Egg shell powder as a potential direct compression excipient in tablet formulation

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ABSTRACT

Background: There is abundance of egg shells which comes as waste from the food industry. Development of this waste into pharmaceutical excipient will have economic gain and provide alternative source of raw materials in tablet manufacture.

Objective: This work is aimed at developing eggshell powder as a direct compressible excipient in tablet manufacture.

Methods: Eggshells collected as wastes from eateries in Enugu State, Nigeria were comminuted, bleached with sodium hypochlorite, washed with water, air-dried, pulverized and sterilised by dry heat. Microbial counts were carried out. Heavy metal analysis was done by atomic absorption spectrometry. Avicel PH 101 for used as a model direct compression excipient. Mixtures of the eggshell powder and Avicel PH 101 in the ratios of 1:0, 0:1, 1:1 and 2:1 respectively were used in preparing ascorbic acid granules and tablets. The flow properties of the granules and tablet properties were evaluated. A step-wise optimisation approach was employed to get the batches with no defects, the least weight and content variation, least friability, hardness in the range of 4-7 kgf, and highest excipient dilution.

Results: The moisture content of the egg-shell powder was 0.68% and microbial analyses revealed compliance with official standards. Heavy metal analysis showed Lead (0.092%), Chromium (0.332%), Copper (0.111%), Iron (2.690%), Cadmium (0.390%), Nickel (1.313%), Arsenic (2.988%), Mercury (0.187%), Zinc (0.705%) and Manganese (0.424%). The eggshell powder had better flow than avicel PH 101. The batch with the best tablet properties selected according to pre-set parameters above contains 30% drug bulked with 1:1 eggshell/avicel pH 101 mixture.

Conclusion: Eggshell powder can serve as a direct compression agent in tablet manufacture.

Keywords: Eggshell powder, ascorbic acid, metal analysis, microbial count, direct compression, powder and tablet properties.

La poudre de la coquille d'oeuf en tant qu'excipient potentiel de compression directe dans la formulation de comprimés

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RESUME

Contexte: Il y a abondance de coquilles d'œufs qui arrivent comme déchets de l'industrie alimentaire. Le développement de ces déchets en excipient pharmaceutique aura un avantage économique et fournira une source alternative de matières premières dans la fabrication de comprimés.

Objectif: Ce travail vise à développer la poudre de coquille d'œuf en tant qu'excipient compressible direct dans la fabrication de comprimés.

Méthodes: Les coquilles d'œufs recueillis en tant que déchets provenant de café-restaurants de l'État d'Enugu, Nigeria ont été blanchies avec de l'hypochlorite de sodium, lavées à l'eau, séchées à l'air, pulvérisées et stérilisées par la chaleur sèche. Des comptages microbiens ont été effectués. L'analyse des métaux lourds a été effectuée par spectrométrie d'absorption atomique. Avicel PH 101 a été utilisé comme modèle d'excipient de compression directe. On a utilisé des mélanges de la poudre de coquille d'œuf et d'Avicel PH 101 dans les rapports de 1:0, 0:1, 1:1 et 2:1 respectivement dans la préparation de granules et de comprimés d'acide ascorbique. Les propriétés d'écoulement des granulés et des propriétés du comprimé ont été évaluées. Une méthode d'optimisation par étapes a été utilisée pour obtenir les lots sans défauts, le poids minimal et la plus faible variation de teneur, le moins de friabilité, la dureté dans l'échelle de 4-7 kgf et la dilution d'excipient la plus élevée.

Résultats: La teneur en humidité de la poudre de la coquille d'œuf était de 0,68% et les analyses microbiennes ont révélé la conformité aux normes officielles. L'analyse des métaux lourds a révélé le plomb (0,092%), le chrome (0,332%), le cuivre (0,111%), le fer (2,690%), le cadmium (0,390%), le nickel (1,313%), l'arsenic (2,988%), le mercure (0,187%), le zinc (0,705%) et le manganèse (0,424%). La poudre de coquille d'œuf a un meilleur écoulement que l'avicel PH 101. Le lot avec les meilleures propriétés de comprimé sélectionnées selon les paramètres prédéterminés ci-dessus contient 30% de médicament gonflé avec un mélange 1:1 de coquille d'œuf/avicel pH 101.

