Dissolution Enhancement & Invitro Evaluation of Ritonavir Inclusion Complexes

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Abstract: Ritonavir is a commonly given protease inhibitor medication that falls under class II of BCS. Because of its poor aqueous solubility, it has a low and variable oral bioavailability. It has difficult formulation and development issues because it is nearly insoluble in water and aqueous fluids. The goal of the current study was to use complicated technologies to increase Ritonavir's solubility and rate of dissolution. Complexation is the process by which two or more molecules join together to create a non-covalent complex that is more soluble than the medication alone. Ritonavir β-cyclodextrin inclusion complexes were prepared by varying the molar concentrations of drug to β-cyclodextrin and Hydroxy propyl β-cyclodextrin in the molar ratio 1:1, 1:2, 1:3 by Kneading method and physical method. The aim of this work was to study the influence of β-cyclodextrin (β-CD) on the biopharmaceutical properties of Ritonavir. Present study deals with the preparation of inclusion complex of Ritonavir β-cyclodextrin as carrier and to evaluate Ritonavir β-cyclodextrin inclusion complex for various parameters. The present research work concludes that the formulation F6 consisting of Ritonavir-hydroxypropyl β-cyclodextrin (1:3) prepared by kneading method showed highest release of Ritonavir in a short period of time.

Keywords: Ritonavir, Inclusion complex, β-cyclodextrin, Hydroxy propyl β-cyclodextrin.

I. INTRODUCTION

About a century ago, cyclodextrins were discovered, and in the first half of this century, the chemistry of cyclodextrins was established. Initially, only trace amounts of relatively impure cyclodextrins could be produced, and their industrial use was hindered by the high cost of synthesis. The efficiency of cyclodextrin production has dramatically increased due to recent biotechnological developments, which has reduced the cost of these materials and made highly pure cyclodextrins and cyclodextrin derivatives available[1]. Therefore, by utilizing the well-established low toxicity of cyclodextrin, poor water soluble medications can be given orally in the complexed form. Because of its distinct cavity size and simplicity of industrialization, beta cyclodextrin seems to be the most practical complexing agent, resulting in a comparatively lower cost for the molecule[2].

Complexation with cyclodextrins is one of the many strategies that has gained popularity recently for improving the solubility and rate of dissolution of medications that are not very soluble. A range of lipophilic medications can be incorporated into cyclodextrins (CDs), which are cyclic torus-shaped molecules with a lipophilic inner chamber and a hydrophilic outside surface. Numerous physicochemical characteristics, including solubility, stability, dissolution rate, and bioavailability, may be positively impacted as a result of the inclusion process[3,4]. Since being approved by numerous regulatory bodies, cyclodextrins have been used more often in pharmaceutical formulations in recent years[5,6]. There is a difference between the amount that can dissolve and the pace of dissolution. "The drug must dissolve in the bodily fluid present at the absorption site before being absorbed into the systemic circulation. The dissolved drug molecules from solution absorb or cross the biological barrier by passive diffusion." The solubility of the pharmacological dosage form is the primary determinant of its dissolution. According to the Noyes-Whitney equation, a drug's solubility and dissolution rate are directly correlated. One important factor that affects a drug's solubility is how quickly it dissolves, which in turn affects how well it is absorbed and how bioavailable it is[7].

Methods of enhancement of the drug dissolution characteristics[8].

1. Increasing the effective surface area of the drug.
2. Incorporation of surface-active agents in the formulation.
3. Alteration of the pH of surrounding medium.
4. Solute-solvent complex reaction.
5. Eutectic mixture and solid solution techniques.
6. Dispersion techniques.
7. Micelle solubilization.
8. Complexation with cyclodextrins.

Recently, cyclodextrin or cycloamylases, a family of cyclic oligosaccharides made from starch by enzymatic degradation have been identified as valuable medicinal excipients. Bacillus marcerans produces the enzyme cyclodextrin-glycosyl transfarase, which reacts with partially hydrolyzed starch to form a mixture of cyclic and acyclic dextrin that can be separated.

Chemistry of Cyclodextrin: Cyclodextrin are cyclic, non-reducing, water soluble oligosaccharides different forms of cyclodextrin known are: α, β and γ. Cyclodextrin are also called schardinger dextrins, cycloglucans or cycloamylases are α-1,4 linked cyclic oligosaccharides obtained from enzymatic conversion of starch. The parent or natural cyclodextrin contain 6, 7 or 8 glucopyranose units.

