

In vitro and *In vivo* Evaluation of Tablets Containing Meloxicam-PEG 6000 Ball-Milled Co-Ground Mixture

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ARTICLE INFO

Article history:

Received on: 20/10/2016

Accepted on: 21/11/2016

Available online: 30/03/2017

Key words:

Meloxicam, PEG, co-grinding, dissolution, tablets, *in vitro*, *in vivo*.

ABSTRACT

This study aimed at a) preparing meloxicam (MLX) tablets containing ball-milled co-ground mixture with PEG 6000 (1:4); b) evaluating the relevant *in vitro* quality parameters and stability; and c) comparing the bioavailability of the developed tablets with that of a reference meloxicam tablet (Mbc[®]) in 6 human subjects. The dissolution rate of the prepared tablets was higher than that containing ball-milled or un-milled drug alone, as well as higher than four marketed meloxicam tablets. Upon storage for 12 months at 25±1°C / 60% RH, no remarkable changes in physical appearance of the prepared tablets, % drug content, thickness, disintegration time and in dissolution rate were observed. The prepared meloxicam tablets showed higher bioavailability which was indicated by significant higher AUC and significant shorter T_{max}. The bioavailability of the developed test tablets was 126.45%, relative to the marketed brand product (Mbc[®] tablets). Grinding of MXL with PEG 6000 resulted in a fast MLX release, which may provide rapid onset of action and allow also a reduction of required dose necessary to obtain the therapeutic effect. This, in turn, may further reduce the most common side effects observed with non-steroidal anti-inflammatory drugs (NSAIDs).

INTRODUCTION

Several factors, other than physicochemical properties of the drug, may influence the therapeutic efficacy of drugs from the dosage forms, such as the dissolution rate. It has been shown by many authors that, the dissolution rate of pure drugs can be altered significantly by the proper selection of formulation components as well as processing methods, e.g. (Blagden *et al.*, 2007; Grau *et al.*, 2000). Meloxicam (MLX) is one of the Cox II inhibitors and considered safer than other NSAIDs. However, the major problem with the drug is its very low solubility in biological fluids. The rate of dissolution can be increased by increasing the surface area of the drug by different methods of size reduction including grinding, co-grinding or solid dispersion methods. Moreover, the dissolution of a drug can also be influenced by the disintegration time of the tablets (Engelhardt *et al.*, 1995). Rapid absorption may also reduce the frequency and severity of gastrointestinal disorders observed after oral

administration of certain drugs such as aspirin and tetracycline, by reducing the contact time in the gastrointestinal tract (Gibaldi 1991). In a previous study from this laboratory, MLX was formulated into co-ground mixtures by ball/vibrational milling in presence of hydrophilic carriers. It was found that grinding of crystalline meloxicam together with a semi-crystalline polymer PEG 6000 led to a drastic reduction in particle size and crystallinity of MLX, as well as enhanced dissolution profile. The co-ground mixture consisting of MLX: PEG (1: 4) using ball mill was found to have superior dissolution compared to all other co-ground mixtures (Etman *et al.*, 2015). Therefore, the aims of this study were: a) to prepare Meloxicam tablets containing ball-milled co-ground mixture with PEG 6000 (1:4); b) to evaluate the relevant *in vitro* quality parameters of the finished tablets such as drug content, hardness, friability, disintegration time, thickness and dissolution; c) to compare the dissolution profile with three marketed Meloxicam tablets; d) to test its stability at 25±1°C / 60% RH for 12 months; and finally e) to compare the bioavailability of the developed tablets with that of a marketed meloxicam tablets (Mbc[®]), using 6 male human subjects in a cross-over design.

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EXPERIMENTAL

Materials

Avicel® (FMC, USA), Polyplasdone® crospovidone (ISP, USA), Aerosil® (Evonik, Germany), magnesium stearate (Siemsgluss & Sohn GmbH & Co., India), Mbil® tablets (Medical Union pharmaceuticals, Abu-Sultan, Ismailia, Egypt.), Mbc® tablets (Boehringer Ingelheim, Germany) and Anti® (ADWIA Co.S.A.E., 10th of Ramadan City, Egypt), clear transparent hard gelatin capsules, size (0) were kindly supplied by Pharco Pharmaceuticals Co. (Alexnadia, Egypt), HPLC grade methanol and acetonitrile (Gliwiceul, Sowinskiogo, Poland), and HPLC grade phosphoric acid and potassium dihydrogen phosphate (Fisher, USA). All materials were used as received from their sources without any further treatment.

Apparatus

Single punch tablet machine, 12 mm flat-faced punches and hardness tester, type TB24 (Erweka, GmbH, Heusenstamm, Germany), micrometer (Moore and Wright Ltd. Britain Tool, Sheffield, UK), differential scanning calorimeter (Pyris 6 DSC, Perkin Elmer, USA) and a friabilator (Erweka, Germany), FT-IR (Thermo Fisher Scientific, Inc., Waltham, MA, USA), scanning electron microscope (LEO, 435 VP, UK), XRD (Rigaku Demki, Rint-2500VL, Tokyo, Japan), a reversed phase C-18 Perkin Elmer HPLC column: Intersil ODS-SP (4.6 x 150mm, 5µm), UV – detector JENWAY 6305 (England), a loop injector equipped with a 25µL loop and vortex (VM-300, U.S.A.).

Methods

Preparation of meloxicam- PEG 6000 tablets

Calculated amount of meloxicam ball-milled co-ground mixture with PEG 6000 in 1:4 ratio (Etman *et al.*, 2015), equivalent to 15 mg of the drug, was employed. Co-ground mixture, Avicel®, crospovidone and Aerosil® were blended thoroughly in a mortar with pestle for 10 minutes to achieve a homogenous mixture. The powder blend (Table 1) was then lubricated with magnesium stearate (0.5 % w/w) for additional 3 minutes and this lubricated blend was compressed into tablets using 12 mm flat-faced round tooling on a single punch tablet machine.

Table 1: Ingredients of the prepared meloxicam tablets.

