Asian Journal of Chemistry; Vol. 35, No. 10 (2023), 2353-2358

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2023.27876

Evaluation of Physico-Chemical Properties Influence on Drug Product *in vitro* Profiles and Solubility Profile of Fourth Generation Antibacterial Drug

GIRISH K. DESHPANDE[1], PARAMESWARI AKSHINTHALA[2], NARESH KUMAR KATARI[3], BALASAHEB DESHMUKH[1], LEELA PRASAD KOWTHARAPU[3], SREENIVAS RAO BATTULA[1,*] and RAMBABU GUNDLA[3,*]

[1]Department of Chemistry, GITAM School of Science, GITAM Deemed to be University, Visakhapatnam-530045, India
[2]Department of Science and Humanities, MLR Institute of Technology, Dundigal, Medchal, Hyderabad-500043, India
[3]Department of Chemistry, GITAM School of Science, GITAM Deemed to be University, Hyderabad-502329, India

*Corresponding author: E-mail: sbattula2@gitam.edu; rgundla@gitam.edu

Received: 6 April 2023; *Accepted*: 16 July 2023; *Published online*: 28 September 2023; AJC-21383

Moxifloxacin hydrochloride drug substance used to treat bacterial infections in many different parts of the body and most widely used worldwide. In this study, various high-end physico-chemical techniques are used like PXRD, DSC, TGA, BET SSA and PSD analysis to check the relative differences on the product behaviour. The present work reports an impact of alteration in particle size due to compaction process where increase in particle size has been observed from 15.912 µm to 751.001 µm, decrease in specific surface area from 1.89 m^2/g to 0.861 m^2/g, decrease in crystallinity from 91.65% to 59.15% and impact of dehydration or loss of water from 3.776% to 1.866%, which were observed during study. All the alterations in the physico-chemical properties impacting the drug manufacturing process and on the bioperformance of the finished product. This research attempt aims to investigate the effects of these modifications on in vitro experiments conducted in the office of generic drugs (OGD) media and the equilibrium solubility profile. The *in vitro* studies resulted about 35% release difference observed in the initial time points, whereas solubility in water observed about 17 mg/mL difference. This research work helps to demonstrate the optimization of finished product development process to meet the biofriendly formulation.

Keywords: Antibacterial Drug, Crystallinity, Dissolution, Solubility.

INTRODUCTION

In order to prevent the action of topoisomerase II (DNA gyrase) and topoisomerase IV, the quinolone/fluoroquinolone antibiotic mofloxacin hydrochloride monohydrate (MHM) acts as an inhibitor for the enzyme complex consisting of a DNA drug and an ATP-dependent enzyme [1]. So, MHM (8-methoxy-quinolone) and quinolones as a whole are effective for treating cancer by inhibiting both enzymes. A major function of gyrase in bacteria is to facilitate DNA unwinding and a major function of topoisomerase IV is to activate decatenation [2-5].

Environmental factors or variables encountered during the manufacturing process may lead to different crystalline hydration states for the active ingredients [6]. These hydrates exhibit diverse physico-chemical properties, such as solubility differences, chemical stability and nature of particle [7]. Water in active/inactive ingredient hydrates can be categorized based on three different structural classes that include those residing in remote lattice sites, lattice channel sites or ion-coordinated sites [8-11]. In case of isolated lattice sites, water molecules are secluded from each other due to contact with drug molecules. Water molecules forming lattice channel sites are in contact with other water molecules of adjoining unit cells along an axis of a unit cell called channel water. Ion-coordinated water takes part in an ion water bond which is usually much stronger than the hydrogen bonds present, called crystal-bound water. It has been shown that under low relative humidity (RH), some channel water containing hydrates may undergo dehydration or absorb water under high humidity conditions [9]. In addition to the generation of different hydrates, a single hydrate form of active or inactive pharmaceutical ingredients may contain more than one structural type of water [8].

Mofloxacin hydrochloride monohydrate (MHM) is available in three forms *viz*. monohydrate, anhydrous and amorphous forms [12]. Thermal and spectroscopic data indicate that the MHM lattice undergoes an adjustment upon removing channel

water and is a little bit compromized in the crystal integrity [13,14]. This study affirms that removing one mole of channel water through heat results in dehydrated MHM with specific physical and chemical changes [15]. These changes are reversible as sample rehydrates and crystal lattice returns to its original state over time.

Understanding the physico-chemical behaviour of active ingredients in the manufacturing process helps to optimize the finished product, enhancing the dissolution and bioperformance in human plasma. Governing the unacceptable properties of active substances during the manufacturing process will help to control the related substances, residual solvents and genotoxic as well as elemental impurities in the final product. This research focuses on the pace of hydration/dehydration phenomena in order to control the quality of the artefact throughout the blending and compression stages of production.

