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Physicochemical Characterisation and Dissolution Properties of Spironolactone Solid Binary Systems

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A B S T R A C T

Objective: To study the physicochemical characterisation and dissolution properties of Spironolactone complexing it with β-Cyclodextrin and Hydroxypropyl-β-Cyclodextrin in different molar ratios (1:1 M and 1:2 M).

Design Interventions: Spironolactone belonging to BCS class II, exhibits low and variable oral bioavailability due to its poor aqueous solubility. Enhancement in solubility and dissolution rate leads to an increase in its oral bioavailability. Solid binary systems were prepared by Physical mixture, Kneading method, Microwave irradiation and Freeze-drying method. They were evaluated by *in vitro* dissolution studies and characterised using different analytical techniques.

Main Outcome Measures: The host guest interactions studied by different techniques confirmed true inclusion of Spironolactone with β-Cyclodextrin and Hydroxypropyl-β-Cyclodextrin at 1:1 M and 1:2 M.

Results: Overall, the rank order of improvement in dissolution properties of Spironolactone with ratios is 1:2 M > 1:1 M, methods Freeze-drying > Microwave irradiation > Kneading method > Physical mixture and complexing agent Hydroxypropyl-β-Cyclodextrin > β-Cyclodextrin.

Conclusion: Among the developed Spironolactone SBSs, the SBS prepared with HP- βCD of 1:2 M ratio by Freeze drying method showed good result in dissolution study which shows the maximum release of drug in the range of 95.6%.

Key Words: β-Cyclodextrin, Hydroxypropyl-β-Cyclodextrin, Kneading method, Microwave irradiation method, Freeze drying method.

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INTRODUCTION

The dissolution of a drug from its solid oral dosage form depends upon its release from the dosage form and its subsequent mixing into physiological fluids. It has been estimated that nearly 35-40% of the drugs suffer from poor aqueous solubility, thereby affecting their absorption from the gastrointestinal tract, which leads to poor oral bioavailability, high intra- and inter-subject variability, increase in dose, reduction in therapeutic efficiency and finally failure in formulation development[1]. The development of solid dosage forms for water-insoluble drugs has been a major challenge for pharmaceutical scientists for decades. The serious problem associated with these drugs is low and erratic bioavailability, which is mainly due to poor aqueous solubility of drugs (< 0.1 mg / ml at 37^0 C)[2]. Various formulation strategies such as micronisation, micellar solubilization, complexation, dendrimers for drug solubilization, formation of solid solutions or dispersions with hydrophilic carriers, self-microemulsifying drug delivery systems, spray drying, nano approaches, pro-drug approaches and salt synthesis[3] have been developed to increase the dissolution rate of water-insoluble drugs. Spironolactone is an aldosterone antagonist and has diuretic and antihypertensive effect.

It is a medication that is primarily used to treat fluid build-up due to heart failure, liver scarring or kidney disease. It is also used in the treatment of high blood pressure, low blood potassium that dose not improve with supplementation, earlypuberty in boys, acne and excessive hair growth in women and as a part of feminizing hormone

therapy in transgender women. It is on the World Health Organization's List of Essential Medicines, the safest and most effective medicines needed in a health system. It is available as a generic medication. Although spironolactone is rapidly absorbed after oral administration, its bioavailability is low due to extensive first-pass metabolism[3-6].

Consequently, the major drawback in executing therapeutic efficacy as oral dosage form is its low aqueous solubility. Hence this work is planned to improve dissolution characteristics of Spironolactone by increasing its solubility and release through inclusion complexation technique using various cyclodextrins by employing different methods.

