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Enhancing solubility and dissolution of celecoxib by freeze drying using β-cyclodextrin

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ABSTRACT

Celecoxib, a selective COX-2 inhibitor, exhibits poor water solubility, dissolution and flow properties. Thus, the aim of the present study was to improve the solubility and dissolution rate of Celecoxib by preparing microparticle by Freeze drying technique. Celecoxib microparticle containing different ratio of drug and β-cyclodextrin were manufactured by Freeze drying using Isopropyl alcohol as solvent to enhance solubility and dissolution rate. The prepared microparticle containing different ratio of drug and polymer were evaluated for in vitro dissolution and solubility. The prepared formulations were characterized by scanning electron microscopy, differential scanning calorimeter, X-ray diffraction and Fourier transform infrared spectroscopy. Dissolution profile of the Freeze dried microparticle was compared with its physical mixture and pure sample. Freeze dried microparticle exhibited decreased crystallinity. The solubility and dissolution of the microparticle containing different ratio of drug and β-cyclodextrin were significantly improved compared with its physical mixture and pure sample of Celecoxib. Dissolution of microparticle containing 3:1 w/w (FD 3) showed higher % release i.e. 98.6 % in 30 min compared to other ratio of microparticle. Consequently, hence, from the above result it can be concluded that Freeze dried microparticle of Celecoxib is a useful technique to improve the solubility and dissolution of poorly water soluble drug like Celecoxib.

Introduction

Celecoxib, 4-[5-(4-methylphenyl)-3-(trifluoromethyl) - 1*H*-pyrazol-1-yl] benzene sulphonamide, belongs to a novel class of agents that selectively inhibit cyclooxygenase- 2 (COX-2) enzymes. The introduction of this first selective COX-2 inhibitor (375-fold selectivity) ([1, 2]) in the pharmaceutical market revolutionized the treatment of osteoarthritis (OA), rheumatoid arthritis (RA), and management of pain. It is one of the top selling molecules (ranked 8th), with a worldwide sales of $2614 million in year 2000, ([3, 4, 5]). US FDA has approved its use in OA, RA, and dysmenorrhea with dose strengths of 100–200 mg once/twice daily. According to the biopharmaceutical classification system (BCS), celecoxib is an extreme example of a class II compound meaning that its oral bioavailability is determined by its dissolution rate in the GI tract ([6-8]). Consideration of the modified Noyes-Whitney equation provides some hints as to how the dissolution rate of very poorly soluble compounds might be improved to minimize the limitations to their oral availability. There have been numerous efforts to improve drug dissolution rates. These include (a) reducing the particle size to increase the surface area; (b) using water-soluble carriers to form inclusion complexes; (c) solubilization in surfactant systems; (d) using pro-drugs and drug derivatization; and (e) manipulation of the solid state of drug substances to improve the drug dissolution i.e. by reducing the crystallinity of drug substances through formation of solid dispersions. However, there are practical limitations to these techniques ([9]). Although particle size reduction is commonly used to increase the dissolution rate, there is a practical limit to the size reduction that can be achieved by methods such as controlled crystallization and grinding. The use of very fine powders in a dosage form may also be problematic because of handling difficulties and poor wettability. Salt formation is not feasible for neutral compounds and the synthesis of appropriate salt forms of drugs which are weakly acidic or weakly basic may often not be practical. Even when salts can be prepared, an increased dissolution rate in the gastrointestinal tract may not be achieved in many cases because of the reconversion of salts into aggregates of their respective acid or base forms. The solubilization of drugs in organic solvents or in aqueous media by the use of surfactants and co solvents leads to liquid formation that is usually undesirable from the viewpoints of patient acceptability and marketing ([10]). Solid dispersions have been widely used to enhance the solubility, dissolution rate, and bioavailability of poorly soluble drugs ([11-14]). There are different types solid dispersion systems categorized according to the physical states of the drug and the carrier in the systems. It may be a molecular solid solution, a dispersion of amorphous or crystalline drug particles in an amorphous carrier matrix, or a combination of a solution and dispersion of solids.

