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Enhancing solubility and dissolution of Mefenamic acid by freeze drying

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ABSTRACT

Mefenamic acid, 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 Mefenamic acid by preparing crystals by freeze drying technique. Mefenamic acid crystals were prepared by freeze drying using THF, isopropyl acetate and water as solvents system to enhance solubility and dissolution rate. The prepared crystals containing Mefenamic acid 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 crystals was compared with its recrystallized sample and pure sample. The samples were stored in stability chamber to investigate their physical stability Freeze dried crystals exhibited decreased crystallinity and the solubility and dissolution of the mefenamic acid crystals were significant improved compared with its recrystallized and pure sample of mefenamic acid. 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. Hence this technique can be used for formulation of tablets of mefenamic acid by direct compression with directly compressible tablet excipients.

Introduction

Mefenamic acid is a potent prostaglandin synthetase inhibitor that is used widely as a non-steroidal anti-inflammatory and analgesic–antipyretic drug (1). It is used in mild to moderate pain including headache, dental pain, postoperative and postpartum pain, dysmenorrheal, osteoarthritis. Since its introduction, there have been numerous manuscripts published that discuss various aspects of the compound including important structural and physical properties (2). It has been suggested that solubility is a key factor in determining bioavailability. Likewise, crystallographic measurements of fenamates, including mefenamic acid, and their complexes have shown that they share a common and invariant feature (3). 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.

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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 [6]. 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 [7].

Therefore, several solubilization techniques were applied and reported to enhance the aqueous solubility of mefenamic acid, formation of Solid Dispersions of mefenamic acid with crospovidone (8), formation of mefenamic acid capsule with sodium lauryl sulphate (9), The Fast-dissolving mucoadhesive micro-particulate containing piroxicam (10).

The formation fast dissolving tablet of piroxicam acid has been proposed (11,12).However, in terms of sales value, sales volume and number of products available on the market, freeze drying (lyophilisation) method has been the most successful (13).

The objective of the present study was to prepare freeze dried crystals of Mefenamic acid using freeze drying teqniques and was evaluated for solvents residual 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 Mefenamic acid freeze dried crystals and investigate their physical stability in a climate chamber at 400C and 75% relative humidity (RH) for 90 days.

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Materials and methods

Mefenamic acid was obtained as a gift sample from Micro Lab. Bangolore, India. All chemicals and buffers used were of analytical grade Preparation of freeze dried crystals of Mefenamic acid Mefenamic acid (3.5g) was dissolved in 20 ml of is tetrahydrofuran (THF) heated at 450 until a clear solution was obtained. The drug solution was poured in to 70 ml solvents consists of water and isopropyl acetate (10ml) maintained at room temperature. Above resulted solution is shifted to 100 ml glass bottle and then transferred to a ultra low freezer at -40 0C and kept in the freezer for 24 hr. the frozen drug solution were placed in a lyophilizer for 72 hr using a Freeze Dryer (IISHIN Lab. Co. Ltd. Korea) with a condenser temperature of -400C and a pressure of 7×10-2 mbar followed by a secondary drying at 25 0C for 24 hr. The resulted crystals were kept in a desiccator's room temperature until further experiment.

Recrystallization of Mefenamic acid (RS)

Mefenamic acid (3.5 gm) was dissolved in 20 ml THF heated at 450C and 10 ml of isopropyl acetate was added. The drug solution was poured quickly in to 70 ml of water maintained at 200C with occasional stirring. The crystals of Mefenamic acid were collected by filtration and were dried at 450C 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 transform infrared (FTIR) spectroscopy

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/cm2 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/cm2 for 1 min. 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/cm2) was calculated using following equation.

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

Where, F, D and t are hardness (kg/cm2), compact diameter (cm) and thickness (cm), respectively.

Solubility studies of crystals

The solubility of Mefenamic acid freeze dried crystals in water and pH 7.2 Phosphate buffer 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 286 nm.

Dissolution studies of crystals

The dissolution of Mefenamic acid 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 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 286 nm.

Determination the physical stability

To determine the physical stability of freeze dried crystals was placed in a climate chamber of 400C and 75% relative humidity (RH). After 90 days, the % drug release of Mefenamic acid in the freeze dried crystals was determined by dissolution study and compare with freshly prepared freeze dried crystals.