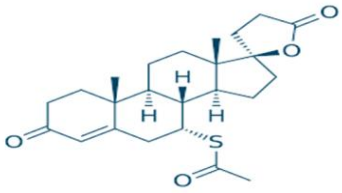

Figure 1: Structure of Spironolactone

Cyclodextrins (CDs) are a class of polymers that form inclusion complexes. Inclusion complexes are formed when a "guest" molecule arrives; it is partially or completely enclosed within a "host" molecule (CD), with no covalent bonds. When inclusion complexes develop, the physicochemical characteristics of the guest molecule gets modified resulting in improved solubility, stability, taste, bioavailability, safety etc. Owing to the limitations of CDs in pharmaceutical utilities, their derivatives were prepared with the aim of improving characteristics such as complexing ability, solubility and safety.

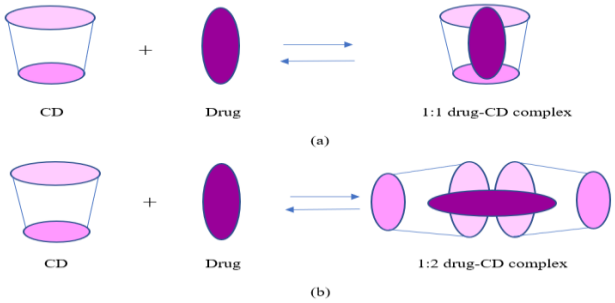

Figure 2: Drug- Cyclodextrin complexation

β- CD is also known as Schardinger's β-CD, cyclomaltoheptaose, cycloheptaglucan, cycloheptaamylose, β- CD, BCD and C7A. The aqueous solubility of natural CDs is lower due to relatively strong intramolecular hydrogen bonding in the crystal lattice. Particularly β- CD shows low aqueous solubility among all naturally occurring CD. Hydroxylation or Methylation of the hydroxyl groups of β-CD enhances solubility and inclusion capacity of parent CDs[7].

HP-β- CD is frequently used to improve the water solubility of insoluble compounds. HP-β- CD cavity is internally hydrophobic and externally hydrophilic. This property permits the full or partial encapsulation of a drug of appropriate size and shape by hydrophobic forces and van der Waals forces without altering the hydrophilicity of HP-β- CD itself. Thus, the inclusion complexes with HP-β- CD could increase the solubility and stability, mask unpleasant taste, reduce adverse reactions, and improve bioavailability of the drug[8].

MATERIAL AND METHODS

Spironolactone (Hetero labs Ltd, Hyderabad), β–Cyclodextrin and Hydroxy-propyl-β-Cyclodextrin (S.D. Fine Chem. Ltd, Mumbai), Methanol, Potassium dihydrogen phosphate, Disodium hydrogen phosphate and all the reagents used were of analytical grade.

METHODS

Standard plot of Spironolactone by UV spectroscopic method: Accurately weighed Spironolactone (10.0 mg) was transferred to 100 ml volumetric flask, dissolved in about 20 ml of methanol and volume was made up-to 100 ml with distilled water to obtain stock solution of 100 µg/ml. From the standard stock solution, 0.5 ml, 1.0 ml, 1.5 ml,2.0 ml, 2.5 ml and 3.0 ml were pipette out into 10 ml volumetric flasks and volume was made up to the mark with 2 ml methanol and distilled water to produce the concentrations ranging from 5-30µg/ml respectively. Then, the calibration curve was plotted in the concentration range of 5-30 µg/ml at 238 nm by taking concentration on X-axis and absorbance on Y-axis. The correlation coefficient (r^2) was found to be 0.9993[9].

Formulation of Spironolactone SBS:

1. **Physical mixtures (PM)**: The physical mixtures of Spironolactone- β-CD and Spironolactone- HP-β-CD at 1:1 and 1:2 M were obtained by mixing individual components together in the mortar for 30 min[10].The mixture was then transferred into a clean beaker, methanol and distilled water were added and mixed well

to prepare the slurry. The slurry was subjected for 24h drying and the dried mass was pulverized and sieved through #120[11].

