



Research Article

ISSN: 0975-7384
CODEN(USA): JCPRC5

Evaluation of Excipient Potentials of Alpha Cellulose Extracted from Rice Husk in Metronidazole Compressed Tablets: Colon Targeted Drug delivery and *In Vitro* Characterizations

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ABSTRACT

The objective of this research demonstrates the excipient potential of alpha-cellulose in metronidazole tablet. Direct compression method was adopted. Powder and tablet properties of the α -cellulose were characterized, evaluated with FTIR, micromeritics, drug content, quality control tests, in vitro drug release and in vitro antimicrobial studies, and the results compared to MCC and Kollidon® VA 64. Chemically, their constituents are almost identical in FTIR study and compatible with API. Batch A powder exhibited a significant good flow property ($p < 0.05$) than others. All the mechanical properties of the batches were within the B.P specification with a significant ($p < 0.05$) low tablet weight and thickness observed in batch A, unlike other batches. Drug contents range within 92.21 ± 0.11 - $98.44 \pm 0.21\%$ and 90.14 ± 0.55 - $95.61 \pm 0.18\%$ for uncoated and coated batches, respectively. In vitro drug release showed gradual drug release except burst effect observed in batch G with a significant ($p < 0.05$) T40 and T75 of 5 and 25 min for uncoated batches, while the coated batches had prolonged T40 and T80, respectively. The MIC was observed around 25 $\mu\text{g/ml}$. Alpha cellulose can be employed as a directly compressible excipient for tablet production.

Keywords: α -cellulose; Metronidazole; MIC; CTDD; Direct compression

INTRODUCTION

Drugs are barely administered alone but frequently formulated into a suitable dosage form with the assistance of excipient(s). These excipients play critical roles such as disintegrant, binder, gelling agent, lubricant, flavorant,

emulsifier, suspending agent, bulking agent, and sweetener and among others [1,2]. Excipients has been defined by the International Pharmaceutical Excipients Council as substances other than the active pharmaceutical ingredients (APIs) of finished dosage form, which have been appropriately evaluated for safety and are incorporated in a drug delivery system to either help the processing of the dosage form during its formulation or provide and enhance bioavailability, patient acceptability, product identification, or enhance the stability during the storage or shelf life of the product [3]. The roles performed by excipients in drug production are very vast and numerous which include protecting the efficacy, safety, stability of APIs in order to achieve therapeutic efficacies, and among others. Availability of excipients can make pharmaceutical products cost-savings and easy usage in drug development which will enhance drug formulation innovations. One of these excipients includes cellulose.

Currently, cellulose and cellulose-based polymers have earned a greater popularity in the pharmaceutical field. They have become more and more important in this area owing to the production of new derivatives with new applications and new chemical moieties. Cellulose has been reported as the most abundant organic polymer in the world [4]. In drug delivery, a wide range of raw materials such as cellulose has been exploited as drug carriers due to their abundance, biocompatibility, economical, safety, renewability, amorphousness and reproducibility [5]. Natural waste products such as agricultural produce significantly generate large amounts of wastes for disposal. The agricultural waste produced in a particular period of the year pose potential pollution problems and therefore, requires efficient utilization after transformation and modification. These wastes significantly contain high quantities of cellulose. Cellulose is a long chain polymer with industrial applications in wood and paper, fibers and clothes, cosmetic and pharmaceutical industries, and veterinary food [6-8]. Celluloses are isolated from natural sources and modified with acid and alkali treatments to produce α -cellulose (cellulose free of hemicellulose and lignin) which can be employed as diluents or filler/binder in tablets production using either granulation or direct compression technique [9]. There are two main cellulose derivatives obtained after chemical modifications. These two main groups of cellulose polymer have different physicochemical and mechanical properties and are ethers and esters cellulose derivatives which are broadly employed in the formulation of pharmaceutical dosage forms. They play important roles as pharmaceutical excipients as had reported such as in extended and delayed release coated dosage forms [10], extended and controlled release matrices [11,12], osmotic drug delivery systems [13], bio adhesives and muco-adhesives [14-16], compressed tablets as compressibility enhancers [17], liquid dosage forms as thickening agents and stabilizers [18,19], granules and tablets-binders/fillers [20,21], semisolid preparations as gelling agents [22,23], disintegrating agent [21], taste masking [24] and many other applications [25]. They can equally be employed to moderate specific drug delivery property pattern such as immediate, controlled/sustained or delay release [5,26]. Cellulose had been isolated from these natural sources such as Agave angustifolia fiber [27], sugarcane bagasse [28,29], chardonnay grape skin [30], rice husk [31], kenaf fiber [32] and onion skin [33].

In the processing of rice (*Oryza sativa*), rice husk is produced as one of the by-products which can be employed after modification as a pharmaceutical excipient. Husk is a dietary fiber consisting of non-digestible carbohydrates and lignin that are intrinsic and intact in plants which contain 25-35% cellulose, 26-31% lignin, 18-21 hemicelluloses 15% pentosan and 21% ash [34,35]. Rice husk possess a great potential for cellulose isolation due to its high concentration through acid hydrolysis. Despite a relative absence of demonstrated metabolic effects of

insoluble cereal fiber such as rice husk, it has been found to be associated with protection in both coronary heart disease and diabetic patient since the insoluble fibers reduce the rate of glucose absorption by the use of low glycemic index foods as shown in cohort studies [36,37]. Researchers believe that the high fiber content of *Oryza Sativa* is what helps in lowering cholesterol [38]. These dietary fibers are used as fillers, diluents and direct compressible excipients in pharmaceutical tablet production for oral route of administration as had stated.

The oral route is considered to be the most convenient for administration to patients. Oral administration of conventional dosage forms normally dissolve in the stomach or intestinal fluids and get absorbed from these regions of the gastrointestinal tract depending on the physicochemical properties of the drugs. Serious problems arise in conditions where localized delivery of the drugs in the colon is required or in conditions where a drug needs to be protected from the hostile environment of the upper gastrointestinal tract (GIT). Dosage forms that deliver drugs into the colon rather than upper GIT confer a number of advantages. Oral delivery of drugs to the colon is valuable in the treatment of colonic diseases whereby high local concentration can be achieved with minimum side effects that occur because of premature drug release of drugs in the upper GIT or unnecessary systemic absorptions. The colon is rich in lymphoid tissues. Colon is attracting interest as a site where poorly absorbed drug molecules may have an improved bioavailability. The colon is considered to have a somewhat less hostile environment with less diversity and intensity of activity than the stomach and small intestine. The colon has a longer reaction time and appears to be highly responsive to agents that enhance absorption of poorly absorbed drugs. The colon is also considered the starting point for the absorption of per orally applied, undigested, unchanged and fully active peptide drugs. As the large intestine is relatively free of peptidases, such special delivery systems will have a fair chance to get their drug sufficiently absorbed after per oral application. The simplest ways for targeting of drugs to the colon is to obtain slower release rates or longer release periods by the application of thicker layers of conventional enteric coatings or extremely slow releasing controlled matrices. The matrices could be formed using soluble or insoluble polymers which include microcrystalline cellulose (MCC), natural cellulose, Kollidon® VA 64, HPMC and others.

Kollidon® VA 64 is a vinyl pyrrolidone-vinyl acetate copolymer with the two components in a ratio of 6:4. It is white or slightly yellowish, free-flowing powder with a faint characteristic odor with no taste. It is soluble in both aqueous and alcoholic medium, though vinyl acetate is not soluble in water which makes the material more hydrophobic with less brittle films. It is traditionally used in the pharmaceutical industries as a dry binder, crystallization inhibitor, granulating agents, and as a film forming agents [39]. Kollidon® VA 64 is a hygroscopic substance [40,41]. Hygroscopic can be an advantage or a disadvantage, depending on the application. It becomes a disadvantage when used in film-coating of tablets but an advantage when used as a binder or adhesive since in direct compression, the moisture content of the tableting powder is important, though, under normal conditions, the usual residual quantity of water in Kollidon® VA 64 is adequate to provide binding effect between the powder particles. It has a plasticity property which gives it, its binding characteristic [42]. Kollidon® VA 64 is regarded as generally recognized as Safe/Self-Affirmed (GRAS/ SA status) by the FDA for use in food and nutritional supplements.

