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COMPARATIVE STUDY ON THE PREPARATION AND CHARACTERIZATION OF INCLUSION COMPLEXES OF BCS CLASS II DRUG WITH CYCLODEXTRINS

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ABSTRACT:
Fenofibrate (FFB) is an effective lipid regulating agent. Chemically it is 2-[4-(4-chloro benzoyl]-2 methyl propanoic acid, 1-methyl ethyl ester. It belongs to BCS class II and require enhancement in solubility and dissolution rate for increasing its oral bioavailability. In the present work, β- Cyclodextrin (β-CD) and Hydroxy Propyl β- Cyclodextrin (HP β-CD) complexes of FFB were prepared and evaluated for solubility and dissolution rate .The aqueous solubility was increased linearly as a function of the concentration of cyclodextrins. Physical characterization of solid inclusion complexes of FFB was studied by DSC and IR. An increase of 21 and 30 fold in the dissolution rate of FFB was observed with FFB- β-CD (1:3) - F6 and FFB- HP β-CD (1:3) - F12 prepared by kneading method respectively. The increase in the dissolution rate of the drug may be due to increase of wettability, the hydrophilic nature of carriers and also due to possibility of reduction in drug crystallinity.

Keywords: Fenofibrate, β-Cyclodextrin, Hydroxy Propyl β- Cyclodextrin, Inclusion complex, Solubility enhancement,

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INTRODUCTION

Fenofibrate (Figure-1) is a lipid regulating agent and is chemically 2-[4-(4-chloro benzoyl]-2-methyl propanoic acid, 1-methyl ethyl ester[1-3]. FFB increases lipolysis and elimination of triglyceride rich particles from plasma by activating lipoprotein lipase and reducing production of apo protein C-III (an inhibitor of lipo protein lipase activity)[1,4]. FFB is almost white powder and is practically insoluble in water. As it belongs to BCS class-II[5] the most effective option for increasing the dissolution rate is an improvement of the solubility through various approaches. Among the various approaches, cyclodextrin complexation has gained good acceptance in recent years. Therefore cyclodextrins are used for converting FFB into FFB-CD inclusion complexes for the study [6,7].

Cyclodextrins are mainly used to increase the aqueous solubility and dissolution rate of drugs. α-CD, β-CD and γ-CD are commonly known as parent cyclodextrins. These consist of 6, 7 and 8 α- (1,4)- linked glucopyranose units, with relatively hydrophobic central cavity and hydrophilic outer surface respectively[8]. Cyclodextrins are used to prevent drug-drug and drug-additive interactions, decrease volatility and mask of objectionable taste or odor of drugs.

The main purpose of the present investigation is to increase the solubility and dissolution rate of Fenofibrate by the preparation of the complex with β-CD & HP β-CD using physical mixing and kneading methods.

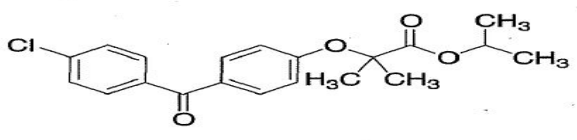

Fig: 1 Structure of Fenofibrate

MATERIALS AND METHODS

Materials: Fenofibrate was obtained as a gift sample from Micro Orgo Chem., Mumbai, β-Cyclodextrin and Hydroxy Propyl β-Cyclodextrin were obtained from Himedia Pvt. Ltd., Hyderabad and all other ingredients used were of analytical grade**.**

Methods: Solid inclusion complexes of FFB- β-CD and FFB- HP β-CD were prepared in the ratios of 1:1, 1:2, 1:3 by using physical mixing method and kneading method (Table 1)[9-11].

a) Physical mixtures: Physical mixtures of the drug (FFB) with β-CD and HP β-CD in 1:1, 1:2 and 1:3 ratios were prepared by mixing them thoroughly for 5 mins in a mortar until homogenous mixture was obtained. This was passed through mesh no # 100 and stored in desiccated environment.

b) Kneading method: Drug with β-CD and HP β-CD were triturated at different ratios in a mortar with a small volume of water: methanol (3:2) solvent blend. The thick slurry was kneaded for 45 minutes, and then dried. The dried product was crushed, pulverized and sieved through mesh no # 100. The solid dispersions thus obtained were stored in a well closed container and kept in a desiccator.