2. **Kneading method (KM):** Spironolactone- β-CD and Spironolactone- HP-β-CD at 1:1 and 1:2 M were triturated in glass mortar with small volume of methanol and distilled water. The thick slurry was kneaded for 1 h and then dried at 45^{0}C. The dried mass was pulverized and sieved through #120[12].

3. **Microwave irradiation method (MI):** Microwave irradiation (MI) is electromagnetic irradiation found between the infrared (IR) and radio frequencies in the range of 0.3–300 GHz. Most of the MI works at a frequency of 2.45 GHz. The aqueous solution of CD (β-CD and HP- β-CD) was added slowly into a solution of Spironolactone dissolved in methanol with constant stirring. The preparations were subjected for irradiation in microwave oven for 90 sec at 60^{0}C. After completion of the reaction, an adequate amount of methanol was added to remove the residual β-CD and HP- β-CD. The resulting mixture was stirred for 1 h and evaporated under vacuum until dry. The dried mass was pulverized and sieved through #120[13,14].

4. **Freeze drying (FD):** The freeze-drying method is an alternative method to the solvent evaporation method. It is suitable for the thermolabile drugs because minimal thermal stress is applied. In this method, the lyophilized molecular dispersion was prepared by dissolving the drug and carrier in a suitable solvent. The calculatedamount of drug and complexing agents was dissolved in methanol and distilled water respectively. The resulting solutions were mixed and magnetically stirred at room temperature. The obtained solution was dried in a freeze-dryer[15].

Detection of solid binary systems in solid state:

FTIR studies: The prepared solid binary systems were analyzed by FTIR spectrophotometer. The sample was placed in an ATR crystal and the tip of the cover was positioned parallel to the sample hole. The absorption spectra were recorded at a wavenumber of 4000–600 cm^{-1}.

DSC: Differential Scanning Calorimetry (DSC) is a thermo analytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference are measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. Spironolactone, βCD, HP-βCD and samples of formulation were placed in aluminium pans and thermatically sealed. The heating rate was 20°C per minute using nitrogen as the purge gas.

XRD: XRD analysisof Spironolactone, βCD, HP-βCD and prepared samples were conducted.Powder were mounted on aluminum stages with glass bottoms and smoothed to a level surface. The XRD pattern of each sample was measured from 10 to 50 degrees 2-Θ using a step increment of 0.1 2 Θ degree and a dwell time of 1 second at each step.

SEM: The powders were imaged by a scanning electron microscope (SEM) run at an accelerating voltage of 10kV**.** The powder in few µg were fixed on to stub by a double-sided sticky carbon tape and kept inside the SEM chamber and analyzed at different magnification such as 60X, 200X, 500X. 1.10X and 2.50X respectively to obtain better clarity on the particle morphology/ topology.

***In vitro* Dissolution studies:** An *in vitro* dissolution study was performed for the formulated SBS in USP paddle type apparatus using phosphate buffer pH 6.8. Dissolution medium was kept at 37º C ± 0.5º C and rotated at 75rpm with 900ml of phosphate buffer pH 6.8. The samples (1 ml) were withdrawn after every 5min and replaced with 1 ml of fresh phosphate buffer pH 6.8 to maintain the sink condition. Samples were then taken and diluted up to 10 ml in volumetric flask. The samples were analyzed for the drug content using UV spectrophotometer at 238nm [16-18].

RESULT AND DISCUSSION

Standard calibration curve of Spironolactone:

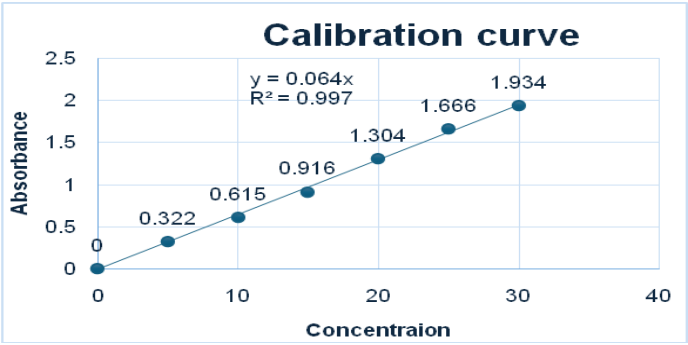

Figure 3: Standard calibration curve of Spironolactone

The standard calibration curve of Spironolactone was found to be linear over a concentration range of 0-30 µg/ml with R^2 value of 0.9993.

