Enhancement of Photostability of Riboflavin by Complexation Techniques

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Abstract
Objective: The present research aims towards studying the formulation and evaluation of Riboflavin-2-hydroxypropy-β-cyclodextrin complexes in increasing its stability and dissolution which is susceptible to degrade in the presence of light.
The present research focuses on the formulation and comprehensive evaluation of Riboflavin-2-hydroxypropyl-β-cyclodextrin (HPβCD) complexes to enhance the stability and dissolution profile of riboflavin, a water-soluble vitamin (Vitamin B2) that is highly susceptible to photodegradation. Riboflavin undergoes rapid degradation when exposed to light, leading to reduced bioavailability and therapeutic efficacy.
Materials and Methods: Riboflavin-2-hydroxypropy-β-cyclodextrin complexes in three ratios 1:1, 1:2 and 2:1 were prepared by kneading method and characterized by FT-IR, DSC and XRD. the pure drug and inclusion complex formulation were simultaneously subjected to accelerated photostability studies according to Q1B ICH 1996 guidelines. The samples were withdrawn in duplicate every day for seven days and were analyzed for the quantitative estimation of riboflavin by UV visible spectrophotometer method and the results were compiled.
Result and conclusion: After the seventh day the percentage drug degraded in the 1:1complexes at 25˚C, 40˚C and 4˚C was 54.15%, 37.03%, and 67.77% respectively. In the end of seventh day pure drug at 25˚C, 40˚C and 4˚C degraded to was 24.05%, 18.35%, and 29.14%. The photo degradation follows1st order kinetics.

***Keywords*:** Riboflavin, photo degradation, kinetics, photostability, cyclodextrin complexes.

Introduction:
In 1872, riboflavin was firstly observed in milk with yellow-green fluorescence by *Alexander Wynter Blyth* & in 1972 it was characterized as riboflavin (1). It is also called as vitaminB_2. Riboflavin was the second co-enzyme to be an isolated & first co-enzyme of vitamin B-complex. In 1939, the researchers came to know an interesting fact that the majority of vitamins are food constituents of humans. Most of the plants and many microorganisms synthesize vitamin B_2 but animals obtained it from nutrient diet like eggs, white cheese & fresh meat. The human body needs vitamin B_2 in small quantity & its deficiency can cause "Ariboflavinosis".(2)
Riboflavin contains a 6,7–dimethyl isoalloxazine ring attached to D-ribitol by a nitrogen atom. Ribitol is an open chain form of sugar ribose. When its aldehyde group reduces then it emits yellow color fluorescence. It has a slight & characteristic odor. Riboflavin appeared as yellow to orange-yellow crystals. It is slightly soluble in water (1gm in 3-5 liter solution) practically insoluble in ethanol, acetone ether & other organic solvents. Riboflavin administration reduces reperfusion injury of different tissues like heart, brain & lungs in the animal models but there is no supportive human study reported so far the area is not yet develop till date. Riboflavin plays an important role to explain the difference in antioxidant pattern of the body. If the deficiency of riboflavin it leads to change in reduction potential of the body mechanism from which its functions to control lipid peroxidation is completely different & glutathione redox cycle process is different. The mechanism for antioxidant action of riboflavin is the conversion of reduced riboflavin to its oxidized form which provides a reducing equivalent for deactivation of hydroperoxidase enzyme.(3)
Mylius, first time in 1886, observed the unusual complexations between several volatile compounds and hydroquinone. He also observed the entrapment of a molecule into another molecule without the presence of any chemical bond. These complexes were earlier known by some other names such as "occlusion compounds, adducts and clathrates" then Schlenk, a scientist, named the complexes as "inclusion compounds". The size of the guest molecule is an important parameter in this technique i.e to form a stable complex with desired physiochemical properties, the size of guest molecule should be equivalent to the host molecule so that it can easily entrap into the cavity of the host molecule (3).
Many researchers have proposed various classifications for the inclusion complexes like, on the basis of organization and structures in complexes, Frank classified the inclusion complexes into polymolecular, monomolecular and macromolecular complexes. Steed and Atwood proposed the compounds, on the basis of topological relationship between host and guest, named as cavitands and clathrands. Dyadin and Terekhova proposed various inclusion complexes like tubulatoclathrates, intercalatoclathrates and cryptoclathrates, on the basis of shape and structure of the

host's cavity (6). In Freudenberg et al. (1935) identified another compound known as γ-cyclodextrin (8). In 1976, a formulation composed of cyclodextrin and prostaglandin (Prostarmon-E™ sublingual tablets) was marketed by Japan for the first time. In1977, Piroxicamor β-CD (Brexin® tablets) was the first formulation which was marketed in Europe andit raconazole/ 2 - hydroxypropyl-β-CD oral solution (Sporanox®) was the first US-approved product (9). Since then cyclodextrins have been used for the improvement of pharmaceutical characteristics of the drugs like aqueous solubility and stability including environmental protection of drugs and prevention of its optical rotation, cyclization etc. Improving photostability is yet another sphere explored by many researchers who were successfully able to formulate cyclodextrin complexes with α-tocopherol (10), Rhein (11), flavonoids and geraldol (12), Nootkatone (13), 13-cis-Retinoic (14), isradipine (15) and could increase their shelf life by protecting them from deleterious effects of sunlight. Presently Aceclofenac-β Cyclodextrin, Ulgut/ Lonmiel, Betahist, Propulsid ,Pansporin-T, Zyrtec, Australian dream, Fluner, Ryndthisol, Stada-Travel, Clear eyes, , Pain relief gel, Mobitil, Transillium, Glymesason, Mena-Gargle, Cycladol/ Brexin/ Flamexin, Opalmon, Prostandin 500, Vitaseptol, Rofizgel, Flogene etc drug cyclodextrin complexes are approved for being marketed throughout the globe (16).