Ingredients (mg)	Un-milled MLX	Milled MLX alone (BM)	MLX : PEG 6000 (1:4) BM
Meloxicam	15	15	75 mg mix = 15 mg MLX
Avicel®	160	160	100
Crosspovidone	20	20	20
Aerosil®	4	4	4
Magnesium stearate	1	1	1
Final weight (mg)	200	200	200

Furthermore, the same amount of Meloxicam ball-milled co-ground mixture with PEG (1:4) and other ingredients used in the preparation of MLX tablets (except magnesium stearate) were

mixed and filled into empty hard gelatin capsules to study the effect of compression, used in tablet manufacture, on drug release.

In vitro evaluation of the prepared MLX tablets

Drug content

Ten Tablets were finely ground in a mortar and the amount of powder equivalent to 15 mg of MLX was taken and dissolved in 100 ml phosphate buffer pH 7.4. Aliquots were filtered through 0.2µm Millipore filter and assayed spectrophotometrically at 362 nm.

Uniformity of Weight

Twenty tablets were weighed collectively and individually, and the average weight (±SD) was calculated.

Hardness test

The hardness test was performed by Erweka hardness tester using 5 tablets, and the average force of fracture (±SD) was recorded in Kg / cm².

Tablet thickness

The thickness of 5 tablets was measured using a micrometer. The mean thickness (±SD) was calculated.

Friability test

Twenty tablets were carefully de-dusted, weighed, loaded in the friability tester, and rotated at 25 rpm for 15 minutes. Tablets were removed from drum, de-dusted and accurately weighed. If obviously cracked, cleaved or broken tablets are present in the tablets sample, the sample fails. The percentage of weight loss was calculated using the following equation:

$$\% \text{ Friability} = [(W1 - W2) \times 100] / W1$$

Where: W1 = weight of tablet before test (initial weight).

W2 = weight of tablet after test (final weight).

The results should be less than 1 % (USP 2006).

Disintegration time test

The disintegration time of the developed MLX tablets was determined by USP disintegration apparatus using one liter distilled water kept at 37± 0.5 °C as disintegrating medium. The average time required for complete dispersion of 6 tablets was calculated (±SD).

Dissolution test

The in-vitro dissolution study was carried out in the USP type II (paddle) dissolution apparatus using 900 ml of phosphate buffer pH 7.4 as the dissolution medium at 37 ± 0.5°C. The speed of the paddle was set at 50 rpm and 5 ml of the dissolution medium was withdrawn for analysis and replaced with the same volume of fresh dissolution medium. The sample was filtered and assayed spectrophotometrically at 362 nm.

Dissolution rate of the prepared tablets was compared with that of 3 marketed tablet brands of MLX (15 mg each);

Mbil[®], Mbc[®] and Anti[®] under the same dissolution conditions described above.

The prepared MLX capsules were also tested for dissolution under the same conditions described before. Each formulation was tested in triplicate and the mean values were calculated.

Scanning electron microscopy (SEM)

Images by SEM were performed to visualize the reduction in particle size of MLX by co-grinding. Prior to examination, samples were gold-coated under vacuum and scanned at 25 kV, using 5000x magnification.

Fourier transform infra-red spectroscopy (FT-IR):

Infra-red spectra of untreated MLX, PEG, and the co-ground mixture were determined, where 2 mg sample was mixed with 200 mg potassium bromide, ground into fine powder, and then compressed into a disc using a hydraulic press. Each disc was scanned over the region of 500 – 4000 cm⁻¹ and the resolution was 4 cm⁻¹. The characteristic bands were recorded for all tested samples.

Differential scanning calorimetry (DSC)

The DSC thermograms were traced for each of the untreated MLX, PEG, and the ground mixture. The powdered sample (5 mg) was hermetically sealed in aluminum pans and heated at a constant rate of 10°C/min, over a temperature range of 25°C to 200°C. Indium standard was used to calibrate the DSC temperature and enthalpy scale under constant purging of dry nitrogen at 30 ml/min.

Characterization of meloxicam tablets by X-ray diffraction (XRD)

XRD analysis of untreated drug, PEG 6000, the ground mixture, and the prepared MLX tablets were performed. Samples were irradiated with monochromatized Cu K α radiation (1.542Å) and analyzed between 2 and 40°. The voltage and current used were 40 kV and 30 mA, respectively. The chart speed was 10 mm/sec.

Stability study

Stability study was carried out by storing the prepared tablets in amber colored screw capped glass bottles which were placed in a thermostatically controlled incubator at 25±1°C / 60% RH over a period of 12 months.

At intervals of 3 months, the stored tablets were subjected to evaluation for any physical changes, in addition to analysis of drug content, dissolution and XRD. Dissolution test was performed in phosphate buffer at pH 7.4 as previously mentioned.

In vivo study

HPLC method of analysis

The MLX concentration in blood was determined by a validated HPLC method, as described below. The mobile phase

consisted of acetonitrile - 20mM potassium dihydrogen phosphate (60:40%, v/v, pH 3.5). Flow rate of degassed mobile phase was 1.2 ml/min (isocratic conditions) at room temperature. Injected sample volume was 25µL and UV detection was set at a wavelength of 355 nm (Velpandian *et al.*, 2000).

Calibration and sample preparation

A primary stock standard solution of MLX (0.1 mg/mL) was prepared in HPLC – grade methanol to obtain a series of working standard solutions of 100, 200, 300, 500, 1000, 2000, and 5000 ng/mL. A stock solution of piroxicam (PIR) (0.1 mg/mL) was also prepared in methanol as the internal standard (IS) solution. Stock solutions were stored at -20°C and working standard solutions used to generate the calibration curve were freshly prepared from stock solutions for each analysis. Blank human plasma from different subjects was collected, and used for preparing the standard samples for calibration curve. Three different MLX standard solutions (100, 500, and 2000 ng/mL) were used for analytical method validation. For construction of the calibration curve, 50 µL of MLX working standard solutions (ranging from 100 to 5000 ng/mL) were freshly prepared and mixed with 250 µL of blank human plasma, pre-thawed at room temperature, plus 20 µL of PIR (internal standard) and 200 µL of acetonitrile acidified with H₃PO₄ (0.1%, v/v), vortexed for 20 seconds, and centrifuged for 15 minutes at 3000 rpm. The clear supernatants were collected in culture tubes and filtered through 0.2 µm Millipore filter.