Solid state characterisation of Spironolactone and its solid binary systems:

FTIR studies: The FTIR spectrum of Spironolactone and its solid binary systems are shown in the Fig.3.

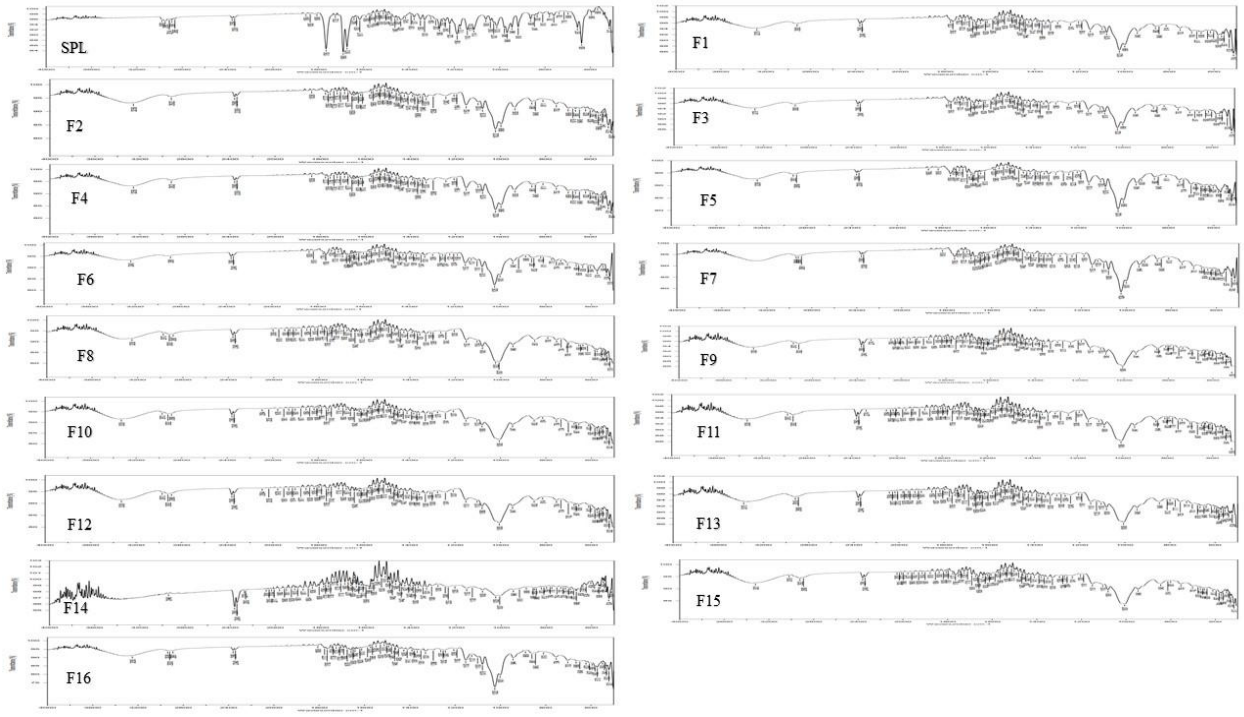

Figure 4: FTIR spectrum of Spironolactone and its SBS.

