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Preparation and Evaluation of Freeze Dried Crystals of Ketoprofen using Lyophilization Monophase Solution Technique for Direct Compression Tablets

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ABSTRACT

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Ketoprofen, an anti-inflammatory drug, exhibits poor water solubility, dissolution and flow properties. Thus, the aim of the present study was to improve the solubility and dissolution rate of Ketoprofen by preparing crystals by freeze drying technique. Ketoprofen crystals were prepared by freeze drying using Isopropyl alcohol (IPA), chloroform and water as monophasic solvents system to enhance solubility and dissolution rate. The prepared crystals of Ketoprofen were evaluated for IPA and chloroform solvent residual by gas chromatography. The prepared formulations were characterized by scanning electron microscopy, differential scanning calorimeter; X-ray diffraction and Fourier transform infrared spectroscopy. Solubility and Dissolution profile of the freeze dried crystals was compared with its recrystallized sample and pure sample. The samples were stored in stability chamber to investigate their physical stability. Solvent residual of IPA and chloroform were found to be within the toxic level in prepared freeze dried crystals. Freeze dried crystals exhibited decreased crystallinity, the solubility of freeze dried crystals increasing nearly about fivefold than the ketoprofen pure sample. Dissolution of the ketoprofen crystals was significantly improved about three times higher than pure sample of ketoprofen. In stability test, the release profile of the freeze dried crystals was almost unchanged as compared with the freshly prepared freeze dried crystals stored at 40 ^{0}C and 75% relative humidity for 90 days. The dissolution profiles of ketoprofen tablets prepared using freeze dried crystals exhibit greater dissolution behavior than tablets prepared by pure ketoprofen sample. Hence this technique can be used for formulation of tablets of ketoprofen by direct compression with directly compressible tablet excipients.

Keywords: Ketoprofen, Freeze drying, compressibility, Solubility, Dissolution, Stability.

INTRODUCTION

Ketoprofen is a non-steroidal anti-inflammatory drug, scarcely soluble in water, which is widely used as analgesic and for the acute and long-term treatment of rheumatoid arthritis and osteoarthritis and adverse effects, like gastrointestinal mucosa ulceration, restrict its oral use and make it a good candidate for transdermal administration[1, 2]. However, due to the excellent barrier function of the skin, the need to use safe and effective enhancers for improving transdermal absorption of drugs is well recognized[3, 4].

Most of the NSAIDs belong to class II category under Biopharmaceutical classification system (BCS) i.e., they are inherently highly permeable through biological membranes, but exhibit low aqueous solubility. Rate of absorption and / or extent of bioavailability for such insoluble hydrophobic drug are controlled by rate of dissolution in gastro-intestinal fluids. However, its oral bioavailability is very low, probably due to poor solubility in water and insufficient dissolution rate[5,6].

Formulation and manufacture of solid oral dosage forms, and tablets in particular, have undergone rapid change and development over the last several decades. One of the most revolutionary technologies is that of direct compression. Direct compression is economical, facilitates processing without the need of moisture, heat and involves small number of processing steps. In direct tabletting method, it is necessary to increase flowability and compressibility of the bulk powder in order to retain a steady supply of powder mixture to the tabletting machine and sufficient mechanical strength of the compacted tablets[7, 8]. In addition to increasing efficiency of the manufacturing process it is also important to increase bioavailability of the drug by improving the solubility of the bulk drug powder. Freeze drying is one of such techniques to improve the micromeritic properties and dissolution of drug because of its crystal in pours in nature and very small in size (nm).

Currently, several solubilization techniques were applied and reported to enhance the solubility of poorly water soluble drugs, However including few literature are available on enhancing the solubility and dissolution of ketoprofen formation of inclusion complexes with cyclodextrin[9] and skimmed milk were carried out[10]. To enhanced the dissolution rate and bioavailability of ketoprofen, a novel dry elixir dosage form was proposed[11]. The formation of ketoprofen solid dispersion, ketoprofen-dextran ester prodrug has been reported to improve ketoprofen solubility and dissolution[12, 13].

