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Inclusion of Montelukast in y-Cyclodextrin: Presenting a Mechanochemical Route to Improve Drug Stability and Solubility †

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Abstract: Montelukast sodium (MLK) is a worldwide anti asthmatic drug. Commercial formulations still have some issues with solubility and instability to light and humidity. To overcome them, the present work reports inclusion compounds of MLK and γ-cyclodextrin (γ-CD). As a molecular capsule, CDs have the ability to protect the inclusion guest from degradation, enhance its solubility and alter the pharmacokinetic parameters. MLK·γ-CD inclusion compounds were prepared by mechanochemistry. Without using any solvent, γ-CD was pre-milled and then co-milled with an equimolar quantity of MLK, in a ball mill at 600 cycles.min-1. After 120 min of milling, the formation of MLK·γ-CD inclusion compounds was confirmed by powder X Ray diffraction and scanning electron microscopy. Additional studies, performed under Pharmacopeia guidelines, showed that the prepared MLK·γ-CD inclusion compounds can indeed increase the dissolution of MLK drug when in ultra-pure water or simulated intestinal fluid (without pancreatin). This way, the MLK·γ-CD inclusion compounds that are presented in this work are a promising solution for improving the therapeutic effectiveness of MLK drug.

Keywords: Montelukast; Cyclodextrins; Mechanochemistry; Inclusion Compounds; Drug Solubility

1. Introduction

Montelukast sodium (MLK) is a widely used anti-asthmatic agent [1-5], that can also be prescribed for other conditions, such as the treatment allergic bronchopulmonary aspergillosis or chronic obstructive pulmonary disease (COPD) [6, 7]. By blocking the cysteinyl-leukotriene 1 receptors, this active pharmaceutical ingredient (API) is able to significantly improve the lung function of these patients [1, 8].

Nowadays, there are already three different dosage formulations of MLK in the market: tablets, chewable tablets and granules. Given this offer, the most suitable formulation can be chosen according to the patient needs concerning drug administration. For instance, MLK granule formulation is quite useful for administering to small babies and elderly patients, which could otherwise be a difficult challenge [9, 10]. Nonetheless, and despite their proved efficiency, MLK formulations still present some limitations to their use, namely MLK poor solubility in water (100 - 1000 mg/mL), as well as its instability to light, temperature, humidity and oxidation [9, 11-13]. In an attempt to overcome these limitations, numerous approaches have been used, among them, the use of cyclodextrins (CDs) as molecular carriers [9, 12, 14, 15]. Cyclodextrins are a class of water-soluble cyclic oligosaccharides, well-known for the capacity to accommodate in their cavity a diversified class of guest molecules, forming the worldwide known inclusion compounds (IC). In the pharmaceutical

industry, IC with CDs are particular interesting, since the inclusion of APIs within the CD cavity allows, in some cases, to protect the pharmacologically active guest molecule from degradation, as well as to modify their physicochemical properties and pharmacokinetic parameters [16-19].

In this way, in this work, we intend to prepare IC between MLK and CDs, in particular gamma-CD (γ-CD), that has been proved to have the ideal cavity size for the inclusion of this guest. Besides, and in contrast with the common procedures [12, 20, 21], in this report the γ-CD·MLK IC will be prepared by mechanochemical grinding, without using any solvent. This method applied to MLK was first described by us in a previous study [22] and it is gaining growing interest in pharmaceutical and organic chemistry applications [23]. In the end, we expect for the preparation of γ-CD·MLK IC to modulate the physicochemical properties of MLK, in particular its solubility and stability. Upon preliminary results described in [22], in this study Pharmacopeia guidelines will be used to determine the physicochemical properties of γ-CD·MLK IC when compared with the pure drug.

2. Experimental Section

2.1. Materials

γ-CD heptahydrate (MW = 1423.11), produced by Wacker Chemie with the commercial name Cavamax W8, was kindly donated by Ashland Industries Deutschland Gmbh. Montelukast sodium (> 98 % of purity, MW = 608.17), hereafter denominated MLK, was obtained from TCI or gently provided by Ashland (being produced by Ria International, India).

