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PREPARATION OF BINARY INCLUSION COMPLEX OF RIVAROXABAN WITH BETA CYCLODEXTRIN; PHYSIOCHEMICAL AND SOLID-STATE CHARACTERIZATION

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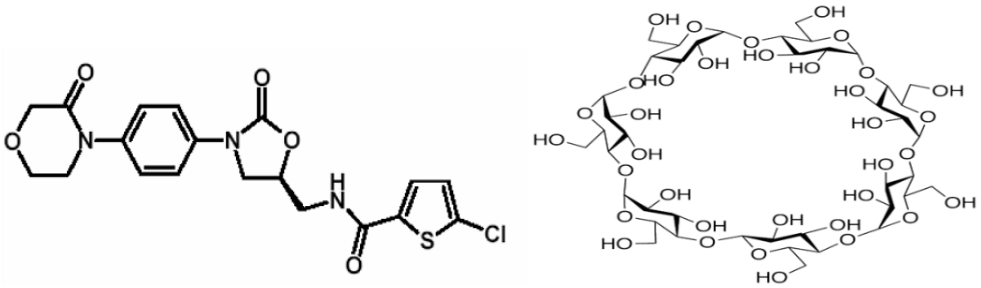
Abstract:
Low aqueous solubility and erratic bioavailability pattern is the limiting factor to achieve desired therapeutic outcomes from a variety of new and existing drugs. This study was aimed to evaluate the effect of the preparation method on the binary inclusion complex of rivaroxaban (RIV). The inclusion complexes were prepared using four different methods (physical trituration, freeze drying kneading and solvent evaporation) at RIV-cyclodextrins weight molar ratios of 1:1, 1:2 and 1:4. Solubility studies in the presence of βCD was performed to determine the quantitative increase in solubility. Dissolution studies were performed to evaluate the drug release behavior from the binary inclusion complex in the aqueous medium. The inclusion complexes were subjected to characterization of fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), X-ray diffractometry (XRD) and differential scanning calorimetry (DSC). The phase solubility studies confirmed that RIV solubility ameliorated proportionally with an increase in β-CD concentration. FTIR, SEM, XRD and DSC confirmed the successful inclusion of RIV into cyclodextrin (β-CD) cavity. Among the four preparation methods (physical trituration, freeze drying kneading and solvent evaporation) used for the inclusion complexes, kneading is the most suitable method for preparation of RIV: βCD inclusion complex with ameliorated solubility and drug release in aqueous medium.

Keywords: rivaroxaban**,** β-cyclodextrin, kneading method, dissolution, solubility

1. Introduction

Solubility is the most crucial physicochemical parameter affecting bioavailability. Solubility issues result in erratic bioavailability patterns, variable absorption, toxicity of gastric lining and problems related to product development. It has been estimated that about forty percent drugs available in the market suffer from solubility issues [1]. Low solubility is the up hilled task for the researchers and scientists. Different solubility enhancement techniques like solid dispersion, solvent deposition, micronization etc. have been employed for rectification of solubility issue in pharma industry [2]. All methods have its own pros and cons. Amongst these, Inclusion complexation is a classical way to ameliorate the solubility of a drug with natural cyclodextrins [3].

Cyclodextrins (CDs) are formed in the result of bacterial degradation of cellulose. These are amphiphilic product (cyclic oligosaccharides) which consist of (α-1,4) linked α-D-glucopyranose units with lipophilic central cavity and a hydrophilic outer surface area [4]. Cyclodextrin inclusion complexation may be a potential approach to ameliorate the saturated solubility ,dissolution profile and ultimately the bioavailability of the BCS class II drugs; and class IV drugs in some cases [5].
Cyclodextrins are a group of oligosaccharides Figure 1 (B), tend to capture the hydrophobic moiety into their cavity and develop host-guest relationship. Their outer surface is hydrophilic due to the presence of hydroxyl groups while the inner cavity is hydrophobic due to the restricted number of water molecules. Their solubilization efficiency is also enhanced in the presence of different hydrophilic polymers [6].
RIV being oxazolidinone derivative Figure 1(A)is used for the prevention and treatment of thromboembolic disorders. RIV belongs to Class II according to the Biopharmaceutics Classification System (BCS) and is notorious for its low solubility. Its low solubility hampers its absorption and bioavailability of drugs in GIT. Many solubility enhancement techniques like solid dispersion, nano formulation and size reduction have been investigated to ameliorate the solubility of the active compound. .Lee et al. (2021) reported novel hot-melt extruded solid dispersion technique to improve the solubility and dissolution of RIV [7]. Sherje et al. (2018) prepared nanocomposites of RIV with β-CD to enhance the solubility of RIV [8].
In this work, binary inclusion complexes of RIV and β-CD were formulated by physical trituration, kneading, solvent evaporation and freeze-drying method. To our best of knowledge, it was the first time that binary inclusion complexes of RIV were prepared with freeze-drying and solvent evaporation methods. Polymer ratio is also a important aspect of inclusion complexation that can influence the solubility and dissolution of RIV. Therefore, the solubility, crystallinity, thermal behaviour, and release of the inclusion complexation were examined in accordance with drug to polymer weight ratio of 1:1,1:2 and 1:4.
Hence, the purpose of the study was to prepare binary inclusion complexes by different methods and to find out the suitable method for the solubility and dissolution enhancement of a water insoluble drug such as RIV. Prepared inclusion complexes were evaluated using Fourier transform infrared (FTIR), scanning electron microscopy (SEM), X-ray powder diffraction (XRD) and differential scanning calorimetry (DSC).