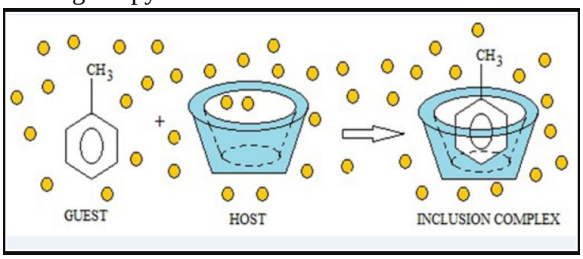

Fig 1: Schematic representation of inclusion complex formation

The above figure provides a schematic representation of the equilibrium involved in forming the inclusion complex between cyclodextrin and toluene in the presence of small amount of water.

In general, there are four energetically favourable interactions that help shift the equilibrium to right:

- The displacement of polar water molecules from the polar cyclodextrin cavity.
- The increase number of hydrogen bond formed as the displays water returns to the larger pool.
- A reduction of the repulsive interactions between the hydrophobic guest and the aqueous environment.
- An increase in the hydrophobic interactions as the guest inserts itself into the apolar cyclodextrin cavity.

While this initial equilibrium to form the complex is very rapid within a minute the finial equilibrium can take much longer to reach. Once inside the cyclodextrin cavity, the guest molecules makes conformational adjustment to take maximum advantage of the weak Vender Waals' forces that exists.

Preparation of Complexes: Cyclodextrin complexes prepared by following methods:

a) Physical mixture
b) Kneading method
c) Common solvent method

II. MATERIALS AND METHODS

Ritonavir was obtained by gift sample from Hetero drugs limited, A.P, β-cyclodextrin, and Hydroxypropyl β-cyclodextrin were obtained by SD Fine chemicals Pvt.Ltd., Mumbai. All other reagents & chemicals used were analytical grades provided by the college.

Preparation of Ritonavir inclusion complexes with β-cyclodextrin and hydroxypropyl β-cyclodextrin:

Physical Mixture: Two distinct molar ratios 1:1, 1:2 and 1:3 of medication and cyclodextrin are combined in a mortar with continual triturating for roughly an hour. The triturated mixture is passed through sieve No.80 and stored in a desiccators. Rapid mass granulators are also used to create the physical combinations on a huge scale for 30 minutes. Following that, the powdered mixes are kept at regulated humidity levels and temperatures of 25 ± 20^0C[9, 10].

Kneading method: A tiny amount of water is added to a mortar along with various molar ratios of drug and cyclodextrin, and the mixture is extensively mixed while being triturated to get a slurry-like consistency. For an hour, the triturating is maintained. After being allowed to air dry at room temperature, the slurry is ground up, run through sieve No. 80, and then placed in desiccators for storage. Granulators with triturating timings ranging from 15 to 1 hour at a controlled humidity of 40–50% are utilized for large-scale production[11,12].

Table 1: Formulation Table for Ritonavir inclusion complexes

Formulation Code	Drug-Polymer	Drug-Polymer ratio	Method Employed
F1	Ritonavir : β-cyclodextrin	1:1	Physical mixture
F2	Ritonavir : β-cyclodextrin	1:2	Physical mixture
F3	Ritonavir : β-cyclodextrin	1:3	Physical mixture
F4	Ritonavir : Hydroxypropyl β-cyclodextrin	1:1	Kneading method
F5	Ritonavir : Hydroxypropyl β-cyclodextrin	1:2	Kneading method
F6	Ritonavir : Hydroxypropyl β-cyclodextrin	1:3	Kneading method

III. RESULTS & DISCUSSION

EVALUATION OF RITONAVIR INCLUSION COMPLEXES

Physical Appearance: All batches of Ritonavir inclusion complexes were evaluated for the colour and appearance.

Table-2: Physical Appearance of the prepared formulations

Formulation code	Colour	Appearance
F1	Creamy	Fine powder
F2	White Creamy	Fine powder
F3	Whitish Creamy	Fine powder
F4	Creamy	Granular Powder
F5	Creamy	Granular Powder
F6	Whitish Creamy	Granular Powder

Solubility Study[13]: Ritonavir's solubility and that of its complexes with β-cyclodextrin and hydroxypropyl β-cyclodextrin were tested in a variety of aqueous solutions. A 100 ml stopper conical flask containing 20 ml of each aqueous fluid was filled with 20 mg of excess Ritonavir and 20 mg of excess Ritonavir-related molar complexes, including β-cyclodextrin and hydroxypropyl β-cyclodextrin. After shaking the mixture for 24 hours at 37±0.5ºC, the sample was filtered through Whatmann filter paper 1002, and aliquots were taken out of the filtered solution and subjected to drug content analysis following the proper solvent dilution.