Methods and Materials:
Riboflavin and 2-Hydeoxypropyl-β-cyclodextrin was procured from C.D.H Pvt. Ltd. New Delhi and Molychem Mumbai respectively. All the chemicals used were of analytical grades.

Preparation of Inclusion Complexes:
Inclusion complexes of riboflavin were prepared by using Kneading method in three different ratios 1:1, 1:2, and 2:1. Drug and Polymer are incorporated in the molar ratio (17, 18).

Kneading Method
1:1 Inclusion complexes:
1.88 g riboflavin and 5.63 g HYP β-CD was weighed to make molar ratio of 1:1. First 2-Hydeoxypropyl-β-cyclodextrin was added to the mortar, small quantity of distilled water was also added while triturating to get slurry like consistency. Riboflavin was incorporated slowly into the slurry and triturated for 1 hour. The obtained paste was dried in hot air oven at 35°C for 24 hours, stored in desiccators over fused calcium chloride.(18)

1:2 Inclusion complexes:
1.88 g riboflavin and 11.26 g β-CD was weighed to make molar ratio of 1:1. First 2-Hydeoxypropyl-β- cyclodextrin was added to the mortar, small quantity of distilled water was also added while triturating to get slurry like consistency. Riboflavin was incorporated slowly into the slurry and triturated for 1 hour. The obtained paste was dried in hot air oven at 35°C for 24 hours, stored in desiccators over fused calcium chloride.(18)

2:1 Inclusion complexes
3.76 g riboflavin and 5.6.3 g β-CD was weighed to make molar ratio of 1:1. First 2-Hydroxypropyl-β- cyclodextrin was added to the mortar, small quantity of distilled water was also added while triturating to get slurry like consistency. Riboflavin was incorporated slowly into the slurry and triturated for 1 hour. The obtained paste was dried in hot air oven at 35°C for 24 hours, stored in desiccators over fused calcium chloride. (18-20)

Characterization of Riboflavin-β-Cyclodextrin Inclusion Complexes
Riboflavin, β-cyclodextrin and the powder obtained after adapting the above mentioned process of complexes preparation was subjected to following studies.(21)

FT-IR Analysis
The FT-IR of the inclusion complexes was performed on (FT-IR Bruker 1206 0280, Germany) instrument by KBr disc technique. The spectra were recorded (Fig 2) over the range of 4000- 400 cm^{-1} and the spectrum was obtained (22-23).

DSC Analysis
DSC measurements were carried out on DSC Q10 V9.9 Build 303. Sample (2mg) were placed in sealed aluminium pan and heated from 35°C to 400°C at a rate of 10°C/minute in an atmosphere of nitrogen gas by passing it at a flow rate of 60 ml/min and an empty pan used as a reference (24-25).

Dissolution Studies
A goal of dissolution testing is to ensure the pharmaceutical quality of the product, which means ability of manufacturing reproducibility, release properties and also the bio pharmaceutical characteristic, such as rate of release and extent of absorption.(2-30).
A well designed in-vitro dissolution test should have following characteristics:
- Wide applicability
- Reliability
- Reproducibility

- Simplicity(15)

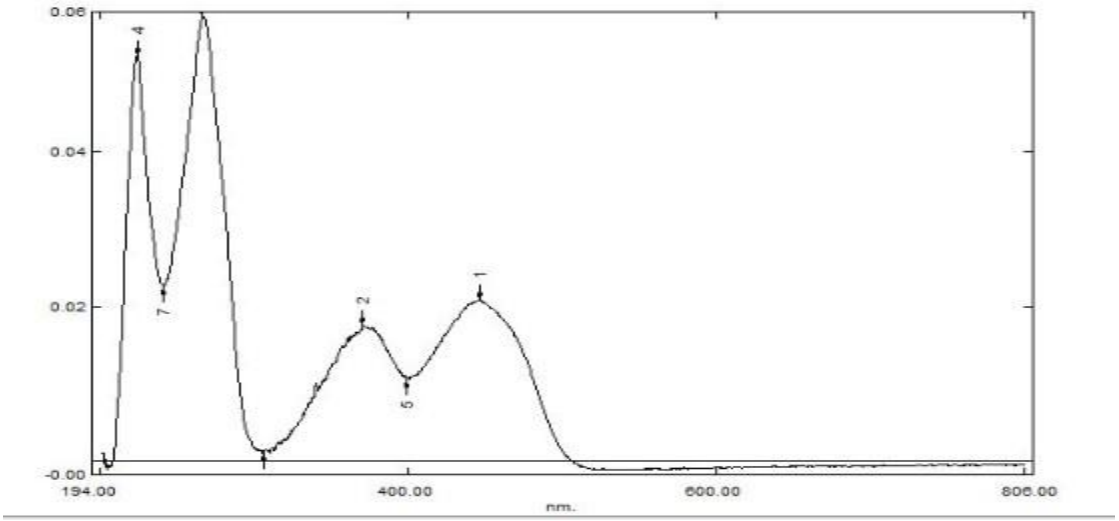
Photostability Studies

Photostability of riboflavin and inclusion complexes was performed in photostability chamber (Thermolab ES2000 UV), equipped with a cool white fluorescent lamp and near UV fluorescent lamp, option 2 according to the Q1B ICH Guidelines for photostability testing (ICH 1996). Irradiance power was set to overall illumination of 1.2 million lux h^{-1} and near UV energy of (1.3W h m^{-2}). Temperature and relative humidity inside the chamber were maintained at 25°C and 60%, at 4°C and 70%, & at 40°C and 40% respectively, throughout the study. A weighed quantity of finely powdered Riboflavin (35 mg) and its complexes (14 gm) were spread as a thin layer in glass Petri dishes (diameter 6 cm). The Petri dishes were placed in the photostability chamber sufficiently apart to avoid shadowing and irradiated with visible lamps (1.2 million lux h^{-1}). The samples were withdrawn in duplicate from the chamber after every 24 hours for up to 7 days. The samples were analyzed in UV Spectrophotometer (UV- 1800, Shimadzu Corp., Japan) (31).