The enhancement in the dissolution rate is obtained by one or a combination of the following mechanisms: eutectic formation, increased surface area of the drug due to precipitation in the carrier, formation of true solid solution, improved wettability, and drug precipitation as a metastable crystalline form or a decrease in substance crystallinity. The type of solid dispersion formed depends on both the carrier-drug combination and the method of manufacture. Freeze drying is one of the techniques for preparing solid dispersion and is widely used as an alternative to milling to reduce particle size ([15, 16, 17]). The

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large surface area of the resulting particle should result in an enhanced solubility and dissolution rate, consequently, improved bioavailability. The aim of the present study was to improve the solubility and dissolution rate of celecoxib by freeze drying technique using different ratio of β-cyclodextrin.

Method And Material

Materials

Celecoxib and β-cyclodextrin were obtained as a gift sample from Micro labs, Bangalore, India. All chemicals and buffers used were of analytical grade.

Preparation of Celecoxib Microparticle

The Freeze dried microparticle was prepared by Freeze-drying technique. The Freeze drying was performed by Mini Freeze Dryer LSD -48; (Jay instrument & systems Pvt. Ltd. Mumbai). The different drug–polymer ratios used for various microsphere formulations were prepared as described in Table 1. The β-cyclodextrin solution was prepared by adding given quantity of β-cyclodextrin to the water and Isopropyl alcohol as solvent system. The given quantity of Celecoxib was added to the β-cyclodextrin solution and the resulting mixture was Freeze-dried. The Freeze drying parameters are described in Table 2.

Preparation of physical mixtures of Celecoxib

Physical mixtures (PM) were prepared by mixing Celecoxib and β-cyclodextrin (in the same ratio as used for Freeze dried) in a mortar for 5 min and then sieving (<355μm).

Evaluation of microparticle

Determination of percentage yield and Drug content

The percentage yield of each formulation was determined according to the total recoverable final weight of microparticle and the total original weight of Celecoxib.

Microparticles (50 mg) were triturated with 10 ml of water. Allowed to stand for 10 min with occasional swirling and methanol was added to produce 100 ml. After suitable dilution, samples were measured at 251 nm using a UV spectrophotometer.

Differential scanning calorimeter (DSC)

A DSC study was carried out to detect possible polymorphic transition during the crystallization process. DSC measurements were performed on a DSC DuPont 9900, differential scanning calorimeter with a thermal analyzer.

Fourier transforms infrared spectroscopy (FTIR)

The FTIR spectral measurements were taken at ambient temperature using a Shimadzu, Model 8033 (USA). Samples were dispersed in KBr powder and the pellets were made by applying 5 ton pressure. FTIR spectra were obtained by powder diffuse reflectance on FTIR spectrophotometer.

Powder X-ray diffraction analysis

X-Ray powder diffraction patterns were obtained at room temperature using a Philips X' Pert MPD diffract-meter, with Cu as anode material and graphite monochromatic, operated at a voltage of 40 *mA*, 45 kV. The process parameters used were set as scan step size of 0.0170 (2θ).

Scanning electron microscopy (SEM)

Scanning electron microscopic (Joel- LV-5600, USA, with magnification of 250x) photographs were obtained to identify and confirm surface nature of the microparticle.

Mechanical Properties

Tensile strength of microparticle was determined by compressing 500 mg of crystals using hydraulic press at different ton/cm^2 for 1 min. The compacts were stored in desiccators for overnight to allow elastic recovery. The thickness and diameter were measured for each compact. The hardness of each compact was then measured using Pfizer hardness tester. The tensile strength (σ) of the compact (ton/cm^2) was calculated using following equation.

$$\sigma = 2F/\pi Dt$$

Where, F, D and t are hardness (ton), compact diameter (cm) and thickness (cm), respectively. Each sample was determined in triplicate.

Determination of solubility

Drug solubility was determined by adding excess amounts of pure Celecoxib, its physical mixture and microparticle to water and pH 7.4 phosphate buffer at 37 ± 0.5°C, respectively. The solutions formed were equilibrated under continuous agitation for 24 h and passed through a 0.8 μm membrane filter to obtain a clear solution. The absorption of the samples were measured using UV spectrophotometric method (UV 1601 A Shimadzu, Japan) at 251 nm and the concentrations in μg/ml were determined. Each sample was determined in triplicate.

Dissolution studies of microparticle

The dissolution of Celecoxib commercial sample, microparticle and physical sample was determined by using USP dissolution apparatus XXIV-Type II (Electro Lab, Mumbai). Dissolution medium was 900 ml pH 7.4 phosphate buffer. The amount of dissolved drug was determined using UV spectrophotometric method (UV 1601 A Shimadzu, Japan) at 251nm. Each sample was determined in triplicate.

