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PRODUCTION AND EVALUATION OF THERMALLY ACTIVATED COW BONE POWDER AS DIRECT COMPRESSIONAL TABLET EXCIPIENT

Emenike IV[1*], Ibrahim YKE[2], Ojile JE[2], Timothy SY[3] and Musa H[2]

[1]Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Gombe State University, Gombe, Nigeria.
[2]Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, Kaduna, Nigeria.
[3]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Maiduguri, Maiduguri, Nigeria.

ABSTRACT

Various excipient materials are used in the direct compression of tablets. These include microcrystalline cellulose, anhydrouse lactose and dicalcium phosphate, marketed as Emcompress. Emcompress is popular because it does not absorb moisture when exposed to highly humid environment, and blend well with other directly compressible materials with excellent results. At present, dicalcium phosphate Emcompress is imported into the country with its attendant logistic problems, and cost implication, there is therefore need to source for local alternatives of dicalcium phosphate. Bones, particularly cow bones (femur, tibia and humerus), known to contain substantial source of calcium phosphate were investigated in this study for the production of directly compressible tablets. Freshly, trashable and therefore more cheaply sourced cow long bones were subjected to furnace heat at 750^{0}C, 850^{0}C and 950^{0}C (which were below the 1670^{0}C melting point of calcium phosphate) for 9 hours. The products were pulverized to form activated bone powder (ABP). The results show that irrespective of the furnace (activation) temperatures (750^{0}C, 850^{0}C and 950^{0}C), the loss in weight of the bone and the mean particle size of comminuted bone through 1.7mm sieve were not significantly different, with an average of 39.17±0.5% and 181 µm respectively. Both ABP and marketed DCP were incompressible at compaction pressure of 3-10 MT force, with 12.5 mm diameter punch and die. However, at equilibrium moisture content of 3.0%, the ABP compressed at 4MT, while the DCP was still incompressible. It would be inferred that the heat activation has turned the bone material more compressible. This work has clearly shown that ABP impact compressibility properties and is useful as a compressible diluent for both low and high dose tablet formulations.

Keywords: Activated Bone Powder, Calcium, Phosphate, Compressibility.

INTRODUCTION

Tablet is the most common oral dosage form used in this century, although the exact statistics were rare. This might not be unconnected to the advantage the tablets affords both the manufacturers and the users [1, 2]. A tablet can also be described as a product of compression and consolidation due to applied pressure to a known weight of powder between two punches in a die cavity, to a coherent unit [3]. In formulation of tablets, beside the active ingredients, other substances generally referred to as excipients are often incorporated to aid the processing of tablets such as flowability, compressibility, elegancy, delivery or release of its active component so as to make it bioavailable [4-6]. One of the most important tableting excipients are the diluents which not only impart bulkness but also facilitate uniform flow and compressibility to the powder/granules mass for compression into tablet matrix [7]. In tablet formulation therefore, diluents such as dibasic calcium phosphate, lactose, starch, microcrystalline cellulose, etc are very important, as they most often determine or modify greatly the characteristics of the

Corresponding Author:- **Emenike IV** Email:- ifeanyivemenike@yahoo.com

resultant tablet [8-10]. In low dose tablets, diluents usually form over 90% of the tablet total weight. To reduce the stages involved in tablet production, and thereby reduce the variables and hence the cost, direct compressible vehicles (diluents) are often used [11]. One of them is calcium phosphate, often marketed as Encompress. This material is currently being imported into the country and it is relatively expensive. There is therefore a need to source local alternatives that possess the good compressional characteristics of Encompress® which will obliviate the problems encountered with importation of pharmaceutical excipients. The general problem associated with importation of drugs and pharmaceutical excipients have made many pharmaceutical firms, companies and researchers to start sourcing for alternatives locally. One good source of calcium phosphate, which is often wasted, is bone. It has become imperative to evaluate this good source of calcium phosphate as a potential alternative to Encompress®.

