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Research Article

Crystallo-co-Agglomeration Technique for Improving Physicochemical Properties, Compressibility and Solubility Characteristics of Metronidazole

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ABSTRACT

The objective of this study is to develop crystallo-co-agglomerates of metronidazole having improved physicochemical, compressibility and solubility properties. The process of agglomeration employed the use of dichloromethane (good solvent), ethanol (bridging liquid) and distilled water (poor solvent). Micromeritic properties of the metronidazole co-agglomerates were studied in terms of bulk density, tapped density, Carr's index, Hausner'sratio, and angle of repose. Co-agglomerates were also subjected to compaction studies using the Heckel and Kawakita compaction models. Characterisation of the agglomerates was studied using Scanning electronic microscopy, Fourier-transformed infrared spectroscopy, and X-ray diffraction. The co-agglomerates were further evaluated for drug release profile. The micrometric properties and dissolution characteristics of metronidazole co-agglomerates were significantly improved when compared to pure metronidazole. The angle of repose for metronidazole co-agglomerates was found to be 29.77°±1.09, Carr's index was13.60±0.35, and Hausner's ratio was 1.15±0.06, indicating good flow ability. The drug released over a period of 30 min was 102.61%, this shows better drug release than the pure drug, this could be due to the solubilization of the drug in presence of hydrophilic polymers. It can be concluded that metronidazole co-agglomerates were successfully prepared using the crystallo-co-agglomeration technique with improved micrometric properties, excellent compressibility, and improved solubility characteristics.

INTRODUCTION

Attempts to design pharmaceutical substances are undertaken in powder technology in order to handle a powder of acceptable flowability and bulk density for direct compression, relatively large particle must be used which may be prone to segregation. However, compressing a drug directly requires good micromeritic properties, such as flowability, good reproducible compression behaviour as it affects it's in vitro and in vivo performance (Paradkar and York, 2011), but it strongly depends upon the quality of the crystals used. To impart these properties the drugs are subjected to particle design techniques, spherical crystallization is one of the techniques of particle design (Kadam and Patil, 2017). By this technique manufacturing process are highly improved and a high degree of particle functionality is achieved. It seems that optimization of crystal properties is an alternative method for modifying the dissolution properties of drugs and therefore their bioavailability (Genikal and Rajendra, 2013).

Spherical crystallization is defined as an agglomeration process that transforms crystalline drugs directly into a compacted spherical form for improving the flowability, solubility and compactability" (Dimartino *et al.,* 2000). Spherical crystallization has been used mainly to obtain compressible agglomerates of a single, water insoluble large-dose drug; and rarely of a drug in combination with a diluent. Most of the excipients,

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such as diluents and disintegrating agents, are hydrophilic in nature; hence incorporation of these excipients in the agglomerates formed using organic bridging liquid is difficult. In order to overcome these limitation of spherical crystallization, crystallo-co-agglomeration (CCA) technique was developed by Kadam *et al.,* 1997.

Crystallo-co-agglomeration enables simultaneous crystallization and agglomeration of two or more drugs or excipients. It is used for size enlargement of all, low dose, high dose, poorly compressible drugs and combination of drugs with or without diluents. In this technique active pharmaceutical ingredient is directly crystallized and agglomerated in combination with an excipient or with another drug with help of bridging liquid simultaneously (Dongare and Bhalekar, 2017).

Basically, it yields complex agglomerates of drug and excipients, which are directly compressible in nature. The term crystallo-co-agglomeration (CCA) indicates crystallization takes place concurrently with another moiety which may be a drug or external inert material. The majority of drugs are hydrophobic, soluble in organic solvents, and poorly soluble in water, whereas many excipients, such as diluents, disintegrants or glidants, are hydrophilic. Therefore, the difference in the physical and chemical properties of drug molecules and the excipient is a major challenge in selecting a solvent system for the crystallo-co-agglomeration and dictates the yield of the process (Paradkar *et al.,* 2010).

Crystallo co-agglomeration is a particle design technique that has been proved to improve the efficiency of the initial steps of the manufacturing operation. It combines the process of crystallization (design of primary particles) and agglomeration (design of secondary particles) and increases the added value of the product by endowing the primary and secondary particles with greater functionality. CCA technique provides a wider application for the agglomerate.

Metronidazole having poor flow (angle of repose 39.92±1.85) and compressibility (Carr's index and Hausner's ratio of 20.13±0.28 and 1.26±0.01 respectively) makes it a least suitable candidate for direct compression. The Crystallo- co- agglomeration technique can be exploited to increase solubility, dissolution and hence bioavailability of poorly soluble drugs such as metronidazole. Metronidazole has poor compressibility and handling qualities; this will prevent the use of direct compressing in tablet production and thus will fail the purpose of the technique. The hydrophilic polymers: poly ethylene glycol (PEG 6000) and polyvinyl pyrrolidone (PVP K30) were used to improve the micromeritic, mechanical, and drug release properties of the agglomerates (Jolly *et al.,* 2016; Patel *et al.,* 2018).

Inadequate solubility and dissolution rate are the problems associated with a stable crystalline form that results in poor oral absorption and less bioavailability. Nowadays crystallo-co-agglomeration technique has been attracting the attention of researchers for increasing the solubility, dissolution and micrometric properties of drugs (Kadam and Patil, 2017). Hence, the aim of this study is to develop directly compressible agglomerates of metronidazole which is geared towards transforming the poor solubility and poor compressibility properties of metronidazole into directly compressible agglomerates having good dissolution, compression and flow behaviour using crystallo-co-agglomeration technique.

MATERIALS AND METHODS

Materials

Metronidazole powder (CDH Chemicals ltd. New Delhi, India), Dichloromethane (Merck ltd. Darmstadt, Germany), Polyethylene Glycol PEG 6000 (Shanghai Yuchuang chemicals tech. co., ltd, China), Poly vinyl Pyrrolidone PVP K30 (Shanghai Yuchuang chemicals tech. co., ltd, China), Ethanol 95% (BDH chemicals ltd Poole, England), Distilled water (Dept of pharm. and ind. Pharm, A.B.U. Zaria).

