EVALUATION OF A CO-PROCESSED EXCIPIENT FROM COW BONE POWDER AND GELATIN FOR USE IN DIRECT COMPRESSION TABLETING

BY

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DECLARATION

I declare that the work in this thesis entitled, 'Evaluation of a Co-processed Material from Cow Bone Powder and Gelatin for Use in Direct Compression Tableting' has been carried out by me in the Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, under the supervision of Prof. A.B. Isah and Prof. (Mrs) A.R. Oyi. The information derived from literature have been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

Yohanah Dauda YERIMA ....

Signature Date

CERTIFICATION

This thesis entitled 'EVALUATION OF A CO-PROCESSED EXCIPIENT FROM COW BONE POWDER AND GELATIN FOR USE IN DIRECT COMPRESSION TABLETING' BY Yohanah Dauda Yerima meets the regulations governing the award of the degree of Master of Science (Pharmaceutics) of the Ahmadu Bello University, and is approved for its contribution to knowledge and literal presentation.

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DEDICATION

This work is dedicated to God Almighty, the Alpha and Omega for His Loving Kindness towards me and for seeing me through the period of this course. To my beloved wife, Mrs. Ruth Y. Yerima and my sweet children: Sharon-Simah, Benita-Bernice, Jason-Joash and Noel- Nice.

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ABSTRACT

The objective of the study was to develop an excipient consisting of cow bone powder and gelatin by the co-processing method and to evaluate its use in direct compression tablet formulation. Co-processing is based on the novel concept of two or more excipients interacting at the sub particle level, the objective of which is to provide a synergy of functionality improvement as well as masking the undesirable properties of individual excipients. Fresh cow femur and tibia bones were collected and cut into pieces using an axe and boiled to about 100 °C to remove all traces of fat, meat and bone marrow and was subsequently washed with hot water. The bones were then dried and subjected to heating to produce the cow bone powder in an industrial furnace at about 600 °C to 800 °C for 6 h and then pulverized. Gelatin was dissolved in a beaker with 20 ml of water using gallenkamp regulator hot plate at a temperature of about 100 °C and incorporated into wet activated bone powder in the ratio of 90 : 10, 95 : 5 and 97.5 : 2.5 respectively. The resultant materials were dried and crushed using an electrical blending machine and thereafter, moisture content and pH were determined using standard methods. The co-processed excipient was physically characterized for flow rate, bulk density, tapped density, angle of repose, carr's index, hausner ratio and moisture content of 12.3(0.06), 1.16(0.02), 1.35(0.02), 21(0.09), 14.1, 1.16 and 4.20 respectively using standard methods. There was no much difference between activated bone powder (ABP) and co-processed in term of flow rate but there was improved mechanical compressibility with co-processed excipient. Fourier Transform Infrared (FTIR) and Differencial Scanning Calorimetry (DSC) spectra data were taken to find out the chemical stability of the excipient. The DSC was performed on all the samples using Perkin Elmer model Pyris-6, USA. The results of FTIR and DSC spectra of pure bone powder, gelatin powder and co-processed excipient indicated that there was no chemical interaction and

only physical changes occurred, since no observable changes in spectra were observed. In the formulation studies, ascorbic acid and metronidazole powders were chosen as model drugs in a binary mix of 30:70 and 40:60 respectively. Tablets of Ascorbic acid and Metronidazole that was prepared by direct compression method employing Activated cow bone powder and gelatin (ABPG95) in ratio 95:5 were made co-processed excipient using punch die set at 8 mm and 12 mm respectively. Good quality tablets were produced with regard to weight uniformity, crushing strength, disintegration time and friability test of 333(0.2), 7.5(0.4), 5.37(0.5) and 1.00 for Ascorbic acid and 501(0.1), 7.6(0.27), 10.0(0.5) and 1.02 for Metronidazole respectively. The drug dissolution test results indicated that there was high release observed within the range of 30-40 minutes for the formulations. The present study concluded that co-processed cow bone powder and gelatin can be used as a direct compression excipient.

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LIST OF ABBREVIATIONS

AA.....Ascorbic acid

ABP.....Activated bone powder

ABPG.....Activated bone powder and Gelatin

API.....Active pharmaceutical ingredient

CCS.....Calcium carbonate and starch

DSC.....Differential scanning calorimeter

FTIR.....Fourier transform infrared

GMIA.....Gelatin manufacturers institute of America

HPMC.....Hydroxypropyl methylcellulose

HR.....Hausner's ratio

MCC.....Microcrystalline cellulose

MET.....Metronidazole

Mg.St.....Magnesium Stearate

MT.....Metric tone

NSAID.....Non-steroidal anti-inflammation drug

PVP.....Povinyl pyrrolidine crospovidone

SMCC.....Silicified microcrystalline cellulose

CHAPTER ONE

1.0 INTRODUCTION

1.1 The Need for Developing Multifunctional Excipients

The continued popularity of solid dosage forms, a narrow pipeline for new chemical excipients, and an increasing preference for the direct compression process created a significant opportunity for the development of high-functionality excipients. The development of new excipients to date has been market driven (i.e. excipients are developed first and market demand is created through marketing strategies) and has not seen much activity as shown by the fact that, for the past many years, not a single new chemical excipient has been introduced into the market. The primary reason for this lack of new chemical excipients is the relatively high cost involved in excipients discovery and development.

The excipients industry today has been an extension of the food industry (Steinberg *et al*, 2001). Increasing regulatory pressure on purity, safety and standardization of the excipients has catalyzed the formation of an international body. However , with increasing number of new drug moieties with varying physicochemical and stability properties, there is growing pressure on formulators to search for new excipients to achieve the desired set of functionalities.

In the recent years, drug formulation scientists have recognized that the single-component excipients do not always give desirable results with most of the active pharmaceutical ingredients. Therefore, the combination of two excipients fall into two broad categories: Physical mixtures and Co-processed excipients.

Physical mixtures are simple admixtures of two or more excipients typically produced by short duration processing at low shear. Excipients with improved functionality can be obtained by developing new chemical excipients and new combinations of existing materials (Moreton, 1996).

Other factors that lead to the search for new excipients are:

- The growing popularity of the direct compression process and demand for an ideal filler-binder that can substitute two or more excipients
- Tableting machinery's increasing speed capabilities, which require excipients to maintain good compressibility and low weight variation even at short dwell times (milliseconds).
- Disadvantages of existing excippients such as loss of compaction of microcrystalline cellulose (MCC) upon wet granulation, high moisture sensitivity, and poor die filling as a result of agglomeration.

- The lack of excipients that address the needs of a specific patient such as those with diabetes, hypertension, lactose and sorbitol sensitivities.
- The ability to modulate the solubility, permeability, stability of drug molecules
- The growing performance expectations of excipients to address issues such as disintegration, dissolution, and bioavailability.

1.2 Pharmaceutical Tablets

Tablets are solid preparation each containing a single dose of one or more active ingredients and obtained by compressing uniform volumes of particles. They are the most preferred dosage forms by pharmaceutical scientists and clinicians because they can be accurately dosed and provide good patient compliance, they are easier for companies to manufacture, and can be produced at a relatively low cost (Rakesh *et al*., 2009). Technological developments in the field of active pharmaceutical ingredients (APIs), excipients and tableting machines during the past decades have made tablet manufacturing a science and the tablets the most commonly used solid dosage form (Rasenack *et al*., 2002).

The low percentage of moisture in tablets affords them the one advantage of microbial stability, without the necessity of including a preservative. Tablets and capsules offer the patient the advantage of ease and convenience of administration when compared with, for example the injectable dosage forms, where administration mostly requires the attention of qualified personnel.

Tablets typically comprise of API admixed with other agents referred to as excipients, which aid or facilitate the compression of the tablet into compact uniform dosage unit. The process of compaction is carried out using a tablet press. The advantages associated with tablets are a result of some meticulous designs (Kumari *et al*., 2013). Some drug powder mixes are compressed without further treatment while others require some tactful engineering. For powders to be directly compressible, they must possess an inherent binding ability or else a binder as excipient, is incorporated in the tablet formula. Where the powder's flowability is poor, the added binder is used to impart granular properties to facilitate the requisite flowability. Uniformity in dosing is governed by uniformity in dosage units which, in tablets is dependent on the appropriate flow of the powder or granules into the die cavity (Aulton, 2007; Jones, 2008) presented the following as the reasons why tablets have an edge over other dosage forms as they equally listed some of the drawback from tablets.

1.2.1. Advantages of tablets

- Tablets are convenient to use and are an elegant dosage form

- A wide range of tablet types are available, offering a range of drug release rates and durations of clinical effects.
- Tablets may be formulated to contain more than one therapeutic agent.
- Tablets may be formulated to release the therapeutic agent at a particular site within the gastrointestinal tract to reduce site effects, promote absorption at that site.
- It is easier to mask the taste of bitter drugs using tablets than for other dosage forms e.g. liquids.
- The chemical, physical and microbiological stability of tablets are superior to liquid dosage forms.
- Tablets are easily manufactured enmass.
- A wide variety of therapeutic agents may be administered orally in the form of tablets, except proteins.
- Accuracy of dose is maintained since tablet is a solid unit dose.
- Easy to transport in bulk and emergency supplies can be carried out by pharmacists.
- Self administration is possible with tablets (Rakesh *et al*., 2009).

1.2.2 Disadvantages of tablets

- The manufacture of tablets requires a series of unit operations and therefore there is an increase level of product loss at each stage in the manufacturing process.
- The administration of tablets to certain groups e.g. children and the elderly may be problematic due to difficulties in swallowing. These problems, however may be overcome by using effervescent, dispersible or solution tablets.
- The absorption of therapeutic agents from tablets is dependent on physiological factors e.g. gastric emptying rate and shows inter-patient variations.
- The compression properties of certain therapeutic agents are poor and may present problems in their subsequent formulation and manufacture into tablets.

- It is difficult to convert a high dose poorly compressible API into a tablet of suitable size for human use.
- Tablet has slow onset of action as compared to parenterals and liquid orals.
- Patient undergoing radiotherapy cannot swallow tablet to avoid chemical interferance.

1.2.3 Types of tablets

Tablets are formulated containing different types or amounts of excipients which generally define where the tablet is intended to disintegrate and be absorbed to elicit its pharmacological effect. Some tablets are intended to quickly dissolve in the mouth while some are desired to disintegrate and dissolve in the colon. Tablets can be broadly classified into these three classes.

- Those designed to instantly dissolve upon contact with a liquid medium like the saliva in the mouth e.g. the orally dissolving tablets (Rao *et al*., 2010).
- Those tablets whose rate of drug release has been modified and the drug is released usually over an elongated period of time running to several hours e.g. the extended release, enteric coated formulations (York., 2005 and Moretone., 2007).
- Those expected to dissolve in the stomach after getting in contact with the gastric fluid and this class of tablets otherwise called conventional immediate release tablets are expected to dissolve within 30 minute (Pathak *et al*., 2011; Govedarica *et al*., 2011) . Tablet 1.1 further displays below types of tablet forms and their properties.

  Below are the types of tablet forms and their properties.

Tablet 1.1 : Types of Tablet Forms and Their Properties

Formulation type	Description
Immediate release	The dosage form is designed to release the drug substance immediately after ingestion
Delayed	The drug substance is not release until a physical event has occurred, e.g. time elapsed, change in pH of intestinal fluids, change in gut flora
Chewable	Strong, hard tablets to give good mouth feel
Effervescent	Taken in water, the tablet forms an effervescent, often pleasant- tasting drink.
Lozenges	Strong slowly dissolving tablets for local delivery to mouth or throat.
Soluble	Tablets taken in water, the tablet forms a solution for ease of swallowing.
Dispersible	Tablets taken in water, the tablet forms a suspension for ease of swallowing.
Pastilles	Intended to dissolve in slowly for the treatment of local infection, usually composed of a base containing gelatin and glycerin

(Adapted from Davies (2009)

1.2.4 Components of Tablets

A tablet as a dosage form is hardly formulated containing only the active ingredient but always with other substances referred to as excipients or aids. In most cases, the excipients while being expected to be inert, collectively account for the largest weight proportion in the tablet formulation formula. Excipients are added to a formulation in order to facilitate its manufacture, function, patient acceptability and drug delivery. (Juan *et al*., 2004). Excipients give a tablet the structure it requires to serve as dosage unit. These specific excipients and their diluents offer a tablet the bulk to make it of compressible size, while binder affords it the requisite adhesive strength to hold together during formulation, transportation and handling by the patient. Disintegrants are included in the formulation to counter the effect of binder by breaking the tablet upon contact with a liquid medium. Lubricants and glidants are to give the tableting process the "smoothness" it requires and ejection of tablets from die cavity (Rudnic *et al*., 2005). The general functions of tablet components are as discussed below:

1.2.4.1 Active Pharmaceutical Ingredient (API)

Almost all tablets, except placebo tablets for experimental purposes, contain one or more active ingredients. This is the ingredient responsible for the desired or observed pharmacological effect. The pharmaceutical ingredient makes the "dose" in tablet as a dosage unit. When the API in the formula is <150 mg it is "low dose" and when it is ≥ 150 mg - 299 mg, it is " medium dose" and the tablet is termed "high dose" when it is ≥ 300 mg (Shiihii *et al*., 2012).

1.2.4.2 Pharmaceutical Excipients

Pharmaceutical excipients can be defined as any substance other than the API that has been appropriately evaluated for safety and is included in API delivery system to either:

- aid processing of the system during manufacturing
- protect, support or enhance stability, bioavailability or patient acceptability
- assist in product identification and
- enhance any other attribute of the overall safety and effectiveness of API product during storage or use (Armstrong,1997).

Ideally, all excipients must be chemically inert, non-hygroscopic, compatible with API, non-toxic, have acceptable taste and be cheap. The pharmaceutical industry classified excipients as follows:

Diluents:

Diluents are excipients commonly used in tablet formulations to bulk up the size of the drug material for ease of compression into tablets. Some drugs, because of their potency e.g. digoxin, are needed in small quantities which becomes a problem and diluents are used to bulk them up to at least a size of 150 mg Rubinstein (1988). Diluents are equally used to promote the flow

properties and compression characteristics of tablet mix (Allen *et al*., 2005). The examples of commonly used diluents in direct compression are dicalcium phosphate, lactose BP, mannitol, microcrystalline cellulose, sodium chloride and sucrose e.t.c.

Requirements of a Good diluent

A good diluent should posses the filling characteristics

- Chemical inertness, biocompatibility and cheap
- Non-hygroscopic with good biopharmaceutical properties.
- an acceptable taste
- optimum particle size or be capable of being size reduced to match the particle size distribution of the API.

Diluents used in wet granulation method are displayed in Table1.2.

Table 1.2 Some Diluents used in Wet Granulation Method

Diluent	**Comments (Remarks)**
Dextrose	Hygroscopic
Dicalcium phosphate	Inexpensive, insoluble in water
Lactose BP	Inexpensive, relatively inert; the most frequently used diluent
Mannitol	Freely soluble; used particularly for chewable tablets
Microcrystalline cellulose	Excellent compression properties; has some disintegrating ability
Sodium chloride	Freely soluble; used for soluble tablets
Sucrose	Sweet taste but hygroscopic; may not be diluted with lactose

Adapted from Rubinstein, 1988

Binders:

Formulation of a good tablet is dependent on the flow properties of the powder particles to be compressed and also their ability to stick to one another after compression. Some powders may possess the ability to flow well and gum together after compression, while other materials need

to have such qualities imparted on them. Optimum concentration of binder is used to form granules that would improve flow ensuring uniform die fill during compression and give the tablet the requisite hardness to withstand rigors of transportation and handling. Insufficient binder gives soft tablets while too much of it leads to the production of too hard tablets that may present with prolonged disintegration time and associated problems with bioavailability. Performance of a binder in tableting is also dependent on the type of binder used. Examples of materials used as binder include: Starch , Glucose, Gelatin, Acacia, Sodium alginate and Sucrose had been and are still in used as binders in tableting (Gohel *et al*., 2005).

Disintegrating Agents:

Tablets after ingestion and upon getting in contact with gastric fluids are expected to disintegrate back to the former granular or particulate state, prior to the API getting dissolved and finally absorbed to elicit the expected pharmacological activity. Disintegration of tablets to granules, facilitated by a disintegrant, presents more surface area and the action of the dissolution medium. A disintegrant, therefore, is that ingredient added in tablets and responsible for break up into small fragments, which promote rapid drug dissolution. The rate of disintegration of tablets is often dependent on type and concentration of disintegrant used. Disintegration process proceeds via two steps as follows:

- The liquid wets the solid and penetrates the pores of the tablet and the tablet breaks into smaller fragments (aggregates of primary particles).
- The aggregate will deaggregate into their primary powder particles.

The mode of action of disintegration can be classified as follows:

- Facilitate water uptake into the pores of tablet, i.e. capillary action e.g. surface active agents.
- Facilitate rupture of tablet by swelling during swelling action, e.g. starch, modified cellulose (Chowdary *et al*., 2012).
- Release of gases to disrupt the tablet structure, normally carbon dioxide, in contact with water, e.g. effervescent tablets.
- Recovery of deformed particles to their original shape in contact with water.

The method of adding disintegrant is as follows:

(1) mixed with other ingredients prior to granulation and thus incorporated with the granules (intragranular addition).

(2) Mixed with dry granules before the complete powder mix is compacted (extragranular addition).

(3) Incorporated as both an intragranular and extragranular portion.

The commonly used disintegrants are: sodium starch glycolate, crosspovidone, microcrystalline cellulose, starch, gums- such as agar, sodium alginate, e.t.c.

Sweetening Agents:

Some therapeutic agents are bitter and so is their tablet after formulation, posing palatability problem especially in children. Bitter tastes in tablets are masked by a process called coating. Sweetening agents are used in chewable tablet to exclude or limit the use of sugar in the tablets. Tablets are coated using three main methods: sugar coating, film coating and compression coating (Davies, 2009). Mannitol and sucrose are examples of materials commonly used to sugar coat tablets.

Colouring Agents:

These are used principally for aesthetic reasons, so as to make the tablet more appealing to the patient. Allam and Kumar (2011) referred to colourants as cosmetics for the pharmaceutical dosage forms. Also colour in tablet helps manufacturer to control product during its preparation as well as serve as a means of identification to the user.

During tableting, colourants are usually incorporated with the binder solution prior to granulation. This is however associated with the problem of colour migration during the process of drying. Solvent migration leads to transfer of colours giving rise to the production of tablets with varying colour shades (mottled appearance). This can however be prevented when the granules are dried slowly with constant stirring. A similar coloured lubricant can also be used to achieve proper colour. Materials used as colouring agents include: Red No.3, Yellow No.6 (Sunset yellow FCF), Blue No.2, e.t.c. (Otto *et al*., 2005).

Lubricating Agents:

Lubricants are agents used to reduce friction between moving parts. In tableting, they perform multiple functions like:

- ensuring smooth ejection of the tablet from the die cavity
- facilitating frictionless movement between the punches and the die during compression and ejection of tablet
- enhance the flow properties of the granules (Davies, 2009)

They are added in small amounts commonly not greater than 1 % of the tablet weight. Insufficient quantity of lubricants may give rise to picking, capping, sticking, binding to the cavity while too much of it may lead to decrease in tablet hardness, increase in tablet

disintegration time and decrease in rate of dissolution of the tablet (Davies, 2009). Some commonly used lubricants are displayed in Table 1.3.

Table 1.3: Lubricants commonly used in tableting

Lubricants	**Concentration (% w/w)**	**Composition**
Magnesium stearate	0.2- 5.0	Not a pure compound. A mixture of magnesium salts of a range of fatty Acids (stearic and palmitic acids)
Stearic acid `	1.0- 3.0	Similar to magnesium stearate. A mixture of stearic and palmitic acids
Glyceryl behenate `	1.0- 3.0	A mixture of glyceryl fatty acid esters
Glyceryl palmitostearate	1.0- 3.0	A mixture of mono-, di - and triglycerides fatty acids containing between 16 and 18 carbon atom.

(Adapted from Davies, 2008)

Glidants:

Glidants improve the flowability properties of a powder mixture; they regulate the flow characteristics of the granules from the hopper into the die cavity. Glidants act by reducing the inter-particulate cohesive forces and consequently permit the granule's smooth and uniform flow. Uniform flow ensures uniform fill of die which eventually gives uniform tablet weights similar to lubricants, they are also added in small quatities. A glidant of choice is fumed silica (Lund, 1994). One with other commonly used glidants are starch and talc.

1.3 Direct compression excipients

It has been estimated that less than 20 percent of pharmaceutical materials can be compressed directly into tablets (Larry, *et al*., 2008). The rest of the materials lack flow, cohesion or lubricating properties necessary for the production of tablets by direct compression. Excipients with optimal functionality are needed to ensure smooth tablet production on modern machines (Jivraj *et al*., 2000).

1.3.1 Ideal Requirements of Direct Compression Excipients

Direct compression excipients should meet the following requirements:

- it should be free flowing, because flowability is required in case of high speed rotary tablet machines, in order to ensure homogenous and rapid flow of powder for uniform die filling.
- It should have high compressibility potential is required for satisfactory tableting.
- A direct compression excipient should have high dilution potential so that the final dosage form has a minimum possible weight.
- The excipient should remain unchanged chemically and physically.
- It should not interfere with the biological availability of active ingredient(s).
- It should be compatible with all the excipients present in the formulation.
- It should not interfere with the disintegration or dissolution of the active ingredient.
- It should be colourless and tasteless.
- It should show low lubricant sensitivity.
- It should have a particle size equivalent to the active ingredients present in the formulation (Jivraj *et al*, 2000).

Many common manufacturing problems are attributed to inadequate powder properties, including non-uniformity in blending, under or over dosage and inaccurate die filling (Smewing, 2002).

The particle size distribution should be consistent from batch to batch. Reproducible particle size distribution is necessary to achieve uniform blending with the active ingredient(s) in order to avoid segregation as outline in Table 1.4.

Table 1.4: Ideal requirements, advantages and limitations of direct compression

Ideal requirement	Advantage	Limitation

Flowability	Cost effective production	Segregation
Compressibilty	Better stability of API	Variation in functionality
Dilution potential	Fast dissolution	Low dilution potential
Reworkability	Less wear and tear of punches	Reworkability
Stability	Simplified validation	Poor compressibilty of API
Controlled particle size	Lower microbial contamination	Lubricant sensitivity.

(Adapted from Jivraj *et al*, 2000)

1.3.2 Methods of preparing direct compression excipients

Direct compression excipients can be prepared by various methods. The outline and main features of the methods are depicted in Table 1.5.

Table 1.5: Summary of Various Methods used to Prepare Direct Compression Excipient

Method	Advantages & Limitations	Example
Chemical modification	Relative expensive, requires toxicological data. Time Consuming.	Ethyl cellulose, Methyl cellulose, Hydrxyproylmethylcellulose, and Carboxy methyl cellulose from Cellulose,cyclodextrin from starch, lacitol.
Physical modification Grinding &/or sieving	Relatively simple and economical, compressibility may alter	Dextrose or compressible sugar, Sorbitol, α-Lactose monohydrate, Dibasic calcium phosphate
Crystallization	Impart flowability to excipients. Requires stringent control on possible polymorphic conversions	β-Lactose, Dipac
Spray drying	Spherical shape and uniform size, Good flowability, poor Reworkability	Spray-dried lactose, Emedex, Fast Flo Lactose, Avicel pH, Advantose 100
Granulation/ Agglomeration	Transformation of small, cohesive Poorly flowable powders into a Flowable and directly Compressible.	Granulated lacitol, Tablettose.
Dehydration	Increased binding properties by thermal & chemical modification	Anhydrous α-Lactose

(Adapted from Shangraw *et al*.,1987)

1.3.3 Advantages and Properties of Multifunctional Excipients

Co-processing excipients lead to the formation of excipient granulates/particulates with superior properties compared with physical mixtures of components or with individual components. The

process is carried out to bring about a synergistic change in the individual undesirable property or improve same. Co-processing is based on the novel concept of two or more excipients interacting at the sub-particle level, the objective of which is to provide a synergy of functionality improvement as well as masking the undesirable properties of individual excipient (Reimerdes, 1993). The following parameters are being adduced for co-processed excipients.

- *Improved flow properties:*

Co-processed excipients show superior flow properties by controlled optimal particle size and particle-size distribution,e.g. cellactose shows better flow characteristics than lactose or a mixture of cellulose and lactose (York, 1992).