(A) **(B)**

Figure 1. Chemical Structure of (A) RIV and (B) βCD

2. Materials and Methods

2.1. Materials

RIV was received as a gift sample from Consolidated Chemical Laboratories (PVT) LTD, Pakistan. β-CD was also received as gift from Roquette (Lestrem, France). All chemicals were of analytical grade.

2.2. Phase Solubility

Compatibility of host and guest relationship was determined by Higuchi-Connors phase solubility method [9]. Excess amount of drug was added in flask containing different molar ratios of βCD mixed

in distilled water. Flasks were placed in water bath (TSSWB15-USA) at 37 ± 2°C with speed of 100 rpm for 72 hours. Centrifugation of the saturated solutions were done at 6000 rpm for 30 min. Supernatant were filtered with 0.45 um syringe filter and suitable dilutions were made to estimate the phase solubility in UV Spectrophotometer (CECIL 7400-S, Cambridge, UK**)** in triplicate manner at 248 nm. Stability constants (K_c) and complexation efficiency (CE) were calculated from the phase solubility diagram.

$$\mathbf{Ks} = \frac{\mathbf{slope}}{\mathbf{So(1-slope)}} \quad \mathbf{(1.1)}$$

Where S_o is the solubility of RIV in pure water; slope is calculated from the plot of RIV concentration against βCD concentrations.

$$\boldsymbol{C.E} = \frac{\boldsymbol{slope}}{\mathbf{1-}\boldsymbol{slope}} \quad \mathbf{(1.2)}$$

Gibbs free Energy can also be used as indicator for complexation process, which can be calculated from following equation

$$\Delta \boldsymbol{G}^{\circ} = -\mathbf{2.303}\boldsymbol{RTlog}(\mathbf{So}/\boldsymbol{Ss}) \quad \mathbf{(1.3)}$$

Whereas, So/Ss is the ratio of the molar solubility of RIV in aqueous solution of cyclodextrin to that of the pure water.

2.3. Preparation of Binary Inclusion Complexes

Binary complexes of RIV with β-CD were prepared in various weight molar ratios of 1:1,1:2, and 1:4. In physical mixing, accurately weighed amounts of RIV and β-CD were added in mortar and pestle to form the consistent mixture. The sample was sieved through sieve number 60 and stored in airtight container [10, 11].

In the freeze-drying method, RIV was dissolved in ethanol and β-CD was dissolved in distilled water and stirred at 50 °C. Then the solution was placed in the freezer at −50 °C and at a reduced pressure. The lyophilized sample sieved and stored [12].

In Kneading method RIV and β-CD were kneaded in mortar for 45 minutes, with equal amounts of water: ethanol (1:1) and dried at room temperature for 24 hrs. Before storing, the kneaded mixture was sieved through sieve number 60 [10].

In the solvent evaporation method, the aqueous phase containing β-CD and organic phase having RIV dissolved in ethanol were thoroughly mixed at a magnetic stirrer. Electric oven was used to evaporate the solvent at 50 °C for 24 hr. Sieving was done prior to its storage [7].

2.4. Solubility Studies

Classical shake flask method was used to carry out the saturated solubility estimation of binary inclusion complexes of drug [13]. Briefly measured amount of inclusion complexes were added in vials containing 5mL of distilled water, after vortex for sufficient time they were placed in a shaking water bath (TSSWB15-USA) for 72 hours. After centrifugation at 6000 rpm for 30 minutes, membrane filters were used to filter the supernatant. Absorbance of the samples was determined in UV spectrophotometer (CECIL 7400-S, Cambridge, UK), after proper dilutions at 248 nm in triplicate manner.