For measurement of meloxicam concentrations in plasma samples, 250 µL of plasma was mixed with 20 µL of the internal standard plus 200 µL of acetonitrile acidified with H₃PO₄ 0.1% (v/v), and treated in the same way for calibration samples mentioned above (Velpandian *et al.*, 2000; Ji *et al.*, 2005).

Analytical method validation

Selectivity of the assay was assessed by analyzing blank human plasma to evaluate interference of endogenous matrix of plasma around the retention times of both MLX and the IS. MLX plasma peak was identified based on retention time and UV spectra of the reference standard. MLX concentrations in plasma samples were quantified according to the plasma calibration curves for which seven MLX concentrations (100, 200, 300, 500, 1000, 2000, and 5000 ng/mL) were used to establish the least square linear regression of the curve:

$$y = ax + b$$

Where “x” is the concentrations of MLX, “y” is the peak area ratio of MLX to IS, “a” and “b” represent the slope and y-intercept of the curve, respectively. According to the pharmacopeial recommendations (USP 2011), the limit of detection (LOD) is defined as the concentration that has a signal – to – noise ratio of 3 : 1, while for limit of quantification (LOQ), the ratio considered is 10 : 1. The limit of quantification (LOQ) was determined based on calibration curves using the formula:

$$LOQ = 10 \times \sigma/S$$

Where “ σ ” is the standard deviation (SD) of the y-intercepts for the regression lines, and “S” is the mean slope of the calibration curves. An acceptance criterion for LOQ was defined as precision less than 15% (coefficient of variation; CV) and accuracy within $\pm 20\%$ of the nominal concentration with repeated analyses.

The within-day (Intra-day) and the between-day (inter-day) precision and accuracy for the method were studied at three concentration levels (100, 500, and 2000 ng/mL) using three replicate determinations for each concentration within one day and repeated on three consecutive days, respectively. Relative difference of the estimated concentrations was expressed as a percentage of the CV. Intra- and inter-day accuracy expressed as bias, which was determined by calculating the percentage difference between the estimated value and nominal value of MLX:

$$\{(\text{estimated value} - \text{nominal value}) / \text{nominal value}\} \times 100$$

Absolute recovery of MLX was measured by comparing the peak area of plasma samples pre-spiked with 10, 500, and 2000 ng/mL MLX with corresponding concentrations of MLX in the mobile phase. Each assay was conducted in triplicate and the obtained results were statically analyzed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test. P-values < 0.05 were considered statistically significant.

Study design

Before starting the *in vivo* study, the protocol was approved by the Ethics Committee, Faculty of Pharmacy, Alexandria University. The study was carried out in two – periods, single – dose, crossover, randomized order. Six healthy male volunteers (24-28 years old and 70-90 kg) participated in this study. The volunteers were informed about the purpose, duration, and risks of the study, and they were requested to sign a written informed consent. The subjects were given the developed meloxicam tablets (test product) and Mbc[®] 15 mg tablet (reference product) in a randomized order, separated by two weeks washout period. Each subject was instructed to abstain from all medications for one week before starting the study or during the study weeks. The subjects were fasted 12 hs before and 4 hs after the administration of tablets. Each subject received the prepared tablet and reference product with 250 ml of water. Venous blood samples (5 ml) were withdrawn at different time intervals of 0, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 hrs post dose administration and were collected in tubes containing EDTA. Precipitation of plasma proteins was performed by addition of 0.5 ml acetonitrile followed by agitation mechanically on a vortex for 30 sec. The blood samples were centrifuged at 3000 rpm for 15 min and plasma was separated and frozen at -20 °C until analyzed.

Pharmacokinetic (PK) evaluation

Plasma concentration – time curves were used for PK analysis of the prepared MLX tablets and the reference product. Area under the curve (AUC) from time 0 to 24 hours (AUC₀₋₂₄) was estimated by the linear trapezoidal method, while AUC from

time 0 to infinity (AUC_{0-inf}) was obtained by adding C₂₄/k_{el} ratio to the AUC₀₋₂₄, where C₂₄ and k_{el} represent the plasma concentration of meloxicam at 24 hours postdose and the elimination constant, respectively. Maximum concentration (C_{max}) and the time to achieve C_{max} (T_{max}) were obtained from direct visual inspection of plasma concentration versus time curves. Other relevant pharmacokinetic parameters including half life (t_{1/2}), elimination rate constant (K_{el}), area under the first moment curve (AUMC) and mean residence time (MRT) were also computed. AUMC was obtained by plotting mean meloxicam plasma concentration multiplied by time versus time. MRT was calculated by dividing AUMC over AUC₀₋₂₄ (Velpandian *et al.*, 2000) and (Ji *et al.*, 2005).

RESULTS AND DISCUSSION

In vitro evaluation of the prepared meloxicam tablets

The post compression parameters such as drug content, weight variation, hardness, friability, thickness, and disintegration time were acceptable. The drug content in various formulations varied between 99.55 and 101.15 %. Tablet weight varied between 199.5 and 201 mg and the hardness varied between 4.5 and 5 Kg / cm². The thickness was in the range 4.07 to 4.24 mm and disintegration time varied between 48 and 396 sec.

Friability is closely related to tablet hardness and is designed to evaluate the ability of the tablet to withstand abrasion in packaging, handling and shipping as friction and shock are the forces that most often cause tablet to chip, cap or break. Friability of all formulations was less than 1%, which indicates a good mechanical resistance of the tested tablets.

Tablets containing co-ground mixture disintegrated rapidly (48 \pm 1.49 sec), while those contained unmilled or milled MLX alone exhibited longer disintegration time (396 \pm 1.5 sec and 225 \pm 1.69 sec respectively). Co-grinding of MLX with PEG 6000 would be expected to increase the wettability, decrease particle size and crystallinity of the drug that resulted in a reduction of disintegration time.

