FORMULATION AND EVALUATION OF CAPSULES FROM ASENA AND ENTERICA PREPARATIONS SUPPLIED BY CENTRE FOR SCIENTIFIC RESEARCH INTO PLANT MEDICINE (CSRPM), MAMPONG, GHANA

By;

Doris Kumadoh B.Pharm [Hons.]

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DEDICATION

This Project is dedicated to God and my parents.

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ABSTRACT

The project sought to transform two decoctions (Asena and Enterica) produced by the Centre for Scientific Research into Plant Medicine for the treatment of arthritis and typhoid fever respectively into capsules. The amount of extract per dose (30ml) of Asena was 400mg. The amount of extract per dose (30ml) of Enterica was 190mg. Adsorbents were used to adsorb water to enhance processing of extracts due to their inability to dry completely in large doses. Five adsorbents (maize starch, light magnesium carbonate, bentonite, kaolin, microcrystalline cellulose) were initially investigated to ascertain their ease of processing into granules with thin viscous extracts of decoctions obtained by drying in the Oven at 60^{O}C. For Asena the amount of adsorbent per dose used for initial investigation was 200mg. This amount was ascertained by determining the amount of extract of Asena that was able to fill a 500mg capsule shell. With Enterica, the amount of adsorbent per dose used for the same purpose was 110mg. This was ascertained by determining the amount of Enterica extract that was able to fill a 250mg capsule shell. The release of the extract in the formulated granules was also determined at 45minutes.

For Asena light Magnesium carbonate, maize starch and bentonite were used at five different weights of 40mg, 80mg, 160mg, 180mg and 200mg per dose of Asena decoction for further investigation. The adsorbent was used to form a paste with thin viscous extracts of the decoction obtained by drying of decoction in the oven at 60^{O}C.The paste was then allowed to dry to a constant weight. The ease of processing of adsorbent extract mix formed after drying into granules was recorded. The percentage loss in weight of granules was determined. The flow properties of the formulated granules were also determined using the fixed height cone, Carr's index and Hausner ratio methods. The

dissolution profiles of the formulated granules were determined using the UV method of analysis. It could be observed that maize starch showed optimum release of extract at all concentrations (87.45 ± 1.82% -97.19 ± 1.46%).

Bentonite also exhibited optimum release at all concentrations (83.94 ± 1.69% -98.15 ± 1.96%). Light magnesium carbonate exhibited poor release of extract with the highest release at 45minutes being37.89 ± 1.54% for 40mg of light magnesium carbonate per dose of Asena decoction. Capsules of Asena were formulated using maize starch at a quantity of 200mg per dose as adsorbent. Maize starch was also used as diluent. Talc was used as a glidant. Two hundred and fifty milligrams capsule shells were used. Each dose of Asena was therefore divided to fill two capsules. The release of extract from formulated capsule at 45minutes was 79.82 ± 2.36%. The disintegration time of capsules was 8.03 ± 0.13 minutes for Asena. The resulting capsules passed the B.P. uniformity of weight tests.

The same procedures were repeated for Enterica using Light magnesium and maize starch at quantities of 22mg, 44mg, 66mg, 88mg and 110mg per dose of Enterica decoction. It was observed that light magnesium carbonate exhibited optimum release (86.08 ± 1.64%) at a quantity of 22mg per dose. Capsules of Enterica were therefore formulated using light magnesium carbonate as adsorbent at a quantity of 22mg per dose of Enterica extract. Lactose and talc were used as diluent and glidant respectively. Two hundred and fifty milligrams capsule shells were used. Each dose of Enterica was filled into one capsule. The release of Enterica extract from formulated capsules at 45minutes was 84.51 ± 1.51%. The disintegration time was4.26 ± 0.34minutes. The B.P. uniformity of weight test for the capsules was satisfactory.

Case studies conducted with Asena capsules at CSRPM on two adult patients suffering from acute pain and osteoarthritis respectively revealed that Asena capsules had encouraging pain relieving effects. A numbered scale of one to ten was used to assess the severity of pain. One represented virtually no pain and ten represented severe pain. The patient suffering from acute pain reported severity of six on the numbered scale. After taking two capsules of Asena three times daily for seven days, the pain had decreased to one on the numbered scale. The patient diagnosed with osteoarthritis reported a decrease in severity of pain from seven to three on the numbered scale by the seventh day using the same dosage of Asena capsules.

A case study conducted on a 24 year old female diagnosed with typhoid fever revealed further studies on Enterica capsules may help to achieve optimum antimicrobial effect. The patient reported with abdominal pains, headaches, nausea and occasional feverishness. The widal test reading was TO: 1/160 and TH: 1/160. After administration of one capsule of Enterica three times daily for a period of two weeks, the abdominal pains, fever, headaches and other symptoms were absent. The widal test still showed a reading of TO: 1/160 and TH: 1/160.

TABLE OF CONTENT

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Herbal medicines have a long and respected history. Many medicaments used in the twentieth century were developed from ancient healing traditions that treated health problems with specific plants. There are over 75,000 plants on earth. Only a few have been studied scientifically. [1]

Herbal medicines includes herbs, herbal materials, herbal preparations and finished herbal products, that contain as active ingredients parts of plants, or other plant materials, or combinations thereof. [2]

According to the World Health Organization (WHO), about 80% of the population of developing countries relies solely on medicinal plants for treatment of various conditions. In developing countries; broad use of herbal medicine is often attributable to its accessibility, affordability and embodiment within wider belief systems. [2]

The WHO recognizes the value of plant medicines in health care delivery and endorses the use of those which have been scientifically proven to be efficacious, safe for use and of good quality. [3]

Quality control for herbal medicines in most countries is, however, poor. Concerns about the quality and safety of herbal remedies are justified because considerable variations in the contents of active ingredients have been reported with batch to batch variations of up to 1000%. [4]

In most countries, the sale and supply of herbal remedies is to a large extent uncontrolled and unregulated so their safety, efficacy and quality may be questionable. Adulteration

and contamination of herbal remedies with other plant materials and conventional drugs have also been documented. [4]

Unfortunately, most countries in sub-Saharan Africa have no regulations, safety monitoring or pharmacovigilance centers for herbal medicines sold on their markets. Documentation of the constituent herbs as well as the active ingredients of local herbal medicines remains poor and shrouded in secrecy. [5, 6]

Herbal formulations may exist as fluid extracts (infusions, decoctions, macerates, and tinctures), dry extracts and special extracts. [7]

Modern pharmacology research into plants normally looks for active ingredient and seeks to isolate it rather than studying the medicinal properties of the whole herb. [1]

Herbalists, however consider that the medicinal properties of a plant lie in the interaction of all ingredients. Plants used as medicines offer synergistic interactions between ingredients both known and unknown. Unlike conventional medicines, herbal medicines must be seen as a complex pharmaceutical preparation and as such should be preferably administered in the form of an extract. Every herbal treatment has specific healing properties, carefully balanced to create a particular action within the body. Herbal preparations take time to act internally. They are generally well tolerated, relatively nontoxic with few if any side effects. [1, 8, 9, 10, 11]

Asena and Enterica are decoctions of the Centre for Scientific Research into Plant Medicine (CSRPM). They are both made up of a combination of several plants to obtain the desired effect. Asena is used in the treatment of fever, arthritis and generalized body

pains. Enterica is used to treat typhoid fever. These conditions are very common in Ghana. [12]

It is important to provide patients with dosage forms which are convenient to their needs and encourage compliance in order to ensure maximum therapeutic effect. Liquid herbal dosage forms such as decoctions, among other things may have stability problems, an unsuitable taste and come in large volumes for the recommended duration of treatment. [7]

For instance, for Asena decoction, the daily dosage is 30ml three times. The total volume in thirty days will be 2,700ml. The decoctions are provided in 300ml bottles, this means that the patient requires nine bottles of the product every thirty days.[12]

Some patients may not comply with the dosage regime because it may not be convenient to carry product everywhere they go. This is particularly disturbing for products like Enterica which are used in the treatment of infections. Transforming these decoctions into solid dosage forms such as capsules will help to address the above concerns and also help to provide a more standardized product. Solid dosage forms can be assessed more easily and batch to batch variation can be reduced. It will also increase confidence in the use of the products by both patients and physicians.

A research conducted on the transformation of two liquid dosage forms from CSRPM, Camber and Bredina used in the treatment of hypertension and diabetes respectively revealed that adsorbents could be used to ensure easy processing of extracts for formulation into granules for encapsulation. [13]

Adsorbent are substances that can adsorb water from aqueous preparations such as herbal extracts to transform them into an apparently dried state. Examples include bentonite,

light magnesium carbonate, maize starch, microcrystalline cellulose, kaolin and fumed silica. The major challenge with the use of adsorbents is whether it would release the extract for the required therapeutic effect.[14]

The idea is to combine ease of processing of adsorbent-extract mix with effective release of extract to ensure optimum therapeutic effect. Adsorbents used for this work are light magnesium carbonate, maize starch and bentonite. These were selected after initial screening of five adsorbents (light magnesium carbonate, bentonite, microcrystalline cellulose, maize starch and kaolin) to ascertain ease of processing and drug release profile.

The aim of the project work is to prepare capsules from decoctions of Asena and Enterica which will release adequate quantity of the extract to ensure optimum therapeutic effect. The most important factors are to ensure that the granules formulated for encapsulation from the adsorbent and extract are easy to process and have good in vitro dissolution characteristics. This will facilitate optimum in-vivo therapeutic effect.