Conclusion: La poudre d'œuf peut servir d'agent de compression directe dans la fabrication des comprimés.

Mots clés: poudre de coquille d'œuf, acide ascorbique, analyse des métaux, comptage microbien, compression directe, poudre et propriétés des comprimés.

INTRODUCTION

Hen egg shell, is the waste material that remains after the removal of egg white and egg yolk. It could be gotten from various sources like poultry farms, hatcheries, egg product factories, restaurants, homes, and homes.[1] From records, in 1997 alone, the Taiwan's egg industry used about 50 million crates of egg and left over 120 tons of eggshell as waste. It was estimated that it would cost between 25,000 and 100,000 USD per annum to dispose of these wastes[2,3] The shells are just discarded and in some cases used for landfilling. This creates more environmental issues because the protein-rich membrane attracts rodents which bore holes to have access to them.[4]

In Nigeria, there is paucity of data on the amount of eggshell waste produced on an annual basis or the cost of its disposal. Large quantities of eggshell form part of the growing menace of municipal refuse. The government spends a large amount of money to remove these wastes. It has been noted that solid waste management is very poor in developing countries. Most developing countries lack both the capacity and the will power to manage solid waste. It is necessary to find a good method of transforming the waste from egg shell into a valuable raw material, giving financial benefits to the egg processing industries, help overcome high disposal costs and negative environmental concerns.[2,5] Many studies have looked for ways to utilize the egg shell waste. For example, egg shell powder has been used as a stabilizing agent to improve soil properties, as coating pigment for ink-jet printing paper additive[6] and as a source of calcium in animal and human nutrition.[1, 7,8,9]

Eggshell has: calcium carbonate (94%), magnesium carbonate (1%), calcium phosphate (1%) and organic matter (4%).[3] This could be a good replacement for calcium carbonate in solid dosage formulation. It is considered safe because of usual very low level of aluminium, cadmium and mercury.[10,11,12] The present work was aimed at investigating the pharmaceutical application of the eggshell powder as a direct compression excipient in tablet formulation. Heating at high temperatures was to destroy organic content as well as microorganisms while preserving the physical characteristics. In the next work after this, other methods of processing will be employed like extraction. The results will be compared to the method used in this work.

METHODS

Sourcing of eggshell material and preparation of the eggshell powder.

Egg shells were sourced from a commercial fast food restaurant in Enugu, Enugu State, Nigeria in July 2014. The eggshells were macerated in 1% sodium hypochlorite solution overnight. The bleached shells were washed and rinsed thoroughly with distilled water. They were drip-dried and then air-dried. The eggshells were pulverised using a domestic manual milling machine (Corona Landers YCIA S.A.). A very fine whitish brown powder was obtained. The powder was screened through a sieve with aperture size of 0.18mm. The powder was then heated in a hot air oven for 1 hour 30 minutes at 160 °C. A report by Halikia *et al* shows that calcium carbonate decomposes at 635 °C and 865 °C. Hence the sample would be expected to be stable at the sterilization temperature of 160 °C.[13] Only one batch of the eggshell powder was obtained.

Determination of the microbial load of the eggshell powder

Sample preparation

Peptone water containing 1% Tween 80 was prepared, sterilized and 9 ml of the solution was aseptically dispensed into sterile test tubes. Also, 90 ml of the solution was put into a sterile conical flask to which was added 10 g of the egg shell sample. This was properly shaken until the sample was evenly dispersed in the solution.

Total viable aerobic (heterotrophic) plate count

The total viable aerobic plate count was carried out using the pour plate method as described by the European Pharmacopeia.[14] Ten (10)-fold serial dilution of the sample solution was prepared in 8 test tubes (labelled 1-8). One (1) mL of each water sample was aseptically pipetted from each test tube into a sterile 9 cm petri plate respectively and 20 mL of sterilized molten agar medium (that has been kept below 45°C) was added to the plate. This was then carefully swirled to properly mix the medium with the inoculum. For bacterial counting, Tryptone Soya Agar (Oxoid, UK), also called Soybean Casein Digest Agar, was used and for fungal enumeration, Sabouraud Dextrose Agar (Oxoid, UK) was used. After the solidification of the agar, plates were incubated in an inverted position at 37±1 °C (for bacterial counting) and 25-27 °C (for fungal counting) for 5 days. The plates were observed daily for presence of countable colonies.