2.2. Equipment

Ball milling was carried out in a Philips MiniMill planetary apparatus, working at a velocity of 600 cycles min^{-1}. Samples were loaded into 50 mL calcium-doped zirconia grinding jars, each containing two yttrium-doped zirconia milling balls with 1 cm of diameter.

Laboratory Powder X-ray Diffraction (PXRD) data was collected at ambient temperature on an Empyrean PANalytical diffractometer, with working wavelengths of $\lambda 1$ = 1.540598 Å and $\lambda 2$ = 1.544426 Å (Cu Kα1,2 X radiation), equipped with an PIXcel 1D detector and a flat-plate sample holder in a Bragg-Brentano para-focusing optics configuration (45 kV, 40 mA). Intensity data were collected by the step-counting method (step 0.01º), in continuous mode, in the ca. $3.5 \leq 2\theta^{o} \leq 50^{o}$ range.

Scanning Electron Microscopy (SEM) images were acquired with a Hitachi SU-70 Schottky emission instrument, working at 10 kV. Samples were prepared by deposition on aluminum sample holders followed by carbon coating in an Emitech K950X carbon evaporator. Energy-dispersive X-ray spectroscopy (EDS) mapping images were recorded using a Bruker QUANTAX 400 microanalysis system.

UV-Vis spectroscopy measurements for the studies of montelukast aqueous dissolution were conducted on a UV-2501 PC Shimadzu spectrometer, at a working wavelength of 346 nm.

2.3. Preparation of γ-CD·MLK IC by mechanochemistry

γ-CD·MLK IC were prepared from a mixture containing amorphous γ-CD and MLK as received from the manufacturer, under the experimental conditions previously described by Barbosa et al. [22].

2.4. Dissolution of γ-CD·MLK IC versus pure MLK: Preliminary in vitro studies

The assays on the dissolution rate of γ-CD·MLK IC and pure MLK, when in ultrapure water, were performed as previously described by Barbosa et al. [22].

2.5. Dissolution of γ-CD·MLK IC versus pure MLK: According to Pharmacopeia guidelines

The dissolution profile of γ-CD·MLK IC and pure MLK were also analyzed under (1) ultrapure water, (2) ultrapure water with 0.5% (m/v) of sodium dodecyl sulphate (SDS), (3) simulated intestinal fluid (without pancreatin), with pH buffered at 6.8, and (4) acetate buffer, pH 4.5; and according to

Pharmacopeia guidelines. For this, a glass vessel, with a capacity of one liter, was filled with 900 mL of one of the aforementioned media. The vessel was immersed in a water bath of suitable dimensions, that allows maintaining not only a temperature of 37 ± 0.5 °, but also a constant and smooth flow of water during the tests. In addition to this, the medium inside the container was kept under continuous stirring with a rotor and a shaft with an agitation paddle attached to it.

10 mg of pure MLK, or an amount of γ-CD·MLK IC that corresponds to the same value of the pure drug, were added to the medium inside the container, that was then covered to delay medium evaporation. Aliquots were then collected at 5, 10, 20 and 30 min, for absorbance measurements under UV-Vis spectroscopy.

3. Results and Discussion

The preparation of γ-CD·MLK IC by co-milling had already been disclosed in one of our previous studies [22]. In this work, first we confirm the reproducibility of that study, by preparing the IC in the same conditions, and then we complement those results with further information regarding the impact of IC formation in the physicochemical properties of MLK drug.

3.1. Preparation of IC through mechanochemistry

γ-CD·MLK IC were efficiently prepared by mechanochemistry, as described by Barbosa et al. [22]. As seen in Figure 1, upon 120 minutes of mechanical grinding an amorphous compound, with no reflections on its diffractogram, was obtained. This could be an initial evidence of the formation of γ-CD·MLK IC, since it is a typical feature for IC prepared by mechanochemical grinding [24-26].

In addition to this, SEM images also showed that in the physical mixture of MLK and amorphous γ-CD there are two distinct morphologies (Figure 2a). While MLK appears as an agglomerate of spheres, γ-CD particles present an irregular shape and size, with round edges. EDS mapping of this mixture further confirms the presence of characteristic elements of MLK, namely S, Na and Cl, only in the agglomerates (Figure 2b). Upon 120 min of grinding, it was reached an uniform distribution of MLK throughout the sample, showing thus a complete interaction between the two components (Figure 2d).