Table: 1 Formulation parameters of Fenofibrate: Cyclodextrin inclusion complexes

Method	Fenofibrate: β-CD	Product Name	Fenofibrate: HP β-CD	Product Name
Physical mixing	1:1	F1	1:1	F7
	1:2	F2	1:2	F8
	1:3	F3	1:3	F9
Kneading method	1:1	F4	1:1	F10
	1:2	F5	1:2	F11
	1:3	F6	1:3	F12

Phase Solubility studies[12]

Phase solubility studies were performed according to the method reported by Higuchi and Connors method. An excess amount of drug was added to 15 ml of triple distilled water with pH 7 containing various concentrations of β-CD and HP β-CD (3-15mM) taken in a series of 25ml stoppered conical flasks and mixtures were shaken for 72hrs at room temperature on a rotary flask shaker. After 72 hrs of shaking to achieve equilibrium, 2ml aliquots were withdrawn at 1hr interval and filtered immediately using 0.45µ nylon disc filters. The filtered samples were diluted suitably and assessed for the drug content by the specific UV spectrophotometric method by using distilled water as a blank. Shaking was continued until three consecutive estimations were the same. The solubility experiments were conducted in triplicate.

Drug content analysis[12-14]

An accurately weighed quantity of solid dispersion equivalent to 50 mg of drug FFB was taken into a 50 ml volumetric flask and dissolved in 40 ml of methanol. The solution was made up to the volume with methanol. The solution was then suitably diluted and assayed for drug content using the UV spectrophotometric method at 290 nm.

In-Vitro dissolution studies[12-19]

In-vitro release rate of FFB from solid inclusion complexes of cyclodextrins were studied by using LABINDIA DISSO2000, an eight stage dissolution rate testing apparatus with paddle stirrer. The dissolution medium consisted of 900 ml of 0.05M Sodium lauryl sulfate (SLS). Solid dispersion equivalent to 60 mg of the drug was spread onto the surface of 900 ml of preheated dissolution medium at 37 $\pm$ 0.5^{0}C at 50 rpm. Aliquots of 5ml were withdrawn at regular intervals of time i.e., 5, 10, 15, 20, 30, 40, 50 and 60 min and the sample were replaced with the fresh dissolution medium each time. The samples obtained were filtered through Whatman filter paper and the absorbance was measured at 290 nm.

Physico chemical compatability studies

a) Differential Scanning Calorimetry (DSC): Seiko, Japan DSC 200 C model differential scanning calorimeter was used. Samples (1-4 mg) in weight were sealed hermetically in flat bottomed aluminum cells (pans). These samples were then heated over a temperature of 25-300 ^{0}C in an atmosphere of nitrogen (30 ml/min) at a constant rate of 10^{0}C per minute using alumina (alumina standard material of DSC supplied by Shimadzu Corporation), as reference standard.

b) Infrared Spectroscopy (IR): H400-84100, Shimadzu, Japan I.R Spectrophotometer was used. Pure drug and its solid inclusion complexes were subjected to I.R Spectroscopy using the above said model. Pellets of samples were prepared after grinding and dispersing the powder in micronized IR grade-KBR powder using a pestle and mortar, and scanned over a wave number of 400 cm^{-1}- 4400 cm^{-1}.

RESULTS AND DISCUSSION

Drug Content: The FFB solid inclusion complexes were tested for drug content and it was found that the drug was within the compendium limits 95-101% w/w. All the solid dispersions were uniform in drug content. The results were shown in Table 2.

Phase solubility studies: The phase solubility diagrams for the complex formation between FFB - β-CD and FFB – HP β-CD were shown in Figure 2 and 3 respectively. The aqueous solubility of the drug increased linearly as function of β-CD and HP β-CD concentration. At all the

concentrations of β-CD and HP β-CD used for the preparation of the inclusion complexes showed significant increase in the solubility of FFB. As the concentration of the β-CD and HP β-CD increased, the solubility of the drug was found to be increased. The phase solubility studies are classified as type A1 according to Higuchi and Connors. The stability constant Kc was calculated and the values were found in the range of 200-500M^{-1} indicated stronger interaction between the guest molecules and the CD and the stability of the complex formed.

