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Research Article

SPHERICAL CRYSTALS OF MOXIFLOXACIN: TO IMPROVE SOLUBILITY, DISSOLUTION RATE AND MICROMERITIC PROPERTIES

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ABSTRACT

Moxifloxacin is a fourth-generation synthetic fluoroquinolone antibacterial agent having poor solubility, bioavailability and micromeritic properties.To improve these properties Agglomerated crystals of moxifloxacin were prepared by a spherical crystallization technique using the ammonia diffusion system (ADS). This technique makes it possible to agglomerate amphoteric drugs like moxifloxacin, which cannot be agglomerated by conventional procedures. When an ammonia-water solution of moxifloxacin is poured into an acetone dichloromethane mixture under agitation, a small amount of ammonia is liberated in the system. The ammonia-water solution plays a role both as a good solvent for moxifloxacin and as a bridging liquid, allowing the crystals' collection to take place in one step. Agglomerates were evaluated for micromeritics properties, drug release, scanning electron microscopy, differential scanning calorimetry, infrared spectroscopy, X-ray diffraction and compressibility properties. The study revealed that Micromeritic Properties, Solubility and In vitro drug release rate is increased when compared with pure Moxifloxacin.The properties of agglomerates were good enough to adopt direct compression technology.

Key words: *Moxifloxacin, spherical agglomerates, solubility, dissolution rate and micromeritic properties.*

INTRODUCTION

The quality and efficiency of a solid pharmaceutical preparation is influenced by primary micrometric properties (shape, size of crystals, etc.) and macrometric properties (bulk-density, flowability, etc.) from active and inactive medical substances, especially when a large amount of nonwater soluble drugs with poor rheologic properties are formulated. The modification of these properties may be useful to reach major active compounds with dissolution improved bioavailability. Accordingly, an appropriate pharmaceutical design is desired.

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Kawashima and co- workers [1-2]established an agglomeration technique which was applicable to amphoteric drug substances like moxifloxacin[3].In this process, as a crystalline solvent, a mixture of three partially miscible solvent, i.e, acetone-ammonia water-dichloromethane was used. By selecting a proper ratio, a small amount of ammonia water was liberated from the system. This ammonia water played two roles, a good solvent for moxifloxacin and a bridging liquid, which collected fine crystals precipitated into agglomerates in one step. Moxifloxacin is an antibacterial agent of the quinolone compounds, which is used to treat acute bacterial exacerbation of chronic Bronchitis, community acquired pneumonia, uncomplicated skin and skin Structure Infections, complicated intra-abdominal infections. It has a zwiterionic molecular structure and thus is only soluble in acid or alkaline solutions. This is the reason a

conventional technique to prepare spherical agglomerates cannot be employed. Given that the rheologic properties (micrometric and macrometric) of this drug are not convenient, they were modified to obtain a proper particle preparation using the spherical agglomeration technique combined with the ammonia diffusion system (applicable to amphoteric drugs).

EXPERIMENTAL

MATERIALS

Moxifloxacin was supllied by Yarrow Chem Products from Mumbai, India as a gift sample. Ammonia, Acetone and Dichloromethane were purchased from Merck Pvt Ltd. Mumbai. All chemicals used were of analytical grade.

Preparation of moxifloxacin agglomerates by ammonia diffusion method

Moxifloxacin (1gm) was dissolved in aqueous ammonia (20ml,25%w/v) and maintained at 20°C to avoid solubility problems. This solution was poured into a mixture of acetone (8.0ml) and dichloromethane (37.0ml) under agitation at 1500 rpm using a mechanical stirrer. The system was thermally controlled at 20 ± 2°C throughout the process. After twenty minutes, the agglomerated crystals were separated out by filtration. The crystals so obtained were dried under vaccum in a decicator and finally kept in a dark and dry container[4-5]. The effect of various parameters on the yield of Spherical crystals was studied. These parameters included the amount of ammonia water used as a bridging liquid (10, 15, and 20 mL);the agitation speed (1000, 1500, and 2000 rpm); the agitation time (30, 60, and 90 min) and the concentration of ammonia in ammonia water (10, 15, 20, and 25%). The effects were tested by changing one parameter at a time while keeping the other parameters constant. The parameters that yielded the best formulation were used for further preparation of the spherical crystals.