Determination of the physical stability

A Long term and accelerated stability study of prepared microparticles (FD-3) was carried out at 25^{0}C and 60% relative humidity for 12 months and 40 ^{0}C and 75% relative humidity for 6 month respectively according to the ICH guidelines. The crystals were packed in high density polyethylene (HDPE) container and placed in stability chamber. The samples were withdrawn at the interval of 0, 1, 3, 6, 9 and 12 month and evaluated for appearance, characterization by FT-IR, XRD, drug content and *in vitro* drug release and compared with freshly prepared crystals.

Results And Discussion

The different ratio of Freeze dried microparticle was collected and found to be free-flowing and white to off white in color. The percentage yield of Freeze dried microparticle of different ratio of β-cyclodextrin and Celecoxib were tabulated in Table no 3. This small yield can be increased by addition of solid substance in large scale production. Drug content for the Freeze dried microparticle of different ratio of β-cyclodextrin and Celecoxib were showed in Table no 3.

DSC curves obtained for pure material, physical mixtures and microparticle are showed Fig. 1. In DSC curve, pure Celecoxib had a sharp endothermic peak at 160°C that corresponded to the melting point of Celecoxib. In DSC study, as the amount of β-cyclodextrin increased in microparticle, the size of the Celecoxib endothermic peak was suppressed. In formulations number Physical mixture 1, 2 and 3, no change in Celecoxib endothermic peak was observed but dispersed. In case of microparticle, the two melting transitions in the system made up of Celecoxib and β-cyclodextrin indicated that both materials formed a separate phase. It was found that Celecoxib was in a crystalline state in the microparticle. The position of the melting peak of β-cyclodextrin remained largely unchanged, while that of Celecoxib shifted depending on the concentration. In formulation of microparticle FD 1, 2 and 3, the endothermic peak of Celecoxib was no longer observed. This could be because Celecoxib was molecularly or amorphously dispersed in the phases suggesting that the absence of crystallinity or presence of amorphous state in the microparticle. On the other hand, the physical mixtures of Celecoxib and β-cyclodextrin

showed an apparent endothermic peak for Celecoxib at around ~160°C.

Fig 1 Shows DSC Spectrum of pure Celecoxib & it's different ratio of physical mixture and microparticle.

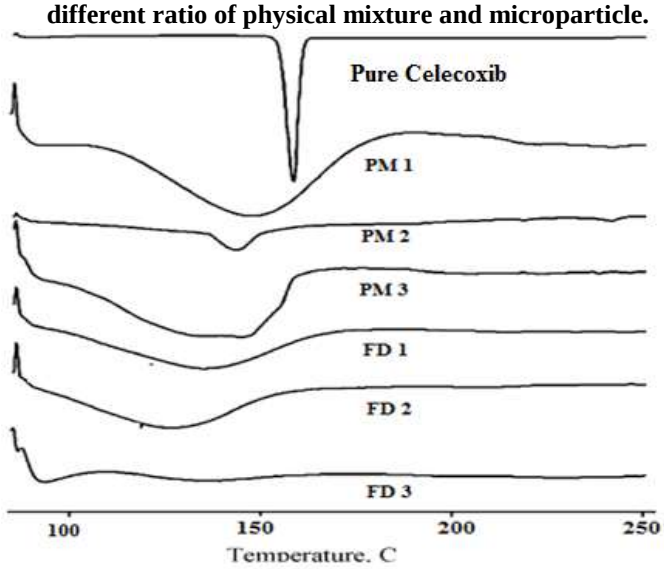
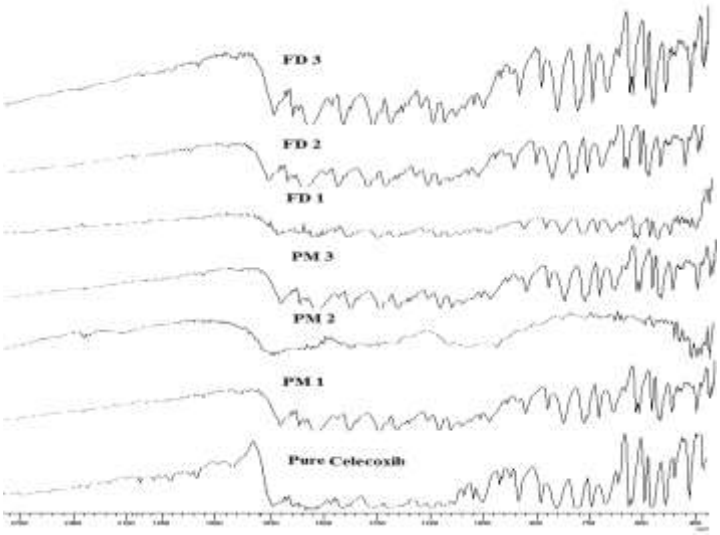
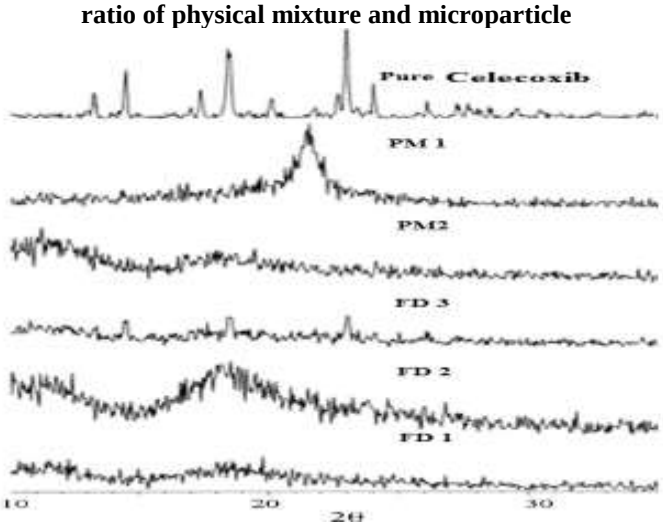