The major goal of any drug delivery system is to supply a therapeutic amount of drug to a target site in the body so that the desired drug concentration can be achieved swiftly and then maintained as obtained with colon targeted drug delivery. Targeted drug delivery implies selective and effective localization of drug into the target at therapeutic

concentrations with limited access to non-targeted sites. A targeted drug delivery system is preferred in drugs having instability, low solubility and short half-life, a large volume of distribution, poor absorption, low specificity, and low therapeutic index. Targeted drug delivery may provide maximum therapeutic activity by preventing degradation or inactivation of the drug during transit to the target site. Meanwhile, it can also minimize adverse effects because of inappropriate disposition and minimize the toxicity of potent drugs by reducing the dose. An ideal targeted delivery system should be non-toxic, biocompatible, and biodegradable and physicochemical stable *in vivo* and *in vitro*. The colon targeted drug delivery is beneficial for the localized treatment of several colonic diseases such as inflammatory bowel diseases, irritable bowel syndrome, colitis, microbial infections, and colonic cancer, and also has potential to deliver macromolecular drugs orally. Metronidazole is a beneficial drug for the treatment of anaerobic microbial infection associated with the colon.

Metronidazole is a nitroimidazole with high active amoebicidal activity with major activity in the colon diseases [43]. This drug has broad-spectrum cidal activity against anaerobic protozoa, including *Giardia lamblia*, *Entamoeba histolytica*, and *Trichomonas vaginitis* [44,45]. Many anaerobic and microaerophilic bacteria, such as *Bacteria fragilis*, *Fusobacterium*, *Clostridium perfringens*, *Clostridium difficile*, *Helicobacter pylori*, *Campylobacter*, *Peptococci*, *Spirochetes*, and anaerobic *Streptococci* are all sensitive to metronidazole. Statistically, approximately 40-50 million cases of amoebiasis has been recorded with 70,000 to 100, 000 deaths annually due to the infection [46]. Drug resistance may occur in the process of inappropriate usage of the drug. Metronidazole is poorly compressible drug with solubility challenge [45,47]. It is almost completely absorbed from the stomach at pH of 1.2 with only little-unabsorbed fraction of the drug reaching the colon. Metronidazole equally undergoes hepatic metabolism [48,49]. High doses of the drug are associated with GIT and nervous side effects [50]. Therefore, in order to address some of these issues of poor bioavailability in the colon, hepatic metabolism, drug resistance, side effects, poor compressibility, and then enhance quality of life of patient with amoebiasis, metronidazole tablet was prepared using direct compression method with compressible excipients. It was coated with Eudragit® S 100 that ionizes at pH greater than 7 to control the drug release to a targeted site (colon) thereby circumventing drug release in the stomach (pH, 1.2). Therefore, a pharmaceutical formulation designed for colon targeted drug delivery has numerous advantages over conventional drug delivery systems which releases drug at the upper part of the gastrointestinal tract. Though some studies have been done on colonic delivery of metronidazole to treat local diseases in the colon but this study was undertaken to evaluate the potentials of natural α -cellulose extracted from rice husk, formulated with metronidazole by direct compression method using *in vitro* properties since natural raw materials are toxicologically harmless, abundant, recyclable and with low cost when compared to the synthetic counterparts.

MATERIALS AND METHODS

Metronidazole (free gift from Juhel Pharmaceutical Company, Enugu, Nigeria), microcrystalline cellulose (Qualikens, India), Kollidon® VA 64 (free gift from BASF), HCl (Sigma Aldrich, Germany), Aerosil 200 (Orion Pharma, Finland), Sodium hypochlorite (Rickitt and Colman LTD, Nigeria), All other reagents and solvents used were of analytical grade. Rice husks were obtained from Abakaliki, Ebony State, Nigeria and was extracted and processed at Drug Delivery Research Unit, Pharmaceutical Technology and Industrial Pharmacy, UNN.

Extraction and Isolation of Alpha-Cellulose from the Rice Husk

About 10 Kg of the rice husks were obtained from Abakaliki in Ebonyi State milled with a hammer mill (500# grinders, China) into a dry powder. Cellulose was extracted from rice husks by adopting the method of Ohwoavworhua *et al.* [51]. A 450 g of the rice husk powder was treated with 2.25 L of 2% w/v of sodium hydroxide immersed in a water bath set at 100°C for 3 h. The resulting material was further digested with 1.8 L of 17.5% sodium hydroxide solution for 1 h at 80°C. This was thoroughly washed with distilled water, filtered and dried in an oven (Gallenkamp, OV110, Germany) at 60°C for 16 h. Thereafter, the product was bleached with 2.55 L of 3.2% v/v sodium hypochlorite in the stainless steel vessel at 40°C for 2 h. The bleached sample was thoroughly washed with distilled water until the pH was 7.1. The bleached cellulose was delignified and hydrolyzed to alpha cellulose with 2.5 N HCl by boiling at 100°C for 30 min. Then, it was thoroughly washed with distilled water until neutral to litmus, filtered with a muslin cloth and dried at 60°C. The dried alpha cellulose was then characterized and stored in an air-tight amber colored bottle for further studies.

Physical Characterization of the Alpha Cellulose

Percentage yield: The percentage yield of the cellulose was determined by comparing the weight of the cellulose after extraction to the weight of the rice husk before extraction using eqn. (1):

$$\text{Percentage yield} = \frac{\text{Weight of extracted cellulose}}{\text{Weight of powdered rice husk}} \times 100 \quad (1)$$

pH determination: The pH of alpha cellulose was determined by preparing a 1% w/v dispersion of the cellulose in water and shaking for 5 min. Then, the pH determined using a pH meter (pH ep® Hauna, Romania) [52,53].

Swelling index: In determining the swelling index, the method in B.P [54] was adopted. A weighed quantity (1.0 g) of the extracted cellulose was placed in 15 ml plastic centrifuge tubes and the volume occupied was noted. Then, 10 ml of distilled water was incorporated and stoppered. The contents were mixed with a vortex mixer (Vortex Gennie Scientific, USA) for 2 min. The mixture was allowed to stand for 10 min and immediately centrifuged at 1000 rpm for 10 min using a bench centrifuge (GallenKamp, England). The supernatant was carefully decanted and the volume of sediment measured. This procedure was repeated for microcrystalline cellulose as a standard. The swelling indices were determined using eqn. (2):

$$\text{Swelling index} = \frac{V_2}{V_1} \quad (2)$$

Where; V1=Volume occupied by the cellulose prior to hydration and V2=Volume occupied by the cellulose after hydration.

Loss on drying: The moisture content of rice husk cellulose was determined using a method specified in the B.P [54] for acacia. A weighed quantity of each sample was transferred into a petri dish and then dried in an oven at 105°C until a constant weight was obtained. The percent moisture content was determined as the ratio of the weight of moisture loss to the weight of the sample expressed as a percentage using relationship 3.

$$\text{Percentage moisture content} = \frac{\text{Amount of moisture loss}}{\text{Amount of original sample}} \times 100 \quad (3)$$

Fourier transform infrared spectroscopy: A 0.4 g of KBr was weighed and incorporated into 0.001 g of the cellulose and both were thoroughly mixed together and molded into a disc. The disc was inserted into the sample compartment of the FTIR spectrophotometer (IRAffinity-1, Shimadzu FTIR-8400, Germany) to determine the IR spectrum. Then the compatibility determined.

Determination of the Flow Properties of the Powder Mixtures of the Formulations

Measurement of bulk and tapped densities: The bulk and tapped densities were determined by weighing 20 g of the cellulose and transferred into a 10 ml measuring cylinder. The volume occupied was noted and recorded as bulk volume and the cylinder was tapped on a hard surface until no further change in volume was observed. The tapped volume was noted. Bulk density and tapped density were calculated using eqns. (4) and (5) [55,56]:

$$\text{Bulk density} = \frac{\text{Weight of sample used}}{\text{Bulk volume}} \quad (4)$$

$$\text{Tapped density} = \frac{\text{Weight of sample used}}{\text{Tapped volume}} \quad (5)$$

Measurement of flow rate and angle of repose: A funnel was clamped on the retort stand at 7.5 cm from the base. A known weight of the mixture (40 g) was gradually placed into the funnel with the orifice of the funnel closed. The orifice was opened and the time taken for the entire powder sample in the funnel to flow out through the orifice was noted and recorded. The height and radius in a centimeter of the powder heap were determined using a meter rule. The flow rate and angle of repose were calculated as shown in eqns. (6) and (7), respectively [57]:

$$\text{Flow rate} = \frac{\text{Mass of powder}}{\text{Time of flow}} \quad (6)$$

Compressibility index and Hausner's quotient: Compressibility index and Hausner's quotient were calculated using eqns. (7) and (8) [58,59]:

$$\text{Carr's Index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100 \quad (7)$$

$$\text{Hausner's quotient} = \frac{\text{Tapped density}}{\text{Bulk density}} \times 100 \quad (8)$$

Formulation of Metronidazole tablets containing alpha cellulose, methylcellulose and Kollidon® VA 64: The tablets were prepared by direct compression technique using the single punch tableting machine (Manesty Type F3, England) with 200 mg of metronidazole incorporated as a marker in each tablet weight (Table 1). And seven batches were produced and coded as batch A-G.