2.5. In Vitro Dissolution studies

Dissolution is a critical factor in preformulation studies, which affects the bioavailability of the moiety. *In Vitro* dissolution studies of pure drug and inclusion complexes were carried out in USP dissolution apparatus type II “PTWS 3CE, Hainburg, Germany” by classical paddle method [14]. Accurately measured quantities of drug and complexes were added in baskets of apparatus containing

900 mL distilled water as dissolution medium at 37 ± 2 °C, speed of 75 rpm for 90 minutes. At prefixed time intervals of 5, 10, 20, 30, 45, 60 and 90 min, a sample of 5 mL was withdrawn and dissolution media quantity was maintained by adding the equal number of aqueous media in it. Absorbance of samples were measured in triplicate manner after filtration.

Dissolution efficiency at 60 min (DE_{60} min), defined as "the area under the dissolution curve up to 60 min, expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time" [15]. It can be calculated by following formula

$$\boldsymbol{DE}(\%) = \frac{\int_0^t \boldsymbol{y\ X\ dt}}{\boldsymbol{y\ 100\ X\ t}}\ \boldsymbol{X\ 100}\% \quad \textbf{(2)}$$

In the above-mentioned equation y is used to denote the % age of dissolve quantity of RIV. DE is used to calculate area of dissolution curve between time points t_1 and t_2 expressed as %age of the curve at maximum dissolution, y_{100}, over the same time period. It was used to compare the performance of variety of carriers in inclusion complexations.

2.6. Fourier Transform Infrared Spectroscopy (FTIR

In FTIR (BRUKER Tensor II-Alpha, Berlin, Germany), samples of pure drug and inclusion complex were scanned over 400 to 4000 cm^{-1} spectral. Potassium bromide disk method adopted to obtain FTIR spectrum of binary inclusion complexes and RIV.

2.7. Scanning Electron Microscopy (SEM)

Surface morphology of pure drug and complexes were elucidated with the help of Scanning electron microscope (JSM-6480, Tokyo, Japan). Shape and particle size of RIV and inclusion complexes were determined with this technique**.**

2.8. X-ray Diffractometry (XRD)

X-ray Diffractometry was employed to analyze the physical state of all samples. The X-ray tube was supplied 30 mA current with a potential of 30kV. The scanning range was from 10°-50° in the range of 2θ, at 0.02°/s increment.

2.9. Differential Scanning Calorimetry (DSC)

Thermograms of pure drug and inclusion complexes were obtained with (Universal V4.2 E TA Instruments, Newcastle, USA). Sample of 5 mg was placed in aluminium pan and heated in the presence of nitrogen (20 ml/min) at a rate of 10 °C/min over a thermal range of (30-300 °C).

2.10. Statistical Analysis

SPSS (version 22) was used for statistical analysis of solubility enhancement data of RIV and binary inclusion complexes. (ANOVA) was employed to analyze the solubility and depicted in mean ± standard deviation (SD).

3. Results

3.1. Phase Solubility

Phase solubility study was performed to analyze the solubility patterns of RIV in aqueous solution of β-CD. β-CD and solubility of RIV had shown linear relationship. By increasing the concentration of β-CD had also enhanced the solubility of RIV as shown in Figure 2. It may be categorized as an A_L type solubility diagram *[16]*. This could be attributed to the solubilization efficiency and cavity dimensions of the β-CD *[17]*.

The higher values of stability constant were also instrumental in determining the existence of strong interaction between RIV and inclusion derivatives *[18]*. In literature, reported values for stability are in range of 200 to 5000 M^{-1} *[19]*. The stability value for binary inclusion complex RIV: β-CD was 699.09 M^{-1} depicting the strong interaction between drug and β-CD. Complexation efficiency is also

a crucial parameter in evaluating the solubility of inclusion complexes. The complexation efficiency for binary inclusion complex was 0.078.

Gibbs free energy could also be determined from phase solubility diagram. The values of Gibbs free energy were critical to understand transfer process of RIV from pure water to aqueous solution of βCD. These values provide knowledge about whether the reaction condition is favorable or unfavorable for drug solubilization in the aqueous carrier solution. Negative Gibbs free energy of transfer values indicate favorable conditions. The values were all negative for βCD at various concentrations as sown in Table 2, thus hinting the spontaneous nature of RIV solubilization. These values decreased with increasing concentration of βCD, thereby demonstrating that the reaction became more favorable as the concentration of βCD increased [20].

Table 1: Gibbs free energy of the RIV: βCD complex in water at 25 ± 2 °C.