Dissolution study

The dissolution rate of MLX from the tested tablet formulation, containing MLX-PEG co-ground mixture, and three different brand products are shown in Fig. 1.

The dissolution profile of tablets containing co-ground mixture with PEG 6000 (1:4) was higher than that containing unmilled or milled drug alone. Also, dissolution rate of tablet containing co-ground mixture of MLX was higher than the other meloxicam tablet brands. This may be due to the use of crospovidone, which causes swelling to 4 - 8 folds in 10 seconds and due to particle size reduction and improved wettability by co-grinding technique. In addition to micronization, conversion of drug to amorphous form during the preparation might have also contributed to the increased dissolution rates observed with the co-ground MLX-PEG mixture.

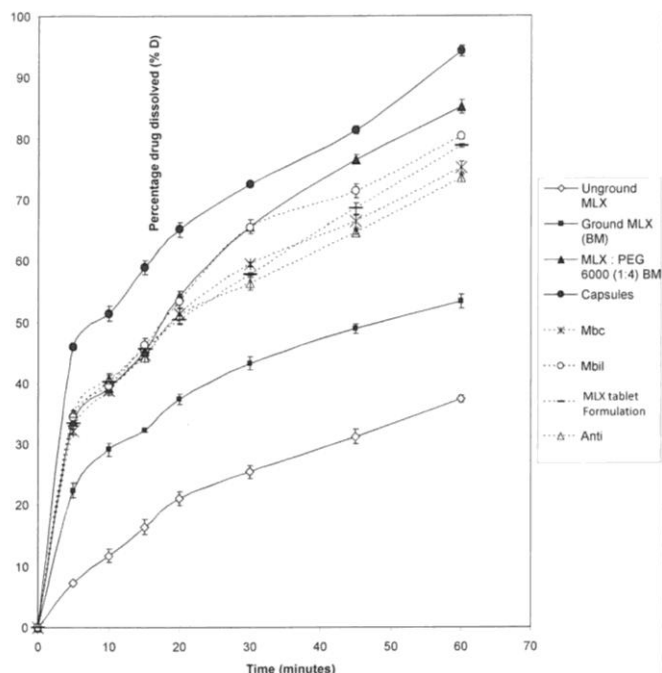


Fig. 1: Dissolution rate profiles of the prepared meloxicam (MLX) tablets, capsules, and the marketed meloxicam brand tablets, in comparison with the untreated MLX, ball milled MLX, and ball milled coground mixture.

Scanning electron microscope (SEM)

The surface morphology of unground MLX, ground MLX alone and its co-ground mixture with PEG (1:4 drug to polymer ratio) using ball mill were examined by SEM. Figures. 2-4 show selected SEM images of representative samples. Drug particles of meloxicam under scanning electron microscope appeared as crystals having the width of about 7 μm and a length of about 21 μm (Fig. 2).

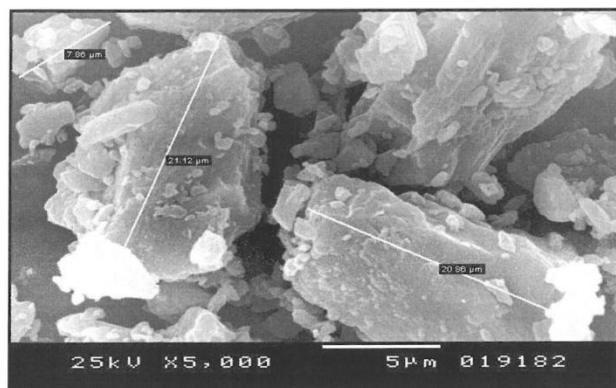


Fig. 2: SEM image of unmilled meloxicam.

Grinding of MLX alone in ball mill resulted in a reduction in particle size (3.5-10.5 μm) (Fig. 3). Co-grinding of MLX with the carrier PEG in a ratio of 1:4 using ball milling technique resulted in a remarkable reduction in particle size to 1.39-2.14 μm width and 2.52-3.59 μm length (Fig. 4). The dimensions measured from the co-ground mixture are the dimensions of particles that constitute a mixture of the drug adhered or fused in the carrier matrix and are not entirely made of

pure drug. This would mean that the particle size of the co-ground drug is much smaller than that of the milled drug alone. The reduction in particle size evidenced by the SEM, in addition to other findings obtained in the IR, XRD and DSC studies hereafter, would explain the observed enhancement in drug dissolution.

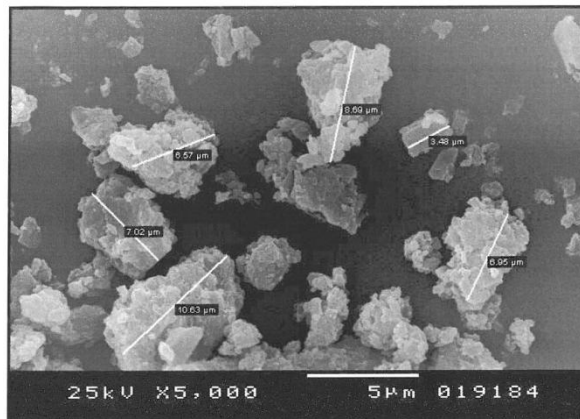


Fig. 3: SEM image of milled meloxicam alone using ball mill.

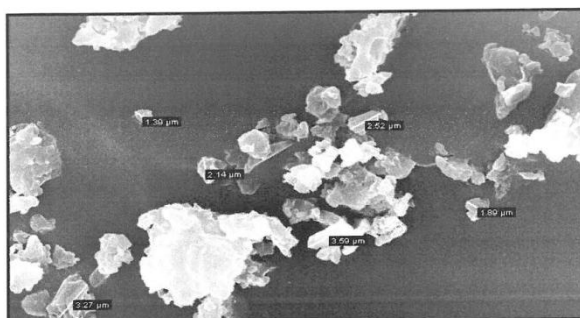


Fig. 4: SEM image of meloxicam with PEG 6000 in ratio 1:4 using co-grinding technique by ball mill.

