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Original Article

Crystallization and Nanostructural Features of Metformin Drug an AFM-Investigations

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<u>ABSTRACT</u>

Objective: Studying the impact of three different types of organic solvent namely toluene, methanol and acetone on the main crystallographic phase of metformin drug.

Methods: Calculations of solubilities rates accompanied with XRD analyses, plus accurate monitoring of metformin microstructural features as function of applied solvent of crystallization process.

Results: XRD analyses indicated that applied solvent has no impact on the crystal structure of metformin. The roughness of metformin surface's were monitored by using high resolution SEM and AFM-microscope.

Conclusion: Surface roughness plays an important role on dissolution rates process and consequently solvation rates. Enhancing or decreasing dissolution rates could be benefit in metformin drug industries.

Keywords: Metformin, XRD, Microstructure, AFM, Roughness, Dissolution rate.

INTRODUCTION

Metformin has applied mainstay of therapy for diabetes mellitus for many years; however. the mechanistic action of metformin still ill-defined. It is well known metformin that is biguanide а antihyperglycemic agent having chemical formula as shown in Fig. 1, used for treating non-insulin-dependent diabetes mellitus (NIDDM)¹⁻⁵. It improves glycemic control

by decreasing hepatic glucose production, absorption decreasing glucose and increasing insulin-mediated glucose uptake. Metformin is the only oral antihyperglycemic agent that is not associated with weight gain. Lower doses should be used in the elderly and those with decreased renal function. Metformin decreases fasting plasma glucose, postprandial blood glucose and glycosylated hemoglobin (HbA1c) levels, which are reflective of the last 8-10 weeks of glucose control. Metformin may also have a positive effect on lipid levels⁶⁻⁸.

The major goal of these investigations is understanding the relationship between roughness of metformin drug as a function of applied solvent used in crystallization process.

EXPERIMENTAL

Crystallization of metformin

Three equivalent weights of highly pure metformin powders (each of 0.3 gm) were dissolved in 30 ml of tolune, methanol and acetone respectively with supporting ultrasonic instrument. The crystallization process was performed using gently microwave assist to avoid any traces from applied solvent. The highly pure crystals were dried in oven the forwarded for structural investigations.

Structural measurements

The X-ray diffraction (XRD): Measurements were carried out at room temperature on the fine ground samples using Cu-K α radiation source, Ni-filter and a computerized STOE diffractometer/ Germany with two theta step scan technique. Rietveld and indexing of structure were made via Fullprof package and Gesas program.

Scannig electron microscopy (SEM): measurements were carried out along abplane using a small pieces of the prepared samples by using a computerized SEM camera with elemental analyzer unit Shimadzu (Japan). Atomic force microscopy (AFM): High-resolution Atomic Force microscopy (AFM) is used for testing morphological features and topological map (Veeco-di Innova Model-2009-AFM-USA). The applied mode was tapping noncontacting mode. For accurate mapping of the surface topology AFM-raw data were forwarded to the Origin-Lab version 6-USA program to visualize more accurate three dimension surface of the sample under investigation. This process is new trend to get high resolution 3D-mapped surface for very small area.

FT-Infrared spectroscopy

The infrared spectra of the solid products obtained were recorded from KBr discs using a Shimadzu FT-IR Spectro-photometer in the range from 400 to 4000 cm⁻¹.

RESULTS and DISCUSSION

Phase identification

Fig. (2) shows the X-ray diffraction patterns of pure metformin extracted from methnol as model of applied solvent which was identical to those measured for metformin extracted form acetone and tolune respectively. These facts and observations confirm that applied solvent applied in extraction process of crystallization has no impact on the internal crystal lattice structure.