EXPERIMENTAL

Mofloxacin hydrochloride monohydrate (MHM, active pharmaceutical ingredient drug substance) was received as a generous gift from the Diacel Chiral Technologies Pvt. Ltd., Hyderabad, India. The sample as supplied was used for the study. The MHM drug reference standard procured from USP (Rockville, Maryland, USA). Dissolution medium chemical hydrochloric acid (HCl) was procured from EMD Millipore (Hydrabad, India). TGA and DSC analysis done by using a TA Instrument Q2000 (DSC) and Q5000 IR (TGA). Merck milli-pore generated milli-Q water used for whole analysis. Bruker D8 Advance X-ray diffractometer used for the XRD testing. SEM anlysis carried conducted by using the JEOL and JSM 6380. Malvern Mastersizer 2000 used for the PSD testing. Surface are testing was done by using the QuadraSorb SI BET instrument. The dissolution release testing was carried by using Lab india 8000 dissolution tester. The dissolution samples were quantified by using Shimadzu UV 1800 spectrophotometer.

Thermal analysis: Simultaneous thermogravimetry (TGA) and differential scanning calorimetry (DSC) analysis thermograms were generated using a TA Instrument Q2000 (DSC) and Q5000 IR (TGA), respectively. Approximate 4 mg (for DSC) and 7 mg (for TGA) of MHM samples were scanned under a dry nitrogen purge from 25 to 250 ºC at 10 ºC/min.

Powder X-ray diffraction (XRD) analysis: X-ray powder diffraction analysis was performed on the samples using the Bruker D8 Advance X-ray diffractometer. The instrument was equipped with a 2.2 kW Cu anode X-ray tube, high-temperature stage and high-speed position sensitive detector (PSD). Cu$K\alpha$ radiation (λ = 1.5418 Å) was used to obtain all powder diffraction patterns. A nickel filter was placed in the receiving path of the X-rays to remove the β-radiation. The samples were mounted and analyzed on a front-loading sample holder without any further sample preparation. All scans were performed over the range of 3.0-45.0º 2θ.

SEM analysis: Images were collected using JEOL and JSM 6380 SEM by mounting samples into the sample chamber of SEM under the magnification of 200X, 500X, 800X, 1000X and 1500X magnifications.

Particle size distribution (PSD) analysis: The particle size distribution of MHM drug substance was determined using the laser diffraction technique. Malvern Mastersizer 2000 instrument was used to analysis using the dry method. Approximately 1 g of each sample was transferred in a dried and clean sample feeder tray and a vibration feed rate of 70% and dispersive air pressure of 3 bar were applied during analysis. For each sample, three measurements were performed to calculate the average particle size at d (0.1), d (0.5) and d (0.9) levels.

BET-specific surface area analysis: The specific surface area of MHM samples was measured using QuadraSorb SI BET automated surface area and a pore size analyser. Analysis was performed using nitrogen gas as an adsorbate with a multipoint model.

Dsssolution profile analysis: The finished oral dosage form tablets dissolution analysis carried on pharamcopeail offfical media 0.1 N HCl (8.5 mL of 36.5% HCl dilued to 1000 mL). The dissolution media volume was 900 mL and appartues was USP II (Paddle) with agitation rate of 50 rpm. Conducted the profile by collecting samples at 10, 15, 30 and 45 min. The collected samples were diluted to target cocentartion (9 µg/mL) by using dissolution medium. Prepared reference standard solution at same concentration and analyzed by using the UV spectrophotometer at 296 nm.

Solubility studies in water: Solubility of drug substances was conducted in water. Different API particle size from different compaction stage was used to investigate the solubility. Equilibrium solubility study was conducted in water, after saturation the samples was diluted to target cocentartion (9 µg/mL) by using water. Prepared reference standard solution at same contration and analyzed by using the UV spectrophotometer at 296 nm.

RESULTS AND DISCUSSION

Thermal studies: Mofloxacin hydrochloride monohydrate (MHM) is in hydrate form and dehydration *via* thermal analysis clearly illustrates that heating results in a loss of 1 mole of channel water [12]. In TG curve (Fig. 1), a mass loss of about 3.776% observed from room temperature to 130 ºC. This decrease agrees with the theoretical value for 1 mole of water in MHM monohydrate (about 4%). Dehydration is assigned to the loss of channel water based on the temperature (above the boiling point of water). The dehydrated sample yielded a flat baseline from 130 to 220 ºC without further weight loss, confirmed by the DSC thermogram. The exothermic peak at about 223 ºC is due to crystallization to an anhydrous form, which melted at about 255 ºC. Subsequently, decomposition was observed in the DSC curve (Fig. 2).

