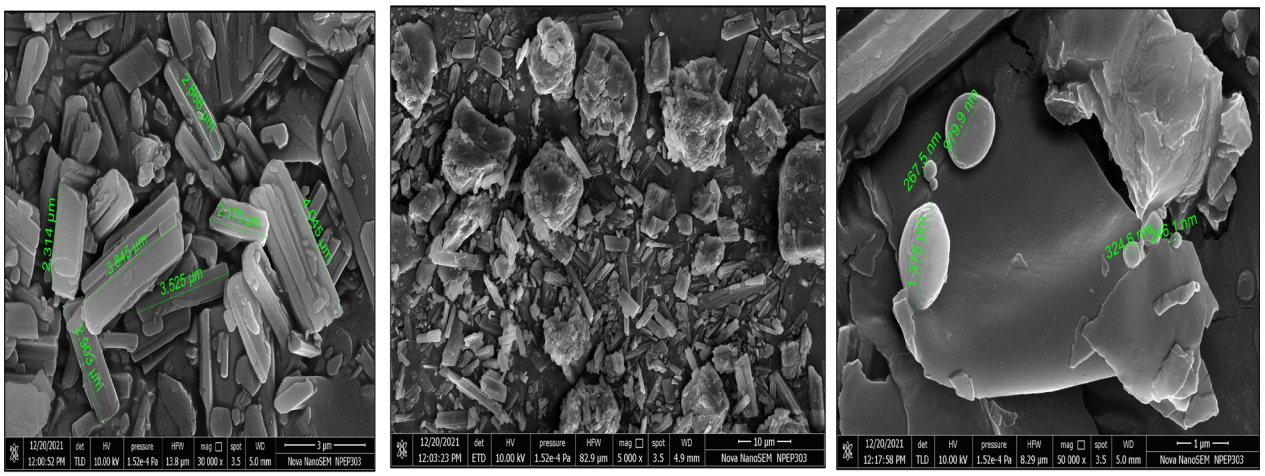


Fig. 11 SEM Photomicrographs of Lapatinib ditosylate and its binary and ternary formulations, LD-βCD-BC, LD-βCD-PVPK30-TC

3.9 Scanning electron microscopy

Figure 11 depicts SEM microphotographs of Lapatinib ditosylate, its binary, as well as ternary, complex prepared by freeze drying. Rod-shaped LD crystals of size about 3 microns could be seen in SEM microphotograph of LD, while surface morphology of the binary formulation, LD-βCD-BC, revealed small, soft, fluffy particles of roughly rectangular shape [20]. Profile of these particles differed significantly from LD crystals, pointing to LD entrapment in cavity of β-CD. However, few crystals of LD were visible in binary formulation indicating its partial engulfment in cavity of β-CD. The ternary lyophilized complex, LD-βCD-PVPK30-TC, exhibited porous structures of diameter 1 µm which does not have a discrete frame indicating attainment of amorphous form in crudely way. Appearance of LD crystals was barely visible

pointing improved entrapment of drug in ternary formulation as compared to binary one.

3.9.1 FTIR spectroscopy

β-CD shows the vibration of free –OH groups between 3300 and 3500 cm^{-1} in its FTIR spectrum. The vibrations corresponding to –CH stretching can also be seen its spectrum at 2926.23 cm^{-1}. Lapatinib ditosylate depicted IR absorption peaks at 3017 cm^{-1}, 1690 cm^{-1}, 1311 cm^{-1}, 1265 cm^{-1}, 1172 cm^{-1}, 1019 cm^{-1}, 1033 cm^{-1}, 678 cm^{-1} and 584 cm^{-1} which could be attributed to aromatic –CH stretch, C=N stretch, S=O stretch, C–O stretch, furan C–O–C stretch, S–O stretch, C–F stretch, C=C bend, and C–Cl stretch, respectively. The characteristic aromatic C=C stretch peaks at 1619 and 1449 cm^{-1} were also visible in FTIR spectrum of LD [21]. In the binary lyophilized complex, LD-βCD-BC, the peaks corresponding to C–O, C–N and C=N stretch have been masked completely indicating entrapment of quinazoline ring in cavity of β-CD. Furthermore, a significant reduction in peak intensity attributed to aromatic C=C stretch and C–F stretch was observed pointing engulfment of fluorophenyl group in β-CD bucket along with quinazoline ring. Since, other explicit LD absorption peaks were present, it could be assumed that the duo, LD and β-CD, has interaction that was occupied up to quinazoline ring and fluorophenyl ring, while other structural entities, i.e., furan ring, sulfonylethylamino group, as well as tosylate salt, remain unentreated. LD-βCD-PVPK30-TC, the ternary freeze-dried complex also exhibited similar FTIR absorption spectra as that of binary freeze-dried complex predicting no involvement of PVP K-30 in chemical interaction of LD and β-CD. This hydrophilic polymer only enhanced the overall solubility of LD with β-CD by wetting phenomenon. All the spectra are depicted in Fig. 12.