Analysis of the corresponding 20 values and the inter-planar spacing d (A°) proved that, the compound mainly belongs to *monoclinic* crystal structure $a \neq b \neq c$ with $P2_1/a$ space group. The calculated lattice parameters were found a= 7.9721, b=13.8765 and c=8.0032Å respectively. These results are fully consistent with those reported by Rodbard *et al.*⁹. Furthermore FT-IR spectra recorded for pure metformin extracted from methanol Fig. 3 confirmed existence of metformin in highly pure state. All characteristics peaks of metformin were observed specially –C=N- and –N-H function groups.

AFM-Surface investigations

Fig. 4_a shows 3D-AFM-mapping surface of highly pure metformin crystallized from methanol as it clear the topological

features of the surface can be divided into five principle zones with different heights namely 1st zone is red zone color which represents ~7% with heights ranged in between 9.64-9.68 μ m, 2nd zone with orange color occupies $\sim 13\%$ and its heights ranged in between 9.57-9.61 μ m, 3rd zone represents 15% of the whole scanned area with yellow color, 4th zone is green color zones (pale and dark color each occypies ~ 12.5 %) with heights ranged in between 9.5-9.57 um and finally 5th zone (blue zones cyan and dark blue) cyan color represents 10% and dark blue color occupies 30% of the whole scanned area with heights ranged in between 9.4-9.43 µm. These heights details are for metformin crystallized in methanol.

Fig. 4_b displays AFM-horizontal and vertical profiles of metformin surface extracted from methanol. As it clear in Fig. 4b the minimum depth in horizontal sector is 9.46 μ m and maximum heights is ~9.6 μ m while minimum depth in the vertical profile is 9.43 μ m and maximum height is 9.6 μ m respectively.

Fig. 4_c shows AFM-Deflection points profile of metformin surface extracted from methanol. The points of deflection distribution levels are important to understand how the grains aggregates and how large could be reached.

Fig. 5 shows 3D-AFM-micrographs recorded for metformin extracted from (a) tolune and (b) acetone respectively. The analysis of the two micrographs indicated that the ratio of heights in case of methanol crystallization is higher than those of tolune and acetone. The calculated AFM- average roughness of metformin was 77 in case of methanol while it is ~ 56 and 39 for acetone and tolune respectively. These results confirmed that methanol as solvent of crystallization enhance the grain size formation to higher size as confirmed in the analysis of surface topology Fig. 4a in which the ratio of heights in case of methanol is

higher than those estimated in tolune and acetone. These results are in partial agreement with those reported¹⁰⁻¹⁴ specially at the point of view effect of solvent type on the crystallization process and consequently impacts on micro-structural features.

Fig. 6 displays the calculated AFMroughness as a function of applied solvent. As clear in Fig. 6 the average roughness of metformin extracted from methanol is the maximum one with average roughness ~ 77 while acetone and tolune recorded 56, 39 respectively. The solubility of the obtained highly pure metformin was tested applying 0.1 gm metformin/10ml solvent, it was observed that metformin crystallized from methanol achieved highest dissolution rate followed by acetone then finally non-polar tolune .These results confirmed that as surface area axposed to solvated molecules increases dissolution or solubility rate will be increased as occurred in our investigations. These results can be used to develop technology of metformin drug industry specially at the point of view controlled released drug as it discussed before¹⁵⁻²¹.

Fig. 7 shows SE-micrograph of pure metformin captured with magnification factor 5 μ m for metformin extracted from tolune and acetone respectively. The grain sizes were estimated and found to be in between 0.44 m and 0.88 m respectively which confirm that applied solvent in crystallization process is correlated with grain size (i.e. grain size of metformin is applied solvent dependent)^{16,17}.

CONCLUSIONS

The conclusive remarks can be summarized in the following points.

1- The crystallographic phase of metformin (monoclinic) is not impacted by solvent applied in crystallization process only surface roughness and topological features are changed and affected remarkably.

- 2- Solubility or dissolution rates are solvent dependent specially in case of polar solvent.
- 3- There are strong correlation between metformin grain size formed and applied solvent of salvation process (i.e. grain size is applied solvent dependent).
- 4- Enhancing or decreasing dissolution rates could be benefit in metformin drug industries.

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