Enhancement of solubility and dissolution of ketoprofen using high dispersion homogenizer[14], The objective of the

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present study was to prepare freeze dried crystals of ketoprofen using freeze drying technique and was evaluated for the solvents residual in freeze dried crystals and DSC, FT-IR, XRD, and SEM analysis were performed to determine the physicochemical properties of the freeze dried crystals and compare with recrystallized sample and pure drug and determined the solubility and dissolution characteristics of the ketoprofen freeze dried crystals and investigate their physical stability in a climate chamber at 40^0C and 75% relative humidity (RH) for 90 days.

MATERIALS AND METHODS

Ketoprofen was obtained as a gift sample from Micro Lab. Bangalore., India. All chemicals and buffers used were of analytical grade.

Preparation of freeze dried crystals of ketoprofen

Ketoprofen (4 g) was dissolved in 25 ml of isopropyl alcohol (IPA) heated at 45^0 until a clear solution was obtained. The drug solution was slowly poured in to 75 ml solvents consists of water (60 ml) and chloroform (15ml) maintained at room temperature. This solution was shifted to 100 ml glass bottle and then transferred to a ultra low freezer at -40^0 and kept in the freezer for 24 h. the frozen drug solution was placed in a lyophilizer for 72 h using a Freeze Dryer (ILSHIN) Lab. Co. Ltd. Korea) with a condenser temperature of -40^0C and a pressure of 7×10^{-2} mbar followed by a secondary drying at 25^0 for 24 hr. The resulted crystals were stored in desiccator at room temperature until further experiment.

Recrystallization of ketoprofen (RS)

Ketoprofen (4 g) was dissolved in 25 ml IPA heated at 45^0 and mixture of 15 ml of chloroform and 60 ml of water was poured quickly in to drug solution maintained at 20^0C with occasional stirring. The crystals of ketoprofen were collected by filtration and were dried at 45^0 for 12 h. crystals were stored in desiccators at room temperature until further experiment.

Determination of residual solvents in freeze dried crystals by gas chromatography (GC)

Gas chromatography (GC) studies were carried out on SHIMADZU model 2014 (Shimadzu Technologies, Japan) coupled with a split/split less injector, operated in a split-mode and FID. The computer with GC solutions software has been used to control the gas chromatograph. Rtx-5 capillary column (cross bond 5%diphenyl/95%dimethylpolysiloxane) with a length of 30 meters and an internal diameter of 0.25 mm was used throughout the study.

Differential scanning calorimetry (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. The samples were heated in an atmosphere of nitrogen and thermograms were obtained by heating at a constant heating rate of 10^0C/min in the range of 20-110^0C. Thermograms for ketoprofen, recrystallized sample and FDT were obtained.

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectral measurements were taken at ambient temperature using a Shimadzu, Model 8033 (USA). About 2 mg of the pure drug, recrystallized and freeze dried crystals were used separately. Pure drug, freeze dried crystals and recrystallized samples were dispersed in KBr powder and the pellets were made by applying 6000 kg/cm^2 pressure. FTIR spectra were obtained by powder diffuse reflectance on FTIR spectrophotometer.

X-ray diffraction analysis (XRD)

X-Ray powder diffraction patterns were used to detect possible polymorphic transition during the crystallization process. X-Ray powder diffraction patterns were obtained at room temperature using a Philips X' Pert MPD diffractometer, with Cu as anode material and graphite monochromator, operated at a voltage of 40 mA, 45 kV.

Scanning electron microscopy (SEM)

Scanning electron microscopic (Joel- LV-5600, USA, with magnification of 250x) photographs were obtained to identify morphological characters of the crystals. The samples were coated with 25-nm-thick gold using a quick carbon coater.

