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Bioavailability Enhancement of BCS Class II Raloxifene Hydrochloride by Inclusion Complex and Solid Dispersion Techniques

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Abstract

Objective: Raloxifene hydrochloride (RLX) is used extensively in the treatment of osteoporosis, only 2% of RLX's bioavailability remains after a significant first pass metabolism. Besides coming from BCS class II, RLX is not very soluble in water. Thus, the goal of the current study was to improve RLX solubility by creating an inclusion complex using β cyclodextrin (β-CD) as a carrier and solid dispersion with Poloxamer 407.

Methods: Inclusion complex and solid dispersion were made using a variety of techniques, including kneading, co-precipitation, and physical mixing and solid dispersion using different drug to carrier ratios (1:1, 1:2 and 1:3).

Results: Inclusion complex made using the co-precipitation method had shown 9-fold improvements in water solubility when compared with plain RLX. In order to assess the optimized complex's compatibility, thermal analysis, and crystallinity, X-ray diffraction, differential scanning calorimetry, and Fourier transform infrared spectroscopy were used. The XRD and DSC study's results indicated that RLX changed from a crystalline to an amorphous state. IC-6 exhibits effective water solubility based on the outcome. However, upon comparison of the two techniques, the β-CD complexation method shown an impressive rise in drug solubility when compared to solid dispersion.

Keywords Osteoporosis, Raloxifene hydrochloride, β-CD, Poloxamer 407, Inclusion Complex, Solid dispersion

1. INTRODUCTION

1.1 Osteoporosis

A disease of the bones that lowers bone mass, bone microarchitecture/mineralization, and/or bone mineral density (BMD) in humans, increasing the risk of fractures. These asymptomatic cases often present until minor damage to the hip, spine, humerus, pelvis, and/or wrist is detected, often resulting in hospitalization.[1]

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1.2 Solubility

Nearly half of the 150,000 novel molecular entities (NMEs) that pharmaceutical companies create each year are poorly watersoluble, and their effectiveness is allegedly reduced by more than 10% of the market. Up to 40% of people using lipophilic drugs will not reach the market. despite potential pharmacodynamic activity.

At the same time, few lipophilic drugs available also require a lot of medicine. Therefore, various formulation methods have been looked upon to increase the solubility, solubility of lipophilic drugs and thus improve oral bioavailability. Osteoporotic fractures cause morbidity and mortality. As the population ages, the incidence and economic impact of osteoporosis will increase. A variety of antiresorptive medications are now available to prevent or treat postmenopausal osteoporosis, including bisphosphonates (alendronate, rosedronate, and ibandronate), calcitonin, estrogens, and the estrogen receptor modulator raloxifene (RLX). In the NEXT (Raloxifene Multiple Evaluation) research the risk of new osteoporosis in women with and without bone marrow was different with raloxifene 60 mg/day compared with placebo.

Raloxifene is an estrogen receptor modulator (SERM) with proven estrogen agonistic effects on bone, improving bone density, reducing bone turnover, and reducing osteoporosis in men after b rth with osteoporosis. It reduces bone loss. Although it is a type II drug according to the Biopharmaceutical Classification System (BCS), with a mere 2% bioavailability. Consequently, it's critical to enhance raloxifene's solubility and solubility, as this will raise its bioavailability from oral dosage forms. In this study, inclusion complexes were prepared by forming a complex with βcyclodextrin (CD) in order to increase the solubility of the drug. Cyclodextrin (CD) has a lipophilic inner cavity and a hydrophilic outer layer and can interact with a variety of guest molecules to form noncovalent inclusion complexes. BCD is widely used in the early stages of medicine due to its ease of use and cavity size suitable for many drugs.In the current research study, poloxamer 407 fragments containing RLX complexes were prepared with βCD to examine the effect of different methods (physical method, coupling method and kneading method) on the water solubility of the drug.The in vitro release of preparations containing complexes was investigated. [2]

2. MATERIALS AND METHODS

2.1 Materials

RLX was received as a sample from Cadila Pharmaceutical Pvt Ltd, Dholka, India. Poloxamer 407 and βCD were obtained as gift samples from ICPA Pharmachem, Ankleshwer. Every additional reagent utilized in the aforementioned study is of analytical grade.

2.2 Determination of solubility of Raloxifene Hydrochloride in various medium

The solubility of raloxifene hydrochloride in various media is determined by the equilibrium solubility method. In this way, 5 ml of each weight is placed in a separate bottle and raloxifene hydrochloride is added to the bottle containing distilled water and pH 6.8 phosphate buffer. Place the vial in a shaker and stir at 37 ± 2°C for 12 hours. Allow the solution to equilibrate over the next 24 hours. Transfer the solution to an Ependroff tube and centrifuge at 2000 rpm for 5 minutes. The supernatant from each vial was filtered through a 0.45 µ filter. Then, appropriate dilution was made and analysis was performed at 287 nm with a UV-visible spectrophotometer (UV-1800, Shimadzu 1800, Japan).

2.3 Phase solubility studies [4-5]

Add raloxifene hydrochloride to 10 mL of water or aqueous β-CD solution (320 mmol L1) in a 25 Ml stoppered Erlenenner flask and shake on a magnetic stirrer (10) at room temperature. After 2 days of equilibration, a portion of the sample was removed, filtered (0.22 µm pore size, Whatman UK), and the chemical content was determined spectrophotometrically at 284.0 nm (Shimadzu UV160A spectrophotometer, Shimadzu, Japan). The apparent 1:1 stability constant of the raloxifene hydrochloride βcyclodextrin complex is calculated from the solubility phase diagram.

$$Kc = (slope/S0) \times (1 - slope)$$

In the formula, Kc is a constant (L·mol·1), the slope is calculated from the linear relationship between raloxifene hydrochloride concentration and βCD, and S0 is the solubility of raloxifene hydrochloride (mmol·1·L). All experiments were performed in triplicate.

