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IMPROVEMENT OF THE SOLUBILITY AND DISSOLUTION OF TOLFENAMIC ACID USING LYOPHILIZATION TECHNIQUE

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Abstract: The aim of the present study was to prepare freeze dried crystals (FD) of Tolfenamic acid (TA) by freeze drying technique. Crystallization medium used for freeze drying of Tolfenamic acid consisted of *N,N*-dimethylformamide (DMF) and Water as solvent systems. The presence of solvents residuals in FD was determined by Gas chromatography and particles were characterized by DSC, FT-IR, XRD and SEM. The respective solubility study and dissolution behaviour studies were carried out. The samples were stored in stability chamber to investigate their physical stabilities. Residual Solvents in FDs were found to be within the limit and exhibited decreased crystallinity as well solubility and dissolution of the Freeze dried crystals was improved than commercial sample of Tolfenamic acid. In stability study, it was found that physical properties and release profile of the freeze dried crystals was unaffected for 3 months. Hence this technique can be used to obtain modified drug raw material for formulation of tablets of Tolfenamic acid by direct compression with directly compressible tablet excipients.

Keywords: Freeze drying, Tolfenamic acid, Crystallinity, Dissolution, Stability study.

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INTRODUCTION

Tolfenamic acid is a potent anti-inflammatory drug, resembling other fenamates in clinical use, namely mefenamic and Flufenamic acid (1). Together with other drugs from the carboxylic acid family, it is used to treat inflammatory and pain-causing diseases of rheumatic and non-rheumatic origin. Tolfenamic acid has also been used extensively in both human and veterinary medicine for its analgesic and antipyretic properties (2).

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 or 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 (3).

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 tab-letting method, it is necessary to increase flow-ability and compressibility of the bulk powder in order to retain a steady supply of powder mixture to the tab-letting machine and sufficient mechanical strength of the compacted tablets. 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. Thus, one of the major challenges to drug development today is poor solubility, as an estimated 40% of all newly developed drugs are poorly soluble or insoluble in water (4). As a result, much research has been conducted into methods of improving drug solubility and dissolution rates to increase the oral bioavailability of hydrophobic drugs (5,6,7).

The objective of the present study was to prepare Freeze dried crystals of Tolfenamic acid by freeze drying technique and to evaluate them for solvent residuals and characterized by DSC, FT-IR, XRD, and SEM analysis. Solubility and dissolution release study of Tolfenamic acid spherical agglomerates and their physical stability at 40°C and 75% relative humidity (RH) for 3 months were also investigated.

MATERIALS AND METHODS

All chemicals and buffers used were of analytical grade.

Preparation of Freeze dried crystals of Tolfenamic acid (FD)

Tolfenamic acid (3 g) was dissolved in 30 ml of DMF and water solvents systems (3:1) heated at 45^0 until a clear solution was obtained. The drug solution was poured in to 10 ml water maintained at room temperature. Above resulted solution is shifted to 100 ml glass vials and then transferred to a ultra low freezer at -40 ^{0}C and kept in the freezer for 12 hr. the frozen drug solution were placed in a lyophilize for 48 hr using a Freeze Dryer (IISHIN 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}C for 24 hr. The resulted crystals were kept in a desiccator's room temperature until further experiment

Recrystallization of Tolfenamic acid (RS)

Tolfenamic acid (3 g) was dissolved in 40 ml of DMF and water systems (3:1), heated at 30°C with occasional stirring. The crystals of Tolfenamic acid were collected by filtration and were dried at room temperature for 12 hours.

Determination of residual solvents in freeze dried crystals by 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% dimethyl polysiloxane) 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.

Fourier transforms infrared (FTIR) spectroscopy

The FTIR spectral measurements were taken at ambient temperature using a Shimadzu, Model 8033 (USA). About 2 mg of the commercial sample, recrystallized sample and Freeze dried crystals were used separately. Commercial sample, freeze dried crystals and recrystallized samples were dispersed in KBr powder and the pellets were made by applying 6000 kg/cm^2 pressure.