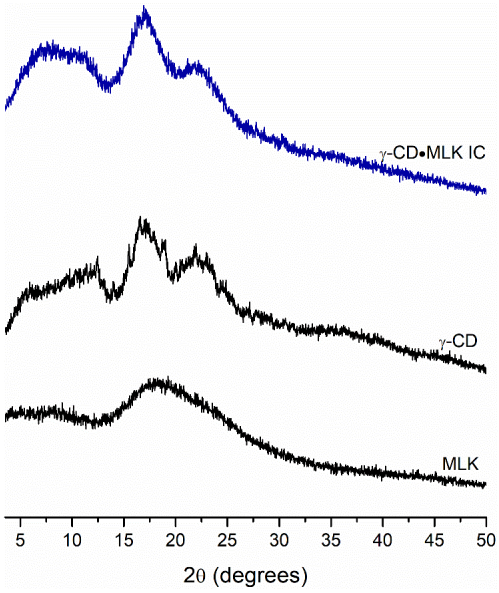

Figure 1. Powder x-Ray diffraction patterns of (bottom) MLK, as received, (middle) amorphous γ-CD heptahydrate, and (top) the γ-CD·MLK IC, prepared by mechanochemistry.

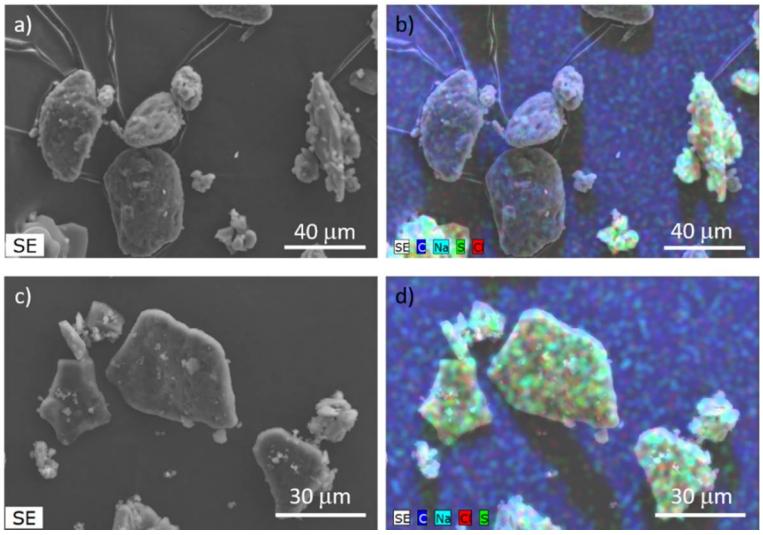

Figure 2. Scanning electron microscopy images and energy dispersive X-ray mapping of: a physical mixture of MLK and amorphous γ-CD (a and b, respectively) and of the γ-CD·MLK IC, prepared by mechanochemistry (c and d, respectively). EDS is shown to access the distribution of MLK characteristic elements: S, Na and Cl atoms.

3.2. Dissolution of γ-CD·MLK IC in pure water

The dissolution profile of both pure MLK and the γ-CD·MLK IC was studied, at first, in ultra-pure water. In a previous study we had reported that till *ca.* 60 min, both the pure drug and the IC showed similar amounts of dissolved MLK in the medium. However, it was possible to notice that the values were slightly higher for the IC [22]. Similar to those results, in this study we noticed that for a period of 30 min, the amount of MLK in ultra-pure water tends to be slightly higher when the dissolved compound is the prepared γ-CD·MLK IC (Figure 3).

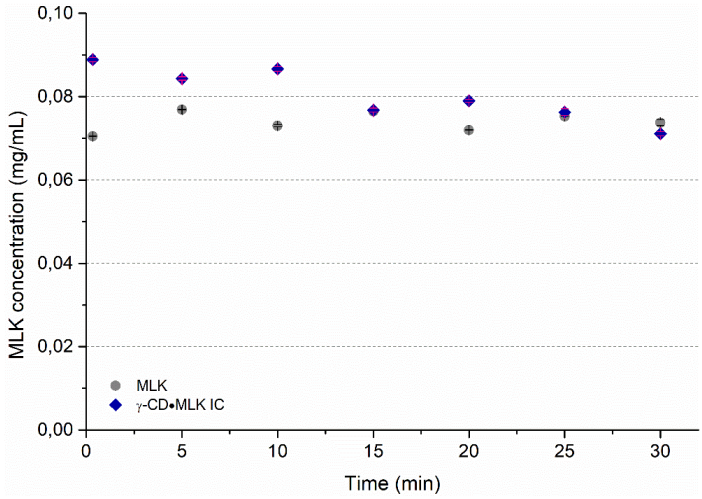

Figure 3. Dissolution profile of MLK, from its pure form and from the γ-CD·MLK IC, when in ultra-pure water.