Table 2: Drug content and Cumulative % drug release of FFB solid inclusion complexes

Product code	% Drug content	% Cumulative drug release (60min)
F1	97.2 ± 1.07	35.2 ± 0.275
F2	98.9 ± 0.04	50.1 ± 0.558
F3	99.6 ± 0.06	70 .6 ± 0.789
F4	99.5 ± 0.06	37.9 ± 0.456
F5	98.4 ± 0.09	58.7 ± 0.534
F6	99.1 ± 0.09	78.8 ± 0.698
F7	96.5 ± 1.2	50.0 ± 0.548
F8	98.3 ± 0.04	70.0 ± 0.558
F9	99.0 ± 0.14	80.2 ± 0.856
F10	98.2 ± 0.132	54.0 ± 0.245
F11	98.6 ± 0.12	76.0 ± 0.368
F12	99.9 ± 0.018	83.6 ± 0.264

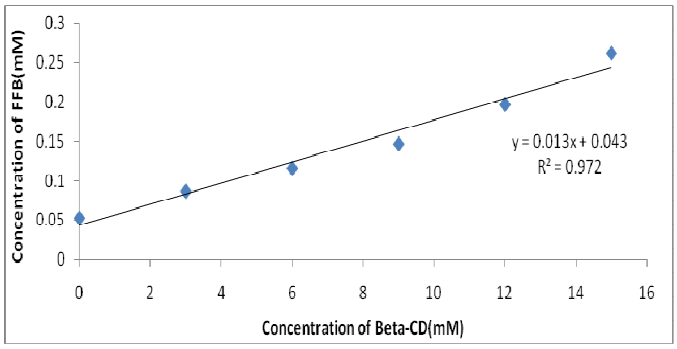

Fig. 2: Phase solubility diagram of FFB in aqueous β-CD solution

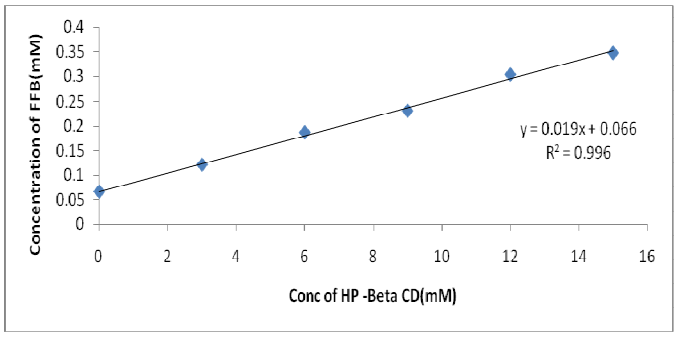

Fig. 3: Phase solubility diagram of FFB in aqueous HP β-CD solution

In-Vitro Dissolution studies: FFB release from the solid inclusion complexes alone was studied up to 60 minutes. The average percentage release of the pure drug was found to be 16.2% in 60 minutes. In the inclusion complexes, cyclodextrins were used as carrier and the dissolution rate increased with the increased amount of β-CD and HP β-CD. The best results among solid inclusion complexes with β-CD were obtained for the FFB: β-CD (1:3) prepared by kneading method Figure-4 and with HP β-CD was obtained for the FFB: HP β-CD (1:3) prepared by kneading method Figure-5. Dissolution parameters of FFB and its cyclodextrin complexes prepared by two methods in different ratios were given in Table 3 (Figure 6). The increased dissolution rate may be due to the higher solubility of the β-CD in dissolution medium and better wettability of FFB in the complex.

Table 3: Dissolution parameters of FFB Solid inclusion complexes prepared by physical mixing (PM) and Kneading methods (KM)

Inclusion complex	T10	T50	T90	DE30%	K
Pure drug	15	>60	>60	24.9	0.0013
F1	5	50	>60	28.4	0.018
F2	3.5	27	>60	40.7	0.026
F3	2.5	20	>60	63.8	0.039
F4	10	47	>60	51.4	0.009
F5	7.5	26	>60	67.7	0.015
F6	5	17	>60	77.0	0.018
F7	6	44	>60	51.8	0.07
F8	3.8	26	>60	72.6	0.013
F9	2.5	24	>60	92.13	0.026
F10	5.8	47	>60	61	0.018
F11	4.6	23.5	>60	79.4	0.020
F12	3	15	>60	95.2	0.024