Fourier Transform Infrared Spectroscopy (FTIR) studies:

FT-IR spectra of the pure drug and spherical agglomerates were obtained on ShimadzuFT-IR-8300 using KBr pellets. Pellets were prepared by slowly grinding the crystals with KBr in a ratio of 2mg of crystals with 100mg of KBr and then applying a pressure of 500psi in a die-punch[6].

Differential scanning calorimetry (DSC)

The thermographs of pure drug and spherical agglomerates were recorded on ShimadzuDSC-60 apparatus calibrated with 8mg indium and zinc at a heating rate of 10ºC/min.The thermal behavior was studied by heating 5mg of the sample at a scan rate of 10ºC/min in a covered sample pan under nitrogen gas flow and the investigations were carried out over the temperature range of 30-360ºC[7]

Powder X-ray diffractometry (PXRD):

The X-ray powder diffraction patterns were recorded on an X-ray diffractometer (PW 1729, Philips, Netherland). The samples were irradiated with monochromatized CuK-α radiation (1.542A°) and analysed between 10-50° 2θ. The voltage and current used were 30kV and 30mA, respectively. The range and the chart speed were $1x10^4$ CPS and 5mm/2θ respectively.

Scanning electron micrographs (SEM) analysis:

The shape and surface topography of agglomerated crystals and conventional crystals were observed through a scanning electron microscope (JEOL USA Inc., Peabody, MA).Dried samples were fixed on aluminum stubs using double sided copper tape and coated with gold palladium in the presence of argon gas using a Hummer I sputter coater (Anatech Ltd., Denver, NC), under vacuum (0.1 mm Hg).

Micromeritic properties

The average particle size distributions of the moxifloxacin powder and its spherical crystals were determined using an optical microscope. The shape and surface of agglomerates were determined using both optical and scanning electron microscopes. The surface areas of the moxifloxacin powder and its spherical crystals were calculated using the formula πd^2, in which *d* represents the diameter of particles. The true densities of pure drug and its

spherical crystals were determined with the use of a relative-density bottle[8]. For the determination of bulk density, 3 g of powder drug or its spherical crystals were placed in a 25-mL graduated cylinder. The cylinder was dropped onto a hard-wood surface from a height of 1 in. at intervals of 2 s. The bulk densities were then obtained by dividing the weight of the samples by the final volume of the samples contained in the cylinders. Flowabilities were measured in terms of angle of repose using the fixed funnel method. Compressibility values were determined by compressing the pure drug powder and the moxifloxacin spherical crystals separately using a single-punch machine. Wettability values were determined indirectly by measuring the densities and surface tensions of the saturated aqueous solutions of drug powder and its spherical crystals[9]. The densities were determined using a relative-density bottle. The surface tension values were determined using a stalagmometer. The porosities of the drug powder and the spherical crystals were calculated from their bulk and true densities. The porosity of the tablets was calculated from apparent density of the tablets using the following equation:

Porosity (ε)= 1-(apparent density/true density)

The contact angles of the saturated aqueous solutions of moxifloxacin and its spherical crystals were determined by measuring the height of a large drop when it was placed on a tablet surface. The contact angle was calculated using the following equation:

$$\text{Cos}\theta = \frac{Bh^2}{\sqrt{3}(1-E)(Bh^2/2)}$$

in which $B=\rho g/2\gamma$; γ is the surface tension of the saturated solution of the sample in water, dyne/cm; ρ is the density of the saturated solution of drug in water, g/cm^3; E is the porosity of the tablet; and h is the height of the liquid drop, cm.

Solubility studies

Solubility studies of pure drug and spherical agglomerates of Moxifloxacin were carried out by using Phosphate buffer (P^H7.4). Saturated solutions were prepared by adding excess drug to the medium and shaking on the shaker for about 4 h at 25 ± 0.5°C under constant stirring. The aliquots were filtered through Whatmann No.41 filter paper. The filtrates were diluted appropriately in phosphate buffer and assayed spectrophotometrically at 287nm. The experiment was conducted in triplicate for each sample.