- *Improved compressibility:*

There is a tremendous improvement in the pressure hardness relation of co-processed excipients, as compared with simple physical mixtures. The co-processed excipents have a marked improvement in their compressibility profiles, e.g. cellactose, SMCC and ludipress shows superior compressible properties than simple physical mixtures of their constituent excipients (silicon dioxide, microcrystaline cellulose) (Sherwood *et al*., 2012 and Decorkar *et al*., 2011).

- *Better dilution potential:*

Dilution potential is the ability of the excipient to retain its compressibility even when diluted with another material. Most active drug substances are poorly compressible, and as a result, excipients must have better compressibility properties to retain good compaction even when diluted with a poorly compressible agent. Cellactose is shown to have a higher dilution potential than a physical mixture of its constituent excipients (Flores *et al*., 2000).

- *Reduced lubricant sensitivity:*

Most co-processed products consist of a relatively large amount of brittle material such as lactose monohydrate and a small amount of plastic material such as cellulose that is fixed between or on the particles of the brittle material (Maarschalk, *et al*, 1999). The plastic material provides good bonding properties because it creates continuous matrix with a large surface for bonding. The large amount of brittle material provides low lubricant sensitivity because it prevents the formation of a coherent lubricant network by forming newly exposed surfaces upon compression, thus breaking up the lubricant network formation to bonds.

- *Fill weight variation:*

In general, materials for direct compression tend to show high fill weight variations as a result of poor flow properties, but co-processed excipients, when compared with simple mixtures or

parent materials, have been shown to have lower fill-weight variation problems (Mukesh *et al*., 2011). The primary reason for this phenomenon is the impregnation of one particle into the matrix of another, which reduces the rough particle surfaces and creates a near-optimal size distribution, causing better flow properties. Fill-weight variation tends to be more prominent with high speed compression machines. Fill-weight variation was studied with various machine speeds for SMCC and MCC, and SMCC showed less fill-weight variation than MCC, Moreton (2009).

Co-processed excipients have the following additional advantages:

- Economy-: the overall product cost decreases because of improved functionality and fewer test requirements compared with individual excipients.
- Co-processing of excipients help in designing tailor-made excipients. They can retain functional advantages and selectively reduce disadvantages.
- Reduction in development timelines and process validation efforts.
- Co-processed excipients by virtue of advantages hold the possibility of patenting the dosage form.

1.3.4 Limitations

- Major limitation of co-processed excipients mixture is the ratio of the excipients in a mixture is fixed and in developing a new formulation, a fixed ratio of the excipients may not be an optimal choice for the API and dose per tablet under development.
- Co-processed excipient lacks official acceptance in Pharmacopoeia.

1.3.5 Co-processed Excipients

A Co-processed excipient is defined as a unique blend of two or more established excipients interacting at sub particle level. Co-processing of excipients could lead to the formation of excipients with superior properties compared to the simple physical mixtures of their components. In Co-processing, the products are treated in a special way without altering the chemical structure (Block *et al*., 2009). The mechanism that occurs during the co-processing procedure is not fully understood but appears to yield a particulate product in which the components are in intimate association with each other. This intimate association cannot be achieved through simple dry blending of components, but rather requires that they can be co-processed by an appropriate process. Development of Co-processed direct compression excipients start with the selection of the excipients to be combined, their target proportion,

selection of preparation method to get optimized product with desired physico-chemical parameters and it ends with minimizing avoidance with batch-to-batch variations (Gohel and Jogani, 2005).

Co-processed excipients designed for modification of physical properties which was not achievable by simple physical mixing. Excipients with improved functionality can be obtained by developing new chemical excipients, new grades of existing and new combinations of materials (Schoneker, 2011).

1.3.6 Selection of the Excipients to be Co-processed

Excipients selection is most important task to go for co-processing technique. Materials, by virtue of their response to applied forces, can be classified as elastic, plastic, or brittle material. But pharmaceutical materials exhibit all three types of behaviour, with one type being the predominant response. This makes it difficult to demarcate which property is good for compressibility. Maarschalk (1999), reported that co-processing performed with a large amount of brittle material and small amount of plastic material, as exemplified by Cellactose (Meggle Corp.) in which 75 % lactose (brittle material) is co-processed with 25 % cellulose (plastic material). This combination prevents the storage of too much elastic energy during compression, which results in a small amount of stress relaxation and a reduced tendency of capping and lamination. However, examples of the other extreme also exist (e.g., SMCC has a large amount of MCC (plastic material) and a small amount of silicon dioxide [brittle material]). These two situations exemplify the fact that co-processing is generally performed with a combination of materials that have plastic deformation and brittle fragmentation characteristics. Hence, co-processing these two kinds of materials produces a synergistic effect, in term of compressibility, by selectively overcoming the disadvantages. Such combinations can help improve functionalities such as compaction performance, flow properties, strain-rate sensitivity, lubricant sensitivity or sensitivity to moisture, or reduced hornification. A distribution of brittle and plastic materials as co-processed excipients, is displayed in Table 1.6.

Table 1.6: Co-processed excipients developed by co-processing brittle and plastic materials.

Excipients Co-processed		**Improved properties compared to physical blend**
Brittle Component	**Plastic Component**	

Colloidal silicon Dioxide	MCC	Novel MCC based excipient is free flowing, posses excellent disintegration properties has improved compressibility relatively to normal off the shelf commercially available MCC.
Dibasic calcium Phosphate	HPMC crospovidone	Has increase flowability, an increase API loading and blending and higher compacaability
Calcium Phosphate	MCC	Novel MCC based excipient has improved compactability and recompactabilty
β lactose	Sorbital	Produce tablet with improved recompactability
Calcium carbonate	MCC	Novel MCC based excipients has improved recompactability
Lactose `	Polyviny Pyrrolidine (PVP) Crospovidone	Novel excipient posses good flowability and good compresibility under low pressure Produce tablets that exhibit excellent disintegration properties coupled with great hardness and low abrasion.

(Adapted from Sherwood *et al*., 2012)

1.3.7 Steps Involved in Co-processing

The actual process of developing a co-processed excipient involves the following steps:

a) Identifying the group of excipients to be co-processed by carefully studying the material characteristics and functionality requirements.

b) Selecting the proportions of various excipients.

c) Assessing the particle size required for co-processing. This is especially important when one of the components is processed in a dispersed phase. Post processing the particle size of the latter depends on its initial particle size.

d) Selecting a suitable process of drying such as spray or flash drying.

e) Development of controlled production parameters such as flow rate, compressibility index to avoid batch to batch variation.

1.4 Methods of Tableting

Several methods of tableting are employed in Pharmacy, and include direct compression, dry granulation or wet granulation. Choice of method is guided by the physical and chemical stability of the active agent, the nature of the excipient, available facilities and operational cost, (Jones, 2008). Most of the pharmaceutical manufacturers are opting for direct compression tableting due to the fact that it requires fewer processing steps, simplified validation, elimination of heat and moisture, economy, and improved drug stability compared with wet granulation technique. Dry granulation requires control of more processing variables than the direct compression. Reproducibility of the product is difficult to achieve in dry granulation. Hence, the current trend in the pharmaceutical industry is to adopt direct compression technology.

1.4.1 Direct Compression

Although simple in terms of unit processes involved, the direct compression process is highly influenced by powder characteristics such as flowability, compressibility and dilution potential. No single material is likely to exhibit all the ideal characteristics. The physico-chemical properties of excipients that ensure a robust and successful process are good flowability, good compressibility, low or no moisture sensitivity, low lubricant sensitivity, and good machineability even in high speed tableting machines with low dwell times (Armstrong and Palfrey, 2001). For a drug to be formulated into high dose tablet using direct compression method, the API would have to be free flowing and compressible (Shiihii *et al*., 2012). On the other hand, the bulking agent needs to possess appropriate flow and good compressibility properties for it to be used in formulating low dose drugs (Muazu *et al*., 2011). The particle size distribution of powder is optimized prior to compression to ensure uniformity in dosage unit. Direct compression is becoming popular as it circumvents lots of unit operation processes associated with other tableting techniques especially the wet granulation method. This makes it the manufactures' preferred method as it offers the advantage of saving cost and time (Davies, 2009).

1.4.1.1 Advantages of Direct Compression

1. A prime advantage of direct compression over wet granulation is economic since the direct compression requires fewer unit operations. This means less equipment, lower power consumption, less space, time and labour leading to reduced production cost of tablets (Chowdary *et al*., 2012).

2. Direct compression is more suitable for moisture and heat sensitive APIs, since it eliminates wetting and drying steps and increases the stability of active ingredients by reducing detrimental effects.

3. Changes in dissolution profiles are less likely to occur in tablets made by direct compression on storage than in those made from granulations (Patrick *et al*., 2000). This is extremely important because the official compendium now requires dissolution specifications in most solid dosage forms (Gohel *et al*., 2004).

4. Disintegration or dissolution is the rate-limiting step in absorption in the case of tablets of poorly soluble API prepared by wet granulation. The tablets prepared by direct

compression disintegrate into API particles instead of granules that directly come into contact with dissolution fluid and exhibit comparatively faster dissolution.

5. The high compaction pressure involved in the production of tablets by slugging or roller compaction can be avoided by adopting direct compression. The chances of wear and tear of punches and dies are less.

6. Materials are ‘’in process’’ for a shorter period of time, resulting in less chance for contamination or cross contamination, and making it easier to meet the requirements of current good manufacturing practices (Chowdary *et al*., 2012).

7. Due to fewer unit operations, the validation and documentation requirements are reduced. Due to the absence of water in granulation, chances of microbial growth are minimal in tablets prepared by direct compression (Ibrahim and Olurinola, 1991).

1.4.1.2 Limitations of Direct Compression

(1) It is prone to segregation due to the difference in density of the API and excipients. The dry state of the material during mixing may induce static charge and lead to segregation. This may lead to problems like weight variation and content uniformity.

(2) Direct compression excipients are relatively expensive because they are special products produced by patented spray drying, fluid bed drying, roller drying or co-crystallization or melt method.

(3) Most of the direct compression materials can accommodate only 30-40% of the poorly compressible active ingredients like acetaminophen which means the weight of the final tablet to deliver the 500 mg of acetaminophen would be more than 1300 mg. The large tablets may create difficulty in swallowing.

(4) All the spray- dried direct compression excipients show poor reworkability since on preparation of tablets, the original spherical nature of the excipient particles is lost. API that has poor flow properties and /or low bulk density is difficult to process by direct compression (Pranav, 2005).

(5) Lubricants have more adverse effect on the filler, which exhibit almost no fracture or shear on compression (e.g. Starch R□1500). The softening effects as well as the hydrophobic effect of alkaline stearate can be controlled by optimising the length of blending time to as little as 2-5 min (Shangraw, 2003).

Table 1.7: Advantages and Limitations of Tablet Manufacturing Methods

Methods	Advantages	Limitations
Direct Compression	Simple, economical process No heat or moisture, so good for unstable compounds	Not suitable for all API, generally limited to lower dose compound Segregation potential Expensive excipient.
Wet Granulation	Robust process, suitable for Most compounds. Impart flowability to Formulation Reduce elasticity problems. Coating surface with Hydrophillic polymer can Improve wettability. Binds API with excipient, thus Reducing segregation potential	Expensive, time and Energy consuming. Specialized equipment required. Stability issues for moisture sensitive and thermolabile API with Aqueous granulation
Wet Granulation (non aqueous)	Suitable for moisture sensitive API Vacuum drying techniques can Remove/reduce need for heat	Expensive equipment Need organic facility. Solvent recovery issues
Dry Granulation	Eliminate exposure to moisture and drying	Dusty procedure. Not suitable for all compounds. Slow process.

(Jayesh et al, 2009)

1.5 Tableting Machine

There are basically two types of tableting machines that are commonly used. Tablets may be compressed in single punch machines which have an output of 60-90 tablets per minute or on multi-station tablet machines exemplified by rotary tablet press which produces up to 50,000 tablets per minute. With new development in machinery, sophisticated tableting machines that can produce as much as 500,000 tablets per minute are now available (Larry *et al*., 2008).

1.5.1 Single Punch Machines

Single punch tablet presses are only indicated in small scale pilot production. They can be driven either manually or electrically. The machine, consist of a frame containing horizontal plate into which the die is fitted. Attached to this plate is the feed shoe containing the granules to be compressed. The lower punch assembly has two collars on it. The bottom collar regulates the dept to which the lower punch descends during a compression cycle, which in turn controls the volume and therefore the weight of granules to be compressed. The upper collar allows the punch to be adjusted so that at the top of compression stroke which is the point of ejection of the tablet, it is levelled with the upper surface of the die. The upper punch is also fitted with a collar which adjust the dept to which the punch descends into die. By this means, the compression pressure can be adjusted as required. A compression cycle in a single punch machine involves the following steps:

- The feed shoe moves over the die-cavity
- The lower punch descends to a preset level
- The granules flow from the hopper into the feed shoe and fills the die cavity
- The upper punch descends into the die-cavity and compresses the granules
- Upper punch and lower punch both ascends and ejects the tablet from the die-cavity.

1.6 Compression and Compaction

Compression is the process of applying pressure to material while compaction is the series of event that occur when the pressure is applied to powders in the die cavity up to the time when the tablet is ejected from the die cavity. In compaction, particles are brought together close enough for the binding forces to bind the particles to form a mass (tablet).

1.6.1 Sequence of Events in Compaction of Powders

According to Hyunjo *et al* (1998) and Handcock and Zografi (1997), the sequence of events during compaction are as follows:

- Transitional repacking or particle rearrangements
- Deformation at points of contact
- Fragmentation and/ or deformation
- Bonding
- Deformation of solid body
- Decompression
- Ejection.

1.6.1.1 Transition or particle rearrangement

Under pressure, particles get rearranged in such a way that the smallest ones fill the void created by the larger particles. This leads to closer packing and a decrease in relative volume, thereby increasing the density of the powder bed. The shape of the particle influences such rearrangement.

1.6.1.2 Deformation at the point of contact

Increase in the applied pressure is likely to lead to deformation of particles at the point of contact. The type of deformation that occurs depends on the nature of particles being compressed. The deformation can either be elastic or plastic in nature.

1.6.1.3 Fragmentation

This is the breaking of the particles into smaller parts especially bigger granules. When the applied pressure is high, particles crack and the new cracks expose fresh surfaces which yield potential bonding areas (Gustafson, 2000).

1.6.1.4 Bonding

This is the permanent attachment of particles to each other resulting from close proximity; the closer the distance between the two particles the greater the attachment.

1.6.1.5 Deformation of solid body

As the applied pressure is increased, the bonded solids consolidate. The deformation can either be elastic or plastic; the ability of formulated powder to form a satisfactory tablet depends on its plastic deformation during compression and elastic recovery during ejection (decompression).

1.6.1.6 Decompression

This is the series of subsidiary events that occur after applied pressure is removed from the upper punch. As the upper punch is removed from the die cavity, the residual pressure confines the tablet. The ability or otherwise to produce intact tablet depends on the pressure exerted by elastic rebound and the associated deformation process during decompression/ ejection.

1.6.1.7 Ejection

This is the process by which compressed tablet is removed from the die as the lower punch rises and pushes the tablet upward. There is continuous die wall pressure and considerable energy may be expended due to die wall friction. When the tablet is removed from the die and lateral pressure is removed, the tablet undergoes plastic recovery with an increase in volume of that portion of the tablet removed from the die**.**

1.7 Evaluation of Tablet

To ensure that tablets meet some physical specifications in terms of quality, tablets are subjected to the following tests:

- Crushing strength
- Disintegration time
- Friability
- Dissolution time
- Weight uniformity
- Thickness and diameter (Armstrong, 1997).

These specifications must be controlled within the production of a batch of tablets as well as batch-to-batch in order to guarantee not only the outward appearance of the product but also its therapeutic efficacy. While some of these quality tests are official, there are others which are not and have only been added by various manufacturers to ensure that their tablets are of very high quality.

1.7.1 Crushing Strength Test

In 1930, a small portable hand tester was introduced by Monsanto. It measures the force required to break the tablet, the force generated by the coiled spring is applied diametrically to the tablet and the force is measured in kilograms force (Kgf). For an acceptable tablet, it is usually 4 to 7 KgF. Strong-Cobb hardness tester was introduced in 1950 and this also measures diametrically applied force required to break the tablet.

Crushing strength is measured throughout the production cycle to detect any need for pressure adjustment. If the tablet is too hard, it may not disintegrate within the required period of time to meet the dissolution specification. And if it is too soft it will not withstand the rigors of handling during subsequent processing such as counting or packing and shipping operation, therefore there is need for a balance in crushing strength.

1.7.2 Disintegration Test

Tablet disintegration test is used as a quality assurance measure. It is not a true predictor on how well the dosage form will release its active ingredient *in vivo***.** The United States Pharmacopoeia (USP) sets standard for tablet disintegration test. The apparatus is relatively simple and it consists of a basket rack holding six plastic tubes which are open at the top and the bottom. The bottom is covered with a 10 mesh screen. The rack is immersed in a suitable liquid at 37± 1 °C. It moves up and down at a specific speed. One tablet is placed into each tube and the time taken by the tablets to disintegrate and pass through the screen is noted. To pass the test, all tablet(s) must pass through the mesh in 15 minutes for uncoated tablets (B.P, 2002).

1.7.3. Friability Test

A tablet must be sufficiently robust to withstand chipping, capping or breaking. These problems arise most frequently as a result of friction and shock. Roche friabilator is usually used to measure resistance to abrassion in tablets. Twenty tablets are weighed and placed in the drum in which a curved baffle is mounted. The drum is rotated at 25 r.p.m for 4 min making the tablets roll and drop. The cylinder section includes self-abrasion of the tablet as it rotates, while they undergo shock as they fall six inches on each turn. The tablets are then removed, dusted and weighed. There should not be more than 1 % decrease in weight. The hardness and friability of tablets are measures of their mechanical strength. They thus give an indication of the extent of cohesiveness among the particles in the tablet (Duberg *et al*., 2000).

1.7.4 Dissolution Test

This test provides a means of determining the rate at which a drug goes into solution and hence its rate and extent of absorption from the tablet formulation. USP and BP (2002) dissolution test apparatus consists of four (4) main parts:

- A water bath
- A cylindrical stainless steel basket made of woven wire cloth
- A covered vessel with opening for withdrawing and replacing samples, inserting a thermometer and passing through the basket into the medium
- Available speed motor.

The temperature is maintained at 37± 0.5 °C and the dissolution medium and volume is specified in the monographs. The dissolution medium is poured into the covered vessel and one tablet is placed in the basket. The basket is lowered into the medium and is situated 2 cm above the bottom of the vessel and rotated at the rate specified in the monograph. Samples are withdrawn at a fixed time intervals and analyzed for drug content.

By plotting the percentage drug release versus time, the time taken for 50 % of the drug to dissolve ($T_{50\,\%}$) can be determined, and ($T_{90\,\%}$) is also usually determined**.** The dissolution area (area under the curve) can also be measured and compared with the dissolution at any point in time to give the extent of dissolution.

1.7.5 Weight Uniformity

This is done by weighing 20 tablets individually; and the mean weight is calculated. Not more than two tablets should deviate from the mean weight. Depending on the weight of the active ingredient, the deviation should be within the range shown in Table 1.8 .

Table 1.8: Average Tablet Weight percentage deviation allowed

Average Weight (mg)	Percentage deviation
130 or less	± 10.0
>130-324	± 7.5
>324	± 5

(Adapted from BP, 2009)

1.7.6 Tablet Thickness

Tablet thickness can vary without necessary variation in weight because of difference in the density of granules and the pressure applied to the tablet as well as speed. Tablet thickness is important because it is desirable for reproducibility and uniformity. It ensures that every batch will be usable with selected packaging component especially sachets. Some filling equipment utilizes the uniform thickness of tablet as a counting mechanism. Tablet thickness is measured by micrometer screw gauge and a deviation of ± 5 % is allowed (Mukesh., 2005).

1.8 Technical Problems During Tableting

An ideal tablet should be free from visual or functional defects, have correct dose of drug, elegant and its weight, size and appearance should be consistent, the drug should be released from the tablet in a controlled and reproducible, have sufficient mechanical strength to withstand fracture and erosion during handling (Mukesh *et al*., 2011).

Tablets without the above quality have defects, which include: binding, chipping and cracking, capping and laminating, sticking, picking and weight variation (Smewing *et al*., 2002)

1.8.1 Binding

This is the adhesion of granules to the die wall and this causes the resistance of the tablet to eject from the die, it is usually due to insufficient lubrication, which produce tablets with rough and vertical score marks on the edges.

Solution: increase lubrication, improve lubricant distribution and increase the moisture content of the granules.

1.8.2 Chipping and Cracking

This is the breaking of tablet edges while the tablet leaves the press or during subsequent handling and coating operations. Chipping may be due to incorrect machine settings, specially mis-set ejection take-off (Rasenack *et al*., 2002).

Solution: the machine for compression should be correctly set before tablet compression.

1.8.3 Capping

This occurs when the upper segment of the tablet separates from the main portion of the tablet and comes off as a cap. It is usually due to air entrapped in the granulation which is compressed in the die during the compression and then expands when the pressure is released.

Reasons for capping: due to large amount of fines in the granulation and/ or the lack of sufficient clearance between the punch and the die wall, in new punches and dies that are tight fitting and too much or too little lubricant or excessive moisture.

Solution: appropriate lubricant be added, punches and die should be well dried.

1.8.4 Lamination

This is due to the same causes as capping except that the tablet splits at the sides into two or more parts. If the tablets laminate only at a certain station, the tooling is usually the cause.

Solution for capping and lamination: increase the binder, add dry binder such as gum acacia, PVP or powdered sugar and then decrease the upper punch diameter (Shangraw *et al*., 1997).

1.8.5 Sticking

Is usually due to improper (incorrectly) dried or lubricated granulation causing the whole tablet surface to stick to the punch faces thereby resulting in→ *dull, scratched, or rough tablet faces* (Rasenack *et al*., 2002),

Solution: the material should be properly dried and right amount of lubricant added.

1.8.6 Picking

Is a form of sticking in which a small portion of granules sticks to the punch face and a portion of the tablet surface is detached from tablet.

Solution for sticking and picking: increase cohesiveness of granules and select lubricant in desired proportion which will minimize this problem, selection of binding agent is also essential to solve the problem of sticking.

1.8.7 Weight variation

Mainly due to the size and distribution of the granules being compressed (presence of too large fine granules), poor flow (cause incomplete filling of the die), poor mixing (sometimes the lubricants and glidants have not been well distributed) and when lower punches are of unequal lengths the fill of each die varies because the fill is volumetric (Gohel *et al*., 2007).

Solution: only a good punch and die control program can provide tooling of uniform dimensions, change lubricant and/ or glidant.

1. 9 Statement of Research Problem

Nigeria depends largely on imported raw materials and finished products for the supply of essential medicines. This has led to the depletion of foreign exchange and nonavailability of jobs, promoting poverty and poor social development.

Direct compression is the most popular because it provide the shortest, most effective and least complex way to produce tablet, but it has been estimated that less than 20 percent of the pharmaceutical materials can be compressed directly into tablets (Larry *et al*., 2008). The rest of the materials lack flow, cohesion or lubricant properties necessary for production of tablets by direct compression which is a big problem to direct compression method. Calcium phosphate currently being imported into the country though relatively inexpensive is not readily available and is a single pharmaceutical excipient which is not compressible alone. This has been a challenge to our local pharmaceutical manufacturer to have readily available excipients for use in pharmaceutical industries with multifunctional properties.

1.10 Justification for the study

In 2005, the African Union (AU) Commission at the Abuja Summit gave a mandate to explore local production of generic medicines in Africa. Local production will save foreign exchange, create jobs, facilitate technology transfer and stimulate exports. Also, raw materials produced locally will be readily available and cheaper. Cow bone is locally available and can be converted from waste to wealth and be use in our pharmaceutical industries.

In order to meet the requirements of the direct compression process, pharmaceutical manufacturers need excipients with optimal functionality to ensure smooth tablet production on modern machines.