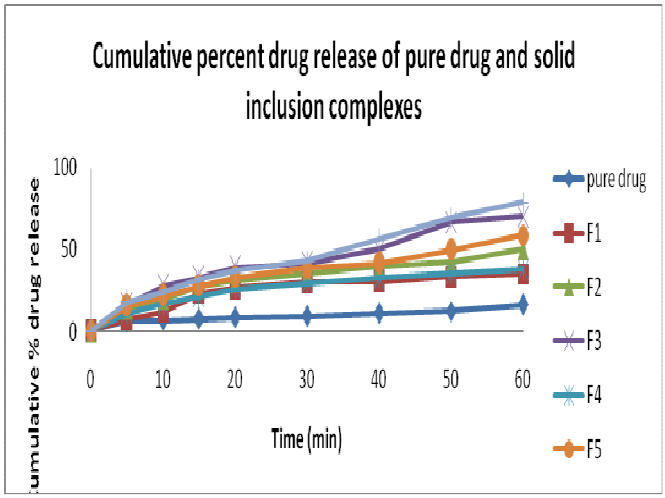

Fig 4: Cumulative % drug release of pure drug and β-CD solid inclusion complexe

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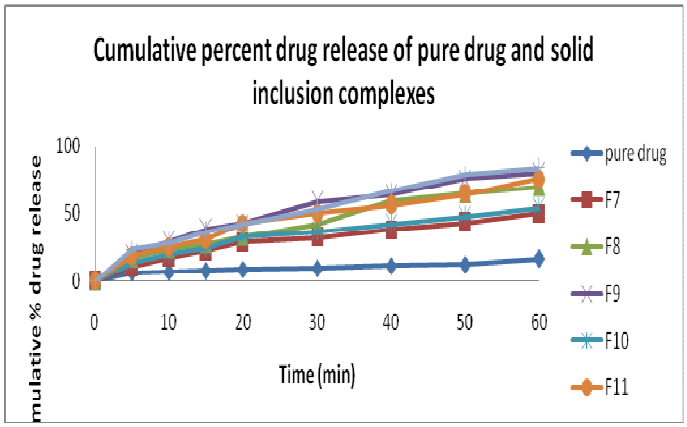

Fig 5: Cumulative % drug release of pure drug and HP β-CD solid inclusion complexes

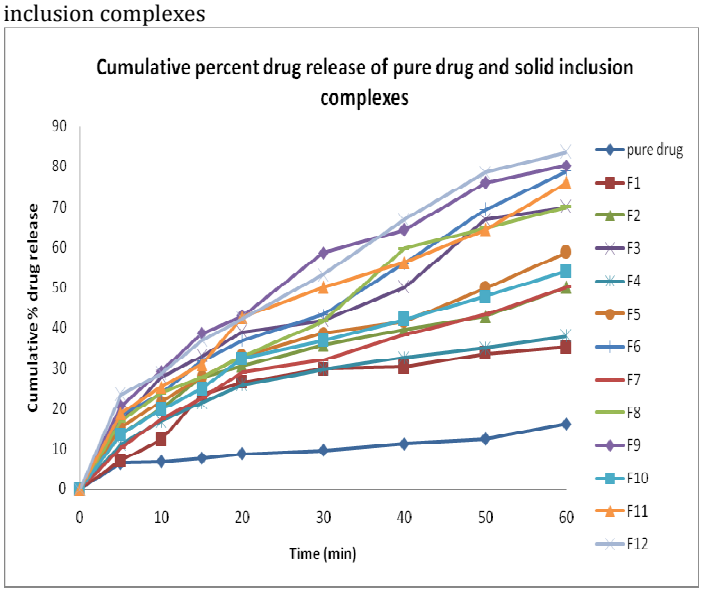

Fig. 6: Cumulative % drug release of pure drug and CD solid inclusion complexes

Differential Scanning Calorimetry (DSC): DSC thermo grams of FFB and its solid inclusion complexes with β-CD and HP β-CD (F3,F6,F9 & F12) were shown in the Figures 7 (a), (b), (c), (d) & (e) respectively.

A DSC thermo gram of FFB exhibited a sharp exothermic peak at 83.3 ^{0}C corresponding to its melting point. In the DSC thermo grams of β-CD inclusion complexes, this peak was shifted to lower temperatures i.e., 81.25 ^{0}C and 79.57 ^{0}C with drug 1:3 complexes prepared by the physical mixing method and kneading method respectively (F3&F6).