Formulation and evaluation of tablets

The spherical agglomerates previously passed through 100 mesh and pure drug (200mg) were separately mixed with specified quantity of microcrystalline cellulose(80mg), magnesium stearate(10mg) and talc(10mg) by geometrical dilution. The mixture was compressed in an electrically driven rotary tablet punching machine (Rimek mini press) using 11/32 punch to obtain tablets. Tablet hardness was determined using a Monsanto hardness tester [9]. Thickness of tablet was measured by using a calibrated vernier caliper The tensile strength (T) of the compact was calculated using the following equation:

$$T = 2F/\pi Dt$$

in which D and t are the diameter and thickness of the compact respectively, and F is the force fracturing the compact.

The friability values of the tablets were determined using Roche-type friabilator. It was rotated at 25 rpm for 4 min. Percent friability was calculated using the following equation:

Friability= ([$W0$ – W]/ $W0$) x 100

in which $W0$ is the weight of the tablets at time zero before revolution,and W is the weight of the tablets after 100 revolutions.

Disintegration test

It was carried by using thermionic disintegration test apparatus as per Indian Pharmacopeia. To test for disintegration time, one tablet was placed in each tube and disintegration testing was carried out in distilled water as a medium at 37 ± 2°C. The time when all the 6 tablets were disintegrated was recorded as the Disintegration Time of the tablets.

Estimation of drug content

Five tablets were weighed and powdered. Amount of powder equivalent to 50mg was

weighed and dissolved in 25ml of methanol and mixed for 5 minutes. Filtered the resultant solution through whatmann filter paper and the methanol was evaporated to about the dryness. The remaining solution was transferred to 50ml standard flask and made up the volume to 50ml with distilled water. The absorbance was measured at 287nm and the drug content was calculated.

In vitro drug release studies

The dissolution test was performed using United States Pharmacopoeia (USP) type II (paddle) apparatus, 900 ml of phosphate buffer P^H 7.4 at 37 ± 0.5℃ and 100 rpm as stirring devices. The dissolution was carried out for 2 hours. Samples (1 ml) were withdrawn at 15 minutes interval. The same volume of medium was replaced immediately to maintain the sink condition. The absorbance of the resulting solutions (after filtering through Whatmann filter paper) was taken at 287 nm after suitable dilutions using buffer P^H 7.4 as a blank.

RESULTS AND DISCUSSION

The amount of bridging liquid is a critical parameter in the spherical crystallization technique. When 10 mL of ammonia water was used, no agglomeration occurred. This may be because only a very small amount of bridging liquid was available for solubilization, leading to incomplete wetting of the agglomerates. When more than 25 mL of ammonia water was used, large agglomerates formed. When 20 mL of ammonia water was used, uniform spherical crystals were produced. This finding indicates that agglomeration might result from the coalescence of agglomerates with the liberated bridging liquid, the rate of which depends on the frequency of the collisions of agglomerates. Therefore, the agglomerates grew in size in proportion to the agitation speed up to a certain limit. Uniform spherical crystals were produced at an agitation speed of 1500 rpm. With increasing agitation speed, the average diameter of agglomerates decreased, and their shape became increasingly irregular. At a high agitation speed, the thickness of the bridging liquid layer adsorbed on the surface mayhave decreased, leading to a reduction in the amount of crystals produced.

Further, the diffusion of bridging liquid from the surface of the particle into the outer medium increased, thereby reducing the growth rate of the primary crystals. The bridging liquid introduced into the dispersing medium after the saturation point was immiscible in the system, and only coalescence of agglomerates with the bridging liquid (liberated from the system) occurred, causing an increase in agglomerate size. When agitation speed was increased, the agglomeration rate increased because of the increased rate of collision and coalescence of particles.

The optimum agitation time for the spherical crystals to remain suspended in the solvent mixture was 55–60 min at 20 ±2º C. With shorter time durations, the formation of spherical crystals was incomplete. When the agitation time was increased beyond this point, a breakdown of the agglomerates took place. Also, as temperature was increased, large agglomerates were formed. This was probably a result of the increased solubility of the drug at high temperatures. Therefore the solubility temperature is an important process parameter for spherical crystallization. When the concentration of ammonia in ammonia water was 25%, spherical crystals were formed, whereas when the ammonia concentration was less than 25%, spherical crystals were not produced.