Concentration of βCD (mM/L)	DG^0 (kcal mol^{-1})
2	-0.454
4	- 0.734
6	- 0.924
8	- 1.068
10	- 1.168
12	- 1.279
15	- 1.405

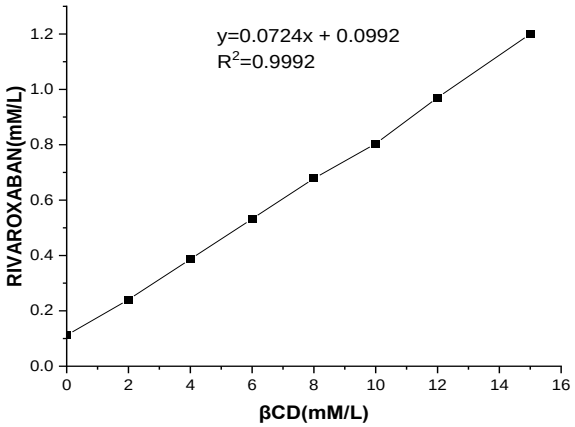


Figure 2. Phase solubility diagram of RIV in aqueous solution of βCD.

3.2. Solubility Study

RIV is practically insoluble in aqueous media having solubility of 5.11 µg/mL in pure water. Solubility of RIV was enhanced by binary inclusion complexes prepared by different methods as shown in Table 2. RIV solubility was significantly increased ($P < 0.05$) with the addition of βCD, in all preparation methods, but to a different extent. Kneading method produced the highest solubility of RIV in βCD, followed by solvent evaporation, freeze drying and lastly physical trituration. Sherje et al. (2018) also prepared the binary complex of RIV by different methods and concluded that method of preparation had a pronounced effect on the solubility enhancement of RIV [19]. βCD concentration was important for each preparation method to solubilise RIV.

For physical trituration, there was noticeable difference in solubility between pure drug and RIV: βCD. RIV: βCD at molar ratio of 1:1 and 1:2 enhanced solubility up to 5.21 and 5.88 times respectively when compared with pure drug. Further increase in cyclodextrin concentration from 1:2 to 1:4 did not enhance further RIV solubilization.

In freeze drying, saturated solubility of RIV/βCD at molar ratio of 1:2 was 38.12 ug/mL, which was superior than physical trituration method (30.05 ug/mL) at the similar ratio. Hence, freeze drying method was more productive than physical trituration in this study.
For solvent evaporation method, the solubility of RIV/βCD at molar ratio of 1:2 was 42.21 ug/mL, which was better than prepared with physical trituration and freeze drying method at the similar ratio. Hence, solvent evaporation method was more productive than physical trituration and freeze drying in this study.
Kneading method was the most effective among the four preparation methods, depicted maximum RIV solubility at similar molar ratios for cyclodextrin. A statistically significant ($p < 0.05$) enhancement in solubility of RIV was seen from 5.11 µg/mL to 46.20 µg/mL for binary derivatives prepared by kneading method at ratio of 1:2. However, no statistically significant increase was observed in RIV solubility with further increase in βCD amount from 1:2 to 1:4 ($P > 0.05$). On the basis of above data, binary complex prepared by kneading and solvent evaporation method were optimized for further characterization of fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), X-ray diffractometry (XRD) and differential scanning calorimetry (DSC) to evaluate the complexation and amorphousness of the RIV.

Table 2. Solubility study of RIV and binary inclusion complexes. Mean ± SD, n = 3.

Methods of Preparation	RIV: βCD (w/w)	Solubility µg/mL
	1:0	5.11 ± 0.12
Physical Mixture	1:1	26.64 ± 0.05
	1:2	30.05 ± 0.32
	1:4	27.23 ± 0.09
Freeze Drying	1:1	33.52 ± 0.31
	1:2	38.12 ± 0.4
	1:4	35.31 ± 0.07
Kneading Method	1:1	40.76 ± 0.03
	1:2	46.20 ± 0.1
	1:4	42.67 ± 0.07
Solvent Evaporation	1:1	38.87 ± 0.61
	1:2	42.21 ± 0.41
	1:4	40.41 ± 0.15

3.3. In Vitro Dissolution Studies

Cumulative dissolution release profile of pure RIV, physical mixture of the drug, binary inclusion complexes prepared by different methods are shown in Figure 3A–D. RIV exhibited only 31.43 % dissolution release after 60 minutes study and physical mixture of drug exhibited slightly improvement and released 38.43 % drug after same interval. This could be attributed to better wettability of molecules due to the companionship of cyclodextrin, which can bring down the interfacial tension between RIV and aqueous dissolution medium [9]. After one hour study binary complexes prepared by kneading method and solvent evaporation method having 1:2 molar ratio depicted 54.25 % and 53.12 % drug release respectively. This can be credited to entrapment of pure drug molecules into the cavity of cyclodextrin. Ameliorated dissolution profiles as compared to parent drug are the characteristics of inclusion complexes [21].
Dissolution efficiency results were manifested in Table. Statistically significant difference ($p < 0.05$) was noticed when analyzing the DE values of inclusion mixtures with the RIV. Keeping in view the above data, it can be concluded that binary complexes of RIV prepared by the kneading and followed by solvent evaporation at ratio of 1:2 had produced the highest solubility and dissolution release of RIV than other preparation methods. This claim may be supported with DSC and XRD analysis.

