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Original article

A NOVEL TECHNIQUE TO ENHANCING THE SOLUBILITY AND DISSOLUTION OF FLUTAMIDE USING FREEZE DRYING

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Abstract

Flutamide, an anticancer drug for prostatic carcinoma has poor aqueous solubility and low oral bioavailability. The study aims to enhance the aqueous solubility and dissolution of flutamide by developing a Flutamide freeze dried tablet (FDT). The FDT was prepared by dispersing the drug in an aqueous solution of highly water-soluble carrier materials consisting of gelatin, lysine, and sorbitol. The mixture is poured in to the pockets of blister packs and subjected to freezing and lyophilization. The drug in FDT is characterized by DSC, XRD and SEM and evaluated for saturation solubility at pH 1.2 and 7.2 .Dissolution studies are carried out and compared with physical mixture (PM) and pure drug. The samples are stored in stability chamber to investigate their physical stability. The DSC and X-ray study implies that crystalline state of Flutamide in FDT is transformed to amorphous state during the formation of FDT. The SEM result suggests reduction in flutamide particle size in FDT (1-4µm) than pure flutamide (19-37 µm). The solubility of Flutamide from the FDT was nine and half times higher (0.0883 mg/mL) than the pure drug (0.0095 mg/mL) in water. The solubility of flutamide from FDT in both pH 1.2 and 7.has increased to twelve and thirteen times higher than the pure drug. The dissolution studies showed that dissolution of drug from FDT (97.76% at 20 min) was significantly improved compared with the pure drug (29.21% at 60 min). The stability test suggests that, the release profile and drug content of the FDT is almost unchanged as compared with the freshly prepared FDT after 90 days storing. Based on these results, it can be concluded that Flutamide can be formulated in to freeze dried tablets with enhanced solubility and dissolution in water.

Key words: *Freeze drying, Freeze dried tablet, Flutamide, Solubility and dissolution.*

Dondurarak Kurutma İle Flutamid'in Çözünürlüğü Ve Çözünme Hızının Artırılması İçin Yeni Bir Teknik

Prostat karsinomasında kullanılan bir antikanser etkin madde olan flutamid, zayıf suda çözünürlüğe ve düşük biyoyararlanıma sahiptir. Bu çalışma, dondurularak kurutulmuş flutamid tabletin (FDT) geliştirilerek flutamidin suda çözünürlüğünün ve çözünme hızının artırılmasını amaçlamaktadır. FDT, etkin maddenin jelatin, lizin ve sorbitolden oluşan yüksek derecede suda çözünebilir taşıyıcı maddelerin sulu bir çözeltisi içinde disperse edilmesiyle hazırlanmıştır. Karışım bir blister ambalajın boşluklarına dökülmüş ve dondurma ve liyofilizasyon işlemleri uygulanmıştır. FDT içindeki etkin madde DSC, XRD ve SEM ile incelenmiş ve pH 1.2 ve 7.2'de doygunluk çözünürlüğü değerlendirilmiştir. Çözünme testleri yapılmış ve saf etkin madde ve fiziksel karışım (PM) ile karşılaştırılmıştır. Numuneler, fiziksel stabilitelerinin incelenmesi için bir stabilite kabininde saklanmıştır. DSC ve X-ışını çalışması, FDT oluşumu sırasında FDT içindeki flutamidin kristal halden amorf hale dönüştüğünü göstermiştir. SEM sonucuna göre, saf etkin madde (19-37 µm) ile karşılaştırıldığında FDT içinde flutamidin partikül büyüklüğü (1-4 µm) azalmıştır. FDT'den flutamidin sudaki çözünürlüğü (0.0883 mg/ml), saf etkin maddenin çözünürlüğüne göre (0.0095 mg/ml) 9.5 kat daha yüksek olmuştur. FDT içindeki flutamidin pH 1.2 ve 7.2'deki çözünürlüğü saf etkin maddeninkinden 12 ve 13 kat daha yüksek bulunmuştur. Çözünme testleri, saf etkin madde (60 dakikada %29.21) ile karşılaştırıldığında, FDT'den etkin maddenin çözünme hızının (20 dakikada %97.76) önemli derecede artırıldığını göstermiştir. Stabilite testleri, taze hazırlanan FDT ile karşılaştırıldığında, doksan gün saklama sonrasında FDT'den etkin madde içeriği ve salımının hemen hemen hiç değişmediğini ortaya çıkarmıştır. Bu sonuçlara dayanarak, flutamidin su içinde artan çözünürlüğe ve çözünme hızına sahip dondurularak kurutulmuş tabletlerinin formüle edilebileceği sonucuna varılmıştır.

