Influence of mechanical milling time on physicochemical properties and stability of cefotaxime sodium

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ABSTRACT

The aim of this study was to examine the effect of mechanical milling time on physicochemical properties and stability of Cefotaxime sodium (CS). CS was micronized by ball milling in five period of time: 30, 60, 120, 240, and 360 min. The powder properties of the samples were examined by HPLC assay, laser diffraction, helium densitometery, IR spectrophotometery, X-ray diffraction (XRD), scaning electron microscopy (SEM), differential scanning calorimetery (DSC), thermogravimetric analysis (TGA) and Karl-Fisher titrimetery. The results showed that ball milling was not an appropriate method for particle size reduction to make solid dosage form such as dry powder inhaler formulation (DPI) of CS and by increase in milling time, degradation of CS increased.

Keywords: Cefotaxime sodium, Ball milling, Particle size reduction, Degradation

INTRODUCTION

Some pharmaceutical dosage forms such as dry powder inhalers (DPIs) are prepared from micronized drug powders. Since 1960s, the development of DPI drug products has tremendously increased because they offer some advantages over the commonly used pressurized metered dose inhaler (pMDI) for respiratory drug delivery such as low sensitivity to microbial growth (1), and suitability for both water soluble and insoluble drugs (2). As a result, preparation of dry powder formulations for inhalation is an interesting and appreciated proposition (3,4). Generally, DPI formulations consist of a micronized drug alone or mixed with carrier particles (5). While particle size of the drugs is very important for preparation of the dry powder formulation for inhalation, the fragile nature of many drugs may be a limitation for application of conventional production processes (6). In order that drug gain access to the lower airways, as a prime requirement it is generally accepted that the drug particles should have an aerodynamic diameter between 1 and 5 μm (7). Consequently, micronized drug particles of about a few micrometers in diameter are formulated preferably for inhalation (8). Fine particles are notoriously difficult to disperse due to their highly cohesive nature (9). Physicochemical properties of the particles such as morphology (10), particle size distribution (11), surface roughness (12) and electrostatic charge (13) are very important for drug detachment from carrier particles and for aerosolisation. The physicochemical properties of the drug must remain unchanged when the compound is reduced to the proper size, and the efficiency of the drug should remain unchanged at the same time. To obtain a powder in the suitable size distribution, some techniques such as milling, spray drying, spray freeze drying, supper critical fluid extraction and crystallization may be applied (14). Optimization of the fine particle fraction has often been sought in advanced particle engineering to generate particles with improved aerosolization behavior (15). The traditional method for production of active drug particles within the respirable range involves high-intensity comminution, usually in a fluid energy (air-jet) mill which provides only limited opportunity to control potentially important particle characteristics such as size, shape, and morphology. Intense milling might also causes unwanted changes in the physicochemical properties of the materials. Another method for production of respirable drug particles is spray drying which is a well-established technology in the food and pharmaceutical industries. However recovering micronized spray-dried materials at an economically acceptable yield is a major challenge which requires extremely high-efficiency cyclone (8). Materials undergoing spray drying are processed through following stages: atomization of the feed material, spray-air contact, drying of the sprayed material, and separation of

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the material from the air stream. This method is a finely controlled technology, with different process variables that results in alteration of product characteristics (16). Ball milling is the metallurgical process for reduction of the size of materials by grinding in a rotating cylindrical mill containing steel balls (17).

Treatment of severe bacterial respiratory tract infections, particularly pneumonia requires the use of two or three antibacterial agents by intravenous administration. In some cases the efficacious systemic intravenous or oral dose, requires doses which are borderline or outright toxic. Thus it would be desirable to have other available modes of delivery routes of antibiotics enabling a targeted delivery of smaller amounts of the antibiotic to endobronchial space of airways for treatment of bacterial infections (18). Anti-infectious agents such as pentamidine antibiotics (mainly colistine and aminoglycosides), and amphotericin B can be administrated by aerosol (19). Cefotaxime represents a very good candidate for the empirical therapy of lower respiratory tract infections. The antimicrobial spectrum of cefotaxime includes most of the clinically relevant Gram-negative and Gram-positive bacterial organisms (20).