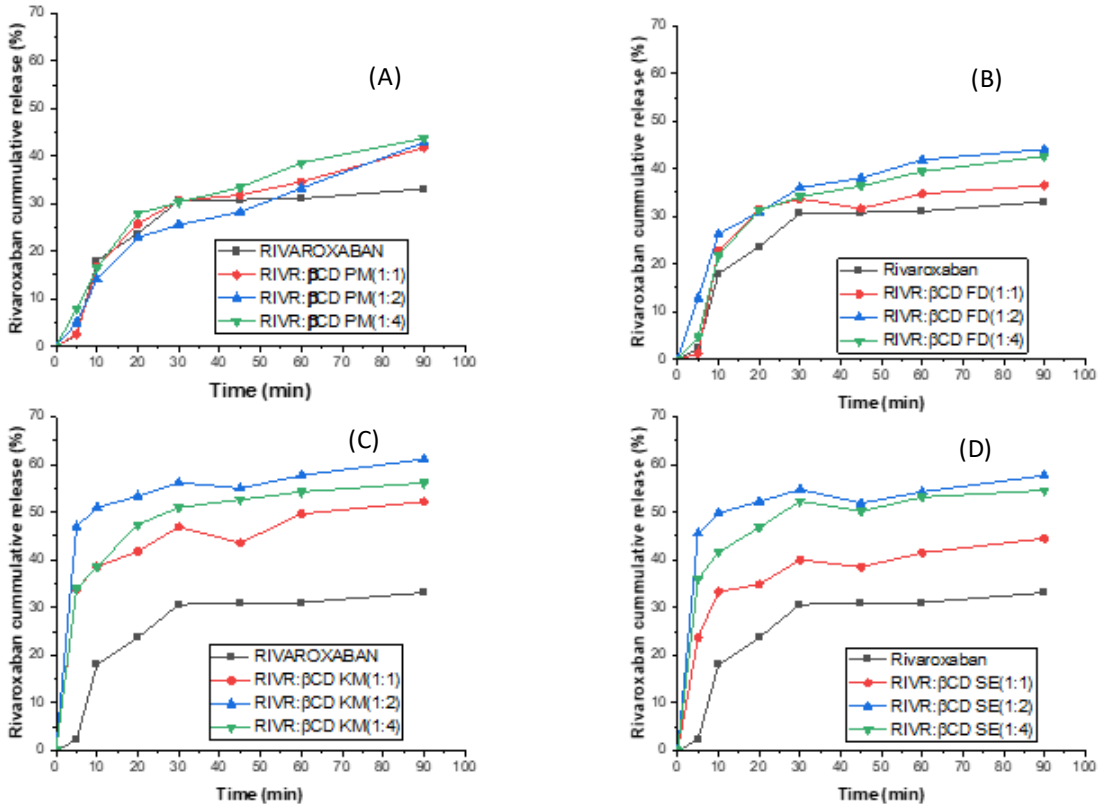


Figure 3. (A) In Vitro Dissolution study of RIV and RIV–βCD inclusion complexes by PT method Mean ± SD, n = 3. **(B)** In Vitro Dissolution study of RIV and RIV-βCD inclusion complexes by FD method Mean ± SD, n = 3. **(C)** In Vitro Dissolution study of RIV and RIV-βCD inclusion complexes KM method. Mean ± SD, n = 3. **(D)** In Vitro Dissolution study of RIV and RIV-βCD inclusion complexes SE method Mean ± SD, n = 3.

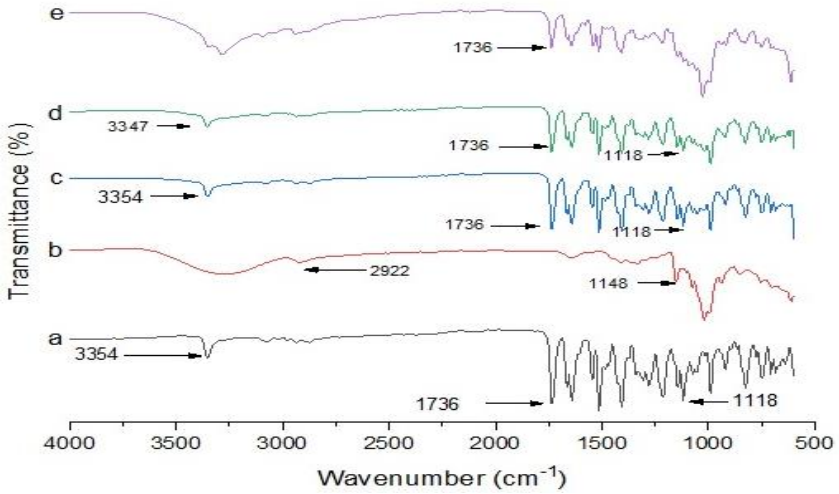
Table 3. Dissolution parameter of RIV and binary systems. Mean ± SD, n = 3.