Standard Curve of Riboflavin in distilled water:

Stock Solution Preparation of Riboflavin: 100 mg of riboflavin was accurately weighed and transferred to a 100 ml volumetric flask containing 50 ml of distilled water. The flask was gently shaken to dissolve its content and volume was finally made up to 100 ml using same solvent and labeled as stock solution (A).

Preparation of Calibration Curve of Riboflavin: 10 ml of this solution (A) was taken into volumetric flask and diluted with distilled water in order to obtained the resulting solution of 100 mcg/ml and labeled as stock solution (B). By using the stock solution (B), solutions of various concentration like 5,10,15,20,….40 mcg/ml were prepared by taking 5,10,15,20,……40 ml of stock solution (B) and diluted with distilled upto 100 ml respectively. These solutions were then subjected to UV-visible spectrophotometric studies and absorbance was measured at 444 nm against distilled water as blank (Figure 1).(32)



No.	P/V	Wavelength nm.	Abs.	Description
1	↑	446.00	0.02	
2	↑	370.50	0.02	
3	↑	266.00	0.06	
4	↑	223.50	0.05	
5	↓	399.00	0.01	
6	↓	305.50	0.00	
7	↓	240.50	0.02	

Figure 1: Peak pick form λ_{max} (Riboflavin)

Results and Discussion

Characterization of Riboflavin-2-Hydeoxypropyl-β-Cyclodextrin Inclusion Complexes

The product obtained after following the procedure for preparation of inclusion complexes by kneading method in 1:1, 1:2 and 2:1 was subjected to FTIR, DSC analysis and the results obtained can be summarized as:

FTIR Studies

Characteristic peak of Riboflavin are 803 cm-1 and 878.17 cm-1 are due to C-H bending alkene and aromatic. These peaks are not present in inclusion complexes of riboflavin and β-cyclodextrin or riboflavin and 2-hydroxyprpyl β-cyclodextrin.

It is evident from the FTIR Spectrum, some of the characteristic peaks are different or absent in the spectrum of inclusion complexes from which we assume the formation of inclusion complexes (33).