Sr. No	Formulation code	F1	F2	F3	F4
1	Raloxifene (mmol/L)	0.39	0.681	0.982	1.33
2	β -CD (mmol/L)	20	40	60	80
3	Distilled water (ml)	20	20	20	20

Table 1: Inclusion Complex Ratio with different methods

Inclusion Formulation Code	Method	Drug Polymer Ratio
IC-1	Physical	1:1
IC-2		1:2
IC-3		1:3
IC-4	Co-Precipitate	1:1
IC-5		1:2
IC-6		1:3
IC-7	Kneading	1:1
IC-8		1:2
IC-9		1:3

Table 2: Inclusion Complex Ratio with different methods

Solid dispersion Batch Code	Ratio
SD-1	1:1
SD-2	1:2
SD-3	1:3
SD-4	1:4

2.4 Preparation of physical mixtures and the solid inclusion complex: [6]

2.4.1Physical mixture

RLX was mixed with βCD in a mortar and pestle for 1 h and ground continuously. Mixture No. It is passed through 1. 1 sieve. It was melted at 80°C and stored in a desiccator over molten calcium chloride.

2.4.2 Co-precipitation method

Carefully measure the amount of raloxifene hydrochloride and β-CD and dissolve them in methanol and distilled water, respectively. The drug was slowly added to the β-CD solution, and the mixture was stirred at room temperature for 1 h and slowly evaporated over boiling water to remove all solvents. The clathrate precipitates into a crystalline powder which is crushed and No. It is passed through 1. 2 sieves. It was stored in a desiccator containing melted calcium chloride at 80° C.

2.4.3 Kneading method

Weight the β-CD accurately and grind it in a mortar with a small amount of 50% methanol to obtain a homogeneous slurry. The solution was added to the slurry and milled for another 1 hour. The mixture is air dried at 25°C for 24 hours, crushed and No. It is passed through 1.25 sieves. It was melted at 80°C and stored in a desiccator over molten calcium chloride.

2.4.4 Preparation of solid dispersion: [7-8]

The dispersion of raloxifene hydrochloride and poloxamer 407 in four different ratios (1:1, 1:2, 1:3, 1:4 w/w) was prepared by evaporating the solvent and physical mixing.

2.4.5 Solvent evaporation method: [9-10]

Dissolve raloxifene hydrochloride and poloxamer 407 in 5 ml of methanol. The solution was stirred on a magnetic stirrer for 30 min and dried in a hot oven at 50°C. After drying, the residue wasground in a mortar and sieved through #60 mesh.

2.5 Characterization of drug and inclusion complex: [11-12]

2.5.1 FTIR

Record the FTIR spectra of raloxifene hydrochloride and all excipients. Samples were analyzed using FTIR spectroscopy with the KBr particle method. Approximately 10 mg of raloxifene hydrochloride is

mixed with an equal weight of potassium bromide. The spectrum was analyzed in the frequency range of 4000-400 cm-1.

2.5.2 Differential scanning calorimetry: [13-14]

In thermometry, DSC is frequently used to monitor endothermic and exothermic reactions. Given that it offers details on drug-excipient interactions in the formulation, it is crucial for pre-formulation investigations. Using a different scanning calorimeter (DSC TA60, Shimadzu, Japan), optimized inclusion complexes with thermograms of RLX, βCD, poloxamer 407, and product dispersions were produced. Samples were measured in-person in aluminum pans and examined in a dry nitrogen environment at 50300°C. A 10°C/min heating rate was applied.

2.5.3 X-Ray diffraction studies: [15-18]

To determine whether a formation is amorphous or crystalline, XRD examinations might be a helpful tool. An X-ray diffractometer (Xpert Pro MPD, Panalytical, Netherlands) was used for X-ray studies of RLX, β-CD, poloxamer 407, physical mixes, optimal inclusion complexes, and solid dispersions. The X-beam source had a radiation wavelength of 1.5405 Ao Lu Cu Kα. The samples in this investigation were positioned on sample glass and scanned at a scanning angular speed of 2°/min at 40kV operating voltage and 30 mA current, covering a range of 2° to 60°.

Table 3: Solubility of Raloxifene hydrochloride in different solvents

Sr. No	Solvent system	Solubility
1	Water	0.0146mg/ml
2	Phosphate Buffer 6.8	0.38mg/ml

3. RESULTS AND DISCUSSION

3.1 Determination of solubility of drug in various mediums

The solubility of raloxifene hydrochloride in various media was examined and the results are shown in Table 3. The results showed that the drug had poor water solubility.

3.2 Phase solubility

Raloxifene hydrochloride is a poorly soluble drug. The solubility of raloxifene hydrochloride in distilled water (pH 6.8) at room temperature is 24.0 µg mL-1 and is significantly affected by the presence of β-CD. The solubility phase diagram is linear (Fig. 1) and can be classified as AL type according to Higuchi and Connors (10). The slope of the resolution graph is less than 1; Therefore, it is speculated that the increase in solubility may be due to the formation of the 1:1 complex.

The stable value of raloxifene hydrochloride β-CD complex is 170.13 L mol-1, indicating that the 1:1 ratio of raloxifene-β-CD complex is stable enough.

3.3 FTIR

FTIR was used to examine the relationship between drug excipients of optimized IC-6 addition, as shown in Figure 2 . 2. Compatibility study showed that the properties of the drug are 905.12 cm-1 (benzene ring), 1464.8 cm-1. 1 (-S-benzothiophene), 1595.92 cm-1 (-C-O-C-stretch) and 1640 cm-1. It is similar to the chemical spectrum in compound IC-6 (C=O stretch). Therefore, it was determined that there was no significant physicochemical interaction between the drug and β-CD during the formation of the inclusions. These results demonstrate the relationship between the drug and the carrier.