X-ray diffraction (XRD) study

The powder XRD patterns of MLX, PEG 6000 and the mixtures thereof (Fig. 5), in addition to the prepared Meloxicam tablets (Fig. 6) were determined. The diffraction pattern of the pure drug showed its highly crystalline nature, as indicated by the numerous distinctive peaks at 10.30, 10.38, 10.87, 13.26, 14.69, 16.22, 17.75, 20.43, 23.44, 24.43, 25.49, 26.42, 27.58, 29.23, 31.90, 33.81, 36.89, 39.50, 43.19, 44.65 and 47.67°. XRD pattern of PEG 6000 alone exhibited two high intensity peaks at 19° and 23°. The lack of the numerous distinctive peaks of the drug in the co-ground mixture could demonstrate that a major quantity of the drug was incorporated in the solid-state carrier matrix in an amorphous structure. The results above indicate that co-grinding of meloxicam with PEG 6000 using ball-mill caused some changes that may include size reduction (Figs. 2-4), reduced crystallinity, and / or conversion into amorphous form (Figs. 5&6). Tablets prepared using this co-ground mixture along with some excipients produced a product with superior properties, compared to the existing marketed products containing meloxicam, as indicated from the bioavailability study hereafter.

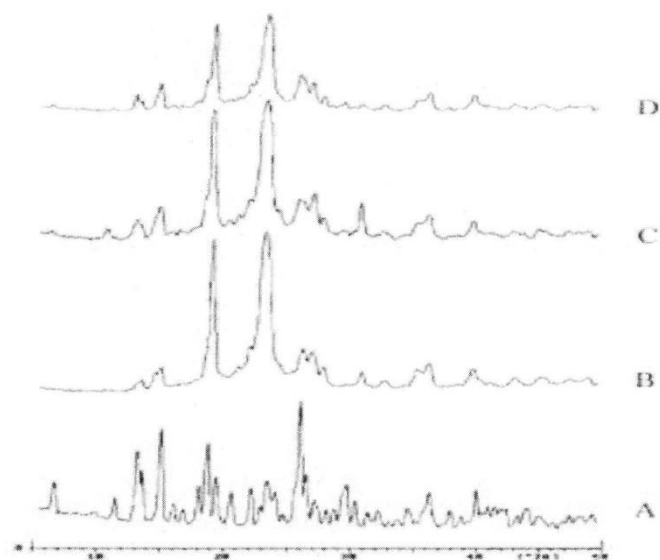


Fig. 5: Powder X-ray Diffraction Spectra of MLX (A), PEG 6000 (B), PM with PEG 6000 (1:4) (C), and Co-ground mixture with PEG 6000 (1:4) (D).

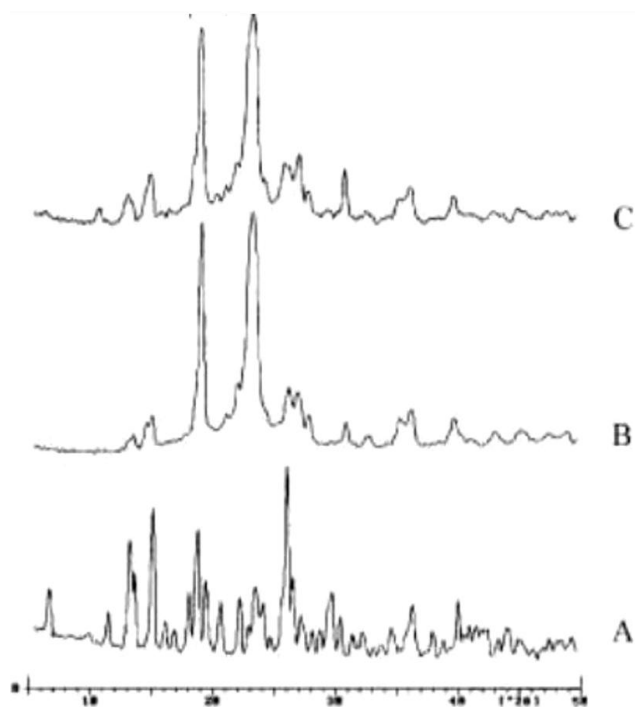


Fig. 6: XRD Patterns of Pure Meloxicam (A), PEG 6000 (B) and the prepared Meloxicam tablets (C).

Infrared (FT-IR) study

The interaction between the drug and the carrier often leads to identifiable changes in the FT-IR profile of the drug. FT-IR spectra for MLX, PEG, and the co-ground mixture were performed (Fig. 7). FT-IR spectrum of MLX showed prominent peaks at 3291, 1620, 1552, 1340, and 1180 cm^{-1} resulting from stretching vibration bands of N-H, C=O, C-C, and two S=O, respectively. The spectrum of co-ground mixture exhibited a decrease in the intensities of N-H and S=O stretching vibration peaks of MLX that ranges from minor to significant changes. The reduction in peak intensities could indicate a sort of hydrogen

bonding, a reduction in crystallinity and / or transformation to amorphous state of the drug, which may explain the better dissolution of the co-ground mixture, compared to the untreated drug. The reduction in peak intensities also would indicate the possibility of intermolecular hydrogen bonding via N-H, S=O and C=N groups of MLX and hydroxyl groups of PEG. In the low frequency region (1000 - 400 cm^{-1}) of the spectrum of the co-ground mixture, the peaks characteristic of MLX were almost unchanged. This indicated that although the drug molecule is hydrogen bonded with the polymer, yet the overall symmetry of the molecule is not significantly affected.