RIV: βCD	DE_{60} (%)
RIV	19.03 ± 0.19
RIV: βCD 1:1(PT)	20.79 ± 0.13
RIV: βCD 1:2(PT)	22.55 ± 0.03
RIV: βCD 1:4(PT)	21.14 ± 0.22
RIVβCD 1:1(FD)	23.04 ± 0.09
RIV: βCD 1:2(FD)	27.47 ± 0.16
RIV: βCD 1:4(FD)	24.63 ± 0.08
RIV: βCD 1:1(KM)	38.24 ± 0.11
RIV: βCD 1:2(KM)	43.77 ± 0.15
RIV: βCD 1:4(KM)	41.91 ± 0.13
RIV: βCD 1:1(SE)	31.85 ± 0.14
RIV: βCD 1:2(SE)	42.09 ± 0.09
RIV: βCD 1:4(SE)	39.84 ± 0.18

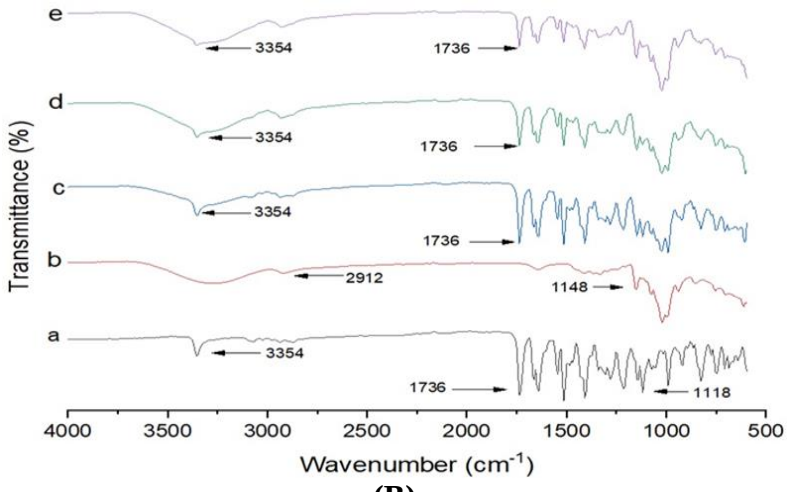
3.4. Fourier transform infrared spectroscopy (FTIR)

The interaction of drugs with cyclodextrin can be evaluated with the help of spectroscopic studies. The FTIR spectrum of pure RIV depicted characteristic peaks of C-O ether stretch at 1118 cm^{-1}, C=O ester bond at 1736 cm^{-1} and secondary amine C-N stretch at 3354 cm^{-1}. The spectrograph of βCD depicted characteristic peaks of vibrational stretching and bending of O-H group, aliphatic C-H group and C-C bonds at 3317 cm^{-1},2922 cm^{-1} and 1155 cm^{-1} respectively as shown in Figure 4.

Kneaded mixtures of binary complex βCD: RIV (1:1) showed reduction in the intensity of peak of RIV in Figure 4A(c), but in βCD: RIV (1:2) visible shift in wave number is detected from 3354 cm^{-1} to 3347 cm^{-1} which could be possible due to entrapment of drug molecule into the cavity of cyclodextrin. On the other hand, it completely disappeared in the binary mixture of βCD: RIV (1:4) as shown in Figure 4A(e), which might be credited to complete complexation. The peak of RIV in the region 3354 cm^{-1} was found in all the mixtures prepared by solvent evaporation method at the same wavelength, but the intensity reduced and no new peaks detected due to the dilution effect. However, C-O ether stretch at 1118 cm^{-1} in all the complexes prepared by solvent evaporation method got disappeared, this might be due to the possible formation of inclusion complex between RIV and cyclodextrin [22].



(A)



(B)

Figure 4. (A) FTIR Spectrum of (a) RIV, (b) βCD, (c) 1:1 RIV: βCD(KM), (d) 1:2 RIV: βCD (KM), (e) 1:4 RIV: βCD (K.M). **(B)** FTIR Spectrum of (a) RIV, (b) βCD, (c) 1:1 RIV: βCD (S.E), (d) 1:2 RIV: βCD (S.E), (e) 1:4 RIV: βCD (S.E).