Table 1. Formulae of Metronidazole tablets

Batch	MT (mg)	α -C (mg)	MCC (mg)	KV (mg)	MS (%)	A (%)
A	200	#####	-	-	-	0.3
B	200	#####	-	-	10	0.3
C	200	99.4	99.4	-	-	0.3

D	200	66.3	66.3	66.3	-	0.3
E	200	99.4	-	99.4	-	0.3
F	200	-	198.8	-	-	0.3
G	200	-	-	#####	-	0.3

MT: Metronidazole, α -C: Alpha Cellulose, MCC: Microcrystalline Cellulose, KV: Kollidon® VA 64, MS: Maize Starch, A: Aerosil.

Coating of Tablets

Each batch of the compressed tablets was divided into two and one half coated to produce batches CA-CG. The coating solution was prepared by dissolving Eudragit® S 100 in acetone at 10% w/v concentration. Approximately 40 tablets from each batch were coated by using the dip method [60]. The coated tablets were dried in the oven (Gallenkamp, OV110, Germany) set at 50°C for 15 min.

Evaluation of Coated and Uncoated Metronidazole Tablets

Physical and mechanical properties: Weight uniformity evaluation test of the tablets was determined by randomly selecting a total of 20 tablets from each batch of the coated and uncoated tablets. The electronic balance (OHAUS, Adventurer, China) was used to weigh the tablets together and the mean weight calculated B.P (2009). The individual weights of the tablets were determined and recorded accordingly and percent deviation obtained.

Tablet thickness and diameter were determined by using vernier caliper (G. T. Tools, Japan), while the hardness or crushing strength test of the tablets was determined using Monsanto hardness tester (Erweka TBH 2B, Frankfurt, Germany) and the average crushing strength obtained in kgF.

Friability test of the tablets was obtained by selecting 10 tablets which were dedusted and weighed (W_0). Then, the dedusted tablets placed in a Friabilator (Eweka Germany) and rotated at 25 rpm for 4 min after which the tablets were removed from the chamber, dedusted and re-weighed (W). The percentage friability was calculated using eqn. (9), while the tensile strength of the tablets was calculated using Newton eqn. (10) [61]:

$$Friability(\%) = 100 \frac{1-w}{w_0} \quad (9)$$

$$T_s = \frac{2P}{\pi dt} \quad (10)$$

Where P is the applied force or pressure (crushing strength), d is diameter and t is the thickness of the tablets. The equation defined tensile strength as being proportional to the diameter and thickness of the tablets.

Disintegration time test: A total of 6 tablets were selected at random from each batch according to the BP [62]. The disintegration medium was simulated gastrointestinal fluid (SGF, pH 1.2) maintained at 37°C in Erweka Disintegration Unit (Type ZT4, Germany). One tablet was placed in each tube in one disintegration unit. The time taken for each tablet to completely break down to particles and pass through the wire mesh was recorded as a disintegration time of the tablet.

Assay of Metronidazole content of the tablet: A standard stock solution was prepared by dissolving 100 mg of metronidazole in 100 ml of simulated gastrointestinal fluid (SGF) pH 1.2 and phosphate buffer (pH of 6.8 and 7.4). A 1 ml of the stock solution was further diluted to 100 ml with the same medium. Aliquots of the solution were

further diluted to obtain 1, 2, 3, 4, 5, 6, 7, 8, 9, mg%. The absorbance readings were determined at predetermined the wavelength of maximum absorption of 276 nm using a UV spectrophotometer, using SGF (pH, 1.2) as the blank. The absorbance values were plotted against the concentrations and the slope of the graph was determined.

In drug content, a total of 10 tablets weighed together and powdered. An amount of powder equivalent to 200 mg of metronidazole was weighed accurately in analytical balance and dissolved in 100 ml of SGF (pH, 1.2). The solution was shaken and filtered. A 1 ml of the above medium was pipetted and made up to 100 ml. The absorbance was measured at 276 nm. The amount of drug in the sample was calculated with reference to the calibration plot. The percentage drug content was calculated using eqn. (11):

$$\text{Percentage drug content(\%)} = \frac{\text{Obtained amount}}{\text{Label claim}} \times 100 \quad (11)$$

***In vitro* release studies:** The *in vitro* release study of metronidazole was carried out using 900 ml of freshly prepared phosphate buffer (pH, 6.8), maintained at $37 \pm 0.5^\circ\text{C}$ and stirring rate of 100 rpm. The tablet was placed inside the dissolution vessel. At different time intervals of 2, 5, 10, 15, 20, 25, 30, 40, 50 and 60 min, a 5 ml sample was withdrawn for the uncoated batches, while for the coated batches the same procedure was followed using SGF first as the dissolution medium for 2 h. In SGF, two hours was chosen to mimic the average gastric emptying time [63]. After 2 h, the dissolution medium was replaced with 900 ml of phosphate buffer, pH 6.8 and dissolution allowed to run for 3 h to simulate average intestinal transit time [64]. After this, the medium was discarded and replaced with a third medium of phosphate buffer pH 7.4 to mimic the ileocecal pH for 7 h [65]. At time intervals of 1, 2, 3, 4, 5, 6, 7 h, a 5 ml sample was withdrawn and replaced with equal volume. The samples were filtered and diluted with a suitable medium. Concentrations of the metronidazole was determined using UV spectrophotometer (Spectrumlab, 752s, Netherlands) at 276 nm with reference to the calibration plot and the same process carried out for all the batches of the tablets. The percentage drug released from each medium was calculated using eqn. (12):

$$\text{Percentage drug released(\%)} = \frac{\text{Amount release}}{\text{Actual drug content}} \times 100 \quad (12)$$

Mechanism of Drug Release

The dissolution data for the tablets were also analyzed to determine the *in vitro* kinetics of release by fitting the dissolution data using linear regression analysis. Four kinetic models including the zero-order release equation, first order equation, Higuchi square root equation, and Korsmeyer-Peppas equation were used:

$$Q_t = K_0 t \quad (13)$$

$$Q_t = \ln Q_0 - K_1 t \quad (14)$$

$$Q_t = K_H \cdot S \sqrt{t} \quad (15)$$

where Q is the amount of drug released in time t, Q_0 is the initial amount of drug in the tablets, K_0 , K_1 , and K_H are the rate constants of zero order, first order and Higuchi rate equations, respectively;

$$M_t/M_\infty = K t^n \quad (16)$$

Where M_t is the amount of drug released at time t , M_∞ is the amount of drug released at the time ∞ , n is the diffusional exponent indicative of the mechanism of drug release, K is the power law constant, M_t/M_0 is the fraction of the drug released. A value of $n=0.5$ indicates Case-I (Fickian) diffusion or square root of time kinetics, $0.5 < n < 1$ anomalous (non-Fickian) diffusion, $n=1$ Case-II transport and $n > 1$ Super Case-II transport [66-70].

***In vitro* Antimicrobial Activity of Metronidazole**

A stock solution of metronidazole was prepared to obtain 1 mg/ml. From the stock solution, serial dilutions of the stock solution were made from 200 mcg to 0.8 mcg/ml. Approximately 3 ml each were put into 10 test tubes. Each of the test tubes was inoculated with about a drop of *Entamoeba histolytica* strain. Extra one test tube containing only the culture medium was prepared and kept to serve as the control. The 11 test tubes were then incubated for 48 h at 37 ° C. After incubation, the test tubes were observed for the presence of growth checking the turbidity.

