The Effect of Modification Methods on the Properties of Lentinus Tuber Regium Powders

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1. Introduction

The pharmaceutical excipients are constituents of medicine supplementary to the pharmacologically active drug (API) contained within the preparation of a therapeutic prescribed amount of a medicinal product [4, 8]. These are added alongside the major medicinal substance to simplify the usage of the pharmaceutical product by the recipient or the patient especially with the necessary route of application. Excipients are extensively used in drug delivery settings in tablets, capsules, etc. and in several uses such as fillers to bulk up medicinal compositions that comprise very effective API, permitting the suitability and precise drug product composition. Excipients serving as fillers or bulking agents include lactose, microcrystalline cellulose, etc. [31]. The improvement of new excipients for use in making pharmaceutical dosage forms is a necessary idea worth devoting attention to. This challenge is taking newer dimension due to the need for more complex excipients in terms of improved performance/functionality and the need to simplify the development of innovative drug transport set-ups [7]. Some of the current drug products and new technologies for drug manufacture, especially tablets where direct compression methods are applied require a model filler-binder that could be used as an alternative for two or more excipients. It also requires to meet up with the high-speed rate of modern tableting machines which demand excipients to retain good compressibility and low weight disparity even at little residence period of compression. This would also improve the inadequacies of some prevailing tableting additives such as loss of compressibility of microcrystalline cellulose (MCC) upon wet granulation, high moisture sensitivity and poor die filling due to agglomeration, etc. [21]. Much need for improved excipients has led to the consideration of modifying the powder obtained from Lentinus tuber-regium Fr (Polyporaceae) Syn. Pleurotus tuber regium. It is a species of complex mushroom that is fit for human consumption which

ARTICLE INFORMATION

Received: Nov. 22, 2018
Revised: January 29, 2019
Accepted: Feb. 28, 2019
Published online: May 10, 2019

ABSTRACT

The current work considered the influence of methods of modification on the physical characteristics of Lentinus Tuber Regium (LTR) powders. The sclerotia of the LTR was pulverized to 250.0 µm and coded as native Lentinus Tuber Regium (NLTR-A). A 500.0 g of NLTR-A was submerged in 3.5 % w/v sodium hypochlorite and stirred continuously for 30.0 min. The resultant slurry was washed severally with purified water until it was neutral to litmus. The mass was dried in an oven at 60.0 °C for 2.0 h, pulverized (250.0 µm) and was noted as the modified Lentinus Tuber Regium powder (MLTR-B). Another 500.0 g of NLTR-A was extracted with 70.0 % v/v ethanol in a Soxhlet extractor. The resultant powder was dried at 60.0 o C for 2.0 h, micronized (250.0 µm) and coded as the modified Lentinus Tuber Regium powder (MLTR-C). Additional 500.0 g of NLTR-A was submerged in 600.0 mL of 0.5 N sodium hydroxide in a 1.0 L beaker and shaken constantly for 30.0 min. The subsequent material was splashed with purified water until the material was neutral to litmus. The mass was freed from water and introduced into 200.0 mL of 0.5 N hydrochloric acid. It was agitated for 30.0 min in a water bath at 100.0 °C. It was flooded in purified water until it was neutral to litmus. The product was dried to constant weight at 60.0 °C and pulverized (250.0 µm). The product was coded as the modified Lentinus Tuber Regium powder (MLTR-D). Generally, NLTR-A, MLTR-B, MLTR-C and MLTR-D were investigated for their organoleptic, solubility, pH, moisture studies, scanning electron microscopy (SEM), x-ray diffractometry (XRD), flow parameters and densities. The results showed that both the native and the modified powders were insoluble in water and most organic solvents. The pH of the derived powders was consistently higher. SEM and XRD revealed morphological differences in each of the derived powders, though, all the powders were non-crystalline. The respective modification methods brought about an improvement in the hydrophilic and flow properties of the modified powders when compared to the native form of LTR.