Following these preliminary results, the dissolution profile of pure MLK and γ-CD·MLK IC was once again analyzed; however, in this turn, under the guidelines recommended by FDA for drugs that, such as montelukast sodium, are classified with a good permeability but low solubility (BCS class II) [27]. In this way, in addition to the 30 min of analysis time, the apparatus, volume of the medium, drug dosage and sampling times were adjusted as described in section 2.5.

When pure MLK and γ-CD·MLK IC were added to ultra-pure water, none to very low amounts of MLK were able to dissolve. In fact, while with pure MLK there was no trace of the drug dissolved in the medium; with γ-CD·MLK IC very low amounts of MLK were able to dissolve and be detected (Figure 4). In a way, these results are in accordance to those that we previously reported and showed that, at lower times, the dissolution profile of pure drug *versus* IC is similar, but the IC allows the dissolution of slightly higher amounts of MLK [22]. A completely different dissolution profile was seen with a solution of ultrapure water with 0.5%(m/v) of sodium dodecyl sulphate (SDS). In this medium, that is considered as a reference for dissolution studies of MLK, almost all the amount of MLK, added either in the form of pure drug or γ-CD·MLK IC, was dissolved in the medium. Besides, the dissolution profiles were very similar, almost equivalent, for both the pure drug and the γ-CD·MLK IC (Figure 5).

Additional studies were also made in media that simulate both the intestinal[28] and the duodenum environments. To mimic the intestinal environment, a solution of simulated intestinal fluid (without pancreatin), with pH buffered at 6.8, was used. In this medium, the behavior of pure MLK and γ-CD·MLK IC was very similar to that previously observed in ultra-pure water. While with pure MLK no drug was dissolved, with the IC a small amount of the drug was able to dissolve (Figure 6). In opposition to this, when in a medium of acetate buffer at pH 4.5, which mimics the duodenum environment, neither pure MLK nor γ-CD·MLK IC evidenced any dissolution, as no traces of MLK were detected in the medium (Figure 7). This may be due to the low pH (4.5), as MLK is a weak and hydrophobic acid that does not dissolve in an acidic medium.

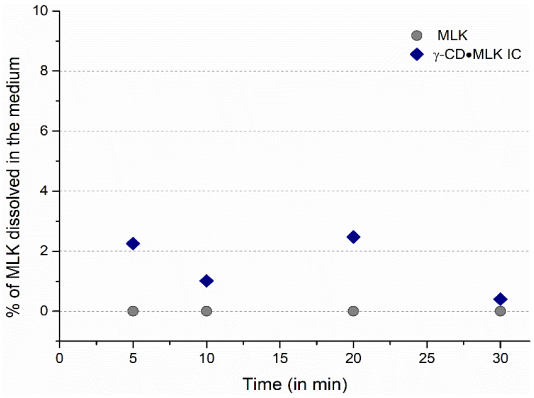

Figure 4. Dissolution profile of MLK, from its pure form and from the γ-CD·MLK IC, when in ultra-pure water, and while following Pharmacopeia guidelines.

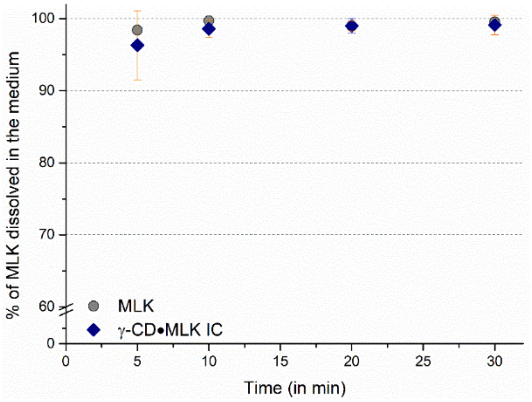

Figure 5. Dissolution profile of MLK, from its pure form and from the γ-CD·MLK IC, when in ultra-pure water with 0.5%(m/v) of sodium dodecyl sulphate (SDS), and while following Pharmacopeia guidelines.