Method

Formulation of Metronidazole Co-agglomerates

Metronidazole was weighed and transferred in to a 250 ml beaker and dissolved in required amount of dichloromethane (good solvent) to make saturated solution at 50 °C. In another beaker PEG 6000 and PVP K30 were dissolved together in sufficient amount of water (non-solvent). The whole system (two dispersions) was then added immediately together under constant stirring using a magnetic stirrer attached with thermometer at 700 revolution per min (rpm).

Table 1. Composition of Metronidazole co-agglomerates.

Ingredients	Amount (g)
Metronidazole	3.00
PEG 6000	0.75
PVP K30	0.75

The stirring continued for 20 min and then ethanol (bridging liquid) was added dropwise to produce agglomerates. The agglomerates were then filtered

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and dried for 24 h in a hot dry oven. The above process was repeated 3 times to observe repeatability.

Evaluation of physical properties of pure metronidazole and metronidazole co-agglomerate powder

1. Micromeritic Studies:

The flow properties of metronidazole bulk and metronidazole agglomerates was determined in terms of angle of repose, bulk density, Carr's Index and Hausnar's ratio. Angle of repose was determined by fixed funnel method, agglomerate size distribution by sieving method, whereas Carr's Index and Hausnar's ratio were calculated from bulk and tapped density.

a) Bulk density (Db):

Accurately weighed 20 g of powder was carefully poured into a 100 ml graduated measuring cylinder through large funnel and the loose volume occupied by the powder was measured which is called initial bulk volume. Bulk density was expressed in g/ml and the studies was conducted in triplicate and is given by,

$$Db = \frac{M}{Vo} \qquad (1)$$

Where, Db = Bulk density (g/ml)
M = mass of powder (g)
Vo = bulk volume of powder (ml)

b) Tapped density (Dt):

Accurately weighed sample of 20 g was introduced into a clean, dry 100 ml measuring cylinder. The cylinder was then tapped on a flat table surface 100 times and the final volume after tapping was recorded. Tapped density was expressed in g/ml and the studies was conducted in triplicate and is given by,

$$Dt = \frac{M}{Vt} \qquad (2)$$

Where, Dt = Tapped density (g/ml)
M = mass of powder (g)
Vt = tapped volume of powder (ml)

c) Compressibility index (CI):

The compressibility of the powder is the percentage difference between the tapped and bulk density (Carr, 1965). the studies were conducted in triplicate and is given by,

$$\boldsymbol{CI = \frac{(Dt-Db)}{Dt} \times 100} \qquad (3)$$

d) Hausner ratio (Hr):

Hausner ratio is calculated as the ratio of the tapped density and bulk density (Hausner, 1967). this study was conducted in triplicate and is given by,

$$Hr = \frac{Dt}{Db} \qquad (4)$$

e) Angle of repose (θ):

An accurately 20 g powder sample was poured into a clean dry glass funnel clamped on a retort stand at 90^o to the flat horizontal surface on which a paper was placed, such that the tip of the funnel was 10 cm from the surface. Powder was carefully poured through the funnel while a powder heap was formed on the paper. Angle of repose was measure as the maximum angle possible between the surface of pile of the powder and the horizontal plane. Studies was conducted in triplicate and is given by,

$$\theta = \frac{h}{r} \qquad (5)$$

where;
θ = angle of repose
h = height of pile,
r = radius of the base of the pile.

f) Flow Rate (Fr):

Flow rate can be measured continuously using an electronic balance with some sort of recording device and a vibrator attached to facilitate flow from the container. A 20 g powder sample was passed through the Erweka flow rate machine and the time taken for each powder to completely pass through the vibrating metal funnel was recorded.

$$Fr = \frac{M}{t} \qquad (6)$$

M= weight of powder (g)

t= time (sec)

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g) Particle Size Analysis:
This method is performed by sifting a powder sample through a stack of wire mesh sieves, separating it into discrete size ranges (500, 250, 150, 90, 75, 45 µm). A 20 g powder sample was sieved using an Erweka vibration sieve through a nest of standard sieves. The vibration rate was set at 200 strokes/min and the sieving time was 10 min.

h) True density
The true density of pure metronidazole and metronidazole co-agglomerates was determined by the liquid displacement method using xylene as the immersion fliud. A 0.5 g powder was transferred into the weighted pycnometer bottle containing xylene. The final weight was recorded, and the density was calculated using the equation:

$$\rho = \frac{W1 \times Sg}{W5 - W7} \qquad (7)$$

W4=W3-W2, W5=W1+W4, W7=W6-W2, W8=W5-W7
Where;
W1 = weight of sample used, W2 = weight of empty bottle (pycnometer), W3 = weight of empty bottle + xylene, W4 = weight of xylene, W5 = weight of sample used + weight of xylene, W6 = weight of empty bottle + xylene + sample, W7 = weight of xylene + sample, W8 = weight of xylene displaced by sample

2. Agglomerates Production yield (%)
The production yields were calculated as the weight percentage of the final product (agglomerates) after drying, with respect to the initial total amount of metronidazole and polymer used for the preparations.

$$\text{Production Yield} = \frac{Practical\ Mass}{Theoretical\ Mass\ (Polymer+Drug)} \times 100 \qquad (8)$$

3. Drug content
An accurately weighed quantity of agglomerates equivalent to 100 mg of metronidazole was taken in a 100 ml volumetric flask. The drug was then extracted by using 0.1 N Hydrochloric acid by subjecting to continuous shaking on a rotary shaker for 4 h. Metronidazole in the extracted fluid was analyzed at 277.0 nm using UV-visible spectrophotometer (UV-1601, Shimadzu, Japan) against 0.1 N Hydrochloric acid solution as blank and with the lowest concentration at 0.781 µg/ml and studies was conducted in triplicates.