1.2 LITERATURE REVIEW

1.2.1 Herbal Product Design and Development

Herbal product design refers to the process of developing, standardizing, processing, and validating an herbal product for the market. Herbal medicine products may be consumed for purposes such as improvement of health, improvement of physical appearance, weight loss, or enhancement of well-being. Herbal products may come in formulations such as decoctions, infusions, tinctures, syrups and elixirs. Other herbal preparations come in the form of emulsions, linctuses, lozenges, pills, tablets, capsules, baths, douches and enemas. Other herbal dosage forms may be ointments, suppositories, liniments, gargles and mouthwashes, inhalants, spray solutions, compresses and poultices.[15, 16]

1.2.2 The Quality of herbal medicines

The quality of herbal medicine is believed to be directly related to its active principles. These constituents are referred to as secondary plant substances or metabolites. However, herbal medicines contain other substances, often neglected and poorly understood. These substances render the ingredients active as medicinal agents. Thus it is often difficult to reproduce the effect of herbal drugs by isolating its individual constituents and recombining them in the laboratory.[8]

1.2.2.1 Effect of manufacturing processes on the quality of herbal products

Variations in the manufacturing processes of an herbal product, such as drying and storage, may affect its quality and medicinal efficacy. Drying factors include the time between collection and drying, the time allowed for drying, and the temperature used for

drying. Storage may also affect the quality of the product as well as moisture absorption during the storage process. Additionally, a herbal product may be inadvertently sterilized or may be infected with moulds, bacteria, or insects during storage. Possibilities for adulteration include the mishandling of the product, and harvesting other plants or plant parts not intended for use in the final product.[16]

Extraction procedures also have a significant effect on the quality and medicinal efficacy of the product. These factors include the type of solvent used, the amount of plant material exposed to the solvent, the degree of agitation, the temperature used during extraction, and the exposure of the solvent and herb to oxygen and light. The degree of quality control depends on the manufacturer, the supplier, and other parties involved in the production process. [16]

1.2.3 Dosage of herbal medicines

Dosage is in general a crucial issue for herbal products. While most pharmaceutical drugs are extensively tested to determine the most effective and safest dosages (especially in relation to patient variables such as body weight, other medications, and allergies), there are few established dosage standards for herbal products. Generally, the dosages recommended may vary, and the exact therapeutic range may not be well known. Experts in the field of complementary and alternative medicine may not agree upon the minimum and maximum dose needed for clinical efficacy. [16] Variations in dosages of herbal products may be attributed to lack of standardization among other factors. [8]

1.2.4 Standardization of herbal medicines

Standardization is a process that manufacturers may use to ensure batch-to-batch consistency of their products. In some cases, standardization involves identifying specific chemicals (known as chemical markers) that can be used to manufacture a consistent product. If the chosen markers are present at about the same amounts between batches of the same product, then it is likely that all of the ingredients in that product are present in equal proportions between these batches. This process provides a measure of quality control. [8, 15, 16]

Standardization is achieved by choosing at least one chemical compound present in the herb and monitoring its concentration in each batch of product. Batches that do not meet the standard are modified accordingly. Standardization is not directly related to efficacy because it does not assess the total chemical composition of the product; rather, it only indicates consistency of contents. Further, consistency of contents is a separate measure from clinical efficacy. [15, 16]

This means that different preparations of the same herbal drug may not be similarly effective. To carry out reliable clinical trials, the herbal medicine must be of standardized quality. The standardization, in the case of a herbal drug, is not simply an analytical evaluation which involves the identification of active principles or of a marker. It must also involve all information and controls that are necessary to guarantee the constancy of activity, of the herbal medicine. [8, 15, 16]

It must be taken into account that the vegetable material to be examined has a complex and inconsistent chemical composition depending on a variety of factors. These include; age and origin, harvesting period, the specific parts of the plant to be processed, the

extraction methods employed, the drying, storage, etc. The use of cultivated plants rather than wild plants may reduce some of these inconsistencies. [8, 15]

The more complex aspect of quality assurance arises with respect to the standardisation of the finished product. The complexity arises because traditional products of different cultures use a wide range of dosage forms, from simple powders made from a single plant to extracts made from many. Standardising all these medicines poses a threefold challenge to modern scientists: first, to identify appropriate tools to tackle the complexities; second, to use tools that are cost-effective and relatively easy to follow; and third, to design tools that are not only for control of quality of ingredients and product, but also for online process control.[17]

1.2.4.1Modern scientific tools for studying traditional medicines

Studying traditional medicines demands a combination of physical, chemical and biological techniques. The importance of taxonomists in the herbal medicines industry cannot be overemphasized, as correctly identifying the plant material is the inevitable starting point for making all herbal medicines.[17]

Using microscopy to authenticate the raw material used in the preparation of medicines has been a useful tool in the herbal sector. Chromatography techniques such as high-performance thin layer chromatography (HPTLC) and high-performance liquid chromatography (HPLC) can be used for 'fingerprinting' herbal products. Other instruments, such as the flame photometer and atomic absorption spectrophotometer

(AAS), are needed for studying traditional medicines that contain metals or minerals. Volatile materials are generally measured through gas chromatography (GC). Higher-end research tools such as nuclear magnetic resonance (NMR), electron spectroscopy for chemical analysis, and mass spectroscopy can be used to characterise compounds in traditional medicines. Molecular DNA-based techniques have become an important tool to study genetic variation between samples in raw drugs derived from plants and animal species. *In vitro* biological assays have also been used in the research, standardisation and quality control of traditional medicines. [8, 17]

1.2.5 Herbal extracts

Extracts are prepared by dissolving medicinal plants in a solvent known as the menstrum to separate their active principles from extraneous substances. The type of formulation depends on the type of solvent used. Water and alcohol are the most popular solvents used in extractions. Extracts may be characterized as dry, soft or liquid depending on the concentration of residual solvent in the final product. The extraction can be accomplished by various methods. The extracts obtained after separation of liquid from the drug residue is called micella. The micella can be converted to ready to use medicinal preparation. [7, 18]

The herb should be reduced to a proper particle size fit for extraction. If the size is more than optimum required in extraction, the extraction will be incomplete. If the size is less than optimum size, there will be canalizations of solvent and the extraction will not be complete. The herb may be soaked with the solvent to be used, prior to extraction.[19]

1.2.5.1 Choice of solvents for extraction

The choice of solvent for extraction is normally based on the nature of the drug to be extracted and the type of preparation desired. Common solvents used for extraction include; water, alcohol, hydroalcoholic mixtures, glycerin, ether and acetone.[20]

1.2.5.2 Water as a solvent for extraction

Water has a wider range as a solvent than any other liquid. It has the advantage of cheapness. It is also a good solvent for plant constituents such as alkaloidal salts, glycosides, sugars and mucilaginous substances. Other compounds easily soluble in water are pectin, plant acids, coloring matter and mineral salts. Water can be used cold or hot. The major disadvantage of water as a menstrum is that it extracts large amounts of inert susbstances and the resulting solutions of plant constituents are usually good media for the growth of yeasts, mould and bacteria.[20, 21]

1.2.5.3 Classification of extracts

Extracts may be classified as; aqueous drug extracts, fluid extracts, thin extracts, thick or viscous extracts, oily extracts, oleo-resins and dry extracts. [18, 22]

1.2.5.3.1 Aqueous Drug Extracts

Aqueous extracts are described as medicinal water preparations intended for use immediately after preparation or to be preserved for the future. There are three methods generally used for their preparation. Decoctions are made by boiling the herb in water for

about ten to sixty minutes. Decoctions are normally suitable for hard plant materials such as barks and roots. Decoctions may also be prepared from herbs with sparingly soluble constituents. Decoction differs from infusion in respect of the fact that the crude drug in infusion is not boiled with the menstrum but only boiling menstrum is poured over the crude drug. Maceration involves placing the solid materials with whole menstrum in a closed vessel and allowing it to stand for several days. Shaking is done occasionally and the preparation is strained, the marc is pressed and the liquid obtained clarified by subsidence or filtration. [7, 18, 22, 23]

1.2.5.3.2 Fluid Extracts

After extraction of herbs, the resulting solutions can be concentrated into fluid Extracts. These are more concentrated and as per recommended of standard texts. Two parts of fluid extract is made from one part of crude drug. Some pharmacopoeia give rigid limits for the ratio of drug to total extract but permits the micella obtained to be adjusted to certain active compounds. In large manufacturing operations, the techniques and machines used ensure that the extracted plant components are not damaged. Thin layer evaporators may be used for this step [22, 23]

1.2.5.3.3 Thin Extracts

Thin extracts are prepared plant liquid extracts concentrates to a honey like consistency by various procedures.[22, 24]

1.2.5.3.4 Thick Extracts

These extracts are thick liquids or viscous material when warm but are not fluid at room temperature. They are plastic masses containing varying quantities of residual moisture and can be adjusted to a defined strength of active substance by addition of calculated quantities of inert substances such as dextrin and lactose. Thick extracts have been completely replaced by dried extracts because of their low stability and susceptibility to microbial growth. [24, 25]

1.2.5.3.5 Oily Drug Extracts

These preparations are made by suspending ground drug material into non-drying oil adopting maceration process. Mild heat can be used for enhancing the extraction in short duration. Examples of oily drug extracts include Aconite, Arnica blossom, Marigold, Rose flower extracts. [26]

1.2.5.3.6 Oleoresins

Oleoresins are prepared by extracting oleoresinous material like plant gum and resins from spices with suitable solvents like ethanol or ethyl acetate. An example of oleoresin is male fern extract.[22, 27]

1.2.5.3.7 Dry herbal extracts

Dry extracts are solid plant preparations obtained by concentrating, condensing and drying fluid extracts under mild conditions. A powdered extract generally contains 95% solids and 5% water residue or moisture. A native dry extract contains only plant material without any additives. [7, 22, 23]A large number of powdered extracts are hygroscopic and

provide problems in processing and handling. Extracts should be stored in air tight containers away from moisture and heat. Dried extracts may be characterized as powdered, standardized or non-standardized.[28]

1.2.5.3.7.1Powdered extracts

These are dry extracts made from the crude by dilution with a solid carrier substrate such as dextrin, celluloses or anticaking agents such as magnesium carbonate. The dry powder is then ground up into powder or granulated for further formulation.[28, 29]

1.2.5.3.7.2 Standardized extracts

These are solid or powdered extracts in which a carrier is added to the crude ensuring that a specified percentage of active ingredient is consumed per unit weight of extract. The amount of active ingredient is therefore known to ensure optimum dosage and consistency is assured. [29, 30]

1.2.5.3.7.3 Solid extracts

These are powdered extracts to which propylene glycol is added to make a semi viscous substance or glucose is added to make a semisolid substance.[29]

1.2.5.3.7.4 Non standardized extracts

These are powdered extracts for which no particular active ingredient has been widely accepted to be responsible for its activity. They are simply expressed in strength in

relation to the dried whole plant since standardization to a particular active ingredient may not be acceptable.[29, 31]

1.2.5.3.7.5 Preparation of dry extracts

In the preparation of dry extracts, the fluid extract is heated and the solvent is allowed to evaporate in a vacuum chamber, frozen or spray dried. Adjuvants, carriers and other suitable inert materials such as highly dispersed silica, lactose, starch and methylcellulose are sometimes added to prevent caking or to adjust the final extract concentration. The resulting mix of adjuvant and extract can then be incorporated into capsules or formulated into tablets. [7, 18, 32]

1.2.5.3.8 Methods of drying of fluid extracts

Drying method for extracts include; drying in Vacuum ovens, spray drying and freeze drying. [27]