Anahtar kelimeler:D*ondurarak kurutma, Dondurularak kurutulmuş tablet, Flutamid, Çözünürlük ve çözünme hızı.*

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INTRODUCTION

Prostate cancer has become one of the most common malignancies in the male population worldwide. Anti androgenic agents are therapeutically effective for benign prostatic hypertrophy and androgen dependent prostate cancer. Flutamide is a non-steroidal fluorine-containing antiandrogens used in prostate chemotherapy. Among the non-steroidal antiandrogens, flutamide is often recommended for immunotherapy; (1). Flutamide is used increasingly as part of total androgen ablation therapy and in neo adjuvant treatment before radical prostatectomy (2). The low bioavailability of flutamide after oral administration may be due to poor wettability, low aqueous solubility, poor permeability, rapid first pass hepatic metabolism, and low concentration at the absorption surface (3). Therefore, it is prudent to develop a novel formulation that mitigates solubility and dissolution and formulation that produce higher concentrations of flutamide in solution at the absorption site, to overcome the solubility-mediated poor bioavailability.

Currently, several approaches are widely used to fabricate Rapid Dissolving tablet (RDT), including techniques like lyophilization, solid dispersion, mucoadhesive micro-particulate, direct compression, and moulding. Several solubilization techniques were applied and reported to enhance the aqueous solubility of poorly water soluble drugs; like formation of solid dispersions with polyvinylpyrrolidone and fast-dissolving mucoadhesive micro-particulate (4-6). Freeze drying (lyophilisation) technique to develop formulations has been the most successful, in terms of sales value, sales volume and number of products available on the market, (6, 7). The fabrication of freeze drying RDTs is based on creating a porous matrix by subliming the water from pre-frozen aqueous formulation of the drug. Aqueous formulation can contain matrix forming agents and other excipients such as preservatives, flavors and lyoprotectants (7, 8). The matrix of the freeze drying RDT consists of two components that work together. The first component is water soluble polymers such as gelatin, dextran, alginate (8) and maltodextrin (9). This component maintains the shape and provides mechanical strength to the tablets (binder). The second constituent is matrix supporting/disintegration enhancing agents such as sucrose and mannitol, which acts by cementing the porous frame work, provided by the water soluble polymer and accelerates the disintegration of the RDT (10). Although there is a wide availability of literature describing the preparation of RDTs by freeze drying, the number of matrix supporting/disintegration enhancing agents used has been limited to saccharides and polyols with majority of the work dedicated to the inclusion of mannitol (8). This is primarily since the incorporation of these matrix forming agents requires fulfillment of stringent characteristics such as reasonable drying time, stability during freeze-drying process, as well as formation of elegant tablets with short disintegration time and adequate mechanical properties. However, high concentration of saccharides and polyols is required to achieve these quality features (8,10), thus restrains their application in delivering drugs for the treatment of long-term chronic conditions especially for children, diabetic and obese patients, due to limited intake requirement. Therefore, this study aims to develop novel excipients by investigating the feasibility of using amino acids as matrix supporting agents (second component) in the fabrication of rapid dissolving tablets prepared by freeze drying in order to produce tablets with enhanced properties and with wider application to pediatric and geriatric patient population.

Amino acids are the basic structural units (monomer) of proteins. An alpha amino acid consists of an amino group, a carboxyl group, a hydrogen atom and a distinctive side chain bonded to a carbon atom (alpha carbon). Basically, the side chains of amino acids are responsible for the variation in their physic -chemical properties. Naturally occurring amino acids can exist in both the L (laevo) and the D (dextro) forms, which are mirror images of each other. However, incorporation of the D form of the amino acid has been limited for pharmaceutical applications due to their potential pharmacological activity, microbiological concerns and toxicity (11–13). On the other hand, the L form of the amino acids have been used extensively in pharmaceutical and cosmetic formulations as pH-sensitive drug carrier (14), cicatrisation topical dermatological preparations (15), in salt conjugate of poorly soluble drug (16), oral tablets, as lubricant (17) and disintegration enhancer (18), in inhalable delivery systems (19) and freeze-dried products, as cryoprotectants (20) and bulking agent (21).