DOI: https://doi.org/10.15415/jptrm.2019.71004
propagates easily in the bush mainly in the hot climates of the world [1]. It breeds commonly on dead woods during damp seasons of the year subject to the environmental condition. It has become possible to propagate it in the laboratory setting [17, 6]. Irrespective of the setting of growth, it produces sclerotia that are fundamentally circular measuring up to 30.0 cm in diameter when fully mature [26, 5]. Taxonomically, Lentinus tuber regium fits into the Basidiomycete sub-division /phylum and Agariceae family [20]. In Nigeria, it is named 'ero usu' (Igbo), 'katala' (Hausa) and 'ohu' (Yoruba). Its dietetic significance has made it an extremely wanted food item having medicinal values and serving for research purposes [15, 2]. In southern Nigeria, its cake is deliciously consumed extensively. It is utilized as a soup thickener either alone or in mixtures with melon seed. It is used medicinally for the management of headache, fever, constipation and cancer of the colon [12, 38]. The sclerotum which is ordinarily seen and picked from decomposing woods has a dark brown outward covering while the inner part is off-white in colour. Findings from collected works indicate that LTR contains potassium, calcium, protein, trace quantities of vitamin E, lipid, alkaloids and tannins [3]. Iwuagwu and Onyekweli (2002) conducted preliminary work on the applicability of LTR powder as a tablet disintegrant. Okoye and Onyekweli (2016) documented the use of the powder derived from the LTR for direct compression of tablets. Several other studies have been documented by other researchers on the LTR in pharmaceutical formulations (36, 35, 33, 34). This research is designed to consider the effect of the approaches of modification on the physico-chemical properties of the LTR powders.

2. Experimental

2.1 Materials

The following reagents were used as procured: sodium hypochlorite (Multipro, Nigeria), ethanol, n-hexane (JHD, China)

2.2 Methods

2.2.1 Procurement and Processing of Samples

The spheres (sclerotia) of Lentinus tuber regium (LTR) were procured from the Mile 3 main market, Port Harcourt, Nigeria. It was identified and allotted a specimen code: OG-ACC-001-UPH-C-057 in the University of Port Harcourt central herbarium. The cleaned sclerotia were cut in bits and pulverized (Binatone, Japan) to 250.0 µm and coded as native Lentinus tuber regium powder (NLTR-A). A 500.0 g of NLTR-A was submerged in 3.5 % w/v sodium hypochlorite and stirred continuously for 30.0 min. The resultant slurry was washed severally with distilled water until it was neutral to litmus. The mass was dried-up in an oven (New Life, DHG, England) at 60 °C for 2.0 h and pulverized (250.0 µm). The sample was noted as the modified Lentinus tuber regium powder (MLTR-B). Another 500.0 g of NLTR-A was extracted with 70.0 % v/v ethanol in a Soxhlet extractor. The resultant powder was dried at 60 °C for 2.0 h. The sample was designated as the modified Lentinus tuber regium powder (MLTR-C). Additional 500.0 g of NLTR-A was submerged in 600.0 mL of 0.5 N sodium hydroxide in a 1.0 L beaker and agitated constantly for 30.0 min. The subsequent material was flooded with purified water until it was neutral to litmus. The mass was freed from excess water and introduced into 200.0 mL of 0.5 N hydrochloric acid. It was shaken for 30.0 min in a water bath at 100.0 °C and later flooded with purified water to have it neutral to litmus. It was dried to a constant weight at 60 °C and pulverized (250.0 µm) and coded as the modified Lentinus tuber regium powder (MLTR-D).

2.2.2 Characterization of the native and the improved Lentinus tuber regium powders

The Properties of NLTR-A, MLTR-B, MLTR-C and MLTR-D were evaluated as follows after they were examined for colour, texture, odour and taste.

2.2.3 pH measurement

The pH of a 2.0 % w/v slurry of each of the powders was evaluated with a pH meter (Corning, model 10, England).