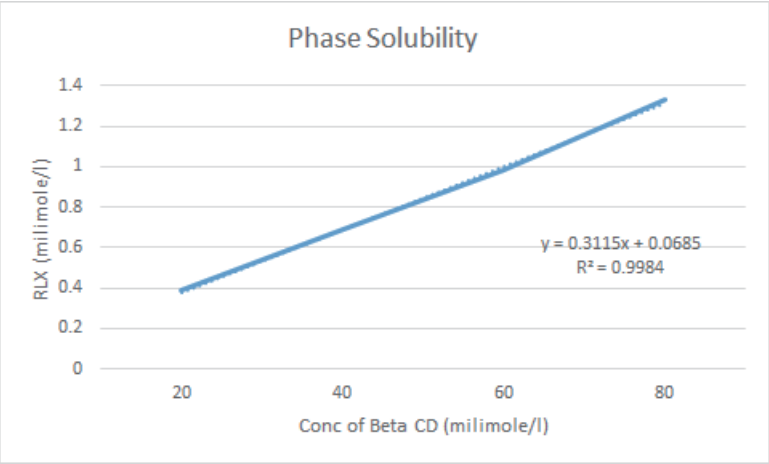
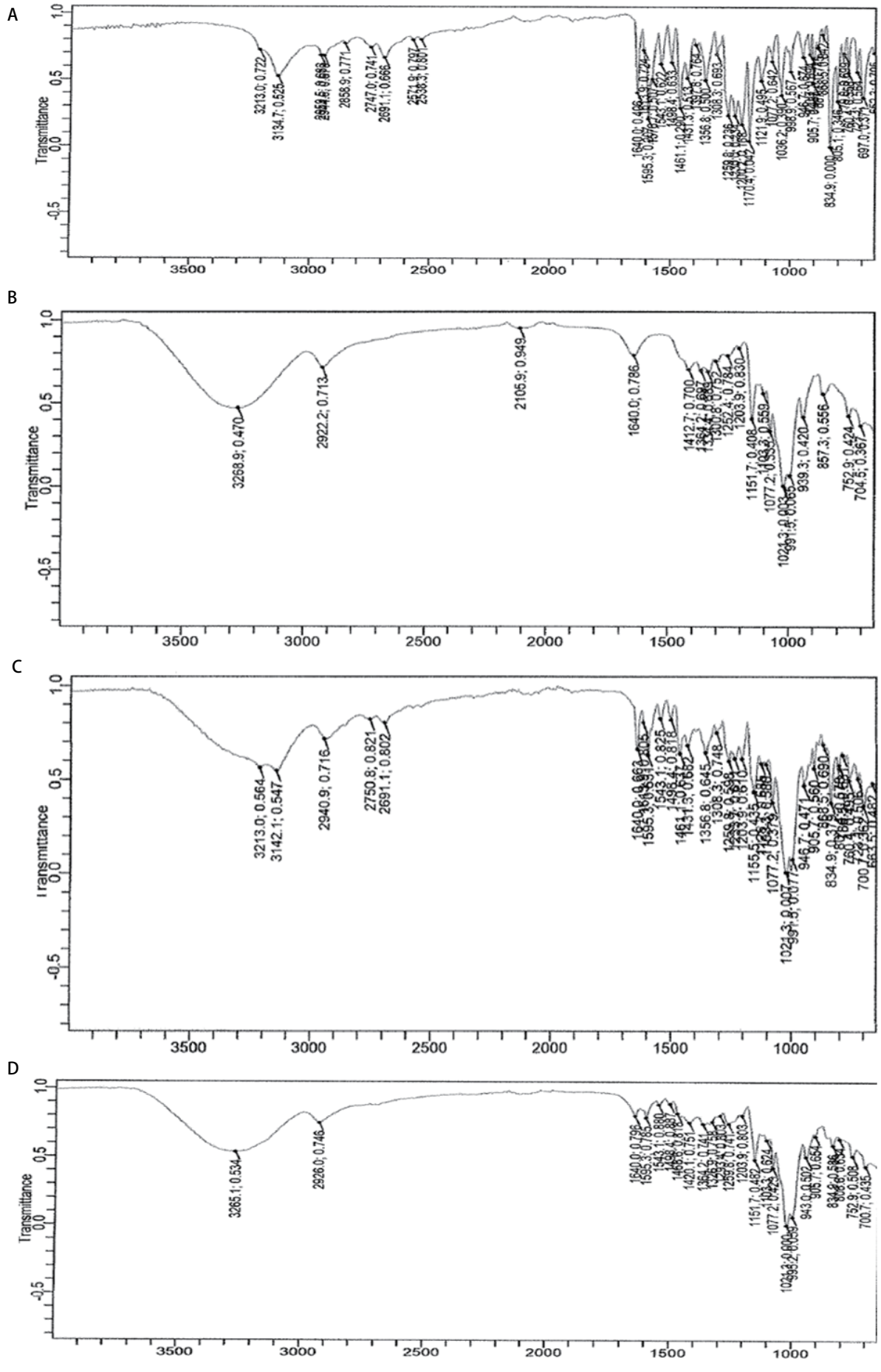


Figure 1. Phase Solubility of Raloxifene Hydrochloride


A
Transmittance
3213.0; 0.722
3134.7; 0.525
2858.9; 0.771
2747.0; 0.741
2691.1; 0.666
1640.0; 0.786
1461.1; 0.633
1356.8; 0.693
1121.9; 0.495
1036.2; 0.642
905.7; 0.567
834.9; 0.000
B
Transmittance
3268.9; 0.470
2922.2; 0.713
2105.9; 0.949
1640.0; 0.786
1412.7; 0.700
1252.4; 0.784
1203.9; 0.830
1151.7; 0.408
939.3; 0.420
857.3; 0.556
752.9; 0.424
704.5; 0.367
C
Transmittance
3213.0; 0.564
3142.1; 0.547
2940.9; 0.716
2750.8; 0.821
2691.1; 0.802
1640.0; 0.662
1543.1; 0.825
1496.4; 0.818
1461.1; 0.662
1356.8; 0.645
1308.3; 0.748
1203.9; 0.810
1155.5; 0.435
1077.2; 0.379
946.7; 0.471
905.7; 0.560
834.9; 0.398
700.7; 0.506
D
Transmittance
3265.1; 0.534
2926.0; 0.746
1640.0; 0.796
1543.1; 0.880
1496.4; 0.881
1420.1; 0.751
1203.9; 0.803
1151.7; 0.462
1077.2; 0.624
943.0; 0.502
905.7; 0.654
752.9; 0.508
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3500
3000
2500
2000
1500
1000