1.2.5.3.8.1 Drying in Vacuum ovens

Vacuum ovens are frequently used in development laboratories for the drying of small samples, particularly when the heat stability of the drug or formulation is uncertain.[33] The general temperature for drying should be between 60 – 70º C. Lower temperatures up to 50º C may be required depending upon the stability of the plant material. [27]

1.2.5.3.8.2 Spray drying

The spray drier can be used for drying almost any substance, in solution or in suspension. It is most useful for thermolabile materials, particularly if handled continuously and in large quantities. The spray drier provides a large surface area for heat and mass transfer by atomizing the liquid to small droplets. The liquids are sprayed into a stream of hot air, so that each droplet dries to an individual solid particle. The particles have a characteristic shape, in the form of hollow spheres sometimes with a small hole. This arises from the drying process, as the droplet enters the hot air stream and dries on the outside to form an outer crust with liquid still in the centre. This liquid then vaporizes, and the internal vapor escapes by blowing a hole in the sphere. [33, 34, 35, 36]

Advantages of the spray drying process include very short drying time, the temperature of the particles are kept low due to rapid evaporation, The characteristic particle form gives the product a high bulk density and, in turn, rapid dissolution (large surface area).The product formed is also free flowing, with almost spherical particles, and is especially convenient for tablet manufacture as it has excellent flow and compaction properties. In addition, labor costs are low because the process yields a dry, free-flowing powder from a dilute solution in a single operation with no handling. [33]

Disadvantages of the spray drying process include the bulky and expensive nature of the equipment used and low overall thermal efficiency, as the air must still be hot enough when it leaves the drier to avoid condensation of moisture. Also, large volumes of heated air pass through the chamber without contacting a particle, thus not contributing directly to the drying process. [33]

1.2.5.3.8.3 Freeze drying

Freeze drying is a process used to dry extremely heat-sensitive materials. It allows the drying, without excessive damage, of proteins, blood products and even microorganisms, which retain a small but significant viability. In this process the initial liquid solution or suspension is frozen, the pressure above the frozen state is reduced and the water removed by sublimation.[28,33]

Thus a liquid-to-vapor transition takes place. There are three states of matter involved: liquid to solid, then solid to vapor.

The major advantage of freeze drying practice is that drying takes place at very low temperatures, so that enzyme action is inhibited and chemical decomposition, particularly hydrolysis, is minimized. [33, 37, 38]

The two main disadvantages of freeze drying are; first, the porosity, ready solubility and complete dryness yield a very hygroscopic product. Unless products are dried in their final container and sealed in situ, packaging requires special conditions. In addition, the process is very slow and uses complicated plant, which is very expensive. It is not a general method of drying, therefore, but is limited to certain types of valuable products which, because of their heat sensitivity, cannot be dried by any other means.[33, 37, 38]

1.2.6 Pharmaceutical dosage form design

Drugs are rarely administered as pure chemical substances alone and are almost always given as formulated preparations or medicines. Excipients are added to provide varied and specialized pharmaceutical functions. Excipients may help solubilize, suspend, thicken, preserve, emulsify, modify dissolution, improve the compressibility and flavour drug substances to form various preparations or dosage forms.[33, 39]

The principal objective of dosage form design is to achieve a predictable therapeutic response to a drug included in a formulation which is capable of large scale manufacture with reproducible product quality. The five basic routes of drug administration are; oral, rectal, parenteral, topical and respiratory.[33]

1.2.6.1 The Oral route of drug administration

The oral route is the one most frequently used for drug administration. Oral dosage forms are usually intended for systemic effects resulting from drug absorption through the various epithelia and mucosa of the gastrointestinal tract. Compared with other routes, the oral route is the simplest, most convenient and safest means of drug administration. Disadvantages of this route include the relatively slow onset of action, the possibilities of irregular absorption and the destruction of certain drugs by the enzymes and secretions of the gastrointestinal tract. The most popular oral dosage forms are tablets, capsules, suspensions, solutions and emulsions. [33, 40, 41]

1.2.6.2 Capsules

Capsules are solid dosage forms containing drug and usually appropriate filler(s), enclosed in a hard or soft gelatin shell. The gelatin shell readily ruptures and dissolves following oral administration, and in most cases the drug is released from a capsule faster than from a tablet.[33]

Hard gelatin capsules consists of two pieces in the form of cylinders closed at one end: the shorter piece, called the 'cap', fits over the open end of the longer piece, called the 'body.

Both soft and hard gelatin capsules contain gelatin, water, colorants and optional materials such as process aids and preservatives; in addition, soft capsules contain various plasticizers.

Capsules may also be manufactured from hydroxypropyl methylcellulose in order to produce a shell with low moisture content. [33, 42]

1.2.6.2.1 Properties of hard gelatin capsule

Hard gelatin capsules are readily soluble in water at 37°C.When the temperature falls below this their rate of solubility decreases. At below about 30°C they are insoluble and simply absorb water, swell and distort. This is an important factor to take into account during disintegration and dissolution testing. Because of this most pharmacopoeias have set a limit of 37 ° ± 1°C for the media for carrying out these tests. Capsules made from hydroxypropyl methylcellulose have a different solubility profile, being soluble at temperatures as low as 10°C. [33]

1.2.6.2.2 Capsule sizes

Hard gelatin capsules are made in a range of fixed sizes; the standard industrial sizes in use today for human medicines are from 0 to 4. For a powder the simplest way in which to estimate the fill weight is to multiply the body volume by its tapped bulk density. For liquids, the fill weight is calculated by multiplying the specific gravity of the liquid by the capsule body volume x 0.8. To accommodate special needs some intermediate sizes are produced, termed 'elongated sizes', that typically have an extra 10% of fill volume over the standard sizes. [33, 43]The capsule size and body fill volumes are shown in Table 1.2.1.

Table 1.2.1 Capsule size and body fill volumes [33]

Capsule size	Body fill volume (ml)
0	0.67
1	0.48
2	0.37
3	0.28
4	0.20

1.2.6.2.3 Capsule shell filling

Hard gelatin capsules can be filled with a large variety of materials of different physicochemical properties. Gelatin is a relatively inert material. The substances to be avoided are those which are known to react with it or those that interfere with the integrity of the shell. Materials for filling into hard gelatin capsules include dry solids, powders, pellets, granules, tablets, semisolids, thermo softening mixtures, thixotropic mixtures, pastes and non-aqueous liquids. If the dose of the drug to be placed in a single capsule is inadequate to fill the volume of the capsule, a diluent is necessary to add the proper degree of bulk to the drug to produce the proper fill. When the amount of drug to be administered in a single capsule is large enough to fill a capsule completely, a diluent may not be required. In many instances, the amount of drug is placed in a single capsule to be taken as a dose of that particular medication.[33,44]

However, when the amount of drug representing a usual dose is too large to be placed in a single capsule, two or more capsules may be required to provide the desired dose of the particular drug.[45]

1.2.6.2.4 Capsule-filling machines

The same set of basic operations is carried out whether capsules are being filled on the bench for extemporaneous dispensing or on high-speed automatic machines for industrial products. The major difference between the many methods available is the way in which the dose of material is measured into the capsule body.[33, 46]

1.2.6.2.5 Filling of powder formulations

There are two basic methods for filling capsules. These are bench scale filling and industrial filling methods. [33]

1.2.6.2.5.1 Bench-scale filling

There is a requirement for filling small quantities of capsules, from 50 to 10,000 in community pharmacy, in hospital pharmacy, or in industry for special prescriptions or trials. There are several simple pieces of equipment available for doing this. These normally consist of sets of plastic or metal plates which have predrilled holes to take from 30 to 100 capsules of a specific size. Empty capsules are fed into the holes, either manually or with a simple loading device. The bodies are locked in their plate by means of a screw and the caps in their plate are removed. Powder is placed on to the surface of the body plate and is spread with a spatula so that it is filled into the bodies. The uniformity of fill weight is very dependent upon good flow properties of the powder. The cap plate is then repositioned over the body one and the capsules are rejoined using manual pressure.[33, 47]

1.2.6.2.5.2 Industrial-scale filling

The machines for the industrial-scale filling of hard gelatin capsules come in great variety of shapes and sizes, varying from semi- to fully automatic and ranging in output from 5000 to 15, 000 per hour. The dosing systems can be divided into two groups:

• Dependent dosing systems that use the capsule body directly to measure the powder. Uniformity of fill weight can only be achieved if the capsule is filled completely. The auger filling system is normally used.[33, 48] Figure 1.2.1 shows an auger capsule filling machine.

• Independent dosing systems where the powder is measured independently of the body in a special measuring device. Weight uniformity is not dependent on filling the body completely. With this system the capsule can be part filled.[33, 49]Figure 1.2.2 shows the independent dosing system.

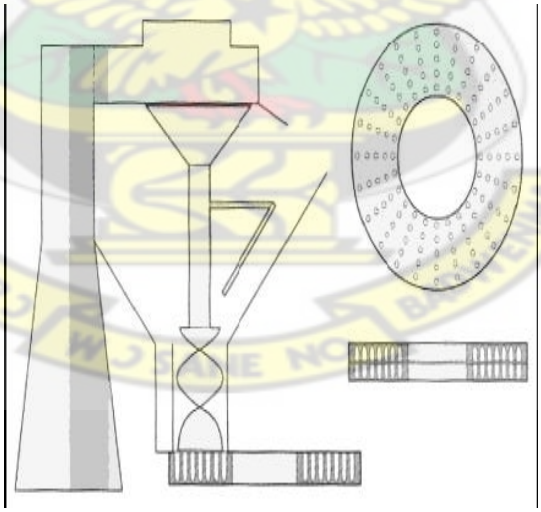


Figure 1.2.1 Auger capsule filling machine using the ring system [33]

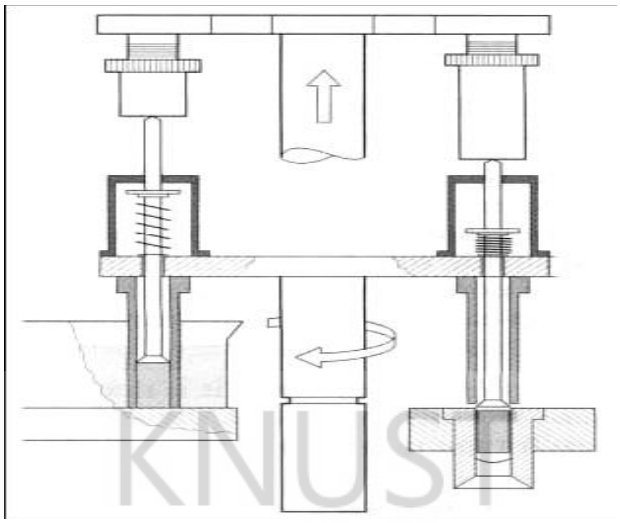


Figure 1.2.2 A dosing tube or dosator-type capsule filling machine[33]