4. Dissolution studies
In-vitro dissolution studies for prepared agglomerates was performed using USP Apparatus I (Basket type) operated at a rotation speed at 100 rpm in 900 ml of dissolution medium (0.1N HCL) maintained at 37 ± 1 °C. Samples of 10 ml were withdrawn at a pre-determined time interval (1min, 3min, 5min, 10 min, 15 min, 20 min, 30 min) and each time the same amount of dissolution medium was added to replace the withdrawn samples. After suitable dilution with 0.1 N HCl, samples were analyzed by UV-spectrophotometer at 277.0 nm using 0.1 N HCl as blank. All the trials were conducted in triplicate.

5. Compaction Studies
The compression behavior of pure drug crystals and prepared Metronidazole agglomerates were evaluated by Heckel and Kawakita plot analysis. Compacts of drug and agglomerates (500 ± 5 mg) were prepared at constant compression speed at different pressures (25, 50, 75, 100, 150, 200, 250, 300 MN/m^2) using a 12 mm flat-faced punch and die set on a Hydraulic Press. The die was lubricated with a dispersion of magnesium stearate in ethanol before each compression. The powder sample (500±5mg) was loaded manually into the die and the compression pressure was applied and a dwell time of 30 s was kept constant for each

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compression. After ejection, the tablets were kept in a desiccator over silica gel for 24 h to allow for elastic recovery.

Tablet dimensions: weight and thickness of tablet for each compression pressure were measured. Determinations were carried out in triplicate and the mean and standard deviation computed. Using the formulas described below, Heckle and Kawakita analysis were carried out. A graph of ln (1/1-D) against P (Heckel plot) and P/C against P (Kawakita plot) were used to analyse the compaction profile.

Porosity (Ɛ) = 1 – D (9)

Apparent density (ρA) = $\frac{weight\ (g)}{volume\ ((\pi r^2 h))}$ (10)

Relative density (D) = $\frac{\rho_A}{\rho_T}$ (11)

where Ɛ represents the porosity of the compressed powder bed at applied pressure *P*,
whereas *k* and *A* are constants. *r* and *h* are the radius and thickness of the tablet respectively.

Heckel analysis
To analyze the compressibility of the agglomerates it was used, with the help of the derivation.

$$\frac{dD}{dP} = K(1 - D) \qquad (12)$$

Where *D* = Relative density of the compact at pressure *P* and *k* is a constant. It is assumed that the change in relative density in respect of pressure is directly proportional to the left-over porosity on further integrating the above equation.

$$\ln\left(\frac{1}{1-D}\right) = Pk + A \qquad (13)$$

Here, "*k*" and "*A*" are constants; *D* and *Py* are the packing fraction and pressure, respectively. The slope, *K* of the Heckel plot gives a measure of the plasticity of a compressed material.

Relative density at zero compression pressure (D_0) is the ratio of the bulk density to the true density of the powder, while the relative density (D_b) describes the rearrangement phase at low pressure, and can be obtained from the relationship;

$$D_b = D_a - D_0 \qquad (14)$$

6. X-ray Differaction
the crystallinity of pure metronidazole and the crystallo co-agglomerates was determined using Bruker XRD (Model: D 8 Advance) with copper target. The conditions are: 40 kV voltages; 40 mA current; at room temperature. The samples were loaded on to the differactometer and scanned over a range of 2θ values from 10 to 800 at a 17 scan rate of 0.050/min.

7. Fourier-transform infrared Spectroscopy FTIR:
To evaluate the drug-polymer compatibility and structural modification of drug during crystallization and agglomeration, FT-IR spectra of pure drug and prepared agglomerates was recorded. Infrared spectra of pure drug and agglomerates were performed using IR Spectrophotometer (FTIR NICOLET IR 100, Thermo Electro-Corporation, USA). The samples (approx. 2-3 mg) was homogeneously mixed with KBr and pressure was applied to compress them into disc or pellet using a hydraulic press at a force of 10T for 2min. The IR Spectra was recorded by placing the pellets in light path. The scanning range was kept from 4000 to 500 cm^{-1}.

8. Scanning Electron Microscopy
The shape and surface characteristics of pure drug crystals and agglomerates was examined by Scanning Electron Microscope (Phenom ProX, Phenomworld, Netherlands).

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Samples were mounted on a double-faced adhesive tape, sputtered with gold. Scanning electron photographs were taken at an accelerating voltage of 15 kV and obtained micrographs were examined at X2000, X5000, X10000 and X15000 magnifications. SEM photomicrographs was used to observe surface characteristics, agglomeration efficiency as well as packing of agglomerated crystals.

9. Statistical Analysis and Data Presentation

Analysis of variance (ANOVA) was used to compare the properties across different categories of data sets using SPSS software version 25. Differences was considered significant for P <0.05. The results obtained was presented as mean, standard deviation and percentages (%) in tables and figures.

RESULTS AND DISCUSSION

Metronidazole was crystallized from dichloromethane-ethanol-water and agglomerated with two hydrophilic polymers viz polyvinylpyrrolidone PVP K30 and polyethylene glycol PEG 6000 in a drug: polymer ratio of 2:1. PEG 6000 is soft and plastic in nature. Thus will aid plastic deformation, thereby providing better compressibility. It also will reduce the interfacial tension between external phase (water and bridging liquid). PVP K30 will result in improved micromeritic properties, solubility and dissolution rate of crystallo co-agglomerates (Jolly *et al.*, 2016; Rahate *et al.*, 2013).

Metronidazole is freely soluble in dichloromethane (good solvent) at 50 °C, but poorly soluble in water (poor solvent). Also, it is soluble in ethanol (bridging liquid) which is immiscible with dichloromethane. Hence, this solvent system was selected for the present study. In this process, crystallization of drug was performed by the addition of drug solution to the anti-solvent phase (water). The dichloromethane solution (maintained at 50 °C) containing drug was added immediately to the aqueous dispersion containing hydrophilic polymers PVP K30 and PEG 6000 and quasi-emulsified droplets of drug solution were produced. The addition of bridging liquid (ethanol) promotes the formation of liquid bridges between the drug crystals to form agglomerates. The metronidazole agglomerated crystals were formed by coalescence of these dispersed crystals.