MATERIALS AND METHODS

Preparation of thermally activated cow bone powder

Three sets of each approximately 1kg of femur, tibia and humerous cow bones were obtained from the municipal abattoir in Zaria, Northern Nigeria. After preliminary studies on the temperature range and time required to produce easily trashable product, the bones were weighed (W_1) accurately on the Mettler balance and some were kept separately on stainless steel tray, set at 750^0C, 850^0C and 950^0C for 9 hours respectively in the electrically operated furnace. The tray was left to cool overnight at the room temperature of about 30^oC. They were weighed (W_2) again on the Mettler balance and the percentage loss (L) in weight was calculated.

Pulverization of thermally activated cow bones

All the bones of the sets were placed in the funnel of the Christy and Norris harmer comminuting machine and it was started. The bones were comminuted and passed through a 1.7 mm mesh sieve into nylon cloth hood to minimize dust pollution.

analysis of the thermally activated cow bone powder (ABP)

A set of Endecott sieves was individually weighed accurately and stacked in the following sieve mesh in descending order: 1000 µm, 500 µm, 355 µm, 250 µm, 180 µm, 90 µm and fines' collecting pan. A weighed amount (300 g) of ABP was placed on the topmost sieve and covered with the sieves cover. The set was clamped on the Endecotts sieves vibrator machine and set to vibrate for 30 minutes. Half way through the sieving action, the vibrator was stopped to brush into the center of sieve any fine particle that lodged to the wall of the sieve. It was reset to complete the 30 minutes sieving action.

Calculation of the mean granule/particle size (MGS)

The mean granule/particle size (MGS) is related to the granule/particle size (S) in micrometer retained on the sieve and percentage weight (W) retained on the sieve as: $MGS = \Sigma(SW)$.

Effect of furnace temperature on particle size analysis of thermally activated bone powder (ABP)

The effect the furnace temperatures of 750^0C, 850^0C and 950^0C had on the mean granule/particle size (MGS) and size distribution of milled thermally activated cow bone powder was obtained by sieving 300 g sample of the ABP after treatment at the respective furnace temperatures, using the sieve analysis as described above. The mean granule/particle size was determined in each case using the formula of MGS above.

Determination of moisture contents of materials

The empty dry weight of 20 ml capacity crucible was weighed accurately (X) on the Mettler balance. The weight of 5 g accurately weighed sample of materials was placed in the crucible (Y) and kept in the Gallen Kamp oven thermostated at 105^0C for 2 hrs. The crucible and its content were then weighed to find the weight (Z) of the dried material. The percent moisture content (Mc) of the material was calculated as: $Mc = 100(1-{}^{Z}/_{Y})$.

Determination of particle size of dibasic calcium phosphate and milled fines of activated bone powder

The sub-sieve sizer equipment was used to determine the particle size according to Ojile *et al* (1980) [12] of the marketed dibasic calcium phosphate (DCP). Part of the fines of ABP collected in the collecting pan was milled in 500 ml capacity porcelain mortar with pestle with full attritive manual force by milling 5 g sample at a time for 5 minutes until enough fine particles similar in size to DCP was obtained. By using the same sub-sieve sizer determination, the mean particle size of the fines was determined.

Chemical analysis of activated bone powder

This was done basically to determine the calcium phosphate and calcium carbonate content of the ABP sample, since both make up 60-70% of bone constituent. Methods used are as described by Kramer and Howland [13].

Direct compression of activated bone powder (ABP) and marketed dibasic calcium phosphate (DCP) alone

The sample weight of 600 mg of fine of each material was compacted with 12.5 mm punch and die assembly on the single station Erweka tableting machine using different compression forces to 10 metric tonnes to see which force compacted the particles into hard compacts.