FT-IR Studies

The interaction between the drug and solvent was studied by IR spectroscopy. From the spectra(Figure1 and2)it was observed that characteristics peaks at 3523. 65 and 3737.09 (–COOH), 1319.01 and 1320.24 (C – F Group), 1106.31and 1106.24(Epoxy Compound), 1319.01 and 1320.24 (Asymmetric Streching of OCH_3), 1703.29 and 1701.78 (C = 0) , 1619.32and 1613.5(N – H bending), 1451.58 and 1447.47 (N – H Streching). The FT-IR spectra of spherical crystal formulation did not show the presence of any additional peaks for new functional groups. These results suggest absence of any

chemical interaction between the drug and solvents used in spherical crystal formulation.

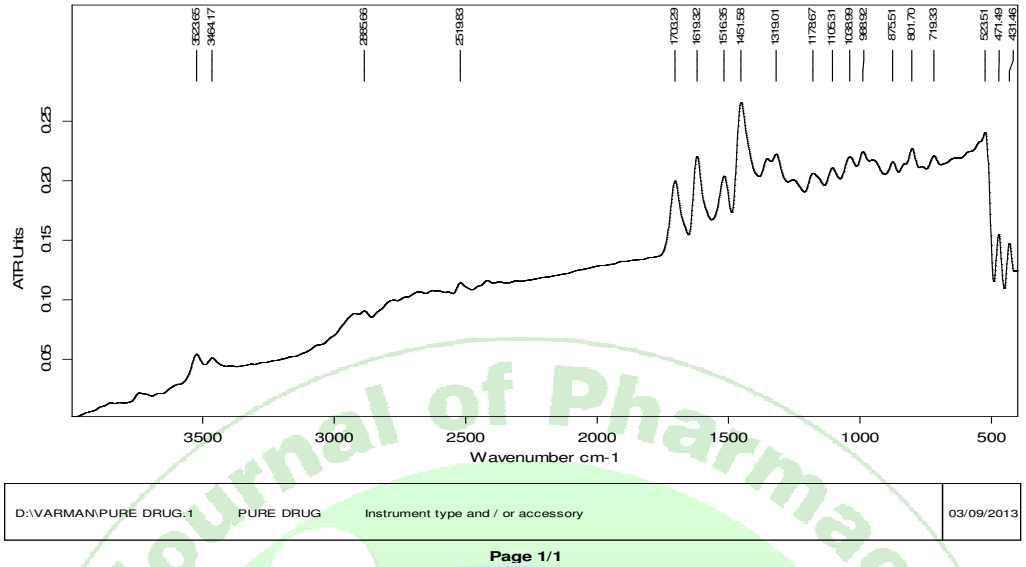

Fig-1: FT-IR spectra of pure drug

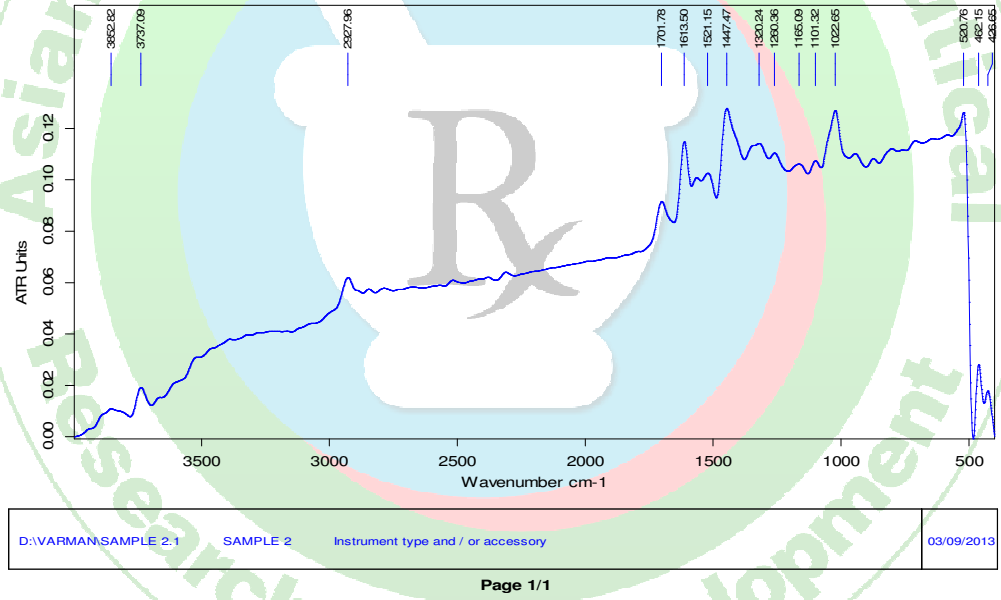

Fig-2: FT-IR Spectra of Spherical Agglomerates

DSC Studies

DSC thermograms were shown in fig.3&4 for pure drug and spherical agglomerates. The DSC study indicates that, the pure form shows endothermic peaks at 74.8°C (72.4mJ/mg) and 206.8℃ (29.4mJ/mg).But in the agglomerated crystals shows only one endothermic peak at 231.3℃ (28.4mJ/mg).The disappearance of endothermic peak may be due to stability of the agglomerated crystals.Both DSC curves shows endothermic peak at (248.4℃, 1.828 mV) and (248.1℃, 0.065mV) of pure drug and agglomerates respectively. The crystal form shows less intense peak, may be due to less crystallinity.