Fig 2. Shows FT-IR Spectrum of pure Celecoxib, its different ratio of physical mixture and microparticle



FT-IR is a very powerful technique in detecting presence of interaction in drug-carrier microparticle. The appearance or disappearance of peaks and/or the shift of their positions are often an indication of interactions such as hydrogen bonding. FTIR spectroscopy has been successfully used for exploring the differences in molecular conformations, crystal packing and hydrogen bonding arrangements for different solid-state forms of an organic compound. Spectral variations originate due to alteration in bonds that exhibit characteristic vibrational frequencies, leading to frequency shifts and splitting in absorption peaks. The FTIR spectra of celecoxib (Fig. 2) showed a characteristic S=O symmetric and asymmetric stretching at 1164 and 1347 cm^{-1}, respectively. Medium intensity bands at 3338 and 3232 cm^{-1} were seen as a doublet, which are attributed to the N–H stretching vibration of $–SO_2NH_2$ group. The C–N stretching band was observed at 1397 and 1388 cm^{-1} for prepared crystals (Fig. 2), but in case of recrystallized sample these same bonds were shifted to lower frequencies at 1374 and 1379 cm^{-1} respectively. The shifts in frequencies indicate the possibility of hydrogen bonding between the –C=O group of solvents and $–NH_2$ group of sulfonamide moiety present in celecoxib. This hydrogen bonding leads to increase in negative charge over oxygen atom caused by shift of electrons of –C=O group, resulting in the weakening of its double bond character. Hydrogen bonding alters the force constant of C=O as well as C–N, thus altering the frequency of stretching and bending vibrations. The bands corresponding to N–H stretching of $–NH_2$ group became diffused and broadened in case of crystals and also a shift to lower frequency (1339 cm^{-1}) was observed in asymmetric stretching of $–SO_2$ group. This clearly indicates the participation of $–NH_2$ and $–SO_2$ groups in intermolecular hydrogen bonding between celecoxib molecules. The spatial arrangement of celecoxib molecules in crystal lattice does not allow intermolecular hydrogen bonding which starts to occur once the orderliness of crystalline lattice is disturbed by formation of amorphous form. Hence above result reveal that there were no significant changes in IR spectra of celecoxib samples. The spectrum of physical mixture shown in Figure 2 was simple summation of pure drug and β-cyclodextrin, revealing no perceptible interaction between the two components. Little evidence could be deduced from the carbonyl band region, probably due to the strong absorption of the carbonyl group of the polymer. In contrast, FTIR spectra of Celecoxib, physical mixture and Freeze-dried microparticle sample display different absorption bands in 3200– 3600 cm−1, with the N–H or O–H stretching vibration completely lost (Fig. 2). This observation, combined with the XRD result, can be attributed to the formation of hydrogen bonding between OH or NH group of Celecoxib with the carbonyl group of β-cyclodextrin. The prepared microparticle containing Celecoxib with β-cyclodextrin showed the characteristic peaks of the drug and the β-cyclodextrin. This suggests the absence of any interaction between the drug and β-cyclodextrin.