Statistical Analysis

Data were analyzed using SPSS Version 16.0 (SPSS Inc. Chicago, IL, US) and one-way analysis of variance (ANOVA). Values were expressed as mean \pm SD (standard deviation). Differences between means were assessed by a two-tailed student's T-test and $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Properties and Characterization of the Alpha Cellulose

The extracted α -cellulose had a percent yield of 17.11% and was observed to be white, bitter, odorless, brittle, and insoluble in an aqueous medium. The obtained pH was 7.0 which are a neutral pH, and good for drug formulation, while the moisture content was 13%. Cellulose has been identified as a hygroscopic compound even though it forms a stable material due to the presence of hydroxyl groups on the chains and relatively large surface to volume ratio of microfibrils as a result of their small sizes [71,72]. This sorption mechanism adsorbs water molecules tightly to accessible hydroxyl groups of anhydroglucose units, followed by a second less tightly bound layer; more additional layers of water molecules. It has been proposed that water only adsorbs in the amorphous areas of cellulose with higher hydrophilicity [7,73].

A research has reported that the total amount of sorbed water is proportional to the fraction of amorphous part and though independent of the surface area [74]. This moisture content has been found to determine the compaction properties, tensile strength and viscoelastic properties [72,74,75]. The moisture within the pores of cellulose may play the roles of internal lubricant, reduce frictional forces, and facilitate slippage and plastic flow within the individual microcrystals [76,77]. It has been reported that the lubricating properties obtained from water can equally reduce tablet density variation by providing a better transmission of the compression force through the compact and by reducing the adhesion of the tablet to the die wall [77,78]. Since the compressibility of powder depends on its moisture content, powder material compressed under the same pressure may not result in the same compact porosity. Therefore, the compaction pressure required to produce a compact at a certain porosity (or solid fraction) decreases with increasing moisture content. Presence of water may reduce intermolecular forces of materials but due to its

plasticizing effect and its positive effect on bonding surface area, tablet ability remains constant or increases for moisture contents between approximately 3 to 5%. Some reports also proposed a transition from the glassy state to the rubbery state of microcrystalline [72,74].

Its swelling capacity was found to be 11% when compared to microcrystalline cellulose that gave 12.4%. This indicated that the extracted alpha cellulose is comparable to MCC without any significant variation in the swelling capacity. Some authors proposed that swelling observed in cellulose are termed ballooning phenomenon, which takes place at certain regions of the cellulose fibers [79-81]. The ballooning phenomenon was explained in most cellulose fibers, that the micro fibrils of the secondary wall are aligned in a helical manner with respect to the long axis of the fiber which brings about the swelling mainly in a transverse direction other than lengthwise [82].

This ballooning phenomenon was observed to depend on nature and quality of the solvent in use, and structural origins, which means that it can be related to the morphological variations within the different walls [83].

Fourier Transform Infrared Spectroscopy (FTIR)

The result of FTIR spectra of the API, excipients and the formulations (Figures 1 and 2) showed that excipients have their peak spectra bands at 1381.64, 3512.36, 2740.36, 2242.42, and 1690.44 cm^{-1} which represented the presence of methyl (-CH₃), H-bonded OH stretch, carbonyl (C=O), and 2° amine for microcrystalline cellulose, extracted α -cellulose, Aerosil, Kollidon® VA 64 and Maize starch, respectively. The presence of methyl (-CH₃), carbonyl (C=O), alkyne (C≡C), aldehyde (CHO), alkenes (C=C) and H-bonded OH stretch were detected respectively at the peaks of 1470.42, 1783.08, 2180.66, 2771.24, 3045.30, 3473.76 cm^{-1} for extracted α -cellulose; 1474.28, 1721.32, 2149.78, 2798.26, 3053.02, 3303.22, cm^{-1} for MCC and 1443.40, 1783.08, 2122.76, 2736.50, 3172.60, 3354.10 cm^{-1} for Kollidon® VA 64. This is an indication that the standard cellulose is analog to both the extracted α -cellulose and Kollidon® VA 64 since the major functional groups were equally present. It should be noted that the good binding sites of cellulose with coating property, were attributed to the presences of free hydroxyl groups on their surfaces which enhanced film adhesion and mechanical strength [84,85]. On this note, the extracted α -cellulose could be used as a coating material, though in contrast, it has been reported that a lubricated MCC lacks the ability to create clean and uncontaminated surfaces during compression which may negatively affect film adhesion [86,87].

It has been reported that metronidazole exhibited peaks using FTIR spectroscopy at 3230, 3105, 1538 and 1375, 1078, and 830 cm^{-1} which were assigned to -OH, -C-CH, -N-O, -C-O, and -C-N, while the observed peaks include 3292.34, 3199.70, 1532.18 and 1366.20, 1011.08 and 825.80 cm^{-1} with the same functional groups respectively. It showed a characteristic absorption peaks at 1258.12, 1690.44, 3446.74, 1690.44, 2987.4, 3188.12 and 2987.4 cm^{-1} representing the presence of C-C stretch, 2° amine, H-bonded OH stretch, C=C stretch, alkyl CH stretch, C=C stretch, and alkyl CH stretch respectively for batches A–G. From the FTIR plots (Figure 2), it was generally observed that the characteristics fingerprints of the functional groups present in the materials were equally identified in the formulations. This showed that there was no interaction between the active ingredient and the excipients used in the formulations and thus pure and compatible.

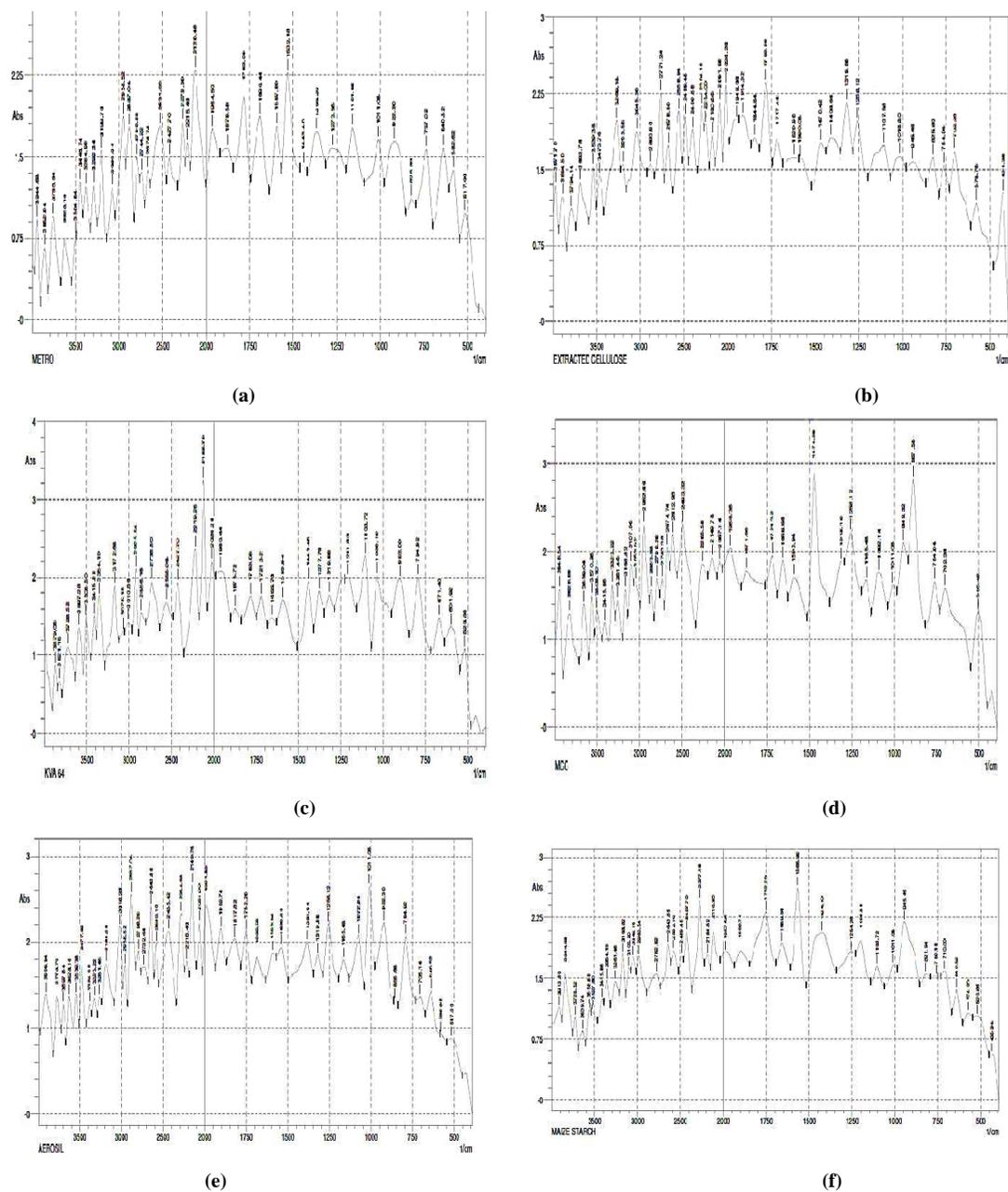
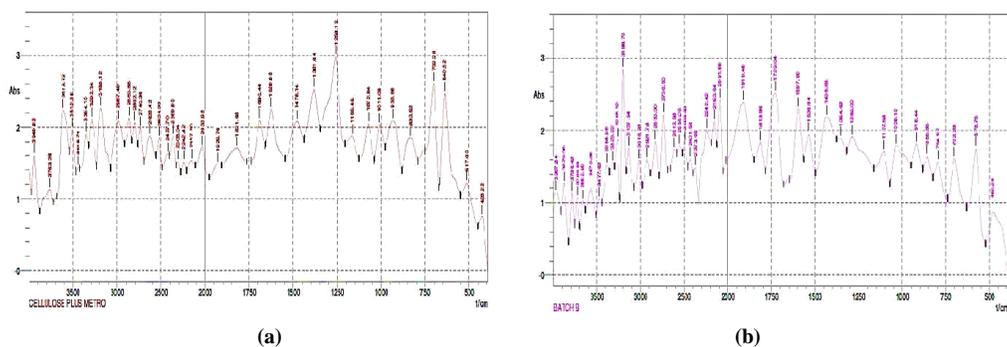