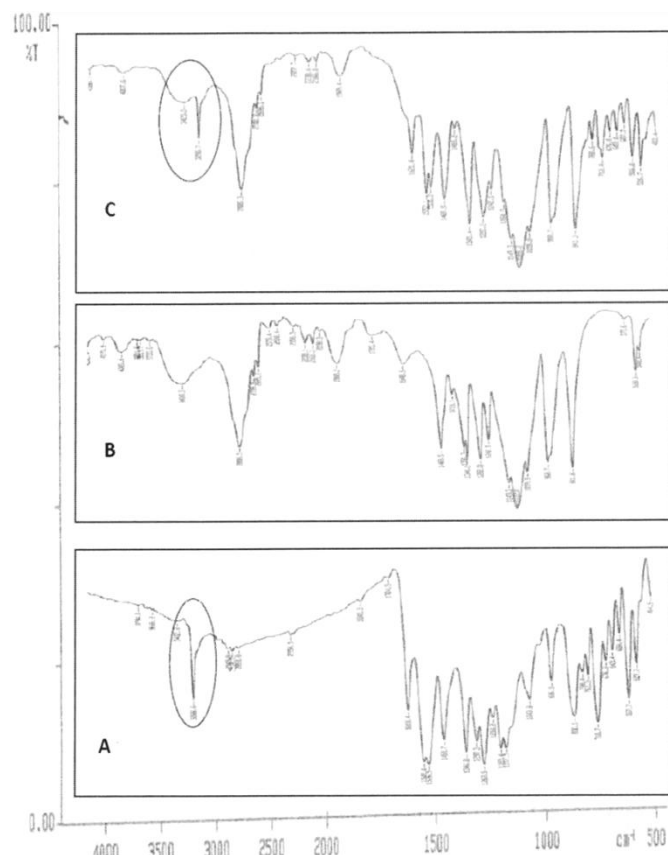


Fig. 7: FT-IR spectra of meloxicam ball milled co-ground mixture with PEG 6000 (1:4) where (A) MLX alone, (B) PEG 6000 (C) co-ground mixture (1:4).

Differential scanning calorimetry (DSC) study

Differential scanning calorimetry (DSC) studies were undertaken to consolidate the IR data explaining the reduction of the crystallinity of MLX with PEG. DSC studies were performed on the individual components and on the freshly prepared co-ground mixture and results are summarized in Table 2. The DSC thermograms of each component exhibited a sharp endothermic peak corresponding to the melting point of MLX (257.38°), and PEG (61.58°) (Fig. 8). A difference in heat of fusion of co-ground mixtures and PMs of MLX with PEG indicates a slight reduction in PEG crystallinity. The decrease in enthalpy (ΔH) value of the co-ground mixture, compared to that of the pure drug, strongly suggests that crystalline drug was transformed at least partly to the corresponding amorphous structure (Table 2). The reduction in

crystallinity and / or transformation to amorphous structure would be responsible for dissolution enhancement of the drug, in addition to the solubilizing effect of the carrier used. The complete disappearance of the melting endothermic peak of MLX and the observed reduction in enthalpy (ΔH) (Table 2) in ball milled meloxicam co-ground mixture with PEG (1:4) strongly suggests a remarkable reduction in MLX crystallinity by co-grinding technique.

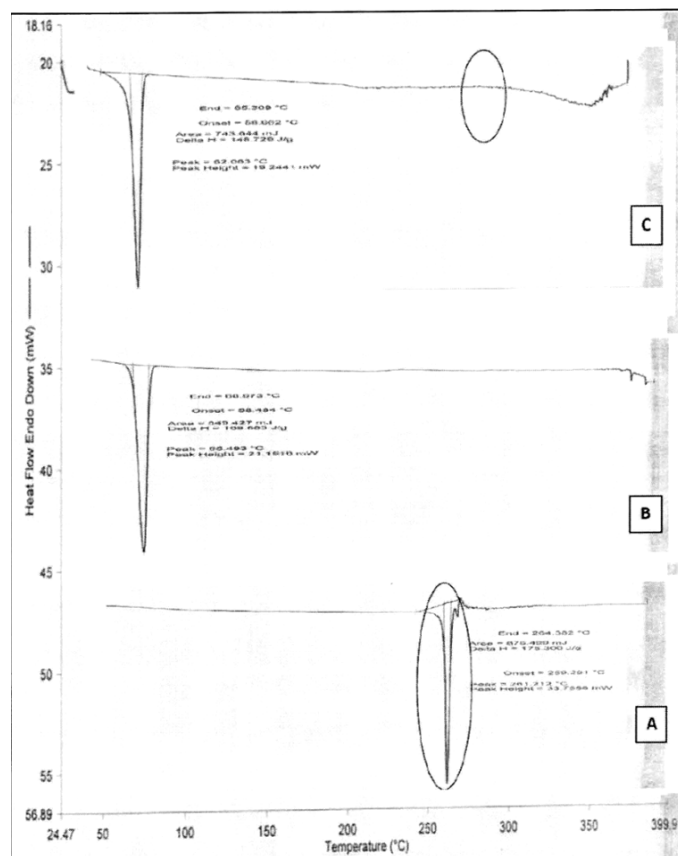


Fig. 8: DSC thermograms of meloxicam (MLX) alone (A), where: (A) MLX alone (B) PEG 6000 (C) MLX- PEG 6000 (1:4) ball milled co-ground mixture.

Table 2: Differential Scanning Calorimetry (DSC) data of meloxicam (MLX), PEG 6000, and MLX-PEG (1:4) ball-milled (BM) co-ground mixture.

Mixture components	Mixture type	Peak Temp. (°C)	Melting range (°C)	ΔH (J / g)
MLX alone	-----	261.212	259.29 – 264.38	175.3
PEG 6000 alone	-----	65.493	58.48 – 68.97	169.885
MLX : PEG	BM (1:4)	62.063	56.62 – 65.30	148.729

Stability study

Stability is one of the critical aspects in ensuring safety and efficacy of drug products. Pharmaceuticals should be stable and maintain their quality until the time of usage or until their expiration date (Yoshiska and Stella, 2002). If a drug product is stable enough to be marketed, it would require relatively long storage time at room temperature or actual temperature at which it will be stored prior to ultimate use, to permit observation of the

rate at which the product decomposes under normal storage conditions. The design of solid-state stability studies is complicated by the fact that, many solid-state degradation reactions don't follow Arrhenius kinetics. Phase changes (glass transition, crystalline melting, polymorphic changes, solvation/desolvation) are expected to be radically changed after different degradation pathways (Koenigbauer *et al.*, 1992). Therefore, stress stability studies beyond certain temperature range could induce phase changes, which could alter the validity of the Arrhenius equation. For example, degradation of an amorphous drug is not expected to follow the Arrhenius relationship when its glass transition temperature is crossed (Duddu and Dal Monte, 1997). In addition, reaction mechanisms could change as temperature is changed (Olsen *et al.*, 1997). Therefore, caution must be taken when the effect of temperature on solid-state degradation is studied. Accordingly, the physical and chemical stability of MLX in the tested MLX-PEG containing tablets were performed at room temperature to avoid the above mentioned limitations.