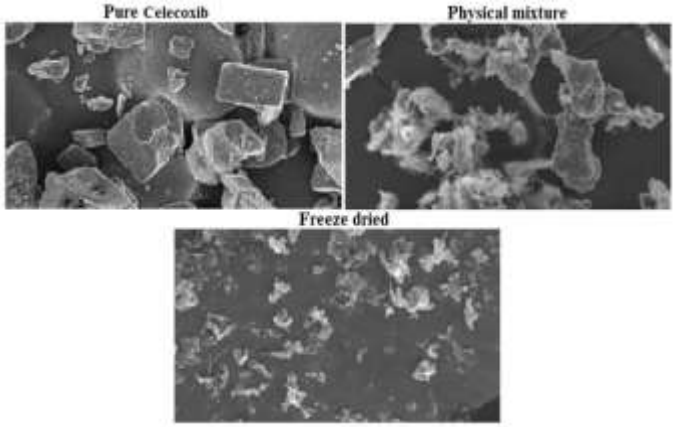
Fig 3. Shows XRD Spectrum of pure Celecoxib & different ratio of physical mixture and microparticle



X-Ray diffraction was used to analyze potential changes in the inner structure of Celecoxib nanocrystal during the formulation. The extent of such changes depends on the chemical nature and physical hardness of the active ingredient. The powder X-ray diffraction patterns of the unprocessed Celecoxib and β-cyclodextrin, their different physical mixture and microparticle formed by Freeze drying are shown in Fig. 3. The characteristic peaks of the Celecoxib appeared in the 2θ range of $10–30^0$ indicating that the unprocessed Celecoxib was a crystalline material. In XRD thermograph of pure Celecoxib powder, physical mixture and prepared microparticle showed that crystallinity of Celecoxib in the formulations was not affected significantly. The x-ray diffraction pattern of the pure drug exhibit its characteristic diffraction peaks at various diffraction angles indicating the presence of crystallinity. The

diffraction study of the different physical mixture of drug and β-cyclodextrin showed the peak corresponding to the crystalline drug molecules present in the mixture, although their intensity was lower than pure drug which may be due to the high percentage of β-cyclodextrin & drug ratio employed. The diffraction pattern of the different Freeze dried microparticle of drug showed absence, broadening, and reduction of major Celecoxib diffraction peaks indicating that mostly an amorphous form (disordered state) existed in the microparticle.

Fig 4. Shows SEM photographs of different sample of Celecoxib



The SEM image of the A) Celecoxib, B) their physical mixture and C) microparticle are shown in Fig. 4. The Celecoxib particles in the physical mixture were broken into much smaller ones, irregular size and result show that Celecoxib particles could be seen in the physical mixture and on the other hand, the shape of microparticle were small in size and micrograph of microparticle shows a matrix formation in which no crystals of Celecoxib could be seen.

Fig 5. Shows Tensile strength of pure Celecoxib, physical mixture and microparticle