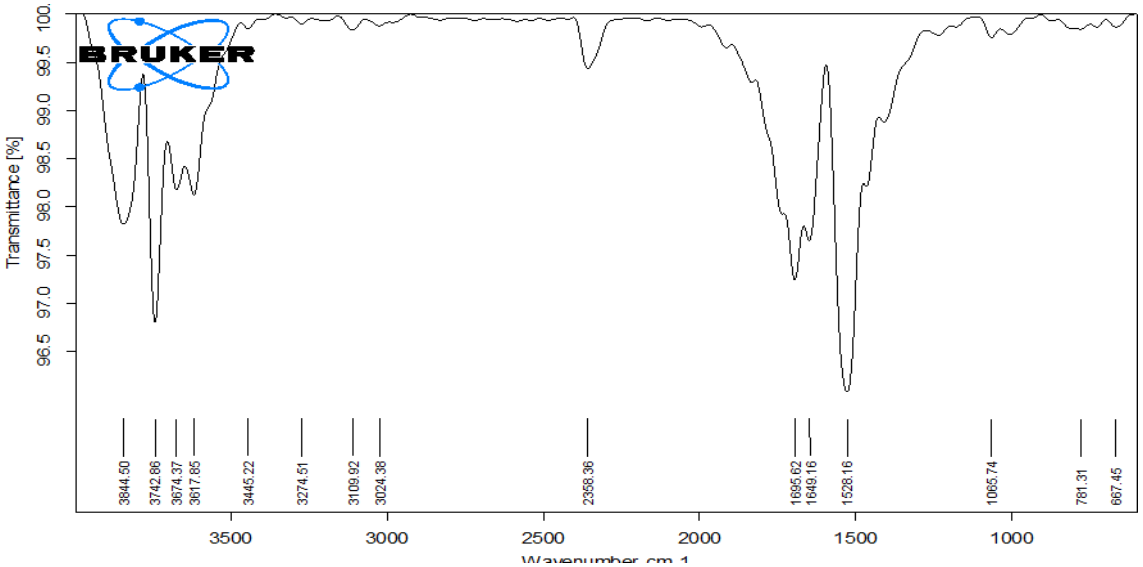


Figure 2a: FT-IR Spectrum of Riboflavin

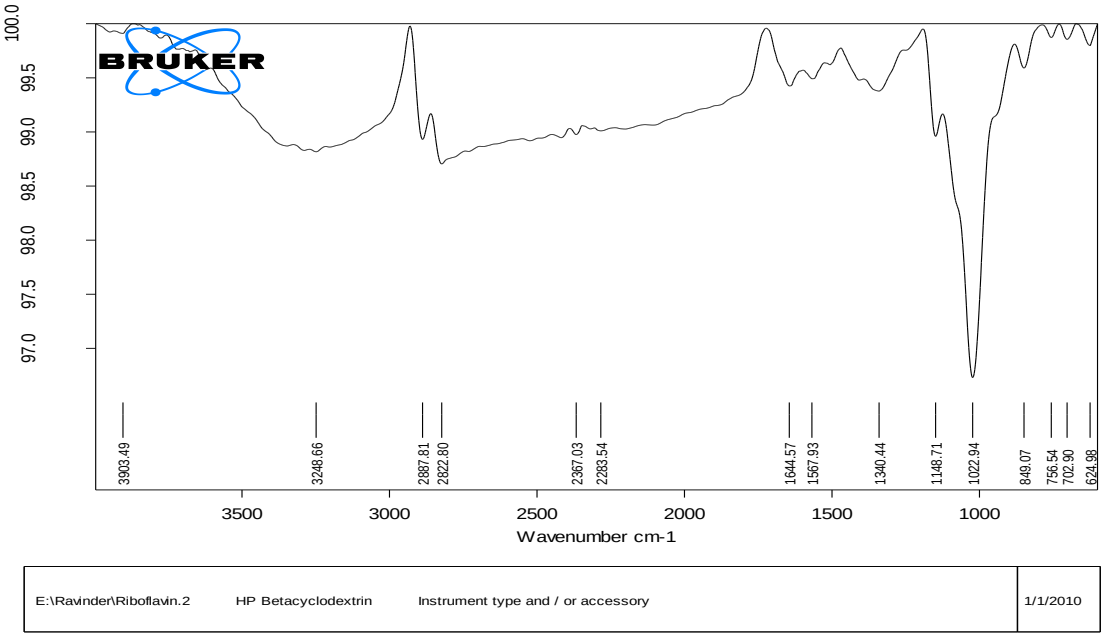


Figure 2b: FT-IR Spectrum of 2-Hydeoxypropyl-β-Cyclodextrin

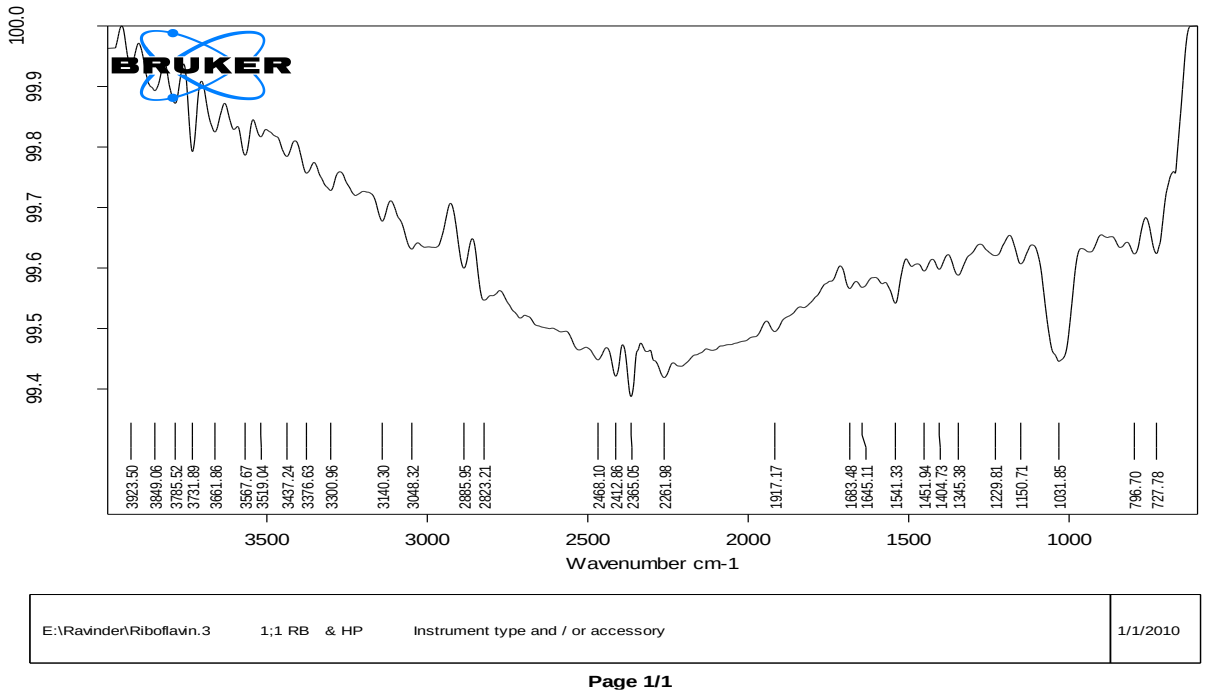


Figure 2c: FT-IR Spectrum of 1:1 Riboflavin-2-Hydeoxypropyl-β-Cyclodextrin complexes

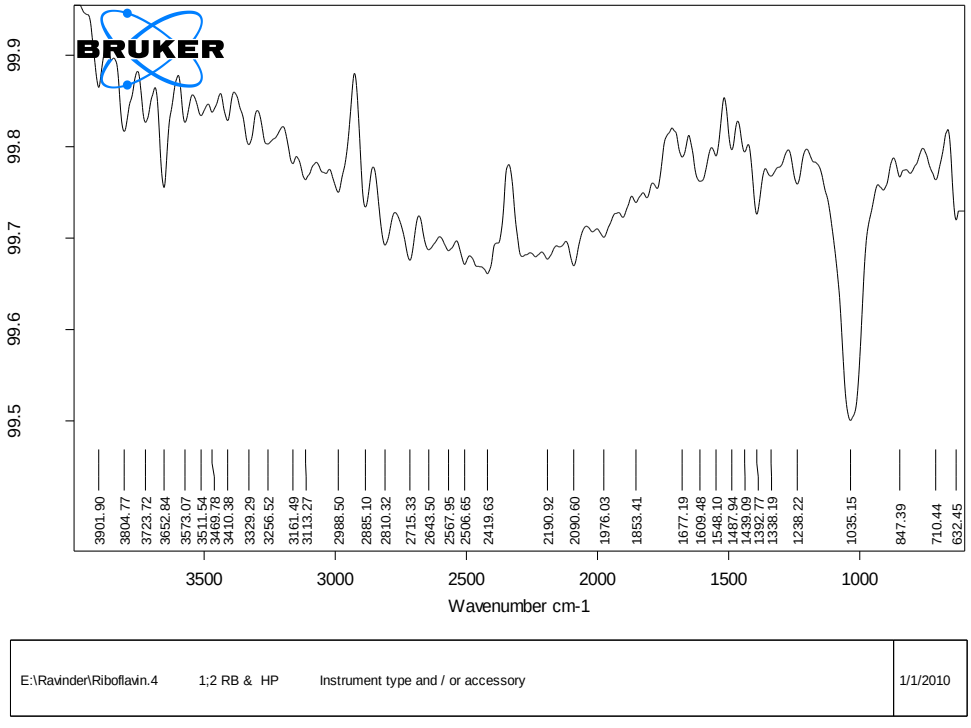


Figure 2d: FT-IR Spectrum of 1:2 Riboflavin-2-Hydeoxypropyl-β-Cyclodextrin complexes