Determination of the equilibrium moisture content of activated bone powder (ABP) and marketed dibasic calcium phosphate (DCP)

The weight of 50 g of ABP was massed with 30% (15 ml) of distilled water in a mortar and pestle, force screened through 1.7mm sieve mesh and dried on the tray to constant weight at 48^{o}C for 3 hours. The same procedure was also carried out for DCP. It should be borne in mind that both ABP and DCP are insoluble in water. The weight of 5 g sample of the dried granules of ABP and DCP were subjected to moisture content determination as shown above respectively. The ABP and DCP dried to constant weight respectively, were now subjected to compaction as described in direct compression of activated bone powder (ABP) and marketed dibasic calcium phosphate (DCP) above.

Shape of granules/powder particles

The ABP and DCP powders were separately placed on a slide of the microscope mounted with a camera and the picture viewed through the eye piece at magnification of x10, where a clear sharp picture was obtained. The picture was snapped using the mounted still camera with Kodak Film. The film was developed and printed, presenting picture of the powder particles.

Treatment of activated bone powder (ABP) by pelletization

Samples of the unsieved powder/granules of the three different batches [750^{0}C (557.4 g), 850^{0}C (548.8 g) and 950^{0}C (680.4 g)] were pelletized using 25 mm punch and die of the Specac Pelletization machine (Energy center, Ahmadu Bello University, Zaria) at three different forces (20, 15 and 10MT). Two parameters of the pellets formed (weight and thickness) were recorded. The pellets were placed on a 1.6 mm (1600 µm) sieve mesh over a thick stainless steel hollow container. With the aid of a pestle, the pellets were micronized through the 1.6 mm mesh to form granules, which were collected in the hollow stainless steel container. Flow rate, bulk density, tapped density and Carr's Index of the granules of the different batches compressed at various forces (MT) were determined.

Determination of flow rate

Flow rates of the granules were determined using Erweka particle flow meter. A known weight of the sample (granules) was introduced into the funnel of the flow meter. The time it took the granules to flow through the funnel (flow time) after operating the machine were recorded. This test was repeated twice and the average calculated. The flow rate was then calculated from the initial weight and the average flow time as; Flow rate = Wt (g) / Time(sec).

Determination of bulk density

Bulk density was determined using a 100 ml plastic cylinder graduated in 1ml divisions. A known weight of the granules was poured into the measuring cylinder. The space occupied by the granules was read, and the bulk density calculated in g/cm^{3} using; Bulk Density (BD) = Mass (g) / Volume (cm^{3}).

Determination of Tapped Density

The measuring cylinder containing the known weight of the granules was gently tapped on top of a wooden table 100 times after which there was no more decrease in volume. The volume was recorded and the process repeated, and the average volume calculated. The tapped density (g/ cm^{3}) was then calculated using; Tapped Density (TD) = Mass (g) / Tapped volume (cm^{3}).

Carr's Index of Compressibility

Carr's Index indirectly measures the ability of the powder to rearrange and pack intimately by reducing the extra-particulate porosity within the packed mass. The lower the value of carr index, the better the flow properties of the powder. Carr's index can be calculated using; $CI = 100(T_D-B_D) / T_D$. Where CI is Carr's Index, T_D =Tapped Density, B_D = Bulk Density [14]..

Statistical analysis

The results were analyzed with Graph Pad Instat software and were expressed as mean ± standard error of the mean.

RESULTS AND DISCUSSION

Effect of heat of furnace maintained for 9 hours at 750^{o}C, 850^{o}C and 950^{o}C on loss in weight, granule size and size distribution of the milled activated bone powder (ABP) are shown in Tables 1 and 2. These furnace temperatures were lower than the melting point of dibasic and tribasic calcium phosphate (1670^{o}C). The dibasic calcium phosphate is believed to be the main constituent of bones [15]. and there could be some tribasic calcium phosphate component [5]. However, it is most probable that the crystalline architecture would be affected by the heat of activation in the furnace. The mean loss in weight was 39.2% with a variation of ± 0.5% (Table 1), showing that the loss from each sample was approximately the same. The loss could be attributed to loss in moisture content and loss of all susceptible organic materials associated with bones that melted and vapourised off. Such organic materials include the bone marrow contents, fats and fatty integuments, outside and within the bones. The heat energy was apparently able to get rid of these constituents. The consequence is that the colour of the bone turned from milky to clearly ash white. The bones were brittle to touch; meaning that the crystallinity of the bone structure must have been weakened considerably.