Table-3: Solubility data for prepared Ritonavir inclusion complexes

Formulation Code	Drug : Polymer	Drug:Polymer Ratio	Method Employed	Percentage Solubility (gm/100 ml)	
				0.1 N HCL	Methanol
F1	Ritonavir : β-cyclodextrin	1:1	Physical mixture	0.0834	0.0757
F2	Ritonavir : β-cyclodextrin	1:2	Physical mixture	0.1000	0.0943
F3	Ritonavir : β-cyclodextrin	1:3	Physical mixture	0.0925	0.0907
F4	Ritonavir : Hydroxypropyl β-cyclodextrin	1:1	Kneading method	0.0834	0.0757
F5	Ritonavir : Hydroxypropyl β-cyclodextrin	1:2	Kneading method	0.0880	0.0832
F6	Ritonavir : Hydroxypropyl β-cyclodextrin	1:3	Kneading method	0.0925	0.0907

Drug Content[14]: In a 50 ml volumetric flask, a precisely 50 mg weighted quantity of the Ritonavir complex was dissolved in a tiny volume of 7.4 pH phosphate buffer, and the volume was adjusted to the appropriate level. In a 10-ml volumetric flask, 1 ml of this solution was diluted with 10 ml of 7.4 pH phosphate buffer, and the volume was increased to 10 ml. using the proper blank, the absorbance of this solution was measured at 290 nm.

Table 4: Drug content estimation of Prepared Ritonavir inclusion complexes.

Formulation code	Drug : polymer	Drug polymer ratio	Method employed	Drug content (%)
F1	Ritonavir : β-cyclodextrin	1:1	Physical mixture	88.73±0.60
F2	Ritonavir : β-cyclodextrin	1:2	Physical mixture	104.03±0.53
F3	Ritonavir : β-cyclodextrin	1:3	Physical mixture	105.73±0.35
F4	Ritonavir : hydroxyl propyl β-cyclodextrin	1:1	Kneading method	106.70±0.28
F5	Ritonavir : hydroxyl propyl β-cyclodextrin	1:2	Kneading method	94.46±0.42
F6	Ritonavir : hydroxyl propyl β-cyclodextrin	1:3	Kneading method	84.15±0.65

In Vitro Dissolution Studies for Ritonavir[15,16]: Ritonavir inclusion complex was dissolved in vitro using USPXX type-II, a paddle stirrer. As the dissolving medium, 900 ml of 7.4 pH phosphate buffer were utilized. A rotational speed of 75 rpm was set for the stirrer. The experiment was conducted using the dissolution media at a constant temperature of 37±1ºC. Each test utilized a complex equal to 100 mg of Ritonavir. After appropriately diluting 5 ml of the dissolving media sample with 7.4 pH phosphate buffer, the absorbance at 290 nm was measured to assess drug release. At each time interval, a new amount of dissolving medium was added to replace the volume that was removed.

Table 5: Invitro Drug release data for Ritonavir complex with β-cyclodextrin

Time (min)	F1	F2	F3
15	26.39±6.52	42.76±2.82	30.10±5.10
30	45.27±12.51	60.23±2.78	47.31±0.84
45	57.38±13.35	78.14±1.67	61.90±3.49
60	66.71±11.41	90.11±1.43	72.11±8.78
75	72.03±11.36	91.67±1.36	76.34±2.54
90	81.29±5.14	94.66±0.15	81.40±0.28
105	83.79±1.80	97.12±0.62	84.89±1.36
120	85.82±1.42	98.81±0.25	90.22±0.32

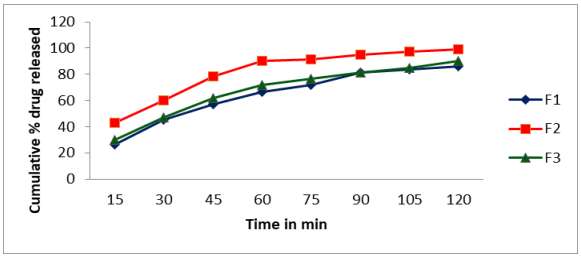

Fig 2: Invitro Drug release for Ritonavir complex with β-cyclodextrin

Table-6: Invitro Drug release data for Ritonavir complex with HPβ-cyclodextrin

Time (min)	F4	F5	F6
15	38.13±3.98	36.04±4.83	81.51±1.25
30	51.27±1.31	55.18±4.94	87.94±1.76
45	61.82±1.12	66.25±3.39	91.38±0.35
60	71.64±3.93	77.72±6.85	93.03±0.02
75	77.72±2.59	80.61±0.79	94.97±1.03
90	82.71±2.02	82.11±0.99	98.41±1.84
105	84.5±1.77	85.70±1.10	99.32±1.77

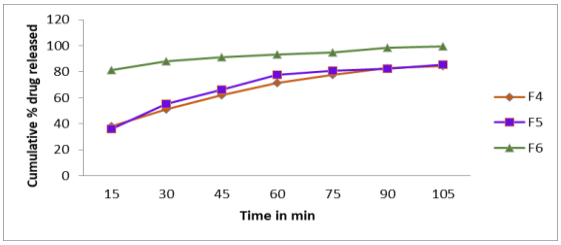

Fig 3: Invitro Drug release for Ritonavir complex with HPβ-cyclodextrin

IV. CONCLUSION

The idea of creating a Ritonavir inclusion complex with beta-cyclodextrin and hydroxy beta-cyclodextrin provides a viable and useful method for achieving the desired goal of enhanced solubility and dissolving properties with higher bioavailability.