The TGA curve of the dried sample at 105 ºC is shown in Fig. 3. After stabilization for 25 min at room temperature, the sample was re-analyzed. The material absorbs moisture and shows mass loss of about 1.856% from room temperature to 130 ºC and dehydration is due to the loss of channel water. The dehydrated sample yielded a flat baseline from 130 to 220 ºC without further weight loss, agreeing with the DSC thermogram. The exothermic peak at about 223 ºC is due to

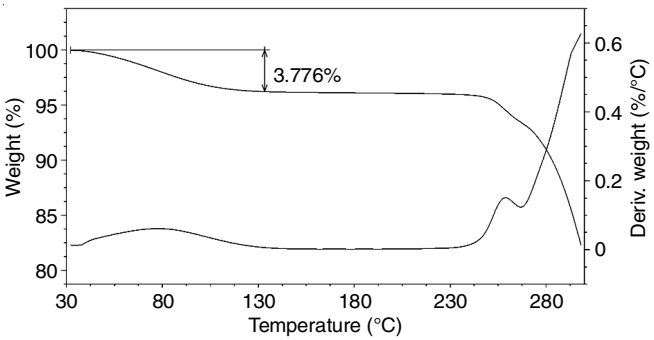

Fig. 1. Comparison of TGA curves for dehydrated sample. Loss of channel water observed from room temperature to 130 ºC

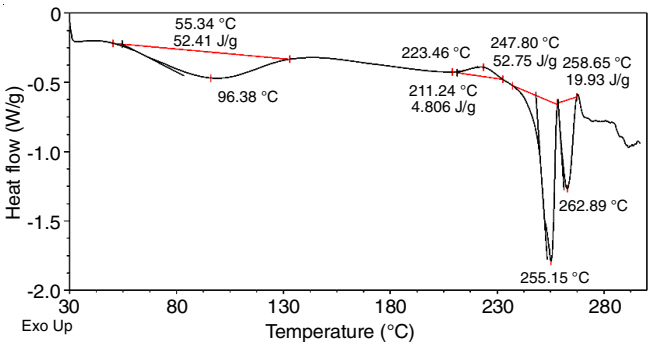

Fig. 2. DSC curves, dehydration endotherm at about 100 ºC in sample with respect to monohydrate/one mole of channel water

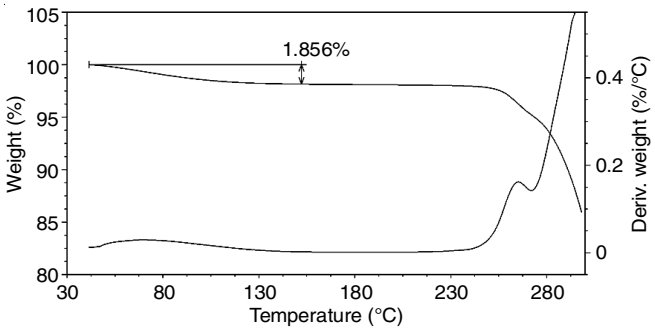

Fig. 3. Comparison of TGA curves for dehydrated sample. Loss of channel water observed from room temperature to 130 ºC

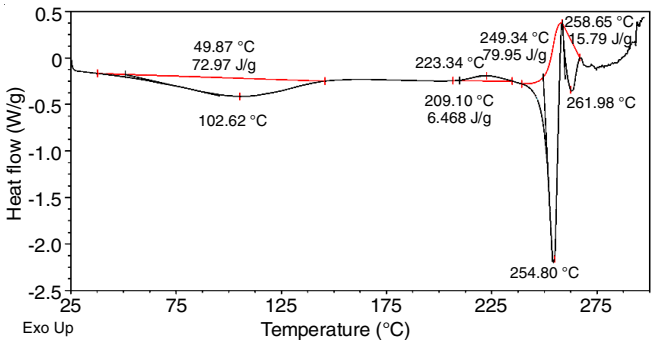

Fig. 4. DSC curves, dehydration endotherm at about 100 ºC in MHM

the crystallization in anhydrous form followed by melting at approximately 255 ºC and subsequently, decomposition is observed in the DSC curve (Fig. 4). Loss and gain of water molecule during the different thermal period indicate the insertion and ejection of water molecule in crystal lattice is due to channel and does not bound to the crystal lattice.