X-ray analysis

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 and confirm spherical nature and morphological characters of the crystals.

Mechanical Properties

Tensile strength of **freeze dried crystals** was determined by compressing 500 mg of crystals using hydraulic press at different kg/cm^2 for 1 min. The compacts were stored in a 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.

Solubility studies

The solubility of Tolfenamic acid 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 shaker. The solution was filtered through Whatmann filter paper No.1 and the drug concentration was determined spectrophotometrically at 290 nm.

Dissolution studies of agglomerates

The dissolution of Tolfenamic acid commercial sample, freeze dried crystals and recrystallized sample was determined by using USP dissolution apparatus XXIV-Type II (Electro Lab, Mumbai). Dissolution medium (900 ml) consisted of pH 7.4 Phosphate buffer and 10 ml of dissolution medium was withdrawn at every 10 min interval for 1 h and replace with fresh media. The amount of dissolved drug was determined using UV spectrophotometric method (UV 1601 A Shimadzu, Japan) at 290 nm.

Determination of the physical stability

To determine the physical stability of freeze dried crystals, a long term and accelerated stability study of prepared Freeze dried crystals was carried out at 40°C and 75% relative humidity for 3 months according to the ICH guidelines. The Freeze dried crystals were packed in high density polyethylene (HDPE) container and placed in stability chamber. The samples were withdrawn at the interval of 0, 1, 2 and 3 months and evaluated for appearance, characterization by FT-IR and drug content and compared with initial results.

RESULTS AND DISCUSSION

A solvent system involved a water and water for a drug. The selection of these solvent depends on the miscibility of the solvents and solubility of the drug in individual solvents. DMF is miscible in any proportion with water (8).

Recrystallization of Tolfenamic acid was done to find out the changes in crystal lattice, being induced by solvents that can influence the physicochemical properties of the substance. Hence the mechanical, micromeritic and dissolution properties of Freeze dried crystals were compared with commercial sample and recrystallized sample. Recrystallization of Tolfenamic acid was carried out using same solvent composition as was used for Freeze drying crystallization (9,10).

Based upon high solubility of Tolfenamic acid in DMF, high viscosity and crystal morphology, DMF was determined to be suitable as Freeze dried crystals medium for Tolfenamic acid because of its high solubility in DMF (1 g/10 ml). The controlling of residual DMF was needed though. DMF is a toxic organic solvent based on their concentration and has little detriment to human body. Therefore, the low level of DMF in the Freeze dried crystals should not be harmful to animals and human (11)

Gas chromatography results confirmed that they were below detection levels of the DMF used in the Freeze dried crystals against the ICH limits i.e. 880 (12). The low level of DMF in the Freeze dried crystals results from its ability to form high surface area crystals and from the fact that the intermolecular forces among DMF molecules are not as strong as those of water. This allows both DMF to evaporate more completely and easily than water

The DSC thermograms showed a sharp endothermic peak for all the Tolfenamic acid crystals. This one step melt might be due to only one crystal form (Triclinic) of the Tolfenamic acid formed during the crystallization process, thus indicating that Tolfenamic acid did not undergo any crystal modification. The temperature range of the endothermic peak of all the Tolfenamic acid crystals lies in the range of 209°C to 214°C (Fig. 1). In DSC curve, commercial sample of Tolfenamic acid had a sharp endothermic peak at 214°C with enthalpy of 166.56 J/g that corresponded to the melting point of Tolfenamic acid (13). Melting points show slight variation as the nature of the crystals might have been affected by the solvent. The melting endotherm for agglomerated Tolfenamic acid was 209.4°C with decreased enthalpy of (163.6 J/g) indicating decreased crystallinity of Tolfenamic acid in FD.