Total coliform count (for *Escherichia coli* and other coliforms)

The total coliform count was performed by the pour plate method using Chromocult Coliform Agar (Merck, Germany). The Chromocult Coliform Agar is a selective and differential chromogenic culture medium that enables the detection, differentiation and enumeration of *Escherichia coli* and other coliforms. It gives reddish or pinkish coloration to coliforms, except for ***E. coli*** which may appear as dark blue or violet colonies. One millilitre (1 ml) of each water sample was transferred aseptically to a sterile 9 cm petri plate and 20 mL of the sterilized molten agar medium (that has been kept below 45°C) was added to each plate. This was then properly mixed and carefully swirled to properly mix the medium with the inoculum. The medium was allowed to solidify before it was incubated at 37±1 °C in an inverted position for 48 hours. After incubation, the plates were examined for the presence of countable colonies of coliforms.[15]

Total *Staphylococcus aureus* and *Salmonella typhi* count

For counting of *S. aureus and S. typhi,* Mannitol Salt Agar and Salmonella Shigella Agars (Oxoid, UK) were used respectively, and the same procedure described above in the total viable aerobic (heterotrophic) plate count was employed.[14]

Isolation and identification of microorganisms

The isolation and identification of fungal isolates were carried out based on their cultural, morphological and microscopic characteristics.[16]

For bacterial isolation and identification, various cultural, staining, and biochemical testing procedures were carried out.[17,18]

Heavy metal content analysis of the eggshell powder

Heavy metal analysis was conducted using Varian AA240 Atomic Absorption Spectrometer (United States) according to standard methods[19] as follows: a series of standard metal solutions in the optimum concentration range were prepared. The reference solutions were prepared by diluting the single stock element solutions (1000 ppm for each element) with water containing 1.5 ml concentrated nitric acid/litre. A calibration blank was prepared using all the reagents ((Fe -20, Cu – 10, Cr, 100 ppm, Ni – 50 ppm, Pb – 20 ppm, Cd – 100 ppm, Hg – 100ppm, As - 80 ppm, Zn – 10 ppm and Mn – 20 ppm) except for the metal stock solutions. Calibration curve for each metal was prepared by plotting the absorbance of standards versus their concentrations as follows: 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ppm.

A 0.5 mg sample of the eggshell powder was thoroughly mixed by shaking, and 100 ml of it was transferred into a glass beaker of 250-ml volume, to which 5 ml of conc. Nitric acid was added and heated to boil till all the residue was completely dissolved. The mixture was cooled, transferred and made up to 100 ml using deionised water. The sample was aspirated into the oxidising air-acetylene flame. When the aqueous sample is aspirated, the sensitivity for 1% absorption is observed.

Determination of the moisture content of the eggshell powder

A porcelain dish with open lid was dried in the oven and cooled in a desiccator. The empty dish was weighed, 3 g of powder was added and weighed again. The loaded dish with open lid was placed in the oven at 105 °C for 3 hours. The closed dish was cooled to room temperature in a desiccator and weighed. The moisture content was calculated using the formula below.[20]

$$Moisture\ content = [(b - c) \div (b - a)] \times 100 \quad \ldots\ldots (1)$$

Where,

a = weight of empty dish

b = weight of dish + powder

c = weight of dish + dry powder

Preparation of powder mixes for different tablet batches

Powder batches were prepared to correspond to the tablet formulae. The drug in each tablet was calculated for 100 mg; lubricant (stearic acid 0.25 %, 0.5% or 0.75 %); direct compression excipients (DCE) - Egg shell powder and Avicel® PH 101 in the ratios of 1:0, 0:1, 1:1 and 2:1. The tablet weight was calculated such that the drug is 30 % of the DCE. A step-wise optimization approach was employed. The tablet weight was computed using the formula below.