XRD studies: The X-ray powder diffraction of MHM indicates the well crystalline form having about 92% crystallinity. The lattice undergoes a transition after losing 1 mole of water,

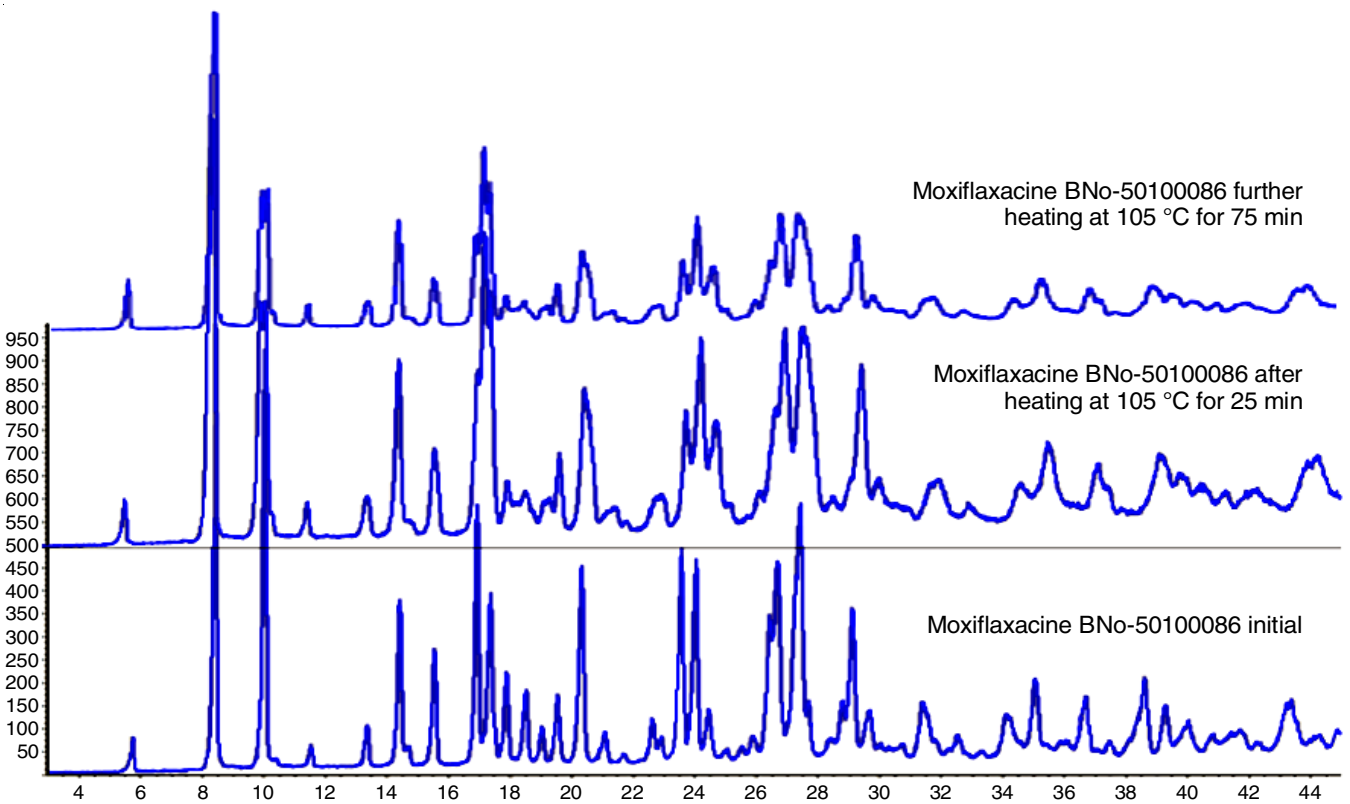

Fig. 5. Overlaid diffraction pattern of moxifloxacin hydrochloride monohydrate dehydrated at 105 °C for 25 and 75 min

which results in a new lattice configuration (Fig. 5). Heating a hydrated sample on a hot plate results in an anhydrous form, demonstrating that the removal of channel water causes a spontaneous lattice readjustment evident from the X-ray signature of the dehydrated monohydrate. The elimination of water within crystal channels results in the amorphization of the crystal structure and a decrease in the intensities of its peaks.

The impact degree of crystallinity of drug on the dissolution profile and other physico-chemical parameters were also investigated systematically. Crystalline MHM samples were compacted at different levels and examined using identical X-ray powder diffraction parameters (Fig. 6). The degree of crystallinity degree decreases with the increase in compaction cycles (Table-1). The findings of the study suggest that the decrease in crystallinity resulting from the loss of water molecules contributes to the formation of anhydrous or amorphous structures, which in turn affects the physico-chemical characteristics of the final dosage form.