Figure 1. FTIR of the API and excipients. Where a: (metronidazole); b: (α -cellulose); c: (Kollidon® VA 64); d: (microcrystalline cellulose); e: (Aerosil); f (maize starch)



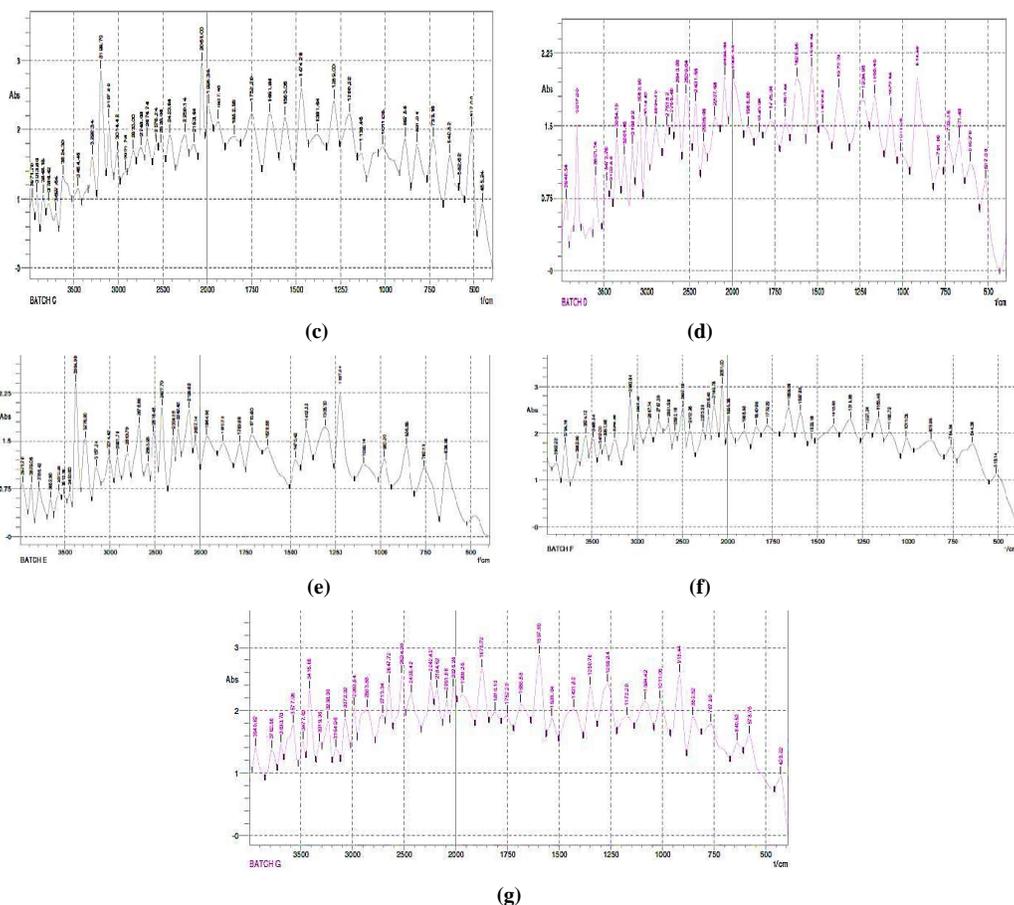


Figure 2. FTIR of the batches A-G. Where a: (Batch A); b: (Batch B); c: (Batch C); d: (Batch D); e: (Batch E); f: (Batch F); g: (Batch G)

Flow Properties of the Powdered Formulations

Flow properties determination of powders is important before going into tableting because variation in particle flow will automatically lead to variation in both tablet weight and active ingredient. Bulk density, particle size, shape, surface area and some other material properties affect powder flow property, while identification of the factor that influences the flow property is not easy since the factors are always interdependent [88]. While friction and cohesion are the two fundamental forces that can influence powder flow [77,89]. The extracted α -cellulose was observed to have a poor flow property, so also MCC (Avicel[®] 102) which may be due to the particle size. In order to enhance the flow property, all the batches were incorporation with a 2% Aerosil powder, a glidant. The results of the flow properties of the powder mixtures are presented in Table 2. The obtained result indicated that the flow properties of the powders mixtures were improved. The angle of repose of $\leq 30^\circ$ indicated good flowability, while poor flowing materials are $\geq 40^\circ$ [90,91]. From the result (Table 2) it showed that the batches confirm to the specification except for batches C and F. Batch A which contained extracted α -cellulose had the best and significant ($p < 0.05$) flow property than others since its Hausner's quotient (H.Q), compressibility index (C.I) and angle of repose values were 1.23, 18.24%, and 25° respectively, while the flow rate had less resistance to flow as shown in Table 2. Hausner's quotient within \leq or ≥ 0.23 and suggests good or poor flow, respectively, while compressibility index values of 5-10, 12-16, 18-21, and 23-28 indicate excellent, good, fair, and poor flow properties of the material, respectively [55,92]. The H.Q (1.46) and C.I (31.34%) of batch F (MCC) was highest among the batches, whereas other batches

containing mixture of α -cellulose and MCC had fewer values than batch F. This showed that the presence of α -cellulose improved the flow properties which may be related to the particle size since finer the particles less likely to flow. A similar C.I result had been highlighted [93]. Therefore, if the Hausner's quotient and compressibility index of the powders are within the required range, the powder will flow at the minimum bulk density and consolidates to maximum density inside the die, prior to compression [94]. A high bulk density (low porosity) will result in a low deformation potential and there will be lack of space for deformation during compression which causes less intimate contact between the particles within the tablets, thereby producing weaker tablets [89,94]. The batches had low bulk and tapped densities in the range of 0.40 ± 0.22 - 0.82 ± 0.12 mg/ml. Bulk and tapped densities of batch A (α -cellulose) were slightly lower than other batches. Hence, low bulk and tapped densities obtained signified good properties required for the production of good quality tablets. A direct compressible excipient must have improvement in compressibility as they are plastically deforming materials, which result in improved tablet ability due to the increased bonding surface area [95]. In materials with low densities, the higher roughness may contribute to their particles interlocking [96]. Reports have proposed that MCC densified by pre-processes (granulation or drying) is typically less tablet able than the original more porous MCC [97,98]. Therefore, low bulk density will lead to higher dilution potential, improve tablet ability, and however, it could hinder flow ability [99,100].