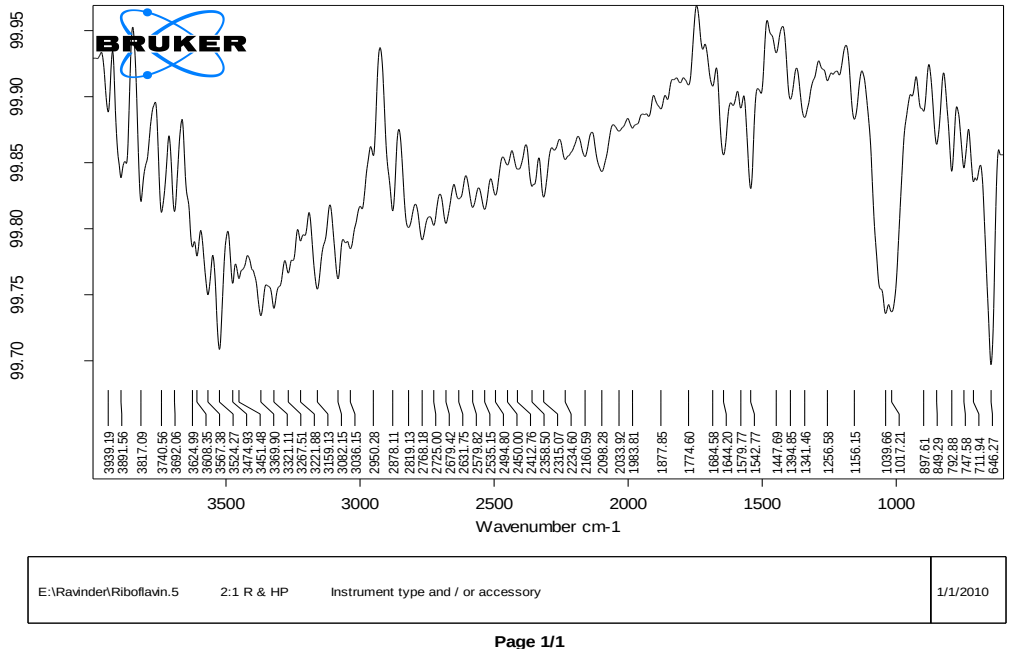


Figure 2e: FT-IR Spectrum of 2:1 Riboflavin2-Hydeoxypropyl-β-Cyclodextrin complex DSC Studies

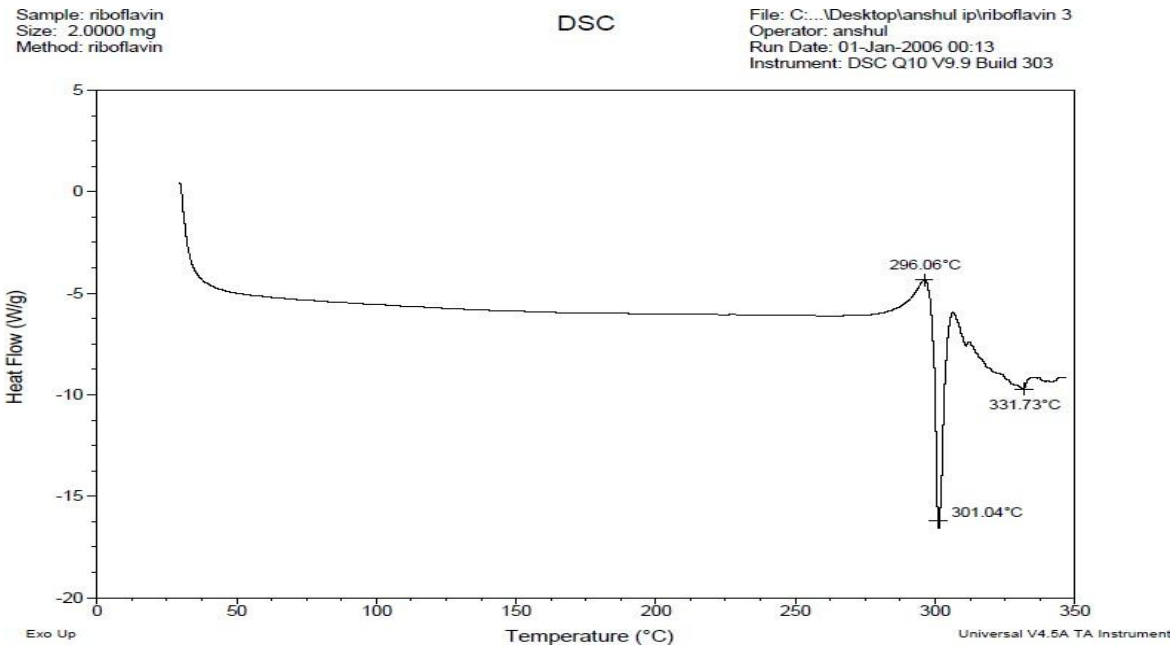


Figure 3a: DSC of Riboflavin(10)