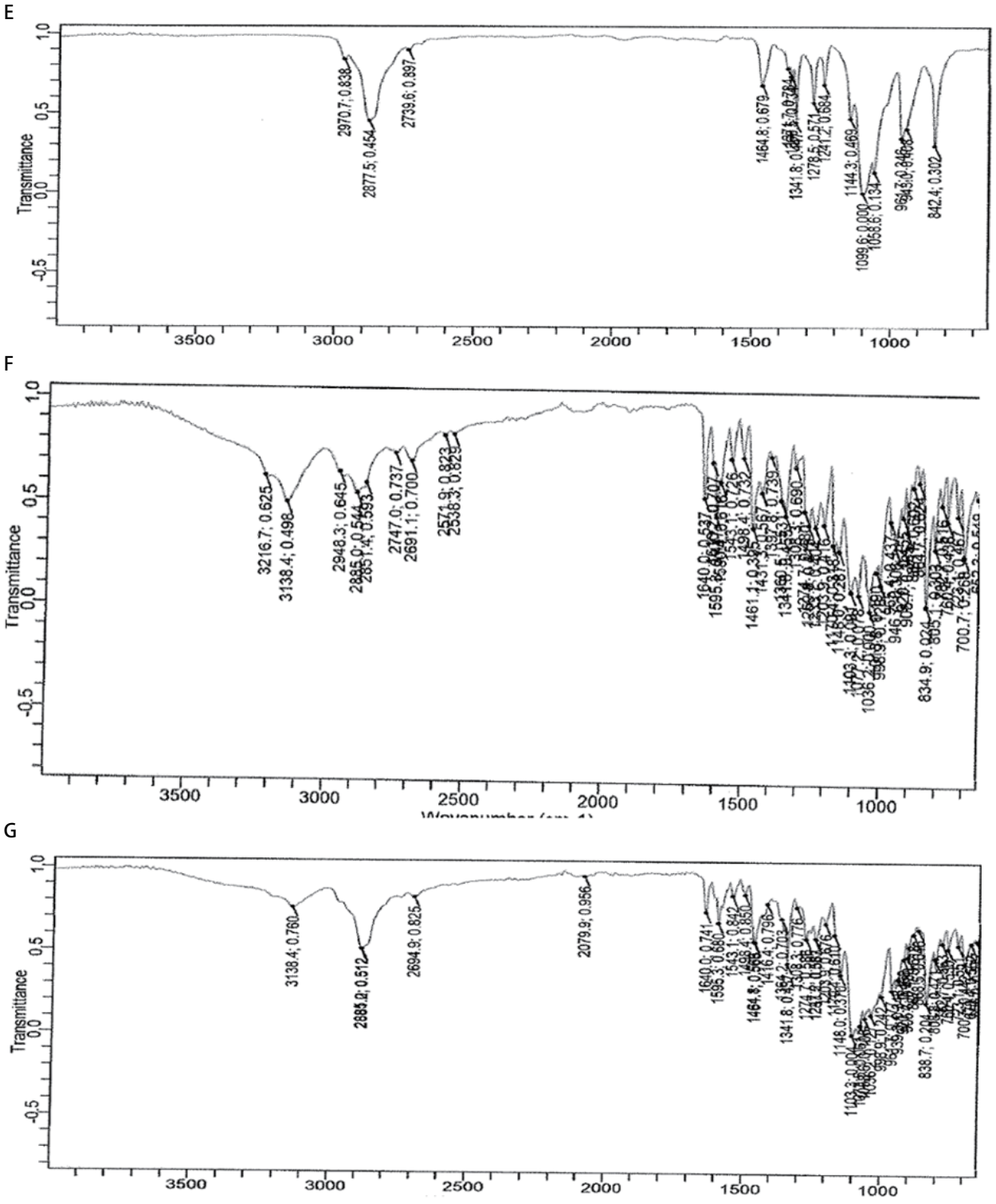
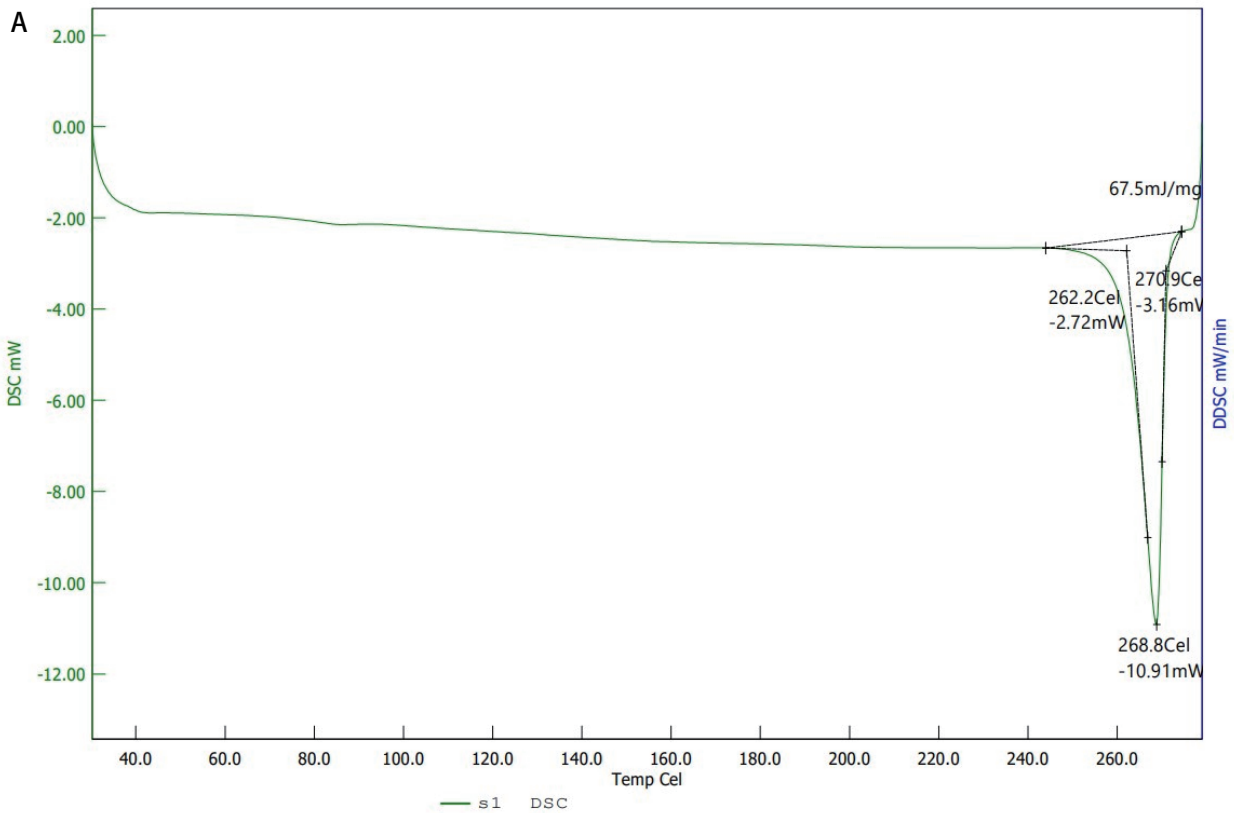