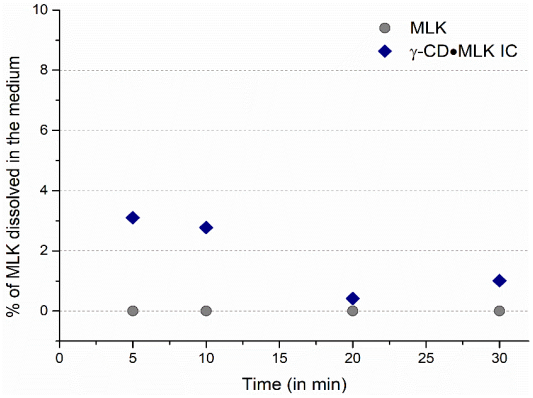

Figure 6. Dissolution profile of MLK, from its pure form and from the γ-CD·MLK IC, when in simulated intestinal fluid (without pancreatin), with pH buffered at 6.8, and while following Pharmacopeia guidelines.

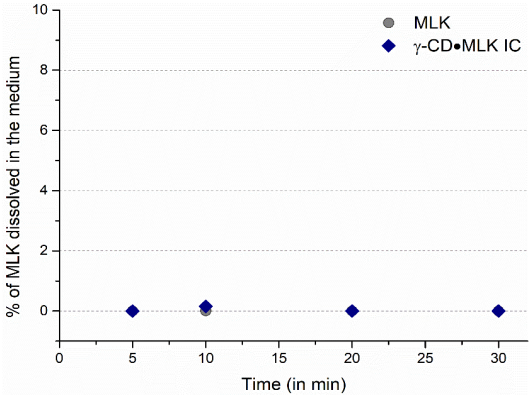

Figure 7. Dissolution profile of MLK, from its pure form and from the γ-CD·MLK IC, when in simulated duodenum environment, with acetate buffer, pH 4.5, and while following Pharmacopeia guidelines.

5. Conclusions

This work begun by confirming the reproducibility of the preparation method for the γ-CD·MLK IC by mechanochemistry, without using any solvents [22]. Advanced characterization techniques, such as PXRD and SEM, allowed confirming the formation of these amorphous compounds.

Following, a more thorough analysis of the dissolution profile of both pure MLK and γ-CD·MLK IC was performed. When in ultra-pure water or in a medium that simulates the intestinal environment, the IC tended to afford a slightly higher dissolution of MLK (*ca.* 3 – 5%). Moreover, when in ultrapure water with 0.5%(m/v) sodium dodecyl sulphate (SDS), the pharmacopoea reference medium for the MLK dissolution test, the dissolution profiles of pure MLK and γ-CD·MLK IC were practically identical, thus indicating bioequivalence. Similar profiles for pure MLK and γ-CD·MLK IC were also seen in simulated duodenum environment, with both compounds showing no dissolution of MLK.

Overall, this work provides insightful results on the use of mechanochemistry as a sustainable methodology to form inclusion compounds between γ-CD and MLK. The prepared γ-CD·MLK IC seem to be able to improve, at some extend, the dissolution of MLK in a few media, while having a dissolution profile equal to pure MLK in the reference medium and thus hinting at bioequivalence, a required trait for its incorporation into oral dosage forms. It is thus possible for γ-CD·MLK IC to help the pharmaceutical industry to overcome current setbacks and develop new MLK formulations with enhanced therapeutic effectiveness

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

γ-CD	**Gama cyclodextrin**
API	**Active pharmaceutical ingredient**
CD	**Cyclodextrin**
COPD	**Chronic obstructive pulmonary disease**
EDS	**Energy-dispersive X-ray spectroscopy**
IC	**Inclusion Compound**
MLK	**Montelukast sodium**
PXRD	**Powder X-ray Diffraction**
SDS	**Sodium dodecyl sulphate**
SEM	**Scanning Electron Microscopy**

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