Effect of micronization method to produce dry powder formulation has been reported in many studies (6, 15). In a previous study the physicochemical properties of the in-situ-micronized drug were compared to those of an unmilled and a jet-milled methods. Differences in the X-ray patterns and amorphous parts could be detected for the jet-milled but not for the in-situ-micronized drug (21). Jet milling and spray drying methods have been used to prepare fine powders of CS for DPIs and it has been reported that micronization method affects physicochemical properties and deposition profiles of CS (22, 23). Also in a recent investigation, air-jet-milling were compared to ball milling to achieve respirable particles of sulbutamol sulphate (24). In this study ball milling were used to investigate the effect of milling time on the stability and physicochemical properties of CS.

MATERIALS AND METHODS

Materials

CS was purchased from Hanemi, Korea. Methanol (HPLC grade), ether, potassium dihydrogen phosphate and disodium hydrogen phosphate were purchased from Merck, Germany.

Methods

Preparation of CS micronized particles

A ball mill apparatus (F.kurt Retsh MM2000 ball miller GMbH & Co.KG, 42781 Hean, Germany) was used to reduce particle size for CS powder. The mill consists of two stainless steel vessels containing a stainless steel ball in each vessel. The vessels were sealed and mounted on an oscillating motor. Six grams of CS was put in each cells. Sampling, were performed after 30, 60, 120, 240, and 360 min. Fine particles of CS were collected after one passage through the instrument and were packed into tightly closed amber bottles and stored in a desiccator over silica gel.

Physicochemical characterization

HPLC analysis of CS

The potency (amount of actual antibiotic activity per amount of powder) of CS samples was determined by HPLC (Waters, Milford, MA, USA) employing a 15 cm × 3.9 mm C-18 Nova-pack column according to BP 2004 (25) with some modifications. The analysis was performed by using mobile phase prepared as follows: 3.5 g of potassium dihydrogen phosphate and 11.6 g of disodium hydrogen phosphate were dissolved in 1000 ml of water at pH 7.0, filtered through a 0.22 µm membrane filter and after addition of 180 ml of methanol was degassed prior to use. The column effluent was monitored at 235 nm. The flow rate was 1 ml/min and the sample size of 20 µl at room temperature was used all over the study. The system was calibrated using standard solutions of CS over the range of 0.25–16 µg/ml ($R^2 = 0.999$).

Reference standard of CS was dissolved in mobile phase to obtain concentrations of 0.25, 0.5, 1, 2, 4, 8 and 16 µg/ml. Prepared samples were injected directly to HPLC column (n=3). Standard curves were constructed using peak areas versus known concentrations of CS. The resulting regression line data were used to determine the concentration of the samples. Sample, containing 2µg/ml of CS, was diluted with mobile phase to obtain an appropriate concentration.

Particle size determination

Particle size distributions were measured by laser diffraction (Malvern Mastersizer X, Malvern, UK) using a 100 mm lens at an obscuration between 0.19 and 0.21.

Samples were prepared by suspending the particles in ether in a water bath with the aid of sonication for 3 min. Each sample was measured in triplicate. The size distribution was expressed by equivalent volume diameters at 10 ($d_{10\%}$), 50 ($d_{50\%}$) and 90% ($d_{90\%}$) cumulative volume.

Scanning electron microscopy

The morphology of representative unprocessed and processed particles was examined using SEM

(Cam Scan MV 2300, England) Prior to scanning. The samples were coated with a thin layer of gold, using a direct current sputter technique.

X-ray diffraction

XRD patterns were collected using an X-ray diffractometer with a rotating anode (Philips, Xpert – Pro, Netherlands).