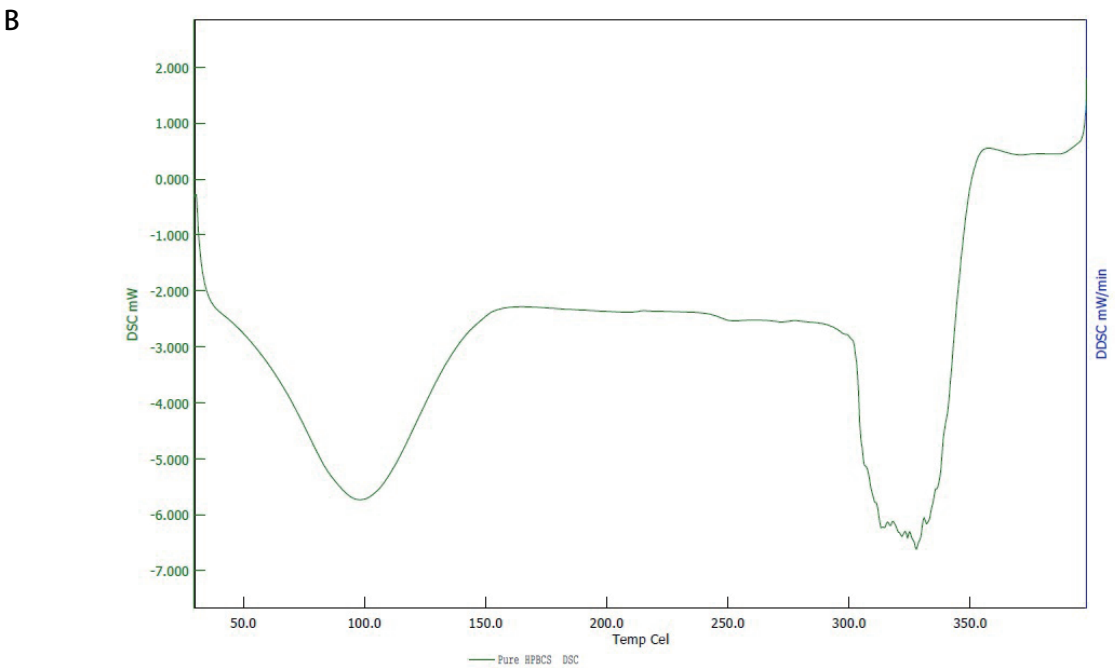
Figure 2. FTIR of (A) Raloxifene HCL, (B) βcyclodextrin, (C) Physical mixture (RLX+ βcyclodextrin) (D) Optimized inclusion complex (IC-6) (E)Poloxamer 407 ,(F) Physical mixture (RLX+ Poloxamer 407) and (G) Solid dispersion (RLX+ Poloxamer 407)

3.4 Differential scanning calorimetry

A more thorough examination of the composite's heat behavior and crystallization is made possible by DSC measurements. Figure 3. 3 displays the DSC thermograms of RLX, β-CD, poloxamer 407, physical mixing, and mixing of RLX and β-CD.

The thermograms of RLX and β-CD have endothermic peaks at 272.92 °C and 268.8. ° C correspond to melting points, respectively. The thermogram of the optimized inclusion complex showed a hydrated β-CD peak at 96.21 °C, while the broad peak of the solution was observed at 258 °C; this indicates the transformation of RLX from crystalline to amorphous form or dissolution. transferring the drug to a molten carrier.