TABLE-1
% CRYSTALLINITY RESULTS FOR MOXIFLOXACIN HYDROCHLORIDE MONOHYDRATE DRUG SUBSTANCE FROM DIFFERENT STAGES OF COMPACTION PROCESS

Sample details	Total net area (Cps x deg)	Background net area (Cps x deg)	Crystallinity (%)
As such API	2362.6	2165.4	91.65
One cycle compaction	2304.8	1844.7	80.04
Two cycles compaction	2247.4	1717.4	76.42
Three cycles compaction	2270.5	1613.1	71.05
Four cycles compaction	2370.4	1402.1	59.15

SEM studies: SEM analysis was employed to acquire images at various magnifications for the purpose of examining the morphology of crystals and detecting any distortions within their lattice structures (Fig. 7). The SEM images inferred that the crystals are in the plate-like geometry and no deformation in crystal lattices observed before physical processing.

Particle size distribution studies: The influence of compaction on the particle size distribution of MHM was also investigated. The results of various stages of the compaction process are summarized in Table-2a. The heating caused a slight increase in particle size due to amorphization and gelatinization (Table-2b). The compaction process in the production of final products results in an increase in particle size when the number of cycles is increased. Temperature induced amorphization and gelatini-zation also leads to increase in particle size.

TABLE-2a
PSD RESULTS FOR MOXIFLOXACIN HYDROCHLORIDE MONOHYDRATE DRUG SUBSTANCE FROM DIFFERENT STAGES OF COMPACTION PROCESS

Sample details	D (0.1)	D (0.5)	D (0.9)
As such API	2.028 µm	5.818 µm	15.912 µm
One cycle compaction	2.365 µm	57.177 µm	203.055 µm
Two cycles compaction	2.846 µm	74.468 µm	258.292 µm
Three cycles compaction	5.788 µm	154.315 µm	454.246 µm
Four cycles compaction	56.826 µm	331.746 µm	751.001 µm

Specific surface area studies: A decrease in the particle size increases the specific surface area. However, the compaction process leads to an increase in the particle size that reduces specific surface area. The surface area of MHM drug substance