1.2.6.3 Bioavailability of Powder-filled capsules

Provided the hard gelatin shell dissolves rapidly in the gastrointestinal fluids and the encapsulated mass disperses rapidly and efficiently, a relatively large effective surface area of drug will be exposed to the gastrointestinal fluids, thereby facilitating dissolution. The overall rate of dissolution of drugs from capsules appears to be a complex function of the rates of different processes, such as the dissolution rate of the gelatin shell, the rate of penetration of the gastrointestinal fluids into the encapsulated mass, the rate at which the mass deaggregates (disperses) in the gastrointestinal fluids, and the rate of dissolution of the dispersed drug particles. The inclusion of excipients such as diluents, lubricants and surfactants in a capsule formulation can have a significant effect on the rate of dissolution of drugs, particularly those that are poorly soluble and hydrophobic.[33,47]

The diluent should exhibit no tendency to adsorb or complex with the drug, as either can impair absorption from the gastrointestinal tract. Both the formulation and the type and conditions of the capsule-filling process can affect the packing density and liquid

permeability of the capsule contents. In general, an increase in packing density of the encapsulated mass will probably result in a decrease in liquid permeability and dissolution rate, particularly if the drug is hydrophobic, or if a hydrophilic drug is mixed with a hydrophobic lubricant such as magnesium stearate. If the encapsulated mass is tightly packed and the drug is hydrophobic in nature, then a decrease in dissolution rate with a concomitant reduction in particle size would be expected, unless a surfactant had been included to facilitate liquid penetration. In summary, formulation factors [33, 47] that can influence the bioavailability of drugs from hard gelatin capsules include:

• The surface area and particle size of the drug (particularly the effective surface area exhibited by the drug in the gastrointestinal fluids);

• The use of the salt form of a drug in preference to the parent weak acid or base;

• The crystal form of the drug;

• The chemical stability of the drug (in the dosage form and in gastrointestinal fluids);

• The nature and quantity of the diluent, lubricant and wetting agent;

• Drug-excipient interactions (e.g. adsorption, complexation);

• The type and conditions of the filling process;

• The packing density of the capsule contents;

• The composition and properties of the capsule shell (including enteric capsules);

• Interactions between the capsule shell and its contents.

1.2.7Formulation of herbal product granules

The development of concentrated herbal extract powders or granules makes them well suited to evidence-based medical research, due to their consistency and easily

quantifiable nature. The portability and convenience of granules dramatically increases patient compliance. [20]

Granules also have a longer shelf life compared to liquid decoctions. They require less space. Manufacture of herbal granules normally involves the following steps. The herbs are decocted in purified water and the decoction is then drained into a container that reduces the liquid by slowly evaporating the decoction at a low temperature. The concentrated decoction is then sprayed as a mist into a machine with a large container that sprays the concentrated decoction into a regulated flow of dry powder, which serves as an excipient for the liquid concentrate. The excipient powder may be maize starch or any adsorbent that will aid the release of the desired substance. A uniform mixture is obtained by mixing the liquid concentrate with the dry excipient which allows the final powder to flow freely with minimal clumping and extends its shelf life considerably. If more than one herb is used. The classical formula for the preparation of its liquid formulation is used and the same process of granulation adopted. [20, 21, 22]

1.2.8 Characterisation of powder flow

When examining the flow properties of a powder it is useful to be able to quantify the type of behavior and many different methods have been described, either directly using dynamic or kinetic methods, or indirectly, generally by measurements carried out on static beds.[33]

1.2.8.1 Importance of characterization

Powders are often inherently unstable in relation to their flow performance. This instability is most obvious when a free flowing material ceases to flow. This transition may be initiated by the formation of a bridge in a bin, by adhesion to surfaces or by any event that may promote compaction of the powder. The ability to predict flow provides many operational advantages such as reducing stoppages and improving product quality by allowing good flow.[50]

1.2.8.2 Indirect methods of characterization of powder flow

The indirect methods used to characterize powder flow are; Angle of repose, bulk density measurements and the shear cell determinations.[33, 51]

1.2.8.2.1 Angle of repose

Angles of repose have been used as indirect methods of quantifying powder flowability, because of their relationship with interparticle cohesion. There are many different methods of determining angles of repose and some of these are the fixed height cone, fixed based cone, tilting table, rotating cylinder, ledge, crater and platform. [33]

The different methods may produce different values for the same powder, although these may be self-consistent. As a general guide, powders with angles of repose greater than 50° have unsatisfactory flow properties, whereas minimum angles close to 25° correspond to very good flow properties.[33] Table 1.2.3 shows relationship between angle of repose and flow properites.

Table 1.2.2Flow properties of powders and corresponding angle of repose [33]

Flow property	Angle of repose (degrees)
Excellent	25-30
Good	31-35
Fair (aid not needed)	36-40
Poor (must agitate, vibrate)	41-45
Very poor	56-65
Very ,very poor	>66

1.2.8.2.2 Shear cell determinations

Powder flowability may be characterized indirectly from the behaviour of powder in a shear cell. A type of shear cell is the cylindrical shear cell that is split horizontally, forming a shear plane between the stationary base and the upper moveable portion of the shear cell ring. After powder bed consolidation in the shear cell, the force necessary to shear the powder bed by moving the upper ring is determined. A flow factor can be obtained by determining the reciprocal slope of a curve or tangent to a curve of unconfined yield stress plotted against the maximum normal stress on a yield locus. [33]

1.2.8.2.3 Bulk density measurements

The ease with which a powder consolidates can be used as an indirect method of quantifying powder flow. In recent years the compressibility index and the closely related Hausner ratio have become the simple, fast, and popular methods of predicting powder flow characteristics. The compressibility index has been proposed as an indirect measure of bulk density, size and shape, surface area, moisture content, and cohesiveness of materials. The aforementioned factors can influence the observed compressibility index. The compressibility index and the Hausner ratio are determined by measuring both the

bulk volume and tapped volume of a powder. While there are some variations in the method of determining the compressibility index and Hausner ratio, the basic procedure is to measure the unsettled apparent volume, (V_0), and the final tapped volume, (V_f), of the powder after tapping the material until no further volume changes occur. The compressibility index and the Hausner ratio are calculated as follows: [22, 33]

$$Compressibility\ Index = 100 \times \frac{V_o - V_f}{V_o} \qquad Equ.\ 1.0$$

$$\text{Hausner } Ratio = \frac{V_o}{V_f} \qquad Equ.\ 1.1$$

Alternatively, the compressibility index and Hausner ratio may be calculated using measured values of bulk density (P_{bulk}) and tapped density (P_{tapped}) as follows:

$$Compressibility\ Index = 100 \times \frac{\rho_{tapped} - \rho_{bulk}}{\rho_{tapped}} \qquad Equ.\ 1.2$$

$$\text{Hausner } Ratio = \frac{\rho_{tapped}}{\rho_{bulk}} \qquad Equ.\ 1.3$$

Table 1.2.3 Flow character of powders in relation to compressibility Index and Hausner ratio[33]

Compressibility index (percent)	**Flow character**	**Hausner ratio**
1-10	Excellent	1.00-1.11
11-15	Good	1.12-1.18
16-20	Fair	1.19-1.25
21-25	Passable	1.26-1.34
26-31	Poor	1.35-1.45
32-37	Very poor	1.46-1.59
>38	Very, very poor	>1.60

1.2.9 Pharmaceutical Adsorbents

These are ingredients, usually solids, with a large surface area which can attract dissolved or finely dispersed substances from another medium by physical or chemical (chemisorption) means. Examples include microcrystalline cellulose, maize starch, magnesium carbonate and fumed silica. [52]Less important adsorbents for use in Direct Powder Blends are talc, magnesium oxide, tricalcium phosphate, magnesium aluminum silicate and clays. [53]

When an ingredient is a liquid it is necessary to convert it into a solid before blending it with the other ingredients to prepare tablets or capsules. Typically the liquid is of oily nature and can be adsorbed onto the surface of a solid. Adsorption, being a surface phenomenon, is most influenced by the available surface area on the solid. Thus, the most efficient adsorbents are very small particles. These materials often have low bulk densities with poor flow and compaction properties. Silicas are high purity sands with specific surface areas in the hundreds of m^2/g. The particle size is typically less than 10 μm and these materials are characterized by very low bulk densities, 0.04-0.08g/cc. These materials can adsorb up to 1.6 ml of liquid per gram. The most common silica used is fumed silica.[53]

1.2.9.1 Microcrystalline cellulose

Microcrystalline cellulose is purified, partially depolymerized cellulose that occurs as a white, odorless, tasteless, crystalline powder composed of porous particles. It is commercially available in different particle sizes and moisture grades that have different

properties and applications.

Its functional category includes use as; adsorbent, suspending agent, tablet and capsule diluents and tablet disintegrant. [54]

Microcrystalline Cellulose is a naturally occurring substance that is proven stable, safe and physiologically inert. [55]

Microcrystalline cellulose has a surface area of about 1.0m^2/g, and much lower adsorptive capacity one tenth of a millilitre per gram, than silicas. Silicified microcrystalline celluloses, microcrystalline cellulose co-processed with silica, have better adsorptive capacities.[53]

1.2.9.2 Maize Starch

Maize starch is obtained from the caryopsis of *Zea mays* L.