Mechanical Properties

Tensile strength

Tensile strength of freeze dried crystals was determined by compressing 500 mg of crystals using hydraulic press at different loads for 1 m. The compacts stored in desiccator 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 (kg/cm^2) was calculated using following equation.

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

Where, F, D and t are hardness (kg/cm^2), compact diameter (cm) and thickness (cm), respectively.

Heckel plot

Compressibility of crystals was evaluated by the Heckel equation (1) in triplicate. Crystals were lubricated with magnesium stearate and were compressed (average mass 500 mg ± 2%), at different pressures, up to constant density of compacts using the 13 mm flat faced punch and die set on a hydraulic press (Spectra lab, India). The range of different

pressures applied to get constant density was 1000-5000 kg cm^{-2}. The tablets were stored in airtight moisture-proof containers for 24 hours to enable elastic recovery and hardening. The compressibility behavior was studied using the Heckel equation:

$$\ln [1/(1-D)] = kP + A \quad (1)$$

Where, D is relative density and k and A are constants.

The slope of the straight line portion, k, is the reciprocal of the mean yield pressure, P_y, of the material. From the intercept A, the relative density, D_A, can be calculated using the following equation:

$$D_A = 1 - e^{-A} \quad (2)$$

Relative density of the powder at the point when the applied pressure equals zero, D_0, is used to describe the initial rearrangement phase of densification as a result of die filling.

Relative density, D_B, describes the phase of rearrangement at low pressures and is the difference between D_A and D_0.

$$D_B = D_A - D_0 \quad (3)$$

Solubility studies of crystals

The solubility of ketoprofen freeze dried crystals in water was determined by taking excess quantity of freeze dried crystals and adding to screw- capped 50 ml glass vials filled with water. The vials were shaken for 24 hours on mechanical shakerat the room temperature. The solution was filtered through Whatmann filter paper No.1 and the drug concentration was determined spectrophotometrically at 260 nm.

Dissolution studies of crystals

The dissolution of ketoprofen pure sample, freeze dried crystals and recrystallized sample was determined by using USP dissolution apparatus XXIV-Type II (Electro Lab, Mumbai). Dissolution medium (900ml) consisted of ph 7.2 Phosphate buffer was used and 10 ml of dissolution medium was withdrawn at every 10 min interval for 1 h. The amount of dissolved drug was determined using UV spectrophotometric method (UV 1601 A Shimadzu, Japan) at 260 nm.

Determination the physical stability

To determine the physical stability of freeze dried crystals were placed in a stability chamber maintained at 40^0C and 75% relative humidity (RH). After 90 days, the % drug release of ketoprofen in the freeze dried crystals was determined by dissolution study and compare with freshly prepared freeze dried crystals.

Preparation of ketoprofen tablets:

Ketoprofen conventional tablets were prepared by mixing the pure ketoprofen sample and freeze dried crystals with Avicel-101 and povidone for a period of 10 min in a cubic mixer. The mixture was mixed with sodium starch glycolate and lactose for 10 m. The mixture was compressed on a tableting machine (Rimek, Mumbai), having a punch diameter of 10 mm and equipped with strain gauge (10-400 kg/cm2). Sufficient compression load between 80-100 kg/cm^2 was applied in order to produce tablets hardness of 5-6 kg/cm^2. The formulation prepared with pure sample and freeze dried crystals was denoted as F and F* respectively and each tablet contains 25 mg ketoprofen. The punched tablets were subjected to dissolution study as described under dissolution study of freeze dried crystals.

RESULT AND DISCUSSION

A solvent system involved an Isopropyl alcohol (IPA), chloroform and water for ketoprofen has been used in previous study[14]. The selection of these solvent depends on the miscibility of the solvents and solubility of the drug in individual solvents. IPA is miscible in any proportion with water and chloroform.

Recrystallization of ketoprofen was done to find out the changes in crystal lattice, being induced by solvents, can influence the physicochemical properties of the substance. Hence the mechanical, micromeritic and dissolution properties of freeze dried crystals were compared with pure sample and recrystallized sample. recrystallization of ketoprofen was carried out using same solvent composition as was used for freeze drying.