Since the presence of three liquids is necessary (good solvent, bridging solvent and poor solvent) for crystallo co-agglomeration, a ternary phase diagram was desirable to select the best quantities (volume) of solvent system. Similar model was used by Kulkarni *et al.*, in there study on the crystallo co-agglomeration of ibuprofen (Kulkarni *et al.*, 2010). The ternary phase diagram represents the phase behaviour or mixtures containing three components in a triangular diagram. Points on the sides of the triangle were excluded. Each triangle in the ternary diagram was representing the volume of one of the solvent systems to be investigated for the crystallization and agglomeration. The optimal ratio for crystallization and agglomeration was found (figure 1). These proportions of Dichloromethane/ethanol/water (10:20:70) were then finally chosen for this study.

Calibration curve of Metronidazole using UV Spectrophotometer:

The calibration curve of Metronidazole was developed in the range of 50 - 0.391 µg/ml at wavelength 277.0 nm. Good linearity with regression coefficient of 0.9795 (r^2 value) was observed.

Micromeritic properties:

Micromeritic properties of the crystallo-co-agglomerates was studied in terms of bulk density, tapped density, Carr's index, Hausnar ratio and angle of repose. the bulk and tapped densities of powder are

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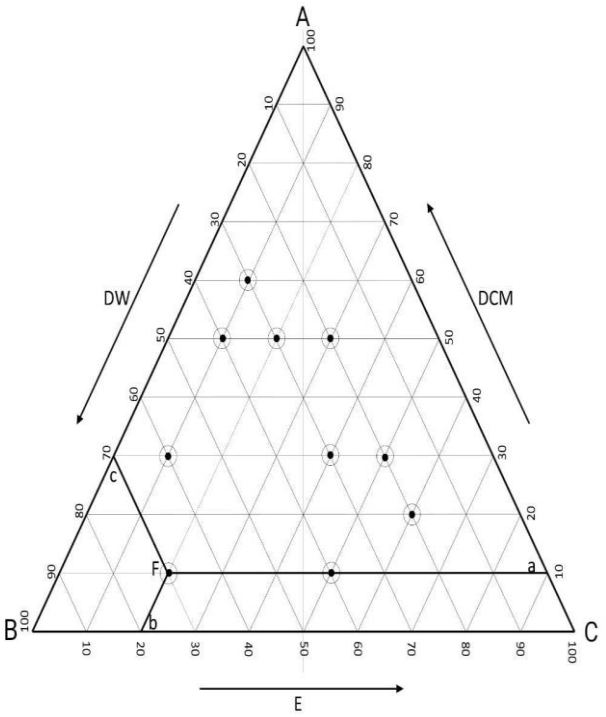
important physical parameters for assessing flowability and compressibility (Alton, 2013). The Carr's index and Hausner's ratio are used to express compressibility and flowability in relation to bulk and tapped densities (Carr, 1965; Hausner, 1967).



Key: E = ethanol, DCM = dichloromethane, DW = distilled water

Figure 1. Ternary phase diagram for optimization of DCM/E/DW solvent system

The different micromeritic properties of pure metronidazole and CCA crystals is shown in Table 2. The differences in the bulk densities may be related to their markedly different crystal habits, leading to different contact points, frictional and cohesive forces between the crystals. These factors affect the sliding of the particle against each other leading to different packing geometry and thus different bulk densities (Krishna e*t al.*, 2013). Flow rates are in agreement with morphology and bulk density data that CCA crystals with low bulk density exhibits better flow properties. Pure metronidazole had a significantly higher angle of repose (39.92^{o}±1.85) which indicates irregular shape of the crystals whereas that of crystallo-co-agglomerates were seen to have an excellent angle of repose of 29.77^{o}±1.85 as shown in table 2.

The Carr's index and Hausner's ratio of metronidazole agglomerates was 13.60±0.35 and 1.15±0.06 respectively compared to that of pure metronidazole (20.13±0.28% and 1.26±0.01. This indicates improvement in the flowability of the agglomerated crystals. The reason for the improved flow of agglomerates is the significant reduction in the interparticle friction because of the polygonal shape and the larger size of the particle (Jolly *et al.*, 2016).

Furthermore, the high values of Carr's index and Hausner's ratio of the pure metronidazole can be attributed to the inherent high cohesive nature of the pure drug powder which hindered its flowability. These could also be related to the particle size, shape and particle distribution of the drug because these are factors that affect the flow of powder material. Generally, as the values of these indices (Carr's index and Hausner's ratio) increases, the flow of powder decrease, and this may lead to formulation problems like weight and content variation (Aulton, 2013).

A statistically significant difference (P value < 0.05) was observed in the mean particle size. The findings indicated 4-folds rise in mean particle diameter of agglomerates compared to pure drug crystals which indicated growth of particles by simultaneous agglomeration after crystallization due to enhanced bonding and bridging formed in presence of polymers. This is in line with studies conducted by Shah and Sorathia on evaluation of spherical agglomerates of Fluvastatin (Shah and Sorathia, 2017). From the particle size analysis of the co-agglomerates, the predominant particle size was 500 µm followed by 90, 250, 150, 75 and 45 µm.

Powders containing predominant particles larger than 250 µm are usually relatively free

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flowing but as the size falls below 100 µm, powders tend to be cohesive and flow problems are likely to occur (Staniforth and Aulton, 2007).