In this study Flutamide FDT were prepared and evaluated. The drug in the formulation was characterized by DSC, XRD, and SEM analysis. Dissolution and solubility of drug in FDT was compared with its physical mixture and pure drug.

EXPERIMENTAL

Materials

Flutamide, micronized gelatin, lysine, and sorbitol were received from IPCA pharmaceutical Ltd. Mumbai, India. De-ionized water and all other materials used were of analytical grade.

Preparation of Flutamide Freeze Dried Tablets

The method consists of the following steps: A 2% w/v solution of gelatin in water was prepared by first soaking the gelatin in water until complete hydration. The hydrated gelatin was stirred using a magnetic stirrer until a clear solution was obtained. Equal weights of lysine (0.886% w/v) and sorbitol (0.886% w/v) were added to the gelatin solution while stirring until completely dissolved. An accurately weighed amount of Flutamide powder (2.5% w/v) was dispersed in the aqueous solution of gelatin, lysine, and sorbitol. The resulted suspension (1 mL equivalent to 25 mg of Flutamide)was poured into each of the pockets of a tablet blister. The tablet blister packs, each containing 8 tablets were then transferred to an ultra low freezer at -40°C, for 24 hours. The frozen tablets were then placed in a lyophilizer for 12 hours using a freeze dryer (IISHIN Lab. Co. Ltd. Korea); with a condenser temperature of -40°C and a pressure of 40 mbar followed by a secondary drying at 25°C for 12 hours. The FDTs were kept in a desiccator room temperature until further experiments were performed. Five blister packs containing a total of 40 tablets were produced in each run. Eight tablets were randomly selected for drug content uniformity. The mean % drug content was found to be 98.76±0.025%.

Preparation of the Physical Mixtures

Flutamide was uniformly mixed with gelatin, lysine and sorbitol in the same percentage as used for the preparation of FDT using a mortar and pestle. The prepared mixtures were kept in desiccators until further use.

Determination of λmax and Calibration Data of Flutamide

Stock solution of 100 µg/mL was prepared by dissolving 10 mg of pure drug in 100 mL of buffer of pH 1.2, buffer of pH 7.2 and distilled water. Solutions were suitably diluted to produce10 µg/mL. The solutions were scanned between 200 to 400 nm. The drug exhibited λ_{max} of 306 nm, 305 nm and 306 nm in buffer of pH 1.2, buffer of pH 7.2 and distilled water respectively, and these values were used for further analysis.

The stock solution was suitably diluted with buffer of pH 1.2, buffer of pH 7.2 and distilled water. The absorbance of these solutions was measured at 306 nm, and a graph of concentration versus absorbance was plotted. The calibration curve of flutamide in distilled water is shown in figure 1.

Determination of Drug Content

The FDTs were triturated with 10 mL of water and allowed to stand for 10 min with occasional swirling and water was added to produce 100 mL. After suitable dilutions, 10 mL of the solution was filtered through a membrane filter (0.45 µm) and the amount of dissolved drug was measured at 306 nm using UV spectrophotometric method. The drug content was determined in triplicate from the standard plot.

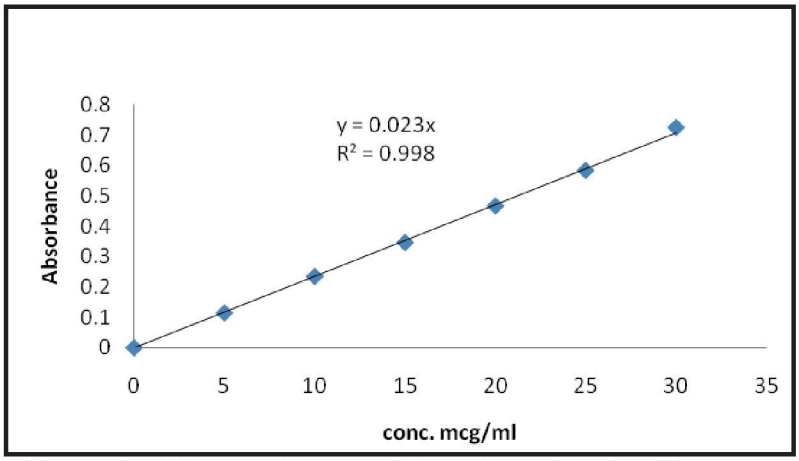

Figure 1. Calibration Curve of Flutamide in Distilled Water.