Calcium phosphate, which is a filler, is practically insoluble in water, with a solubility of 0.02 g per 100 ml at 25 °C and incompressible. It is a dibasic calcium phosphate (usually found as dihydrate, with the chemical formula of $CaHPO_4.2H_2O$) that is converted to anhydrous form and

can be obtained in both a fine particulate form, mainly used in granulation, and an aggregated form. The latter possesses good flowability and used in tablet production by direct compression. Activated cow bone powder (ABP) at 700 °C, 800 °C and 900 ° contained calcium phosphate and was found to be incompressible at a low percentage moisture content, but became compressible in the presence of high percentage moisture content by direct compression (Emenike, 2004). Therefore, co-processing ABP with gelatin is expected to improve compressibility properties and probably better dilution potential and reduced lubricant sensitivity, thereby promoting the direct compressional property of ABP.

1.11 HYPOTHESIS

Ho: The co-processed material from cow bone powder (ABP) and gelatin does not significantly improve its direct compression property.

Hı: The co-processed material from cow bone powder (ABP) and gelatin does significantly improve its direct compression property .

1.12 AIM OF THE STUDY

The aim of the study is to develop an excipient consisting of cow bone powder and gelatin by co-processing method and to evaluate its use in direct compression method of tableting.

1.13 OBJECTIVES

- To collect cow bone from Abbatoir in Samaru-Zaria, Kaduna State, Nigeria and cleaned to remove any attached tissues and of activate it at 600 °C - 800 °C for 6h using electrical operated furnace.
- Pulverization and physicochemical characterization of the activated bone powders (ABP)
- To co-process the powders (Bone powder and gelatin) using the following ratios 90:10, 95:5 and 97.5:2.5.
- To characterise the co-processed powders (Angle of repose, Flow rate, Bulk & Tapped densities, Carr’s Index, Hausner's ratio, Swelling capacity etc)
- To determine the compatibility of the powders via Fourier Transform Infrared (FTIR) and Differential Scanning Calorimeter (DSC).

- To carry out dilution potential studies using ascorbic and metronidazole as model drugs
- To also determine the lubricant sensitivity ratio on the co-processed material.
- To carry out formulation studies using the co-processed excipient using ascorbic acid and metronidazole as model drugs, evaluate the properties of the tablets produced and evaluate the statistical analysis.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. Recent Research Work Done on Co-processed Excipients

The actual process of developing a co-processed excipient involves a sequence of steps such as identifying the group of excipients to be co-processed, by studying the material characteristics and functionality requirements, selecting the proportions of various excipients, assessing the particle size required for co-processing, selecting a suitable process of drying and optimizing the process (Chowdary *et al*., 2013).

2.1.1 Co-processing of Lactose

2.1.1.1 Ludipress®

Uma and Naheed (2014) reported that co-processing of 93.4 % α-lactose monohydrate with 3.2 % povidone and 3.4 % crospovidone resulted in a suitable filler used for direct compression tableting on high speed presses. Ludipress is odourless, tasteless, white free- flowing granules especially developed for direct compression, but is also suitable as filler for hard gelatin capsules. The binding properties of Ludipress, both unlubricated and lubricated with 1 % magnesium stearate, are good and were found to be much better than those of the physical mixture, although Ludipress contains a disintegrant, the disintegration of tablets takes longer than tablets containing α-lactose monohydrate, anhydrous β-lactose, spray- dried lactose or tablettose (Sumit and Aliasgar, 2009). Ashrafi *et al*., (2011) reported that Ludipress, when used in high amount, can extend the sustaining effect of the formulation to some extent.

Baykara *et al* . (2008), reported that Ludipress has stable flow proerties but its dilution potential with Acetaminophen is lower than the Avicel PH 101, Elcema G250 or Elcema P050. Sujatha *et al.,* (2013), also evaluated the powder and tableting properties of Ludipress and found it exhibited a good batch-to-batch uniformity and flow characteristics compared with the physical blends and the other excipients investigated.

2.1.1.2 Microcelac100®

This is another marketed spray-dried product, containing a lactose monohydrate (75 %) and MCC (25 %). Armand *et al.* (2002), reported that Microcelac® has both filling properties of lactose and binding capacity of MCC which provides a better tableting performance at lower cost. Muzikova and Zvolankova (2010) found that the tablet strength from pure Cellactose 80® was lower than that of those from Microcelac 100® both without and with lubricant in the compression forces of 6 and 8 kN. Michoel *et al.,* (2002), showed that Microcelac 100® has superior flow and binding properties and do not change even on addition of folic acid. These improved characteristics were attributed to spray-drying (Sumit and Aliasgar., 2009).

Awasthi *et al.,* (2010), developed a direct compression co-processed excipient of solid dosage forms using Lactose and Mannitol by melt granulation method using Acetaminophen (NSAID) as a model drug and found out that the tablets manufactured showed relatively better disintegration time and high compressibility profile.

2.1.2 Co-processing of Starch

2.1.2.1 Starlac®

The latest material on the market is Starlac®, a co-processed filler-binder consisting of 85 % α-lactose monohydrate and 15 % native corn starch (Gohel *et al*., 2003). Starlac® is a bi- functional excipient, used as binder and disintegrant; however, it exhibits the lowest elastic recovery at high binding capacity (Hauschild *et al*., 2004). The advantages of Starlac® are its good flowability depending on the spray-drying process, an acceptable crushing force due to its lactose content, its rapid disintegration depends on starch (Hauschild *et al*., 2004).

Ravi *et al*. (2011) evaluated the co-processed properties of pregelatinized starch obtained from potato with microcrystalline cellulose and found that the properties were superior to their physical mixture and individual excipient.

Shittu *et al*. (2012) also evaluated the co-processed properties of microcrystalline tapioca starch, α-lactose monohydrate and microcrystalline cellulose and found that the properties were superior to their physical mixture of an individual excipient.

Sumit and Aliasgar (2009) also found out that Starlac® is used in low-dosage and fast dissolving formulations, direct compression, dry granulation and homeopathic formulation.

Olowosulu *et al*. (2011) have developed an efficient direct compression tableting excipient (starAc) of co-processed particles of maize starch (MS) and acacia gum (Ac) by co-drying their well dispersed aqueous mixtures for direct compression.

Chukwuma *et al.* (2012) developed and evaluated cassava starch (tapioca starch) as a co-binder with gelatin using wet granulation method to formulate different batches of sodium salicylate tablets with varying binder concentration ratios (25:75, 50:50, 75:25 and 0:100). The results showed that the formulations prepared with gelatin had lower granule friability than those produced with cassava starch-gelatin binder mixture. Moisture uptake studies showed that gelatin batches absorbed more moisture than cassava starch-gelatin batches.

Nidal *et al.* (2010) reported that preparation and characterization of a Novel co-processed excipient of chitin and crystalline mannitol using pregelatinized starch-polyvinyl pyrrolidone in a method of gelatinizing potato starch in the presence of PVP with Ritonavir, Efavirenz, Stavudine (antiretrovirals) as a drug. The results exhibited excellent to good flow properties.

2.1.3 Co-processing of Cellulose

2.1.3.1 Vitacel®

Co-processing of 75 % MCC with 25 % calcium carbonate was carried out in a weight ratio from about 75:25 to 35:65 by Sujatha *et al*. (2013). The product exhibited low lubricant sensitivity; its compression profile (tablet hardness versus tablet compression force) remains relatively unchanged when various lubricants are employed. Jacob *et al*. (2007) fabricated composite particles of rice starch and MCC by spray-drying technique and evaluated its direct compressibility. These composite particles exhibited good compressibility and flowability whereas its tablets showed low friability and good self-disintegrating property. Thus, these developed composite particles could be introduced as a new co-processed excipient for direct compression (Hauschild *et al.*, 2004). Thoorens *et al*. (2011) co-processed microcrystalline cellulose and calcium carbonate, resulting in a product with improved recompactability.

2.1.3.2 Prosolv®

Co-processing of MCC (98 %) with colloidal silicon dioxide (2 %) into SMCC (Prosolv) resulted in improved strength of tablet compacts and reduced sensitivity to wet granulation (Sherwood *et al*., 2012). Deorkar *et al*. (2011) reported that there is no discernible chemical or polymorphic difference among the SMCC, MCC and dry mixes of MCC and silicon dioxide, indicating that the material produced by 'silification' process is chemically and physically very similar to standard MCC. In spite of being very similar structures, analytical techniques such as infra-red (IR) could not provide an explanation for the improvements in compressibility of SMCC over MCC (Rao *et al*., 2012). Also, Amelia *et al. (*2007), co-processed microcrystalline cellulose, colloidal silicon dioxide and sodium starch glycolate, the resulted product have excellent flow properties, high compressibility, render low disintegration time to tablets and have better binding properties.

2.1.4 Co-processing of inorganic fillers

Co-processing of calcium carbonate (70 %) with sorbitol (30 %) (Formaxx®) offers a distinct advantage of producing directly compressible calcium carbonate. Chowdary *et al*. (2013) and Aundhia *et al*. (2011) reported that formaxx® offers improved flowability, superior compaction properties at low compression forces and has low friability compared to calcium carbonate. The unique processing of calcium carbonate with sorbitol masks the chalky and gritty taste of calcium carbonate. Freitag *et al*. (2005) also showed that co-processing of magnesium carbonate with 5 % powdered cellulose seems to be a promising excipient for direct compression. This co-processed product combines to give good flow and tablet properties, and is superior to pure magnesium carbonate or their physical mixture (Ajay *et al*., 2012).

Dicalcium phosphate is the most common inorganic salt used in direct compression as a filler-binder (Autamashi *et al*., 2011). The advantage of using dicalcium phosphate in tablets for vitamin and mineral supplement is the high calcium and phosphorous content. Mourya *et al.* (2012), developed co-processed excipient of dicalcium phosphate and starch (25:75) for the

formulation and evaluation of fast dissolving tablets using Acetaminophen. The excipient) showed optimum compressibility characteristics and tablets showed fast disintegration

Mukesh *et al.* (2011) developed a novel co-processed excipient of physically modified (Nitric acid treated) wheat starch and dicalcium phosphate for the preparation and evaluation of material properties of co-processed diluents containing modified starch and dicalcium phosphate using Acetaminophen and found that there was improved flow and compressibility characteristics. Russell (2004), also co-processed calcium carbonate and starch (Barcroft®), the result gave a great advantages in term of flowability, compression profile and offer advantage to developers and formulators.

2.2 Materials for Co-processing

2.2.1 Cow Bone

Bone is a rigid tissue that performs mechanical, biological and chemical functions. In development of bones, both chemical and physical properties are affected by age, nutrition, hormonal status, and disease (Loveridge, 1999). The skeletal system forms the external structure and appearance of mammalian vertebrate species and has the obvious functions of locomotive, structural support of the body, and protection of soft tissue (brain, heart, spinal cord and lung). Bone also serves as a metabolic reservoir for Calcium, Phosphorous, and other minerals and it houses cells responsible for bone formation and resorption. Bone remodelling provides calcium to meet the demands of egg shell formation in poultry (Dacke *et al*., 2000).

All bone consists of certain minerals; primarily hydroxyapatite (Ca_{10} $(PO_4)_6$ $(OH)_2$ deposited in an organic matrix, of which collagen is the major constituent (Keene *et al*., 2004). Bone mineral is deposited in large number of small crystals, giving bone a large surface area. The surface area of one gram of bone mineral is estimated to be $>100\ m^2$(Lawrence *et al*., 2004). This surface area allows for a rapid interchange of ions between interstitial fluid and bone. Mucalo (2010), found out that cow bone when heated up to a very high temperature of about 1000 °C, produces a by-product that was purely Hydroxyapatite (calcium phosphate) an important mineral constituents that is needed by the body.

Cow bones are waste materials and commonly litter in abattoirs because they have little or no value to the people (Emenike, 2004). However, it has been discovered that cow bone could be used as bone meal to treat bone deformation (Nickola *et al*., 2004).

Emenike (2004), pulverized activated cow bone powder (ABP) and used it to formulate low and high dosage forms of folic and metronidazole as model drugs. He found out that ABP could be used as direct compression excipient to formulate both high and low dose drugs with about 3 % moisture content. Sri *et al*. (2009), carried out studies on the properties of dense hydroxyapatite-extract from cow bone by heating it up to 800 °C before pulverizing and mixing together with binder to form dense hydroxyapatite. The properties were analyzed by scanning electron microscope which showed phase of pure hydroxyapatite up to 1250 °C and fracture strength

result indicated that the mechanical properties of specimen increased as the temperature increased.

Bone char, which is used to process sugar, is made from the bones of cattle mostly from Afghanistan, Argentina, India and Pakistan (Laura, 2012). The bones are sold to traders in Scotland, Egypt, and Brazil who then sell them back to the United State of American's sugar industry. The bones are only accepted in countries that are deemed free of Bovine Spongiform Encephalopathy (BSE) also known as mad cow disease (Keene *et al*., 2004). To prevent the spread of mad cow disease, the skull and spine are never used. The bones are also heated to high temperature in the range of 400-500° C (Wilson *et al*., 2004).

Bone char- often referred to as natural carbon-is widely used by the sugar industry as a decolorizing filter, which allows the sugar cane to achieve its desirable white colour.

Hiller *et al*. (2003), studied the cow bone minerals changes during experimental heating using an X-ray scattering method and found out that as the heat increased the minerals in the bone decomposes, leaving pure Hydroxyapatite .

2.2.2 Industrial Uses of Cow bone:

- Bone char (calcium triphosphate) is used to remove fluoride from water and to filter aquarium water.
- Tricalcium phosphate is used in powdered spices as an anticaking agent.
- Calcium phosphate is also used as rinsing agent (food additive).
- It is often used in the sugar refining industry for decolourizing (a process first reported by Louis Constant in 1812).
- It is used to refine crude oil in the production of petroleum jelly.
- Bone char is also used as a colouring agent.
- It is sometimes used for artist's paint, printmaking, calligraphic & drawing inks as well as other artistic application because of its deepness (Keene, 2003).

2.2.3 Cow bone for human use.

A small, shaped piece of cow bone can be used as a bone substitute in certain types of bone replacement operation (Michael, 2010).The bone is heated in muffler furnace for up to 1000°C to evaporate all organic matter leaving only calcium phosphate. The bone is grounded into powder form and passed through a spraying apparatus which consists of plasma torch with a very high

temperature (Keene *et al*, 2004). The bone powder is passed through this high temperature in which some are partially melted and form splats, build up make a high rough surface of hydroxyapatite called a plasma coating, in the same manner implants are treated to improve their biocompatibility (Keene *et al*., 2004) .

Bone powder is used in direct compression of tablets when pelletized, just like dibasic calcium phosphate (Emcompress®) (Emenike, 2004).

2.3 Gelatin

Gelatin is defined as the product obtained from the acid, alkaline, or enzymatic hydrolysis of collagen, the chief protein component of the skin, bones, and connective tissue of animals including fish and poultry. Gelatin is a natural product, a solid substance, tasteless, colourless and translucent, obtained from partial hydrolysis of collagen.

Gelatin is water soluble with high molecular weight from 15,000 to 250,000 protein. It is a refined and purified protein that functions as a stabilizer, binder and gelling agent, commonly used in soft and hard gel capsules (GMIA, 2012).

Gelatin derived from an acid-treated precursor is known as Type A and gelatin derived from an alkali-treated process is known as Type B (GMIA, 2012).

The commercial production of gelatin started in Holland around 1685, followed shortly by England in 1700 and Massachusetts (USA) in 1808 (GMIA, 2012). Gelatin is an important material, finding application in the food, pharmaceutical and photographic industries as well as diverse technical uses (GMIA, 2012).

Gelatin, in terms of basic elements is composed of 50.5 % carbon, 6.8 % hydrogen, 17 % nitrogen and 25 % oxygen (Ward, 1977).

2.3.1 Health Benefits of gelatin

- It supports skin, hair and nail growth
- Good for joints and can help in recovery from arthritis
- It helps tighten loose skin (like the one from babies in five years)

- It can improve digestion since it naturally binds to water and helps food move more easily through the digestive track.
- It is a source of protein (though not a spectacular one) but its specific amino acids can help build muscle.
- Gelatin is - largely composed of the amino acids, glycine and proline, which many people do not consume in adequate amounts as they are found in the bones, fibrous tissues and organs of animals which many people do not take (Chowdary *et al*., 2010).
- The amino acids found in gelatin are used for optimal immune function and weight regulation.
- Glycine, which makes up to about 1/3 of the amino acids in gelatin powder is anti-inflammatory and this evidence is from its wound healing property (GMIA, 2012).

2.3.2 Pharmaceutical uses of gelatin

- Ademola *et al*. (2011), evaluated the binding property of Cissus (*Cissus polpunea*) gum and gelatin gum in paracetamol tablet formulation. When gelatin was used as a binder, the tablets showed higher crushing strength than those containing the Cissus gum. Also formulations containing Cissus gum had lower disintegration times than the ones containing gelatin.

- As binders in pharmaceutical tablet production, Emeje *et al*. (2007), evaluated the binding property of gelatin and okra (*Hibiscus esculentus*) gum in paracetamol tablet formulation and discovered that gelatin exhibited higher crushing strength.
- For making soft and hard capsules shells
- As stabilizer in production of pharmaceutical suspensions (GMIA, 2012)

- Used for tablet coating to reduce dusting, mask unpleasant taste and allow for printing and color coating for product identification.
- Glycerinated gelatin is used as a vehicle for suppositories for insertion into the rectum, vaginal or the urethra.
- Solution of modified gelatin (3.0-5.5 %) and salts are commonly used as plasma substitute during emergency surgery (Kragh, 1977).

2.3.3 Photographic uses of gelatin

- Gelatin uses for photographic is primarily Type B alkaline processed gelatin, especially for emulsion preparation.
- It serves several functions in the preparation of the silver emulsion.
- Gelatin is used for surface sizing and for coating purposes during paper manufacture.
- Gelatin is used almost universally as the binder for the complex mixture of chemicals used to form the head of a match (Finch, 1989).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3 .1 MATERIALS

3.1.1 Chemicals and Reagents

- Cow bones used were femur and tibia fresh from the abattoir (Samaru-Zaria, Kaduna State, Nigeria)
- Gelatin powder, ascorbic acid powder and xylene (BDH Chemical Ltd, Poole England)
- Magnesium stearate (Hopkin and Williams Ltd, England)
- Metronidazole (Roche, Nigeria PLC)
- Calcium carbonate and starch (Bacroft®) (SPI Pharma, Rockwood Park 503 Carr Road, Wilminton, USA)
- Sodium Starch Glycolate (CHP Carbohydrate E PIRNA GmbH & CoKG, Germany)
- Hydrochloric Acid (Sigma-Aldrich GmbH, Germany)
- Sodium hydroxide pellets (Avondale Laboratories Ltd, Banbury, England)

3.2 Methods

3.2.1 Preparation of Thermally Activated Cow Bone Powder (ABP)

The method of Emenike, (2004) was adopted with modifications to prepare thermally activated cow bone powder. One kilogram of fresh cow femur and tibia bones were collected from the abattoir in Samaru-Zaria, Kaduna State, Nigeria. They were cut into pieces with axe and heated to about 100 °C for 1 h to remove all traces of meat, fat and bone marrow and subsequently cleaned with hot water. The bone pieces were then weighed (w) on Gallenkamp mettler balance (Serial No: 647624, Type P163, CH-8606 Grefensee-Zurich, Switzerland), then allowed to dry for about two days and weighed after drying.

3.2.2 Activation of the Bone at High Temperature

The sample (dried cow bone) was then heated in an industrial furnace (Project F 442 No: 91-442, Type CFR, Holland) at Industrial Development Centre (IDC) Samaru-Zaria, Nigeria at 600 °C and 800 °C for 6 h respectively. It was allowed to cool for 5h to a room temperature of 37 °C and weighed (w_1) on a mettller balance (serial No: 647624, Switzerland).

The percentage loss (% L) in weight was calculated as:

$$L = 100\left(1 - \frac{w_1}{w}\right) \%$$ 1

The % yield (Y) was calculated as

$$Y = 100 \times \frac{w_1}{w} \%$$ 2

Where W = weight of initial bone before drying and

W_1 = final weight of bone after heating in furnace

3.2.3 Pulverization of Thermally Activated Cow Bone Powder

The ABP were placed in an electrical laboratory blender (Model 38 BL 40, Christison, Germany). The machine was started and the bones were grinded for 5 min and passed through a 1.7mm mesh sieve.

3.2.4 Sieve Analysis of the Thermally Activated Cow Bone Powder

A set of Endecott sieves (Endecott, London, UK) was accurately stacked in the following descending order: 500 µm, 250 µm, 180 µm, 90 µm and the collecting pan. A weighed amount (30 gm) of ABP was placed on the topmost sieve and covered with the sieve cover. The set of sieves was clamped on the Endecott sieve shaker (Endecott, London, UK) and set to vibrate for 15 min. The weight of ABP retained on each sieve was taken and percentage cumulative weight oversize was plotted against sieve size.

3.2.5 Calculation of the Mean Particle Size (MPS)

MPS is related to the granule/particle size(s) in micrometer retained on the sieve and percentage weight (w) retained on the sieve as:

$$MPS = \Sigma SW$$ 3

Where S = sieve size

W = weight of the particles retained

3.2.6 Organoleptic Properties of ABP

The colour, odour, taste and texture of the powder (ABP) were observed and noted.

3.2.7 Chemical test for presence of Calcium in the ABP

A 5 gm quantity of ABP using Triple Acid Digestion method adopted from Maria *et al*, (2010). A 30 ml of mixed acid (concentrated nitric acid (HNO_3), concentrated perchloric acid ($HClO_4$) and concentrated sulphuric acid (H_2SO_4) in a ratio of 65 : 6 : 2 respectively) was added into 50 ml volumetric flask containing the sample of ABP and shaken, then made up to the volume with distilled water.

The concentration of calcium was analysed using an Atomic Spectrophotometer (Type AA-700, Shimaduz, Japan) while concentration of phosphorous was detected using UV/VIS Spectrophotometer (Type 160A, Shimaduz, Japan).

The same method was used to detect the concentration of Ca^{2+} and $P_{O4}{}^{2+}$ at 600 °C and raw bone.

3.2.8 pH Determination

Two gram of the ABP was dispersed in 100 ml of distilled water and shaken vigorously for 5 min and allowed to stand. The pH of the supernatant liquid was read off using a pH meter (Oaklon, pH1100, USA).

3.2.9 Determination of moisture Content (MC)

The weight of 20 ml capacity crucible was taken accurately (X) on the Mettler balance. Five grams of the sample was then placed in the crucible (Y) and kept in the Gallenkamp oven and thermosted at 105 °C for 1 h. The crucible and its content was then weighed to find (Z) of the dried the percentage MC of the material was calculated as:

$$MC = 100\left(1 - \frac{Z}{Y}\right) \quad \text{.....4}$$

Where Z = final weight and

Y = initial weight of powder + crucible

Z-Y = wt loss

3.2.10 Determination of Bulk and Tapped Densities

A 50 gm quantity of the material (ABP) was placed in a 100 ml measuring cylinder and the volume occupied by the material was recorded as the bulk volume. The bulk density was obtained using the equation below:

$$\text{Bulk density} = \frac{\boldsymbol{mass}\ of\ material\ (gm)}{\boldsymbol{bulk}\ volume\ of\ material\ (ml)} \quad \text{.....5}$$

The cylinder was then tapped on a wooden platform by dropping the cylinder from a height of 3 cm at 2 s intervals until there was no significant change in volume reduction. The volume occupied by the sample was then recorded as the tapped volume. The tapped density was calculated using the formula:

$$\text{Tapped Density} = \frac{\boldsymbol{mass\ of\ material}\ (\boldsymbol{gm})}{Tapped\ volume\ (ml)} \quad \text{.....6}$$

3.2.11 Flow Rate

A diameter funnel was properly clamped onto a retort stand. A funnel of 10 cm diameter and efflux tube length of 3 cm orifice base was used for the study. A 50 gm quantity of the material was weighed out and gradually transferred into the funnel with the funnel orifice closed with a shutter. The time taken for the entire material to flow through the orifice was recorded. The flow rate was obtained by dividing the mass of the sample material by the time of flow in seconds (Aulton, 2007). The mean of three determinations was recorded.

$$\text{Flow Rate} = \frac{weight\ of\ material\ (g)}{Time\ of\ flow\ in\ seconds} \quad \text{.....7}$$

3.2.12 Angle of Repose

Fifty (50) grams of the material was placed in a plugged glass funnel set at a distance of 10 cm from the flat surface. The material was then allowed to flow through the funnel orifice by removing the cotton plug from the funnel orifice. The height of the heap formed as well as the diameter of the heap was noted. The angle of repose (θ) for each material was calculated using equation below:

$$\text{Tan } \theta = \frac{H}{R} \quad \text{.....8}$$

Where θ is the angle of repose

H is the height of the heap and

R is the radius of circular base.