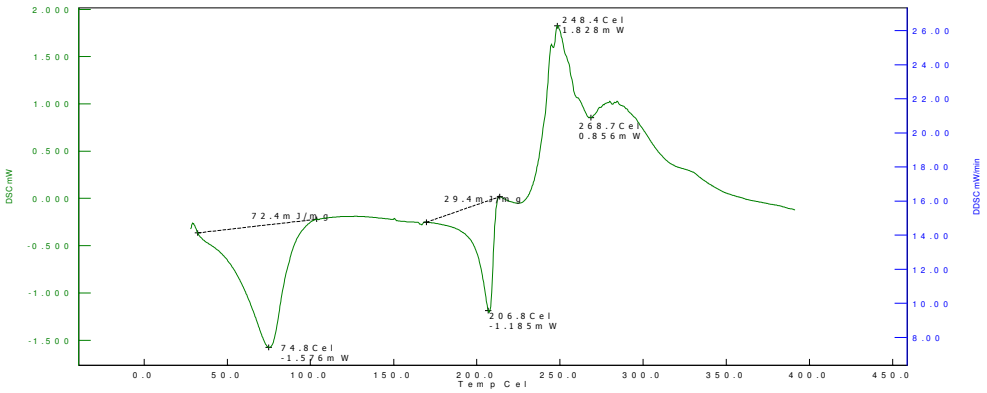

Fig-3:DSC Thermogram of pure drug

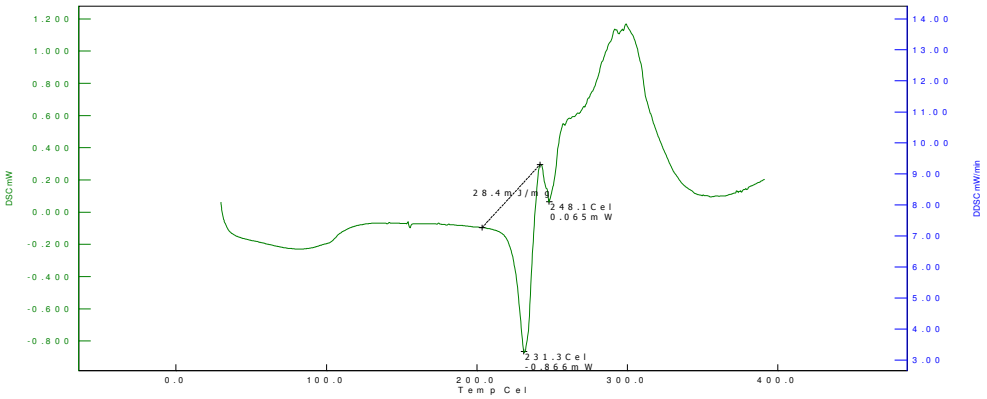

Fig-4:DSC Thermogram of Spherical Agglomerates

XRD Studies

XRD is a powerful technique for the identification of crystalline solid phase. Table.2 gives the XRD data obtained for the pure drug and as well as the spherical agglomerates in terms of lattice spacing and the relative intensities. The XRD patterns were showed in Fig.5&6. The characteristic diffraction peaks of pure drug (moxifloxacin) which were still detectable in the spherical agglomerates, indicates that the particles get crystallized and did not undergo any structural modifications. The pure form of drug exhibited high intense and long peaks . Whereas spherical agglomerates showed less intense peaks when compared to the pure form(Table.1). It indicates that considerable decrease in crystallinity of the drug in the form of spherical agglomerates.

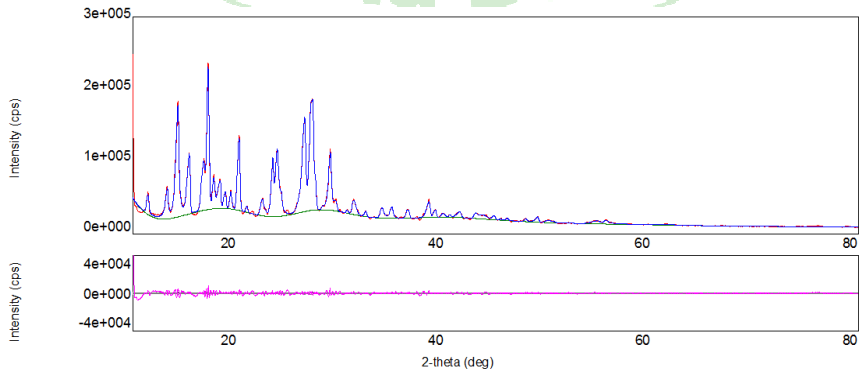

Fig-5: XRD Spectra of Pure Drug