3.5. Scanning Electron Microscopy (SEM)

SEM images of RIV and binary inclusion complexes are shown in Figure 5(a-h)**.** RIV consists of deposition of small and large particles on the surface as shown in Figure 5(a),which might be result of micronization or any other size reduction method used at the time of production [23]. βCD has a rough and irregular surface with rhomboidal crystals as shown in Figure 5(b). 1:2 RIV: βCD complex prepared by kneading method shown in Figure 5(d) had small particles dispersed on the surface

denoting the presence of RIV in the complex. On the other hand, 1:2 RIV: βCD binary complex synthesized with solvent evaporation method had denser and fused particles on the surface indicating less crystallinity showed in Figure 5(g). SEM analysis helps in assessing the probable nature of the complex and the presence of single component in the complex. It was also evident that in the SEM images it was impossible to identify crystals of both components and this might be due to the better interaction of RIV with cyclodextrin [24].It can be concluded that binary complexes prepared by solvent evaporation and kneading method revealed less crystallinity.

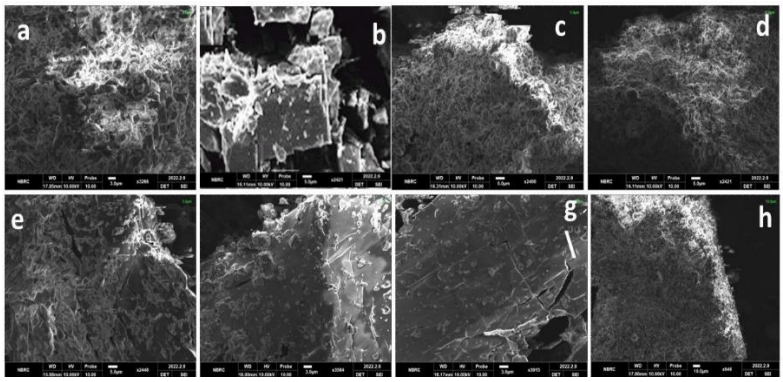


Figure 5.SEM of (a) RIVR, (b) βCD, (c) 1:1 RIVR: βCD(KM), (d) 1:2 RIVR: βCD (KM), (e) 1:4 RIVR: βCD (K.M), (f) 1:1 RIVR: βcd (S.E), (g) 1:2 RIVR: βCD (S.E), (h) 1:4 RIVR: βCD (S.E).

3.6. X-ray Diffractometry (XRD)

XRD of the pure RIV manifested its crystalline nature, indicated by distinctive peaks at diffraction angles of 2θ. RIV depicted characteristics peak at 2θ value of 22° in Figure 6 (a). The x-ray diffractogram of βCD has sharp peaks at diffraction angles (2θ) 14.1°,17.7° and 22.2° showing a typical crystalline pattern. The RIV-βCD inclusion complexes in Figure 6 depicted less crystallinity and diminished peak strength as compared to pure drug. The KN method could influence crystal structure in the inclusion powder. Decreased peak intensity with slight shift of RIV in 1:2 RIVR: βCD mixture can be due to dilution of drug with cyclodextrin. The less crystallinity resulted in ameliorated dissolution and solubility [25].

For solvent evaporation, 1:1 RIVR: βCD (S.E) depicted a reduction in the number of crystalline peaks while 1:2 RIVR: βCD (S.E) exhibited less crystalline structure with negligible crystalline peaks. This could be supported that βCD was able to alter the crystalline nature of RIV using solvent evaporation method.

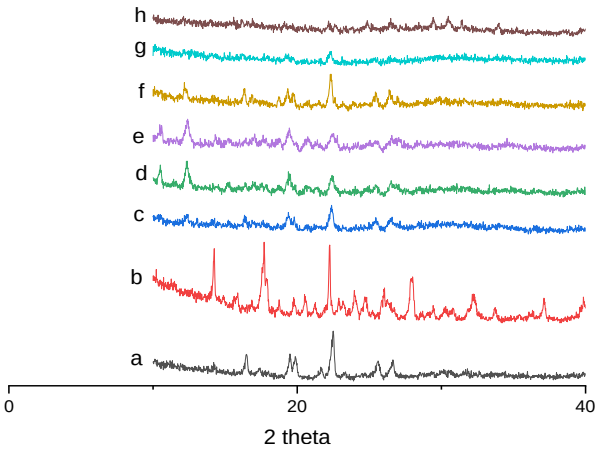


Figure 6. XRD Diffractograms of (a) RIV, (b) βCD, (c) 1:1 RIVR: βCD(KM), (d) 1:2 RIV: βCD (KM), (e) 1:4 RIV: βCD(KM), (f) 1:1 RIV: βCD (S.E), (g) 1:2 RIV: βCD (S.E), (h) 1:4 RIV: βCD (S.E).