Differential scanning calorimetry (DSC)

DSC measurements (PL-DSC, Polymer Laboratories, Surrey, UK) were performed on an accurately weighed samples (5–10 mg) at a heating rate of 10 ^{o}C/min under nitrogen gas purge.

Thermo gravimetric analysis (TGA)

The weight loss of vehicle as a function of temperature in each sample was determined using a thermo gravimetric analyzer (Perkin-Elmer, USA). About 5mg of each material was weighed and heated at the temperature range of 50–300 ^{o}C at a scanning rate of 10 ^{o}C/min under nitrogen purge.

Infrared spectroscopy

Infrared spectra of samples were obtained with a Nicollet spectrophotometer (Magna 550, Nicollet Instrument Corporation, Madison, WI, USA) in the near-infrared (4000–6000 cm^{-1}) region using compressed KBr disc technique. The spectra were obtained by averaging 64 scans at a resolution of 4 cm^{-1}.

Determination of water content

The water contents of samples were analyzed according to the Karl Fisher moisture method of the United State Pharmacopoeia, method A (26). The measurements were performed with a Toledo® DL38 KF Titrator (Mettler Ltd, Switzerland).

Statistical analysis was performed using a one-way analysis of variance (one way ANOVA) with multiple comparison data using a Tukey honest significant difference test (Statistica, StatSoft, Tulsa, USA).

RESULTS AND DISCUSSION

Table 1 shows particle size distribution, water contents, and true densities of unmilled CS (UM), and ball milled CS after 30 (BM1), 60 (BM2), 120 (BM3), 240 (BM4), and 360 (BM5) minutes. As shown in this table, 90% of particles ($d_{90\%}$) of UM, BM1, BM2, BM3, BM4, and BM5 were less than 69.22, 29.35, 23.37, 22.59, 30.22 and 35.11 µm respectively.

XRD scans of the UM, BM1, BM3 and BM5 are shown in Fig 1. The XRD data of UM showed some sharp peaks at diffraction angles from 13^{o} to 38^{o}. This data confirmed that UM have definitely crystalline shape. Although for BM3 and BM5 the XRD profiles, confirmed that these samples were predominately amorphous, but it could be related to the increase of heat during ball milling process. The substantial point was about XRD scan for BM1 which was a combination of UM and BM3 profiles and indicated that BM1 started transformation from crystalline to amorphous state. The effect of jet milling and spray drying processes of CS on crystallinity was also investigated (22). The spray drying process also transformed CS from crystalline to amorphous status, but jet milling process had no effect on the crystalline form of CS.

As shown in Fig 2, by increase in milling time, volume median diameters ($d_{50\%}$) of samples reduced until 60 min, and after that not only it did not decrease, but also increased at 240 and 360 minutes. According to XRD profiles increase of milling time leads to destruction of CS crystalline form and changes it to an amorphous state. These phenomena could be related to the production of heat due to rubbing and attrition between particles and consequently adhesion of particles to each other. Clearly all the particle size distributions of samples are not within the respirable ranges. As it was stated, a generally accepted aerodynamic particle size range for respiratory drug delivery is 1-5 µm. (6). There was no significant ($p>0.05$) differences in water contents and true densities of all samples (Table 1).

Fig 3 shows scanning electron micrographs of UM, BM1, BM3 and BM5 respectively. Unmilled samples indicated coarse powders with uniform shape that covered by fine particles, but ball milled samples consisted of agglomerates particles with different size especially in BM5. It is clear that irregular shapes of particles could be affected by method of micronization (15, 27).

As shown in Fig 4, DSC scan of UM shows a broad endotherm approximately at 80 ^{o}C that could be related to the presence of water in the samples. This endotherm peak could be observed at about 70 ^{o}C in DSC scans of BM1, BM3 and BM5. These diagrams show exothermic peaks at about 175 ^{o}C, which is consistent with recrystalization of amorphous CS. This exotherm was absent in DSC scans of UM and intensity of exothermic peak for BM1 were lower than other samples. These results may suggest that the BM1 sample was a combination of crystalline and amorphous states. All samples have a sharp exothermic peak at about 270 ^{o}C that depend on decomposition of CS.