IPA was found to be suitable freeze drying medium for ketoprofen because of its high solubility in IPA (2g/12.5ml). The controlling of residual IPA was needed though. IPA is a toxic organic solvent based on their concentration and has little detriment effect to human body. Therefore, the low level of both IPA and chloroform in the freeze dried crystals determined by GC and that should not be harmful to both animal and human[16, 17].

Gas chromatography results confirmed that there were 5.7 and 2.57 ppm residual of IPA and chloroform present in the freeze dried crystals spectively, which was much lower than the toxic level 400 & 60 ppm respectively[16, 17].

The low level of both IPA and chloroform in the freeze dried crystals results from its ability to form high surface area crystals and from the fact that the intermolecular forces among both IPA and chloroform molecules are not as strong as those of water. This allows both IPA and chloroform to sublime more completely and easily than water.

The DSC thermograms (fig. 1) show a sharp endothermic peak for all the ketoprofen crystals. The temperature range of the endothermic peak of all the ketoprofen crystals lies in the

range of 94^{0}C to 96^{0}C. Melting points show slight variation as the nature of the crystals might have been affected by the solvent. The melting endotherm for pure ketoprofen sample was 96.58° with enthalpy of (181.01 J/g)[4] whereas in the case of freeze dried crystals melting endotherm was 94.23° with decreased enthalpy of (174.31 J/g) indicating decreased crystallinity. This one step melt is be due to only one crystal form (Triclinic) of the ketoprofen formed during the crystallization process, thus indicating that ketoprofen did not undergo any crystal modification.

All the crystals have exhibited general characteristic peaks at 2983-2930 cm^{-1} (Aromatic C-H stretch carboxylic acid O-H stretch), 1695-1649 cm^{-1} (C=O stretch), 1595 cm^{-1} (Aromatic C=C stretch), 1437 cm^{-1} (CH-CH_3 deformation), 2891 cm^{-1} ((C-H) stretch plus O-H deformation), 1690 cm^{-1} (Carboxylic O-H out of plane deformation), 860-640 cm^{-1} (C-H out of plane deformation for substituted aromatic) (fig. 2)[9]. Specific changes in IR spectra are not very clear, could be due to variations in the resonance structure, rotation of a part of a molecule or certain bonds. Alteration could be due to minor distortion of bond angles, or even a result of the presence of a solvent of crystallization.

X-Ray diffraction was used to analyze potential changes in the inner structure of ketoprofen crystal during the formulation of freeze dried crystals. The characteristic peak of the ketoprofen appeared in the 2θ range of 10–50^0, and all crystal were found triclinic type (a#b#c, α#β#γ) indicating that the unprocessed ketoprofen was a crystalline material. All the samples showed similar peak positions (2θ) in X-ray diffraction, formation of different polymorsphs of ketoprofen was ruled out. However relative intensities of XRD peaks were modified (Fig. 3). The relative intensities of freeze dried crystals reduced more than two times than pure ketoprofen. This could be attributed to the markedly different crystal habits of the samples. Therefore the relative abundance of the planes exposed to the X-ray source would have been altered, producing the variations in the relative intensities of the peak or may be due to differences in crystal sizes[5, 6]. The pure drug exhibits its characteristic diffraction peaks at various diffraction angles indicating the presence of crystallinity. The X-ray diffraction of the RS of drug showed the peak corresponding to the crystalline drug molecules present in the RS, although their intensity was lower than pure drug due to the differences in crystal sizes. The X-ray diffraction pattern of the freeze dried crystals showed that ketoprofen peak intensity was much lower than the pure drug and RS samples of ketoprofen. This could be due the increasing the wettability of freeze dried crystals. These results could explain the observed enhancement of solubility and dissolution of ketoprofen in freeze dried crystals.