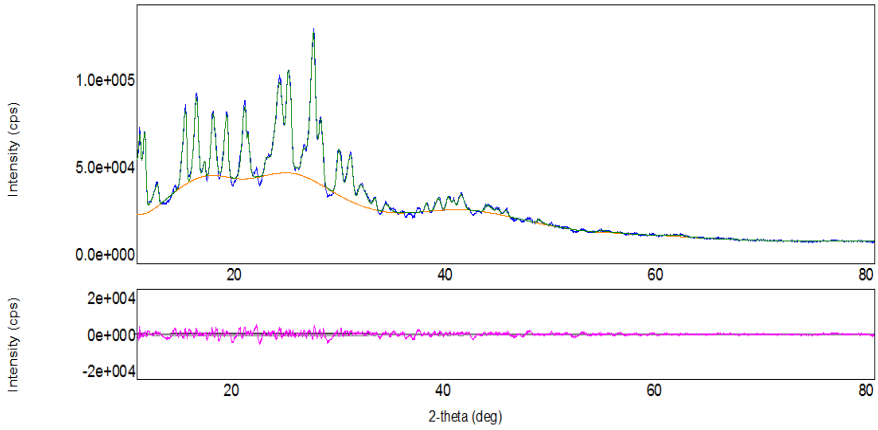

Fig-6: XRD Spectra of spherical agglomerates.

Table-1: X-ray diffraction data in terms of (°2) theta and intensities of pure drug and spherical agglomerates

Puredrug		Spherical agglomerates	
°2 theta	Intensity	**°2 theta**	Intensity
10.00	38050	10.22	11433
10.02	37272	10.24	11735
10.98	4548	10.72	11334
14.02	10464	11.82	7001
14.36	28100	15.18	8513
14.44	22485	15.68	14260
17.14	22452	18.48	13644
17.28	36215	19.46	7616
20.12	15021	20.22	14057
20.26	20832	22.46	9443
23.50	16235	23.50	16205
23.94	18049	24.42	16448
26.58	24830	25.80	10278
27.34	28684	26.96	13262

Optical and Scanning Electron Microscopy

The surface morphology of the agglomerates was accessed by scanning electron microscope(Fig.7&8). The pure form of drug shows rod shape crystals having poor flow and compression properties. The surface morphology of the prepared agglomerates shows that spherical shape with slight rough surface. It will give good flow and compression properties.