Table 2. Granule properties of the powder mixture

Parameter	A	B	C	D	E	F	G
Flow rate (g/sec)	0.55	0.62	0.53	0.93	1.01	0.60	1.25
Angle of repose	25.63	34.99	48.74	36.13	37.95	44.42	27.47
Bulk density (g/ml)	0.40	0.44	0.47	0.53	0.59	0.46	0.65
Tapped density (g/ml)	0.49	0.59	0.65	0.67	0.82	0.67	0.82
Compressibility index (%)	18.37	25.42	27.69	20.90	28.05	31.34	20.73
Hausners' quotient	1.23	1.34	1.38	1.26	1.39	1.46	1.26

Physical and Mechanical Tests of Coated and Uncoated Metronidazole Tablets

Direct compression persists to be the most economical technology in the large-scale production of tablets. This technique produces tablets with more stability and faster dissolution and has less wear and tear of punches with simplified validation. Metronidazole is a poorly compressible drug which was formulated by direct compression method using compressible excipients. In the process of pharmaceutical powder compression, particles will undergo rearrangement followed by elastic deformation. Previous work has reported that cellulose undergoes plastic deformation which is independent of their particle sizes [93,101]. The physical and mechanical properties of the compressed metronidazole tablets were tested using weight uniformity, thickness, hardness, friability, and disintegration test as presented in Table 3.

Weight uniformity test is an official test in which tablets formulated in the same batch are expected to maintain uniform weight or fall within a specified range without significant deviation. B.P specification stated that tablets weighing 250 mg and above are expected not vary by 5%. In line with B.P, the result showed that all the batches were within the specified range [62] since not more than two tablets out of the twenty tablets deviate by 5% as shown in Table 3.

In the thickness of the tablets, the thickness of the uncoated and coated ranged between 0.520 ± 0.01 - 1.030 ± 0.02 and 0.546 ± 0.05 - 1.091 ± 0.05 , respectively (Table 3). Batches C and F (uncoated) were found to have the highest thickness, while batch CF (7.79 ± 0.31) was the highest from the coated batch at a significant level ($p < 0.05$) than others. It was observed that tablets formulated with extracted α -cellulose (batch A and CA) had significant low tablet weight and thickness ($p < 0.05$) which may be presumed to be as a result of high plasticity and mechanical interlocking nature of the material. This indicated that during compression, plasticity deforms and increased the area of interparticle bonding [102], which enhanced the binding quality. As it has been reported that porous agglomerates deform on a macro scale due to the presence of slip planes and microcrystalline cellulose dislocates on a microscale [4,103]. Also, the α -cellulose had a good mechanical interlocking property of irregularly shaped and elongated particles which improved the tableting property. This is consistent with some reported researches that stated good mechanical interlocking property of microcrystalline cellulose [75]. Furthermore, it means that α -cellulose had more brittleness than MCC since its compression behavior requires higher compression forces for deformation to occur which resulted in the production of tablets with lower weight and thickness (Table 3).

The results of the crushing test of the batches are shown in Table 3. Crushing strength test of the tablets was within the B.P [62,97] acceptable specification of ≥ 5 kgF. From the result, it could be seen that all the batches possessed good crushing strength, while batch F from the uncoated and coated batch (CF) having the highest hardness of 7.79 ± 0.31 and 7.95 ± 0.33 , respectively though not significant from batches A and CA (uncoated and coated batches with α -cellulose) and batches C and CC (uncoated and coated batches with combination of α -cellulose and MCC), respectively. It has been highlighted that the presence of hydrogen groups on adjacent cellulose molecules led to the formation of multiple hydrogen bonds which provides the mechanical strength and cohesiveness of tablets even with the application of low pressure [104]. The tensile strength of the uncoated and coated tablets was obtained in the range of 23.51 ± 0.11 - 46.04 ± 0.21 MPa and 25.43 ± 0.13 - 51.31 ± 0.22 MPa, respectively. Batches A and CA (uncoated and coated) had a significant low ($p < 0.05$) tensile strength than others. This indicated that the extracted α -cellulose had higher brittleness, low ductility, and low elasticity than MCC with highest crushing strength and tensile strength since low brittleness, high ductility and elasticity are prerequisites for the formation of strong tablets. This has been reported by previous works [105,106].

Friability test measures the resistance of tablet to abrasion. Friability test results obtained from the batches as presented in Table 3 indicated that the uncoated and coated batches were significantly $\leq 1\%$ ($p < 0.05$), where the coated batches had almost 0% friability which is expected for a coated tablet due to added mechanical strength provided by the film coat. Therefore, it could be inferred that all the direct compressible binders (α -cellulose, microcrystalline cellulose and Kollidon® VA 64) employed in this research have enough plasticity which provides less susceptibility to the capping of the tablets, less brittleness and even less tendency to sticking to the punches of

the tableting machine. A tablet batch is rejected if any tablet caps, laminates, or breaks upon the course of the test or the percent friability values of 0.8-1.0% frequently quoted as the upper level of acceptance of pharmaceutical products are exceeded [107]. This implied that these formulations can withstand handling, packaging, and transportation without cracking the coat [108].

The results of disintegration time of uncoated batches include 14.20 ± 1.55 , 5.05 ± 2.43 , 11.13 ± 1.10 , 14.76 ± 2.40 , 12.68 ± 1.22 , 16.19 ± 2.43 , and 10.83 ± 1.13 min, respectively for batches A-G. Disintegration time is the time taken for the tablet to reduce completely to smaller particles. This time is very important as it is a rate-limiting step for dissolution to take place. Conventional tablets (uncoated) are expected to disintegrate within 15 min and from the result, all the batches passed the test except batch F which contains MCC (16.19 ± 2.43) and may be due to higher mechanical strength (7.79 ± 0.31 KgF) of that formulation since its porosity was slightly above the extracted α -cellulose. However, it was reported that MCC densified through an extrusion process tends to disintegrate very slowly in the absence of a superdisintegrant or a pore former [109]. Also, high compaction pressure has been reported to increase disintegration time since water penetration into the tablets decreases [73,88]. Batch B (contains MTZ+ α -cellulose+maize starch) had a significant low disintegration time of 5.05 ± 2.43 ($p < 0.05$) than others which may be due to the presence of a disintegrant, maize starch in that batch since maize starch, a disintegrant had created a hydro-channel which decreases the disintegration time thereby disentangling its particles. All the extracted α -cellulose batches had their disintegration time within the acceptable range which may be as a result of its porosity level since porosity enhanced disintegration and swelling as water penetrates tablet matrix by means of the capillary action of the pores with a subsequent disruption of the hydrogen bonds. Moreover, the faster disintegration time may also be related to the faster breaking of fewer hydrogen bonds formed during the compression process unlike in MCC that had higher hydrogen bond during the plastic deformation period. This is in agreement with a previous report [110,111].

Drug Content of Uncoated and Coated Batches of Metronidazole Tablets

The drug contents of the uncoated and coated batches were found to be in the ranges of 92.21 ± 0.11 - $98.44 \pm 0.21\%$ and 90.14 ± 0.55 - $95.61 \pm 0.18\%$, respectively (Table 3). In uncoated batch, A and G had the highest and equal drug content of 98%, while 96% was the highest drug content from batch CG (coated batch). In the assay of metronidazole content, the acceptable B.P limit for absolute drug content is 90-110% [62]. The result obtained showed that all the batches passed the test as the percentage drug contents were within the acceptable range.