- The solubility tests were conducted in two distinct fluids, including methanol and 0.1N HCl. We can infer from the results that Ritonavir is more soluble in methanol than in 0.1N HCl.
- Ritonavir is uniformly distributed throughout the formulation, according to the results of the drug content uniformity study.
- The Invitro release study, which was conducted for different inclusion complexes using 7.4 pH phosphate buffer as a dissolution medium, demonstrates a greater release of the drug from the complexes.
- When compared to complexes prepared with β-cyclodextrin, the complexes prepared with hydroxypropyl β-cyclodextrin exhibit a higher release rate among the different inclusion complexes that were prepared.
- The formulations made using the kneading approach exhibit a faster release than the complexes made using the physical mixture method.
- The Invitro release study also demonstrates that when the polymer concentration rises, so does the drug's release from the complex.
- All formulations InVitro data showed reasonably linear graphs when plotted, suggesting that the release mechanism adhered to first-order kinetics.

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REFERENCE

[1] Loftsson T and Brewster ME. Cyclodextrins as pharmaceutical excipients. Pharmaceutical Technology – Europe, 1997; 9(5): 26-34.

[2] Loftsson T and Morcus E Brewster. Pharmaceutical application of cyclodextrins. Journal of Pharmaceutical Sciences, 1996; 85(10): 1017-1026.

[3] Holvoet C and Heyden YV. J.Pharmazie., 2005; 60(8): 598.

[4] Ueda H and Ou D. Drug Dev. Ind. Pharm., 1998; 24(9): 863.

[5] Thompson DO. Crit. Rev. Ther. Drug Carrier Syst., 1997; 14:1

[6] Hedges AR. Chem. Rev., 1998; 98:2035.

[7] Chowdary KPR, Vijayasrinivas S. Biopharmaceutical classification system and its significance in pharmaceutical research. The Pharma Review, Jan-Feb. 2005; 121-125.

[8] Brahmankar DM, Sunil B Jaiswal. Bioavailability and bioequivalence. Biopharmaceutics & Pharmacokinetics – A Treatise. First Edition, Vallabh Prakashan, New Delhi, Indian 1995; 269-302.

[9] Agarwal, R & Gupta, V 2012, Cyclodextrins - A review on pharmaceutical applications for drug delivery, International Journal of Pharmaceutical Research, vol. 2, no. 1, pp. 95-112.

[10] Shimpi, S, Chauhan, B & Shimpi, P 2005, Cyclodextrins: application in different routes of drug administration, Acta Pharmaceutica, vol. 55, no. 2, pp. 139-156.

[11] Liu, L & Guo, Q 2002, 'The driving forces in the inclusion complexation of cyclodextrins', Journal of Inclusion Phenomena and Macrocyclic Chemistry, vol. 42, pp.1–14.

[12] Singh, R, Bharti, N, Madan, J & Hiremath, SN 2010, 'Characterization of Cyclodextrin Inclusion Complexes – A Review', Journal of Pharmaceutical Science and Technology, vol. 2 no. 3, pp. 171-183.

[13] Soniwala MM, Patel PR, Mansuri MS, Parikh RK and Gohel MC. Various approaches in dissolution enhancement of rofecoxib. Ind. J. of Pharm. Sci., 2005; 67(1): 61-62.

[14] Indop MA, Sunita C Bhosle, Tayade PT and Vavia PR. Evaluation of toxicity and antitumor effect of a hydroxypropyl β-cyclodextrin inclusion complexes of quercetin. Ind. J. of Pharm. Sci., Aug, 2002; 64(4): 349-343.

[15] Chowdary KPR et al. Investigation of dissolution enhancement of intraconazole by complexation with β and hydroxypropyl β-cyclodextrin. Indian Journal of Pharmaceutical Sciences, Sept-Oct. 2001; 438-441.

[16] USP24 / NF-19, Asian Edition Published by United states pharmacopoeial convention inc, 12601 Twinbrook Rock Ville MD, 20852, 2000; p. 1941-1943.