Result and discussion:

A solvent system involved a THF, isopropyl acetate 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 and chloroform.

Recrystallization of Mefenamic acid 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 spherical crystals were compared with pure sample and recrystallized sample. Recrystallization of Mefenamic acid was carried out using same solvent composition as was used for freeze drying.

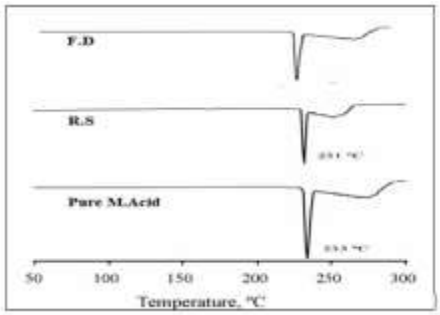
Based upon high solubility of Mefenamic acid in THF, high viscosity and crystal morphology, THF determined to be suitable freeze drying medium for Mefenamic acid because of its high solubility in THF (1.75gm/10ml). The controlling of residual THF was needed though. THF is a toxic organic solvent based on their concentration and has little detriment to human body. Therefore, the low level of both THF and isopropyl acetate in the freeze dried crystals should not be harmful to both animal and human [14, 15].

Gas chromatography results confirmed that there were 5.7 and 3.9 ppm residual of THF and isopropyl acetate present in the freeze dried crystals respectively, which was much lower than the toxic level i.e. 200 & 100 ppm respectively [14,15]. The low level of both THF and isopropyl acetate 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 THF and isopropyl acetate molecules are not as strong as those of

water. This allows both THF and isopropyl acetate to sublime more completely and easily than water.

The DSC thermograms showed a sharp endothermic peak for all the Mefenamic acid crystals. This one step melt might be due to only one crystal form (Triclinic) of the Mefenamic acid formed during the freeze drying process, thus indicating that Mefenamic acid did not undergo any crystal modification. The temperature range of the endothermic peak of all the Mefenamic acid crystals lies in the range of 2330 C to 229 0C (Fig. 1).

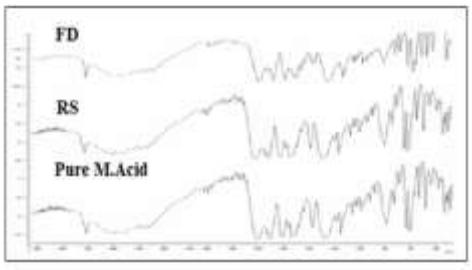
Fig 1 Shows DSC Spectrum of different samples of mefenamic acid



In DSC curve, pure Mefenamic acid had a sharp endothermic peak at 233°C with enthalpy of 184.32 J/g that corresponded to the melting point of Mefenamic acid. Melting points show slight variation as the nature of the crystals might have been affected by the solvent. The melting endotherm for freeze dried Mefenamic acid was 229°C with decreased enthalpy of (164.43 J/g) indicating decreased crystallinity of Mefenamic acid in freeze dried crystals [1, 2].

Infrared spectra of mefenamic acid commercial, recrystallized and freeze dried crystals showed characteristic peaks at 1255 cm-1 (-OH group bending and vibrations of COOH), 1647 cm-1 (N-H stretching vibration), 1572 cm-1 (C=O stretching), 1504 cm-1 (Aromatic C-H plane deformation), 1163 cm-1 (Aromatic-O-CH3) and 757(Aromatic C-C vibration for ortho substitution). Spectrum of recrystallized mefenamic acid was slightly different from pure sample in the region of wave number between 3350 and 3300 cm-1. This suggests that the recrystallized mefenamic acid from the mixture of water, isopropyl acetate and THF has a different crystalline form than its crystalline form in pure sample and in freeze dried (Figure-2).

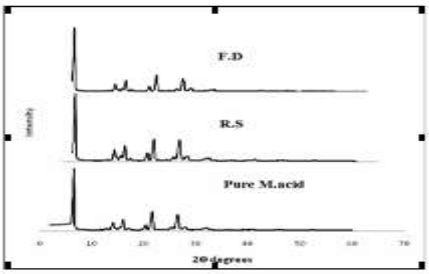
Fig 2 Shows FT-IR Spectrum of different samples of mefenamic acid



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 solvents of crystallization.