3.9.2 Molecular docking studies

In order to study the molecular interaction between LD and β-CD, in silico study was performed and conformation of ligand, LD with β-CD was examined. These docked conformations are shown in Fig. 13a and b. The central quinazoline ring and fluorophenyl ring of LD were found to occupy cavity of β-CD, while the furan ring and sulfonylethylamino groups were lying in the periphery. The hydrogen bonding interactions between quinazoline ring and hydroxyl units of sugar moieties of β-CD were commonly observed. Also, the 4-amino group

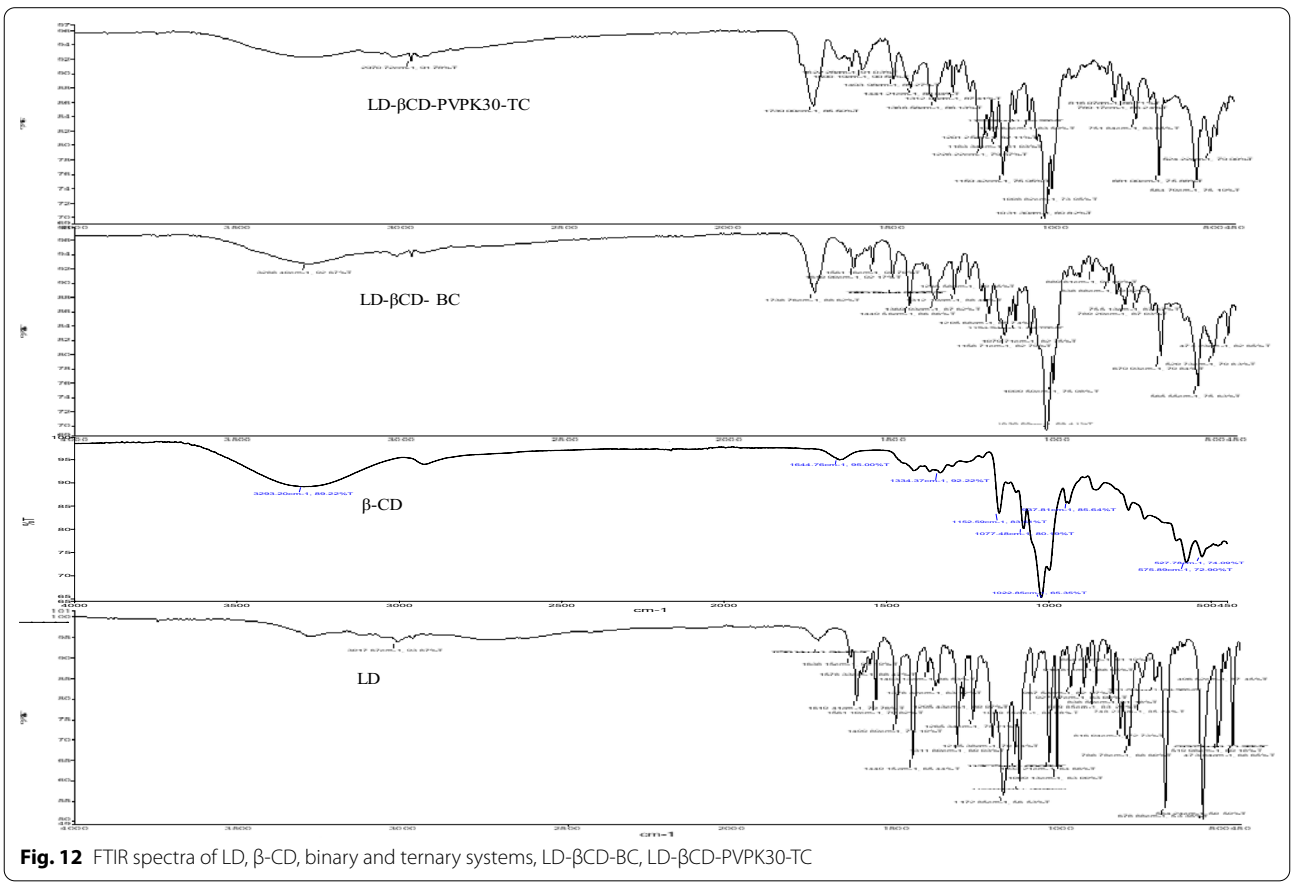


Fig. 12 FTIR spectra of LD, β-CD, binary and ternary systems, LD-βCD-BC, LD-βCD-PVPK30-TC

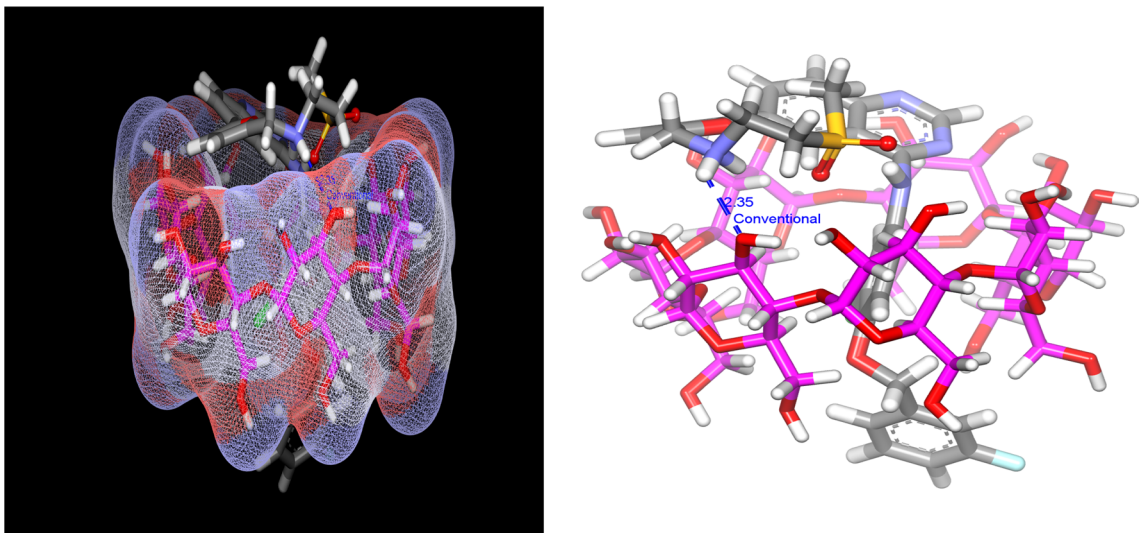


Fig. 13 Binding mode of the Lapatinib ditosylate binary inclusion complex from side [Ball and stick model of LD (gray color) and Wire-mesh model of β-CD] and Binding pose of LD with β-CD. [Ball and stick model of LD (gray color) β-CD (pink color)]

connecting quinazoline ring to chlorophenyl ring was detected to be involved in forming the hydrogen bond with -OH group of β-CD, further making the interaction more stable. In addition, pi-pi stacking interactions were present between aromatic quinazoline ring, as well as fluorophenyl ring of LD and sugar rings of β-CD [6]. The chemical group, sulfonylethylamino was observed to be protruded from β-CD cavity indicating its lack of involvement in complex formation. Binding affinity of LD with β-CD was found to be -6.751 kcal/mol in docking studies. No stearic hindrance was seen when LD interacted with β-CD pointing formation of stable complex in the duo.