Table 3. Evaluation properties of uncoated and coated tablets

Batch	WU (mg)*	T (mm)*	CS* (kgF)	FB (%)*	D.C (%)*
A	292.7 ± 16.9	0.520 ± 0.01	7.68 ± 1.04	0.420 ± 0.04	98.58
B	383.7 ± 7.9	1.024 ± 0.01	6.65 ± 1.36	1.030 ± 0.13	96.89
C	382.95 ± 4.3	1.030 ± 0.02	7.51 ± 0.79	0.420 ± 0.05	92.21
D	395.85 ± 4.7	1.027 ± 0.01	6.38 ± 0.76	1.041 ± 0.11	93.77
E	366.2 ± 10.8	1.025 ± 0.02	6.13 ± 0.51	1.090 ± 1.34	90.66
F	383.4 ± 4.9	1.029 ± 0.02	7.79 ± 0.31	0.250 ± 0.01	97.98
G	362.3 ± 6.7	1.028 ± 0.02	6.36 ± 0.42	0.320 ± 0.32	98.44

CA	338.1 ± 18.1	0.546 ± 0.05	7.88 ± 1.05	0.003 ± 0.01	91.21
CB	443.1 ± 8.5	1.075 ± 0.03	6.78 ± 1.36	0.000 ± 0.00	90.61
CC	442.3 ± 4.6	1.082 ± 0.05	7.76 ± 0.80	0.002 ± 0.01	90.1
CD	457.3 ± 5.0	1.078 ± 0.05	6.51 ± 0.77	0.000 ± 0.00	90.91
CE	424.1 ± 11.6	1.066 ± 0.05	6.25 ± 0.52	0.000 ± 0.00	90.14
CF	442.85 ± 5.3	1.091 ± 0.05	7.95 ± 0.33	0.002 ± 0.01	91.7
CG	418.45 ± 7.2	1.079 ± 0.05	6.49 ± 0.42	0.000 ± 0.00	95.61

Mean ± SD, n=3; Wu (Weight uniformity); T (thickness); CS (crushing strength); FB (friability); D.C (disintegration test).

***In vitro* drug release of metronidazole coated and uncoated tablets:** The results of the *in vitro* drug release of metronidazole tablets studied in simulated gastric fluid (pH, 1.2), and simulated intestinal fluid (pH, 6.8) with uncoated batches, while for coated batches, phosphate buffer (pH, 7.4) was included to the former two fluids as presented in Figures 3-5. The *in vitro* drug release of all the uncoated batches showed gradual drug release except burst effects observed in batch G (metronidazole and Kollidon® VA 64) in SGF (pH, 1.2) with significant variation ($p < 0.05$) in the T40 and T75 (time to release 40 and 75% of the drug) of 5 and 25 min respectively as shown in Table 4. Burst release is rapid drug release where 15-30% of the drug is released within the first hour which is undesirable since it can have adverse pharmacological effects and can make the delivery systems to be economically ineffective [110]. This burst effect may be due to a higher affinity of Kollidon® VA 64 to hydrophilic and hydrophobic surfaces which enhanced the faster dissolution of the drug. Due to the good solubility of Kollidon® VA 64, drugs normally dissolve rapidly whether in gastric or intestinal juice [112], which is frequently relatively independent of the compression force employed during tableting. This result is consistent with a report of a previous finding [112]. Also, Kollidon® VA 64 has been found to improve the solubility of many drugs such as griseofulvin [112]. Whereas, a significant ($p < 0.05$) higher T40 of about 20 min was observed in the uncoated batches C and F in SGF (pH, 1.2) which may be as a result of higher crushing strength (7.79 ± 0.31 kgF) and tensile strength (46.04 ± 0.21 MPa) of the formulation exhibited by the component (MCC). In SIF (pH, 6.8), all the batches of the uncoated batches had significant prolonged T75 ($p < 0.05$) with only batch E and G to release up to 75% of the drug content which may be due to solubility issue of the drug in medium since such was not encountered during dissolution in the SGF (pH, 1.2). This is in agreement with a report that metronidazole had more solubility affinity in SGF (pH, 1.2) [113]. As a result of this, the batches were coated and targeted to the colon for the effective management of the colon related disease. The *in vitro* drug release of the coated batches had T40 and T80 (time to release 40 and 80% of the drug, respectively) between the ranges of 4-5 h and 6-7 h, respectively. In SGF, there was almost no observable drug release in all the batches except in batch A that had <10% which will not benefit colon targeted drug delivery. The maximum drug release (75.91 ± 0.19 - $91.21 \pm 0.22\%$) in all the batches were observed in the phosphate buffer (pH, 7.4) which is demonstrating colon pH showing that the more drugs will be available for effective therapeutic achievement in the colon. The batches did not comply with BP specifications which stated that at least 80% of drug release should occur within 60 min of dissolution rate test [114] which may be attributed to the gelling property of coating polymer and its suitability in controlling drug release for colon targeted formulation.

Finally, it is noteworthy that the fast disintegration time observed in batch B had no significant variation in the dissolution rate of the batch.

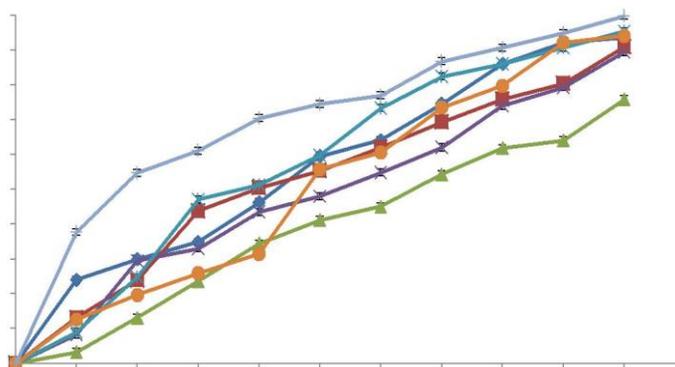


Figure 3. A graph of % drug release against time of all the batches of metronidazole tablets in simulated gastrointestinal fluid (SGF)

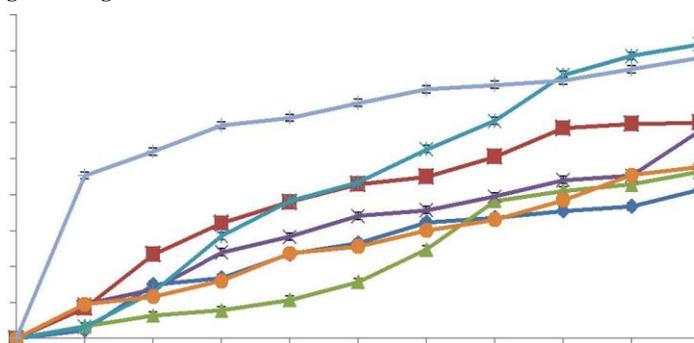


Figure 4. A graph of % release against time of all the uncoated batches of metronidazole tablets in simulated intestinal fluid (SIF)

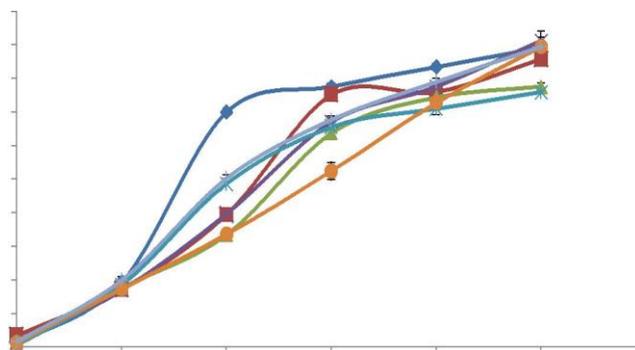


Figure 5. The dissolution/ release study results of the metronidazole coated batches. Where, CA, CB, CC, CD, CE, CF and CG are for coated batches, respectively

Table 4. Release profiles of the metronidazole tablets

Batch	Uncoated tablets				Coated	
	(SGF)		(SIF)		T40 (h)	T80 (h)
	T40	T75	T40 (min)	T75 (min)		
A	15	40	60	-	4	6
B	10	40	20	-	5	7
C	20	60	40	-	5	-
D	15	50	40	-	5	7

E	10	30	20	50	4	-
F	20	40	50	-	5	7
G	5	25	22	60	4	7

Kinetics and Mechanism of Metronidazole Release from the Compressed Tablets

The results of the *in vitro* release kinetics studied with four models are presented in (Figures 6-9) and showed that all the batches significantly ($p < 0.05$) followed the zero order plots since the regression analysis ($R^2 > 0.9$) were obtained and highly linear. The first order plots of the batches also exhibited good linearity since the $R^2 > 0.9$ except in batches CD and CF. This showed that batches CA-CC, CE, and CG followed mixed order drug release which may be due to control release pattern. Higuchi and Peppas' plots had $R^2 > 0.9$ in all the batches, where the n values of the Peppas' plot were ≥ 0.85 , a case II transport (zero order). This indicated that case II Fickian diffusion predominated. Therefore, metronidazole tablets formulated with α -cellulose and others predominately followed zero order which indicated that the drug release rate is independent of time.