X-Ray diffraction was used to analyze potential changes in the inner structure of Mefenamic acid crystal during the formulation of freeze dried crystals. The characteristic peak of the Mefenamic acid appeared in the 2θ range of 10–600, indicating that the unprocessed Mefenamic acid was a crystalline material. All the samples showed similar peak positions (2θ) in X-ray diffraction, formation of different polymorphs of Mefenamic acid was ruled out. However relative intensities of XRD peaks were modified (Fig. 3).

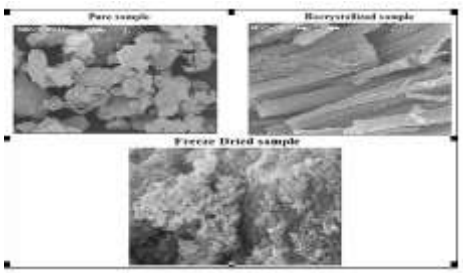
Fig 3 shows XRD Spectrums of different samples of mefenamic acid



The relative intensities of freeze dried crystals reduced much lower than pure Mefenamic acid. 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 (1, 2). 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 Mefenamic acid peak intensity was much lower than the pure drug and RS samples of Mefenamic acid. This could be due the increasing the wettability of freeze dried crystals. These results could explain the observed enhancement of solubility and dissolution of Mefenamic acid in freeze dried crystals.

In SEM study showed that crystals of pure sample are of the smallest size (5-10 µm) and they have irregular shapes. Recrystallization crystals with intermediate size (7-18 µm) which had rod like shapes. The resultant freeze dried crystals had a smooth surface covered with numbers of small crystals (average particle size 257 nm) (Fig. 4).

Fig 4 Shows SEM photographs of different samples of mefenamic acid

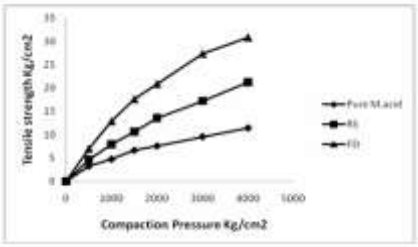


Freeze dried crystals exhibited superior compressibility characteristics compared to conventional drug crystals (Fig. 5). It could be due to the fact that during the process of compression fresh surfaces are formed by fracturing crystals. Surface freshly prepared by fracture enhanced the plastic inter particle bonding, resulting in a lower compression force required for compressing the freeze dried crystals under plastic deformation compared to that of single crystal[10, 11].

Freeze dried crystals showed increased solubility than the pure sample in water and increased nearly more than twofold higher (0.1237 mg/ml) than pure Mefenamic acid (0.0526

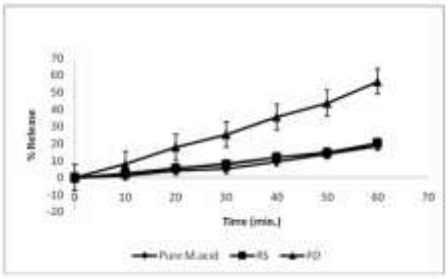
mg/ml). The higher solubility of Mefenamic acid from freeze dried may be due to the reduction in particle size and increased wettability of Mefenamic acid in freeze dried crystals [12, 13].

Fig 5 Tensile strength of different samples of mefenamic acid



The dissolution profiles of Mefenamic acid (fig. 6) 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 amount of drug dissolved in 60 min greatly varied for Freeze dried crystals.

Figure 6 Dissolution profiles of different samples of mefenamic acid



The results of the stability study of Freeze dried crystals stored at 40 0C and 75% relative humidity for 90 days. The influence of Freeze dried crystals on the physical stability of Mefenamic acid was investigated. The % of drug release from Freeze dried crystals almost same i.e. (55.63%) after 90 days of storing when compare with the freshly prepared freeze dried crystals i.e. (56.72 %). Above result shows that Freeze dried crystals of Mefenamic acid was stable after 90 days at 40 0C and 75% relative humidity.

Conclusion

Freeze dried crystals of Mefenamic acid were prepared by freeze drying technique. 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 Mefenamic acid during the crystallization process i.e., polymorphism has not occurred. The dissolution of the freeze dried crystals was improved compared with recrystallized sample and pure Mefenamic acid sample. Hence this technique can be used for formulation of tablets of Mefenamic acid by direct compression with directly compressible tablet excipients.

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