3.2.13 Carr's Compressibility index and Hausner's Ratio

Carr's compressibility index (%) of the material was obtained using the formula:

$$\text{Carr's Index (\%)} = \frac{Tapped\ density - Bulk\ density}{Tapped\ density} \text{ x 100 \%} \quad \text{.....9}$$

Hausner's quotient (HQ) was obtained using the formula below:

$$\text{HQ} = \frac{Tapped\ density}{Bulk\ density} \quad \text{.....10}$$

3.2.14 Preparation of Co-processed Excipients

A binary mix of ABP and gelatin was carried out in the following ratios: 90:10, 95:5, 97.5:2.5 using beaker and stirring rod after dissolving the gelatin with water (20 ml) on Gallenkamp Regulator Hotplate (No; HL-052, App No: 6B 8547 E, England) at a temperature of about 100 °C. Wet method was used to prepare the ABP and Gelatin (ABPG-95). The various ratios were properly mixed using stiring rod and spread on a tray (40 cm Diameter stainless steel, China) at room temperature for two days for proper air drying. Thereafter they were homogenised using a blender (Model 38 BL40, Christison, Germany). A preliminary evaluation (compressibility, crushing strength and disintegration) of the co-processed materials were carried out on all the ratios to select the sample that formed good compacts

Table 3.1: Ratios of combination excipient (ABP:Gelatin)

ABP : Gelatin	Co-processed excipient
90 : 10	ABPG90
90 : 5	ABPG95
97.5 : 2.5	ABPG97.5

100 : 0	ABP

3.2.15 Fourier transform infrared (FT-IR) spectroscopy

Fourier transform infrared (FT-IR) spectral data were taken on a Shimadzu (model FTIR-8300, Japan) instrument to find out the chemical stability of the excipients. FTIR spectral of the pure cow bone powder, pure gelatin powder and co-processed excipient were obtained. All the samples were crushed with potassium bromide (KBr) to get pellets at 1 ton/cm^2 Spectral. Scanning was done in the range between 4000 to 500 cm^{-1} .

3.2.16 Differential Scanning Calorimetry (DSC)

The differential scanning calorimetry, was performed on the pure ABP, Gelatin and co-processed excipient (ABPG95) at National Institute of Pharmaceutical Research and Development (NIPRD)- Abuja, using Perkin Elmer model pyris-6, USA. DSC measure exothalmic and endothalmic peaks of the materials to see if there are changes in peaks of the materials.

3.4 Characterisation of the Co-processed Excipient

3.4.1 Particle Density

The method described by Odeku, *et al. (*2008*)* was adopted. The particle density was determined with a pycnometer bottle using xlene as the displacement fluid. An empty bottle was weighed (w), filled with xylene and the excess liquid wiped off. The filled bottle was weighed (w_1) and the difference between w_1 and w obtained as w_2. A two gram quantity of the powder (ABPG-95) was weighed (w_3) and transferred into the filled bottle. The excess solvent was wiped off and the bottle weighed again (w_4). The particle density, ρ□ (g/cm^3) was then calculated (after three replication) from the equation given below.

$$\rho\square = \frac{w_2 \, x \, w_3}{50(w+w_4+w_2+w)} \quad \text{.....11}$$

Where w = empty bottle

w_1 = empty bottle + liquid

w_2 = wt of xylene

w_3 = weight of the powder

w_4 = wt of powder +bottle +liquid

3.4.2 Microscopy

The microscope was calibrated using the eye-piece and stage micrometer. A little quantity of the powder samples (ABPG-95 and CCS-90) were mounted individually on a slide in glycerol and viewed under the microscope. Two hundred (200) particles were counted to determine the particle size distribution. A photomicrograph of the powder samples were captured using a

compound microscope (Fisher, Rochester- New York), at 400x magnification and a plot of percentage frequency distribution against particle size was drawn for each powder.

3. 5 Comparative Analysis of ABPG95 and CCS90

The ABPG95 and standard (CCS90) were subjected to the following tests: sieve analysis, moisture content, angle of repose, bulk and tapped densities, Carr's index and Hausner's ratio. The same procedures used as earlier described for powdered ABP section 3.3.

3. 6 Dilution Potential

A binary mix of the drug and co-processed excipient (ABPG95) and Bacroft® (CCS-90) were mixed separately in the following ratios: 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, and 80:20. It was then compressed at varying compression loads on the single punch tableting machine (Type EKO, Erweka- Apparatebau- G.m.b.H Heusenstamm, Germany). The crushing strength of each of the compacts from the binary mix was determined and recorded.

3.6.1 Compaction of the individual powders to form tablets

Before the mixing of the powders; ABP, ABPG95, AA, MET, Gelatin and CCS-90 were test compressed at various compression pressures of 4.5 -7.5 metric tonnes and crushing strength, disintegration and friability tests were carried out.

Compacts of each material weighing 350 mg for AA and 500 mg for MET, Gelatin and ABPG95 were prepared by compressing them for 30 s with pre-determined pressure at 4.5 MT using 8 mm and 12 mm respectively in a normal concave-face punches on a single punch tablet machine (Type Eko Erweka Apparatebau. G.m.b.H Heusentamm, Germany). Before each compression, the die flat-faced punches was lubricated with 1 %w/v dispersion of magnesium stearate in ethanol solution.

3.7 Formulation Studies

3.7.1 Compression of Tablets

Four different batches of tablets were prepared by direct compression method having a batch size of 60 tablets for each batch at compression pressure of 4.5 MT. The tablet formula for each batch is given below (Table 3.1)

Table 3.2 Tablet Formular

	Batch No			
	I	**II**	**III**	**IV**

Active ingredient	AA	AA	MET	MET
(30 %)	100	100	-	-
(40 %)	-	-	200	200
ABGP95 (69 %)	230	-	-	-
CCS90 (69 %)	-	230	-	-
ABPG95 (59 %)	-	-	295	-
CCS90 (59 %)	-	-	-	295
Mg. Stearate (1 %)	3	3	5	5
Total (mg)	**333**	**333**	**500**	**500**

Batch:

I= Ascorbic Acid/ABPG95

II= Ascorbic Acid/CCS90

III = Metronidazole/ABPG95

IV = Metronidazole/CCS90

Mg.St =Magnesium Stearate

3.8 Evaluation of Tablet Properties

Tests to evaluation tablet properties were conducted for all batches produced after 24 h of tablet production. These tests evaluations include:

i Weight uniformity

ii Thickness and diameter

iii Crushing strength

iv Friability test

v Disintegration time test and

vi Dissolution test

3.8.1 Weight Uniformity Test

Ten tablets from each batch were selected randomly and weighed individually and as a whole using an analytical balance (Mettler Analytical Balance, Philip Harris Ltd, England). Their mean weights and standard deviations were determined as stipulated in BP (2002).

3.8.2 Tablet Thickness and Diameter

Tablet thickness and diameter of five (5) tablets were measured using a screw gauge micrometer (Moore and Wright, England). The mean of five determinations was calculated and recorded.

3.8.3 Crushing Strength Determination

Ten tablets randomly selected from each batch were assessed for crushing strength using a Monsanto hardness tester (Manesty Machines Ltd, Speke, Liver pool, England). The tablets were placed between the spindle and anvil of the tester. The knob was then screwed gradually and gently until the tablet was held in position. The reading pointer was adjusted to zero on the scale. Pressure was applied by turning the knob until the required pressure that crushed the tablet was read in terms of kilogram force (KgF) on the scale. The mean of ten determinations of each batch was recorded.

3.8.4 Friability Test

The tablets (10) were selected at random from each batch, dusted and weighed together using the analytical balance (No: xo 27555, Arvada CO 80004, USA) and then subjected to abrasive shock in an Erweka Friabilator (Type TA3R, Erweka- Apparatebau- GmbH Heusenstamm, Germany) operated at 25 rpm for 4 mins and then stopped. The tablets were dusted again and reweighed. The percentage loss in weight was calculated for each batch of the tablets.

3.8.5 Disintegration Test

The disintegration time for each of the tablets was determined in distilled water at 37 ± 0.5 °C using the Erweka disintegration test apparatus (Type ZT3, Erweka-Apparatebau-G.m.b.H Heusenstamm, West Germany). Six tablets from each batch were tested and the time taken for each tablet to break into small particles and pass through the mesh was recorded as the disintegration time.

3.8.6 Dissolution Studies

Before the dissolution studies, the following were carried out:

a. Calibration Curve for Ascorbic Acid

A 0.1 mg/ml stock solution of ascorbic acid was prepared by dissolving 100 mg of ascorbic acid in 1000 ml of 0.01 N HCl. A serial dilution was performed to yield solutions of concentration ranges of 3.125-100 μg/ml. the absorbance of each concentration was taken at 244 nm and plotted against the various concentrations to obtain the calibration curve for ascorbic acid. The linear regression equation for the graph was resolved from the plot and used to calculate the amount of drug released with time during dissolution studies.

b. Calibration Curve for Metronidazole

A 0.2 mg/ml stock solution of metronidazole was prepared by dissolving 200 mg of metronidazole in 1000 ml of 0.1 N HCl. A serial dilution was performed to yield solutions of concentration range of 0.3125-10 μg/mL. the absorbance of each concentration was taken at 277 nm and plotted against the various concentrations to obtain the calibration curve for metronidazole. The linear regression equation for the graph was resolved from the plot and used to calculate the amount of drug released with time during dissolution studies.

The dissolution rates of the tablets were determined using an Erweka dissolution apparatus (Type DT, Erweka-Apparatebau- GmbH Heusenstamm, Germany). The dissolution medium was 1000 ml of 0.1 N HCl for metronidazole and 0.01 N HCl for ascorbic acid, maintained at 37 ± 0.5 °C. The revolution of the basket containing the test tablets was 100 rpm. Ten millilitre of the sample was withdrawn from a position half way between the surface of the dissolution medium and the top of the rotating basket at 10 min intervals for 1 h. Each volume of sample withdrawn was replaced with an equivalent volume of dissolution medium maintained at the same temperature. A tenfold (1:9) dilution with the dissolution medium was done for each sample withdrawn before absorbance of the sample was read at 224 nm for ascorbic acid and 277 nm (BP, 2002) for metronidazole using a UV/VIS Spectrophotometer (Helios Zete UV-VIS Spectrophotometer, Thermo Fischer Scientific Inc., Cambridge, UK). The percentage drug release was plotted against time to generate a dissolution curve profile.

3.9 Statistical Analysis

Statistical analysis was carried out to compare the tableting properties of ABPG-95 with CCS-90 in the formulation of ascorbic acid and metronidazole tablets using the student's t-test as a statistical tool. At 95 % confidence interval, $p \leq 0.05$ was considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Preliminary Investigation on ABP

The results of preliminary investigation on the activated bone powder are presented in Table 4.1. The organoleptic examination of the ABP revealed that it was odourless, ash white and tasteless with brittle texture upon feeling between the fingers. The yield of ABP was 72 % .The pH was slightly basic (7.8) and the percentage content of calcium and phosphorous determined by using digestion method (Maria *et al*., 2010), gave 40.21 % and 18.5 % on ABP for calcium and phosphorous respectively, while the raw bone (without heat from furnace) gave 49.5% and 26.25 % for calcium and phosphorous respectively and It was also observed that bone powder activated at 600 °C gave a concentration of 45.12 % and 21.4 % for calcium and phosphorous . The shape of the activated bone powder was spherical.

Table 4.1: Results of the preliminary investigations o ABP

Properties	Results
Odour	odourless
Colour	ash white
Taste	tasteless
Texture	brittle
Yield (% w/w) after activation	72
pH	7.8

Loss on weight (% w/w) after activation	28
At 800 °C ABP (calcium content %)	42.1
At 800 °C BP (phosphorous %)	18.5
Raw bone (calcium content %)	49.5
Raw bone (phosphorous content %)	26.25
At 600 °C ABP (calcium content %)	45.12
At 600 °C ABP (phosphorous %)	21.4
Particle shape	Spherical

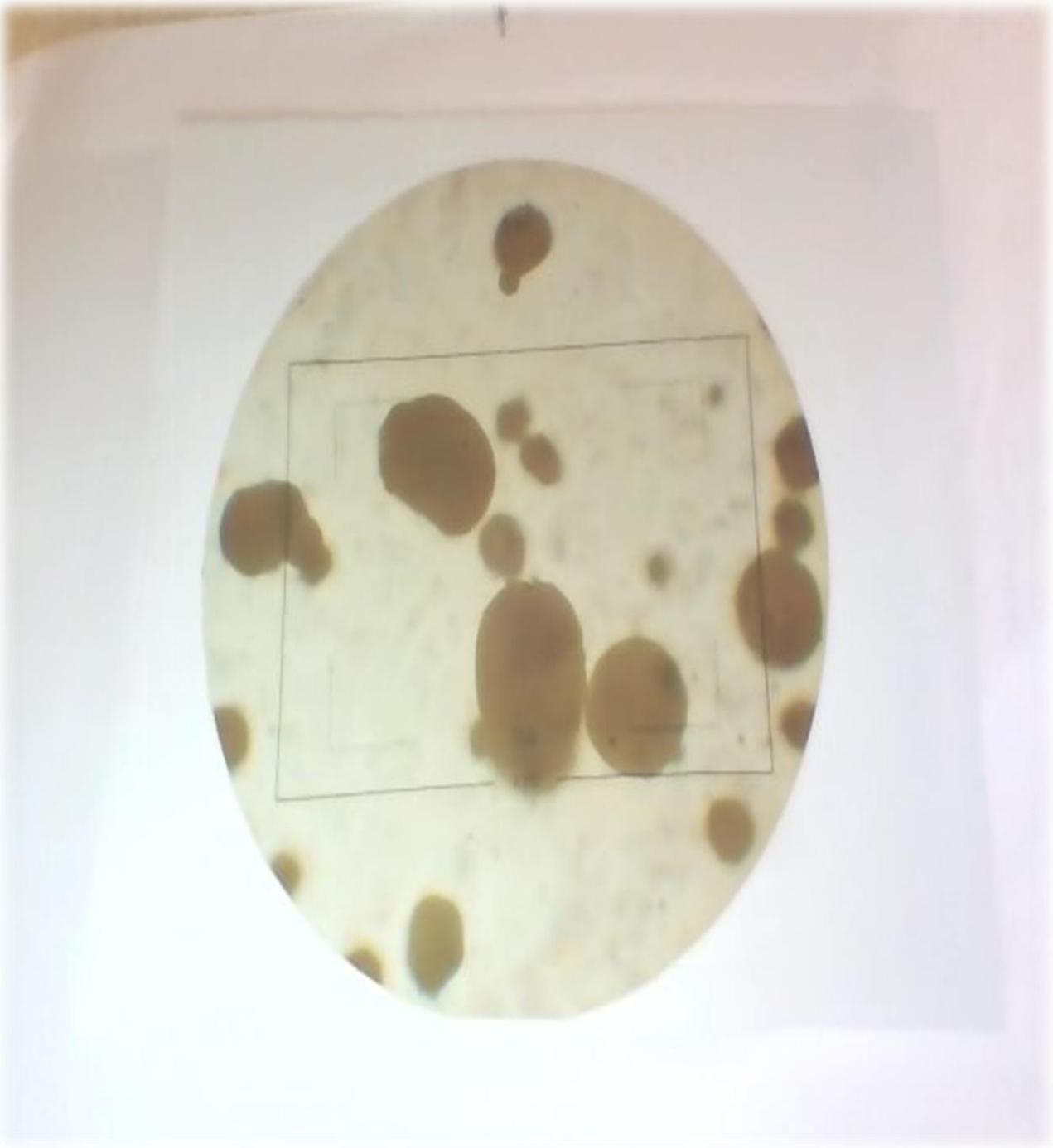
4.2 Microscopy

Plates 4.1,4.2 and 4.3 showed photomicrographs of the ABP, ABPG95 and CCS90 respectively when viewed under the microscope at 400 x magnification. On examination ABPG95 and CCS90 appeared to be spherical in shape with a particle size range of 2-23 µm for ABPG95 and 5-21 µm for CCS90



Plate 4.1: Photomicrograph of Activated Bone Powder (ABP) using microscope (Magnification X 400) (Fisher, Rochester - New York).

Plate 4.3: Photomicrograph of ABPG95 (Magnification X 400) (Fisher, Rochester - New York).



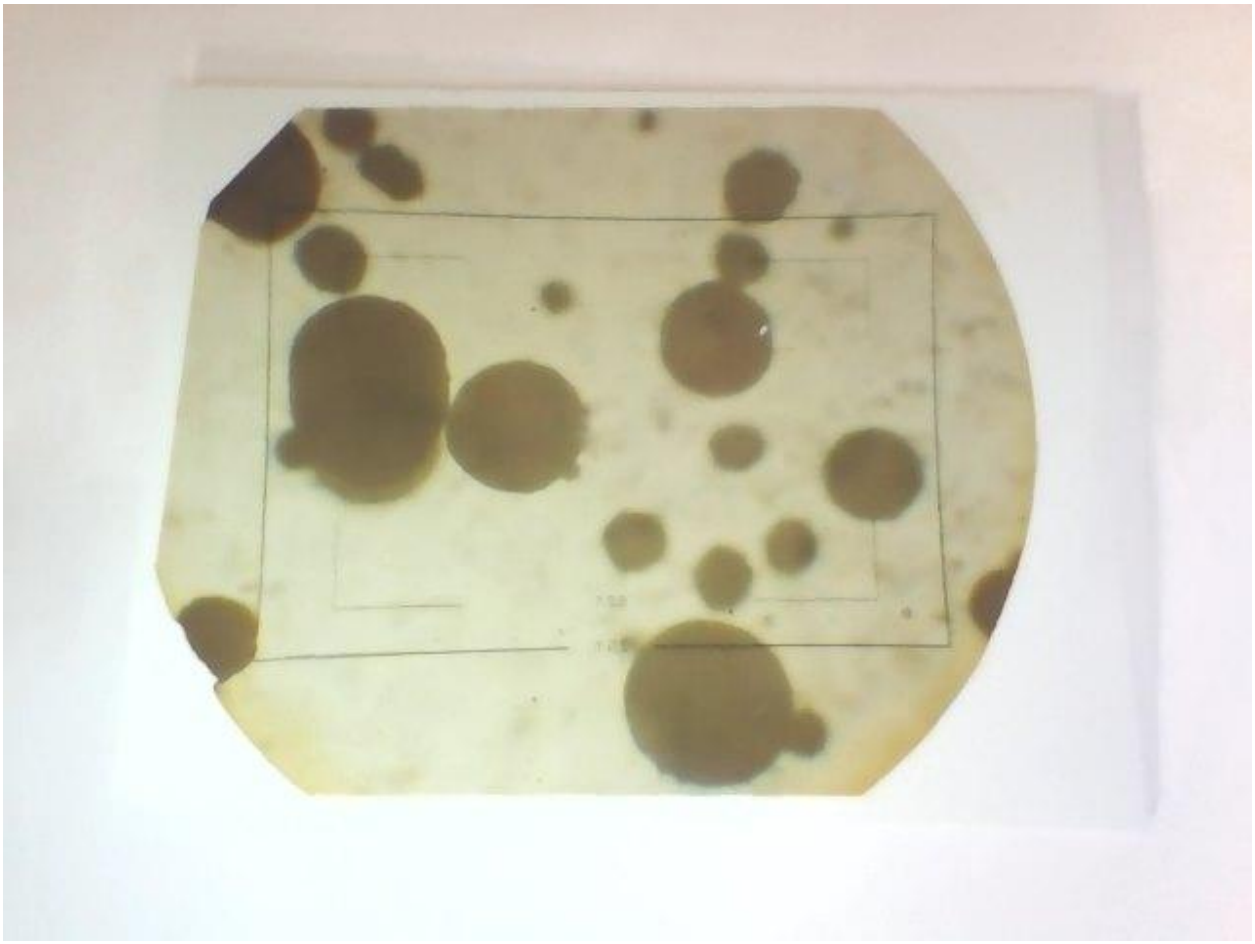


Plate 4.3: Photomicrograph of CCS90 (Magnification X 400) (Fisher, Rochester-New York) (Fisher, Rochester-New York).

4.3 Particle size and percentage distribution

On sieving, ABP, ABPG95 and CCS90 and weighing each sieved fraction, the sieve analysis was obtained and percentage distribution was as shown in Figure 4.1 and 4.2. The graphs of percentage weight retained against the particle sieve size and percentage cumulative distribution oversize against the particle sieve size respectively for ABP, ABPG95 and CCS90 are shown in Figure 4.2 and 4.3.

Particle size distribution curve was normal for both materials ABPG95 and CCS90 with average a mean particle size of 7µm and 6 µm respectively as shown in Figure 4.3.

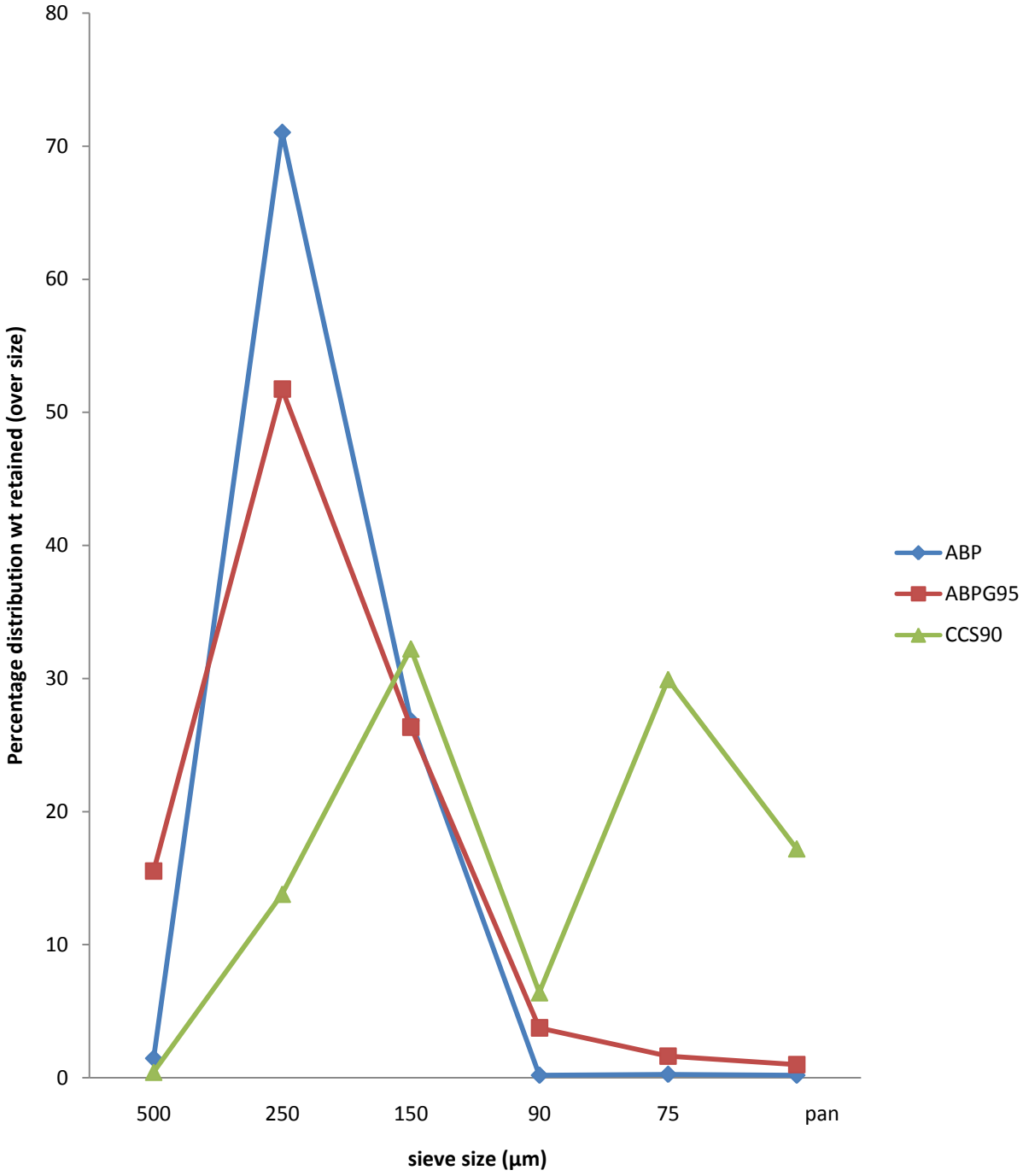


Figure 4.1: Graph of percentage wt retained (over size) against sieve size (µm) for ABP, ABPG95 and CCS90