Table 2. Micromeritics for Metronidazole Co-agglomerates and Pure Metronidazole powder

Parameters	Metronidazole co-agglomerates	Pure Metronidazole powder
Bulk Density (g/ml)	0.520±0.09	0.673±0.08
Tapped Density (g/ml)	0.616±0.13	0.842±0.13
Flow Rate (g/sec)	4.92±0.13	3.45±0.07
Angle of Repose (θ)	29.77±1.09	39.92±1.85
Carr's Index	13.60±0.35	20.13±0.28
Hausner's Ratio	1.15±0.06	1.26±0.01
Mean Particle size (µm)	288.40	69.72
Percentage (%) fines	0.35	45.05

Particle size and particle size distribution of metronidazole co-agglomerates and pure metronidazole powder

The percentage powder retained against mesh sieve size is presented in Figure 2 below. The predominant particle size for metronidazole co-agglomerates was 500 µm followed by 250, 90, 150, 75 and 45 µm respectively.

Drug content and percent yield:

The yield of agglomeration was 71.45% wt/wt. The amount of metronidazole entrapped in agglomerates was 91.50%. The percentage of fines generated in the CCA batch was 0.35% wt/wt. Fines might have been generated because of sticking of the wet powder mass to the vessel, thermometer and stirrer during agglomeration. The loss of metronidazole to supernatant was statistically significant (P value < 0.05). This loss may have been due to the poor solubility of the drug in water, resulting in migration of metronidazole from DCM into water.

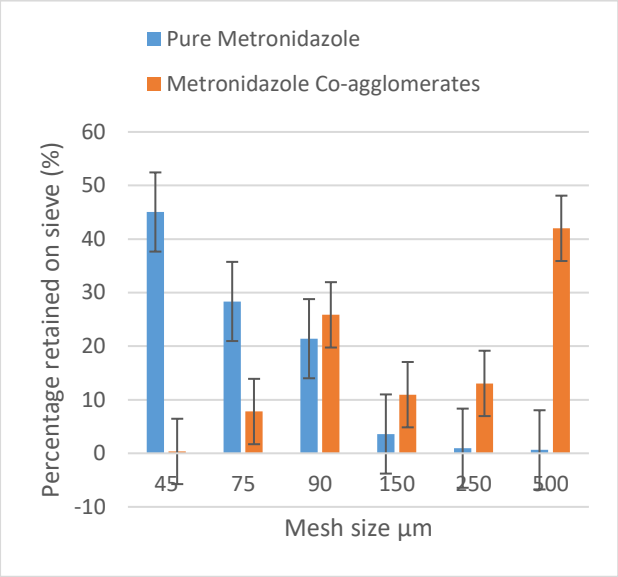

Figure 2. Percentage weight of powder retained versus mesh size (particle size)

Table 3. Drug Content and percentage yield of Metronidazole co-agglomerates

Parameters	Co-agglomerates
Drug content (%)	91.50
Percentage Drug Yield (%)	71.45

Heckel plot analysis

The Heckel equation shown as equation 13 is one of the most frequently used equations for characterizing the compressibility of pharmaceutical powders. This is probably due to its simplicity. Heckel derived mathematical relationship for the densification of powder bed at an applied pressure (Heckel, 1961a; Sonnergaard, 1999) is the most commonly employed.

The slope of Heckel plot "*K*" of optimized batch was an indicative of plastic behavior of the material (Kawashima *et al.*, 2003). The larger the value of "*K*," the more the plasticity of the material. Table 4 shows parameters of Heckel plot. "*K*" value of pure metronidazole drug and optimized Crystallo co-agglomerates obtained were 4.47×10^{-5} and 1.37×10^{-3} respectively.

The constant *A* is obtained from the intercept of the linear regression equation of the Heckel plot. It is related to the particle rearrangement during die filling

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(Sonnergaad, 1999) and has been used to obtain the value of D_a. D_a is the total densification of the plastic deformation before interparticle bonding becomes appreciable (Hsu *et al.*, 1997). The D_a values, pure metronidazole was shown to have the highest value while metronidazole co-agglomerates exhibited low D_a values. This can be attributed to the low particle size and low inter particle spaces in the pure drug than the agglomerates.

A value of the optimized agglomerates (1.226) was less than the pure drug (2.348). This finding suggested that a low compression pressure was required to attain the closest packing of the agglomerates, fracturing of the texture, and densifying of the fractured particles (Raval et al., 2013).

D_o is used to describe the densification due to the movement and rearrangement of particles (densification of fragmentation). The result indicates that pure metronidazole exhibited higher degree of packing in the die that the co-agglomerates (Odeku and Itiola, 2007).

The D_b value represents the particle rearrangement phase in the early compression stage and tends to indicate the extent of particle fragmentation, although fragmentation can occur concurrently with plastic and elastic deformation of constituent particles (Salim *et al.*, 2018, Heckel, 1961b). Values of D_b for pure metronidazole and metronidazole co-agglomerates are 0.23 and 0.19, respectively. The D_b values were higher in pure metronidazole that metronidazole co-agglomerates. This indicates that metronidazole co-agglomerates require less pressure to undergo fragmentation and rearrangement while pure metronidazole may require higher pressure.

The low mean yield pressure (P_y) values suggest plastic deformation as the predominant mechanism of consolidation. This type of mechanism is seen in comparatively softer material (Pawar *et al.*, 2004). The fragmentation leads to formation of new surfaces which on further compression come into closer contact of each other leading to strong bonding (Kachrimanis *et al.*, 2000). This gives rise to a thicker compact. The table 4 shows various parameters of heckel analysis.

The reciprocal of *k* is the mean yield pressure (P_y), which refers to the pressure at which materials begin to deform plastically (Rojas and Kumar, 2011). The P_y values showed that metronidazole co-agglomerates have the lowest value while the pure metronidazole exhibited the highest value, this indicates plastic deformation of the agglomerates (Patra *et al.*, 2007). Here, value of yield pressure (*Py*) for pure drug and optimized batch of metronidazole co-agglomerates was 22370.11 and 732.50, respectively. Thus, the Heckel plot data suggested that the agglomerated crystals were fractured easily and the new surface of particles generated contributed to promote plastic deformation under applied compression pressure (Kawashima *et al.*, 2003).