In the DSC thermo grams of HP β-CD inclusion complexes, this peak was shifted to lower temperatures i.e., 79.56^{0}C and 79.83^{0}C with drug 1:3 complexes prepared by the physical mixing method and kneading method respectively (F9&F12).

The intensity of peak is reduced in the case of inclusion complexes when compared to the pure drug. The DSC patterns thus suggest an interaction between FFB with β-CD and HP β-CD and physical conversion of FFB into a solid solution form of higher concentration of the carriers. The rapid dissolution and higher dissolution efficiency values observed with solid inclusion complexes is due to this interaction and physical conversion of FFB into solid solution form.

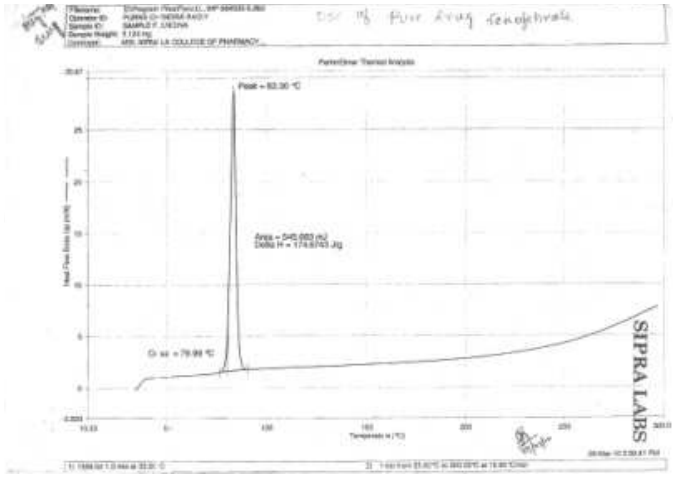

Fig. 7 (a) DSC thermogram of Fenofibrate

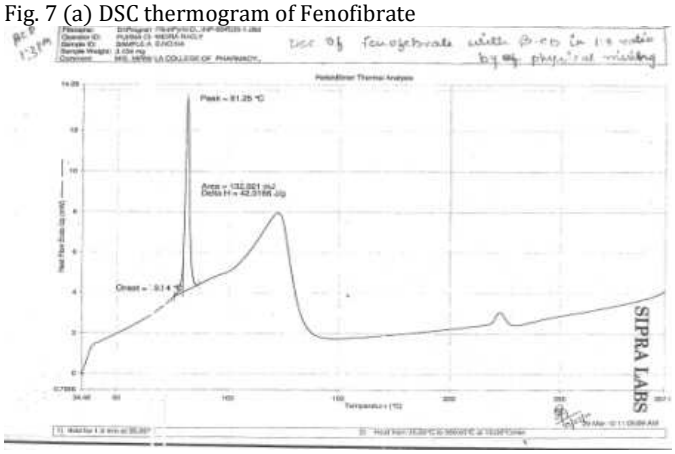

Fig. 7 (b) DSC thermogram of F3 - FFB: β-CD PM (1:3)

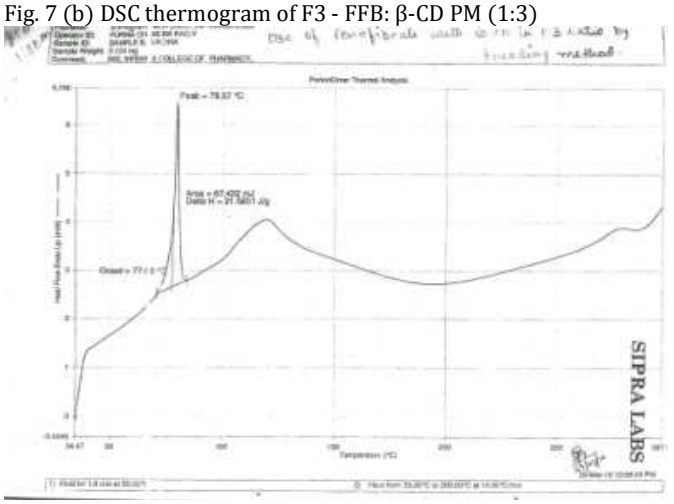

Fig. 7 (c) DSC thermogram of F6 - FFB: β-CD KM (1:3)