It appears as a matt, white to slightly yellowish, very fine powder which creaks when pressed between the fingers. It is practically insoluble in cold water and in ethanol (96 %).The presence of granules with cracks or irregularities on the edge is exceptional.[22] It is normally used as a pharmaceutical excipient (filler, diluents, glidant, disintegrant). [56]

Maize Starch exhibits all the properties of native starch with some special features such as non-foaming and non-thinning characteristics of boiling solution.[57]

Maize starch has been used for a long time as an adsorbent. Specific surface area for maize starch is around 0.4m^2/g, which is the highest of the common starches. [53]

1.2.9.3 Magnesium Carbonate.

Magnesium carbonate, $MgCO_3$, is a white solid that occurs in nature as a mineral. Several hydrated and basic forms of magnesium carbonate also exist as minerals. In addition, $MgCO_3$ has a variety of uses. The most common magnesium carbonate forms are the anhydrous salt called magnesite ($MgCO_3$) and the di, tri, and pentahydrates known as barringtonite ($MgCO_3 \cdot 2H_2O$), nesquehonite ($MgCO_3 \cdot 3H_2O$), and lansfordite ($MgCO_3 \cdot 5H_2O$), respectively. Some basic forms such as artinite ($MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$), hydromagnestite ($4MgCO_3 \cdot Mg(OH)_2 \cdot 4H_2O$), and dypingite ($4MgCO_3 \cdot Mg(OH)_2 \cdot 5H_2O$) also occur as minerals. Magnesite consists of white trigonal crystals. The anhydrous salt is practically insoluble in water, acetone, and ammonia. All forms of magnesium carbonate react in acids. Magnesium carbonate crystallizes in the calcite structure where in Mg^{2+} is surrounded by six oxygen atoms. The dihydrate one has a triclinic structure, while the trihydrate has a monoclinic structure. References to 'light' and 'heavy' magnesium carbonates actually refer to the magnesium hydroxy carbonates hydromagnesite and dypingite respectively. [58] Light Magnesium carbonate is defined as hydrated basic magnesium carbonate with a content of 40.0 % to 45.0 %, calculated as Magnesium Oxide. It appears as white or almost white powder and practically insoluble in water. It dissolves in dilute acids with effervescence.[22]

Light magnesium carbonate is used as adsorbent in preventing the formation of eutectic mixtures in capsules and to diffuse oils in the preparation of aromatic water. [59]Magnesium carbonate has specific surface area of about6.15 m^2/g. [52]

1.2.9.4 Bentonite

Bentonite is natural clay containing a high proportion of montmorillonite, a native hydrated aluminium silicate in which some aluminium and silicon atoms may be replaced by other atoms such asmagnesium and iron. The appearance is very fine, homogeneous, greyish-white powder with a more or less yellowish or pinkish tint. Bentonite is practically insoluble in water and in aqueous solutions. It swells with a little water forming a malleable mass. [22]

Bentonite is a high performance desiccant used to protect a variety of products from moisture degradation, maintaining product quality and shelf life and widely used in pharmaceutical, nutraceutical and diagnostic packaging applications. Because of its adsorptive properties, environmental benefits and cost effectiveness, bentonite is an ideal desiccant and viable alternative to traditional desiccants such as silica gel for healthcare packaging applications. [59]Bentonite is used as an adsorbent additive to tablet formulations to allow oils, fluid extracts and eutectic melts to be incorporated into tablets.[60]

1.2.9.5 Kaolin

Kaolin is a weathering product of silicate rock naturally occurring hydrated aluminium silicate which is white, yellowish-white, earthy, nonporous and odorless to dull material having a plastic touch and slightly oily feel. It is also almost tasteless and practically insoluble in water. The approximate chemical formula of kaolin is $Al_4Si_4O_{10}$ $(OH)_8$. The

hydrophilic surface of kaolin allows it to be easily dispersed in water at neutral pH of 6-8. [22, 61, 62]

The most preferred kaolin for pharmaceutical formulations is the finely divided particles because they yield a very large surface area that adsorbs a wide variety of compounds. The characteristics chemical composition of kaolin deposit often determines its industrial utilization.

Kaolin has been employed to adsorb toxic substances from the alimentary canal and in the treatment of diarrhoea associated with food poisoning.[62, 63] Kaolin is also used as an adsorbent agent following the ingestion of toxins. [61, 64]

1.2.10 Asena: herbal product used in the treatment of arthritis and fever.

The decoction of Asena produced by the Centre for Scientific Research into Plant Medicine (CSRPM), Mampong Akuapem, Ghana has been used in the management of arthritis in patients presenting at its outpatients clinic for over two decades. It is made from seven medicinal plants. These are *Khaya senegalensis, Kigelia africana, Nauclea latifolia, Clausena anisata, Piliostigma thonningii, Trichilia monodelpha and Strophanthus hispidus.* No adverse reaction has been reported with the use of the product. [65] The analgesic and anti-inflammatory activities of *Khaya senegalensis*[66] and *Kigelia Africana*[67]used in the preparation of Asena are well documented.

The phytochemical constituents of the aqueous Asena extract of are; saponins, phenolics, reducing sugars and polyamides.[65]

Arthritis is the inflammation of one or more joints, with pain, swelling and stiffness. The most common form is osteoarthritis which normally involves the knee, hips and hands. Fever is an elevation of body temperature above normal. Normal body temperature is 37^{o}C in the mouth and 0.6^{o}C lower in the axilla or armpit.[68, 69]

The anti-inflammatory and anti-nociceptive effect of the aqueous extract of Asena has been investigated in rats and it was observed that doses at 20-40 mg kg^{-1} had anti-nociceptive and anti-inflammatory effects comparable to therapeutic doses of standard drugs like diclofenac (10 mg kg^{-1}), aspirin (100 mg kg^{-1}) and morphine (10 mg kg^{-1}). The analgesic effects may be mediated via both peripheral and central mechanisms.[65]

1.2.11Enterica: herbal product used in the treatment of typhoid fever.

Enterica is a decoction of twelve plants used by the CSRPM in the treatment of typhoid fever for over two decades. These plants are; *Spondias mombin, Persea americana, Psidium guajava, Trema orientalis, Cnestis ferruginea, Momordica charantia, Vernonia amygdalina, Latana carnara, Paullinia pinnata, Citrus aurantifolia, Morinda lucida and Bidens pilosa*. [12]

Typhoid fever is a bacterial disease, caused by *Salmonella typhi*. It is transmitted through the ingestion of food or drink contaminated by the faeces or urine of infected people. Healthy carrier state may follow acute illness. Typhoid fever can be treated with antibiotics. However, resistance to common antimicrobials is widespread. [68, 69, 70]Orthodox antibiotics used in the treatment of typhoid fever include azithromycin,

chloramphenicol, third-generation cephalosporins, and trimethoprim-sulfamethoxazole. All these antibiotics have associated adverse effects.[69]

No adverse reaction has been reported since use of Enterica in the treatment of typhoid fever. The product is however not recommended for pregnant women, nursing mothers and children under six years. [12]

The phytochemical constituents of extract of the product include saponins, tannins and reducing sugars. [12]Antimicrobial assay of the plants used in the preparation of Enterica are well documented.

CHAPTER TWO: MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Plant materials

Premixed and milled plant materials for the formulation of Asena and Enterica were all obtained from CSRPM, Mampong in the Eastern Region of Ghana.

2.1.2 Adsorbents and excipients

Laboratory grades of Lactose, Talc, Light magnesium carbonate, Bentonite, Microcrystalline cellulose, Kaolin and Maize starch were obtained from the chemical store of the Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana.

2.1.3 Reagents

Concentrated hydrochloric acid, concentrated sulphuric acid, Iodine solution, Fehling's solutions A and B. 2M acetic acid solution, Barium Hydroxide solution, 10M sodium hydroxide solution, ammonium chloride solution, iodinated Zinc chloride solution, iodine solution.

2.1.4 Equipment

Cooking pans

Retsch laboratory sieves

Analytical balance

UV-Vis spectrophotometer (Cecil 7200)

General laboratory glass ware (beakers, pipettes, conical flasks, etc.)

Whatman filter paper

Erweka disintegration apparatus, ZT 3/1, GmbH Heusenstamm, Germany, Nr 68318

Erweka dissolution apparatus, DT 6, GmbH Heusenstamm, Germany, Nr 68045

Gallenkamp hot air oven,OMT150. XXX2.C Serial No. SG 96/02/151

2.2 METHODS

2.2.1 Collection of plant materials and processing

The plant materials were processed after initial combinations of the various plant materials used for the preparation of the decoctions of Asena and Enterica according to classified formula used by CSRPM.

2.2.2 Preparation of decoction from plant Material.

Asena: To prepare 1L of decoction, 100g of the provided plant material was weighed into a cooking pan. 1300ml (1.3L) of purified water was added and the mixture boiled for thirty minutes. The mixture was allowed to stand for twenty four hours to macerate. The mixture was strained using calico (four folds or layers) after decanting. The residue was washed with hot boiling water to make up the product to a volume of 1L. Other required quantities of the product were prepared by scaling up or down.

Enterica: 50g of the provided plant material was weighed into a cooking pan. 1,250ml (1.25L) of purified water was added and the mixture boiled for thirty minutes. The mixture was allowed to stand for twenty four hours to macerate. The mixture was strained using calico (four folds or layers) after decanting. The residue was washed with hot boiling water to make up the product to a volume of 1L. Other required quantities of the product were prepared by scaling up or down.

Quantities of plant materials used in the preparation of Asena and Enterica decoctions are according to classified formula used by CSRPM. These quantities provide decoctions with required therapeutic effects.

2.2.3 Determination of maximum wavelength of absorption of extracts.

One gram of the crude dried extract of Asena was weighed and dissolved in a quantity of distilled water in a 100ml volumetric flask. More distilled water was added to the 100ml mark and the solution shaken. Serial dilutions of the solution were then made to obtain several concentrations (0.0001, 0.001, 0.010, 0.1 %w/v). The solutions were scanned to obtain the maximum wavelength of absorption using a UV- Vis Spectrophotometer. The same procedure was repeated for the dried extract of Enterica.

2.2.4 Calibration curve for Asena and Enterica extracts

Solutions of concentrations (0.0175, 0.015, 0.0125, 0.01, 0.0075, 0.0050, 0.0025%w/v) were prepared from the crude dried Asena extract and their corresponding absorbances were recorded at a wavelength of 278.5nm using UV- Vis spectrophotometer. A Calibration curve was then plotted. The same procedure was repeated for the dried extract

of Enterica using solutions of concentrations of 0.0250, 0.0225, 0.0200, 0.0175, 0.0150, 0.0125, 0.0100, 0.0075, 0.00625 %w/v.

2.2.5 Determination of amount of extract per dose of decoction

The doses for both Asena and Enterica decoctions are 30ml. To obtain the amount of extract per dose. 30ml portions of each of the decoctions of Asena and Enterica were accurately measured and transferred into two sets of three weighed clean and dry petri dishes. The preparations were evaporated to complete dryness and the weight of the extract and dish recorded. This was repeated for two other batches of decoctions. The weight of the extract per dose was then determined.