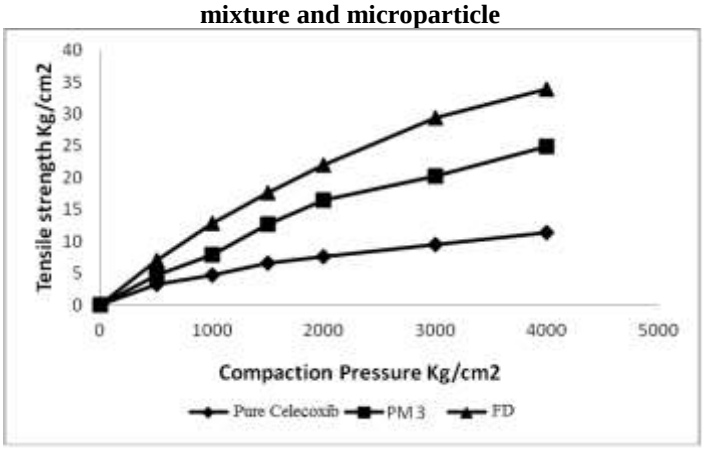
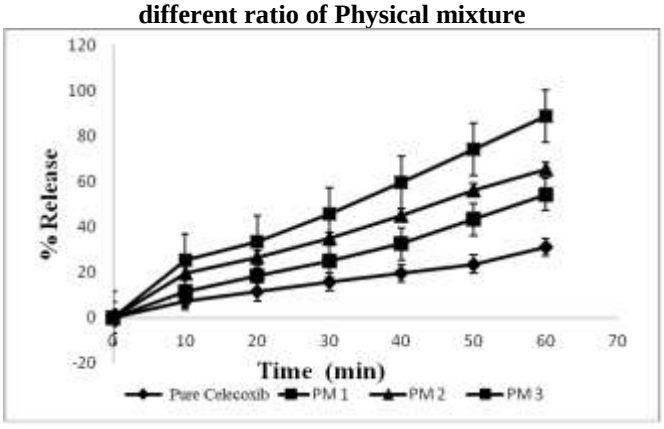


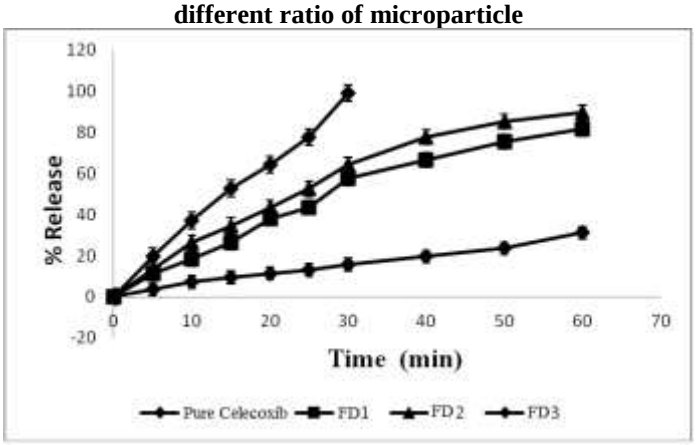
Figure 6. Shows Dissolution of pure Celecoxib and its different ratio of Physical mixture



Microparticle exhibited superior compressibility characteristics compared to Physical mixture and pure sample of Celecoxib drug crystals (fig. 5). It could be due to the fact that during the process of compression fresh surfaces are formed by fracturing crystals. Surface freshly prepared by fracture enhanced the plastic inter particle bonding, resulting in a lower compression force required for compressing the microparticle under plastic deformation compared to that of single crystal. Tensile strength of the same ratio of microparticle and physical mixture (3:1 w/w) showed that tensile strength of microparticle is higher than physical mixture. But tensile strength of microparticle ratio of 3:1 (FD 3) show much higher than any ratio of microparticle, physical mixture (PM 3) and pure sample this may be due to the increasing in the plastic inter particle bonding of microparticle.

The solubility of Celecoxib, their physical mixture and microparticle in water and in pH 7.4 phosphate buffer is shown in Table 3. The dissolution curve of Celecoxib in pH 7.4 phosphate buffer is shown in Fig. 6. These results show that the solubility of Celecoxib increased on increasing the concentration of β-cyclodextrin in microparticle. The solubility of Celecoxib from the microparticle was significantly higher than from it is physical mixture. When the microparticle and physical mixture contained the same weight ratio of Celecoxib i.e. 3:1 w/w(FD 3 & PM 3), it was found that the solubility of Celecoxib from microparticle is much higher than physical mixture of same % in pH 7.4 phosphate buffer as well as in water. The higher solubility of Celecoxib from microparticle may be due to the increase in surface area, wet-ability of microparticle and solubilizing effect of the β-cyclodextrin as carrier to microparticle.