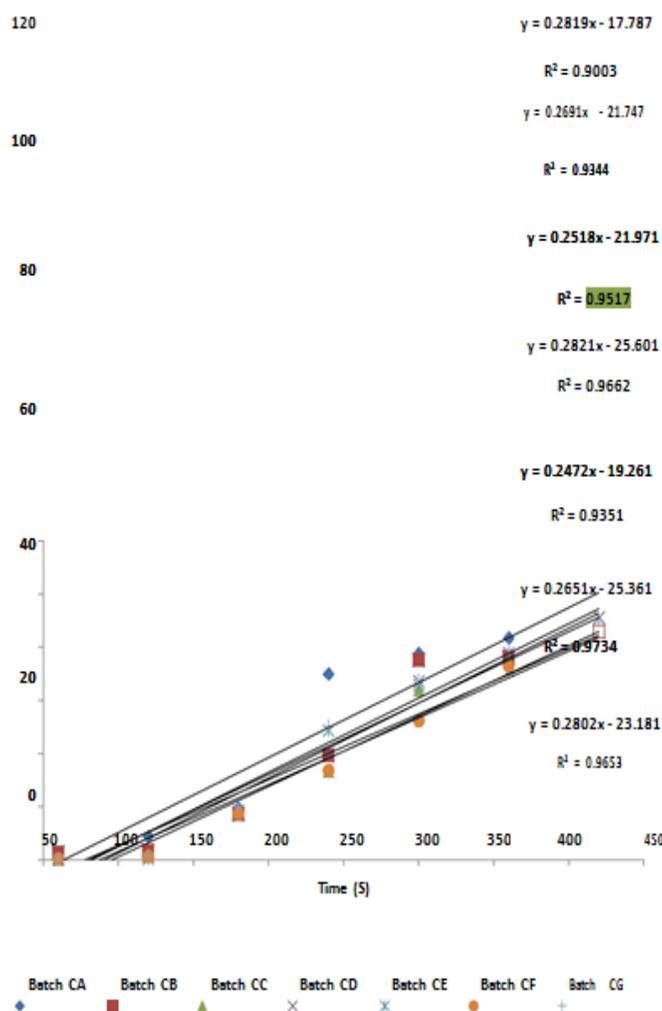


Figure 6. Zero order model for metronidazole tablets

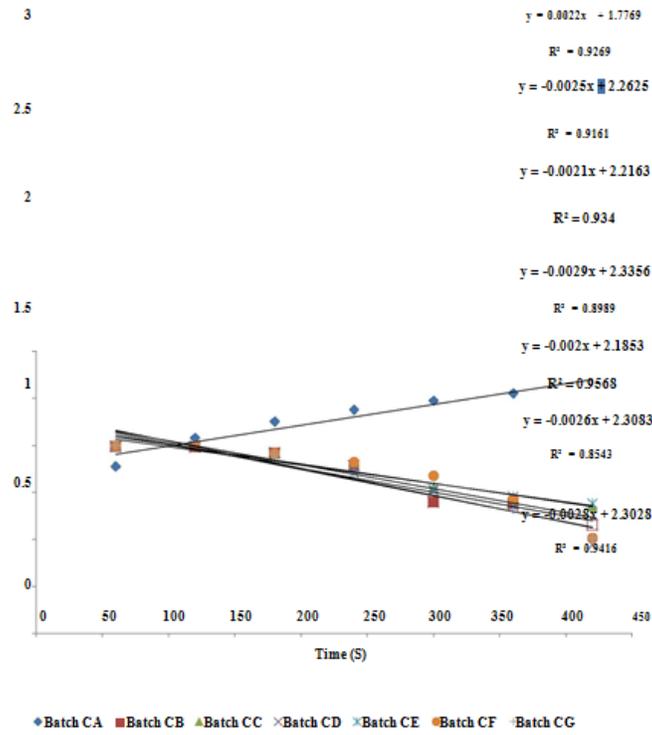


Figure 7. First order model for metronidazole tablets

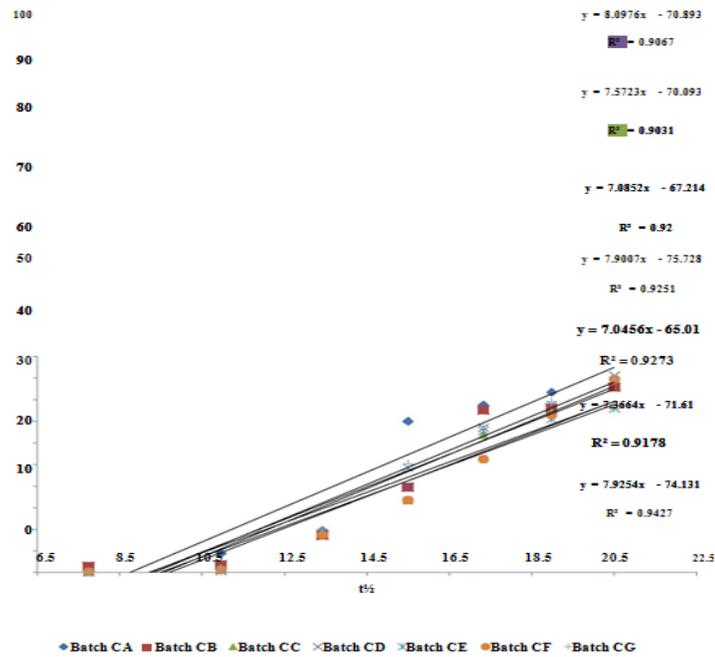


Figure 8. Higuchi model for metronidazole tablets

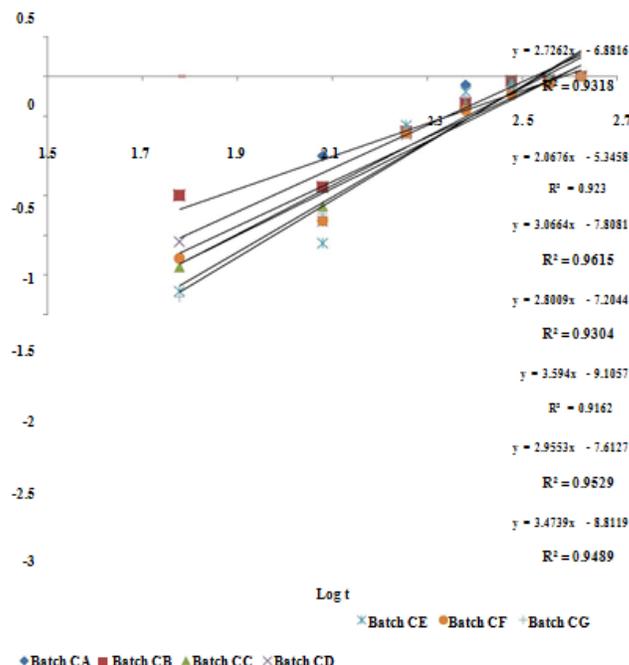


Figure 9: Pepper’s model for metronidazole tablets

In Vivo* Activity of Metronidazole (Minimum Inhibitory Concentration) against *Entamoeba histolytica

Minimum inhibitory concentration (MIC) is the lowest concentration of a drug that will inhibit the visible growth of an organism after an incubation period. The result of the MIC presented in Table 5 showed that the minimum inhibitory concentration of the drug is around 25 µg/ml against *Entamoeba histolytica* (T4). This finding was in agreement with a report that the MIC ranged from 12.5-25 µm [115]. There was an absence of microbial growth in T1-4 due to a higher concentration of the drug. There was a significant difference (p<0.05) between the test tubes (T5-10) that had growth when compared to the control (T) which had on the culture medium. This showed that even below the MIC the metronidazole tablet still had antimicrobial inhibition significantly (p<0.05) higher than the control. This showed that the formulation did not inhibit the activity of the drug.

Table 5. Minimum inhibitory concentration of metronidazole against *Entamoeba histolytica*

Concentrations (µg/ml)	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T
200	-										
100		-									
50			-								
25				-							
12.5					+						
6.25						+					
3.125							+				
1.625								+			

0.8										++		
Control	+++++											

KEY: T1-10 are test tubes containing the test solution and the inoculums in their order of decreasing concentrations, T is the control containing only the culture medium.

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