Fig 5 presents infrared spectra of UM, BM1, BM3 and BM5. The patterns of all samples were not

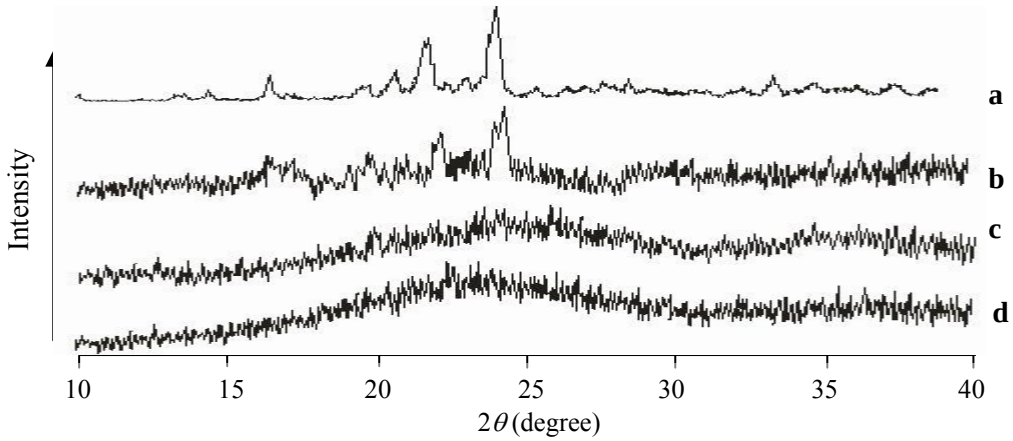

Figure. 1 X-ray diffraction patterns of (a) UM, (b) BM1, (c) BM3 and (d) BM5.
UM = Unmilled cefotaxime, BM = Ball milled cefotaxime.

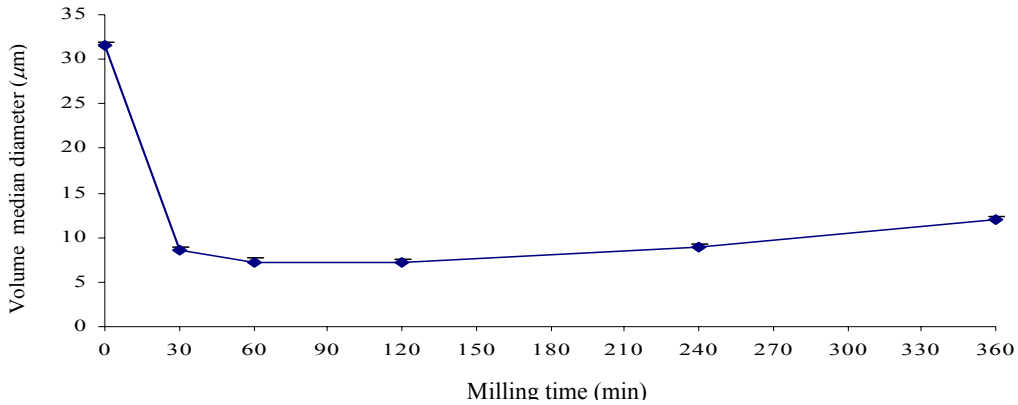

Figure 2. Effect of milling time on volume median diameter of the samples.