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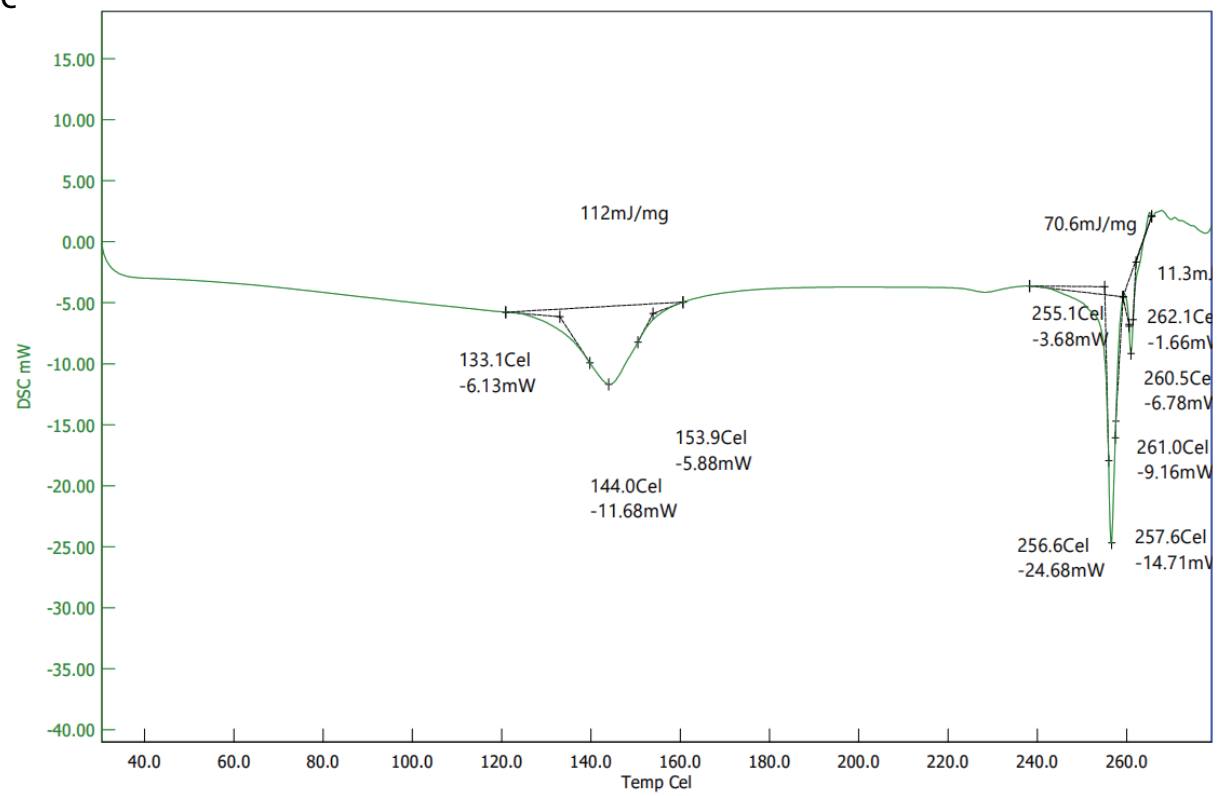
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-40.00
DSC mW
112mJ/mg
70.6mJ/mg
133.1Cel
-6.13mW
153.9Cel
-5.88mW
144.0Cel
-11.68mW
255.1Cel
-3.68mW
262.1Ce
-1.66mW
260.5Ce
-6.78mW
261.0Cel
-9.16mW
256.6Cel
-24.68mW
257.6Cel
-14.71mW
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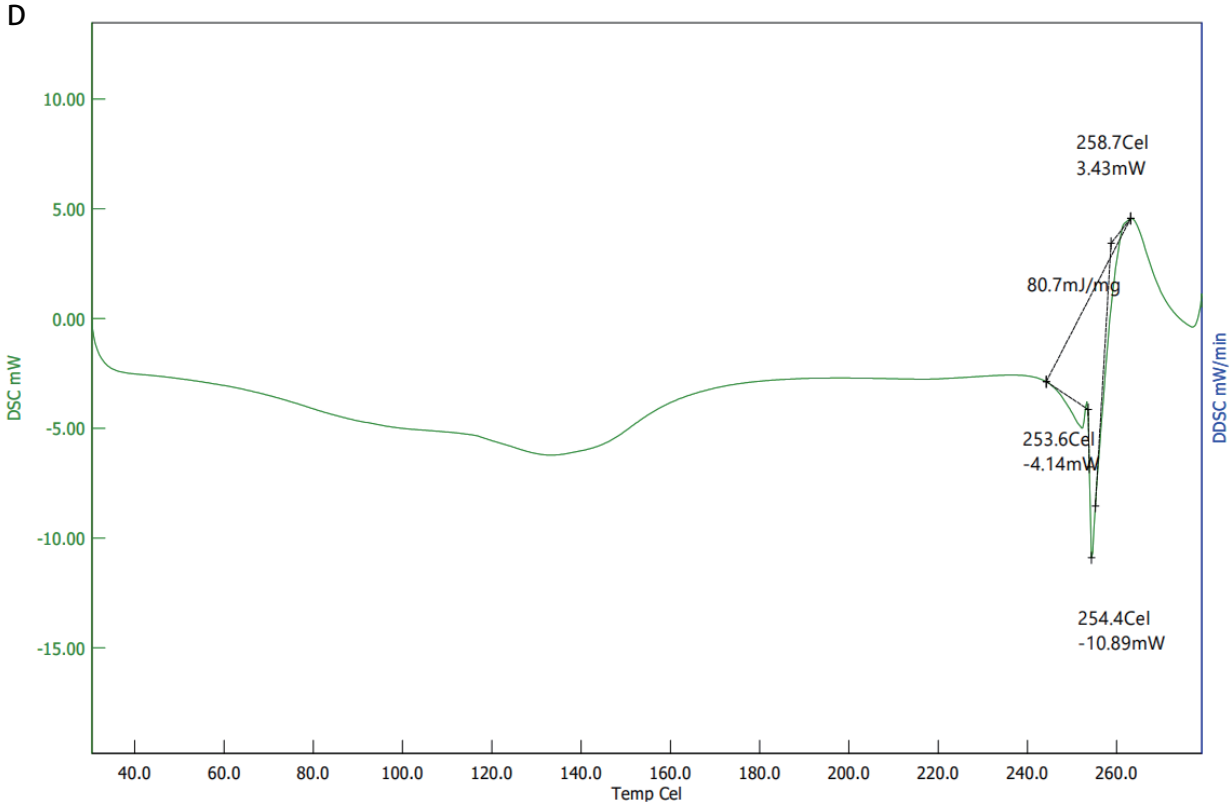
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DSC mW
DDSC mW/min