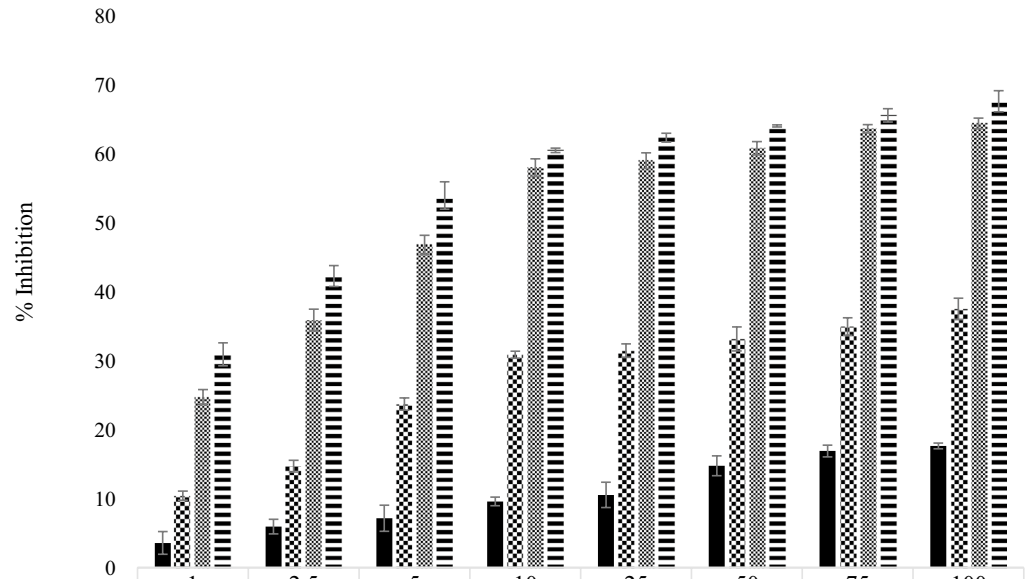
3.9.3 In vitro anticancer activity

At concentrations of 1–100 µg/ml, the percentage inhibition of β-CD, LD, LD-βCD-BC, and LD-βCD-PVPK30-TC on MCF-7 cells was investigated, and the findings are presented in Fig. 14. Carrier, β -CD had a dismal anticancer action on selected cell line. Both binary and ternary inclusion complexes showcased significantly higher percentage inhibition values as compared to pure drug, LD. The improved drug availability due to API's complexation with β-CD resulted in its amplified solubility and accounts for the stronger cell growth inhibition with inclusion complexes compared to pure drug. Among the inclusion complexes, ternary cyclodextrin complex, LD-βCD-PVPK30-TC, exhibited higher percentage cell inhibition as compared to binary complex which could be ascribed to its enhanced solubilization efficacy. Thus, the ternary complex demonstrated its superiority in terms of in vitro anticancer activity.

However, it can be observed from Fig. 14 that the percentage cell inhibition values were prominently higher up to concentration 10 µg/ml. Afterward, the cancer cell growth inhibition increased but in a gradual manner. Absence of dose-dependent response of drug, as well as formulation, in the concentration range of 25–100 µg/ml, could be attributed to the saturation of HER2 receptors on the MCF-7 cell line [22].

4 Discussion

Lapatinib ditosylate, a potent anticancer agent for breast neoplasm, has limitations for oral delivery due to its restricted solubility. The medicament is efficacious for HER2 + type of breast cancer and acts by inhibiting two major targets involved in its pathogenesis, namely tyrosine kinase and epidermal growth factor receptor. The current study targeted enhancement in the anticancer activity of LD by means of augmenting its aqueous solubility and dissolution. The solubility boost was achieved by the use of β-cyclodextrin and a ternary co-complexing agent. Different hydrophilic polymers and organic acids were screened for this purpose and the best synergistic agent with β-CD, PVP K-30 was ultimately chosen for final formulation purpose. The binary complex of LD with β-CD was prepared by kneading and freeze drying method, while the ternary complex was prepared by LD, β-CD, and PVP K-30 by later technique. The complex formation was confirmed by the use of techniques namely DSC, PXRD, FTIR and SEM. Also, in silico



	1	2.5	5	10	25	50	75	100
β-CD	3.57	5.94	7.14	9.58	10.53	14.75	16.88	17.63
LD	10.41	14.68	23.68	30.84	31.42	33.12	34.92	37.46
LD-βCD-BC	24.69	35.85	46.74	57.93	58.93	60.72	63.54	64.32
LD-βCD-PVPK30-TC	30.89	42.22	53.89	60.42	62.24	63.94	65.48	67.51

Concentration (µg/ml)

Fig. 14 Percentage inhibition values of β-CD, LD, LD-βCD-BC and LD-βCD-PVPK30-TC with respect to concentration

docking studies were carried out to study the geometry of LD in cavity of β-CD. The evaluation of formed complexes was also carried out by in vitro means for parameters, aqueous solubility, drug dissolution and anticancer activity using MCF-7 cell line. This cell line is the most commonly used in breast cancer research and can also be used of for HER2 + cancer since it expresses HER2 proteins and tyrosine kinase. The percentage inhibition of cancer cells was studied with LD and its synthesized formulations [23].