Total Direct Compression Excipient Weight (W_E) = (egg shell alone or Avicel® 101 alone or mixture of Egg shell and Avicel® PH 101)

Tablet weight (W_T)= 100 mg + W_E + (Magnesium stearate or stearic acid % of W_E) ..... (2)

The tablet thickness, after some preliminary runs, was

set as the minimum thickness that does not cause jamming of the rotary tableting machine (Proton Minipress – Rotary Tableting Machine, Proton Engineering, India). The compression pressure was set such that the minimum tablet hardness recorded with the Monsanto hardness tester was 4 kgf. Batches were selected with the following criteria: the highest dilution potential, minimal tablet defaults e.g. capping and lamination, chipping, picking, sticking to dies cavity or breaking, tablets with minimal weight variation, batches with minimal tablet breakages, tablets with hardness not less than 4 kgf and tablets with minimal Avicel® PH 101 in the powder mix.

Table 1: Formula for the preparation of ascorbic acid tablets

Batch	Ascorbic acid (mg)	Eggshell powder (mg)	Avicel PH 101 (mg)	Stearic acid (mg)	Tablet weight (mg)
1A	100	333.3	-	1.1	434.4
1B	100	333.3	-	2.2	435.5
1C	100	333.3	-	3.3	436.5
2A	100	-	333.3	1.1	434.4
2B	100	-	333.3	2.2	435.5
2C	100	-	333.3	3.3	436.5
3A	100	166.7	166.7	1.1	434.4
3B	100	166.7	166.7	2.2	435.5
3C	100	166.7	166.7	3.3	436.5
4A	100	222.2	111.1	1.1	434.4
4B	100	222.2	111.1	2.2	435.5
4C	100	222.2	111.1	3.3	436.5

Stearic acid= SA, Batches A = 0.25%SA, Batches B = 0.5%SA, Batches C = 0.75%SA,
Batch 1 = Egg shell alone, Batch 2 = Avicel PH 101 alone, Batch 3 = Eggshell/Avicel PH 101 (1:1),
Batch 4 = Eggshell/Avicel PH 101 (2:1).

Determination of the flowability of the eggshell powder and the powder mixes

Flow rate and angle of repose

A dry glass funnel was supported by a retort stand at 4 cm distance from the tip of the funnel to the table. A paper was placed below the funnel assembly. A sheet of fiber board was used to block the funnel outlet. Then 30 g of the powder mix was introduced without compacting into the funnel. The fiber board sheet was withdrawn and the timer started simultaneously. The timer was stopped when all the powder has passed through the funnel.

The time needed for the entire powder to flow out of the funnel was recorded. Three determinations were carried out. In each determination, the height of the heap was measured using a ruler and tape. A pencil was used to trace the contour of the base of the powder on the paper. The angle of repose of the conical heap so formed was determined as follows.[21]

$$Tan\,\theta = {}^{Height\ of\ Powder\ Heap}/_{Radius\ of\ Powder\ Heap} \quad \ldots\ldots (3)$$

$$Angle\ of\ Repose\ (\theta) = Tan^{-1}\left[\frac{Height\ of\ Powder\ Heap}{Radius\ of\ Powder\ Heap}\right] \quad \ldots\ldots (4)$$

$$Flow\ Rate\ (FR) = {}^{Mass\ of\ Powder}/_{Time\ of\ Flow} \quad \ldots\ldots (5)$$

Determination of bulk density, tapped density, compressibility index and Hausner ratio for eggshell and the powder mixes.

The method described in the United States pharmacopoeia was used.[22] A 30g sample of each powder was weighed out and gently introduced into a 100-ml measuring cylinder. The volume occupied by the powder was noted. This represents the bulk volume of the powder. The cylinder was tapped 200 times on the wooden platform to a constant volume of the powder. The volume occupied by the powder after tapping was noted. This represents the tapped volume. The procedure was repeated three times. Therefore, the bulk and tapped densities, Hausner's ratio and Carr's compressibility index were calculated by using the equations 6, 7, 8,, and 9:[23,24]

$$Bulk\ Density\ (BD) = {}^{Mass}/_{Bulk\ volume} \quad \text{..... (6)}$$

$$Tapped\ Density\ (TD) = {}^{Mass}/_{Tapped\ volume} \quad \text{..... (7)}$$

$$Hausner's\ ratio = {}^{TD}/_{BD} \quad \text{..... (8)}$$

$$Carr's\ compressibility\ index = \left[{}^{TD - BD}/_{TD}\right] \times 100 \quad \text{..... (9)}$$

Compression of the powder mixes

Using the batch 2A powder mix, the target weight was first weighed out using an electronic weighing balance (Ohaus, England). The powder mix was carefully filled into the die cavity of a Proton Minipress Rotary Tableting Machine.[25] The die volume was adjusted until the powder was flush with the top surface. Other die cavities were filled and levelled. The compression pressure was adjusted to give a tablet hardness of 4 kgf (measured using the Monsanto Hardness Tester) at a compression dwell time of 41.9 ms. The pressure that gave this hardness was recorded as 49 kN. This pressure was maintained for all other batches. Only the volume corresponding to the set tablet weight was adjusted for each batch. Higher compression pressures caused the punches to jam and so were discouraged.