Differential Scanning Calorimetry (DSC)

A DSC study was carried out to detect possible polymorphic transition during the crystallization process. The 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°C/min in the range of 20°C to 140°C. Thermograms for flutamide, PM and FDT were obtained.

X-ray Diffraction Analysis

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. 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 topography of the crystals. Cross-section of FDT was made to study their inner structure. Photographs were taken at magnification of 1000X.

Solubility Studies

Flutamide (100 mg), its FDTs and PMs (equivalent to 100 mg Flutamide) were placed in glass stopper flasks containing 100 mL of water, buffer of pH 1.2, buffer of pH 7.2. The flasks were shaken in a water bath at 25°C for 12 hours. After suitable dilutions, 10 mL of the solution was taken and filtered through a membrane filter (0.45 μm), and the dissolved drug in triplicates was measured spectrophotometrically at 306 nm.

Dissolution Studies

The dissolution profile of pure Flutamide was compared with the PM and FDT, using USP dissolution apparatus XXIV-Type II (Electro Lab, Mumbai). All tests were conducted in 900 mL of distilled water maintained at 37±0.2°C with a paddle rotation speed at 100 rpm. After specified time intervals, 10 mL of samples of dissolution medium were withdrawn and replaced by equal amount of fresh medium and then filtered through a membrane filter (0.45 μm) and the percentage of dissolved drug was determined using UV spectrophotometric method (UV 1601 A Shimadzu, Japan) at 306 nm.

Determination of the Physical Stability of FDT

Ten FDTs were covered in aluminum foil and placed in a climate chamber of 20°C and 45% relative humidity (22). After 90 days, the drug content and percent drug release of Flutamide from the FDT were determined by dissolution study and compared with freshly prepared FDT.

RESULTS AND DISCUSSION

Different fast dissolving carrier materials were used in the preparation of Flutamide FDT. Lysine was used to prevent shrinkage of the tablet during manufacture and it was possibly due to the plasticizing effect of lysine. This is in line with previously reported research, which suggests that the freeze-dried systems, upon inclusion of solutes in the formulation results in lowering of the glass transition temperature and is dependent on the interactions between the added excipient and unfrozen water (22). Addition of plasticizing agents potentially reduced the intermolecular forces between binder molecules and increases polymer chain mobility, thereby providing a cushioning effect. However, the degree of plasticizing varied between the amino acids, which can be attributed to the differences in their physicochemical properties (23) and the total number of moles added. The use of sorbitol to impart crystallinity, hardness, and elegance to the tablet is well-known and are acceptable materials used in preparing of freeze-dried tablets. Previous research has shown that the two common methods to enhance the mechanical strength of the lyophilized FDTs are the inclusion of higher concentration of the binder and addition of excipients such as matrix supporting agents (saccharides and polyols) (8). In this study, the use of 5% (w/w) gelatin solution as a binder provided high resistance to friability. The percentage excipients used was optimized during the formulation process to result in a strong and elegant tablet that could be handled with ease. Generally, the mechanical properties of tablets are mainly influenced by the intermolecular bonding force and contact points between the excipients (24, 25).

DSC thermograms for pure Flutamide, physical mixtures and FDT were shown in Figure 1. Pure Flutamide had a sharp endothermic peak at 113.54°C that corresponded to the melting point of Flutamide (2). The thermogram of the PM showed the endothermic peak of Flutamide, although broader, spitted, and slightly shifted to the left, indicating that the crystalline state is maintained but decreased in the PM and shows sharp endothermic peak at 112.31°C. However, the melting endotherm was absent on the DSC thermogram of the FDT, suggesting absence of crystallinity and presence of amorphous state of the drug. This could be because Flutamide was molecularly or amorphously dispersed in the FDT (26).