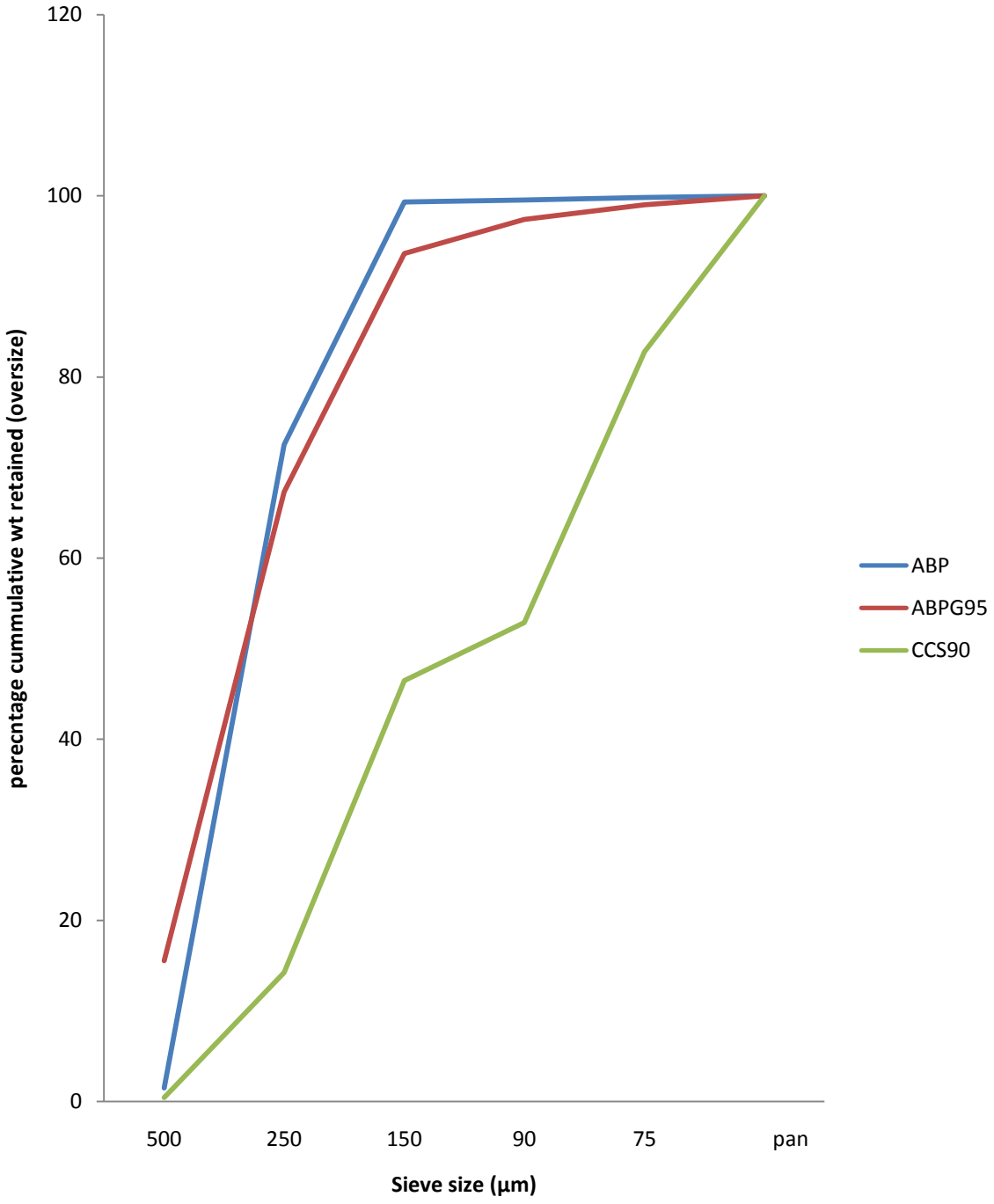


Figure 4.2: Graph of percentage cumulative wt retained (over size) against sieve size (µm) for ABP, ABPG95 and CCS90

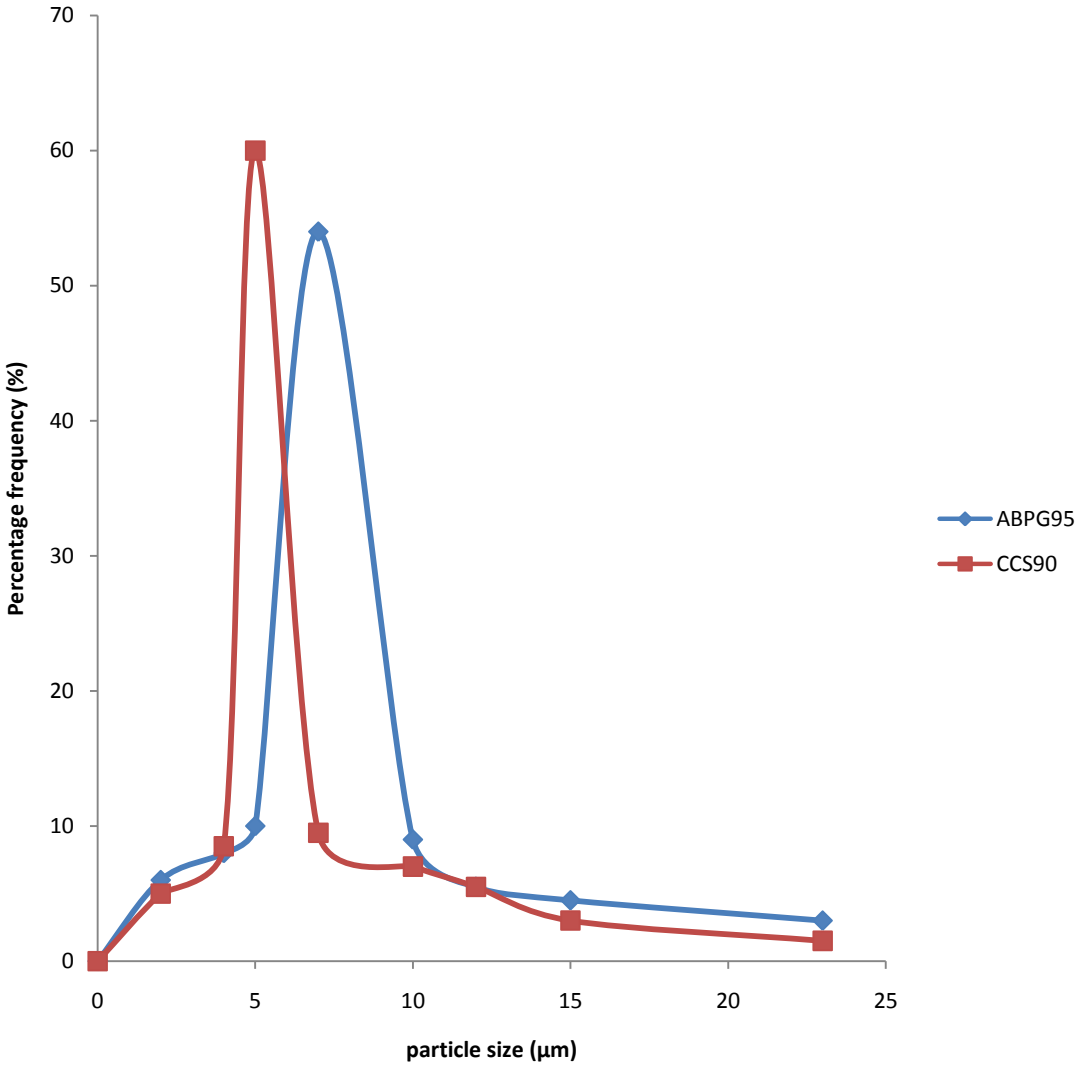


Figure 4.3: Graph of Percentage Frequency Distribution against Particle size (µm) for ABPG95 and CCS90

4.4 Physicochemical Properties

The results for the flow properties and angle of repose are presented in Table 4.2. The highest angle of repose was obtained with ABPG-95 (26°)and the lowest was obtained with CCS-90 (13°). Bulk and tapped densities values are also presented in Table 4.2.The values are presented in the following order; Bulk density: CCS-90>ABPG-95>ABP and Tapped density: CCS-90>ABP>ABPG-95.

The Hausner's ratio and Carr's index were computed from the figures obtained from bulk and tapped densities and each material and the values for these parameters confirmed the good flow properties of the three materials. The materials were in the following order; ABPG-95>CCS-90>ABP as shown in Table 4.2.

4.5 Fourier transform infrared (FT-IR) spectral studies

The FT-IR spectra of pure activated ABP, pure gelatin powder and co-processed (ABPG95) materials are depicted in Figure 4.4, 4.5 and 4.6 respectively. It is expected that ABPG95 peaks will not change compared to individual powder peaks ensure chemical compatibility.

4.6 Differential scanning calorimetry (DSC)

The differential scanning calorimetric spectra are displayed in Figure 4.7, 4.8, 4.9 and 4.10 of pure ABPG, pure gelatin powder, ABPG95 and superimposed of the three samples with their characteristic peaks respectively.

Table 4.2: Physico-chemical Properties of ABP,ABPG95 AND CCS90

Physicochemical Properties	ABP	ABPG95	CCS90
Flow rate (g/sec)	11.6±0.5	12.3 ±0.06	14.4±0.12
Angle of repose (^{0})	24.5±0.8	21.0±0.09	13.5±0.01

Tapped density (g/ml)	1.28 ±0.00	1.35±0.02	1.47±0.02
Bulk density (g/ml)	1.18±0.00	1.16±0.02	1.15±0.01
True density (g/ml)	0.03	0.02	0.02
Carr's index (%)	15.0	14.1	8.16
Hausner's ratio	1.14	1.16	1.08
Moisture content (%)	3.60	4.20	4.05

Key:

ABP represent activated bone powder, ABPG95 represent Activated Bone Powder and Gelatin (95:5) CCS90 represent Calcium Carbonate and Starch (90:10).

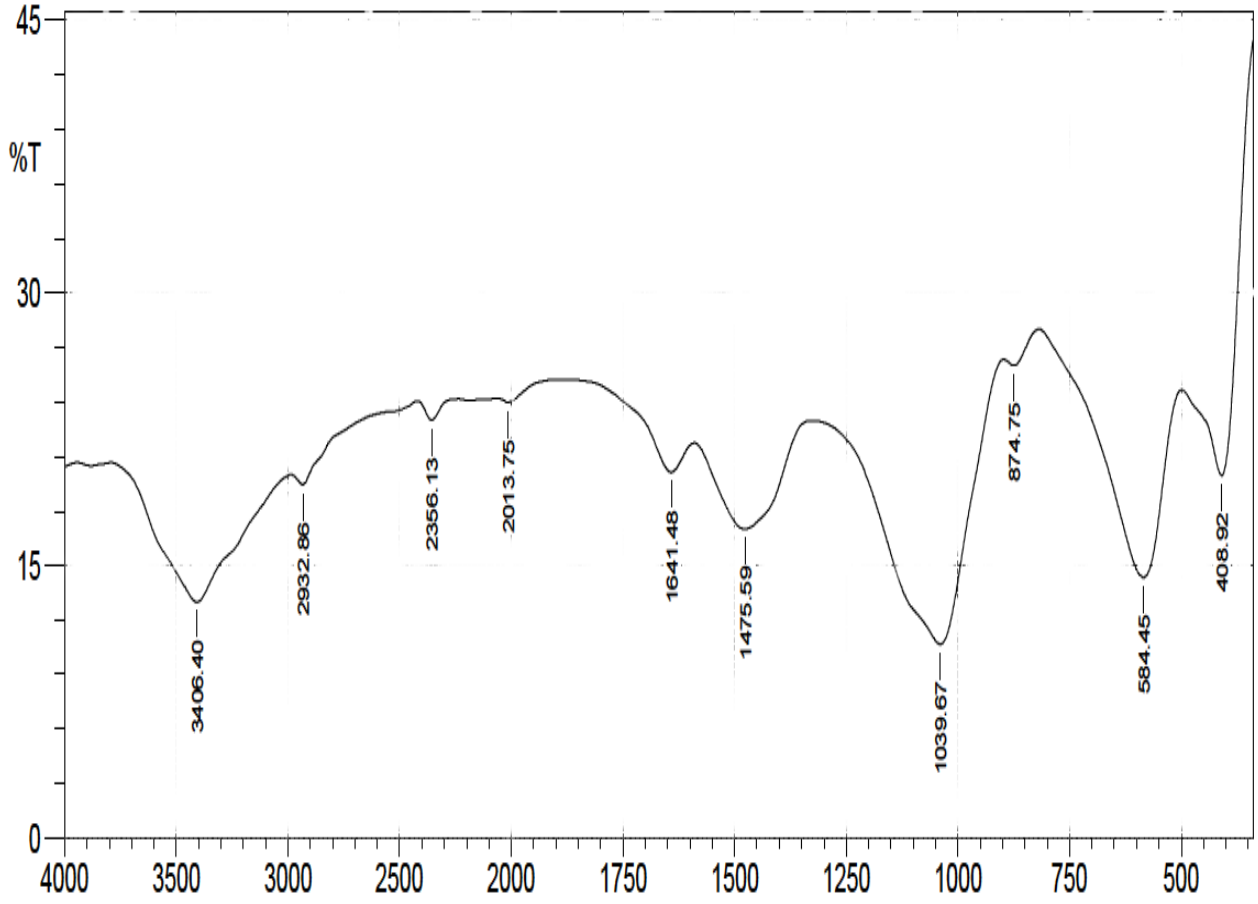


Figure 4.4: FT-IR spectra of pure cow bone powder (ABP)

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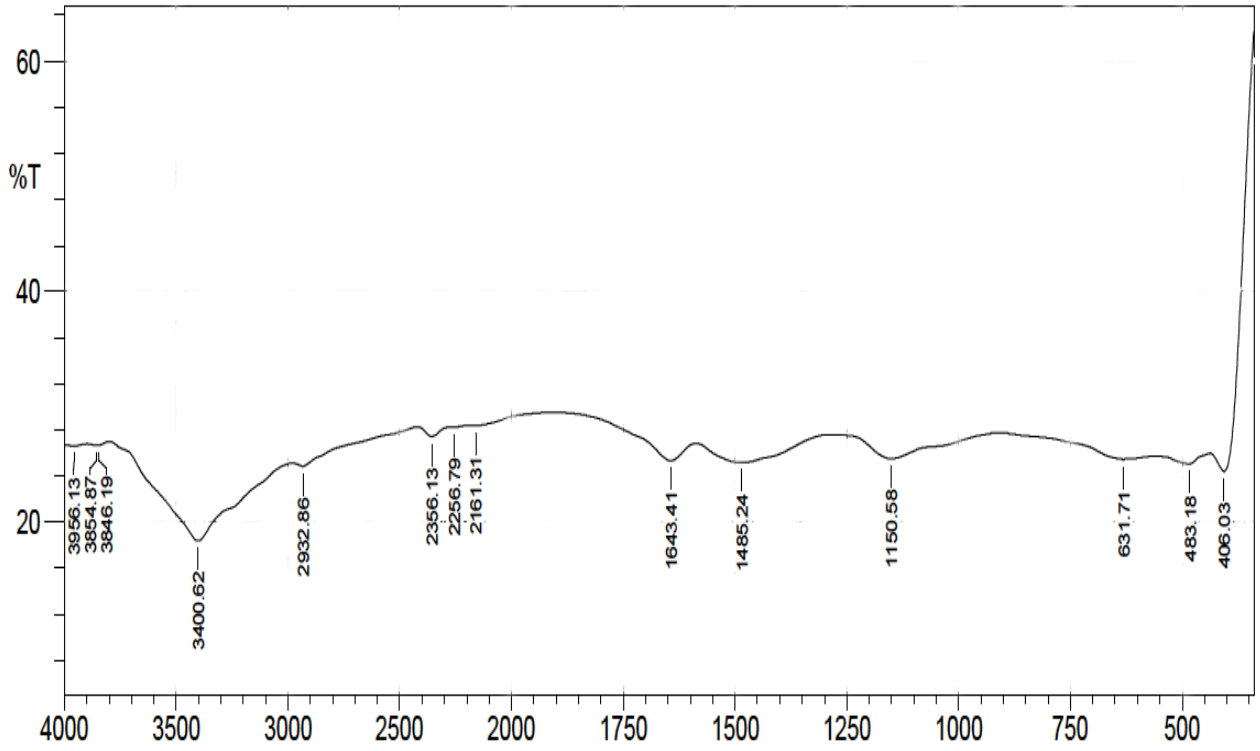


Figure 4.5: FT-IR spectra of pure gelatin powder

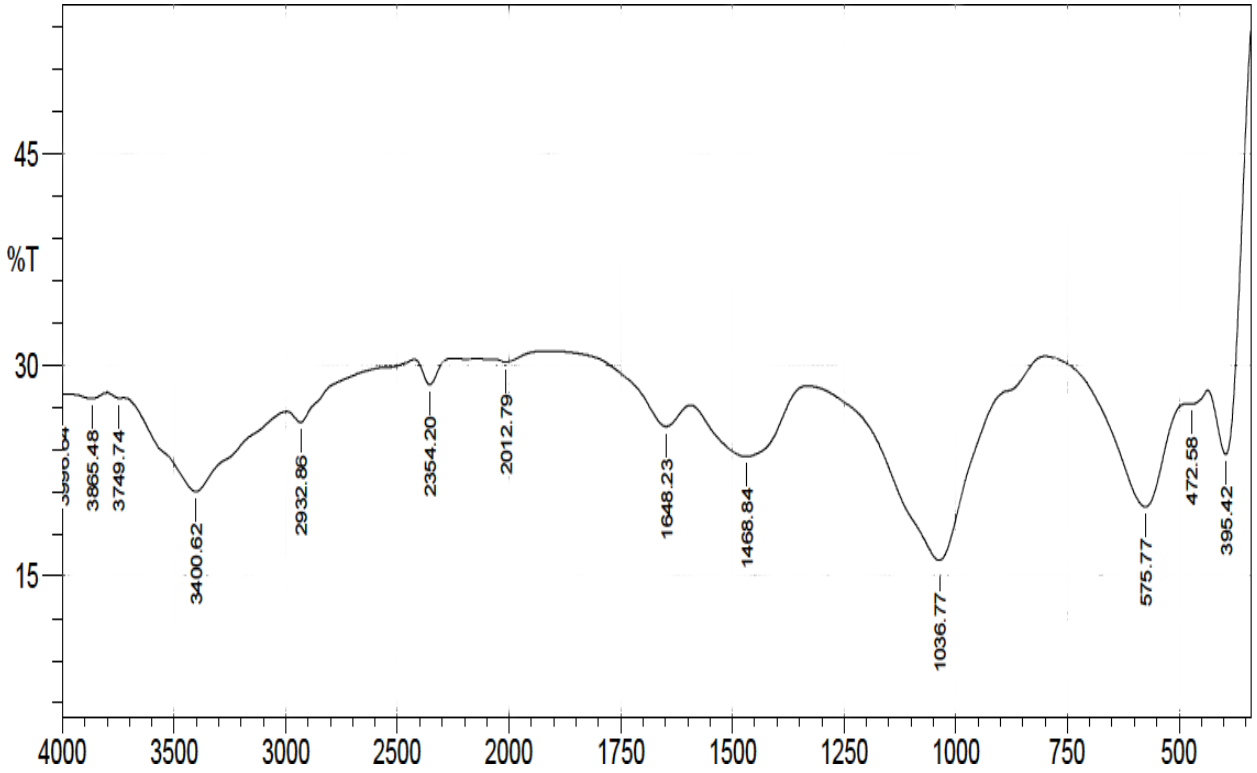


Figure 4.6: FT-IR spectra of co-processed excipient (ABPG95)

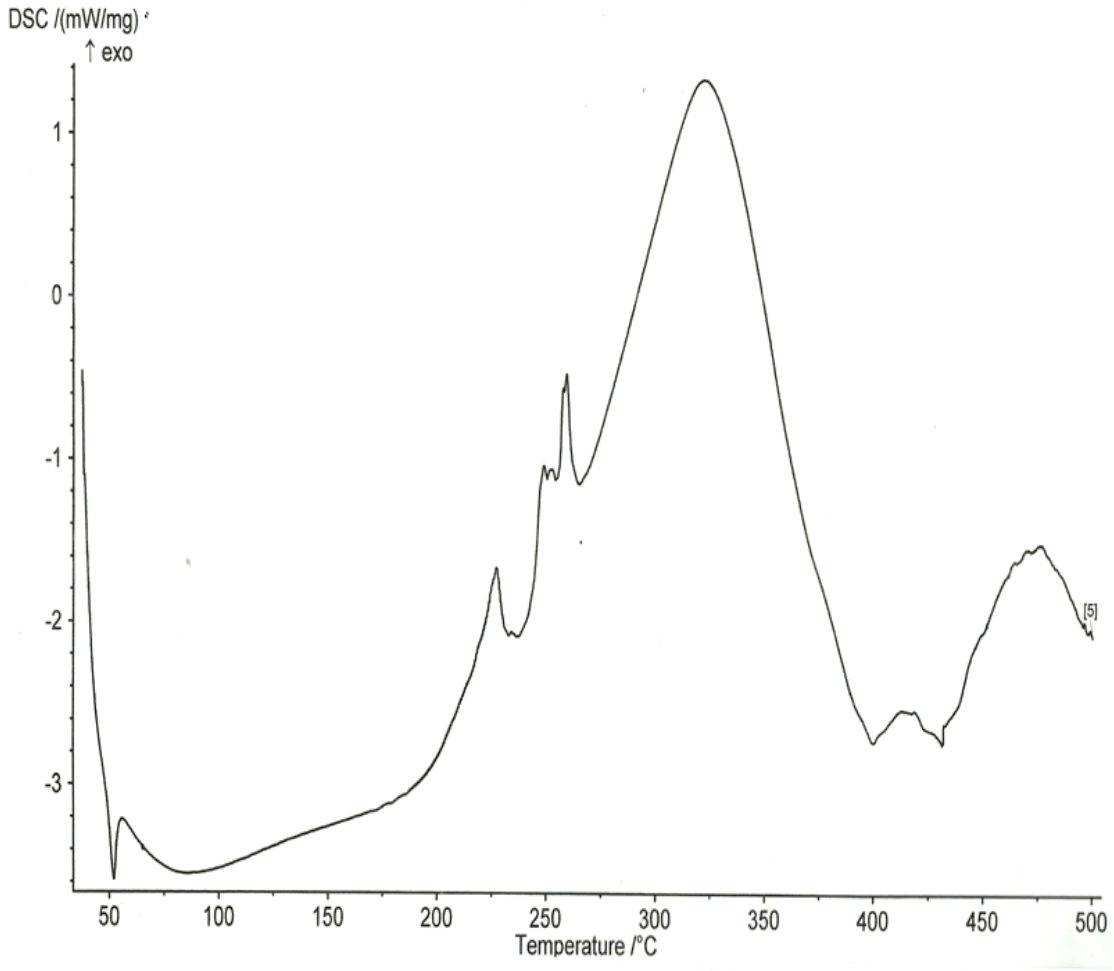


Figure 4.7: DSC spectra of pure activated bone powder (ABP)