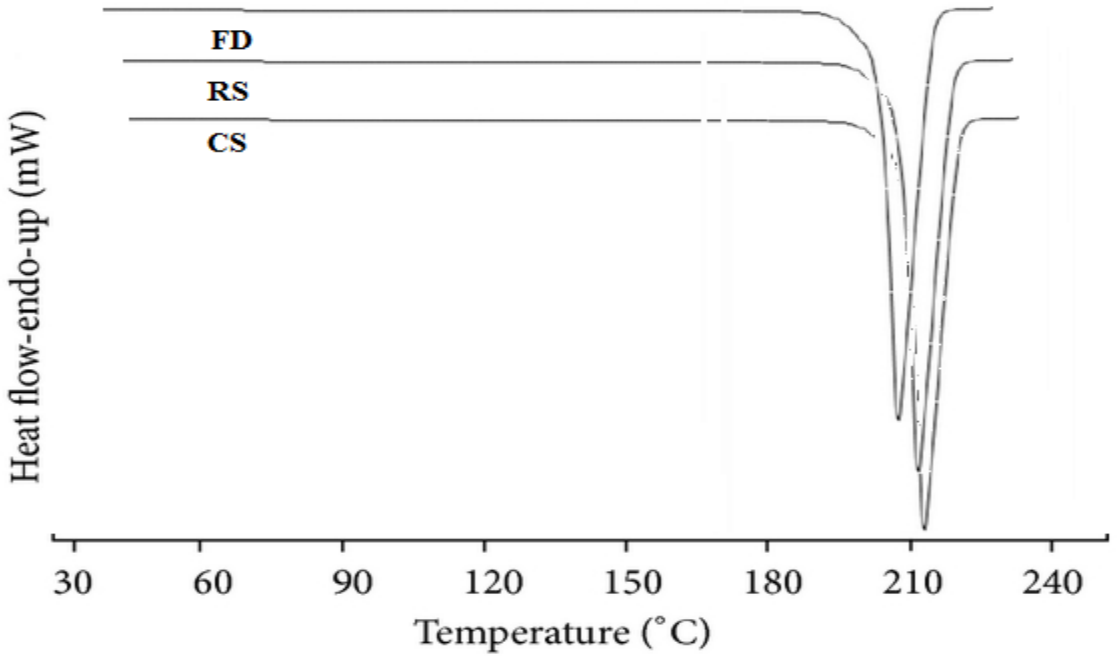


Fig. 1: DSC thermograms of Tolfenamic acid crystals

Spectroscopic methods due to their nondestructive nature can be used along with other solid-state techniques for quantitative analysis of pharmaceutical solids. FTIR spectroscopy is considered as a valuable technique to study the degree of crystallinity based on the measurement of characteristic peak intensity for its particular polymorphic crystal state[19]. Infrared technique is also very useful to study the hydrogen bond formation of polymers with different drugs (14). Therefore, FTIR spectroscopy was used in this study to identify the possible interaction between TA and solvent. It is envisaged that TA contain Peaks at 1661, 1590, 1575, 1500, 1270, and 749 cm^{-1} (14) are the most intense, characteristic peaks of TA (Fig-2). The FTIR spectrum of pure TA shows NH-stretching vibrations at around 3342–3340 cm^{-1}. Since commercial TA and prepared crystals share the same spectral region to exhibit their characteristic peaks of NH-stretching vibrations, it was difficult to identify the changes taking place in the peak height of TA in the prepared crystals at the spectral region of 3350–3300 cm−1. However, the prepared SAs still show broadening of the peak in the same region that could be due to variations in the resonance structure, rotation of a part of a molecule on certain bonds. Alteration could be due to minor distortion of bond angles, or even a result of the presence of solvents of crystallization.