Evaluation of tablet properties

After the compression, some physicochemical tests were performed on all the tablet batches which include:

Hardness test

Ten (10) tablets were selected randomly from each batch and their hardness were determined using Monsanto hardness tester. The mean and standard deviation for each batch was calculated.[27]

Uniformity of weight test

Twenty (20) tablets were randomly selected from each batch. The tablets were weighed together using an electronic weighing balance (OHAUS, England). The mean tablet weight was calculated. The tablets were weighed individually for each batch. The batch relative standard deviation (RSD) and percentage deviation for each tablet were calculated using the following formulae;

$$RSD = 100\left[{}^{S}/_{\mathbf{Xmean}}\right] \quad \text{..... (10)}$$

Where S is the sample standard deviation defined by:

$$S = \left[\sum (X - X_{mean})^2 / n - 1\right]^{1/2} \quad \text{..... (11)}$$

X_{mean} is the mean of the values obtained from the tablets tested expressed as a percentage of the label claim; "n" is the number of tablets tested. X_1, X_2, X_3,.....Xn are the individual values (X) of the tablets tested expressed as a percentage of the label claim).[26]

Friability test

Ten (10) tablets were selected at random from each batch and dedusted. The ten tablets were weighed together in the electronic weighing balance. The dedusted tablets were placed in a friabilator (DBK, India) and set to rotate at 25 rpm (rotations per minute) for 4 minutes. The tablets were removed from the friabilator, dedusted and weighed again.[27] The percent friability was calculated using the formula below.

$$\%\ Friability = {}^{[Initial\ Weight\ -\ Final\ Weight]100}/_{Initial\ Weight} \quad \text{..... (12)}$$

Assay of drug content

Redox titration method using iodine solution was used to do the assay as described by University of Canterbury Chemistry Department. A 100-mg sample of ascorbic acid in solution was titrated with the standard iodine solution (0.005mol/L) to determine the quantity of the standard iodine solution needed to react with the 100mg ascorbic acid in solution. A 16.2 ml of the standard iodine solution was used to titrate 100 mg ascorbic acid. As the iodine is added during the titration, the ascorbic acid is oxidized to dehydroascorbic acid, while the iodine is reduced to iodide ions.

Ascorbic acid + I_2 → 2 I^- + Dehydroascorbic acid ..... (13)

Once all the ascorbic acid has been oxidized, the excess iodine is free to react with the starch indicator, forming

$$\frac{16.2\ ml\ Iodine\ Solution}{100\ mg} = \frac{Titrant\ (ml)}{X\ mg\ of\ Ascorbic\ Acid\ in\ Tablet} \quad \text{..... (14)}$$

$$X\ mg\ of\ Ascorbic\ Acid\ in\ Tablet = \frac{Titrant\ (ml)\times 1\times 100}{16.2\ ml\ Iodine\ Solution} \quad \text{..... (15)}$$

Determination of content uniformity of tablets

Ten (10) tablets were randomly selected from each of the batches and each was dissolved in 200 ml of distilled water in a volumetric flask. A 20-ml aliquot of the sample solution was pipetted into a 250-ml conical flask and about 150 ml of distilled water and 1ml of the starch indicator solution was added to the flask. The sample was titrated with 0.005mol/L iodine solution. The endpoint of the titration was identified as the first permanent trace of a dark blue-black color due to the starch-iodine complex. The content of ascorbic acid in each tablet was calculated using the following formula in equation-12 above. The mean drug content was calculated and the coefficient of variation determined.[28]

RESULTS

Colour and texture of egg-shell powder

The color of the egg-shell powder was whitish-brown and it was gritty to the touch.

Moisture content of egg-shell powder

The moisture content calculated as percentage loss in weight was 0.68%.