2.2.4 Hydration capacity

Utilizing the technique of [19], the hydration capability of each of the powders was determined. A 1.0 g quantity was retained in a 15.0 mL plastic centrifuge tube. A 10.0 mL volume of water was introduced and later agitated. The set-up was additionally shaken once in a while for the next 2.0 h period and kept still for 30.0 min. It was centrifuged at 3000.0 revolutions per minute (rpm) for 10.0 min. The supernatant water was decanted and the mass of the wet powder was determined. The procedure was repeated trice. The hydration capacity was calculated using equation 1 as the mass of the damp powder, x against that of the dry powder, y.

\[ \text{Hydration capacity} = \frac{x}{y} \]  

(1)

2.2.5 Swelling index

The swelling index of the samples were determined using a slight modification of the methods established by [9].
5.0 g of the powders in turn was retained in a 100.0 mL graduated measuring cylinder which was tapped, taking note of the tapped volume as Vx. An 85.0 mL volume of water was used to disperse the powder. The volume was adjusted to 100.0 mL using water. The set-up was kept still for 24 h. The capacity of the deposit formed, Vv, was evaluated. This process was repeated thrice. The swelling index was calculated from equation 2.

\[
\text{Swelling index} = \frac{V_v}{V_x}
\]  

(2)

2.2.6 Moisture absorption test

Saturated solutions of potassium sulphate, potassium chloride, sodium chloride and magnesium nitrate were retained in relative humidity flasks to simulate different relative humidity of 96, 84, 75 and 52 % respectively at ambient conditions (29 ± 1°C) (The Pharmaceutical Codex, 1994). A 1.0 g mass of each powder in a watch glass was stored in each of the relative humidity for 7 days. Moisture gain was estimated in terms of percentage moisture gain with reference to the initial weight of powder.

2.2.7 Moisture content

A tarred empty crucible was weighed and 1.0 g of the sample was transferred into it and left in the oven at 105°C. The set up was weighed intermittently until a constant weight was reached [24]. This was performed in triplicates. The proportion of water contained was calculated from Equation 3.

\[
\left( \frac{w_i - w_f}{w_i} \right) \times 100
\]  

(3)

Where, \( w_i \) is the final weight of powder after drying, and \( w_i \) is the initial weight of powder before drying.

2.4.6 Scanning Electron Microscopy (SEM)

The scanning electron microscopies of the samples were evaluated with a scanning electron microscope (Phenom Prox, (PhenomWorld, Eindhoven, Netherlands).

2.2.8 X-ray diffractometry

The X-ray diffractions of the powders were determined using an X-ray diffractometer (Thermo Scientific, ARL X'TRA, Australia).

2.2.9 Determination of bulk, tapped and particle densities

A 20.0 g quantity of each of the samples was used in the evaluation of bulk and tapped densities by means of a Stampf volumeter (STAV 2003 JEF, Germany). The procedures were repeated thrice for each powder. The bulk and tapped densities were calculated from equations 4 and 5 respectively.

\[
\text{Bulk density} = \frac{\text{Weight of powder}}{\text{Bulk volume,} V_o}
\]  

(4)

\[
\text{Tapped density} = \frac{\text{Weight of powder}}{\text{Tapped volume,} V_f}
\]  

(5)

The particle density of each of the powders was established by the solvent displacement technique employing \( n \)-hexane as non-solvent (Odeku et al, 2005). An unfilled 25.0 ml density bottle was balanced (\( W \)). It was occupied with \( n \)-hexane and reweighed (\( W_{\text{f}} \)). The variance among \( W_i \) and \( W \) was considered as \( W_{\text{f}} \). A 0.5 g amount of the sample was assessed (\( W_i \)) and cautiously conveyed into the density bottle. The superfluous liquid was dabbed and the density bottle was reweighed (\( W \)). This was conducted three times. Particle density, \( P_t (g/ml) \) was computed from equation 6.

\[
P_t = \frac{W_{\text{f}} \times W_i}{V \left(W_{\text{f}} - W_i + W_{\text{f}} + W \right)}
\]  

(6)