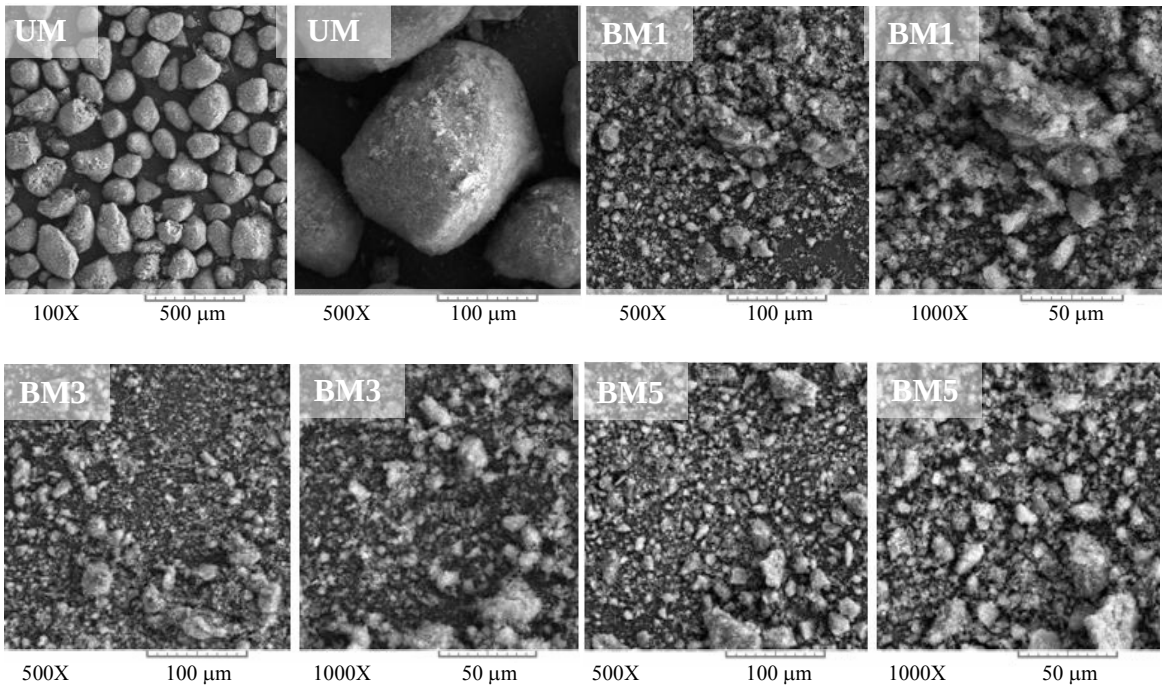

Figure 3. Scanning electron micrographs of the samples. 1. Unmilled cefotaxime, 2. BM = Ball milled cefotaxime.

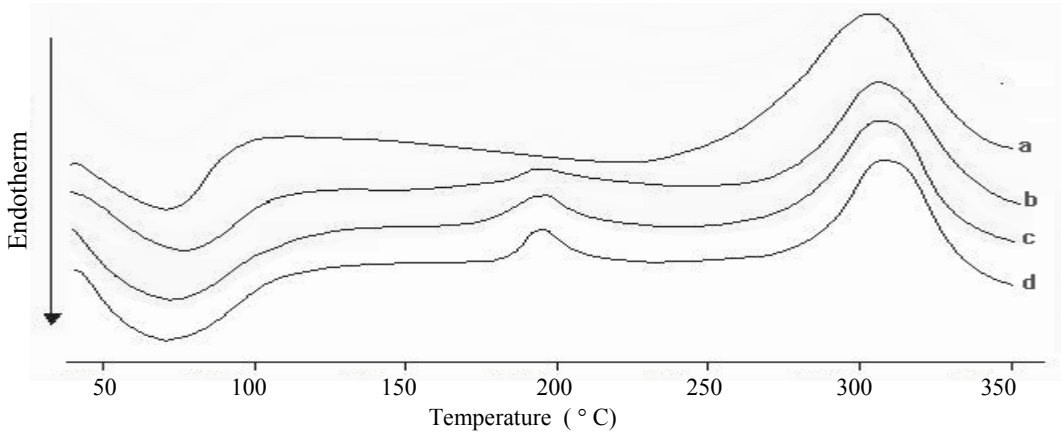

Figure 4. DSC scans of (a) UM, (b) BM1, (c) BM3 and (d) BM5.
UM = Unmilled cefotaxime, BM= Ball milled cefotaxime.