Comparison of spectra shown in Fig. 3, suggests that the FTIR spectra for Spironolactone showed characteristic peak at 1690 cm^{-1} (–C=O stretching of thioacetyl carbonyl group), 1765 cm^{-1} (–C(=O) –O– stretching of lactone ring), 2960 cm^{-1} (–C–H (alkyl) stretching of α, β-unsaturated ring) and 2891 cm^{-1} (–C=C stretching of α, β unsaturated ring). The Spironolactone has strong absorption peak at 1690 cm^{-1}. The SBS prepared with βCD by KM, MI and FD methods in both the ratios, the band was shifted towards lower wavelength from 1022-1025 cm^{-1} and SBS prepared with HP-βCD by KM, MI and FD methods in both the ratios, the band was shifted towards lower wavelength from 1005-1007 cm^{-1} indicating the formation of inclusion complex and thus suggesting the formation of hydrogen bonds between the carbonyl groups of Spironolactone and the hydroxyl groups of the host cavities during complexation process.

DSC Studies: The DSC spectrum of Spironolactone and its solid binary systems are shown in the Fig.5

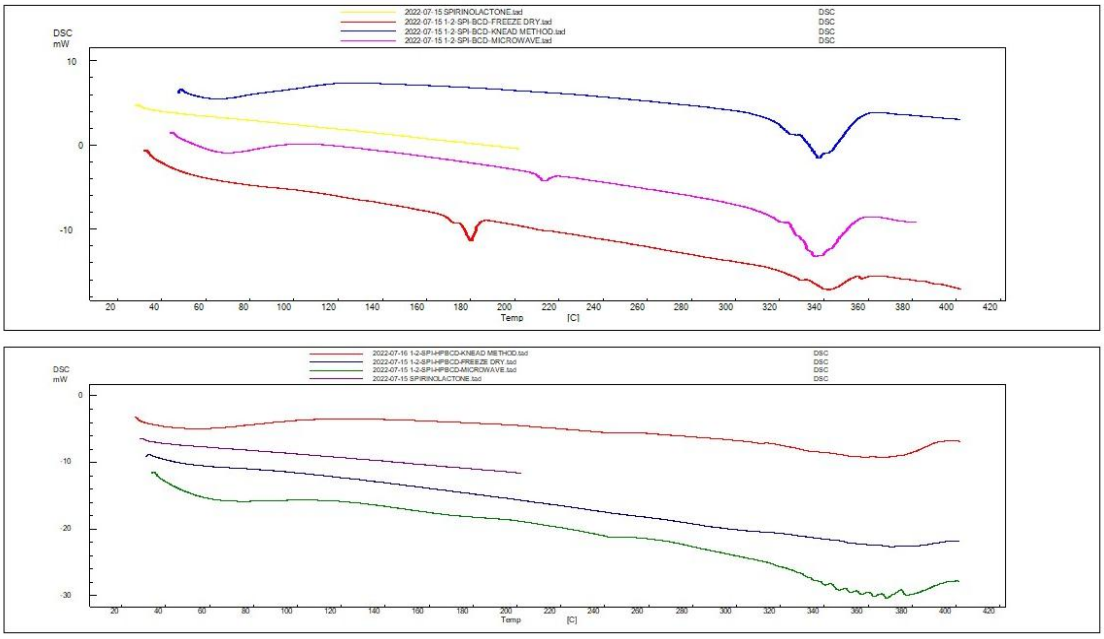

Figure 5: DSC spectrum of Spironolactone and its SBS

SPL has an endothermic peak at 29.2 °C corresponding to its melting point indicating its typical crystalline and anhydrous nature. F4, F12 and F14 showed two endothermic peaks, while F6 and F8 showed three endothermic peaks. Whereas F16 showed smaller peaks indicating that there is good interaction and resulted in the formation of true inclusion complexes.

XRD Studies: The XRD spectrum of Spironolactone, F4, F6, F8, F12, F14 and F16 are shown in the Fig. 6.