3.7. Differential Scanning Calorimetry (DSC)

DSC is a thermo analytical technique employed to evaluate the phase transitions of free drug (RIV) and binary inclusion complexes prepared by different methods. This study reveals about the physicochemical characteristics of the guest -host relationship between drug and cyclodextrin. Absence or change in endothermic peak of pure drug may provide the information about changes in the crystal lattice and physical characteristics [26]. In DSC thermogram, RIV exhibited a sharp endothermic peak at 234 °C Figure 7(a) which conformed with its melting point [23] whereas βCD indicated broad endothermic peak at 138 °C Figure 7(b)which might be due to exsiccation. 1:1: RIV: βCD prepared by kneading method depicted reduced intensity of endothermic peaks as compared to RIV while 1:2:RIV:βCD complex depicted peak at 231°C and showed a significant drop in peak strength of RIV which might be credited to entrapment of RIV into the cavity of cyclodextrin [23]. However, 1:4: RIV: βCD binary complex prepared by solvent evaporation method delineated the perceptible shift in peak from 234 °C to 215 °C. Similarly, 1:2: RIV: βCD binary complex prepared by solvent evaporation method depicted reduced intensity of endothermic peak which might be anticipated as a result of complexation. DSC thermographs revealed that pure RIV was losing its fusion peak, which might be due to the drug molecules participation in complexation. Hence it can be concluded that binary complexes prepared by kneading and solvent evaporation methods have prominent shift and reduction in the intensity of endothermic peaks, which might be due to possible interaction of RIV with cyclodextrin and resulted in reduced crystallinity [27].

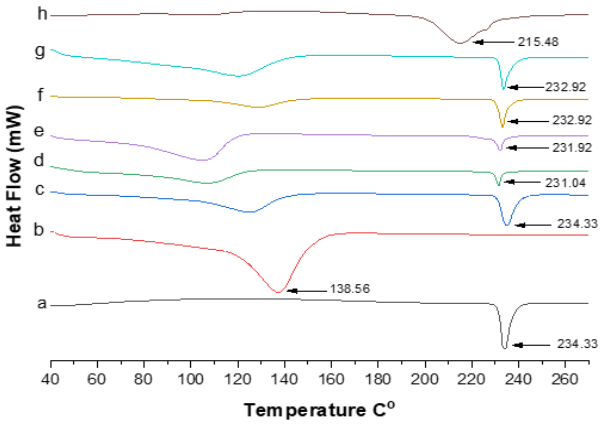


Figure 7. Thermograms of (a) RIV, (b) βCD, (c) 1:1 RIV: βCD(KM), (d) 1:2 RIV: βCD (KM), (e) 1:4 RIV: βCD (K.M), (f) 1:1 RIV: βCD (S.E), (g) 1:2 RIV: βCD (S.E), (h) 1:4 RIV: βCD (S.E).

3.*9. Comparison of different preparation methods*

When different four preparation methods, PM, FD, KM and S.E were compared, PM was the simplest preparation method. In simple PM, cyclodextrin could ameliorate the solubility and dissolution profile of of RIV about 5.81 times. Through the increment in solubility of pure drug with PM was less as compared to other methods as shown in Table 1. In the FD method, RIV was dissolved in ethanol and β-CD was dissolved in distilled water and sample was kept under vacuum to remove the solvents from the sample. When the FD was compared with other preparation methods, it was found that RIV solubilisation was higher with FD than physical PM but less than KM and S.E method. There was an increase of about 7.42 times in the solubility of RIV.This could be due to partial interaction of RIV with βCD.

In S.E two solvents were used, one organic solvent, ethanol to dissolve RIV and water to dissolve βCD. Both solvents were miscible when mixed together. A fine powdered form of the complex was obtained after drying. There was an increase of about 8.26 times with βCD in the solubility of RIV, greater than PM and FD but less than KM. There was a further reduction in the crystallinity of RIV with βCD, indicating the inclusion of pure drug in the cavity of βCD.

In KM, a wet paste was formed by adding water with βCD. The consistency of the paste increased after mixing with RIV.When the kneading method compared with other preparation methods, it was concluded that RIV solubilisation was maximum with KM. There was an increment of about 9.04 times with βCD in the solubility of RIV. KM has proven to be the best method among the four methods used in the present study to produce an inclusion complex with amorphous appearance.

4. Conclusions

The four preparation methods, physical trituration, freeze drying, kneading and solvent evaporation, could increase the solubility and dissolution rate of RIV via formation of inclusion complex with βCD. Kneading method was the most effective method in terms of RIV solubilisation. Advance characterization of FTIR, SEM, XRD and DSC results of prepared binary inclusion complexes has confirmed the successful inclusion complexation of RIV with cyclodextrin. In future, these inclusion complexes prepared by kneading and solvent evaporation method could be effectively used to ameliorate the solubility and dissolution release of other hydrophobic drugs.

Author Contributions.

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