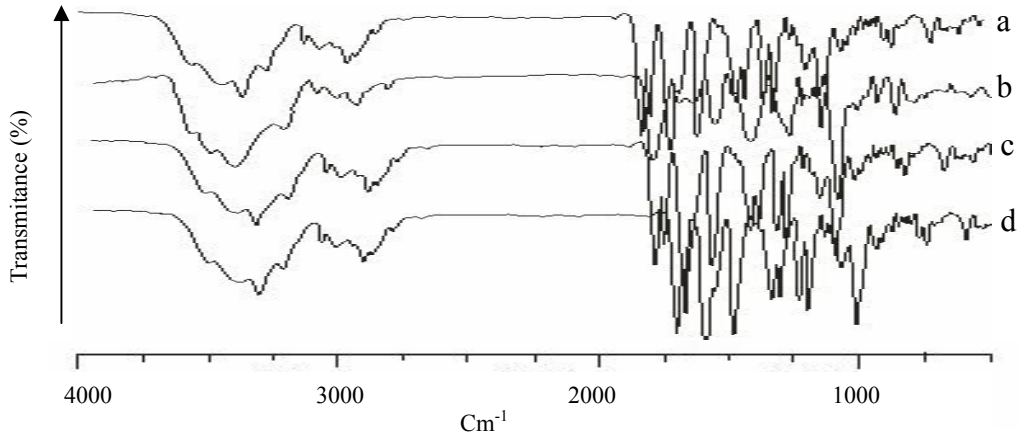

Figure 5. Infrared spectra of (a) UM, (b) BM1, (c) BM3 and (d) BM5.
UM = Unmilled cefotaxime, BM = Ball milled cefotaxime.

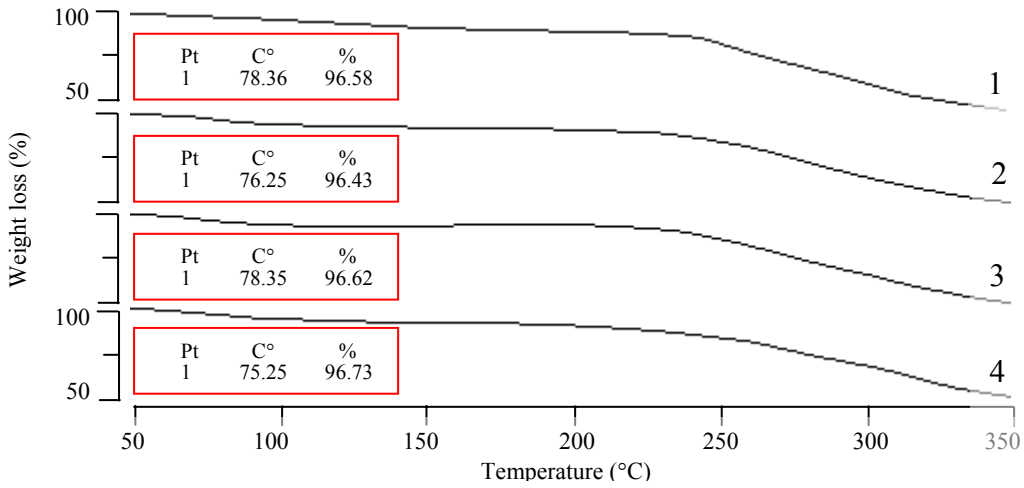

Figure 6. TGA thermo grams of (a) UM, (b) BM1, (c) BM3 and (d) BM5.
UM = Unmilled cefotaxime, BM = Ball milled cefotaxime.

Table 1. Particle size distribution, water contents, and true densities of the ball milled samples (mean ± SD, n=3).