SEM study showed that crystals of pure sample are of the smallest size (7-15 μm) and they have irregular shapes. Recrystallized crystals with intermediate size (9-22 μm) and had irregular shapes. The freeze dried crystals were formed by microcrystalline precipitates, so the resultant freeze dried crystals had a smooth surface with small in size ((average particle size 136.8 nm) (Fig. 4).

Freeze dried crystals exhibited superior compressibility characteristics compared to conventional drug crystals (Fig. 5). It could be due to the fact that during the process of

Fig. 1: DSC Thermograms of Ketoprofen Sample.

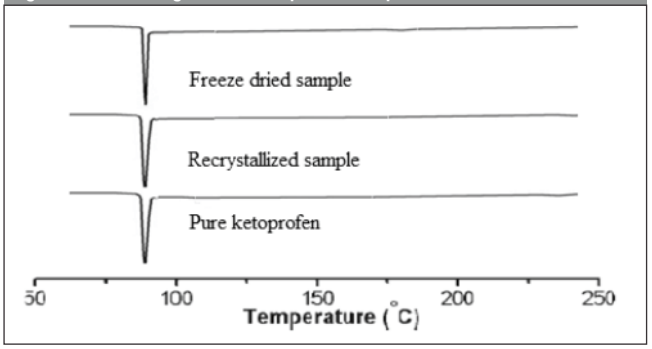


Fig. 2: FT-IR Spectra's of Ketoprofen Samples.

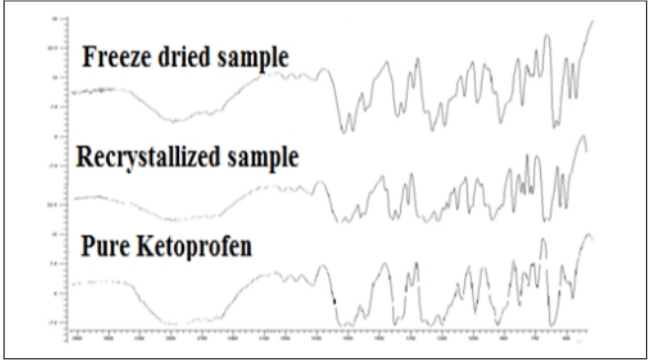
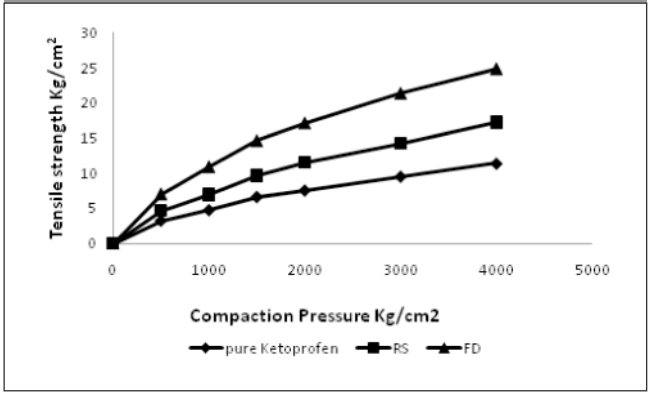


Fig. 5: Tensile strength of pure ketoprofen, Recrystallized sample and Freeze dried crystals



compression fresh surfaces are formed by fracturing crystals. Surface freshly formed by fracture enhanced the plastic inter particle bonding, resulting in a lower compression force required for compressing the freeze dried crystals under plastic deformation compared to that of single crystal[8]. Ketoprofen crystals prepared freeze drying show higher tensile strength compared to recrystallized sample and pure drug sample, hence are suitable for tabletting.