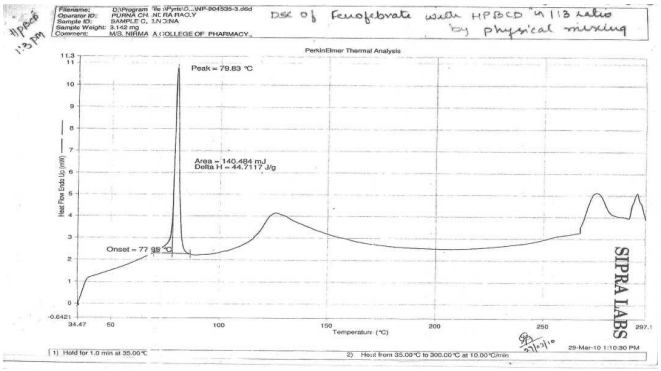

Fig. 7 (d) DSC thermograms of F9 - FFB: HP β-CD (1:3) PM

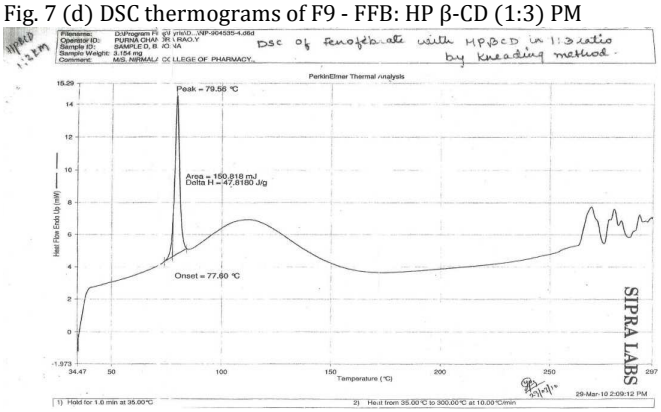

Fig. 7 (e) DSC thermograms of F12 - FFB: HP β-CD (1:3) KM

Infrared Spectroscopy (IR): IR Spectra of optimized inclusion complexes of FFB and its solid inclusion complexes with β-CD and HP β-CD (F3,F6,F9 & F12) were shown in the Figures 8 (a), (b), (c), (d) & (e) respectively.

IR Spectra of optimized inclusion complexes of FFB with β-CD and HP β-CD 1:3 kneading method and physical mixing method with pure drug showed the characteristic peaks of FFB as stretching bond of the CO group at 1729.66cm^{-1} and aromatic CH stretching at 3395 cm^{-1} ,C=C stretching at 1599.16 cm^{-1} and aliphatic C=H stretching at 2900 cm^{-1}. This indicates that there is no chemical modification of drug with excipients.

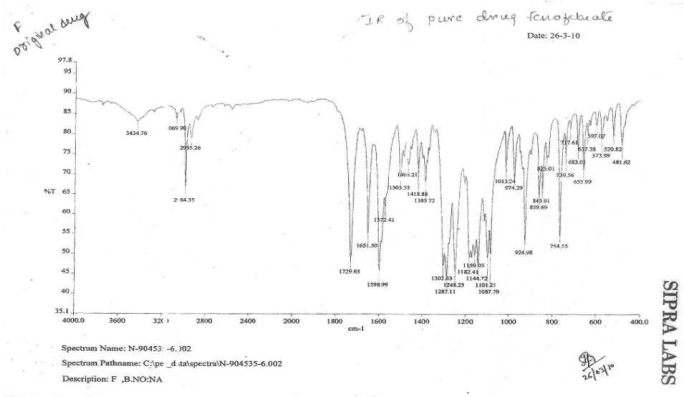

Fig. 8: (a) FTIR Spectra of Fenofibrate

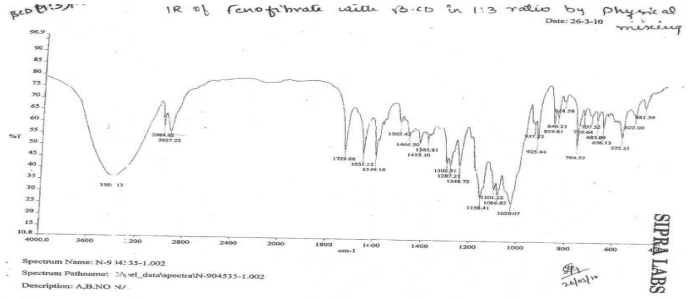

Fig. 8: (b) FTIR Spectra of F3 - FFB: β-CD PM (1:3)