Physical stability

The tested tablet formulation did not show any change in their appearance (such as color and odor) during the stability study when stored at 25°C/ 60 % RH for 12 months.

In addition, no remarkable changes were observed in the disintegration time, thickness and drug content of the test Meloxicam tablets during storage for 12 months.

Dissolution rate

Pharmaceutical scientists, e.g. (Moore and Flanner 1996) and (Duan *et al.*, 2011), used the concept of similarity factor and difference factor to compare between dissolution profiles of different dosage forms, rather than comparing the dissolution tolerance limit specified in the Pharmacopeia.

The similarity factor (f_2) is a logarithmic transformation of the average of the squared vertical distances between the test and reference mean dissolution values at each dissolution time point. It was evident that the dissolution rate of MLX from the tested tablets was not significantly affected, as indicated by the calculated similarity factor (f_2) and difference factor (f_1). The observed similarity factor (f_2) was 85 (>50) and difference factor (f_1) was 2 (<15), clearly indicates that there is a significant similarity and no significant difference in dissolution rate of the prepared tablets before and after the stability study.

Powder X-ray diffraction pattern

Figure 9 shows that no changes in the XRD pattern of MLX were found after storage for 12 months, indicating the absence of significant reversion of MLX from its proposed amorphous state to its crystalline state during the period of storage at 25°C.

The results of stability study of meloxicam tablets prepared using the co-ground mixture with PEG 6000 revealed a proper resistance of the prepared tablets to storage at 25±1°C /

60% RH for a period of 12 months without showing any appreciable change in its properties.

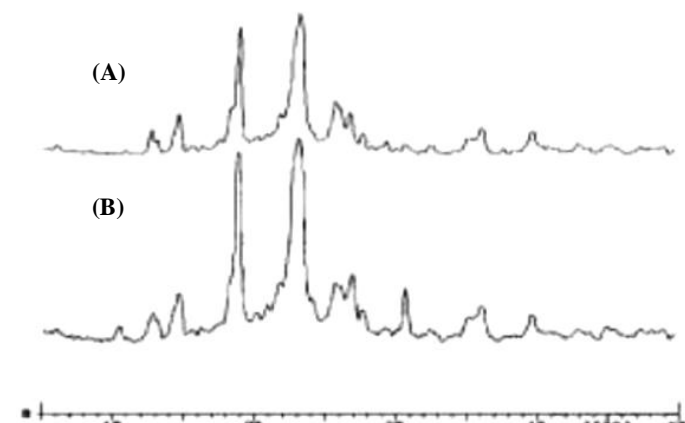


Fig. 9: X-ray diffractograms of the prepared meloxicam tablets before (A) and after (B) storage for 12 months at $25\pm 1^\circ\text{C}$ / 60% RH.

In vivo Study

HPLC method

Both the MLX and the internal standard peaks were well resolved and no endogenous interference was observed. Acetonitrile was selected as the organic modifier of the mobile phase as this reagent produces the least base-line noise compared to methanol. Optimal separation was achieved with 60% acetonitrile in 20 mM potassium phosphate buffer at pH 3.5. The retention times of MLX and IS were approximately 5.75 and 4.53 minutes, respectively.

The mean regression calibration curves were expressed as $y = 12.362 (\pm 0.27) x + 0.0006 (\pm 0.06)$ with the correlation coefficient value (r) = 0.9997. Based on the SD of the y-intercepts and slope of the curves, the LOQ of MLX was estimated to be 100 ng/mL. Subsequently, repeated analysis with this LOQ (100 ng/mL) revealed a signal-to-noise ratio greater than 10: 1 with a level of accuracy within 20% and a CV less than 15%. Therefore, 100 ng/mL of MLX in a 250 μL sample volume was identified as the lowest quantifiable concentration of the calibration curve for our study conditions.

Intra- and inter-day precision expressed as CVs ranged from 0.12 to 3.95 % and 0.11 to 3.46%, respectively. Intra- and inter-day accuracy expressed as a percentage of the bias ranged from 0.23 to 2.47% and 0.14 to 3.78%, respectively. These values satisfied the ICH guidelines (ICH 1996) regarding assay reliability. The absolute recovery rates of MLX from the (100, 500 and 2000 ng / ml) samples were 91.67 ± 1.49 , 93 ± 1.12 and $92.32\% \pm 1.5$, respectively. The absolute recovery rate of IS was 97.3%

Pharmacokinetic assessment

The developed MLX tablets were tested for their in vivo performance against a marketed reference product (Mbc® tablets) in six healthy male volunteers. Plasma MLX concentrations at different time intervals for the test and reference product in the

individual volunteers were calculated, and the mean values vs. time are graphically presented in Fig. 10. The relevant pharmacokinetic parameters including C_{\max} , T_{\max} , K_e , $T_{1/2}$, AUC_{0-24} and $AUC_{0-\infty}$ of the test and reference product in the six volunteers are illustrated in Table 3. The obtained results revealed that, meloxicam tablets containing co-ground mixture with PEG 6000 showed higher C_{\max} ($1.49 \pm 0.58 \mu\text{g} \cdot \text{mL}^{-1}$), higher AUC_{0-24} ($37.56 \pm 1.07 \mu\text{g} \cdot \text{hr} \cdot \text{mL}^{-1}$) and higher $AUC_{0-\infty}$ ($44.637 \pm 1.94 \mu\text{g} \cdot \text{hr} \cdot \text{mL}^{-1}$), compared to the reference Mbc® tablets; $1.3 \pm 1.99 \mu\text{g} \cdot \text{mL}^{-1}$, $28.72 \pm 1.53 \mu\text{g} \cdot \text{hr} \cdot \text{mL}^{-1}$, and $35.3 \pm 1.56 \mu\text{g} \cdot \text{hr} \cdot \text{mL}^{-1}$, respectively.