Heckel profile of pure sample and prepared crystals are shown in Fig. 6, and characteristic values of P_Y, D_A, D'_0 and D'_B and elastic recovery are reported in Fig.6 & Table 2. The compression of prepared crystals beginning at lower relative density, while the initial rearrangement phase without pressure increase for pure sample this corresponds to different D'_0 values. D'_B is greater for prepared crystals; indicate a greater brittle fracture tendency of these materials. Elastic recovery is relatively high for a brittle material, but it must be noted that tablets survive the decompression phase and show no sign of capping. Prepared ketoprofen crystals exhibited higher porosity compared to pure sample, hence require lower compression force for compressing under plastic deformation compared to commercial sample.

Freeze dried crystals showed increased solubility than the pure sample in water and increased nearly more than fivefold higher (0.9262 mg/ml) than pure ketoprofen (0.1722 mg/ml). The higher solubility of ketoprofen from freeze dried crystals may be due to the reduction in particle size and increased wettability of ketoprofen in freeze dried crystals[8].

The dissolution profiles of ketoprofen (fig. 7) exhibited improved dissolution behavior for freeze dried crystals than pure sample. The reason for this faster dissolution could be linked to the better wettability and reduction in particle size of the freeze dried. The % drug dissolve in 60 min greatly varied for freeze dried crystals than recrystallized sample and pure sample of ketoprofen.

The results of the stability study of freeze dried crystals stored at 40 ^{0}C and 75% relative humidity for 90 days. The percentage of drug release from freeze dried crystals almost same i.e. (97.18%) after 90 days of storing when compare with the freshly prepared freeze dried crystals i.e. (97.23 %). Indicating that the freeze dried crystals of ketoprofen was stable for 90 days at 40 ^{0}C and 75% relative humidity.

The dissolution of ketoprofen tablets (fig. 8) containing freeze dried crystals exhibited improved dissolution behavior than tablets prepared by pure ketoprofen sample.

CONCLUSION

Freeze dried crystals of ketoprofen were prepared by freeze drying technique. The solvent residual found to be well below

Fig. 6: Heckel plot of pure ketoprofen, Recrystallized sample and Freeze dried crystals

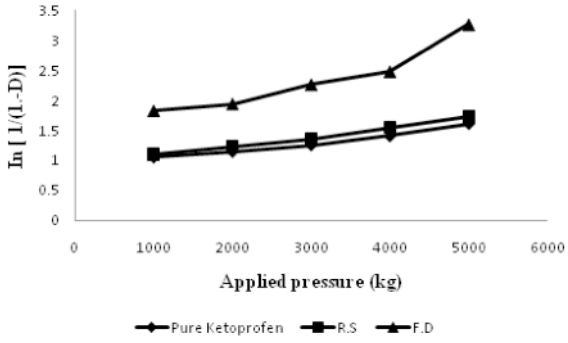


Fig. 3: X-ray diffraction Spectra of Ketoprofen Samples.

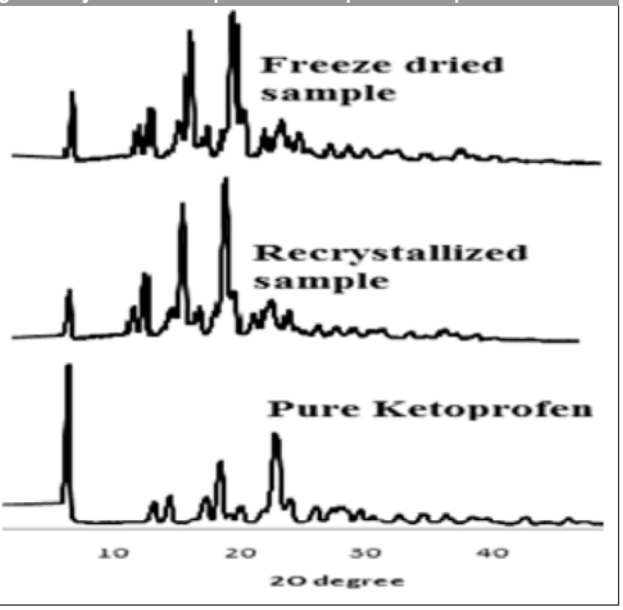


Fig. 4: SEM of Different sample of ketoprofen.