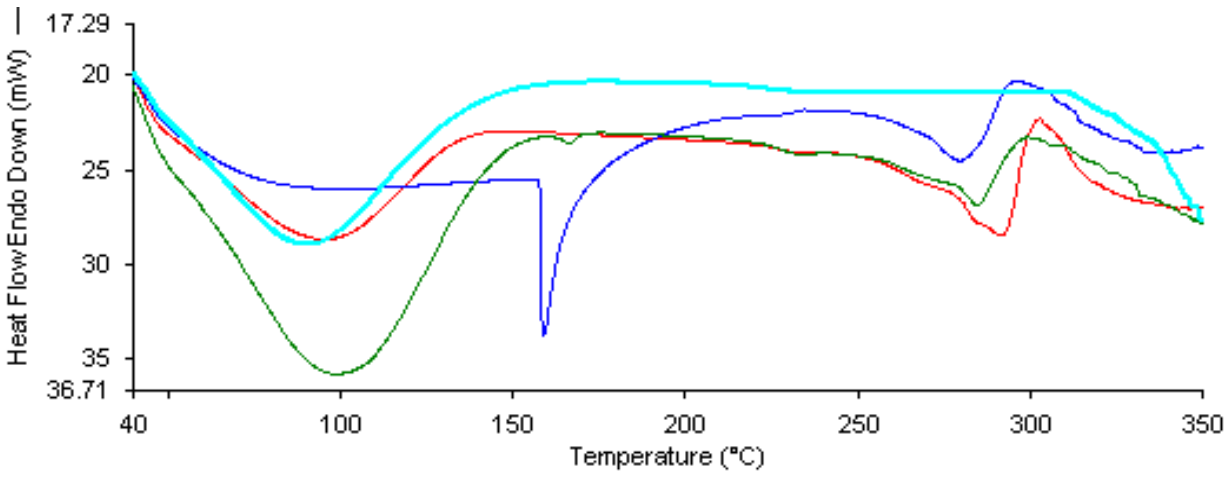


Figure 3b Overlay of DSC of Inclusion Complex

The DSC thermogram of riboflavin shows a sharp endothermic peak at 301°C (Fig 3a) which is also evident in the DSC thermogram of riboflavin-2-Hydeoxypropyl-β cyclodextrin inclusion complexes of ratio 1:1, 1:2 and 2:1 at 279.9°C, 288.422°C and 291.59°Crespectively (Fig 3b).The DSC thermogram of Hypdroxypropyl-β-cyclodextrin shows a broader peak at 88°C (Fig 3b) which is absent in the thermogram of all inclusion complexes of all the three different ratios 1:1, 1:2, and 2:1. However broad endothermic peak at 89.96°C, 97.4°C and 98.24°C respectively(34-36).

Standard Curve of Riboflavin: The standard curve of riboflavin was prepared by following method:
Different concentrations of riboflavin 5, 10, 15, 20….40 mcg/ml was prepared in distilled water and absorbance was measured at 444nm (Fig 1). λ_{max} obtained after scanning 10 mcg solution of riboflavin for 200-800 nm. Each absorbance value rep resents a mean of three consecutive readings. Standard curve of riboflavin with regression coefficient value (R^2 = 0.9994) was obtained. (Fig 1 andTable1)(37)

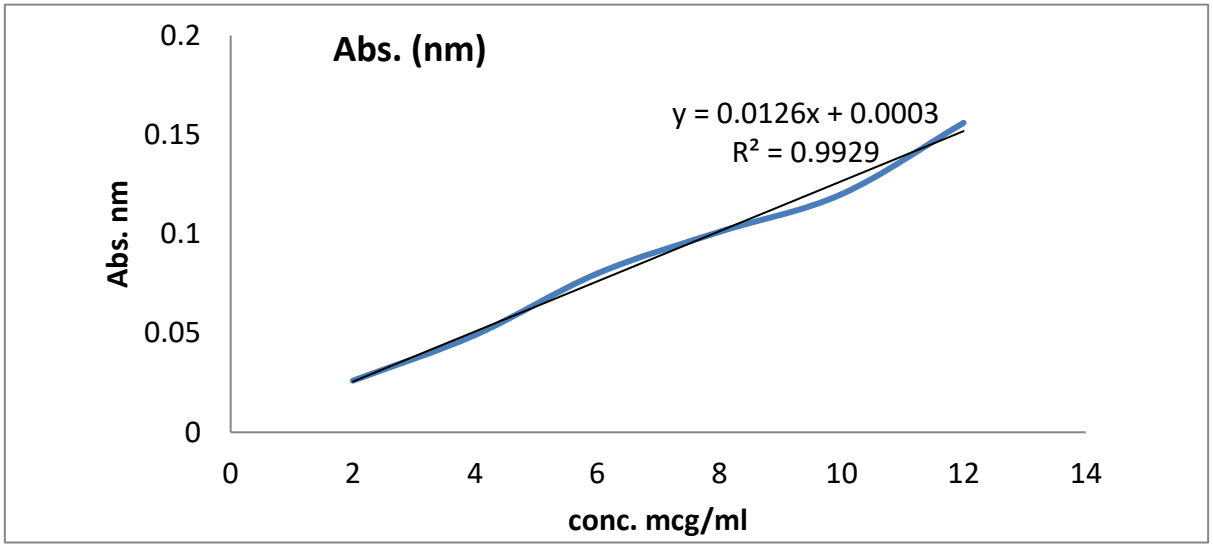


Figure 1: Standard Curve of riboflavin

Table 1: Absorbance of Riboflavin at 444 nm

Conc.(mcg/ml)	Abs. of riboflavin (444 nm)
5	0.194
10	0.301
15	0.410
20	0.523
25	0.638
30	0.747
35	0.869
40	0.994

Dissolution Studies
Dissolution studies were performed on inclusion complexes and the drug (riboflavin) to determine how the dissolution profile of the drug changes in the inclusion complexes in contrast to the drug alone.
All the dissolution test were performed in triplicate. The *in-vitro* dissolution test was performed for pure riboflavin, 1:1 inclusion complex, 1:2 inclusion complex, 2:1 non-inclusion complex using USP dissolution test apparatus type II (Lab India DF 8000) with a paddle rotation speed adjusted to 50 rpm. A 900ml of distilled water was used as dissolution medium, for dissolution studies of pure drug riboflavin & its complexes in different ratios 1:1, 1:2 and 2:1. Sample were drawn from the dissolution media at regular interval and replaced with equal amount of distilled (37) water to maintain the sink condition. Withdrawn samples were then tested using UV- Visible spectroscopy to determine the dissolved amount of drug in the dissolution media. The absorbance was measured at a wavelength of 444 nm. Cumulative drug release of pure riboflavin and complexes of various ratios in distilled water are given in table 2 and fig. 2 respectively (38-40).