Sample	Cumulative percent (undersize) [1]			Water content (%) [2]	True density (g/ml)
	$d_{10\%}$ (μm)	$d_{50\%}$ (μm)	$d_{90\%}$ (μm)		
UM[3]	10.22 (0.2)	31.56 (0.4)	69.22 (0.9)	3.4 (0.1)	1.56 (0.01)
BM[4]1	1.07 (0.1)	8.56 (0.3)	29.35 (0.9)	3.7 (0.1)	1.56 (0.01)
BM2	1.09 (0.2)	7.21 (0.5)	22.37 (0.8)	3.3 (0.1)	1.55 (0.01)
BM3	1.01 (0.2)	7.27 (0.2)	22.59 (0.7)	3.3 (0.1)	1.56 (0.01)
BM4	1.56 (0.1)	8.99 (0.3)	30.22 (0.8)	3.2 (0.1)	1.56 (0.01)
BM5	1.88 (0.3)	11.95 (0.4)	35.11 (0.8)	3.2 (0.1)	1.56 (0.01)

1. d_{value} = The size distributions are expressed by equivalent volume diameters at 10 ($d_{10\%}$), 50 ($d_{50\%}$) and 90% ($d_{90\%}$) cumulative volume.
2. Determined by karl fisher method. 3. Unmilled cefotaxime. 4. Ball milled cefotaxime.

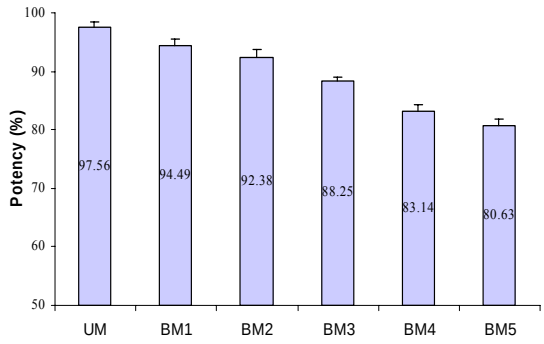

Figure 7. Potency (%) of all samples (mean± SD, n=3). UM= Unmilled cefotaxime, BM= Ball milled cefotaxime.

different. Flattering of peaks in BM3 and BM5 could be correlated to transformation of CS from crystalline to amorphous state. While UM and BM1 exhibited a significant lactam band at 1760 cm^{-1}, this band was shifted to about 1715 cm^{-1} in BM3 and BM5 and their intensities were lower than those of UM and BM1. These results could be related to degradation of some molecules of CS due to the break of lactam chains.

TGA thermograms of samples are shown in Fig 6. All samples at 75– 78 ^{o}C had one step in the TGA plot that could be related to lose of water. The weight lose for UM, BM1, BM3 and BM5 were 3.43%, 3.57%, 3.39% and 3.27% respectively. TGA outcomes confirmed presence moisture content that were determined by Karl Fisher appratus (Table 1).

The potency of samples were determined by HPLC method and results are shown in Figure 7. The potency decreased from 98.56% for UM to 80.63% for BM5 by increase of milling time. The potency of processed CS samples was out of acceptable range of credible pharmacopoeias (23). This significant ($p<0.05$) decrease of potency is possibly related to increase of heat due to attrition during process. These results recommend that ball milling method of micronization is not an appropriate technique to reduce particle size of CS to prepare DPI formulations.

CONCLUSION

The effects of ball milling technique in various periods of time for micronization to prepare DPI were investigated. Particle size distributions of all samples were not appropriate for respiratory drug delivery and SEM photographs confirmed it. On the other hand, determination of all samples showed none of them had potency in acceptable range of pharmacopoeias and increase of milling time resulted in significant ($p<0.05$) degradation. TGA and DSC analysis showed that ball milling could transform CS from crystalline to amorphous state.

In conclusion these results suggest that ball milling method of micronization is not appropriate technique to reduce particle size of CS to prepare DPI formulations.

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