Table 2: Results of metal analysis

Metal	Concentration in Egg shell powder (ppm)	Recommended Limits[19]
Lead (Pb)	0.092	1 ppm
Chromium (Cr)	0.332	5 ppm
Copper (Cu)	0.111	10 mg/day
Iron (Fe)	2.690	13 mg/day
Cadmium (Cd)	0.390	15 ppm
Nickel (Ni)	1.313	5 µg/kg/day
Arsenic (As)	2.988	60 ppm
Manganese (Mn)	0.424	0.14 mg/kg/day (10 mg/day considered safe in diet, based on assumed 70 kg body weight)
Mercury (Hg)	0.187	1 ppm
Zinc (Zn)	0.705	10-15 times the RDA (in the 100 to 250 mg/day range)

Metal content analysis

Table 1 below shows the metal composition of the egg-shell powder in comparison to the limits recommended by the American Society for Testing and Materials, USA.[19].

Microbial content analysis

The result of microbial content analysis is shown in table 3.

Table 3: Total bacterial, coliform, staphylococcal and fungal counts of the eggshell powder

Parameters	Pre-treatment (organism/ml)	Post-treatment (organism/ml)	Standard Specifications[14] (Organism/ml)
Total viable aerobic bacteria count	10 x 10^3	2x10^1	Not more than 10^4 bacteria per gram or ml
Total viable aerobic fungal count	8 x10^3	4x10^1	Not more than 10^2 fungi per gram or ml
Total coliform count	0	0	Not more than 10^2 per gram or ml
S. aureus	2 x10^3	0	Absence of *S. aureus* per 1g or 1ml
E. coli	0	0	Absence of *E. coli* per 1g or 1ml
Salmonella spp.	1x10^2	0	Absence of salmonella per 10g or 10ml

Table 4: Flow properties of powder granules

Batch	Flow Property			
	Carr's index (%)	Hausner's ratio	Angle of repose (°C)	Flow rate (g/sec)
1A	12	1.1	15.8±0.5	44.0±5.8
1B	11.5	1.1	16.7±0.7	34.8±0.6
1C	12	1.1	17.5±0.0	43.3±6.6
2A	23.4	1.3	24.5±0.7	29.9±2.1
2B	21.2	1.3	24.5±1.8	32.2±1.1
2C	19.6	1.2	24.3±1.0	28.9±3.0
3A	9.4	1.1	19.7±0.8	36.6±0.4
3B	11.4	1.1	19.1±0.5	36.9±0.3
3C	11.4	1.1	18.4±0.5	36.0±0.7
4A	10	1.1	20.7±0.3	40.3±2.8
4B	10	1.1	20.7±0.6	41.2±4.4
4C	10	1.1	21.3±1.1	41.9±3.7

Stearic acid= SA, Batches A = 0.25%SA, Batches B = 0.5%SA, Batches C = 0.75%SA, Batch 1 = Egg shell alone,
Batch 2 = Avicel PH 101 alone, Batch 3 = Eggshell/Avicel PH 101 (1:1), Batch 4 = Eggshell/Avicel PH 101 (2:1).
n = 3

Results of tablet compression

Only powders of batches 2A, 2B, 2C, 3A, 3B and 3C were compressible. The powders belonging to batches 1A, 1B, 1C, 4A, 4B and 4C were incompressible and so, work on those batches was discontinued.

Tablet properties

The results of tablet weight uniformity, hardness, friability and content of active ingredient (assay) are shown in table 5, 6 and 7.