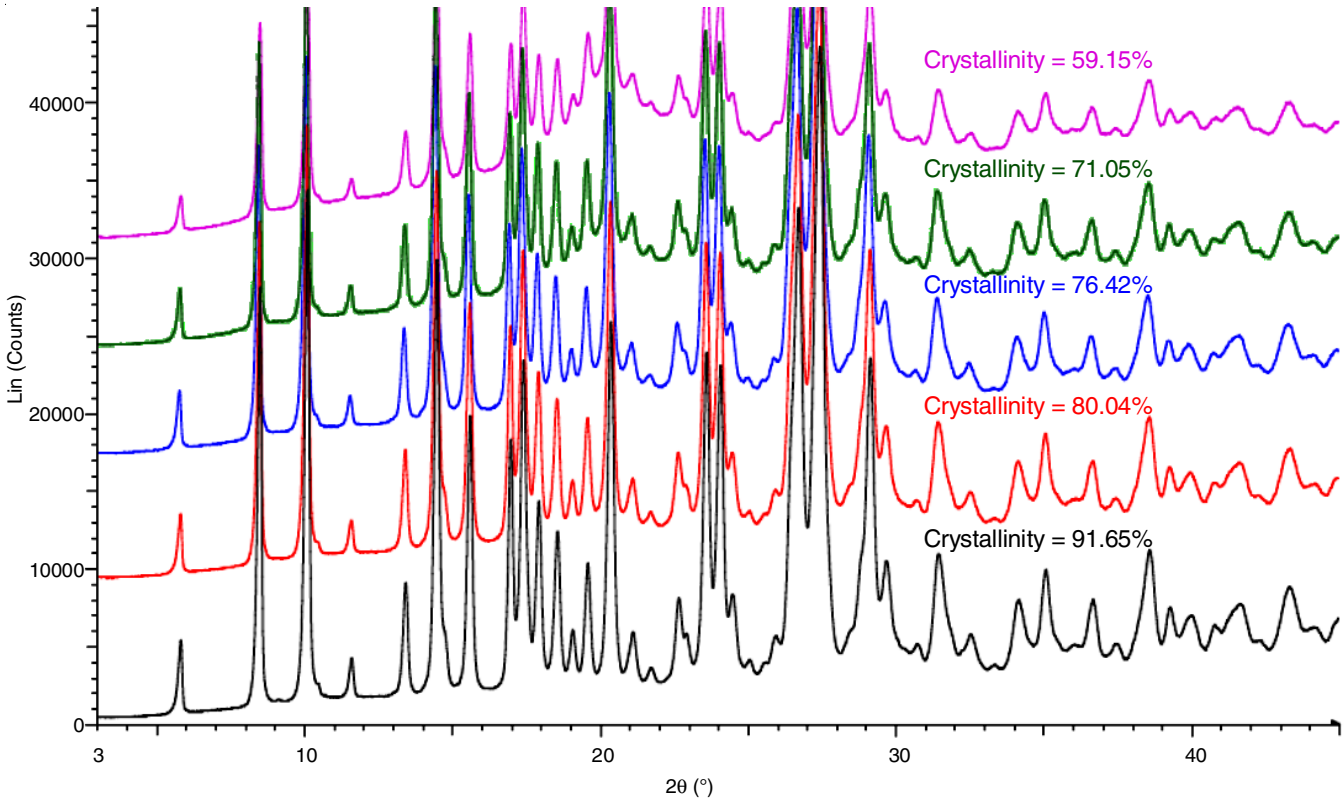

Fig. 6. Moxifloxacin hydrochloride monohydrate shows the impact of compaction on % crystallinity

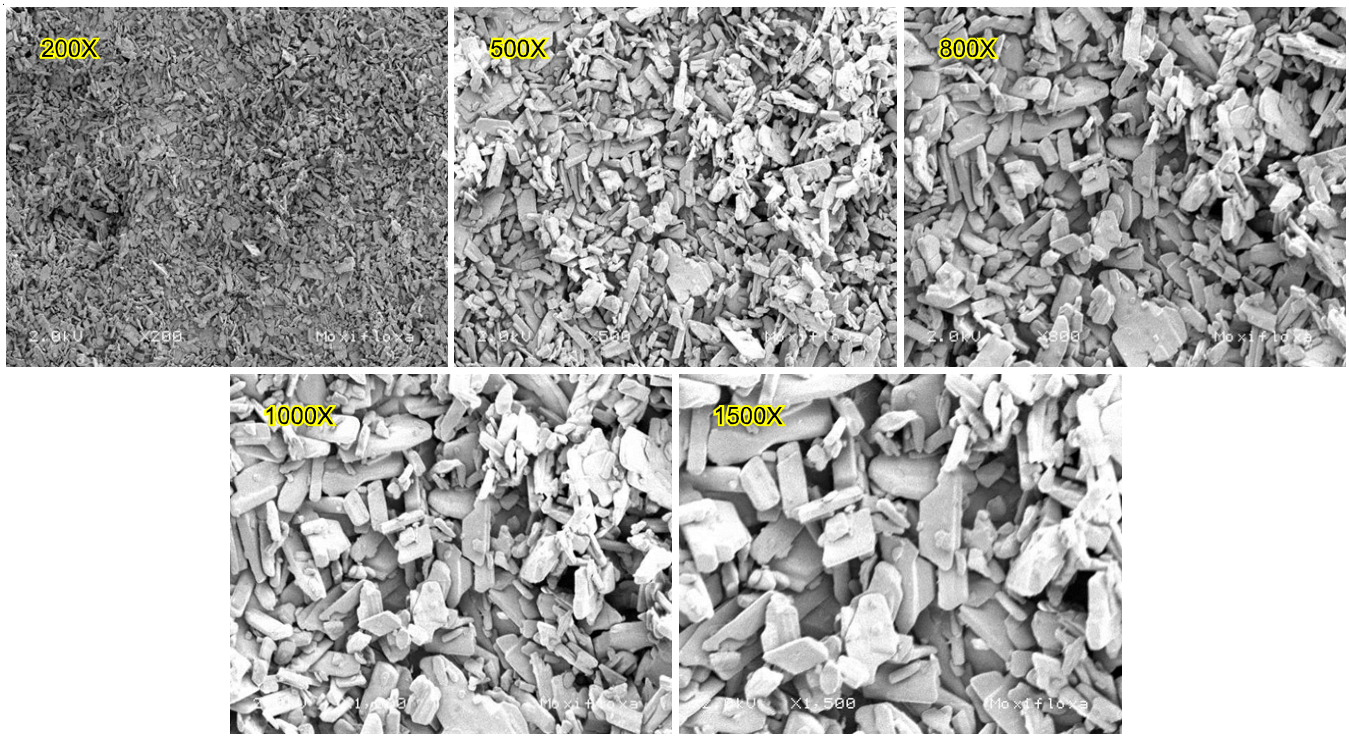

Fig. 7. Scanning electron microscopy images of crystal lattices

TABLE-2b
PSD RESULTS FOR MOXIFLOXACIN HYDROCHLORIDE MONOHYDRATE DRUG SUBSTANCE AFTER DEHYDRATION

Sample details	D (0.1)	D (0.5)	D (0.9)
Dehydration at 105 °C for 25 min	5.432 µm	12.722 µm	25.564 µm
Dehydration at 105 °C for 75 min	5.260 µm	11.879 µm	22.598 µm