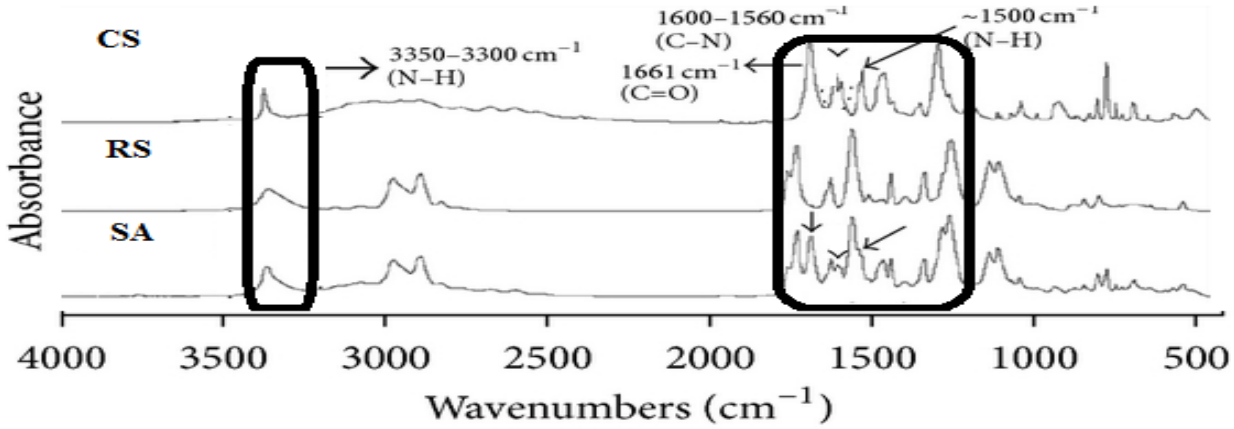


Fig. 2: FT-IR spectra of Tolfenamic acid crystals

In order to confirm the changes in the physical properties of the crystalline TA and prepared FDs were studied using XRD technique. XRD is usually considered as the most definite method of detecting and quantifying the crystalline/amorphous nature of the sample. XRD shows strong characteristic diffraction peaks for crystalline solids whereas diffused and hallow diffraction patterns for amorphous powders (14). The pure crystalline TA shows characteristic diffraction peaks at 2θ values of 5.21°, 11.58°, 15.72°, 18.70°, 19.77°, 24.90°, 25.32°, 25.90°, and 26.81°, respectively (Fig-3). In case of The X-ray diffraction of the RS and prepared FDs, both the samples showed same characteristic diffraction peaks although their intensity was lower than commercial drug may be due to the differences in crystal sizes. This could be due to the increasing the wettability of FDs. These results could explain the observed enhancement of solubility and dissolution of Tolfenamic acid in Freeze dried crystals.

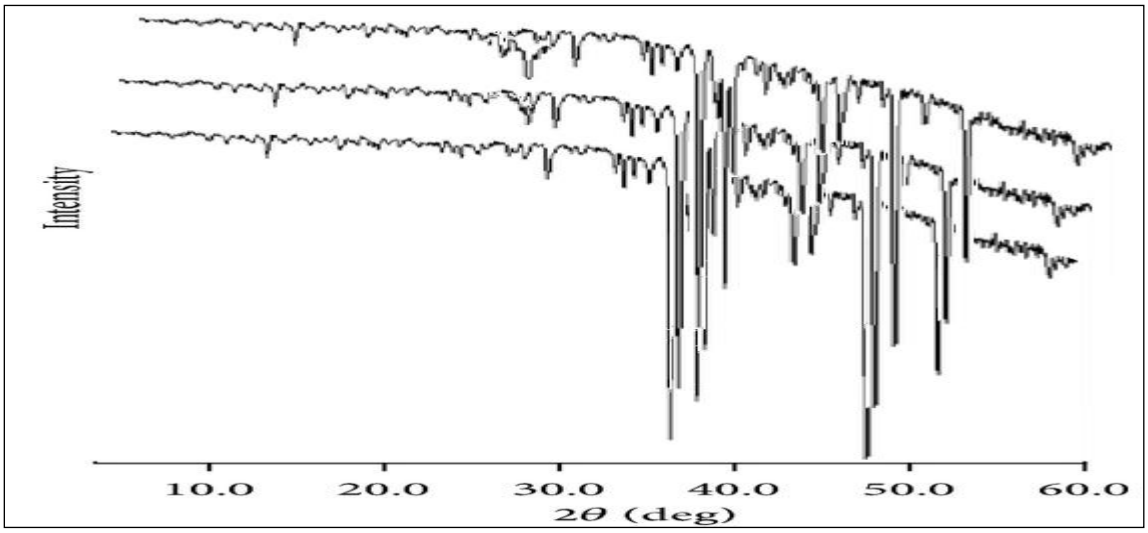


Fig. 3: X-ray diffraction spectra of Tolfenamic acid crystals

SEM study showed that crystals of commercial sample are of the smallest size (11-16 µm) and irregular shape and size. Recrystallization leads to crystals with intermediate size (7-21 µm) which 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 (~0.75 µm) (Fig. 4).