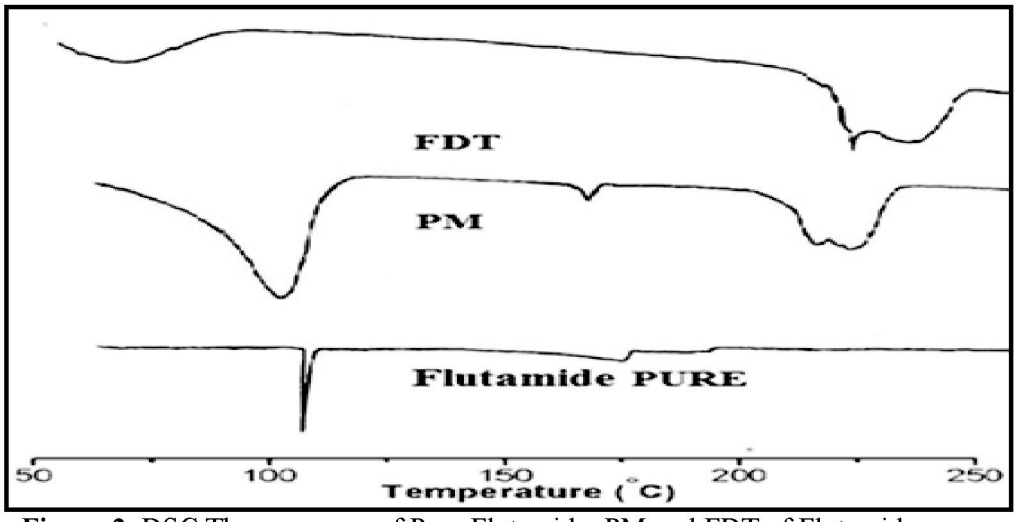

Figure 2. DSC Thermograms of Pure Flutamide, PM and FDT of Flutamide.

X-Ray diffraction was used to analyze potential changes in the inner structure of flutamide nanocrystal during the formulation of FDT. 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 pure drug, physical mixture and FDT are shown in Figure 3. The results of DSC studies were further

conformed by X-ray diffraction studies. The characteristic peak of the Flutamide appeared in the 2θ range of 0–30^0, indicating that the unprocessed Flutamide was a crystalline material (2, 3). The pure drug exhibits its characteristic diffraction peaks at various diffraction angles indicating the presence of crystallinity. The X-ray diffraction study of the PM of drug and excipients showed the peak corresponding to the crystalline drug molecules present in the mixture, although their intensity was lower due to the high excipients-drug ratio employed. The diffraction pattern of the FDT of the drug showed absence, broadening and reduction of major Flutamide diffraction peaks indicating that mostly an amorphous from (disordered state) existed in the FDT. These results could explain the observed enhancement of solubility and rapid dissolution of Flutamide in FDT.