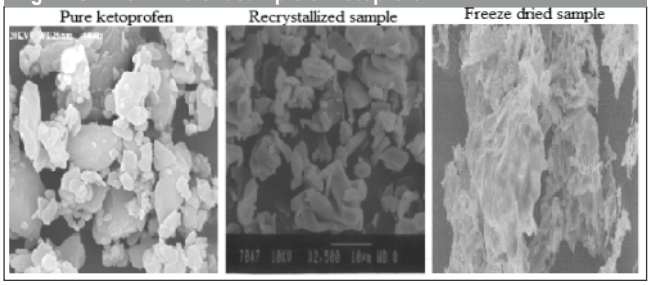


Table 1: Composition of Ketoprofen Tablets

Ingredients	F	F*
Ketoprofen (mg)	25	25
Avicel-101 (mg)	100	100
Sodium starch glycolate (mg)	5	5
Aerosil (mg)	5	5
Lactose (mg)	65	65

Figure 7: Dissolution of pure ketoprofen, Recrystallized sample and Freeze dried crystals

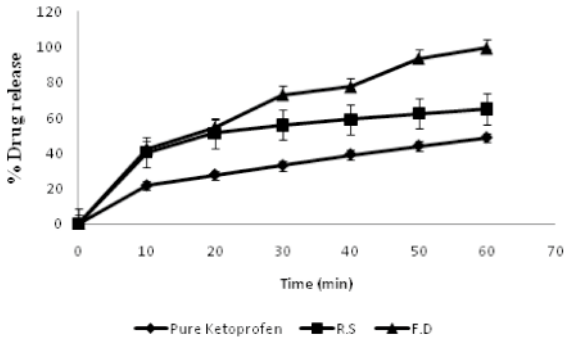


Fig. 8: Dissolution of tablets containing pure ketoprofen and Freeze dried crystals

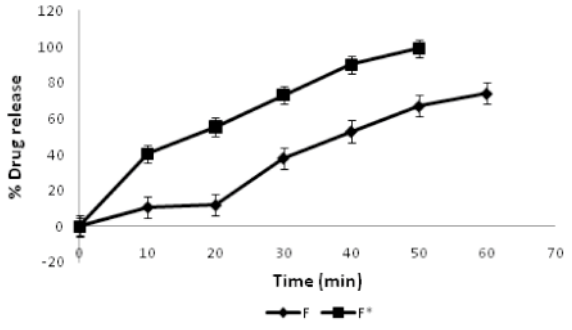


Table 2: Heckel's parameters and elastic recovery of ketoprofen crystals

Parameters	Pure sample	Recrystallized crystals	Freeze dried crystals
P_Y	64.4 ±0.21	66.01±0.31	68.2 ± 0.72
D'_0	0.554 ±0.07	0.484±0.02	0.442 ±0.03
D_A	0.689 ±0.03	0.713±0.02	0.761 ±0.03
D'_B	0.152 ±0.02	0.182±0.01	0.237 ±0.01
Elastic recovery (%)	3.83 ± 0.12	4.37±013	5.30 ± 0.01

the toxic level. Freeze dried crystals exhibited decreased crystallinity and improved mechanical properties. DSC FT-IR and XRD studies showed that there is no change in the crystal structure of ketoprofen during the crystallization process i.e., polymorphism has not occurred. The solubility and dissolution of the freeze dried crystals was improved compared with recrystallized sample and pure ketoprofen sample because of crystals in pours in nature and reduction in particle size of freeze dried crystals . Stability result shows that freeze dried crystals of ketoprofen was stable after 90 days and showed almost same drug release as compare to fresh prepared freeze dried crystals. The dissolution of tablet containing freezes dried crystals shown more drug release than compare to tablets containing pure sample. Hence this technique can be used for formulation of tablets of ketoprofen by direct compression with directly compressible tablet excipients.

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