Where, \( v \) is the volume of density bottle, 25.0 ml

2.2.10 Flow properties

A 50.0 g amount of each of the powders was used to define the flow rate using the funnel technique [11]. The angle of repose of NLTR-A was estimated by pouring 50.0 g of each powder into a two open-ended tubular plastic pipe stationed on a level surface of noted diameter. The tubular pipe was gently drawn up so as to constitute a cone of powder on the surface. The altitude of the cone of the powder made was valued. This is an adjusted technique of [18] and the angle of repose got is designated as the static angle of repose. The angle of repose of MLTR-B, MLTR-C and MLTR-D were found as follows. A clean glass funnel was fastened on a retort stand such that a regular perpendicular height of the tip of the funnel was 3.0 cm from a horizontal flat base with a clean graph sheet of paper. Each powder was, in turn, discharged into the funnel till the powder pile constituted reached the funnel tip and extra discharge of powder from the funnel outlet was clogged [37]. The processes were repeated thrice and the boundaries of the powder mass made in each case were indicated and the diameter of the perimeter of each powder stack was valued with a ruler. The following calculations were carried out:
Flow rate = \frac{\text{Mass of powder}}{\text{Time}} \quad (7)

\text{Angle of repose, } \theta = \tan^{-1}\left(\frac{2h}{d}\right) \quad (8)

Where, \(\theta\) is the angle of repose, \(h\) is the height of powder heap, \(d\) is the diameter of powder heap.

2.2.10.1 Carr's Index (CI)
Carr's Index \[10\] was computed by means of bulk and tapped densities data when fitted into the equation:

\text{Carr's Index} = \frac{(\text{Tapped density} - \text{bulk density})}{\text{Tapped density}} \times 100 \quad (9)

2.2.10.2 Hausner's ratio
The Hausner's ratio \[14\] was considered as the ratio of tapped and the bulk densities of the powders.

\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \quad (10)

2.2.10.3 Porosity
The porosity of the respective powders was calculated from the equation:

\text{Porosity} = 1 - \left(\frac{\text{bulk density}}{\text{true density}}\right) \times 100 \quad (11)

2.2.11 Statistical analysis
All statistical analysis of data was performed using the IBM SPSS Statistics 20 software at a 95% confidence interval with the mean difference significant at 0.05 level.

3. Results and Discussion
3.1. Organoleptic, pH and moisture characteristics of the powders
The results of the properties of the native (NLTR-A) and the modified \textit{Lentinus tuber regium} powders (MLTR-B, MLTR-C and MLTR-D) are presented in Table 1. Both the native and the modified samples were off-white in colour, tasteless and odourless powders. They were insoluble in water and several organic solvents. All the modified powders showed a significant increase in pH in comparison with the native sample (\(p<0.05\)). A marked increase in swelling index, moisture content (Figure 1) and hydration capacity were observed in all the modified powders when compared with the NLTR-A (\(p<0.05\)). However, there was an insignificant difference in the results of the hydration capacity of NLTR-A and MLTR-C (\(p>0.05\)). The moisture absorption capacity of the powders were in the order MLTR-D>MLTR-C>MLTR-B>NLTR-A (Figure 2). This shows that the methods of modification adopted improved the hydrophilic property of the LTR.