A number of approaches are available for solubility enhancement of pharmaceutical actives like development of nanoparticles, dispersions, liposomes, phytosomes, niosomes to name a few, yet complexation approach was preferred in this study by keeping in mind its advantages over the others; like formulation stability, cost effectiveness, simplicity of process, compatibility, and usefulness [24]. β-cyclodextrin was chosen as a complexing agent over modified ones since better results were obtained with it when combined with ternary agent. In addition, β-CD enjoys the status of being listed in GRAS excipients.

Phase solubility analysis of LD with β-CD produced A_L type of curve indicating linear rise in LD concentration with increase in β-CD concentration. The curve established a molecular stoichiometry of 1:1 between LD, and β-CD in forming a stable complex with a stability constant of 209.275 M^{-1}. Hydrophilic polymers, namely Polyvinyl pyrrolidone K-30, Polyethylene glycol-4000 and Hydroxypropyl methyl cellulose E5, and organic acids, namely citric acid and tartaric acid, were assessed as the ternary agents with β-CD. The highest synergistic effect was observed with PVP K-30, hence it was chosen for further experiments. It showed greatest stability constant and complexation efficiency as compared to other screened co-complexing agents; the values being 444.03 M^{-1} and 0.009591, respectively. Effect of pH on complexation was studied in the pH range of 3.2 to 11.2. Maximum solubility enhancement was achieved at pH 3.2 where LD existed in ionized form, yet it entered in the inner void of β-CD. This unexpected result could be accredited to harmonious action of proton acceptor, PVP K-30 with LD and β-CD which in turn did not interrupt their molecular interaction. Hence, binary complex of LD was prepared with β-CD at 1:1 molar ratio at a pH of 3.2 and similarly ternary complex of LD was formulated with β-CD in 1:1 molar ratio followed by addition of 0.5%w/v PVP K-30 and adjusting the pH of formulation to 3.2. The weighed amount of PVP K30 was kept low since it may add weight burden in formulating its final dosage form.

The prepared formulations were evaluated for solubility enhancement and promising results were achieved with about 1.38 and 2.86 times increase in aqueous solubility of LD as compared to pure drug in binary and ternary formulations, respectively, that are prepared by lyophilization technique. Similarly, a solubility rise of 1.25 times was seen in binary complex prepared by kneading method. Thus, superiority of lyophilization technique over kneading method was evident from results of this study. The dissolution study performed in designated dissolution media also provided similar results and maximum drug release was obtained with ternary formulation, LD-βCD-PVPK30-TC which released 65.36% of LD at last time point, 45 min and the drug release was 2.01 times greater than pure LD. Higher drug release was also seen in binary complexes prepared by both kneading and lyophilization techniques, and a drug release of 52.88% and 59.53% was achieved with them, respectively, which was prominently higher than LD drug release; 32.43% at 45 min.

The inclusion complex formation was studied and confirmed by various solid-state techniques. Differential scanning calorimetric analysis of LD revealed its highly crystalline nature with appearance of a sharp melting event at 253 °C. Prepared physical mixtures and synthesized formulations of LD when analyzed by DSC displayed shifts in melting events of LD associated with significant changes in enthalpy values. So, the study concluded entrapment of LD in β-CD cavity while retaining its crystallinity, though the crystallinity was significantly reduced in ternary formulation, LD-βCD-PVPK30-TC. Parallel results were obtained with PXRD studies, where sharp peaks were present in diffractogram of LD at 2Θ values of 18.2, 20.9, 24.5 and 26.9 due to its crystallinity. This peak intensity was remarkably reduced, and an almost diffused diffraction pattern was observed in all prepared binary and ternary formulations indicating alteration at solid-state level of LD due to complexation. DSC investigations along with PXRD confirmed absenteeism of attainment of amorphous form of LD though processed by lyophilization.