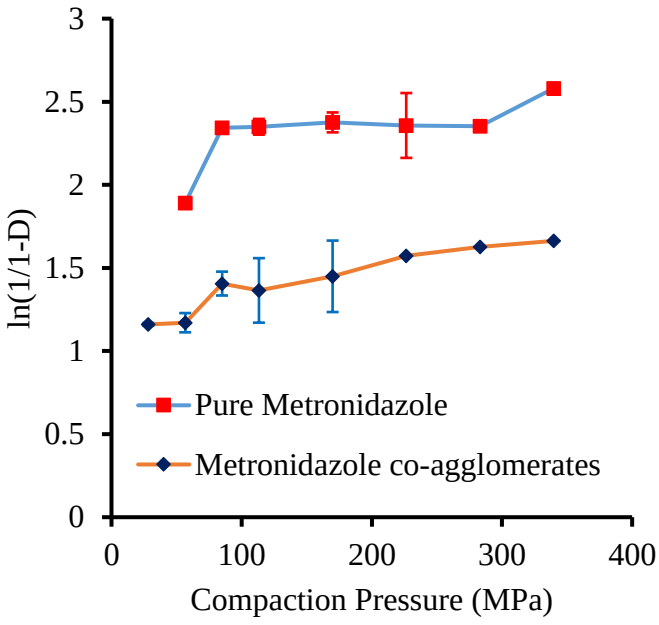

Figure 3. Heckel plot of pure metronidazole and metronidazole co-agglomerates

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Table 4: Mean yield pressure and relative density derived from Heckle plot.

	Metronidazole	Metronidazole co-agglomerates
K	4.5E-05	0.00137
P_y	22370.1	732.5
A	2.35	1.26
Do	0.67	0.52
Da	0.9	0.71
Db	0.23	0.19
R^2	0.9182	0.9015

Powder yield pressure (P_y), powder total densification (A) (g/ml), relative density due to die filling (D_o) , total powder relative density (D_a) , relative density due to particle rearrangement/fragmentation (D_b), correlation coefficient (R^2)

Kawakita plot analysis

The study of the extent of volume reduction of the pure drug and co-agglomerates as a function of applied pressure was graphically illustrated in the Kawakita plots in figure 4. A linear relationship was obtained at all compression pressures employed with correlation coefficient R^2 of 0.9998 and 0.9995 for pure metronidazole and metronidazole co-agglomerates respectively. Values of *a* and *b* were obtained from the slope and intercept of the plots respectively.

The extent of volume reduction of the materials under the applied compression load were denoted by the constant *a* (Ghori and Conway, 2016). Pure metronidazole has higher volume reduction tendency than metronidazole co-agglomerates, this can majorly be attributed to its particle size (69.72 µm). The P_k parameter provides a quantitative estimation of the pressure that is required to reduce the volume of the powder by 50% (Kawakita *et al.*, 1984; Nordström 2008) and it correspondingly quantifies the inverse plastic deforming capacity of the composites (Adedokun and Itiola, 2010). At low compression pressure all the samples can undergo significant volume reduction.

A comparison of the results on compressional characteristics (P_y and P_k) of the pure metronidazole and metronidazole agglomerates is noteworthy. Odeku and Itiola (Odeku and Itiola, 1998) have shown that P_y relates essentially to the onset of plastic deformation during compression, while P_k relates to the amount of plastic deformation occurring during the compression process. It is also notable that pure metronidazole showed the lowest amount of plastic deformation, while metronidazole agglomerates showed the highest amount of plastic deformation during compression. This is probably responsible for the higher tensile strength (Ts) values of metronidazole agglomerates since higher amount of plastic deformation would lead to more contact points for inter-particulate bonding (Odeku and Itiola, 2007).

In-vitro dissolution studies

This is an essential tool to evaluate drug release from a dosage form from which gives an overview of drug release in the GIT (Genikal and Rajendra, 2013). There was a positive correlation between the disintegration time and dissolution rates.

Dissolution behaviour of pure Metronidazole and agglomerates was studied using 0.1N HCl as dissolution medium (figure 5). The amount of pure metronidazole dissolved in 0.1N HCl was 65.36 % at 30 min. Metronidazole co-agglomerates showed maximum drug release of 102.61% in 5 min. Dissolution rate enhancement of metronidazole agglomerates were due to the presence of polymers, which may have improved the wettability of the drug (Jadhav *et al.*, 2010; Pawar *et al.*, 2004).

The metronidazole co-agglomerate was subjected to dissolution studies in order to understand the pharmacokinetics and thereby bioavailability of the obtained product. The British Pharmacopoeia stipulates that not less than 70% of metronidazole must be dissolved in 45 min

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(British Pharmacopoeia, 2010). In-vitro dissolution rate affects the solubility of a drug molecule within a formulation which consequently influence in-vivo absorption and bioavailability of the drug (Alshehri *et al.*, 2017). Figure 8 shows the percent metronidazole dissolved with time. At 5 min, metronidazole co-agglomerates had more than 90% drug dissolved while for pure metronidazole at 30 min less than 70% of the drug dissolved. Low percent drug dissolved will result in poor bioavailability of the drug thereby leading to therapeutic failure. All the drugs may still dissolve eventually, but coming after 45 min may not be acceptable (British Pharmacopoeia, 2010).

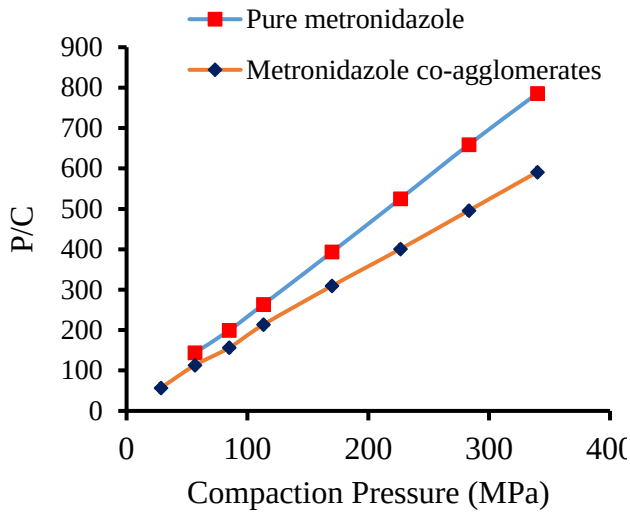

Figure 4. Kawakita plot of pure metronidazole and metronidazole co-agglomerates.