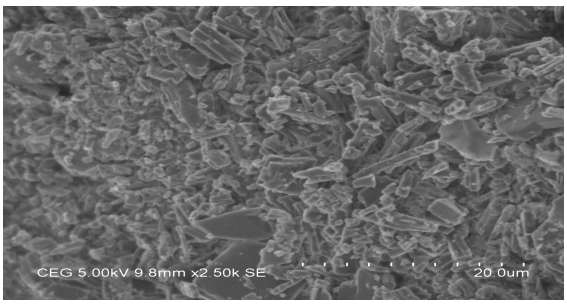

Fig.7: Scanning Electron Micrographs (SEM) of Moxifloxacin -pure

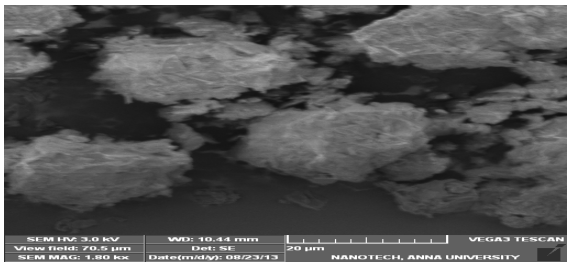

Fig.8: Scanning Electron Micrographs(SEM)of Moxifloxacin Sphericalagglomerates

Micromeritic properties

The loose bulk density (LBD) and tapped bulk density (TBD) were used to assess the packability of the crystals. The pure drug powder was more bulky and fluffy, which was indicated by the low LBD value (0.184± 0.00058 gmL-1, n=3). The highest TBD value (0.278± 0.001gmL-1, n=3) of pure drug indicates a high inter granular space between particles. In contrast, the spherical agglomerates exhibited higher LBD (0.247± 0.00057 gmL-1, n=3) and TBD (0.276± 0.00058gmL-1, n=3) values. These results indicate good packability of the prepared spherical crystals when compared with pure moxifloxacin. The Carr's index, Hausner's ratio and angle of repose are the parameters which are used to assess the flow and compressibility properties of the agglomerates. Carr's index, Hausner's ratio and angle of repose of pure drug were 33.57±0.0058% , 10.50 ±0.0058 (n =3)and 37.56±0.015^{o} (n=3), respectively, indicating extremely poor flow properties. The powder could not pass through the funnel during the angle of repose experiment. The poor flow of moxifloxacin could be due to the irregular shape and high fineness of the powder, which posed hurdles in the uniform flow from the funnel. On the other hand, the prepared spherical agglomerates exhibited low Carr's index, Hausner's ratio and angle of repose values, indicating excellent flow properties and compressibility(Carr's index: 10.5 ±0.0058%,n=3: Hausner's ratio: 1.11 ±0.0058,n=3;angle of repose: 27.40±0.015^{o},n=3). The spherical crystals had greater wettability than did the moxifloxacin powder, as indicated by the lower contact angle and surface tension. This result may have been caused by the lower crystallinity of agglomerated crystals as compared with the bulk drug. A study of the surface area followed the micromeretic principle. Spherical crystallization also increased the aqueous solubility of moxifloxacin.

Table2:Physical properties of Powder and spherical crystals of Moxifloxacin

Parameter	Moxifloxacin (Powder)	Moxifloxacin (Spherical agglomerates)
Particle size(µm)	72.24±2.12	484.88±12.62
Surface area(cm^2)	1.64x10^{-4}	88x10^{-4}
True density(g/cm^3)	1.4713±0.064	0.8987±0.068
Bulk density(g/cm^3)	0.564±0.045	0.403±0.035
LooseBulk density(g/cm^3)	0.184± 0.00058	0.247± 0.00057
TotalBulk density(g/cm^3)	0.278± 0.001	0.276± 0.00058
Carr'sindex(%)	33.57±0.0058	10.5 ±0.0058
Hausner's ratio	10.50 ±0.0058	1.11 ±0.0058
Angle of repose	37.56°±0.015^{o}	27.40 °±0.015 ^{o}
Density(g/cm^3)	0.9645±0.25	0.9885±0.15
Surface tension (dyne/cm)	54.67±0.85	47.35±0.85
Aqueous solubility (mg/ml)	0.16±0.98	0.26±1.82