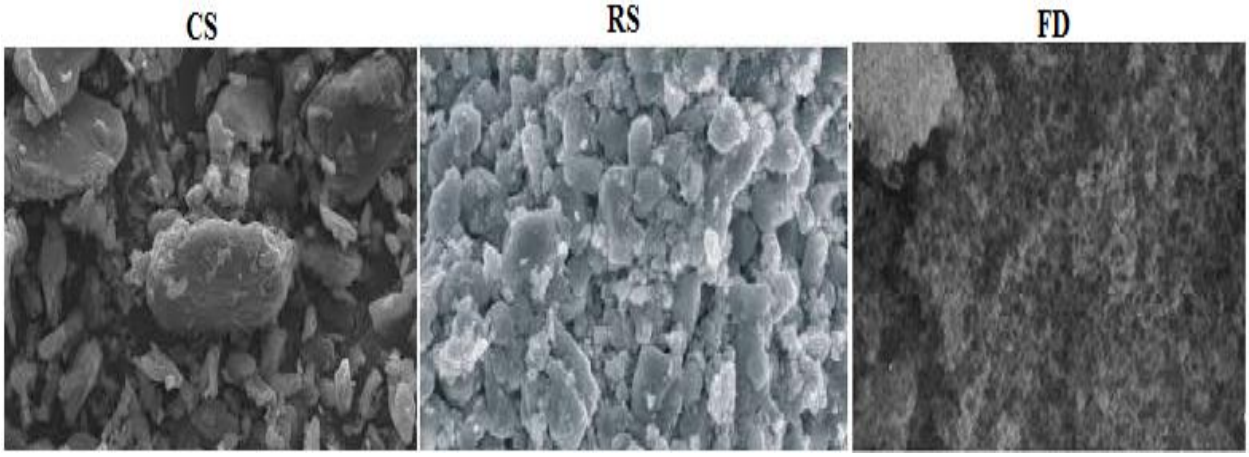
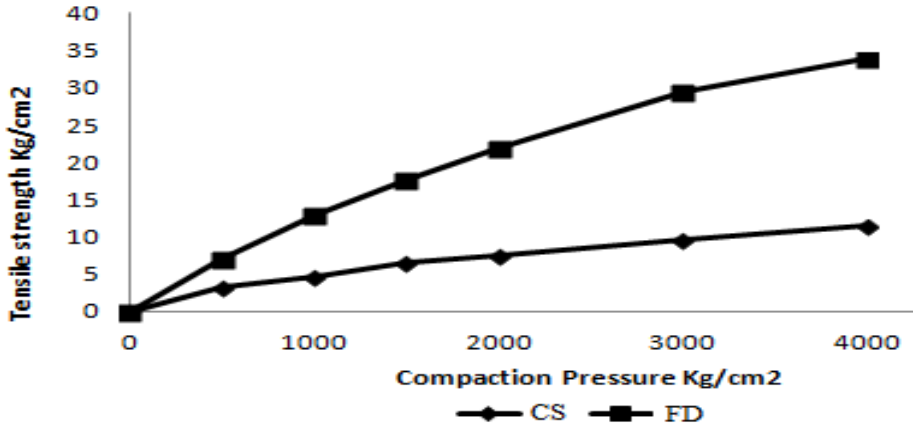


Fig. 4: SEM of Different crystals of Tolfenamic acid.

Freeze dried crystals exhibited superior compressibility characteristics compared to Recrystallized and commercial 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 freeze dried crystals under plastic deformation compared to that of single crystal (9, 10).