2.2.6 Determination of volume per area of decoction that dries to give a dried extract

Two sets of containers of different sizes were used. 500ml, 1000ml and 1500mls of decoction of Asena were transferred into three containers each with area of base of $0.0201m^2$. The procedure was repeated for a container of area of base $0.0707\ m^2$ with an addition of one more container of the same area for 2000ml of decoction. The preparations were evaporated at 60^oC for 48 hours. The characteristics of the resulting extracts were then recorded and the volume of decoction per area of container to give dry extract of moisture content less than 5%w/w was calculated.

Dry extracts were then obtained by scrapping and grinding of preparations with moisture content less than 5% w/w into fine powder. Enough dried extract was obtained for further work. The same procedure was repeated for Enterica decoction.

2.2.7B.P. tests for identification of adsorbents (B. P, 2009)

2.2.7.1 Test for light magnesium carbonate

100 milligrams of the powder was dispersed in 2ml of water in a test tube. Three millilitres of2M acetic acid was added and the mouth of the test tube immediately fitted with a stopper. The mixture was gently heated and the gas collected into 5ml of 4.78%w/v solution of Barium Hydroxide. The resulting precipitate formed was dissolved in concentrated hydrochloric acid solution.

2.2.7.2 Test for bentonite

One gram of potassium nitrate and 3g of sodium carbonate were added to 0.5 g of bentonite placed in a metal crucible. The mixture was heated until it melted and allowed to cool. Twenty millilitres of boiling water was added, mixed with the residue and filtered. The insoluble residue was washed with 50ml of water. One millilitre of concentrated hydrochloric acid and 5ml of water were added to the resulting residue. The resulting mixture was filtered and 1ml of 10M sodium hydrochloride solution was added and filtered. 3ml of 10.7%w/v ammonium chloride solution was added to the filtrate. The precipitate formed was observed.

2.2.7.3 Test for kaolin.

The procedure for the identification of Kaolin is the same as that of Bentonite and same results are expected.

2.2.7.4 Test for microcrystalline cellulose

Fifty milligrams of microcrystalline cellulose was placed on a watch glass and dispersed in 10ml of iodinated Zinc chloride solution. The colour change observed was noted. The iodinated Zinc chloride solution was prepared by dissolving 40g of Zinc chloride and 13g of potassium iodide in 21ml of water. One gram of iodine was then added and the preparation shaken for about 15 minutes. The resulting mixture was then filtered.

2.2.7.5 Test for maize starch

One gram of maize starch powder was suspended in 50ml of purified water, boiled for 1 minute and cooled. Two drops of iodine solution was added to 1ml of the thin cloudy mucilage obtained. The colour change was noted. The preparation was then heated and the resulting colour change noted.

2.2.8 Phytochemical tests on extracts

2.2.8.1. Test for phenolic compounds

A few drops of ferric chloride solution were added to about 2mls of prepared decoction of Asena and Enterica.

2.2.8.2 Test for saponins

5mls of decoction of Asena was shaken and allowed to stand. The same was repeated for the decoction of Enterica.

2.2.8.3 Test for reducing sugars

2mls each of Fehlings solution A and B was added to about 1ml of decoction of Asena in a test tube and heated. The resulting colour change was noted. The same procedure was repeated for the decoction.

2.2.9 Determination of appropriate adsorbents for formulation of granules

Five 300ml portions representing 10 doses of freshly prepared decoction of Asena was transferred into five different containers and placed in the oven at a temperature of 60^{o}C. The preparation was allowed to evaporate in order to obtain a thin viscous extract. Two grams of maize starch representing 200mg of maize starch per dose of Asena was added to the thin extract in one container and used to form a paste. The paste was allowed to dry completely at 60^{o}C. The ease of scrapping and processing of the adsorbent extract mix was recorded. Processing involved passing the adsorbent extract mix through sieve 20 (850µm). The percentage of extract released at 45minutes in a dissolution test of the processed mix was also determined. The weight of adsorbent used was based on the determination of the amount of Asena extract that was able to fill a five hundred milligram capsule shell. The weight of Asena extract per dose was subtracted from the total to determine the maximum amount of adsorbent to use per dose.

The same procedure was repeated for the other four containers using 2g of kaolin, light magnesium carbonate, microcrystalline cellulose and bentonite respectively.

For Enterica, the same procedure was repeated using 1.1g of adsorbents representing 110mg of adsorbent per dose of Enterica. The weight of adsorbent used was based on determination of the amount of Enterica extract that filled a two hundred and fifty

milligram capsule shell. The weight of Enterica extract per dose was then subtracted from the total to determine the maximum amount of adsorbent to use per dose.

The appropriate adsorbents to be used for further study were then determined based on ease of scrapping and processing as well as the percentage release of Asena and Enterica extracts from the adsorbent extract mix at 45minutes in the dissolution tests.

2.2.10 Formulation of Asena and Enterica granules using selected adsorbents

Each batch of granules was made using 100 doses (3L) of decoction.

Five 3L portions of freshly prepared decoction of Asena were transferred into five different containers and kept in the oven at a temperature of 60^{o}C until thin concentrated extracts were obtained. This normally took about 36 hours when a container of area of base 0.0707 m^2 was used. 4g, 8g, 16g, 18g, 20g representing 40mg, 80mg, 160mg, 180mg, and 200mgrespectively per dose of product of maize starch was then added each to the thin concentrated extract into each container and used to form a paste. The paste was dried at 60^{o}C and the product formed(cakes), scraped and processed into granules by the use of sieve No. 20 (850µm). The ease of scrapping and processing was noted as well as the percentage loss in weight. The coarse and fine granules were separated using sieve No.40 (425µm) and the size distribution determined. The flow properties of the granules were also determined. The granules were packed into tightly fitted glass jars and kept away from light and moisture. The procedure was repeated using light magnesium carbonate and bentonite.

The same procedure was repeated for the formulation of granules of Enterica using 2.2g, 4.4g, 6.6g, 8.8g and 11g of maize starch and light magnesium carbonate representing

22mg, 44mg, 66mg, 88mg and 110mg of adsorbent per dose of product. In the case of the formulated granules of Enterica, the flow properties were not determined due to inadequate weight of granules. This resulted because the plant material provided was not enough to produce more granules. The flow properties of the granules formulated for encapsulation was however determined.

2.2.11 Determination of flow properties of granules

A known weight of granules of Asena was gently poured down the side of a 100ml clean dry measuring cylinder and the initial fluff volume V_o was noted. A retort stand was arranged such that its arm was 5cm above the mouth of the cylinder. The cylinder was then tapped for about 30 times by raising it to touch the arm of the stand and allowing it to drop whiles guiding it with the hand. The final tapped volume, V_f of the granules was also noted. The Carr's index and Hausner ratio were then calculated.

The angle of repose of the granules was determined using the Fixed Height Cone method. A quantity of the granules was allowed to flow through a funnel clamped at a fixed height unto a horizontal surface. The Height and diameter of the resulting cone were measured and the angle of repose calculated.

2.2.12 Dissolution of extracts, granules and capsules

The procedure described by the BP 2009 (Appendix XII B1) [22] was used.

The experimental conditions adopted for the dissolution of extract and various granules were as follows

Medium 900ml of Distilled water

Paddle speed	50 revolutions per minute(rpm)
Sampling times	5, 10, 15, 30, 45 and 60 minutes
Temperature	$37^{o}C \pm 0.5^{o}C$

The six vessel dissolution apparatus was used. The water bath was filled to the maximum mark and the compartments labeled A, B, C, D, E and F. The round bottom beakers were each filled with 900ml of distilled water, placed in their respective compartments and held firmly in the bath. The thermostat was set at $37^{o}C$. The height of the paddles was set at about 2cm above the bottom of the beakers and revolutions set at 50rpm. The dissolution medium was allowed to reach temperature equilibrium of $37^{o}C \pm 0.5^{o}C$. Six samples of 400mg of Asena extract were weighed out for the procedure. The time was set at 0.00 and samples introduced into the consecutive round bottom beakers at 4 minute intervals. At 5 minutes, 20ml of the dissolution medium was withdrawn from vessel A and 20ml of fresh medium replaced. The first few milliliters was discarded and the rest filtered into a test tube. The filtrate was then diluted with distilled water to obtain a concentration of about 0.010%w/v of the extract. The procedure was repeated at times 10, 15, 30, 45 and 60 minutes for Vessel A. Vessels B, C, D, E and F were also taken through the same process.

The whole procedure was repeated for capsules of Asena and Enterica and respective weights of granules of Asena and Enterica formulated using different adsorbents. However in the case of all preparations containing Enterica extract 5ml of dissolution medium was withdrawn from the beakers at the appropriate time intervals and filtered. 5ml of medium was used for replacement. The filtrate was not diluted.

The absorbance of the test solutions were then measured in a 1cm cell at wavelength of 278nm for all Asena preparations and 356nm for all Enterica preparations. Distilled water was used as the blank solution. The mean absorbance of the six values obtained for each sample was then calculated together with its standard deviation. The concentrations of the samples were calculated using their respective calibration curves. The percentage release of each sample was also calculated. A dissolution profile plot was then established.

Immediate (optimum) release means that 75% of the API is dissolved within 45 minutes. [71]

2.2.13 Uniformity of weight of formulated capsules

The test was done for both Asena and Enterica capsules according to the BP 2009 (Appendix XII C1) [22] method for Uniformity of weight of capsules. The weight of an intact capsule was determined using an analytical balance. The capsule was carefully opened making sure not to lose any shell material and the content removed totally. The difference between the weight of the intact capsules and the empty shell was calculated. The procedure was repeated for nineteen more capsules. The mean weight of the twenty capsules was calculated and the percentage deviations from the mean determined.

2.2.14 Disintegration test of formulated capsules

The test was done for both Asena and Enterica capsules. The procedure contained in the BP 2009 (Appendix XII A1)[22] was used. The bath was filled with water to the desired mark and the temperature set at $37^{o}C \pm 0.5^{o}C$. The beaker was filled with 600ml of distilled water and suspended in the main bath. The temperature was allowed to reach

equilibrium with that of the bath. One capsule was put into each of the six tubes. A disc was placed on each capsule to prevent it from floating. A watch clock was set and the apparatus operated until all six capsules had disintegrated leaving only remnants of gelatin shell on the mesh. The time was recorded. The procedure was repeated twice and the mean disintegration time of capsules calculated.

2.2.15 Clinical case studies

Case studies were conducted at the Centre for Scientific Research into Plant Medicine clinic. Two adult patients were used to check the therapeutic efficacy of each product. For Asena, one patient diagnosed with acute pain and another diagnosed with osteoarthritis were used for the study. The consent of patients was sought for the study. Patients were allowed to rate the degree of pain on a numbered scale of one to ten with one representing virtually no pain and ten representing severe pain. Asena decoction was replaced with Asena capsules for these patients. The prescription was given for a period of seven days. The dosage regimen was two capsules taken three times daily. The patients were monitored for seven days. Alleviation of pain was used as the measure of efficacy.