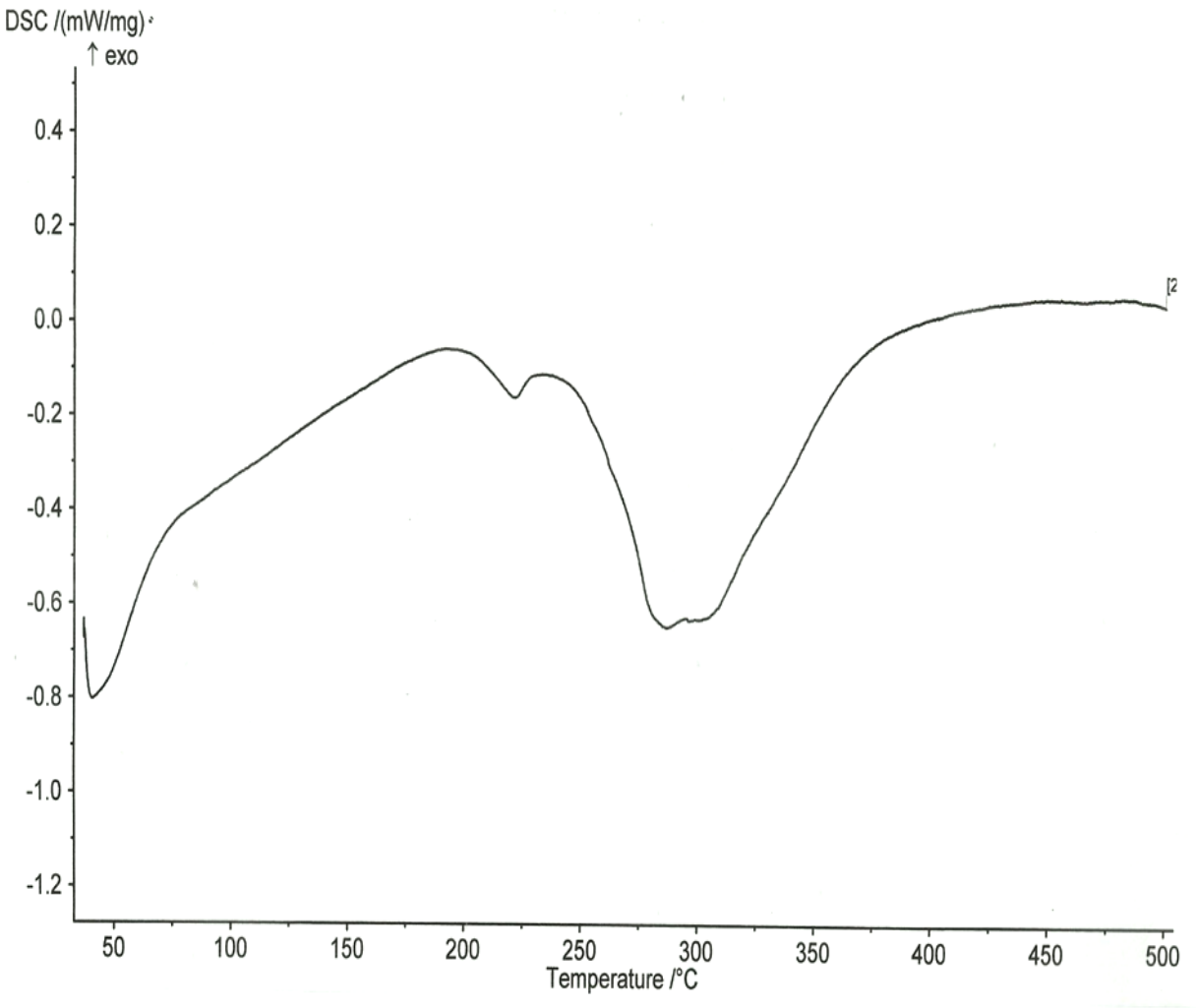


Figure 4.8: DSC spectra of pure gelatin powder

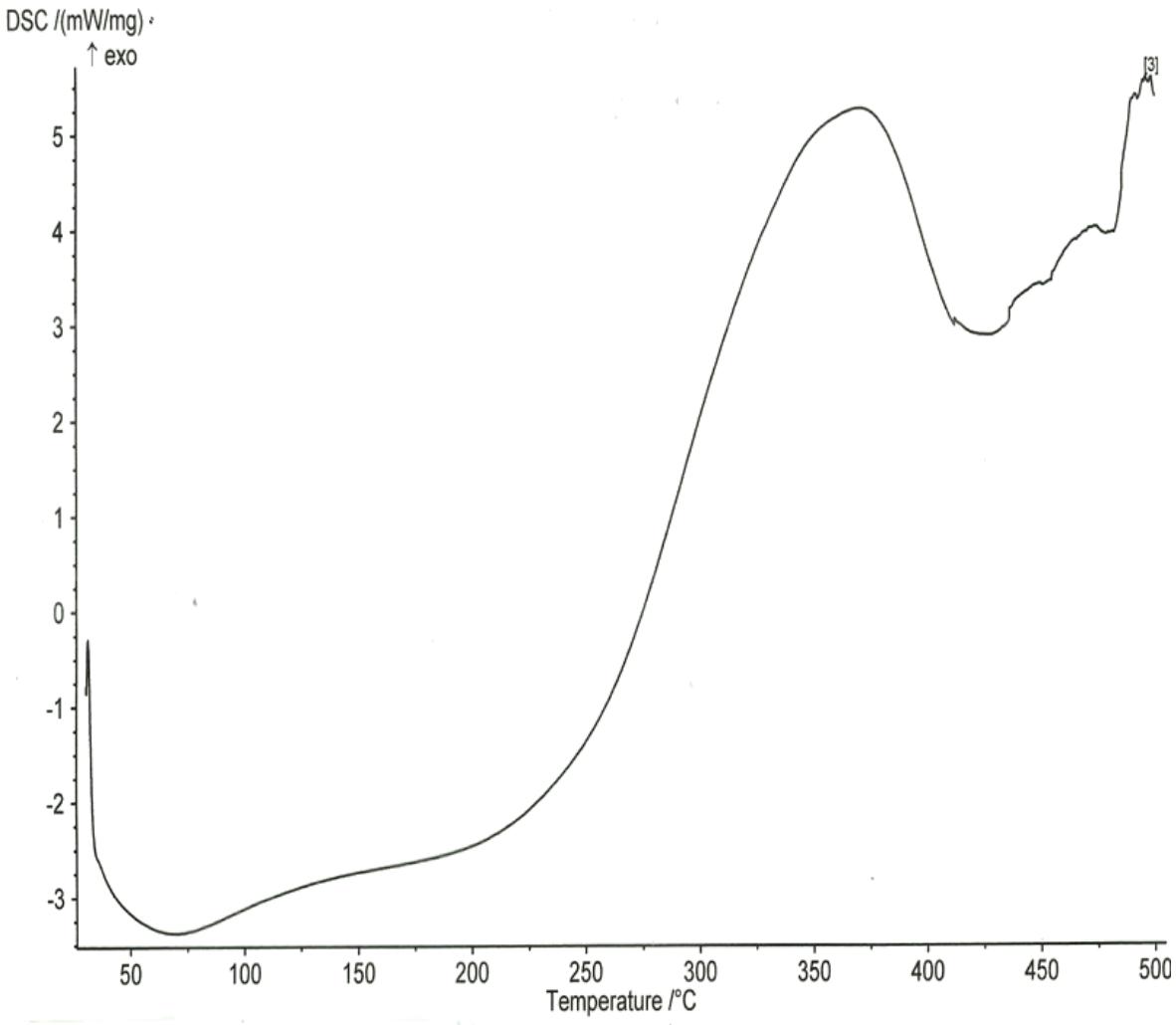
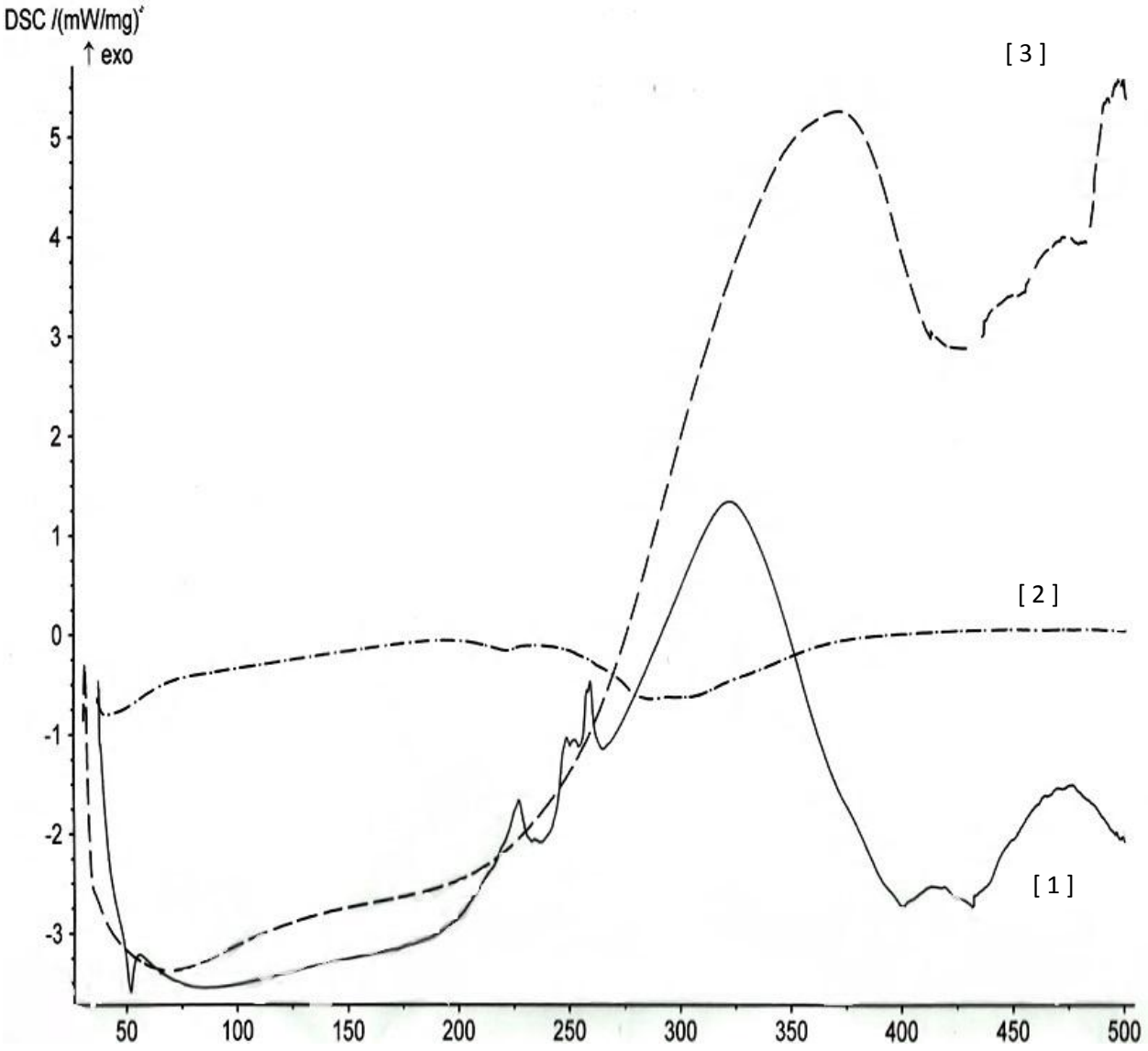


Figure 4.9: DSC spectra of co-processed excipient (ABPG95)



Key:

1: pure cow bone (ABP)

2: pure gelatin

3: Co-processed excipient (ABPG95)

Figure 4.10: Super-imposed DSC spectra of Gelatin, ABP and ABPG95

The results of individual tablet properties obtained from ABP, ABPG95, Gelatin, CCS90, AA and MET are presented in table 4.3.

4.7 Dilution potential

The results for dilution potential are displayed on Table 4.4 and Table 4.5. The results reviewed that both ABPG95 and CCS90 were able to bind up to 40 % active drug (MET) and 30 % for ascorbic acid and still retained their compressibility properties.

Table 4.3 Mechanical and Disintegration Times of Individual and Material Tablets

Tablet	Comp Pressure (MT)	Crushing Strength (Kgf)	Disintegration (min)	Friability(%)	Compressibility (CS/F)
ABP	5.5	1.75	0.6	-	-
Gelatin	6.5	7.5	>45	23	0.33
ABPG95	4.5	8.25	>40	1.35	6.11
CCS90	4.5	7.5	35	1.05	7.14
AA	3.0	2.25	2.25	24.0	0.09
MET	3.5	3.1	3.8	5.92	0.52

Table 4.4:Crushing Strength, Disintegration Time, Friability and Compressibility for Binary Mix (MET: ABPG95)

MET: ABPG95	Crushing strength KgF	Disintegration time	Friability %	Compressibility CS/F
20:80	5.3	24.14	2.36	2.25
*30:70	5.6	22.23	1.05	5.33
*40: 60	6.5	16.17	1.0	6.5
50:50	3.5	6.5	4,34	0.80

* = selected binary ratios.

Table 4.5 Crushing Strength, Disintegration Time, Friability and compressibility for Binary Mix (MET:CCS90)

MET:CCS90	Crushing strength KgF	Disintegration time	Friability %	Compressibility CS/F
20:80	5.3	24.14	2.45	2.16
*30:70	5.6	22.23	1.03	5.44
*40: 60	6.5	15.10	0.95	6.84
50:50	3.5	6.5	3.65	0.96

***** = selected binary ratios

Table 4.6: Crushing Strength, Disintegration Time and compressibility for Binary Mix (AA: ABPG95)

AA:ABPG95	Crushing Strength KgF	Disintegration Time (min)	Friability %	Compressibility CS/F

20:80	4.5	22.30	1.85	3.0
30:70*	5.6	14.15	1.05	6.0
40:60	2.0	3.45	2.45	1.0
50:50	1.0	1.23	3.70	0.27

*Selected binary ratio

Table 4.7: Crushing Strength, Disintegration Time and compressibility for Binary Mix (AA:CCS90)

AA:CCS95	Crushing Strength KgF	Disintegration Time (min)	Friability %	Compressibility CS/F
20:80	4.6	22.23	1.45	3.17
*30:70	6.5	13.12	1.03	6.3
40:60	2.5	3.45	2.5	1.0
50:50	1.0	1.25	3.65	0.27

***** = selected binary ratios

4.8 Evaluation of Tablet Properties

Four batches of tablets were formulated using ascorbic acid and metronidazole as active drugs with ABPG95 and CCS90 as direct compression filler/binders (Table 3.1). The properties of the tablets formulated were evaluated and the results obtained are displayed on Table 4.8

Table 4.8 Evaluation of Physical properties of Tablets formulated

	Batches of Tablet			
Properties	**I**	**II**	**III**	**IV**
Uniformity of weight (mg)	333±0.2	333±0.5	501±0.1	500±0.2
Crushing strength (Kgf)	7.5±0.4	7.1±0.45	7.6±0.27	7.3±0.32
Friability (%)	1.00	0.98	1.02	0.5
Thickness (mm)	3.43±0.12	3.41±0.1	4.5±0.1	4.45±0.1
Diameter (mm)	7.5±0.18	7.2±0.5	12.1±0.5	12.2±0.1
Disintegration time (min)	5.37±0.5	3.3±0.01	10±0.5	2.6±0.1
$T_{50\,\%}$ (min)	15	12	18	14
$T_{90\,\%}$ (min)	31	22	36	27

Key:

Batch I ABPG95/AA 70:30

Batch II CCS90/AA 70:30

Batch III ABPG95/MET 60:40

Batch IV CCS90/MET 60:40

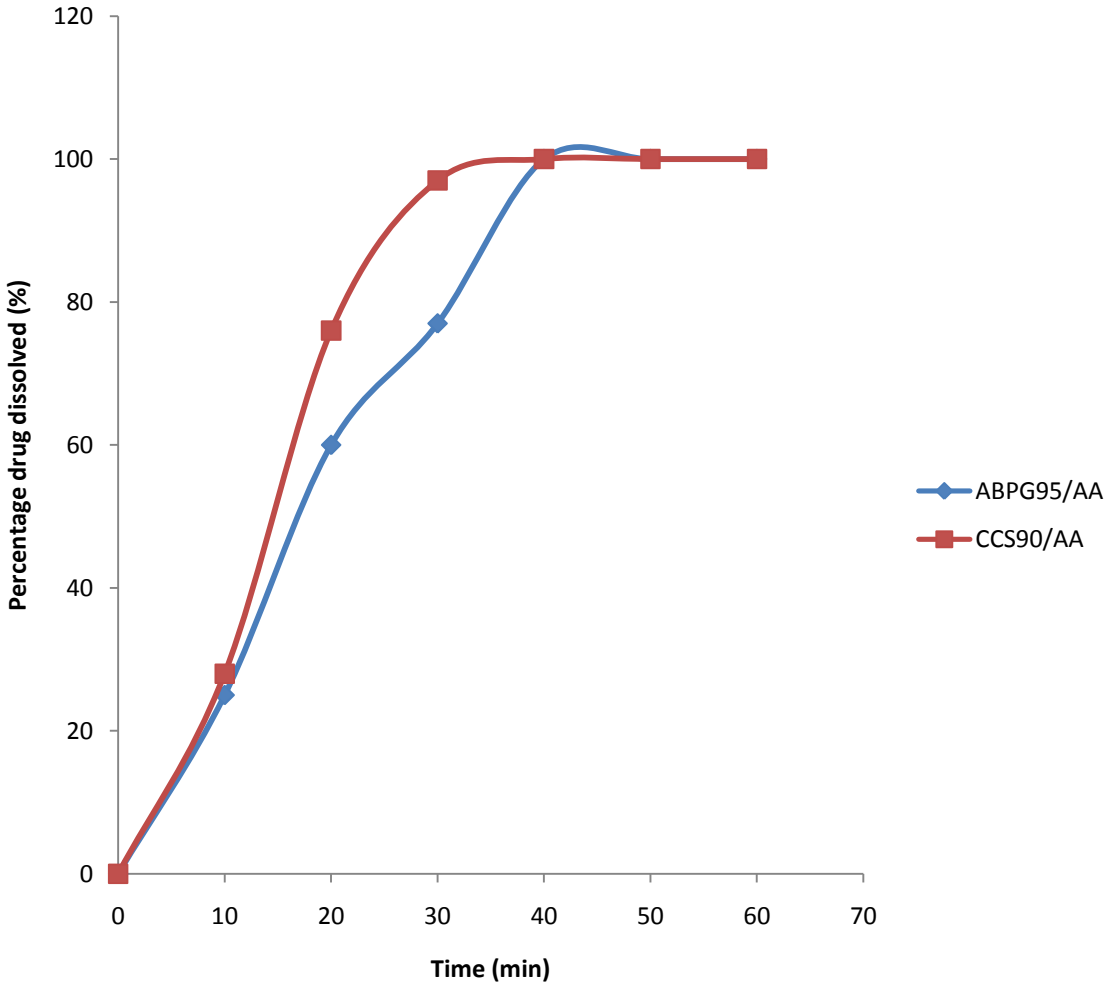


Figure 4:11:Graph of Percentage drug release against time (min) for batches I and II

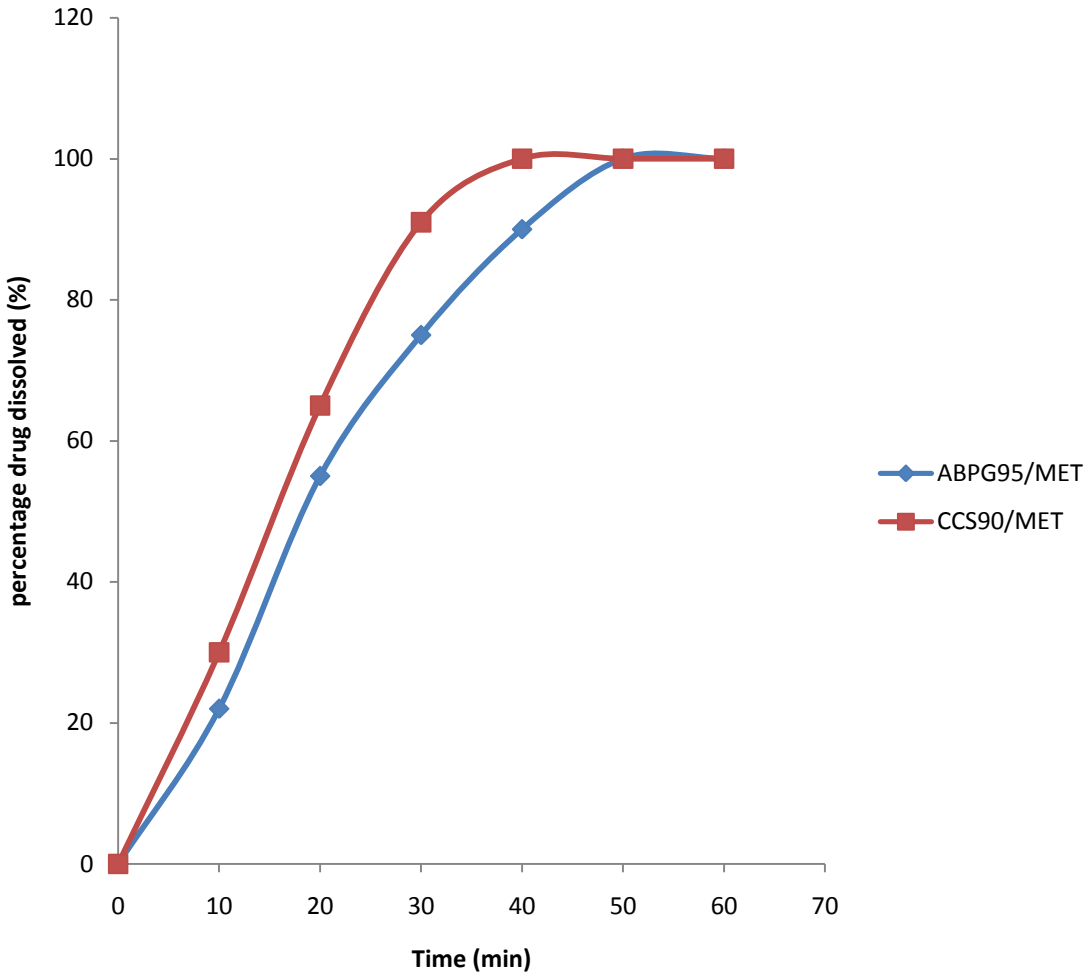


Figure 4.12: Graph of Percentage drug release against time (min) for Batches III and IV

CHAPTER FIVE

5.0 DISCUSSION

5.1 PRELIMINARY INVESTIGATION

The application of heat at high temperature was for extraction process and it got rid of organic components of the bone. The percentage loss in weight of bones after drying and heating in furnace for 6 h at 800 ^{0}C was 28% while the yield gave 72% as presented in Table 4.1. Temperatures above 750 ^{0}C has been confirmed not to offer any advantage in term of activation of bonds in the bone does not offer any advantage in term of activation of breaking bonds (Emenike, 2004). The loss in weight could be attributed to loss in moisture content and all susceptible organic materials associated with bones that decomposed . Such organic materials include the bone marrow contents, fats and fatty integuments within the bones. The colour of the bones turned from milky colour to ash white. The bones were brittle to touch; meaning that the crystallinity of the bone structure must have been weakened considerably (Hoon and Seon-Hong, 2001). Chemical analysis was performed to ascertain the content of calcium phosphate in both raw bone and ABP at 800 ^{0} C, the percentage content of calcium and phosphorous is indicated in Table 4.1 revealedd that raw bone has the highest percentage (%) of calcium and phosphorous as the heating temperature increases, the percentage decreases respectively. This could be due to decomposition of elements during heating or most probable that the crystalline architecture of the bone is affected by heat of activation in the furnace (Emenike, 2004). ABP at 600 °C also showed a considerable amount of Calcium and phosphorous compared to the amount at 800 °C.

5.2 Microscopy

The microscopical examination revealed a particle size ranges of 2-23 µm of ABP, ABPG95 and CCS90 and there were more spherical particles shape with ABP. on examination, ABPG95 and CCS90 appeared to be spherical/ovoid in particles shape as seen in the photomicrograph (X 400 magnification) displayed in plates 4.2 and 4.3. The arithmetic mean particle size was computed as 108 µm for ABPG95 and 120 µm for CCS90. Sieved analysis of ABP, ABPG95 and CCS90 indicated that ABPG95 has the highest particles size retained at 500 µm while CCS90 has the lowest particle size at 250 µm aperture. ABP retained more particles followed by ABPG95. A useful guide to the choice of the number of sieve in a nest, and their respective aperture size, is that not more than 5 % w/w of the test sample should be retained on the coarse sieve and not more than 5 % w/w should pass through the finest (Mukesh *et al*., 2011). The percentage retained on each sieve and cumulative percentage weight retained was calculated as displayed in Figure 4.1 and 4.2. Maximum particles were found between 150-250 µm range in both the samples indicating good flow. The spherical/irregular shape of ABP indicated that the powder will be more closely fitted and more densely packed. Particle size and particle size distribution of the samples have considerable impact on the flow properties of powder (Saha and Shahiwala, 2009). There was no much difference from normal distribution curve and there was similarity between the sample and the reference standard in term of particle size distribution.