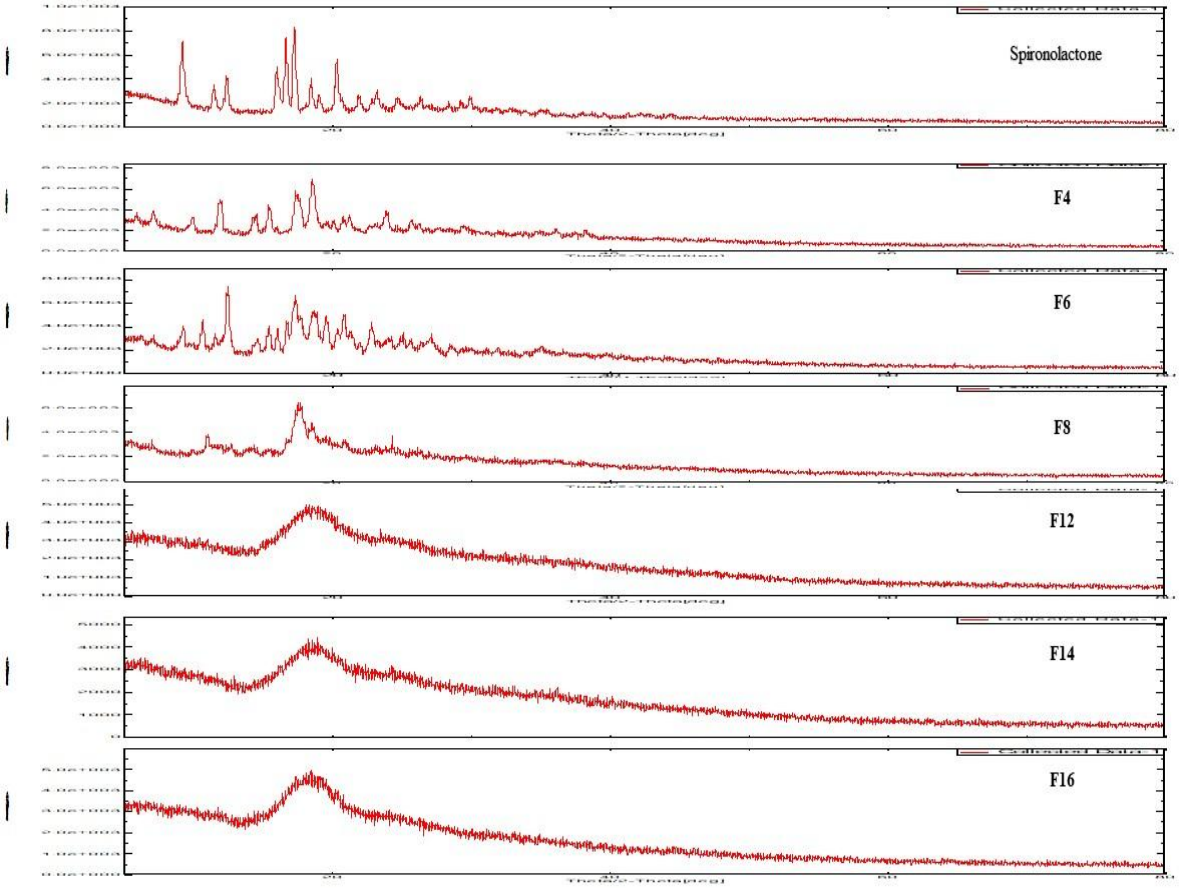

Figure 6: XRD spectrum of Spironolactone, F4, F6, F8, F12, F14 and F16

The pure drug Spironolactone exhibited its characteristics diffraction peaks in the $2^{\circ}\Theta$ range of 9° to 27° indicating the presence of drug in its highest crystalline state. X-ray diffraction patterns of F4, F6, F8, F12, F14 and F16 showed all the principle peaks of Spironolactone. The diffraction patterns are the sum of each component, indicating the presence of Spironolactone in crystalline state. However, the peak intensities of SBS 1:2 M Spironolactone and βCD were lower than the SBS 1:2 M Spironolactone and HP-βCD. The results suggest that the crystallinity has been modified whereas the crystal structure of Spironolactone remains unaltered. These results indicate moderate to true inclusion complex between SPL and βCD as well as SPL and HP-βCD in solid state at 1:2 molar ratios in all the methods.