For Enterica, a case study was conducted on a 24 year old female diagnosed with typhoid fever. The patient reported with abdominal pains, headaches, nausea and occasional feverishness. On examination tenderness was observed around the epigastrum and the umbilical region. All other organs were not palpable. A widal test showed a reading of

TO: 1/160 TH: 1/160. The patient was administered with one capsule of Enterica three times daily for a period of two weeks.

CHAPTER THREE: RESULTS, CALCULATIONS AND COMMENTS

3.1 RESULTS FOR ASENA PREPARATIONS

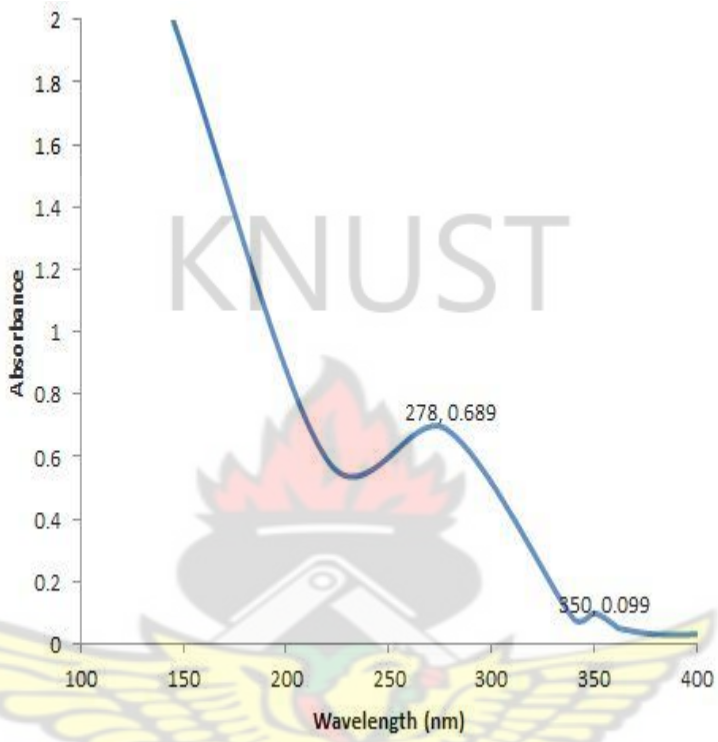
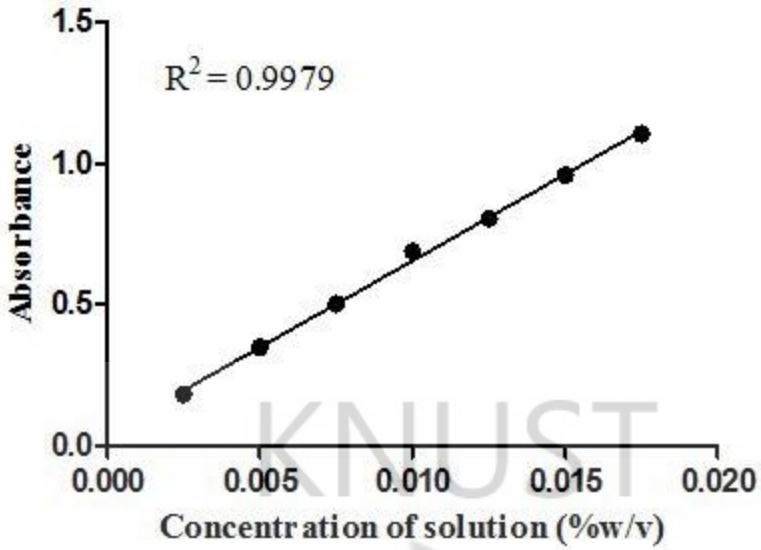


Figure 3.1.1 UV spectrum graph of Asena extract

Comment

A solution of native extract of Asena in distilled water of concentration 0.010%w/v gave an observable peak at a wavelength of 278nm. This peak was used as a marker for quantification in all dissolution studies of Asena preparations.



Best-fit values	
Slope	61.26 ± 1.266
Y-intercept when X=0.0	0.04386 ± 0.01416
X-intercept when Y=0.0	-0.0007160
1/slope	0.01632

Figure 3.1.2 calibration curve for solution of Asena extract

Comment

Equation of curve is Y= 61.26X + 0.0438

The absorbances used for the calibration curve were obtained at a wavelength of 278nmwhich was the maximum wavelength of absorption of the extract. The equation of the calibration curve was used to determine the concentration of all Asena dissolution samples. The calibration data is recorded in Appendix A (Table A-5: Calibration data for solution of Asena extract without Adsorbent).

Table 3.1.1 Identification test for adsorbents

Test	Observation	Inference
Magnesium carbonate	A white precipitate was formed. This dissolved when concentrated HCL was added	Magnesium carbonate may be present
Bentonite	A white gelatinous precipitate was formed	Bentonite may be present
Kaolin	A white gelatinous precipitate was formed	Kaolin may be present
Maize starch	A dark blue colour was formed which disappeared on heating	Starch may be present
Microcrystalline cellulose	A bluish violet colour was formed	Microcrystalline cellulose may be present

Comment

The five adsorbents used in the study were chosen based on their availability, costs and evidence of their use as adsorbents in pharmaceutical preparations. The identification tests were conducted to ensure that the available laboratory grade powders provided could be identified as labeled.

Table 3.1.2 Phytochemical test of Asena

Test	**Observation**	**Inference**
Phenolics	Dark green colouration of solution	Phenolics may be present
Saponins	Froth formed did not break readily on standing	Saponins may be present
Reducing sugars	A brick red precipitate was formed	Reducing sugars may be present

Comment

These three constituents were those contained in the preparation as stated in the document obtained from the CSRPM.

Table 3.1.3 Determination of weight of extract per dose of Asena Decoction

Dish	**A**	**B**	**C**
Weight of dish +extract (g)	43.171	43.675	28.087
Weight of empty dish (g)	42.769	43.275	27.682
Weight of extract (g)	0.402	0.400	0.405

Calculation

$$\text{Mean weight} = \frac{0.402 + 0.400 + 0.405}{3}$$

$$\text{Mean weight} = 0.4023 \pm 0.002517 \text{ g}$$

Comment

30mls of decoction of Asena was used for this procedure because that was the dose stated on the label of Asena decoction produced by CSRPM. The weight of extract per dose used for subsequent calculations was 400mg.

Table 3.1.4 Determination of quantity of decoction of Asena per unit area which dries completely

Container	Diameter of base (m) of container	Area of base(m^2) = πr^2	Quantity of decoction (L)	Nature of extract after 48 hours of drying
A	0.16m	0.0201	0.5	Dried
B			1.0	Gummy
C			1.5	Gummy
D	0.30m	0.0707	0.5	Dried
E			1.0	Dried
F			1.5	Dried
G			2.0	Gummy

Calculation

r= radius = diameter of base/2

For containers of area, 0.0201m^2, a dried extract is obtained with 0.5L of decoction

Quantity (volume) of decoction per unit area = 0.5/ 0.0201 = 24.88L/m^2

For containers of area 0.0707m^2, a dried extract is obtained with 1.5L of decoction.

Quantity (volume) of decoction per unit area = $\frac{1.5}{0.0707}$ = 21.22L/m^2

Mean = $\frac{4.88+21.22}{2}$ = 23.05L/m^2

Standard deviation = 1.30

Quantity (volume) of decoction per unit area = 23.05 ± 1.30L/m^2

Comment

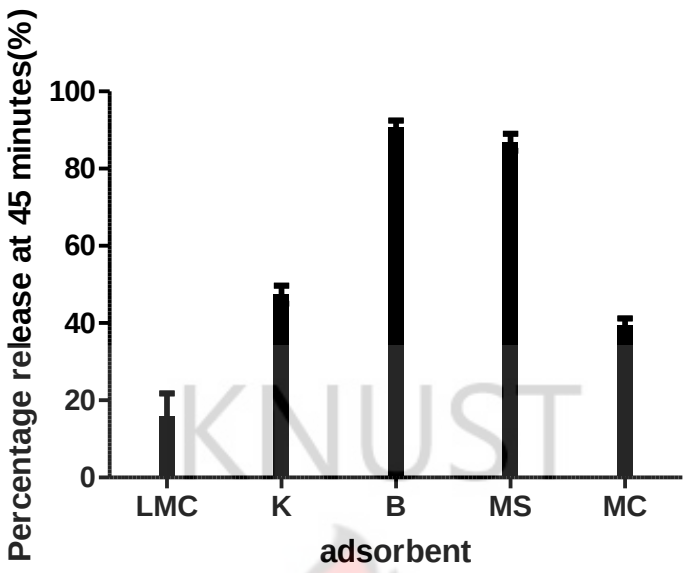
This procedure was done to determine how completely dried extract of the decoction could be obtained with an optimum moisture content for analytical work and to establish the need for the use of adsorbents to enhance processing of extract into granules for encapsulation.

Table 3.1.5 Ease of scrapping and processing of extract Adsorbent mix of Asena after drying using different adsorbents

Adsorbent	**Weight of adsorbent per dose**				
	40mg	80mg	160mg	180mg	200mg
Magnesium carbonate	Very easy	Very easy	Very easy	Very easy	Very easy
Bentonite	Difficult	Easy	Very easy	Very easy	Very easy
Maize starch	Very difficult	Very easy	Easy	Very easy	Very easy

Comment

These three adsorbents were chosen for processing of Asena after initial screening of five adsorbents with relation to ease of scrapping and processing as well as dissolution characteristics of the five adsorbents. Microcrystalline cellulose and kaolin were eliminated from further study due to difficulty in scrapping and processing- Appendix A **(**Table A-1Ease of scrapping and processing of formulations of Asena containing five different adsorbents)



LMC – Light magnesium carbonate, K – Kaolin, B – Bentonite, MS – Maize starch, MC – Microcrystalline cellulose

Figure 3.1.3: Dissolution (percentage release at 45minutes) of Asena adsorbents mix using five different adsorbents at 200mg per dose.