Figure 6 Shows Dissolution of pure Celecoxib and its different ratio of microparticle



The dissolution rate profiles were plotted as the % release from the different freeze dried microparticle, physical mixture and pure Celecoxib versus time in minutes. The rate of dissolution of pure Celecoxib was slow Compared to physical mixtures and different microparticles in 60 min. The % release of microparticle containing ratio of 3:1(FD 3) showed high drug release when compared to other ratios microparticle, its physical mixture and pure Celecoxib. There was a significant difference in the drug release between the microparticle, physical mixture and pure sample. The increase in dissolution from the Freeze dried microparticles was probably due to the wetting and solubilizing effect of the β-cyclodextrin, which could reduce the interfacial tension between the Celecoxib and the dissolution medium and large surface area of the resulting microparticle thus leading to a high dissolution rate and thereby improve the bioavailability.

Table 1. Freeze-Dried microparticle formulation

Numbers	Formulation numbers	Different ratio of β-cyclodextrin and drug (w/w)
1	FD 1	1:1
2	FD 2	2:1
3	FD 3	3:1
4	PM 1	1:1
5	PM 2	2:1
6	PM 3	3:1

Table 2. Freeze-Drying Parameters

Inlet temperature (°C)	Feed pump speed %	Vacuum (mm Wc)	Aspirator level (kg/cm2)
115	12	-75	2.0

Table 3. Solubility of Celecoxib microparticle at different ratios of β-cyclodextrin in water and pH 7.4 phosphate buffer

Different formulations containing polymer: Drug ratio(w/w)	Concentration of Celecoxib microparticle in water (µg/ml) SD±3	Concentration of Celecoxib microparticle in pH 7.4 µg/ml SD±3	Percentage yield%	Drug content SD ±3	Particle size determination (µm) SD±3
Pure drug	0.037	1.84			
FD 1	01.01	22.29	58.78	96.11±0.02	1-2
FD 2	15.67	28.93	59.64	97.94±0.23	1-3
FD 3	21.98	39.73	85.53	98.73±-0.21	2-4
PM 1	3.04	11.87	-	97.88±0.22	-
PM 2	7.76	18.43	-	99.34±0.01	-
PM 3	10.84	22.73	-	99.63±0.03	-

Table 4: Stability data of Freeze dried microparticle (FD-3) of Celecoxib

Testing interval	Description of Drug	FT-IR Study	XRD Study	Drug content (±SD)	Dissolution Study (±SD)
Sample name: Celecoxib Freeze dried microparticle Storage condition: 40^0C /75% RH					
Initial	White to off white	As standard	As standard	99.87±0.022	98.60±0.011
1 month	Complies	Complies	Complies	99.64±0.021	97.35±0.041
3 month	Complies	Complies	Complies	99.73±0.011	97.33±0.01
6 month	Complies	Complies	Complies	99.57±0.043	97.62±0.05
Sample name: Celecoxib Freeze dried microparticle Storage condition: 25^0C /60% RH					
Initial	White to off white	As standard	As standard	99.12±0.01	98.60±0.011
1 month	Complies	Complies	Complies	99.08±0.02	98.39±0.040
3 month	Complies	Complies	Complies	99.04±0.01	97.28±0.027
6 month	Complies	Complies	Complies	98.89±0.03	98.89±0.013
9 month	Complies	Complies	Complies	98.42±0.02	98.57±0.023
12 month	Complies	Complies	Complies	98.37±0.04	97.77±0.037

The best way to guarantee stability is by maintaining their physical state and molecular structure. The results of the stability study of prepared microparticles (FD-3) of Celecoxib stored at 25 ^{0}C and 60% relative humidity for 12 month and 40 ^{0}C and 75% relative humidity for 6 month is presented in table 4. The influence of physical stability on the prepared crystals was investigated. Prepared Freeze dried microparticles of Celecoxib were stable and complied with all the properties when compared to initial results of prepared microparticles of Celecoxib.

Conclusion

In this present study, an increased solubility and dissolution rate of Celecoxib were achieved by Freeze dried microparticle using different ratio of β-cyclodextrin. Freeze dried microparticle exhibited decreased crystallinity compare to its physical mixture and pure Celecoxib. DSC and XRD studies showed that there is no change in the crystal structure of Celecoxib during the Freeze drying process i.e., polymorphism has not occurred. The solubility and dissolution of the Freeze dried microparticle was improved significantly compared with its physical mixture and pure sample. The drug dissolution rate from microparticle was highest at the polymer-drug ratio of 3:1 w/w (FD 3). Hence this Freeze drying technique was very simple method & can be used for formulation of tablets of Celecoxib by direct compression without further process like (mixing, granulation) with directly compressible tablet excipients.

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