The calcium content was found to be about 60%, while the calcium carbonate content was below 5% and other compounds like Magnesium oxide below 1%. Calcium phosphate and calcium carbonate constitute approximately 60-70% of bone weight. From Table 1, it is

seen that approximately 40% of the total bone was lost during ashing, leaving about 60% of the initial weight of the bone, expected to be mainly calcium phosphate and calcium carbonate. Calcium carbonate has a melting/decomposition points of between 700-800^{o}C and about 1760^{o}C respectively. At the activation temperature of between 750^{o}C to 950^{o}C, most if not all of the calcium carbonate most have decomposed and vapourized leaving mainly calcium phosphate.

On sieving the pulverized bone, and weighing each sieved fraction, the sieve analysis was obtained as shown in Table 2. The interesting result is that, irrespective of the furnace temperature of 750^{o}C, 850^{o}C and 950^{o}C, the mean particle size was 181 µm. Also, the difference in percentage particles retained at different sieve sizes for the different temperature was not much (Table 2). The significance of this is that the heat activation from 750^{o}C up to 950^{o}C was able to produce the same texture of bone powder, giving the same particle size distribution. It is possible that at 750^{o}C all bonds might have been broken and so no more bonds left to be broken at 950^{o}C. Temperature above 750^{o}C does not therefore apparently offer any advantage. The granule size and size distribution of the milled ABP compared to dicalcium phosphate (DCP), indicates that the MGS of DCP (153 µm) is lower than that of ABP (181 µm), while the percentage fine (<90 µm) of DCP (about 50%) is much higher than that of ABP (about 30%), with higher MGS. ABP is therefore expected to have better flow properties than DCP, while DCP should have better compressibility, and should thus form harder compacts on compression than ABP, due to its higher percentage of fines. Finer particles are already more densely packed, so on compression, the energy needed for densification is expected to be lower than for larger particles. This is in agreement with the reports of Wells and Langridge and Rawlins [10] in which comminuting, sizing and handling of powders especially dicalcium phosphate dihydrate microcrystalline cellulose system in direct compression tablets was highlighted. The rest of the energy of compression is employed in rupturing of bonds and binding of particles, resulting in the formation of harder compacts. While for larger particles, part of the compression energy is used for densification, leaving lower energy for bond fracture and binding of particles, when compared to the fines, the resultant compact is therefore expected to be weaker than those from the fines.

Table 3 shows the bulk and tapped densities of untreated (before pelletization and comminution) ABP and DCP. Both the bulk densities of ABP were higher than those of DCP. This translated to lower Carr's Index (CI) values for ABP than DCP calculated. A lower value of CI indicates better flow properties and compressibility of powders. ABP from the result has better flow properties and is expected to also be more compressible than DCP. Hersey and Rees and Heckel [15, 16] reported on the density- pressure relationship in powder compaction. The higher the bulk density and the lower the CI indicate better flow properties and compressibility.