| Table 1. Properties of the native and the modified \textit{Lentinus tuber regium} powders |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Parameter                      | Sample          | NLTR-A          | MLTR-B          | MLTR-C          | MLTR-D          |
| Colour                         | Off white       | Off white       | Off white       | Off white       |
| Taste                          | Tasteless       | Tasteless       | Tasteless       | Tasteless       |
| Odour                          | Odourless       | Odourless       | Odourless       | Odourless       |
| pH                             | 6.80±0.01       | 6.82±0.01       | 10.56±0.01      | 9.28±0.01       |
| Hydration capacity             | 4.05±0.02       | 4.11±0.01       | 4.07±0.02       | 4.37±0.02       |
| Swelling index                 | 3.06±0.01       | 3.70±0.01       | 4.10±0.02       | 5.15±0.02       |
| Moisture content (%)           | 1.19±0.01       | 1.68±0.02       | 1.92±0.03       | 2.91±0.02       |
| Bulk density (g/ml)            | 0.30±0.01       | 0.36±0.02       | 0.44±0.01       | 0.54±0.02       |
| Tapped density (g/ml)          | 0.41±0.01       | 0.46±0.02       | 0.50±0.12       | 0.60±0.01       |
| Particle density (g/ml)        | 1.18±0.02       | 1.61±0.02       | 1.71±0.02       | 1.85±0.02       |
| Flow rate (g/s)                | 0.00±0.00       | 10.30±0.02      | 11.20±0.01      | 12.24±0.02      |
| Angle of repose (deg.)         | 51.46±0.04      | 29.72±0.03      | 29.24±0.03      | 27.41±0.03      |
| Carr's index                   | 29.51±0.03      | 12.06±0.12      | 11.94±0.05      | 9.49±0.10       |
| Hausner's ratio                | 1.40±0.02       | 1.13±0.01       | 1.13±0.01       | 1.08±0.01       |
| Porosity (%)                   | 77.83±0.12      | 70.25±0.06      | 69.72±0.02      | 60.48±0.03      |
3.2. Morphological properties

The morphological studies of the NLTR-A, MLTR-B, MLTR-C and MLTR D respectively using the SEM showed that the respective powders were amorphous and irregularly shaped and contain striations. The scanning electron micrographs of the various samples were different from one another (Figures 3-6). This shows that the methods of modifications used resulted in different morphological outcomes on the native powder of LTR. The results of the x-ray diffractions of the native and the modified samples of the LTR powders showed that both the native LTR as well as the modified powders were non-crystalline (Figures 7-10). The clusters of short peaks in the x-ray diffractions are an indication that both the native and the modified powders are natural with multiple constituents.
3.3. Flow properties

Considering the flow properties of the powders, the results gotten revealed that the NLTR-A was not flowable whereas a consistent increase in flow rate was practical in all the modified samples (p<0.05). Looking at other flow parameters such as the angle of repose, the value got for the NLTR-A was above 50 °, a proof of its poor flow properties. On the other hand, a very substantial decrease in the values of angle of repose (< 30.0 °) were noted for each batch of the modified powders, showing an enhanced flow properties. The improvement in the flow characteristics of the derived powders are further observable from their values of Carr’s index and Hausner’s ratio. The flowability of powder could be established from the particle packing or storing, where particles in free-flowing solid material generally form a close association upon pouring resulting in a powder bed of low porosity. The values of porosity for the LTR powders decreased steadily from the NLTR-A through all the batches of the modified powders with MLTR-D having the lowest value. Literature shows that particle size, shape and surface texture to a pronounced extent control powder flowability. The existence of moisture in the powder or granule bed may also build up the inter-particle cohesion and thereby cause a decline in flowability [10, 14, 27, 28, 13, 22].

3.4. Powder densities

There were consistent increase in values across the modified LTR powders in comparison with the NLTR-A for bulk, tapped and particle densities respectively. A statistically significant variation was noted between the respective bulk and tapped densities of the native and all the modified powders. Powders with insufficient flow properties exhibit larger interparticulate interfaces with more difference between the bulk and tapped densities [29]. This was the case with the NLTR-A which was not flowable. Though, the results obtained for the comparison of the bulk and tapped densities for the modified batches of LTR did not completely fall in line with this literature. A consistent increase in flow parameters at any point of comparison of the modified and the native powders showed a significant difference (p<0.05) except between MLTR-B and MLTR-C (p>0.05). Particle size increase, through granulation, co-processing or particle engineering is frequently used to minimize the influence of the cohesive forces, and hence boost the flowability of powders [30]. The results obtained revealed that the various methods adopted in the modification of the NLTR-A brought about a great improvement in the hydrophilic and flow characteristics of the MLTR-B, MLTR-C and MLTR-D with MLTR-D exhibiting the best result.
4. Conclusion

An attempt was made to modify the native form of *Lentinus tuber regium* powder. The results obtained showed that the methods used resulted in the enhancement of the hydrophilic and flow properties of the native powder.

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