Table 5: Tablet weight (mg) variation test results

Batch	2A	2B	2C	3A	3B	3C
Mean ± SD	418.0±5.8	419.9±4.7	425.7±2.0	429.8±1.5	433.2±2.2	420.9±6.9
Coeff var	1.4	1.1	2.0	1.5	2.2	1.6
Tablet no	Deviation of weight from mean					
1	1.91	-0.02	3.69	2.28	-1.57	0.21
2	1.91	2.36	-3.36	2.28	0.74	-4.54
3	1.91	-0.02	-1.01	-0.05	-0.18	0.21
4	1.20	0.45	1.34	-0.05	0.74	0.21
5	-0.48	-0.02	-0.07	-0.05	0.74	-0.26
6	0.72	-0.02	-0.54	-0.05	0.74	0.21
7	-0.48	-0.02	-1.01	-0.05	-0.65	0.21
8	0.24	-0.02	1.34	0.42	0.74	0.21
9	0.24	-0.50	1.34	0.42	0.74	0.21
10	-0.48	0.45	-0.07	-0.05	0.74	-0.74
11	0.48	-0.02	0.40	-0.05	0.28	0.21
12	-0.96	-0.02	1.34	-0.05	0.28	0.21
13	-2.87	-0.02	-3.36	-0.05	-1.57	0.21
14	1.91	-0.02	1.34	-0.05	3.05	0.21
15	-2.87	-0.02	-1.01	-2.37	-1.57	-0.26
16	-0.48	-0.02	3.69	2.28	-1.57	0.21
17	-0.48	-2.41	1.34	-2.37	-6.19	0.21
18	-0.48	2.36	-1.01	-2.37	5.36	4.97
19	-0.48	-0.02	-3.36	-2.37	-1.57	0.21
20	-0.48	-2.41	-1.01	2.28	0.74	-2.16

Stearic acid= SA, Batches A = 0.25%SA, Batches B = 0.5%SA, Batches C = 0.75%SA, Batch 2 = Avicel PH 101 alone, Batch 3 = Eggshell/Avicel PH 101 (1:1).

Table 6 : Hardness, friability and drug content

Tablet Property	Batch 2A	2B	2C	3A	3B	3C
Hardness (kgf)	10.4±0.4	10.3±0.5	9.9±0.9	5.8±0.5	4.7±0.6	5.2±1.2
Friability (%)	0.262	0.238	0.283	0.281	0.407	0.274
Drug content (%)	90.7	94.3	101.6	94.1	100.1	99.3

Stearic acid= SA, Batches A = 0.25%SA, Batches B = 0.5%SA, Batches C = 0.75%SA, Batch 2 = Avicel PH 101 alone, Batch 3 = Eggshell/Avicel PH 101 (1:1).

Table 7: Uniformity of content test results

Batch	Drug Content (mg)									
	I	II	III	IV	V	VI	VII	VIII	IX	X
2A	98.7	95.3	99.3	96.0	96.7	98.7	98.7	99.3	99.3	98.0
2B	96.7	104.7	110.0	102.7	98.7	108.0	104.7	101.3	99.3	106.7
2C	106.7	108.7	109.3	106.7	108.0	108.0	107.3	108.0	106.7	108.7
3A	96.0	96.7	96.7	98.0	94.7	97.3	99.3	95.3	95.3	97.3
3B	108.0	113.3	108.7	110.0	111.3	112.0	111.3	111.3	110.7	110.7
3C	108.7	109.3	111.3	108.7	112.0	111.3	110.0	106.0	108.7	108.7

Stearic acid= SA, Batches A = 0.25%SA, Batches B = 0.5%SA, Batches C = 0.75%SA,Batch 2 = Avicel PH 101 alone, Batch 3 = Eggshell/Avicel PH 101 (1:1).

DISCUSSION

Metal content analysis is important because heavy metals are implicated in toxicity. Hence, limits have been placed on the levels of these elements in food. The results of the metal analysis (table 2) show that all the metals are within tolerable limits. Thus, the egg shell powder could be regarded as being safe for human consumption.[29]

Microbial content analysis showed that the sample conformed to European Pharmacopoeial specifications for total viable aerobic bacteria count, total coliform count and *E. coli* count. The sample initially failed the specifications for Total viable aerobic fungal count, *S. aureus* count and Salmonella count. After heating the sample at 150 °C for 1hour 30 minutes and subsequent microbial investigation (post-treatment), the sample conformed completely with the compendial specifications.[30] Eggshells could be contaminated by microorganisms when they pass through the multifunctional birth canal of the birds, which is also used for urinary and defecation purposes by the birds.[31, 32,33]

Staphylococcus bacteria can also be transmitted through infected wounds on parent birds as can staph. Infections found on the hand of aviculturists, if the egg comes in contact with lesions. Artificial incubators will grow staph readily, and it can spread horizontally in this manner.