from different stages of compaction cycles was measured using QuadraSorb SI BET automated surface area and a pore size analyser (Table-3a). The dehydration process leads to crystal rearrangement and directly impacts the specific surface area of the sample (Table-3b). After dehydration, the surface area of drug substance was measured using QuadraSorb SI BET automated surface area and a pore size analyser. A decrease in specific surface area is due to dehydration temperature, leading to gelatinization in crystal lattices, particle size increase and pore size reduction. Decrease in particle size leading to increase in specific surface area and *vice-versa*. Compaction cycles and gelatinization due to thermal events leads to reduce the surface area due to increase in particle size.

TABLE-3a
BET SPECIFIC SURFACE AREA RESULTS (m^2/g) FOR MOXIFLOXACIN HYDROCHLORIDE MONOHYDRATE DRUG SUBSTANCE FROM DIFFERENT STAGES OF COMPACTION PROCESS

Sample details	Specific surface area (m^2/g)	Correlation coefficient
One cycle compaction	1.89	0.9997
Two cycles compaction	1.449	0.9996
Three cycles compaction	0.890	0.9993
Four cycles compaction	0.861	0.9997

TABLE-3b
BET SPECIFIC SURFACE AREA RESULTS (m^2/g) FOR MOXIFLOXACIN HYDROCHLORIDE MONOHYDRATE DRUG SUBSTANCE AFTER DEHYDRATION

Sample details	Specific surface area (m^2/g)	Correlation coefficient
Dehydration at 105 °C for 25 min	0.813	0.999322
Dehydration at 105 °C for 75 min	0.674	0.998866

Impact of the particle on dissolution profile: The tablets using MHM active pharmaceutical ingredient were formulated with different particle sizes (Table-2a) at D 15.912 µ, 203.055 µ, 258.292 µ, 454.246 µ and 751.001 µ a(0.9). The dissolution of the samples was measured using the analytical method outlined in the United States Pharmacopoeia (USP-NF 2022). The results indicate that the drug release rate slows with the increase in particle size (Table-4). Therefore, the parameters of particle size and surface area influence the dissolving process. The results help modify, control and improve desired dissolution drug release patterns to meet the bioavailbility.

Impact of particle on solubility profile: MHM samples were prepared in triplicate for initial (untreated) and under 90% RH at 20 ± 0.5 ºC by transferring excess quantity (approximately 300 mg) into 10 mL volumetric flask containing 5 mL water (Milli-Q water). The flasks were shaken at 150 rpm for 24 h at room temperature using a tabletop shaker. After shaking, the suspensions were filtered through a 0.50 µm PTFE hydrophilic filter, adequately diluted the filtrate and quantified using an ultraviolet spectrophotometer (Table-5).

Conclusion

The alteration of the crystal lattice was influenced by the inadequate hydration, absence of water molecules and lack of

TABLE-4
RESULTS FOR % DRUG RELEASE PROFILE FOR MOXIFLOXACIN HYDROCHLORIDE MONOHYDRATE DRUG SUBSTANCE TABLETS PREPARED BY DIFFERENT STAGES OF COMPACTION PROCESS

Sample details	Particle size (μm) D (0.9)	Dissolution % Drug release profile at different times			
		10 min	15 min	30 min	45 min
As such API	15.912	65	70	97	100
One cycle compaction	203.055	44	62	95	99
Two cycles compaction	258.292	40	58	93	97
Three cycles compaction	454.246	32	52	89	92
Four cycles compaction	751.001	30	45	85	90

TABLE-5
SOLUBILITY RESULTS FOR MOXIFLOXACIN HYDROCHLORIDE MONOHYDRATE DRUG SUBSTANCE FROM DIFFERENT STAGES OF COMPACTION PROCESS