Values are represented as mean ±SD,n=3

Slugging was required to make a coherent tablet form of Moxifloxacin powder, whereas spherical crystals formed a coherent tablet after one compression. Thus the spherical crystals are directly compressible, which could be a result of the new clear surface formed during compression. A hardness study of tablets showed that the tablets prepared from spherical crystals had greater mechanical strength than those prepared from the powder (Table.3). This may be a result of the stronger bonds formed between newly formed crystals of agglomerates. A friability study showed lower friability of the tablets prepared from the spherical crystals, possibly owing to the better compaction of the spherical crystals. The solubility study showed that spherical crystals have good solubility in water as well as in other solvents. This increase in solubility may be a result of the decreased crystallinity and increased wettability of the agglomerates of crystals. Disintegration tests showed that the tablets of spherical crystals disintegrated more rapidly than the tablets made from simple crystals of moxifloxacin. The amount of drug present in the tablets was found to be 88.21% and 97.99% for pure drug and spherical agglomerates respectively by UV spectrophotometricaly. The result indicates, the tablets prepared from spherical agglomerates having more amount of active ingredients when compared to the pure form. Fig.9 shows that the dissolution rate of tablets of spherical crystals was higher than that of tablets made from simple Moxifloxacin, owing to the greater wettability of the spherical crystals.

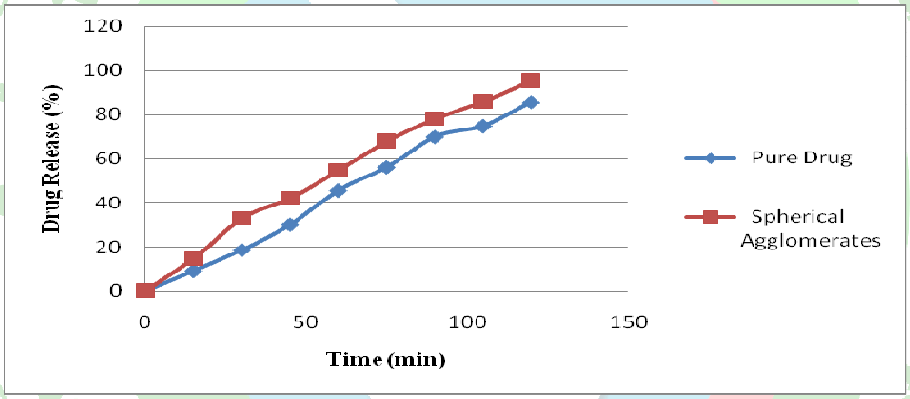

Fig. 9: Comparative Dissolution Study of Prepared Tablets of Pure Drug and Spherical Agglomerates.

Table3:InvitroComparison of tablets of plain powder and spherical crystals of Moxifloxacin

Parameter	Formulation	
	Tablets of plain powder	Tablets of spherical crystals of moxifloxacin
Porosity	0.26±0.82	0.192±0.62
Contact angle	82.4±0.96	26.3±1.52
Hardness(kg)	4.6±0.12	6.2±0.22
Tensile strength(kg/cm^2)	6.22±1.42	8.64±0.95
Friability(%)	4.78±0.98	0.86±0.96
Disintegration time	6min28s±20s	4min22s±18s
Drug Content(%)	94.21±0.94	97.99±1.54

Values are represented as mean ±SD,n=3

CONCLUSION

The present investigation reveals that spherical agglomerates of moxifloxacin prepared by ammonia diffusion system exhibited improved micromeritic properties which are essential requirements for direct tableting. Hence in

addition to improve the Solubility enhanced dissolution rate was observed compared with pure drug moxifloxacin. So this technique may be applied for producing oral solid dosage forms for moxifloxacin with improved dissolution rate and improved bioavailability.

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