The moisture contents and compressibility of ABP and DCP in their original states and when massed with water and dried to constant weight are given in Table 4. As indicated in the Table, ABP had a moisture content of zero much below that of DCP of 2.8. Interestingly, however, is the similarity of the equilibrium moisture content of the two powders, which did not translate to similarities in their compressibility behaviours. ABP with an equilibrium moisture content of 3% was able to compress, while DCP of same equilibrium moisture content did not form compact. It is important to note that neither ABP nor DCP could form compact when compressed in their original form i.e. before massing with water. Bone is largely calcium phosphate salt in the divalent form [15]. This heat activated bone powder alone was compressible at equilibrium moisture content of 3.0% but its marketed counterpart, DCP alone could not compress even with the equilibrium moisture content of 3.0% [18]. It could therefore be inferred that the heat activation has turned the bone material, into a more compressible form. From the brittleness to touch of the activated bone, it is possible that the heat activation could have weakened the crystallinity and intergrity of the bone structures. This effect must have caused the bone to become more compressible. The reports of Esezebo and Garr and Rubinstein amply justifies the result of this study in which compression and tabletting of pharmaceutical powders is always carried out at elevated temperatures.

The shape of granules / powder particles shows that ABP are more amorphous or irregularly shaped while DCP was more spherical like shaped. With the amorphous nature of ABP, it is expected that the particles will be more closely fitted and therefore more densely packed than DCP particles on densification [19]. It is possible therefore, for ABP though with lower percentage of fines, to be more compressible and form harder compacts than DCP, since densification is expected to be more with ABP due to the amorphous nature of the granules [20].

Table 1. Effect of furnace temperature maintained for 9 hours each on loss in weight of cow bone samples

Parameters	Bone Sample			Mean ± SEM
	I	II	III	
Furnace temperature (^{O}C)	950	850	750	850 ±
Initial bone weight (W_1) (g)	1120.0	905.0	911.0	978.7 ±
Final bone weight (W_2) (g)	680.4	548.8	557.4	595.5 ±
Loss in bone weight (L) (%)	39.3	39.4	38.8	39.2 ± 0.5

$L = (W_1-W_2/W_1)100$

Table 2. Effect of furnace temperature maintained for 9 hours each on granule size and size distribution of the milled activated bone powder compared with dicalcium phosphate

Sieve sizes (µm)	Percentage distribution of particles (%)			DCP (%)
	750 °C	850 °C	950 °C	
1000	1.5	1.4	1.6	
500	8.8	8.5	9.0	16.6
355	8.0	8.4	7.9	
250	13.2	13.5	13.0	5.6
180	13.3	13.7	12.5	
150				2.9
125				6.1
90	22.8	23.3	22.8	14.6
75				16.3
50	32.4	31.2	33.2	37.9
MGS≥∑μw	181	181	181	153

DCP = Dicalcium phosphate

Table 3. The Density (bulk and tapped) and Carr's Index of activated bone powder

Material	Density (g/cm^3)		Carr's Index (%)
	Bulk	Tapped	
ABP (750°C)	1.33	1.82	26.90
ABP (850°C)	1.30	1.65	21.20
ABP (950°C)	1.25	1.54	18.80
DCP	0.44	0.67	34.30

ABP = Activated bone powder, DCP = Dicalcium phosphate

Table 4. Diluent powder moisture content and its compressibility all alone

Material	Moisture content of stock* (%w/w)	Equilibrium moisture content# (%w/w)	Compressibility▲
ABP	0	-	Incompressible (no compact formed)
	0	3.0	Compressible with crushing strength of 6kg
DCP	2.8	-	Incompressible (no compact formed)
	2.8	3.0	Incompressible (no compact formed)

*= Moisture content from stock, # = Equilibrium moisture content after massing powder with 30%v/w distilled water and drying to constant weight at 48°C, ▲ = 600 mg powder was compressed by variation of the compression force from 4 to 10 metric tone force in 12.5mm punch and die set

CONCLUSION

The heat activation between 750 °C and 950 °C in the furnace for 9 hours disrupted the crystalline architecture of the cow bone to produce activated bone powder which has been shown to be much more compressible and flowable compared with its marketed counterpart dibasic calcium orthophosphate. It should be realized that as at now, cow bones litter as waste in many abattoirs in the country requires only heat activation in a furnace to convert them to a suitable diluent for formulating low and high dose drugs.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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