CS-Commercialized sample and FD- Freeze dried crystals

Fig. 5: Tensile strength of Freeze dried crystals and commercial sample as a function of compaction pressure

Freeze dried crystals showed increased solubility than the commercial sample in water and increased near Fourfold higher (0.673 mg/ml) than commercial Tolfenamic acid (0.171 mg/ml). The higher solubility of Tolfenamic acid from FD may be due to the increased wettability and reduction in particle size of Tolfenamic acid in Freeze dried crystals (8, 10).

The dissolution profiles of Tolfenamic acid (fig. 6) exhibited improved dissolution behavior for FDs than commercial sample. The reason for this faster dissolution could be linked to the better wettability and reduction in particle size of the Freeze dried crystals. The amount of drug dissolved in 60 min greatly varied for Freeze dried crystals.

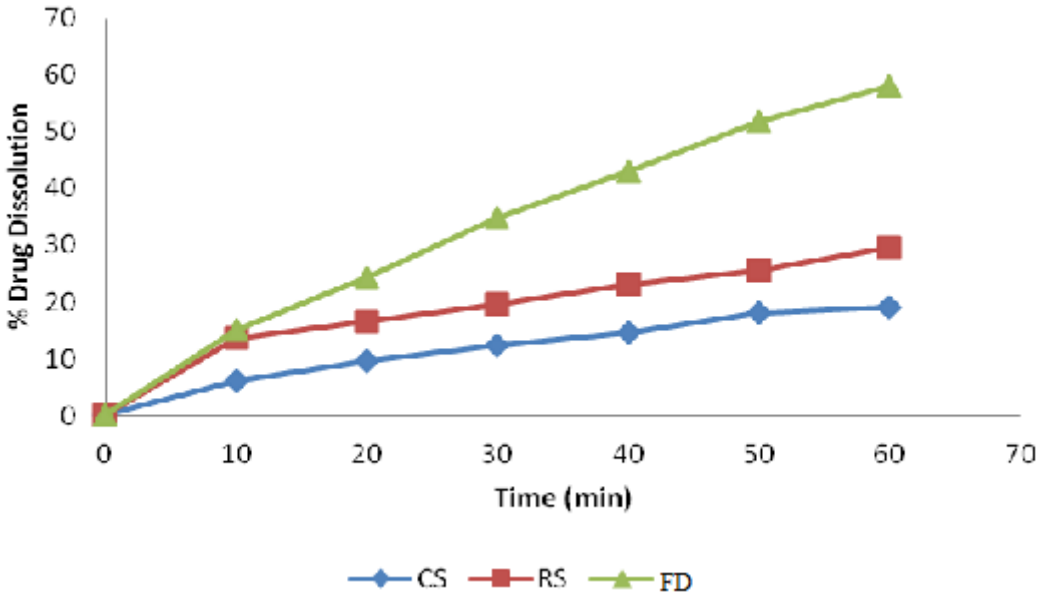


Fig. 6: Dissolution profile of Tolfenamic acid crystals

CS-Commercial sample, RS.-Recrystallized sample, FD-Freeze dried crystals

With respect to the influence of FDs on the physical stability of prepared FDs of Tolfenamic acid stored at 40°C and 75% relative humidity for 3 month. The influence of physical stability on the prepared Freeze dried crystals was investigated. Prepared Freeze dried crystals of Tolfenamic acid were stable for 3 month and complied with all the selected properties when compared to initial results of prepared Freeze dried crystals of Tolfenamic acid.

CONCLUSION

Freeze dried crystals of Tolfenamic acid were prepared by solvent change technique. FD exhibited decreased crystallinity and improved mechanical properties. DSC and XRD studies showed that there is no change in the crystal structure of Tolfenamic acid during the crystallization process i.e., polymorphism has not occurred. The dissolution of the Freeze dried crystals was improved compared with commercial sample of Tolfenamic acid. Stability showed

that prepared Freeze dried crystals were stable for 3 month. Hence this technique could be used for formulation of tablets of Tolfenamic acid by direct compression with directly compressible tablet excipients.

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