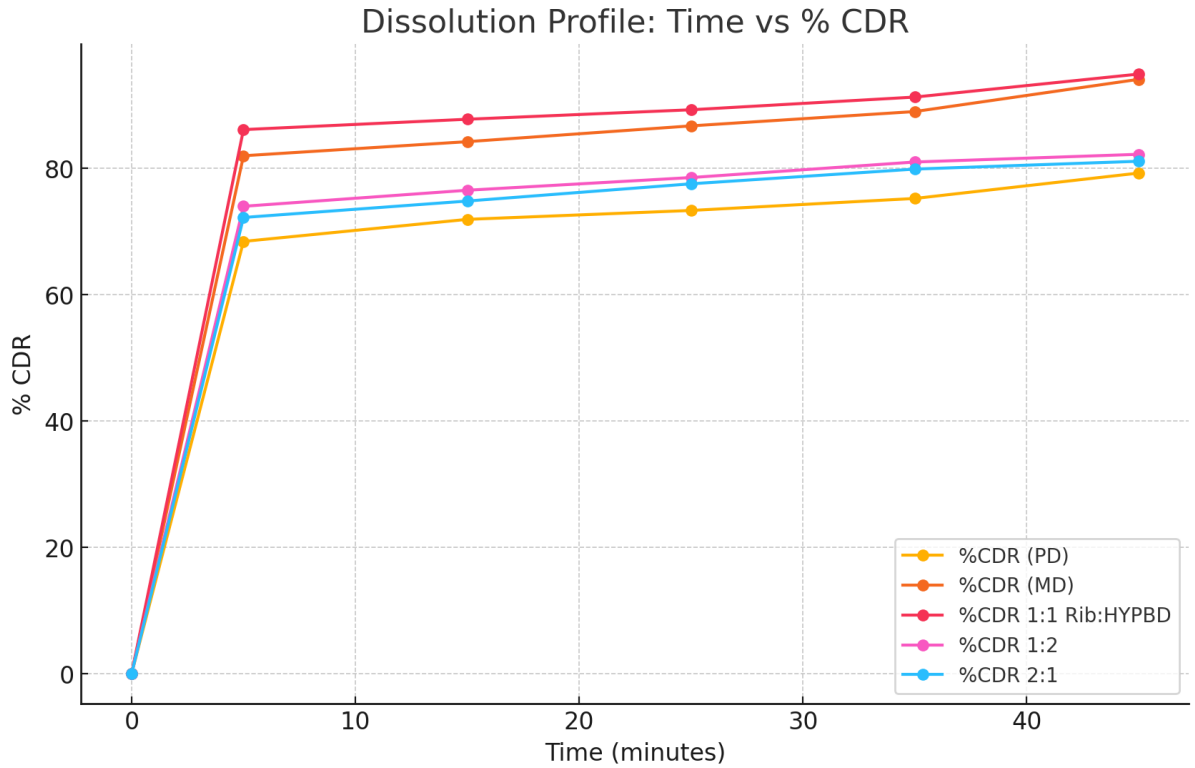


Figure 2. % CDR of Riboflavin, Market Formulation 1:1, 1:2 and 2:1 Complexes

Table 2

Time (min)	% Cumulative Drug Release (CDR)				
	Riboflavin	**1:1 complex**	**1:2 complex**	**2:1 complex**	**Market Formulation**
5	68.44	77.24	74	72.23	82
15	71.93	86.14	76.54	74.83	84.43
25	73.34	89.28	78.55	77.56	86.73
35	75.24	91.29	81.01	79.89	89
45	79.26	94.93	982.23	81.13	94.1

Photostability Studies

In these investigations the photostability of riboflavin in the pure state and the 1:1 riboflavin-β-cyclodextrin complexes by prepared by kneading method was observed at 25±2°C, 4±2°C and 40±2°C in Photostability chamber for 7 days and the amount of drug decomposed was calculated everyday using UV Spectrophotometer (UV- 1800, Shimadzu Corp., Japan). At 25°CTotal drug percentage remaining in the pure form was 24.15 % while in the complexes state was 54.25 %. respectively after 7 days of the study (Table 2 and figure 4). At 40°C, the total drug percentage remaining in the pure form was 18.35 % while in the complexes state was 37.35 % (Table 3) respectively after 7 days of the study and at 4°C the total drug percentage remaining in the pure form was 29.15% while in the complexes state was 67.11 % (Table 3) respectively after 7 days of the study. Photo-degradation of riboflavin analysis at 25±2°C, 4±2°C and 40±2°C Table 3 shows the relationship between time (in days) and percentage drug remaining which represents zero order kinetics while Table 3 shows the relationship between time (in days) & log % drug remaining which represents 1^{st} order kinetics. The slope of degradation of pure drug changed sharply after 3^{rd} day which shows a steep increase in drug degradation after 3^{rd} day. This can be attributed to the conversion of riboflavin into lumiflavin which play their role in accelerating the photo-degradation of riboflavin). However this trend is not seen in the degradation pattern of inclusion complexes. This could be because the total drug degradation of complexes in all the three temperature is comparatively very less leading to the formation of lumiflavin and lumichrome in smaller quantities that could not do a noticeable acceleration in the photo-degradation of riboflavin. This shows that complexation of drug by β-cyclodextrin protected it significantly from deteriorating effects of light. Temperature plays a very important role in degradation of riboflavin. It was observed that degradation increased with increasing temperature (41-42).