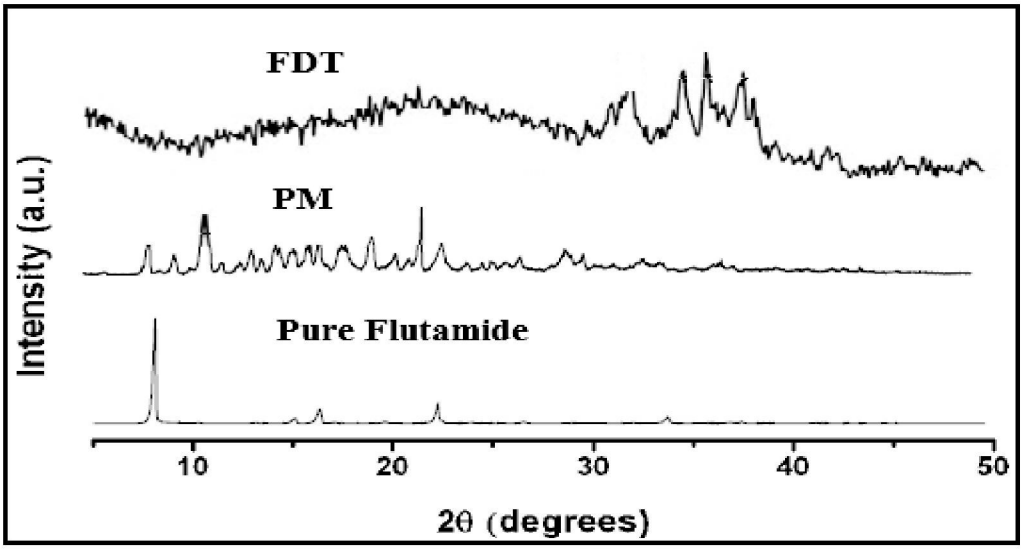

Figure 3.X-ray Powder Diffraction Spectrums of Pure Flutamide, PM and FDT of Flutamide.

SEM micrographs of pure Flutamide, PM, and FDT are shown in Figure 4. The Flutamide crystals could be seen in the PM while the micrograph of FDT shows a matrix in which no crystals of Flutamide could be seen (6). The SEM micrograph of FDT suggests that the particle size of the drug might have been reduced (1-4 μm) during dissolution in the gelatin-lysine-sorbitol solution than the pure drug (19-37 μm). This could therefore indicate that Flutamide particle size has been reduced which also accelerates solubility and dissolution (10).

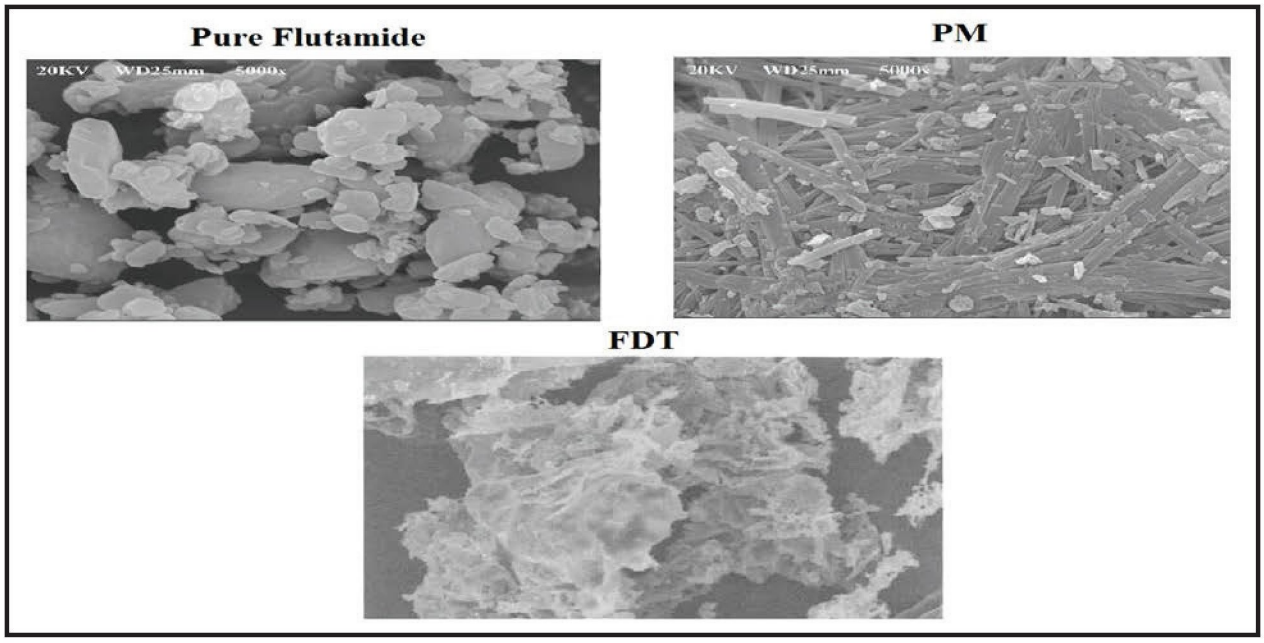

Figure 4. Scanning Electron Micrographs of Flutamide Samples.