SEM analysis: SEM images of Spironolactone, F8 and F16 are shown in the Fig. 7.

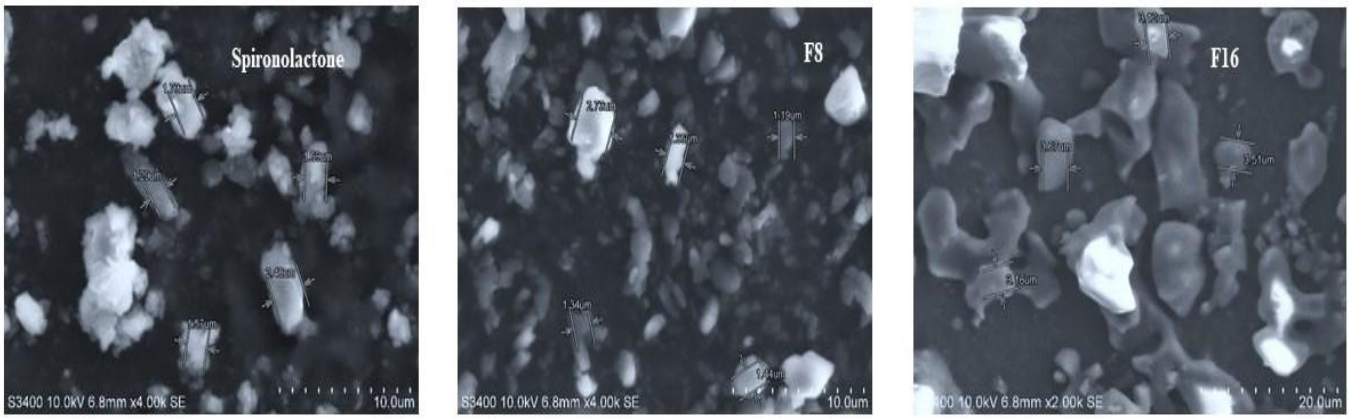

Figure 7: SEM images of Spironolactone, F8 and F16

SEM images of pure drug Spironolactone, F8 and F16 are shown in the Fig. 8. SEM revealed that Spironolactone has rough surface while the Spironolactone SBS has smooth surface. Although SEM photographs do not give 100 % information about complex formation, it was observed that the alteration in particle size and shape confirmed the formation of inclusion complexes of SPL-βCD and SPL- HP-βCD.

In vitro dissolution studies:

Table 1: *In vitro* Dissolution profile of 1:1 M & 1:2 M of SPL: βCD solid binary system by PM, KM, MI and FD.

Sl. No.	Time (min)	% CDR								
		SPL	F1	F2	F3	F4	F5	F6	F7	F8
1	5	25.2	29.2	35.2	36.1	37.4	39.4	43.6	45.8	46.1
2	10	27.6	33.6	36.8	37.3	41	43.2	45.1	47.7	49.3
3	15	30.3	37.3	38.8	39.8	43.2	44.1	46.2	50.3	53.2
4	20	34.7	39	41.9	42.7	47.2	48.1	49.3	52.4	58.7
5	25	37.8	41.8	44.9	47.6	49.8	51.7	53.7	58.3	61.5
6	30	40.1	45.8	47.4	49.3	53.1	54.3	57.1	61.1	66.5
7	40	44.2	48.3	51.9	53.6	57.3	58.3	63.2	69.7	75.3
8	50	47.9	55.6	57.2	58.4	64.2	67.8	71.1	75.8	84.6
9	60	50.1	58.3	61.3	65.9	72.8	76.4	80.6	86.3	92.6

Table 2: *In vitro* Dissolution profile of 1:1 M & 1:2 M of SPL: HP-βCD solid binary system by PM, KM, MI and FD.