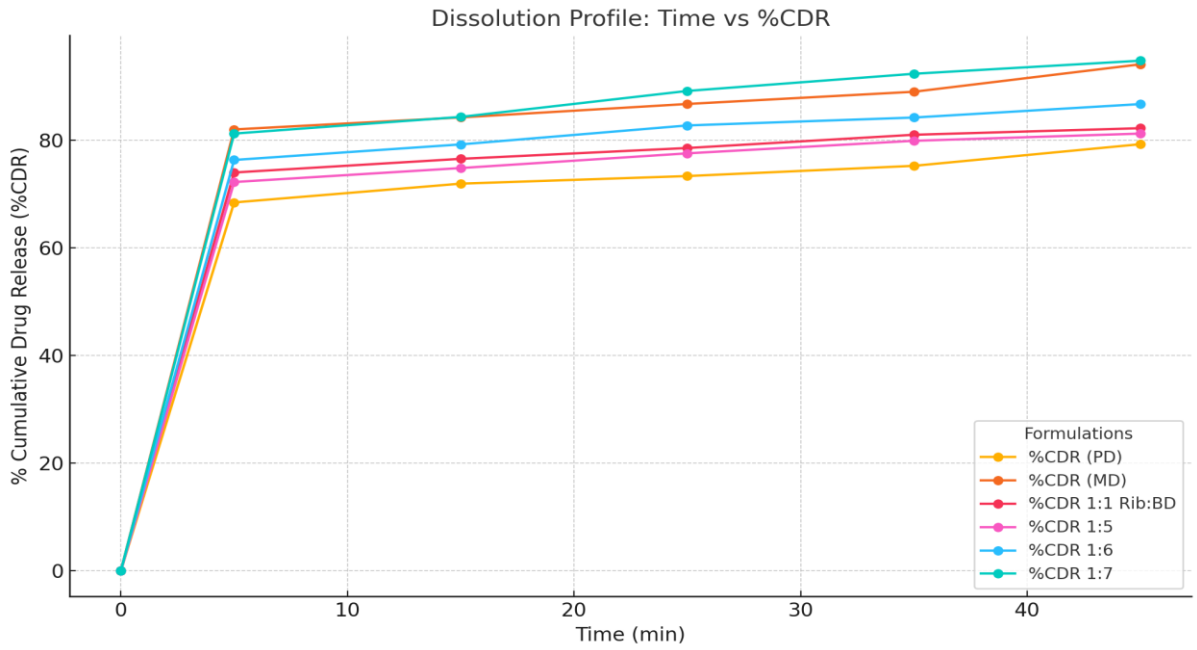


Figure . Degradation Data

Table 3: Photostability Data of Riboflavin and 1:1 Riboflavin-2-Hydroxypropyl-β-Cyclodextrin

Time (In Days)	% Remaining PD at 25 °C	% Remaining PD at 4°C	% Remaining PD at 40°C	% Remaining HYPBCD at 4°C	% Remaining HYPBCD at 40 °C	% Remaining1:1 HYPBCD at 25 °C
0	100	100	100	100	100	100
1	74.38	85	74.47	95.66	81	90.69
2	67.77	76.2	65.16	88.69	73.18	85.08
3	55.8	64.05	55.78	79.01	65.18	78.18
4	51.72	55	38.64	77.12	54.18	71.18
5	44.20	45.71	35.40	75.20	48.62	67.44
6	28.11	38.75	28.45	70.83	41.92	62.45
7	19.47	29.14	22.24	67.7	37.03	54.39

Conclusion: The study focuses on formulating and evaluating Riboflavin-2-hydroxypropyl-β-cyclodextrin (HPβCD) inclusion complexes using the kneading method in 1:1, 1:2, and 2:1 molar ratio. FTIR analysis confirmed successful complex formation by the disappearance of riboflavin's characteristic peaks at 803 cm^{-1} and 878.17 cm^{-1}, associated with C-H bending of alkene and aromatic groups. DSC analysis revealed a shift in riboflavin's endothermic peak from 301°C to 279.9°C, 288.42°C, and 291.59°C for the respective complexes, indicating molecular interactions and improved stability. The absence of HPβCD's broad peak at 88°C and new peaks at 89.96°C, 97.4°C, and 98.24°C further support complex formation, enhancing riboflavin's dissolution and stability.

The dissolution profile of riboflavin and its inclusion complexes (1:1, 1:2, 2:1 ratios) was evaluated over 45 minutes and compared to a market formulation. At 5 minutes, riboflavin showed 68.44% cumulative drug release (CDR), while the inclusion complexes exhibited higher release: 1:1 (77.24%), 1:2 (74%), and 2:1 (72.23%). By 45 minutes, the 1:1 complex reached 94.93%, and the 1:2 complex showed 92.23%, while riboflavin released 79.26%. The market formulation showed the highest release across all time points, with 94.1% CDR at 45 minutes, indicating faster dissolution compared to the complexes.

The photostability of riboflavin and its 2-hydroxypropyl-β-cyclodextrin (HPβCD) inclusion complexes was analyzed over seven days at 4°C, 25°C, and 40°C. Pure riboflavin showed rapid degradation, with only 19.47% remaining at 25°C and 22.24% at 40°C by day seven, whereas storage at 4°C improved stability (29.14%).

The 1:1 riboflavin-HPβCD complex exhibited better stability, retaining 67.7% at 4°C and 37.03% at 40°C. The 1:1 riboflavin-HPβCD complex demonstrated superior protection, maintaining 54.39% at 25°C, significantly higher than free riboflavin. These findings confirm that HPβCD inclusion enhances riboflavin's photostability, offering a promising strategy for improved formulation stability.

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