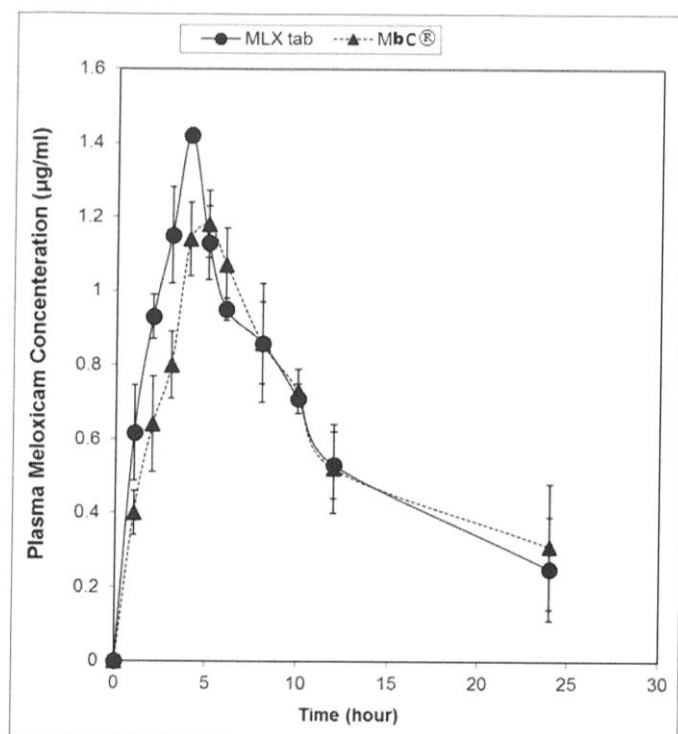


Fig. 10: Mean Plasma concentration time curve of meloxicam following oral administration of 15 mg prepared meloxicam tablets and the reference Mbc® tablets to 6 healthy human male volunteers.

It could be observed from the results presented in Fig. 10 that the prepared meloxicam tablets showed higher mean plasma concentration in most of the time intervals tested. The mean calculated T_{\max} of test product was shorter (4.5 h) than that of the reference one (5.17 h), which could mean a faster onset of action of the developed product and better control of inflammation and pain.

The calculated MRT of the test product (7.403 h) was higher than that of the reference one (6.53 h) which could mean a longer duration of action of the developed product.

Statistical analysis of the results indicated significant differences ($P < 0.05$) between the developed tablet and the reference product. The AUC_{0-24} , $AUC_{0-\infty}$ and C_{\max} values of MLX from test tablets were higher than the corresponding values of the reference tablets; namely 1.31, 1.26, 1.15 folds respectively.

Table 3: Pharmacokinetic parameters of the prepared meloxicam tablets and Mbc tablets (reference product) in 6 healthy human male volunteers.

Volunteer abbreviation	C _{max} (µg/ml)		T _{max} (h)		T _{1/2} (h)		K _{el} (h ⁻¹)		AUC ₀₋₂₄ (µg.h.ml ⁻¹)		AUC _{0-∞} (µg.hr.ml ⁻¹)	
	MLX tab	Mbc	MLX tab	Mbc	MLX tab	Mbc	MLX tab	Mbc	MLX tab	Mbc	MLX tab	Mbc
M	1.471	1.256	6	6	21.5	22.7	0.032	0.031	40.65	30.4	49.4	39.11
W	1.26	1.16	4	5	19.8	20.4	0.035	0.034	36.1	26.3	44.243	33.4
S	1.64	1.14	4	5	21.2	18.1	0.033	0.038	42.58	24.3	50.99	30.88
H	1.23	1.24	4	5	17.35	19.3	0.04	0.036	34.88	31.9	42.88	38.29
K	2.047	1.82	4	4	18.2	19	0.038	0.036	35.87	34.34	40.08	39.89
Z	1.27	1.17	5	6	19.1	18.9	0.036	0.037	35.3	25.1	40.23	30.24
Mean ± SD	1.49 ±0.58	1.3 ±1.99	4.5 ±0.55	5.17 ±0.82	19.20 ±0.64	20.7 ±1.05	0.036 ±0.04	0.0335 ±0.05	37.56 ±1.07	28.72 ±1.53	44.637 ±1.94	35.3 ±1.56

CONCLUSION

Meloxicam tablets containing ball-milled co-ground mixture with PEG 6000 in ratio of 1:4 exhibited superior dissolution rate, short disintegration time and good hardness. Dissolution rate of the prepared tablets was higher than the three marketed meloxicam tablets. The observed fast drug release from the developed tablets will provide rapid onset of action and also allow a reduction of drug dose necessary to achieve the therapeutic effect. This, in turn, reduces the most common side effects observed with non-steroidal anti-inflammatory drugs.

The stability study did not show any significant changes in physical appearance of the prepared tablets, drug content, thickness, disintegration time, and dissolution rate during and after the end of 12 months storage period at 25±1°C / 60% RH.

The prepared meloxicam tablets showed higher bioavailability, as evidenced by significant higher AUC and significant lower T_{max} which could indicate higher extent of absorption and rapid onset of action. The bioavailability of the test developed tablets was 126.45%, relative to the marketed brand product (Mbc[®] tablets) which will be reflected on better pain and inflammation relief.

Financial support and sponsorship: Nil.

Conflict of Interests: There are no conflicts of interest.

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How to cite this article:

Etman MA, Shekedef M, Nada AH, Ismail A. *In Vitro* and *In vivo* Evaluation of Tablets Containing Meloxicam- PEG 6000 Ball-Milled Co-Ground Mixture. *J App Pharm Sci*, 2017; 7 (03): 031-039.