In summary, the microbial counts showed that the egg shell powder complied with the European Pharmacopoeial specifications after heat sterilisation.[14]

The compressibility indices, Carr's index and Hausner's ratio reveal particle-particle interaction and powder bed consolidation. The more the ability to consolidate the greater the likelihood of poorer flow. The result of compressibility indices (Table 4) shows that there was not much difference in the indices when the powder beds were repeatedly compressed and decompressed. When Avicel alone was the DCE, the compressibility increased significantly. In fact, the Hausner's ratios were above 1.18 and the Carr's indices above 20, which indicate not very good flow. When egg shell was used either alone or at a 1:1 ratio with Avicel, the Hausner's ratio remained the same at 1.1, with the Carr's indices below a value of 15, which indicate good flow. Generally, all the selected batches had good flow. The batches with more of the egg shell showed lesser compressibility (more flowability) than the batches with more of Avicel. Thus, this could have accounted for the better flow gotten with the egg shell powder than with Avicel. The change in the lubricant concentration from 0.25 % w/w to 0.75 % w/w did not show any clear trend of effect on the powder bed compressibility or flow.[24,26]

The flow rate measurements revealed that all the batches had acceptable flow rates within the range of 28 – 44 g/s which can sustain tableting speed of any capacity. However, there was better flow when the egg shell was used either alone or with Avicel.[24,26]

The United States Pharmacopoeia (2005) states that for tablets of more than 324 mg weight, no two tablets should vary from the mean by more than 5 %. From the table 5, no single tablet varied from the mean by up to 5 %. This shows uniform tablet batch weight and hence, uniform die filling. Thus, this is a good indication that the quantity of drug contained in one tablet will be similar to that in another tablet of the same batch. This is important in patient care.[25]

Crushing strength of tablet is another important

parameter that could help to decide if a drug would be delivered from a tablet within the standard time limit. The tablet hardness result in table 6 above shows that tablets with higher Avicel content were harder than tablets containing more of the egg-shell powder which were either poorly compressed or too brittle leading to tablet deformities. The tablet batches made with 1:1 ratio of egg-shell/Avicel PH 101 had hardness values within the set limits of 4 – 7 kgf. With egg shell powder and with Avicel, as the concentration of stearic acid increased from 0.25 % w/w to 0.75 % w/w, the tablets softened. Stearic acid was used to improve granule flow which should lead to more uniform tablet properties.[25]

Friability testing is carried out to determine how well tablets can withstand the stress that comes with transportation. It is common to see particles from tablets at the bottom of the container which is as a result of attrition of weakly bonded particles. The USP (2005) prescribes a friability of 0.5 - 1 %. Therefore, the friability result in table 6 shows that all the tablet batches considered had friability values within standard and tolerable limits.[26]

The United States Pharmacopoea (2005) states that the ascorbic acid content in a tablet should be within the range of 90 – 110 % of the tablet claim. From table 6 above, the least deviation was 0.1% and the highest was 9.3%. These values are within the tolerable limits of drug content. Therefore, all the tablets conformed to the acceptable limits. This shows that the formulations contain the label claim of ascorbic acid 100 mg/dose.

In the uniformity of content test, United States Pharmacopoea[22] states that, unless otherwise specified in the individual monograph, the requirements of dosage uniformity are met if the amount of the active ingredient in not less than 9 out of the 10 dosage units as determined from the content uniformity method lies within the range 85.0% to 115.0% and no unit is outside the range 75.0% to 125.0% of the label claim. The results in table 7 show that no single tablet had a deviation outside of ± 15 %. Thus, they all conformed to acceptable limits of uniformity of content test. This show that there was proper mixing during the formulation.[27]

The result of tablet properties discussed above shows batches 3A, 3B and 3C had the best tablet properties. These tablet batches are composed of 1:1 ratio of egg-shell/Avice PH 101, 30% Drug/ DCE ratio and 0.25 – 0.75% stearic acid content.

CONCLUSION

Egg-shell powder can be developed from the wastes from food and confectioneries with low risk of microbial degradation, infection and low heavy metal content for use as pharmaceutical excipient. Egg-shell could serve as a good direct excipient when combined with Avicel PH 101 at a ratio of 1:1.

ACKNOWLEDGEMENT

We wish to acknowledge the contributions of Mr Ikechukwu Boniface Nwobu, a technologist in the Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria. He handled all the tablet compression. This work was personally funded and there is no conflict of interest.

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