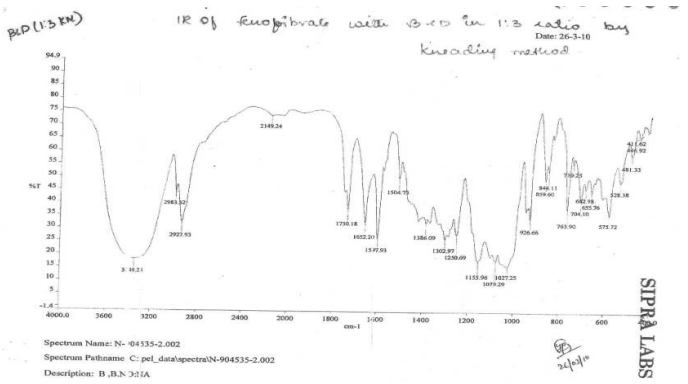

Fig. 8: (c) FTIR Spectra of F6 - FFB: β-CD KM (1:3)

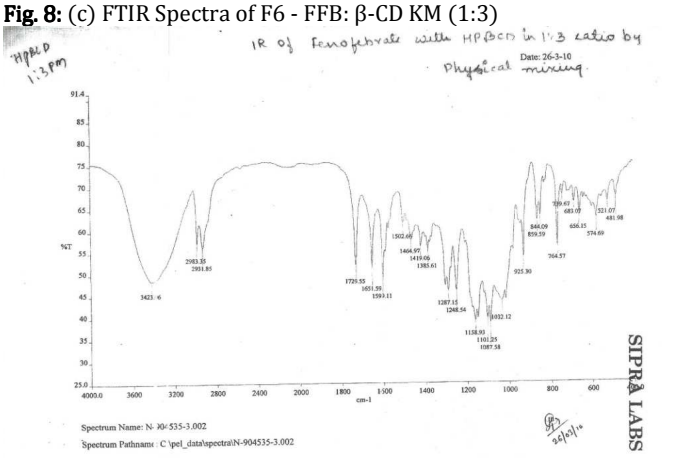

Fig. 8: (d) FTIR Spectra of F9 - FFB: HP β-CD (1:3) PM

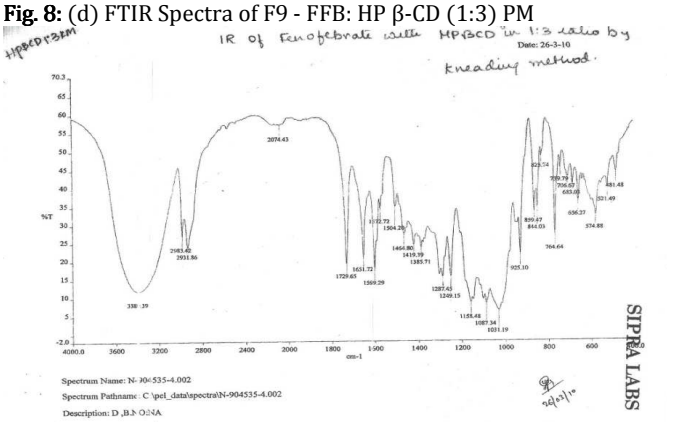

Fig. 8: (e) FTIR Spectra of F12 - FFB: HP β-CD (1:3) KM

CONCLUSION

The aqueous solubility of FFB was increased linearly as function of concentration of the CD in each case. The phase solubility diagram is classified as type AL. The stability constants were in the range 200 to 500 M indicating that complexes formed between drug and CD are quite stable. The dissolution of FFB from complexes followed first order kinetics. All the dissolution parameters indicated rapid and higher dissolution of FFB from CD complexes when compared to pure drug. Solid complexes prepared by kneading method exhibited higher dissolution rate and dissolution efficiency values than those prepared by physical method. A 21 fold increase in dissolution rate of FFB was observed with β-CD 1:3 inclusion complex prepared by kneading method and 30 fold increases in dissolution rate of FFB was observed with HP β-CD 1:3 inclusion complex prepared by kneading method. Thus cyclodextrin complexation could be employed for the enhancement of solubility and dissolution rate of the selected drug Fenofibrate.

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