5.3 Organoleptic properties

The result of organoleptic examination of ABPG95 and that of CCS90 showed that colour, taste, odour and texture were retained (Table 4.3). Texture was gritty in ABPG95 and slightly smooth for CCS90. This is an indication that the sample significantly maintained the integrity of the starting material. Since there was no change in term of colour, odour and taste, suggest that the excipient could easily be accepted by patient and probably give good functional properties. The

medication adherence often closely related to the odour, taste and colour of the product (Guru *et al*., 2011). While formulating, it is necessary to consider colour, odour, texture and taste together and not in isolation. The pH of ABPG95 and CCS90 fund where 7.8 and 7.3 respectively suggesting that they were both alkaline.

5.4 Physicochemical properties

The physicochemical properties of ABP, ABPG95 and CCS90 are presented in Table 4.2. The values recorded for flow rate (g/sec) for the three materials range from 11.6±0.5 to 14.4±0.12. This indicates that the values obtained were within the specified limits for the production of good quality tablets (Aulton, 2007). ABP, ABPG95 and CS90 have good flow properties in the following order: CCS90>ABPG95>ABP. The flow of powder during manufacturing of tablets dictates the quality of the product in terms of its weight and content uniformity (Prescott and Barnum, 2000). The weight variation is minimized if the formulation exhibit good flow property (Adedokun *et al*., 2009).

The values recorded for the angle of repose for the three materials ranged from 13±0.01 to 26.0±0.09 with CCS90 having the smallest angle and ABPG95 having the largest angle. The angle of repose is an index of flowability of a powder or granular substance. Value of the angle of repose is affected by the cohesive nature of the powder (Bhimte *et al.,* 2007). Powder with angle of repose ranging from 25 ° - 30 ° is considered excellent flow properties, whereas powder with angle close to 36 ° - 40 ° correspond to fair and aid needed to flow well (Carr, 1965). This revealed that the three materials have excellent flow properties which will give a content uniformity of the tablet during compression. Particles larger than 250µm are usually relatively free flowing but as the size falls below 100µm, powders become cohesive and those having a particle size less than 10µm are usually extremely cohesive and resist flow under gravity (Aulton, 2007). From the results, the three powders have good flow properties and should not present flow problem during formulation.

The values of bulk and tapped densities provide information on the flowability of powders and are used to calculate the Carr's index which is a measure of the flowability and compressibility of a powder. The lower the Carr's index of a material, the better the flowability and compressibility of powder (Odeku *et al*., 2009). The Hausner's ratio provides an indication of the degree of densification, which could result from vibration of the hopper (Adedokun *et al*., 2009). According to Hausner (1967), for powder that possesses good flow properties, the angle of repose, Carr's index and Hausner's ratio should not be greater than 35 °C, 16 % and 1.2 respectively. This is confirmed in the three materials given in Table 4.2. The values of bulk and tapped densities occurred in descending order as follows CCS90>ABPG95□ABP for tapped and CCS90□ ABP□ ABPG95 for bulk The tapped density is usually higher than the bulk density because of diminished void spaces as a result of a change in bulk volume. The change in bulk volume is produced by a rearrangement of the packing geometry of the particles resulting in a tightly packed powder bed (Staniforth and Aulton, 2007). Also, the bulk density is always less than the tapped density of its component particles because the bulk powder contains inter particulate pores or voids (Staniforth and Aulton, 2007). The bulk density of a powder is dependent on particle packing and changes as the powder consolidated. A consolidated powder

is likely to have a greater arch strength than a less consolidated one and may therefore be more resistant to flow, this is in agreement with the findings of Apeji (2010).

The moisture content for both materials did not exceed the limit of 15% specified by the BP (2002) for calcium. Moisture content plays a critical role in tablet formulation and is a very important physico-chemical property that affect other properties of a material such as flow and stability of product.

5.5 FT-IR Study

FT-IR studies as displayed in Figures 4.4, 4.5 and 4.6. The FT-IR absorption spectra of pure cow bone powder and pure gelatin powder showed peak bands at 4306.4 cm^{-1} and 3400.62 cm^{-1} , respectively. The FT-IR absorption peak band of co-processed is 1039.67 cm^{-1}, showed that no significant shift and no disappearance of characteristic peaks found in pure bone powder and gelatin powder , suggesting that there is no detectable chemical interaction or complexation between pure bone powder and gelatin powder, only physical changes occurred.

5.6 Thermal Analysis by DSC

There was a sharp endothermic peak of pure cow bone powder at 52.0 °C, gelatin at 40.7 °C and co-processed at 70.6 °C. The endothermic peak of pure activated cow bone powder and pure gelatin powder has very high intensity, showing the crystalline form of cow bone powder and gelatin similar to the finding of Kumar *et al*. (2011). No peaks corresponding to the melting point of pure bone powder and gelatin powder were observed in the co-processed. The endothermic peaks of co-processed lost its sharpness and distinctive appearance, an indication that no possible interaction was found between ABP and Gelatin. The DSC spectra, revealed that the changes were only physical and not chemical.

5.7 Compression of individual materials into tablets

The compression of ABP, Gelatin, ABPG95, CCS90, AA and MET as showed in Table 4.3 gave the compressibility profile in the following order: Gelatin, ABPG95 and CCS90 suggesting that they have good crushing strength, while ABP, AA and MET did not. All failed friability test, gelatin and ABPG95 has disintegration time (min) more than 40 min while CCS90 has more than 35 min to disintegrate . AA, MET and ABP did not give good compression profile alone.

5.8 Dilution potential

One of the requirements of a directly compressible excipient is that it should possess high dilution capacity. The dilution potential of an excipient is its ability to retain its compressibility and form a coherent compact when mixed with a poorly compressible drug (Hauschild *et al*., 2004).

Ascorbic acid (AA) and Metronidazole (MET) are poorly compressible in nature and so were used as model drugs to determine the dilution potential of ABPG95 and CCS90. The result is presented in Table 4.4, 4.5, 4.6 and 4.8. ABPG95 and CCS90 were able to bind up to 40% of MET compared to AA which accommodated a maximum of 30%. ABPG95/AA and CCS90/AA

still have a low dilution potential among other directly compressible fillers and this can be linked to its brittleness and strong cohesive properties of AA (Shittu *et al*., 2012).

A high bulk density, which is a low porosity, will result in a low deformation potential, a lack of space for deformation during compression will cause less intimate contact between the particles within the tablets, resulting in weaker tablets (Yuksel, *et al*, 2007 and Momoh *et al*., 2012).

5.9 Evaluation of Tablet Properties

The results for the uniformity of weight, thickness and diameter for the various batches of tablets formulated are presented in Table 4.8. The values for the uniformity of weight for the tablets range from 333 ± 0.2 to 333 ± 0.5 and 500 ± 0.2 to 501 ± 0.1 mg for AA and MET respectively. This falls within the official limits specified by the BP (2002). This implies that all the batches passed the weight variation test and revealed that co-processed excipient must have enhanced the flow of the formulation, ensuring that uniform volumes of the powder blend were fed into the die cavity resulting in tablets uniformity weight. A reasonable assumption is made that the variation in the weight of individual tablets is a valid indication of corresponding variation in drug content (Rawlins, 2004).

The results of tablet hardness also displayed in Table 4.8 confirmed that AA and MET tablets had harness profiles. Although, there is no official limit for tablet hardness, values falling within the range of 4.5-8 Kgf are generally acceptable. Results obtained for batches of tablets containing ABPG95 and CCS90 all falls within 7.1 ± 0.45 and 7.5 ± 0.4 for AA and 7.3 ± 0.32 Kgf and 7.6 ± o.27 Kgf for MET. The results of the study gives an assurance that the mechanical properties of the tablets would not be compromised during packaging, transportation and use.

The results of tablets friability test presented in Table 4.8 revealed that the tablets score ranged from 0.5 to 1.02 for both AA and MET. According to BP (2009) specifications, values of friability $\leq$ 1 % are acceptable for tablets formulated by wet granulation method, but for tablets prepared by direct compression, values of friability of up to 2 % are acceptable (Chowdary *et al*., 2012). The results therefore show that the tablets could be able to withstand shock and vibrations during the packaging, transportation and use.

The tablet disintegration time results presented in Table 4.8 show that the tablets complied with BP (2009) specifications for the disintegration time of normal release tablets. Tablets disintegration time ranged from 2.6 ± 0.1 to 10 ± 0.5 min for I (ABPG95/AA) and III (ABPG95/MET) have longer disintegration time compared to batch II (CCS90/AA) and IV (CCS90/MET) which has disintegration time of 3.3± 0.01 and 2.6 ± 0.1 min, respectively. This indicated that the binding properties of gelatin is higher than that of starch. All the tablets disintegrated in less than 15 min, this is in conformity with most compendia that disintegration time for uncoated tablets should not exceed 15 min (BP, 2009). Though the disintegrant property of ABPG95 was more pronounced in formulation containing poorly compressible and water insoluble API (MET) than in formulation containing highly water soluble moisture sensitivity API (AA) (Shittu *et al*., 2012).

The results of the drug release profile of AA and MET tablets formulated with ABPG95 and CCS90 of batches I to IV are shown in Figure 4.7 for ABPG95/AA and CCS90/MET and Figure 4.8 for ABPG95/AA and CCS90/MET, respectively. The dissolution efficiency (D.E.) i.e. percentage of drug released after 15-36 min was 100 % with ABPG95 andCCS90, respectively. CCS90 has the highest percentage drug released after 15 minutes in comparison to ABPG95. The alternates hypotheses is therefore accepted and null hypotheses rejected.

5.10: Contribution to Knowledge

The study established that:

1. Calcium Phosphate ($CaPO_4$) was derived from activated cow bone at 600 °C and 800 °C respectively

2. Direct Compression Properties of $CaPO_4$ derived from activated cow bone was improved by co-processing with gelatin.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

Cow bones especially the femur and tibia which are the largest bones rich in calcium were considered in this research work. Application of furnace heated from 600 °C up to 800 °C was a separation process which got rid of organic components of the bones:- bone marrow, fat tissues and other components of the bone. The heat application in the furnace therefore served as a dual purpose of melting and vaporising all organic components as well as activating the bone crystalline structure to become more amorphous.

Co-processing of ABP with gelatin (ABPG95) at various ratios showed improved functionality over direct physical individual excipient. In terms of binder ratio, the crushing strength values followed an approximate order of 90:10>95:5>97.5:2.5. This is in agreement with the findings of Uma *et al*. (2014). The stability and compatibility via FTIR and DSC was carried out to confirmed the co-processed material compatibility and stability.

The co-processing ABP and gelatin (ABPG95) was characterized for flow rate, angle of repose, bulk and tapped densities, Carr's index and Hausner ratio. The flow rate and angle of repose indicated that both the ABP and ABPG95 have good flow properties which will lead to uniform die filling of the powder blend during tableting and ensure content uniformity. The value of Carr's index and Hausner ratio reviewed that the co-processed material has improved functionality over direct physical mixture of individual material. This is line with the findings of Ajay *et al*., (2012) that co-processed material has improve functionality in direct compression tableting compared to an individual excipient. ABP and gelatin were incompressble, even when the compaction pressure was varied between 4 to 10 MT force with 12 mm punch and die single punch tableting tooling.

The dilution potential obtained for ABPG95 and CCS90 using AA as model drug was 30 % while that of MET was 40 %. ABPG95 can therefore be employed in the formulation of tablet containing poorly compressible and soluble API since the dilution potential were similar in functionality with Bacroft® (CCS90)

The tablet properties evaluated for ABPG95 and CCS90 as direct compression filler-binders revealed relatively similar properties except that drug -release from CCS90 was much faster when compared to drug-release from ABPG95.

6.2 Conclusion

The modification of ABP by co-processed method with gelatin improves the compressibilty profile. Therefore, modification of ABP makes it a suitable excipient as a direct compression filler-binder in direct compression tableting.

The absence of chemical changes in FTIR and DSC will help to reduce company's regulatory concerns during the development phase of the co-processing excipient.

6.3 Recommendations for Future work

i. Future work should be carried out on (ABPG95) economic potential for its industrial and pharmaceutical applications

ii. Further work with other binders such as natural gums and starches should be carried out to compare their feasibilities in dilution potential.

iii. Wider ratios of ABP and gelatin should be investigated e.g. ABP:G 96:4, 97:3, 98:2, 99:1.

REFERENCES

Ajay, S.C., Amrita, D. and Tushar, T. (2012). Formulation Development Techniques of Co-processed Excipients, *Journal of Advanced Pharmaceutical Sciences;* **2**(2). Pp234- 240

Alderborn, G. (2002). Tablets and compaction In: Aulton, M.E, (Ed), *pharmaceutics: The science of dosage form design,* 2nd edition. Churchil Livingstone, New York, pp 397-440.

Adedokun, M., Itiola, o. (2009). Material properties and compaction characteristic of natural and pregelatinized forms of four starches. Carbohydrate polymers, **79**: 818-824.

Ademola, O.A., Michael, A.O., and Kolawole, T.J. (2011). Evaluation of Cissus gum as Binder in a Paracetamol Tablet Formulation, *Farmacia*, **59**(1): 85-96

Alderborn, G. (2007). Tablets and compaction In: Aulton, M.E (Ed), *Aulton's pharmaceutics: The design and manufacture of medicines,* Churchil Livinstone Elsevier, London, 3rd Edition, Chapter 31, Pp 441-482.

Alebiowu, G. and Itiola, O.A. (2001). Compression characteristics of native and pregelatinized sorghum, plantain and corn starches and the mechanical properties of their tablets. *Drug Devevlopment and Industrial Pharmacy,* **28**(6):663-672.

Amelia A; Ahire V.J. (2007). Characterization and Evaluation of Spray Dried Co-processed Excipients and their Application in solid Dosage Form. *Indian Journal of Pharmaceutical Sci*ences; **69**:85-90.

Armand, M., Patrick, R. (2002). Comparative Evaluation of co-processed Lactose and Microcrystalline Cellulose with Their Physical Mixtures in the Formulation of Folic Acid Tablets, **7**(1): Pp79-87.

Armstrong, NA. (1997). Functionality Related Tests for Excipients. *International Journal of Pharmacy*; **155**:1-5.

Armstrong, N.A., Palfrey, L.P. (1989). The effect of machine speed on the consolidation of four directly compressible Tablet Diluents. *Journal of Pharmacy and Pharmacol*ogy, **41**:149-151.

Aulton , M.E. (2007). Pharmaceutics: The Science of Dosage Form Design 3rd Edn. Churchil Living Stone. Edinburg ; 197-210.

Apeji, Y.E. (2010). Tableting properties of Microcrystalline Starch Derived from Cassava (*Manihot esculenta Crantz*) starch by Enzymatic Hydrolysis Using α-amylase enzyme. MSc Thesis. Ahmadu Bello University, Zaria.

Ashford, M. Aulton, M.E. Second Edition (2007). Pharmaceutics. The Science of Dosage Form Design. Churchill. Living Stone New York, Pp234-252.

Aundhia, C.J., Raval, J.A., Patel M.M., Shah, N.V., Chauhan, S.P., Sailor, G.U., Javial, A.R., Mahashwari, R.A. (2011). "Spray drying in the pharmaceutical industry-A review" *Indo American Journal of Pharmaceutical Research;* **7**(1): 125-138.

Autamashih M, Isah A B, Allagh T S, and Ibrahim M A. (2011). Use of anhydrous calcium phosphate and selected binders in the tablet formulation of a deliquescent crude plant extract: *Vernonia galamensis* (Asteraceae); *Journal of Applied Pharmaceutical Science* **01**(08): 118-122.

Awasthi, RD, Garg, G, Pawar, V, Sharma, G, Kulkarni, GT. (2010). Development of Directly Co-processible Co-processed Excipients for solid Dosage forms. Scolar Research Library, *der pharmacia letter*; **2**(6):151-165.

Ayyappan J, Umapathi P, Darlin quine. (2010). Development and Evaluation of a Directly Compressible Co-processed Multifunction Sustained Release Agent for Tablets. *International Journal of Pharmceutics and Pharmaceutical Sciences; **2***(4):201-205.

Bakre, L.G., and Jaiyeoba, K.T.(2009). Effect of Drying Methods on the Physicochemical and Compressional properties of Okra Powder and the Release Properties of its `Metronidazole Tablet Formulation. *Archive of Pharmacal Research;* **32**(2): 259- 267

Bansal, A.K. (2003). Improved excipients by solid-state manipulation. *The industrial Pharmacy, issue* 31.

Bansal, A.K, Nachaegari, S.K., (2004). Co-processed excipients for solid dosage forms. *Pharmaceutical Technology* ,**28** : 52-64.

Baykara, T., Duman, G., Ozesener, K.S., Ordu, S., and Ozates, B. (2008). Comparing the compressibilty of Ludipress with the other Direct Tableting Agents by Using Acetaminophen as an Active Ingredient, *Drug Development and Industrial Pharmacy*, **17**:2359-2371.

Bisrat, M, Anderberg, E.K, Barnett, M.I and Nystrom, C.(1992). Physicochemical aspects of drug release. XV. Investigation of diffusional transport in dissolution of suspended, sparingly soluble drugs. *International Journal of Pharmacy,* **80**: 191-201.

Block, L., Moreton, R., Apte, S., Wend, R., Munson, E., Creekmore, J., Persaud, I., Sheehan, C., Wang, H. (2009). Co-processed Excipients. *Pharmacopeia Forum*; **35**:4-10.

British Pharmacopoeia (BP). (2009). The Commission Office London, 111: 6578-6585.

British Pharmacopoeia (2002, vol I and II: Her Majesty's Stationery Office, University Press, Cambridge.

Chowdary KPR, and Ramya k. (2012). Recent Research on Co-processed Excipients for Direct Compression-A Review; *International Journal of Comprehensive Pharmacy*, 0976-8157.

Chowdary K P R, Udaya C D, Sandana N and Madhavi P. (2012). Preparation, Characterization and Evaluation of Cellulose-Ethyl cellulose Co-processed Excipient in the Formulation Development of Tablets. *International Journal of Chemical and Research,* **2**(05): 21- 30.

Chowdary KPR, Franklin S.I. and Satyanarayana A.R. (2012). Preparation, Characterization and evaluation of Starch-PEG 1500 co-processed excipient as directly compressible vehicle in tablet formulations. *Pharmacie globale, IJCP*; **03**(11):1-4.

Chowdary, KPR., Ramya, K. (2013). "Recent Research on co-processed excipients for direct compression- A REVIEW." *Pharm Globale (IJPC),* Vol. **04,** Issue 02 ISSN 0976-8157. 1-5

Christopher C, DeMerlis. (2002). The IPEC-Americas Excipient Master File Guide. *Pharmaceutical Technology*; 36-44.

Chukwuma, O. A, Godwll C.O, Calister E.U, Saome A.C. (2012). Physical and Mechanical effects of Starch gelatin binary binder mixtures on sodium salicylate tablets. *Journal of Chemical and Pharmaceutical Research*; **4**(3): 1625-1628.

Dacke, CG., Arckle, S, Cook, DJ., Wormstone, IM., Jones, S., Zaidi, M., and Bascal, ZA; (2000). Medullary bone and avian calcium regulation. *Journal of Experimental Biology* **184**: 63-68.

De Boer, A.H., Bolhuis, G.K. and Lerk, C.F. (1999). Bonding Characteristics by Scanning Electron Microscopy of Powders Mixed with Magnesium Stearate. *Powder Technology.*, **20**: 75-82

Deorkar, N., Farina, J., Randive, S. (2011). Directly compressible high functionality granular microcrystalline cellulose bas excipient, manufacturing process and use thereof; US20110092598.

Duberg, M. and Nystrom, C. (2000). Studies on direct compression of tablets. *Acta Pharmaceutical Sciences;* **19**: 421-436.

Emeje, M.O., Ismil, C.Y. and Okunle, O. (2007). Evaluation of Okora gum as Dry Binder in Paracetamol Tablet Formulation, *Continental Journal of Pharmaceutical Sciences,* **1**: 15-22.

Emenike, I .V. (2005). Tableting Properties of Thermally Activated Cow Bone Powder in High and Low Dose Formulations, a PhD Dissertation, Ahmadu Bello University, Zaria, Nigeria.

Feintag, k. (2005). Co-processed of powdered cellulose and magnesium carbonate. Direct tableting versus tableting after all compaction/ dry granulation. *Pharmaceutical Development Technology,* **10**, Pp 353-362

Finch, C.A. (1989). Gelatin, U//mann's Encyclopedia of industrial chemistry, Vol. A **12** chapter, 2: 315-VCH, Publishers. Weinheim.

Flores, L.E., Arellano, R.L., and Esquivel, J.J.D. (2008). Study of Load Capacity of Avicel PH-200 and Cellactose in Direct- Compression Ex cipients Using Experimental Design. *Drug Development and Industrial Pharmacy,* **26**(4): 465-469.

FORMAXX® pdf is available online the given link 85.238.144.18/lifescience/Formaxx %20 Co processed %20 Calcium %20 Carbonate.pdf.

Fuhrer C. (2001). Substance behaviour in direct compression. *Labo- Pharmaceutical Problem Technology*, **269**: 759-762.

Gelatin Manufacturers Institute of America (GMIA) (2012).

Gohel, M,C. (2005). A review of co-processed directly compressible excipients. *Journal of Pharmaceutics and Pharmaceutical Sciences,* **8**(1): 76-93.

Gohel, MC and Jogani, PD. (2003). An Investigation in Direct Compression Characteristics of Co-processed Lactose-Starch using Experimental Design, *Indian Journal of Pharmaceutical Sciences,* **65**: 31- 38.

Gohel, M C., Parikh, B K and Shah, A R. (2007). Preparation and Evaluation of the Material Properties of Co-processed Diluents Containing Modified Starch and Dicalcium Phosphate. *International Journal of Pharmaceutical Sciences Research;* **2**(3): 543-551.

Gustafsson,C. (2000). Solid State Characterisation and Compaction Behavior of Pharmaceutical Materials. Comprehensive summaries of Uppsala Dissertation from Faculty of Pharmacy, Sweden.

Hancock, B.C., Carlson, G.T., Ladipo, D.D., Langdon, B.A. and Mullarney, M.P. (2002). Compaction of the mechanical properties of the crystalline and amorphous forms of a drug substance. *International Journal of Pharmacy,* **241**: 73-85.

Hauschild, K. and Picker, K.M. (2004). Evaluation of a new Co-processed Compound Based on Lactose and Maize Starch for Tablet Formulation. *American Association of Pharmaceutical Scientist and Pharmaceutical Science,* **6**(2):1-12.

Hausner, N.H. (1967). Flow properties of some pharmaceutical powders. *International Journal of Powder Metallurgy* **3**: 7-11.

Hiller, J.C., Thompson, T.J.U., Evison, M.P., Chamberlain, A.T., Wess, T.J. (2003). Bone mineral change during experimental heating: an X-Ray scattering. *Journal of Biomaterials,***24**: 5091-5097.

Hoon Jang and Seon-Hong Kang. (2002). Phosphorus removal using cow bone in hydroxyapatite crystallization, *water Research,* **36**: 1324-1330.

Hyunjo,K., Gopi, V., Fassihi, R. (1988). Compatibility Characterisation of Granular Pectin for Tableting operation using a Compaction Simulator. *International Journal of Pharmaceutics,***161:** 149- 159.

Ibrahim Y K, and Olurinola P F. (1991). Comparative Microbial Contamination Levels in Wet Granulation and Direct Compression Methods of Tablet Production. *Pharm Acta Helv.* **66**:298- 301.

Isah, A.B., Abdulsamad A., Gwarzo M.S. and Abbah H.M. (2009). Evaluation of the disintegrant Properties of Microcrystalline Starch Obtained from cassava in Metronidazole tablet formulations. *Nigerian Journal of Pharmaceutical Science, **8***(2): 26-35.

Jacob S, Shirwaikar A.A., Joseph A., Srinivasan K.K. (2007). Novel co-processed excipients of mannitol and microcrystalline cellulose for preparing fast dissolving tablets of glipized. *Indian Journal of Pharmaceutical Science,* **69**:633-639.

Jayesh, P. and Manish, R. (2009). Tablet Formulation Design and Manufacture: Oral Immediate Release Application. *Pharmacy Times,* **41**(4): 21-23.

Jivraj, M, Matini, L.G, and Thomson, C.M. (2000). An Overview of the Different Excipients Useful for the Direct Compression of Tablets. *Pharmaceutical Science Technology,* **3**:58-63.