The solubility of Flutamide increase from FDT (0.0883 mg/mL), nearly nine and half times higher when compared to the solubility of the pure drug (0.0095 mg/mL), suggesting the presence of high amount of amorphous form of the Flutamide in FDT (6). Increase in solubility of Flutamide from the PM (0.0231 mg/mL), nearly two and half times higher than the pure drug. This could be due to the solubilizing effect of highly water soluble carrier materials used in the formulation, such as lysine and sorbitol (13, 25). The solubility of flutamide in buffer of pH 1.2 and buffer 7.2 from FDT increased almost near to twelve and thirteen times higher than pure drug, respectively. The solubility data of different samples are shown in Table 1. The higher solubility of Flutamide from FDT may be due to the increase in surface area, wettability and solubilizing effect of highly water soluble carrier materials used in the formulations.

Table 1. Solubility of Flutamide Pure Drug, PM and FDT in Distilled Water at 25°C.

Flutamide Samples	Solubility (mg/mL ± SD*) In water	Solubility (mg/mL ± SD*) In pH 1.2	Solubility (mg/mL ± SD*) In pH 7.2
Pure Flutamide	0.0095±0.013	0.0143±0.010	0.0173±0.020
PM	0.0231±0.021	0.0432±0.011	0.0563±0.013
FDT	0.0883±0.018	0.1771±0.010	0.2337±0.014

(n=3, mean ± SD*).

The dissolution curves of Flutamide in distilled water are displayed in Figure 5. The dissolution rate profiles are plotted as the percent release from the FDT, physical mixture and pure Flutamide versus time in minute. The rate of dissolution of pure Flutamide was very slow compared with physical mixtures and FDT. Flutamide in the FDT was immediately dispersed and almost completely dissolved (97.76%) in 20 minutes. Initial dissolution rate of Flutamide in the FDT increased markedly (about

tenfold) compared to pure Flutamide. The dissolution rate was also higher and faster in FDT than in PM. The percentage of dissolved amount of Flutamide from its PM at 60 minutes (80.45%) increased approximately three fold compared to flutamide pure drug (29.21%).

The increased dissolution rate of Flutamide from its FDT suggests that Flutamide FDT might have a rapid oral absorption following disintegration in the mouth and dissolution in the saliva, since solubilized Flutamide is absorbed rapidly and completely from the gastrointestinal tract after oral administration. The enhancement in solubility and dissolution rate of Flutamide in its FDT may be attributed to the formation of amorphous state in the FDT of the fast dissolving carrier materials (6, 25).

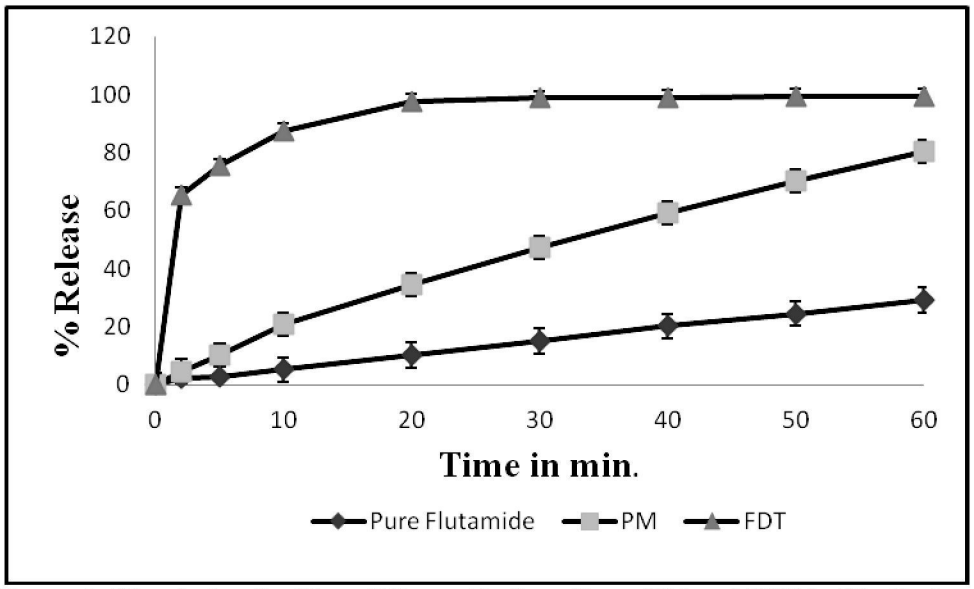

Figure 5. Dissolution Profiles of Flutamide Pure Drug, PM and FDT in Distilled Water at 37^{0}C. n=3 with mean ± SD.