The dissolution profiles of metronidazole (Figure 5) exhibited improved dissolution behaviour for co-agglomerates than pure drug. The reason for this faster dissolution could be linked to the better wettability of the metronidazole co-agglomerates, attributed to hydrophilic nature of PVP K 30 and PEG 6000 used in co-agglomeration technique. In addition, change in crystalline to partial amorphous form of metronidazole during crystallo co-agglomeration process could have contributed to increase in drug release (Deshkar *et al.*, 2017). These observations were in agreement with XRD results.

Table 5. Parameters obtained from Kawakita analysis of the Pure metronidazole and metronidazole co-agglomerates.

	Metronidazole	Metronidazole Co-agglomerate
a	0.44	0.59
b	0.3	0.12
$b^{-1}(P_k)$	3.32	8.64
D_i *(1-a)*	2.29	1.7
R^2	0.9998	0.9995
True Density	1.31	1.51

Total volume reduction/compressibility (*a*), compression co-efficient (*b*), yield strength (P_k), packed initial relative density (D_i), correlation coefficient (R^2).

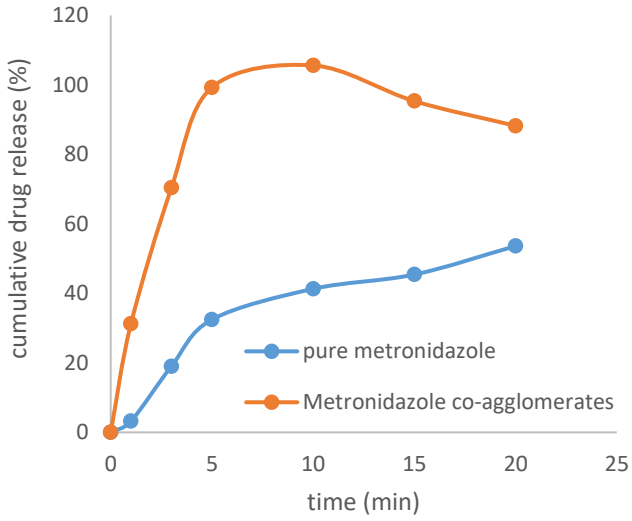

Figure 5: Comparison of in-vitro dissolution profile data of metronidazole in pure form and as co-agglomerates.

Characterization of metronidazole-polymer agglomerates:

Fourier Transform Infrared Spectroscopy

Infrared spectrum of pure metronidazole is shown in figure 6. The characteristic absorption peaks of metronidazole were obtained at 3658.80 cm-1 due to Secondary Amine N-H stretch, 2109.70 cm-1 due to aromatic C-H stretch, 1990.40 cm-1 due to C=O stretch, 1580.40 cm-1 due to C - C (S) stretch and at 1073.5 cm-1 due to C-N stretch.

By comparing the FTIR spectrum of metronidazole with the drug-polymer agglomerates (Figure 7), it was concluded that all the characteristic absorption bands and bonds of various functional groups

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present in metronidazole were retained. There was no considerable change in the positions of characteristic absorption bands and bonds of various functional groups present in the drug. The results of the FTIR spectra indicated the absence of any well-defined interaction between metronidazole, solvents and hydrophilic polymer (Rahate *et al.*, 2013, Dixit *et al.*, 2011).

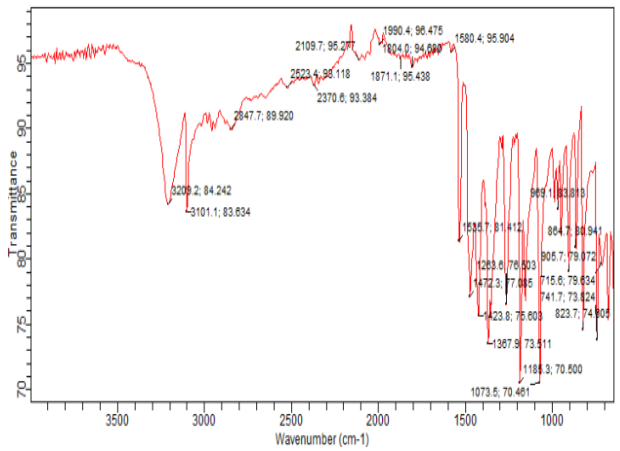

Figure 6: FTIR of Pure Metronidazole.

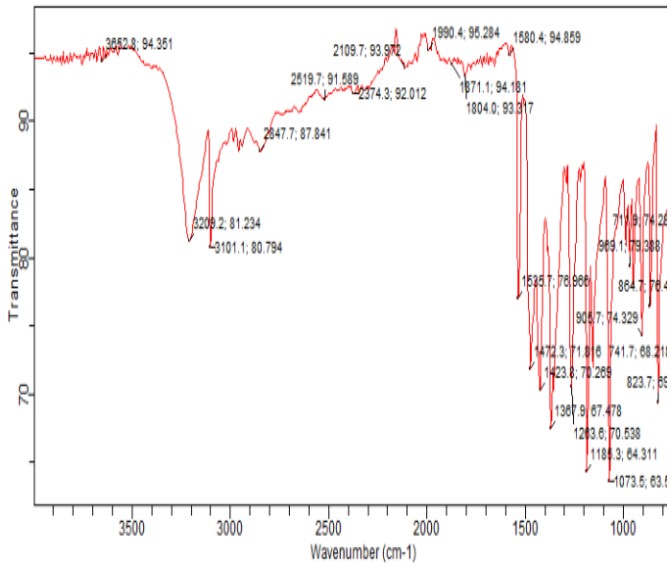

Figure 7: FTIR of Metronidazole co-agglomerates.