Sl. No.	Time (min)	% CDR								
		SPL	F9	F10	F11	F12	F13	F14	F15	F16
1	5	25.2	32.8	35.6	36.7	38.4	42.5	45.6	48.8	49.6
2	10	27.6	35.6	38.5	39.6	43.2	46.2	49.3	53.7	54.2
3	15	30.3	38.1	40.7	43.2	47.8	50.1	54.8	57.3	59.7
4	20	34.7	41.7	43.3	46.9	50.2	55.1	58.1	61.4	65.1
5	25	37.8	44.2	46.5	49.4	54.8	59.7	62.5	68.3	69.9
6	30	40.1	47.6	49.7	54.7	58.1	63.3	66.2	73.1	75.7
7	40	44.2	49.3	55.2	59.9	64.3	67.3	71.9	77.7	81.4
8	50	47.9	54.5	61.8	65.6	68.2	74.8	77.4	83.8	87.6
9	60	50.1	59.7	69.4	71.2	74.8	79.9	82.4	88.3	95.6

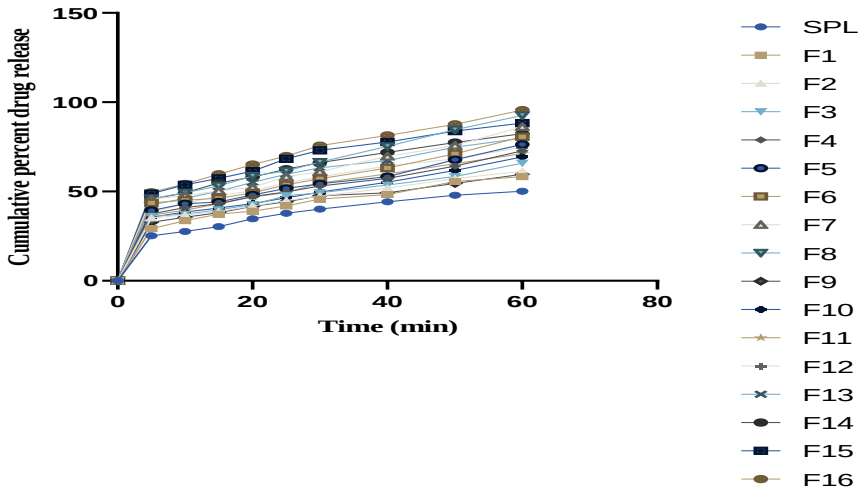

Figure 8: Comparative release profiles of Spironolactone and its SBS.

***In vitro* Dissolution studies:** *In vitro* dissolution profile of Spironolactone solid binary system prepared by PM, KM, MI and FD are shown in Fig. 8 and Table 1 and 2. It is clearly seen that the SBS of 1:2 M in all the methods has increased dissolution rate compared to that of SBS of 1:1 M in the time interval of 5-60 min. Predominantly, the Spironolactone SBS using HP-βCD of 1:2 M prepared by Freeze drying method has greater dissolution rate compared to other methods.

CONCLUSIONS

The current aim was to study the dissolution properties and characterisation of Spironolactone solid binary system using β – Cyclodextrin and Hydroxypropyl – β – Cyclodextrin. Spironolactone SBS were prepared using βCD and HP-βCD at 1:1 M and 1:2 M by PM, KM, MI and FD. The studies showed that the complexation of Spironolactone with HP-βCD at 1:2 M by Freeze drying method (F16) has better dissolution rate. In addition, the characterisation studies such as FTIR, DSC, XRD and SEM showed that there was a formation of true inclusion complexes. As per the excipient profile HP-βCD is highly soluble in water and alcohol, well tolerated in humans and has no adverse effects on kidney function. The observations have shown that the SPL formulation prepared by using HP- βCD (1:2 M) by FD was acceptable with reasonable limits.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest.

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