The dissolution behavior of FDT must remain unchanged during storage. The best way to guarantee this is by maintaining their physical state and molecular structure. For optimal stability of amorphous FDT, the molecular mobility should be as low as possible. However, FDT, partially or fully amorphous, are thermodynamically unstable and will have a natural tendency to crystallize, because the crystalline state has a lower energy compared to amorphous material. However, amorphous material can be kinetically stable, which implies that the equilibrium state, i.e. crystalline, is not reached within the timeframe of the experiment or shelf life of the product. Therefore, the physical state should be monitored because changes therein are likely to alter the drug release (27, 28).

The results of the stability study of FDT stored at 20°C and 45% relative humidity for 90 days are shown in Figure 6. The influence of FDT on the physical stability of Flutamide was investigated. The percentage of drug release from FDT is almost same i.e. (99.32%) after 90 days of storing when compared with the freshly prepared FDT i.e. (99.47 %) at 60 minutes. The drug content of FDT was found to be 98.62% after 90 days of storing when compared with the drug content of freshly prepared FDT i.e. 98.87%. The above result shows that FDT of Flutamide was stable after 90 days at 20°C and 45% relative humidity.

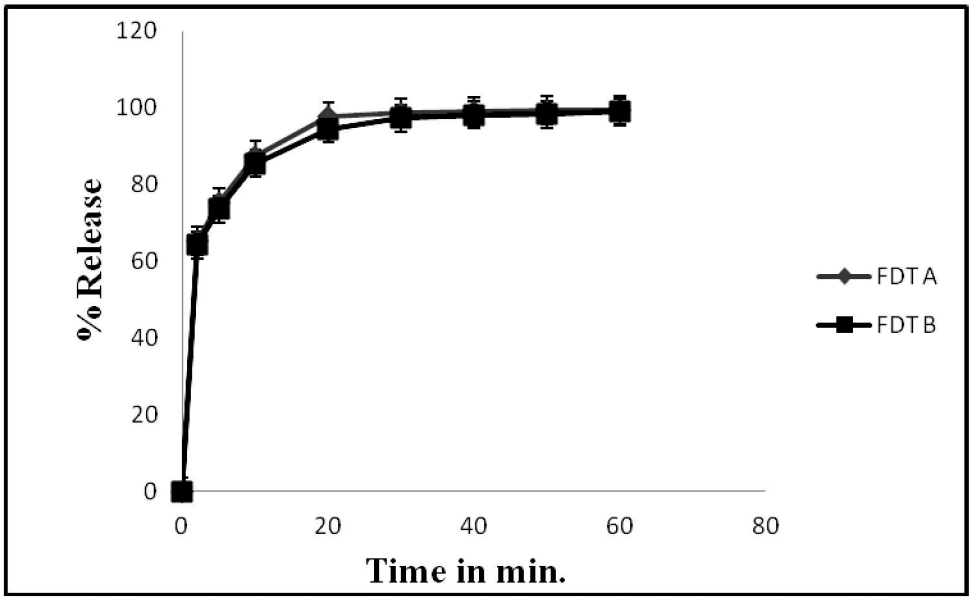

Figure 6. Dissolution Profiles of Flutamide Samples for Stability Tests: a) Freshly Prepared FDT, b) FDT After 90 days of Storing. n=3 with mean ±SD.

CONCLUSION

The study revealed that the FDT formulation of Flutamide made of widely used, safe, water-soluble excipient is feasible for enhancing the solubility and dissolution rate of Flutamide. These results are attributed to the formation of an amorphous state of the Flutamide at FDT and probability to reduction of Flutamide particle size. The result of physical stability tests showed that FDT was stable due to the unchanged dissolution profile of Flutamide after 90 days. Based on these results, it can be concluded that the FDT could be suitable in terms of solubility and dissolution in water. This technique is a promising procedure for the formulation of Flutamide tablets by direct compression with directly compressible tablet excipients without mixing or further formulation steps.

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