The effect of β-CD concentration on crystallinity of complex was studied by increasing the proportion of β-CD in complex, and the complexes were investigated by DSC and PXRD studies. The binary lyophilized complexes of LD:β-CD prepared in 1:2, 1:3, and 1:4 molar ratio showed decreased crystalline nature of complex. The DSC curves of these prepared complexes showed widened melting events associated with enhanced enthalpy values. Also, the PXRD patterns of these complexes showed weak intensity diffraction peaks where the peak intensities decreased with increased β-CD concentration. The binary complex prepared in 1:4 ratio of LD: β-CD showed almost diffused diffraction pattern in PXRD studies, indicating attainment of disordered state of the complex. This effect can be ascribed to enhanced entrapment of LD in β-CD cavity as a greater number of later molecules were present for interaction.

SEM photomicrographs of LD presented its micron-sized, rod-shaped crystals, while its binary and ternary formulations exhibited soft, fluffy particles of roughly rectangular shape indicating their verge of transition from crystalline state to amorphous state. The peaks corresponding to aromatic C=C stretch, C-F stretch, as well as to C–O, C–N and C=N stretch, were veiled in FTIR spectrum of prepared binary and ternary complexes indicating entrapment of fluorophenyl group and quinazoline ring of LD in β-CD cavity. Other functionalities of LD; furan ring, sulfonylethylamino group as well as tosylate salt stay un-interacted with βCD and were projected outward the rim of carrier. Docking study revealed similar findings and confirmed involvement of hydrogen bonding interactions between -OH group of β-CD and quinazoline ring of LD. Also, pi-pi stacking interactions present among carrier ring and fluorophenyl ring of LD also contributed to the thermodynamic stability of complex.

The in vitro anticancer activity of all formulations performed on MCF-7 cell line revealed stronger cell growth inhibition as compared to LD alone. Greater inhibition was achieved with ternary formulation as compared to binary one representing superiority of the former which was attributed to its enhanced solubility and drug release behavior. The heightened wettability of formulation due to addition of PVP K30 resulted in overall improvement in physicochemical behavior of inclusion system.

5 Conclusion

The present study used kneading and freeze drying methods to form binary and ternary inclusion complexes of Lapatinib ditosylate, LD-βCD-KBC, LD-βCD-BC, and LD-βCD-PVPK30-TC. Based on phase solubility research findings, PVP K30 was chosen as a ternary co-complexing agent among the evaluated hydrophilic polymers and organic acids. The increased aqueous solubility of the medicine due to complexation resulted in substantial upsurge in drug dissolution. The complex formation was confirmed using a variety of solid-state characterization techniques, including DSC, PXRD, FTIR, and SEM. During DSC experiments, shifts in melting endothermic events coupled with changes in its enthalpy relative to pure drug suggest complex formation of LD with β-CD and PVP K30. This result was backed up by PXRD measurements, which showed that complexation is achieved and the formed complexes has severely declined crystallinity. SEM morphological

analysis of inclusion complexes demonstrated the formation of small, microscopic sized, irregular, fluffy particles which suggest LD's improved release tendency in the cyclodextrin carrier. FTIR analysis demonstrated interaction of the quinazoline ring of LD with β-CD in binary as well as ternary complex. Similar results were obtained in docking study that confirmed entanglement of fluorophenyl and quinazoline ring of LD in inner void of β-CD, while the remaining functionalities were projected outwards. In vitro anticancer activity studies employing the MCF-7 cell line revealed that both binary and ternary inclusion complexes inhibited cell growth better than pure LD. The ternary inclusion complex of LD with β-CD and PVP K30 produced superior results in terms altered solid-state characteristics of LD which resulted in notable rise in water solubility and drug release.

Abbreviations

DSC: Differential scanning calorimetry; HPMC E5: Hydroxyl propyl methyl cellulose E5; LD: Lapatinib ditosylate; PEG 4000: Polyethylene glycol 4000; PVP K30: Polyvinyl pyrrolidone K30; PXRD: Powder X-ray diffraction; SEM: Scanning electron microscopy; β-CD: β-Cyclodextrin.

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Author contributions

In the conceptualization, writing, data collection, rewriting, and editing, all authors contributed equally. The final manuscript was read and approved by all authors.

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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