Sample details	D (0.1) (μm)	D (0.5) (μm)	D (0.9) (μm)	Water medium (mg/mL)
As such API	2.028	5.818	15.912	24
One cycle compaction	2.365	57.177	203.055	17
Two cycles compaction	2.846	74.468	258.292	15
Three cycles compaction	5.788	154.315	454.246	11
Four cycles compaction	56.826	331.746	751.001	7

defined structure in the drug's poorly hydrated, anhydrate and amorphous forms. Upon removal of a single mole of channel water from mofloxacin hydrochloride monohydrate (MHM), a novel and stable semi-crystalline phase is observed to emerge. The aforementioned phase has the potential to be distinct from the initial hydrates that are formed in MHM. It does not instantly come to its initial state/phase under relevant conditions. The observed alterations in the XRD pattern of the initial MHM sample suggest the emergence of a novel phase accompanied by the process of dehydration. Dehydrated drug substance also shows differences in the thermal transitions than that of pure monohydrate form. These transitions show sharper with more change in enthalpy endothermic peaks due to the loss of a mole of water from the crystal lattice. This rearrangement in the crystal lattice affects the physical strength of dehydrated crystal form due to fractures. It produces crystals with slightly larger particle sizes due to amorphization, leading to gelatinization. This increase in particle size is evident from the particle size distribution data. Similarly, compaction during the manufacturing process plays a vital role and can impact the physicochemical properties of the final drug product on its dissolution and bioperformance. An increase in particle size due to compaction decreases the specific surface area and reduces the degree of crystallinity. Based on the results of dissolution and solubility profile, particle size plays a vital role as its increase lowers the dissolution rate. Understanding this physical consequence of the change in particle size during manufacturing is crucial to understanding the chemical profile and performance of the final commercial product.

ACKNOWLEDGEMENTS

The authors thank the Management, Department of Chemistry, GITAM deemed to be University, Vishakhapatnam, India, for providing the the research facilities.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. D.C. Hooper and G.A. Jacoby, *Cold Spring Harb Perspect Med.*, **6**, a025320 (2016); https://doi.org/10.1101/cshperspect.a025320
2. R.E. Ashley, A. Dittmore, S.A. McPherson, C.L. Turnbough Jr., K.C. Neuman and N. Osheroff, *Nucleic Acids Res.*, **45**, 9611 (2017); https://doi.org/10.1093/nar/gkx649
3. S.J. McKie, K.C. Neuman and A. Maxwell, *BioEssays*, **43**, 2000286 (2021); https://doi.org/10.1002/bies.202000286
4. E.L. Zechiedrich and N.R. Cozzarelli, *Genes Dev.*, **9**, 2859 (1995); https://doi.org/10.1101/gad.9.22.2859
5. K.D. Corbett, R.K. Shultzaberger and J.M. Berger, *PNAS*, **101**, 7293 (2004); https://doi.org/10.1073/pnas.0401595101
6. F. Tian, H. Qu, A. Zimmermann, T. Munk, A.C. Jørgensen and J. Rantanen, *J. Pharm. Pharmacol.*, **62**, 1534 (2010); https://doi.org/10.1111/j.2042-7158.2010.01186.x
7. C. Lester, G. Lubey, M. Dicks, G. Andol, D. Vaughn, R.T. Cambron, K. Poiesz and N. RedmanFurey, *J. Pharm. Sci.*, **95**, 2631 (2006); https://doi.org/10.1002/jps.20662
8. S.L. Price and S.M. Reutzel-Edens, *Drug Discov. Today*, **21**, 912 (2016); https://doi.org/10.1016/j.drudis.2016.01.014
9. D.M. Raut, R. Allada, K.V. Pavan, G. Deshpande, D. Patil, A. Patil, A. Deshmukh, D.M. Sakharkar, P.S. Bodke and D.T. Mahajan, *Der Pharm. Lett.*, **3**, 202 (2011).
10. D.E. Braun, L.H. Koztecki, J.A. McMahon, S.L. Price and S.M. Reutzel-Edens, Mol. Pharmaceutics, 12, 3069 (2015); https://doi.org/10.1021/acs.molpharmaceut.5b00357
11. L. Borka, *Pharm. Acta Helv.*, **66**, 16 (1991).
12. J.H. Kirk, S.E. Dann and C.G. Blatchford, *Int. J. Pharm.*, **334**, 103 (2007); https://doi.org/10.1016/j.ijpharm.2006.10.026
13. N.L. Redman-Furey, W.J. Collins and M.A. Burgin, Thermoanalytical Characterization of the Hydration States of Risedronate. In Proceedings of the 30th North American Thermal Analysis Society Conference, pp. 733-738 (2002).
14. D. Giron, *Thermochim. Acta*, **248**, 1 (1995); https://doi.org/10.1016/0040-6031(94)01953-E
15. N. Redman-Furey, M. Dicks, A. Bigalow-Kern, R.T. Cambron, G. Lubey, C. Lester and D. Vaughn, *J. Pharm. Sci.*, **94**, 893 (2005); https://doi.org/10.1002/jps.20308