Jogani P.D, Gohel M C, Bariya S H. (2003)." Development of Agglomerated Directly Compressible Diluent Consisting of Brittle and Ductile Materials" *pharmaceutical Development and Technology,* **8**(2); 143-151.

Juan C.E, Arturo,S.O, Montserrat, G.L. (2005). Modified calcium phosphate excipient; Wo 2005004916 $A_{2.}$

Keene B.E, Knowlton K.F, McGilliard M.L, Lawrence L.A, Nickols-Richardson S.M, Wilson J.H, Rutledge A.M, McDowell L.R, and Van Amburgh M.E. (2004). Measures of Bone Mineral Content in Mature Dairy Cows; *Journal Dairy Sciences;* **87**: 3816-3825.

Keene, B.E., Knowlton, K.F., Wilson, J.H., McGilliard, M.L., and Holtaway, C. (2003). Bone breaking strength in mature dairy cows. *Transactions of the American Society of Agricultural Engineers.*

Kragh, A.M. (1977). The Science and Technology of Gelatin. A.G. ward and A. Courts, eds, p450. Academic Press, London.

Kumari M.S., Prasanthi E.H., Sadha b., Praveena M.K., Ushari S. (2013). Reassessment of Novel co-processed multifunctional excipient; *International Research Journal of Pharmaceutical and applied Science;* **3**(4):122-128.

Larry L, Augsburger, Stephen W H. (2008). Pharmaceutical dosage forms: Tablets; Unit 5, Direct compression and the role of filler-binders, 3rd ed; 2; 173.

Lerk,CF. (2001). Consolidation and Compaction of Lactose, *Drug Development Industrial Pharmacy,* **19**: 2359-2398.

Loveridge, N. (1999). Bone: More than a stick. *Journal of Animal Science* **77**(Suppl.2): 190-196.

Maarschalk, K.V.D.V., and Bolhius, G.K., (1999). improving properties of Material for direct compaction: *Pharmaceutical Technology,* **23**(5):34-48.

McDowell,LR., Williams, SN., Lawrence, LA., Wilkinson, NS., Ferguson, PW and Warnick, AC. (2000). Critarial to evaluate mineralization in cattle: II. *Noninvassive techniques. Journal of Animal Science.* **69**: 1243-1254.

Momoh, M.A., Brown, S.A., Onunkwo, G.C., Chime, S.A., Adedokun, M and Akpabio, E.I. (2012). Effect of hydrophillic and hydrophobic binders on the physico-chemical properties of sodium salicylate tablet formulation. *Journal of Pharmaceutical Sciences* **5**(4): 2045-2048.

Moreton, R.C. (1996). Tablet Excipients to the year 2001: A look into the crystal Ball. *Drug Development Industrial Pharmacy,* **22**(1): 11-23.

Moreton,R.C. (2009)."Cellulose , Silicified Micricrystalline," In Hand Book of Pharmaceutical Excipients, Wade V and Weller PJ Eds. (Pharmaceutical Press, London). 110-111.

Mourya K, Prajapati S.K; Jain S K, Kumar A, Thakran A K and Alok S. (2012). Formulation and Evaluation of fast Dissolving Tablets of Acetaminophen. *International Journal of Pharmaceutical Science Research;* **3**(2): 610-614.

Muazu,J., Musa, H., Isah, A.B., and Bhatia, P.G. (2011). Effects of processing variables on distribution of low dose hydrophilic and hydrophobic drug in granules prepared from various diluents. *Nigerian Journal of Pharmaceutical Science,* **10**(1): 65-72.

Mukesh C G, Rajesh K P, Manali D P and Bhavika H Z. (2011). Preparation and Evaluation of Material Properties of Co-processed Diluent Containing Modified Starch and Dicalcium Phosphate. *International Journal of Pharmaceutical Science and R*esearch, **2**(3):543-551.

Musa, H. Ochu, S. N , and Bhatia, P.G (2010). Evaluation of the Tablet Binding Properties of Barley (Hordeum vulgare) Starch. *International Journal of Applied Pharm*acutics, Volume **2**,Issue 4.

Nachaegari, S.K, and Bansal, A.K. (2004). Coprocessed Excipients for Solid Dosage Forms. *Pharmaceutical Technology;* **28**:52-64.

Nidal D, Iyad R, Mahmoud M.H.A, Stephen A.L, Babur Z.C and Adnan B. (2010). Preparation and Characterization of a Novel co-processed Excipient of Chitin and Crystalline Mannitol. *American Association of Pharmaceutical Scientists and pharmaceutical Technology, **11***(4):1558-1571.

Nystrom, G, Alderborn, G, Durberg, M, and Karehill, P.G. (1993). Bonding surface area and bonding mechanisms- two importantfactors for the understanding of powder compactability. *Drug Development and Industrial Pharmacy,* **19**: 2143-2196.

Odeku, O.A. (2005). Assessment of *Albicia zygia* gum as a binding agent in tablet formulations. Acta Pharmaceutica Sep, **55**(3) 263-276.

Odeku, O., Picker-Freyer, K. (2009). Characterisation of acid modified dioscorea starches as direct compression excipients. *Pharmaceutical Development Technology,* **14**: 259-270.

Okafor, I.S, Ofoefuke, S.I and Udeala, O.K. (2000). A comparative study of modified starch in direct compression of a water soluble drug- chloroquine phosphate. *Bollettino Chimico Farmaceutico,* **139**(6): 252-5.

Olowosulu, A.K., Oyi, A.R., Isah, A.B., and Ibrahim, M.A. (2011). Formulations and Evaluation of novel Co-processed Excipients of Maize Starch and Acacia gum (StarAc) For Direct Compression Tableting. *International Journal of pharmaceutical Research and Innovation,* **2**: 39-45.

Onyishi, I.V., Chime, S.A. and Ugwu, J.C.(2013). Evaluation of binder and disintegrant properties of Starch derived from *Xanthosoma Sagittifolium* in metronidazole tablets. *African Journal of Biotechnology,* **12**(20): 3064-3070.

Oyeniyi, Y.J., and Itiola, O.A. 2012). The physico-chemical Characteristics of Micro crystalline cellulose, Derived from Sawdust, Agricultural waste product. *International Journal of Pharmacy pharmaceutical Science;* **4**: 197-200.

Oyi, A.R, Apeji, Y.E, Musa, H. (2009). Compact Analysis of Microcrystalline Cassava starch. A direct compression binder. *Nigerian Journal of Pharmaceutical Science*

Panda D, Swain S.,Gupta, R., Si S., Kanungo S.K., (2006). Preparation and evaluation of gels from gum of Moringa oleifera; *India Journal of Pharmaceutical Science* Vol. **68**(6), Pp 777-780.

Patel, SS, Patel N M. (2009). Development of directly compressible co-processed excipient for dispersible tablets using 3^2 full factorial design; *International Journal of Pharmacy & Pharmaceutical Sciences;* **4**(1):37-43

Patel, SS., Patel, NM, Soniwala, MM.(2009). Statistical Development of a Multifunctional Directly Compressible Co-processed Excipient Using the Melt Agglomeration Technique. *Asian Journal of Pharmaceutical Sciences;* **4**(6): 340-566.

Pranav, D, Gohel, M.C. (2005). A review of coprocessed directly compressible excipients; *Journal of Pharmacy and Pharmceutical Sciences;* **8**(1): 76-93.

Rakesh, P.P. (2009). "Directly Compressible Materials vial Co-processing." *International Journal of Pharmaceutical Technology Research,* ISSN:0974-4304; **1**(3): 745-753.

Ramani R G. (2010). Development of Novel Coprocessed Excipients for the Design and Evaluation of Fast Dissolving Metoclopramide Hydrochloride Tablets; Deparment of pharmaceutical technology, H.K.E. Society's college of pharmacy;5-105.

Ramya, K., Chowdary, K.P.R. (2013). Preparation, characterization and evaluation of a new co-processed excipient as Directly Co-pressible in Tablet Formulation; *Journal of Glob Trends in Pharmaceutical Sciences;* **4**(4): 1322-1328.

Rao, V.U., Balasubramaniam, J., Haldar, R.K. (2012). Co-processed excipient compositions. US20120178822.

Rasenack, N., and Muller, B.W.,(2002). Crystal Habit and Tableting Behaviour. *International Journal of Pharmacy;**244***:45-57.

Reimerdes, D. (1993). The Near Future of Tablet Excipients. *Manuf. Chem;* **64**:14-15.

Rubinstein, M.H. (1998). Tablets Pharmaceutics: the Science of Dosage Form, Churchill, UK, 1st ed., 304-321.

Rawlins, E.A (2004). Tablets and Capsules. In: Rawlins, E.A. (Ed), *Bentley's textbook of pharmaceutics,*8th edition, AITBS Publishers, India, Chapter 19, Pp 269-318.

Rudnic , E.M, and Kottke, M.K. (2008). Tablet dosage forms In: Banker, G.S, Rhodes,C.T, (Eds), Modern Pharmaceutics, 3rd Edition revised and expanded. *Marcel Dekker Inc*;
New York, USA Pp 333-394.

Russell, R.(2004). Synthetic excipient challenge all-natural organics offer advantages/challenges to developer and formulators. *Pharmaceutical Technology,* **27**: 38-50.

Sam, A. P., and Fokkens, J.G..(1997). Drug Delivery System: Adding Therapeutic and Economic Value to Pharmacotherapy. Part 2, *Pharmaceutical Technology European,* **9**: 58-66.

Sanjay, S.P., Natvarlal, M.P. (2009). Development of Directly Co-pressible Co-processed Excipient for Dispersable Tablets using 3^2 factorial Design.

Schoneker, D. (2011). Co-processed Excipients: IPEC guidelines. http//ipecamericas.org/content/excipientfest.

Shangraw R F, Wallace J W and Bowers F M. (2003). Morphology and Functionality in Tablet Excipients for Direct Compression. *Pharmaceutical Technology,* **11**: 136-143.

Shangraw, R F. (1997). Emerging Trends in the use of Pharmaceutical Excipients. *Pharmaceutical Technology,* **21**(6): 36-42.

Shangraw, R.F. (1987). Direct Compression Tableting, Encyclopedia of Pharmaceutical Technology. 4, Marcel Dekker, USA, 2nd ed., 85-160.

Sherwood, B.E., Hunter, E.A., Staniforth, J.H. (2012). Pharmaceutical excipient having improved compressibilty. US5585115.

Shittu, A.O., Oyi, A.R., Isah, A.B., Ibrahim, M.A. (2012). Formulation and evaluation of 2-component composite excipient "microcrystarlac" as a fiiler-binder for direct compression; *International Journal of Scientific and Technology Research ,* **1**(3): 2277-8616.

Silverstein, L. (2002). Excipient GMP Quality Standards One is Enough; *Pharmaceutical Technology .,* **25**: 46-52.

Smewing , J. (2002). Powder flow analysis- the solution, *Manufacturing Chemist,* **2**(5)32-33.

Sonnergaard, J M. (1999). A critical evaluation of the heckel equation. *Int Journal of Pharmacy* **193:** 63- 71.Sotton, E and Obiorah, B.A.(2000). The effect of particle shape and Crystal habit on properties of sodium chloride. *Journal of Pharmacy and Pharmacology,* **25**: Suppl. 37p-43p.

Sri Aliza, M.A., Zaheruddin,K., Shahriza,H. (2009). Study the properties of dense Hydroxyapatite - extract from cow bone; *Journal of Nuclear and Related Technology* vol.**6**, No.1.

Staniforth, J.N., Sherwood, B.E., Hunter, E.A., Davidson, C.M. (2002). Process for preparing directly compressible solid dosage form containing microcrystalline cellulose. US 6395303.

Steinberg, M., Blecherl; and Mercill, A. (2001). From inactive Ingredient to Pharmaceutical Excipients; *Pharmaceutical Technology,* **25**(7): 62-64.

Sujatha, M.K., Prasanthi, C.H., Sudha, B., Praveena, M.K., Ushasri,S. (2013). Reassessment of Novel Co-processed Multifunctional Excipients. *International Research Journal of Pharmaceutical and Applied Sciences; (IRJPAS)*;**3**(4): 122-128.

Sumit, S., and Aliasgar, F.S. (2009). Multifunctional co-processed excipients for improved tableting performance: *Expert Opinion Drug Delivery.*,**6**(2): 197-208.

Swarbrick, J., Boylan, J. Encyclopedia of pharmaceutical technology. Sieving of pharmaceuticals, 14:67.

Thoorens, G., Lelercq, B., Ruszkay, T. (2011). Microcrystalline cellulose and calcium phosphate compositions useful as pharmaceutical excipients. WO2011056775.

Uma, R.G. and Naheed, B. (2014). Over View of Co-processed Excipients Used To Improve Tableting Performance, *Journal of Advanced Drug Delivery* (JADD), **1**(6): 2348-3278.

Ward, A.G. and Courts, A. (1977). The Science and Technology of Gelatin Academic press New York, NY.

York, P. (1999). Crystal Engineering and Particle design for the powder compaction process. *Drug. Development Industrial Pharmacy;* **18**(6,7): 677.721.

Yuksel, N., Turkenen,B., KKurdoghu, A.H., Basaran, B., Erkin, J., Baykara, T. (2007). Lubricant efficiency of magnesium stearate in direct compressible powder mixtures comprising cellactose® 80 and pyridoxine hydrochloride. *FABAD.Journal of Pharmaceutical Sciences;* **32**: 173-183.

APPENDIX

A1 Particle size distribution for ABPG95

Particle size (µm)	Frequency	FX	% Frequency	Cumulative Frequency

2	12	24	6	6
4	16	64	8	14
5	20	120	10	24
7	108	756	54	78
10	18	220	9	87
12	11	216	5.5	92.5
15	9	210	4.5	97
23	6	230	3	100

A2 Particle size distribution of CCS90

Particle size (µm)	Frequency	FX	% Frequency	Cumulative Frequency
3	10	30	5	5
4	17	68	8.5	13.5
6	120	720	60	73.5
8	19	152	9.5	83
11	14	154	7	90
14	11	154	5.5	95.5
18	6	108	3	98.5
20	3	60	1.5	100

A3 Particle size distribution of ABP

Sieve sizes (µm)	wt retained (gm)	% distribution	% cumulative
500	0.44	1.47	1.47
250	21.31	71.03	72.5
150	8.05	26.83	99.33
90	0.06	0.2	99.53
75	0.08	0.27	99.80
Pan	0.06	0.2	100.00

A4 Particle size distribution of ABPG95

Sieve size (µm)	wt retained (gm)	% distribution	% cumulative
500	6.24	15.53	15.53
250	20.7	51.75	67.28
150	10.54	26.35	93.63
90	1.50	3.75	97.38
75	0.65	1.63	99.01
Pan	0.39	0.99	100.00

A5 Particle size distribution of CC90

Sieve size (µm)	wt retained (gm)	% distribution	% cumulative
500	0.13	0.43	0.43
250	4.14	13.8	14.23
150	9.67	32.23	46.46
90	1.92	6.4	52.86
75	8.98	29.93	82.79
Pan	5.16	17.21	100.00

A6 Result of dilution potential (MET: ABPG95/CCS90

MET: ABPG95/CCS90	Crushing strength KgF	Disintegration time	Compressibility description
20:80	5.3	24.14	good
30:70	5.6	22.23	good
40: 60	6.5	17.17	good
50:50	3.5	6.5	poor
60:40	2.0	1.45	poor

A7 Result of dilution potential (AA: ABPG95/CCS90)

AA:ABPG95/CCS90	Crushing strength KgF	Disintegration time	Compressibility description
20:80	4.5	22.37	good
30:70	5.6	18.17	good
40:60	2	3.45	poor
50:50	1.0	1.23	poor

A8 Dissolution test result for ABPG95/AA

Time (mins)	Abs (nm)	Amount dissolved (mg/1000ml)	% drug dissolved
10	0.985	50.65	25

20	2.327	78.5	60
30	3.012	160.4	77
40	3.895	200	100
50	3.894	200	100
60	3.895	200	100

A9 dissolution test results for CCS90/AA

Time (mins)	Abs (nm)	Amount dissolved (mg/1000ml)	% drug dissolved
10	1.102	56.7	28.3
20	2.951	151.7	76
30	3.785	194.6	97
40	3.879	200	100
50	3.886	200	100
60	3.885	200	100

A10 Dissolution test results ABPG95/MET

Time (mins)	Abs (nm)	Amount dissolved (mg/1000ml)	% drug dissolved
10	0.960	43.9	22.0
20	2.391	109.4	55
30	3.263	149.3	75
40	3.935	180	90
50	4.376	200	100
60	4.375	200	100

A11 Dissolution test results for CCS90/MET

Time (mins)	Abs (nm)	Amount dissolved (mg/1000ml)	% drug dissolved
10	1.305	60	30
20	2.851	130	65
30	3.962	181	91
40	4.371	200	100
50	4.373	200	100
60	4.372	200	100

A12 Conentration vs absorbance values for Ascorbic acid

Conc. (µm/ml	Absorbance (nm)
1.534	3.125
0.815	1.5625
0.363	0.7813
0.172	0.3906
0.12	0.1953
0.06	0.0977

A13 Concentration vs absorbance values for Metronidazole

Conc. (µm/ml	Absorbance (nm)
1.909	3.125
0.883	1.563
0.377	0.781
0.148	0.391
0.092	0.195
0.047	0.098

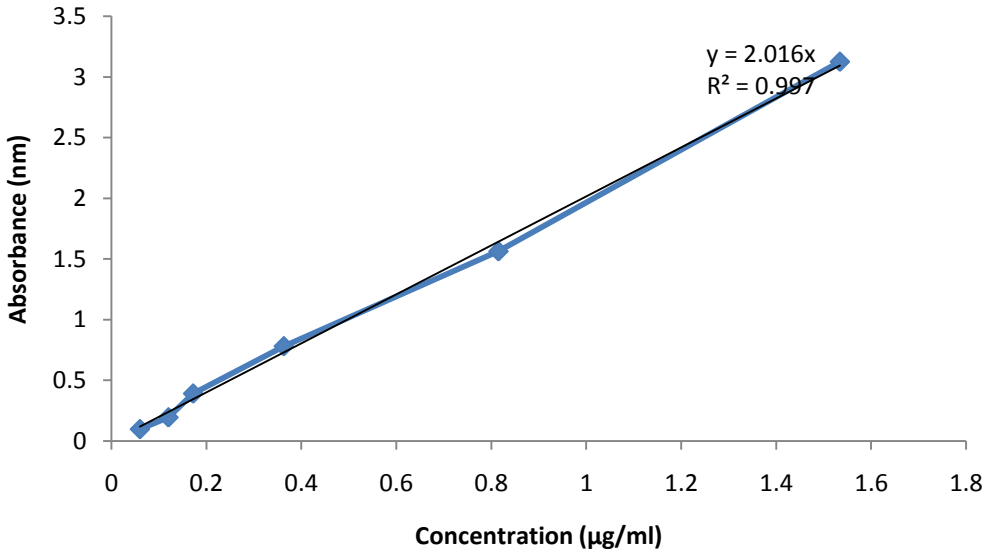


Figure A1 Calibration curve for Ascorbic acid

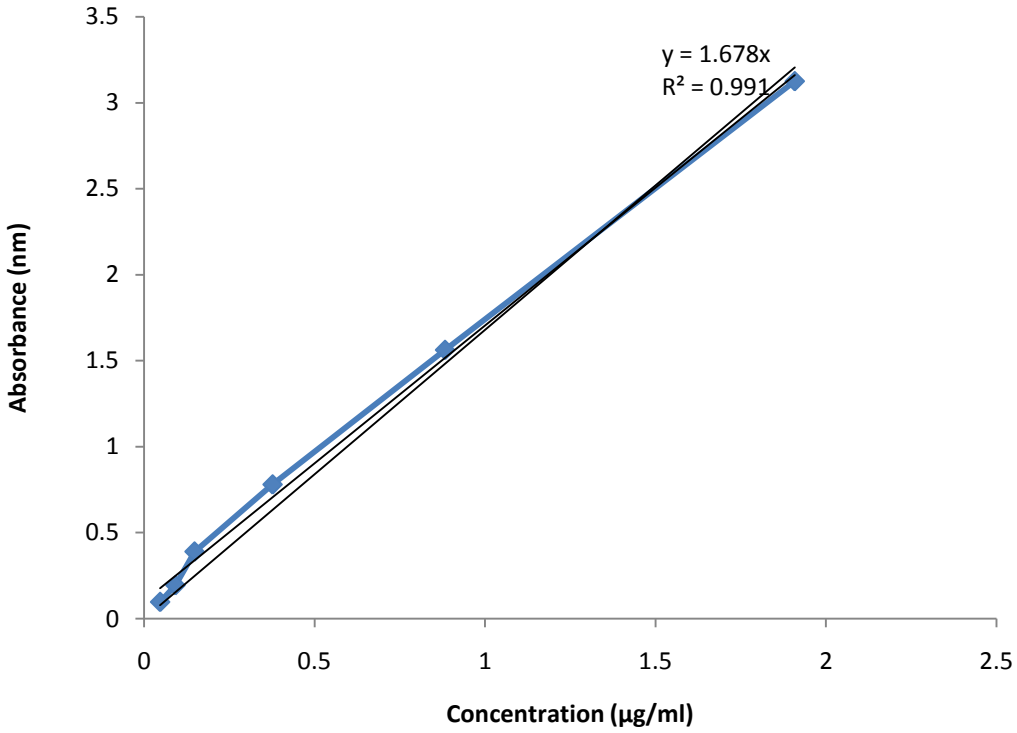


Figure A2 Calibration curve for Metronidazole

A14 pure cow bone powder

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	408.92	19.969	15.527	498.62	339.48	94.253	17.435
2	584.45	14.35	11.224	818.81	498.62	217.128	31.366
3	874.75	25.999	0.819	896.93	818.81	44.696	0.48
4	1039.67	10.672	14.56	1327.07	896.93	327.495	65.591
5	1475.59	17.006	5.284	1590.36	1327.07	187.393	16.119
6	1641.48	20.146	2.207	1887.41	1590.36	188.285	1.126
7	2013.75	23.977	0.534	2062.94	1887.41	106.922	0.429
8	2356.13	23.023	1.078	2419.78	2235.57	115.116	1.345
9	2932.86	19.464	0.931	2986.87	2419.78	370.345	-3.234
10	3406.4	12.987	7.369	3793.14	2986.87	626.09	68.436

A15 pure gelatin powder

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	406.03	24.402	12.944	435.93	339.48	46.132	8.295
2	483.18	25.073	0.037	485.11	436.89	28.554	0.012
3	631.71	25.457	0.646	909.47	564.2	198.48	0.925
4	1150.58	25.494	2.152	1291.39	910.43	217.962	5.369
5	1485.24	25.161	1.954	1586.5	1292.35	171.515	5.319
6	1643.41	25.343	1.996	1903.8	1587.47	176.141	2.549
7	2161.31	28.399	0.127	2190.24	1906.7	152.879	0.402
8	2256.79	28.241	0.034	2271.26	2191.21	43.864	0.02
9	2356.13	27.456	0.813	2421.71	2272.22	82.867	0.839
10	2932.86	24.853	0.567	2981.08	2422.67	320.456	0.696
11	3400.62	18.382	7.728	3797	2982.05	528.45	52.595

A16 co-processed excipient

	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	395.42	23.6752	15.2638	435.93	339.48	49.6611	10.1592
2	472.58	27.234	0.269	485.11	435.93	27.5822	0.1844
3	575.77	19.9235	8.3138	799.52	485.11	185.9544	16.4786
4	1036.77	16.1423	13.5595	1331.89	799.52	329.8552	48.1312
5	1468.84	23.5198	4.2909	1593.25	1331.89	155.746	10.5826
6	1648.23	25.6402	2.1963	1906.7	1593.25	169.3815	0.976
7	2012.79	30.1943	0.4365	2069.69	1906.7	83.8877	0.3357
8	2354.2	28.6359	1.8057	2424.6	2258.72	87.5135	1.8414
9	2932.86	25.9445	1.1862	2995.55	2424.6	309.9866	-1.1857
10	3400.62	21.0147	6.2384	3721.77	2995.55	447.7573	37.0629
11	3749.74	27.6535	0.0094	3798.93	3748.78	27.8374	-0.0013
12	3865.48	27.6188	0.0205	3868.37	3804.72	35.3832	0.0246
13	3996.64	27.9417	0.0047	4000.5	3987.96	6.9421	0.0006