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253.6Cel
-4.14mW
254.4Cel
-10.89mW
40.0
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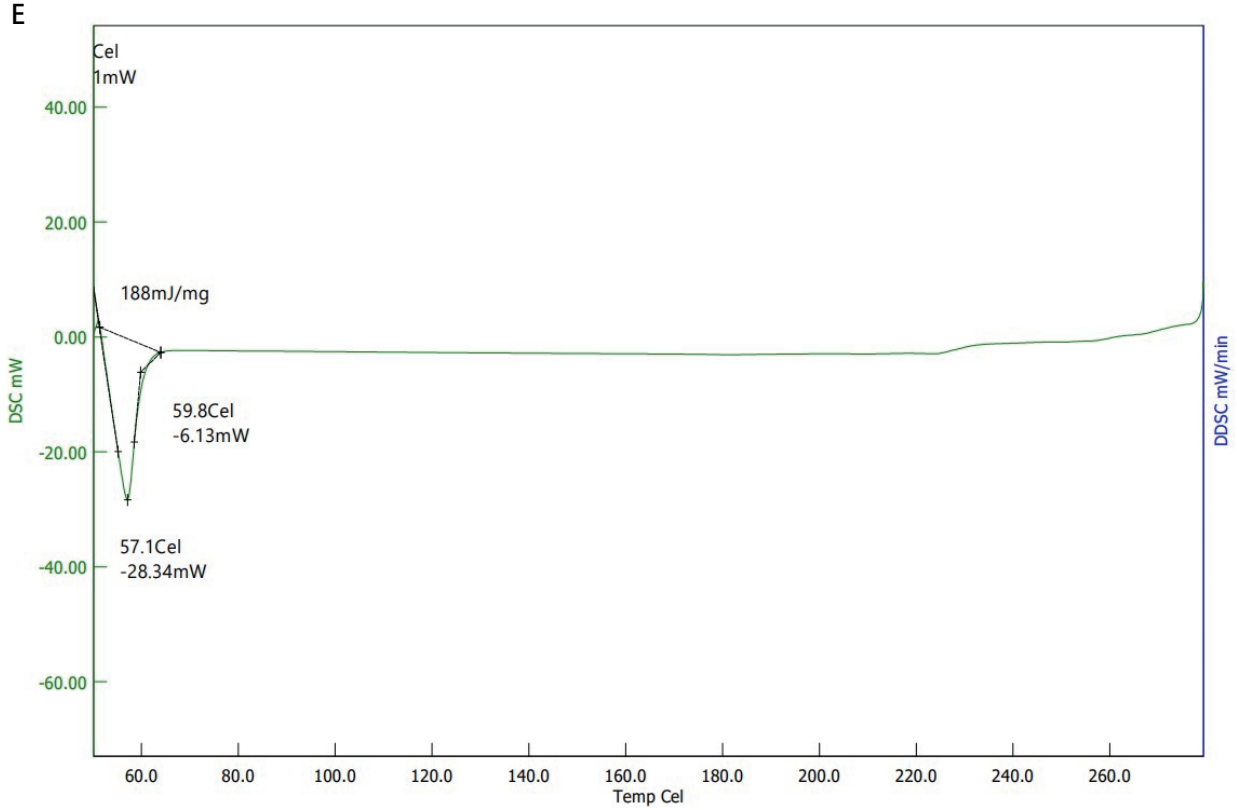
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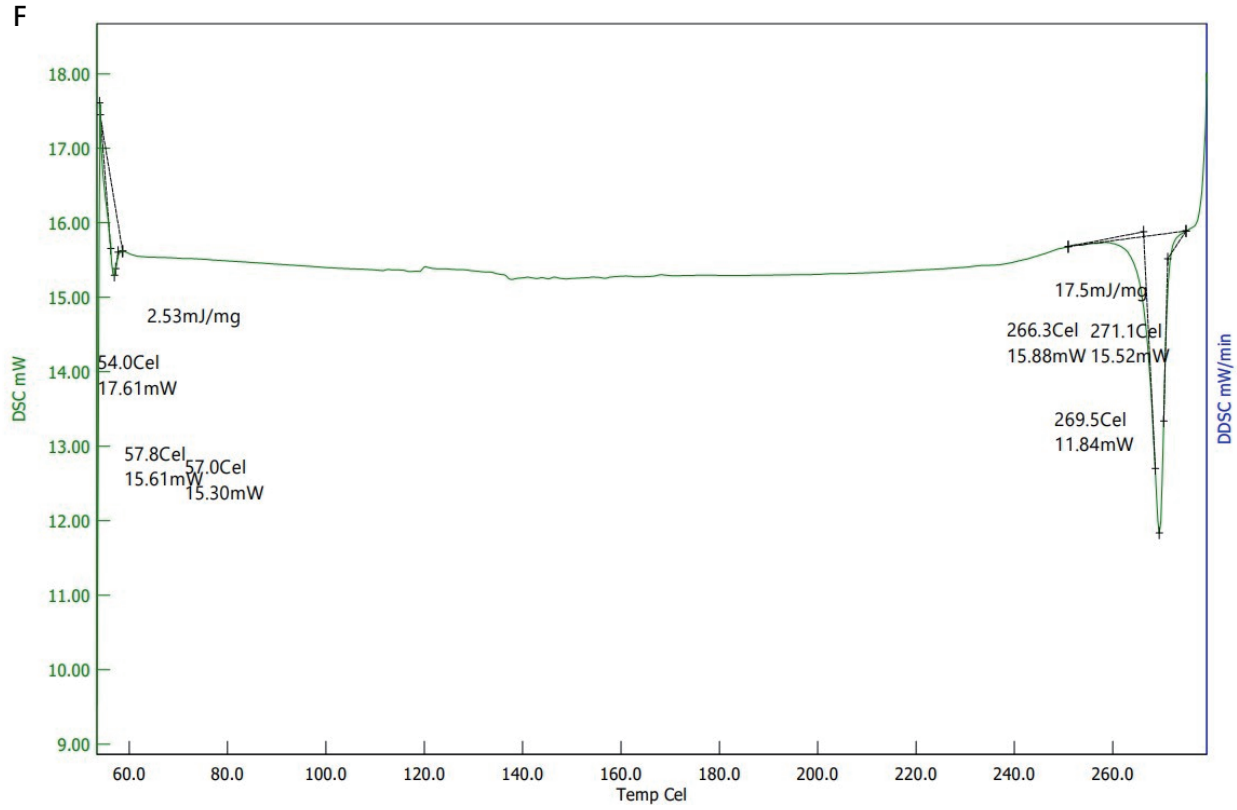
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15.30mW
17.5mJ/mg
266.3Cel 271.1Cel
15.88mW 15.52mW
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DSC mW
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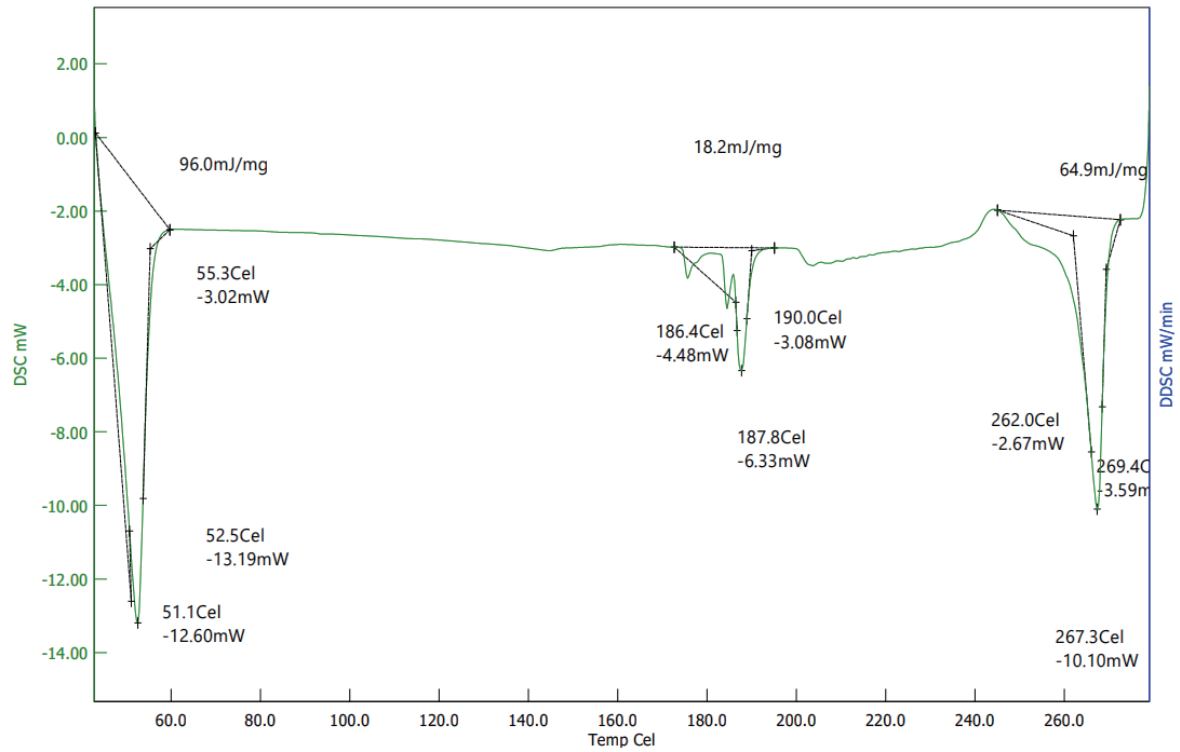