Scanning Electron Microscopy (SEM):

Figure 8 and 9 shows the photomicrograph of pure drug and agglomerates respectively. The image of pure drug shows cohesive rod-shape and elongated form of morphology. The images of the agglomerates show polygonal shaped morphology which suggested that the possible elongated rod-shape fine crystals of metronidazole (Figure 8), was converted to compact agglomerates by the process of Crystallo co-agglomeration technique.

Figure 8: SEM of Pure Metronidazole

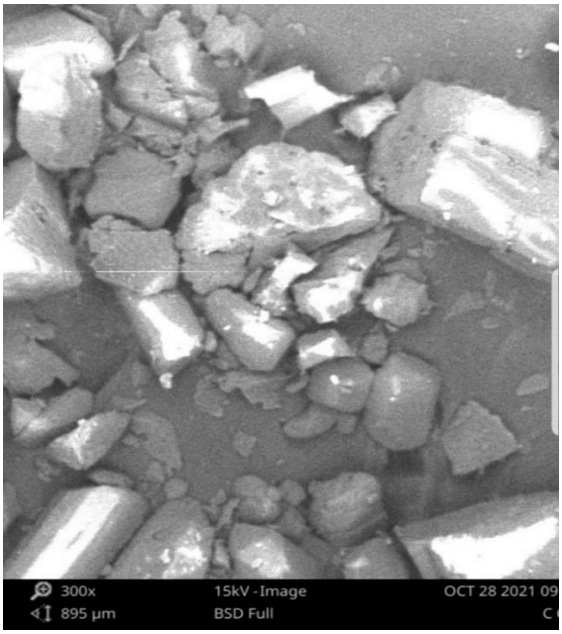

Figure 9: SEM of Metronidazole co-agglomerates

XRD

The XRD pattern in the 10-70°, 2θ range showed that the diffraction peaks, characteristics of pure metronidazole (Figure 10) were detectable as showed from the intense peaks, which was transformed to

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halo pattern with less intense in sample of crystallo co-agglomerates (Figure 11). This suggested the possible decrease in crystalline nature or partial amorphous nature (presence of amorphous regions in the crystals) of developed spherical agglomerates (Chandran *et al.,* 2016; Chavda & Maheshwari, 2008).

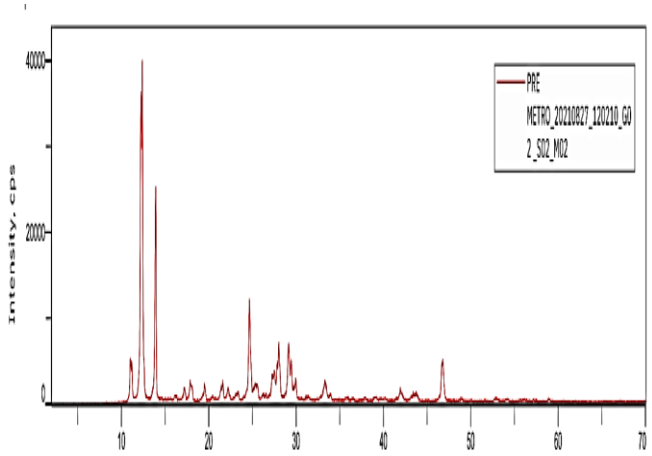

Figure 10. XRD of Pure Metronidazole

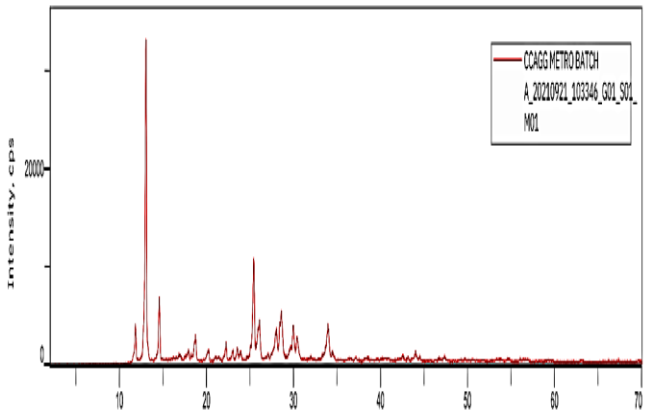

Figure 11. XRD of Metronidazole co-agglomerates

Though, the intensity of peaks in XRD was reduced (notably at 13, 15 and 47 degrees 2θ) while peaks above 30 degrees 2θ were less intense, there is no considerable change in d-spacing values suggesting no change in crystal form of drug, but crystal habit of the drug might be changed thus suggesting the absence of a polymorphic transition. Therefore, the presence of different polymorphs of metronidazole in these samples was ruled out (Chavda and Maheshwari, 2008).

However, the difference in the relative intensities of their peaks may be attributed to the difference in the crystallinity or particle size of the sample and because of dilution and adsorption of the polymer to drug crystals (Kadam and Patil, 2017; Jolly *et al.,* 2016; Maghsoodi and Barghi, 2011).

Conclusions

Metronidazole was successfully prepared by crystallo co-agglomeration technique using hydrophilic polymers viz PVP K30 and PEG 6000. Metronidazole co-agglomerates exhibited improved micromeritic properties, excellent compressibility, high flow rate, good angle of repose, excellent flowability and packability due to particle size enlargement and reduced interparticulate friction compared to pure drug. FTIR, and XRD studies showed there was no change in the crystal habit and structure of metronidazole during the crystallization process i.e., polymorphism has not occurred. The solubility of the co-agglomerated metronidazole shows significant improvement compared to the pure metronidazole. Thus, crystallo-co-agglomeration technique is a tool for particle engineering, which has transform the poorly flowable metronidazole powder into co-agglomerates that can be best suited for direct compression.

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