Figure 3. DSC Thermogram of (A) Raloxifene HCL, (B) βcyclodextrin, (C) Physical mixture (RLX+ βcyclodextrin) (D) Optimized inclusion complex (IC-6) (E)Poloxamer 407 ,(F) Physical mixture (RLX+ Poloxamer 407) and (G) Solid dispersion (RLX+ Poloxamer 407)

3.5 X-Ray diffraction studies

X-ray diffraction patterns of pure RLX and RLX-ð ½ CD inclusion complexes were recorded, as shown in Figure 3. DSC and XRD studies were performed to determine the decrease in crystallinity of RLX. The diffraction patterns of the chemical samples have clear peaks at 2° angle at 12.711°, 14.387°, 15.690°, 19.078°, 21.139° and 22.610°. All these peaks were found to be present in the RLX-ð½CD complex, although their intensities were small. It was concluded that the substance transformed from crystal form to amorphous form.

4. CONCLUSIONS

FTIR, DSC, and XRD results showed that the water solubility of the optimized (IC-6) complex (1:3 molar ratio) increased by more than 9-fold compared to RLX, including complexes and fragments. Further studies are needed to use this complex to prepare immediate-release vaginal formulations of raloxifene hydrochloride.

Acknowledgement

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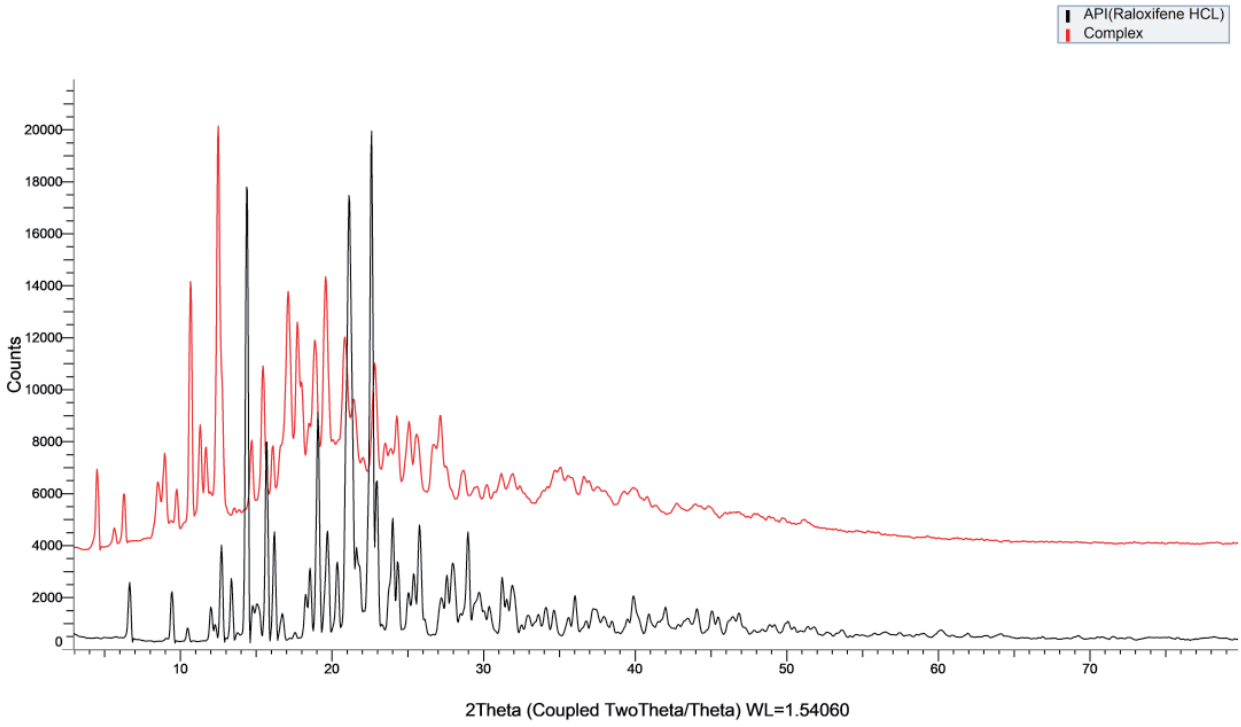

Figure 4. XRD of Overlay of Raloxifene and Complex

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