Comment

This was done in order to enable the selection of appropriate adsorbents for further study in the formulation of Asena granules for encapsulation. The detailed results are shown in Appendix A (Table A-6 Dissolution data of granules of Asena formulated using 200mg per dose of five different adsorbents at 45minutes). Microcrystalline cellulose and Kaolin were eliminated from further study due to poor dissolution characteristics. Light magnesium carbonate was included in further study due to its ease of processing and to ascertain if variation in quantity of adsorbent used per dose of Asena could cause significant change in dissolution characteristics.

Table 3.1.6 Percentage loss in weight of Asena granules formulated using different adsorbents

Adsorbent	Weight of adsorbent per dose (mg) / percentage loss in weight (%)				
	40mg	**80mg**	**160mg**	**180mg**	**200mg**
Light magnesium carbonate	3.32	3.54	2.57	1.76	5.88
Bentonite	8.07	9.06	1.61	5.93	8.58
Maize starch	9.36	12.42	8.96	3.66	6.20

Comment

The percentage loss in weight was established based on the formulation of granules of Asena using different adsorbents for a hundred doses of product –Appendix A (Table A-2 Percentage loss in weight of Asena granules using different adsorbents).

Table 3.1.7 Size distribution of Asena granules formulated using different adsorbents

Adsorbent		Weight of adsorbent per dose				
	Percentages (%)	**40mg**	**80mg**	**160mg**	**180mg**	**200mg**
Light Magnesium carbonate	**Coarse**	72.57	70.26	57.88	55.98	54.49
	Fines	27.43	29.74	42.12	44.02	45.51
Maize starch	**Coarse**	61.43	64.86	67.51	65.49	61.91
	Fines	38.57	35.14	32.49	34.51	38.09
Bentonite	**Coarse**	64.39	50.14	55.08	53.72	67.16
	Fines	35.61	49.86	44.92	46.28	32.84

Granules were formulated using hundred doses of decoction

Comment

The detailed results are outlined in Appendix A (Table A-3 Size distribution of Asena granules using different adsorbents)

Table 3.1.8 Flow properties of Asena granules formulated from different adsorbents using Hausner ratio, Carr's index and Angle repose

Absorbent	weight of adsorbent per dose (mg)	H.R	C.I (%)	Angle of repose
Light magnesium carbonate	40	1.15	12.71	45
	80	1.19	15.79	41
	160	1.22	18.26	42
	180	1.18	15.27	37
	200	1.11	10.00	35
Bentonite	40	1.15	12.96	28
	80	1.17	14.29	37
	160	1.13	11.76	30
	180	1.11	9.68	30
	200	1.10	8.82	23
Maize starch	40	1.06	5.45	39
	80	1.19	16.13	37
	160	1.13	11.59	41
	180	1.19	16.25	43
	200	1.12	10.98	43

Comment

The flow properties of formulated granules were generally good. The flow properties according to Carr's Index and Hausner ratio were obtained from calculation from results in Appendix A (Table A-4.1 Flow properties of Asena granules formulated from different adsorbents using Hausner ratio and Carr's index). The flow properties using angle of repose was obtained from calculation from results in Appendix A(Table A-4.2 Flow properties of Asena granules by the fixed height cone method)

Dissolution profiles of extract and granules of Asena formulated using different adsorbents

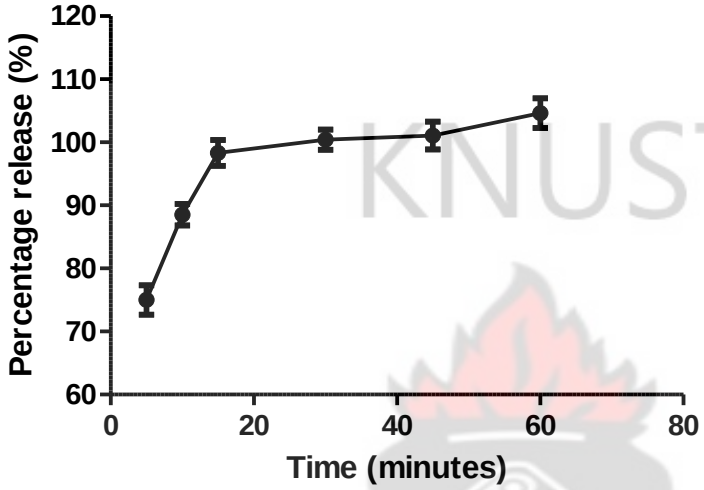


Figure 3.1.4: Dissolution profile of Asena extracts without adsorbents – Appendix A (Table A-7.1 Dissolution data of Asena extract without adsorbent)

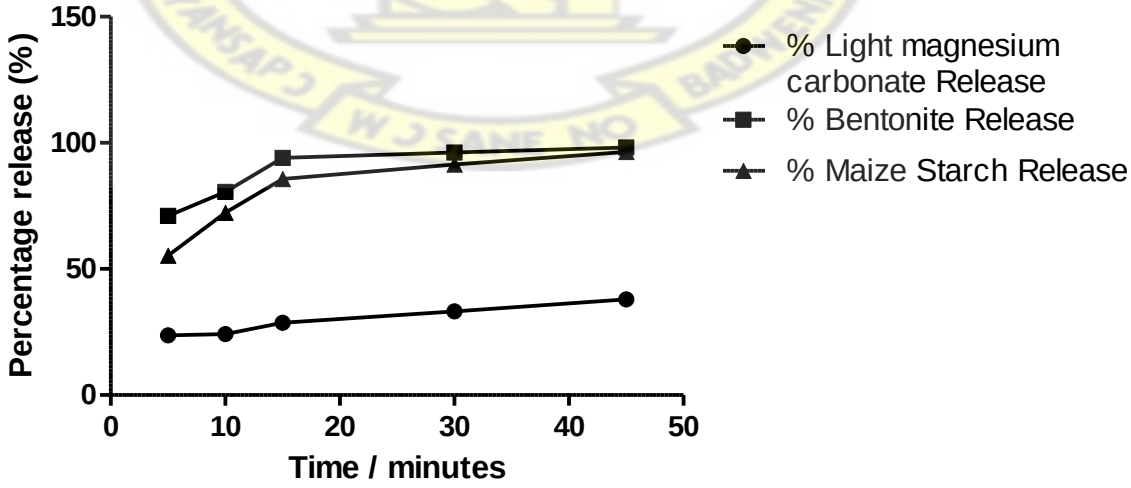


Figure 3.1.5: Dissolution profile of Asena granules using 40mg per dose of different adsorbents – Appendix A (Table A-7.2 Dissolution data of Asena granules using 40mg per dose of different adsorbents)

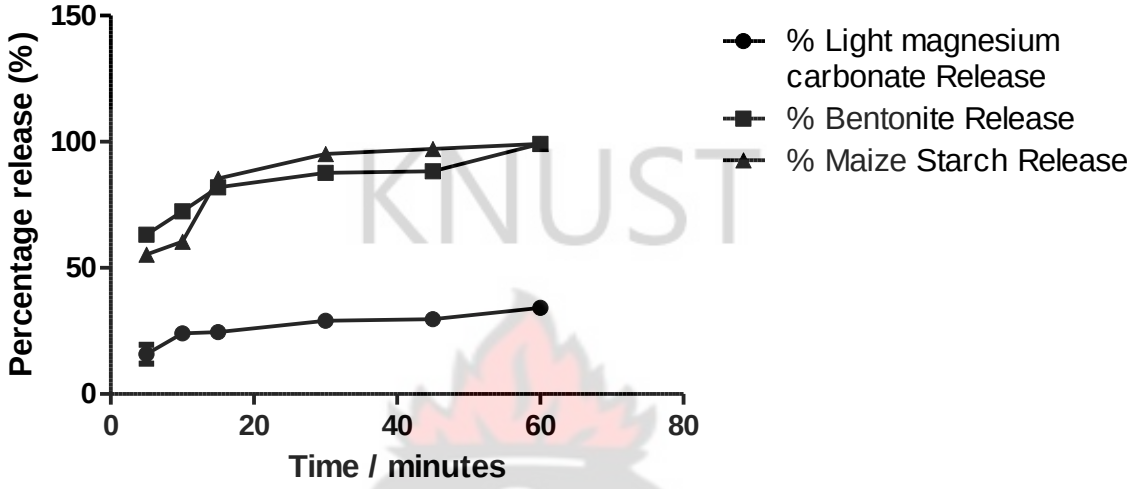


Figure 3.1.6: Dissolution profile of Asena granules using 80mg per dose of different adsorbents – Appendix A (Table A-7.3 Dissolution data of Asena granules using 80mg per dose of different adsorbents)

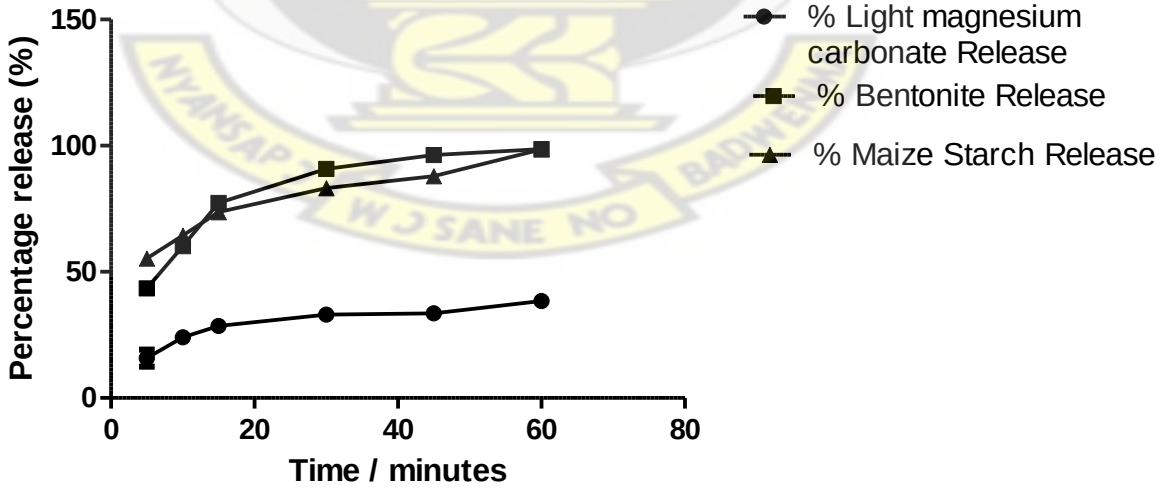


Figure 3.1.7: Dissolution profile of Asena granules using 160mg per dose of different adsorbents – Appendix A (Table A-7.4 Dissolution data of Asena granules using 160mg per dose of different adsorbents)

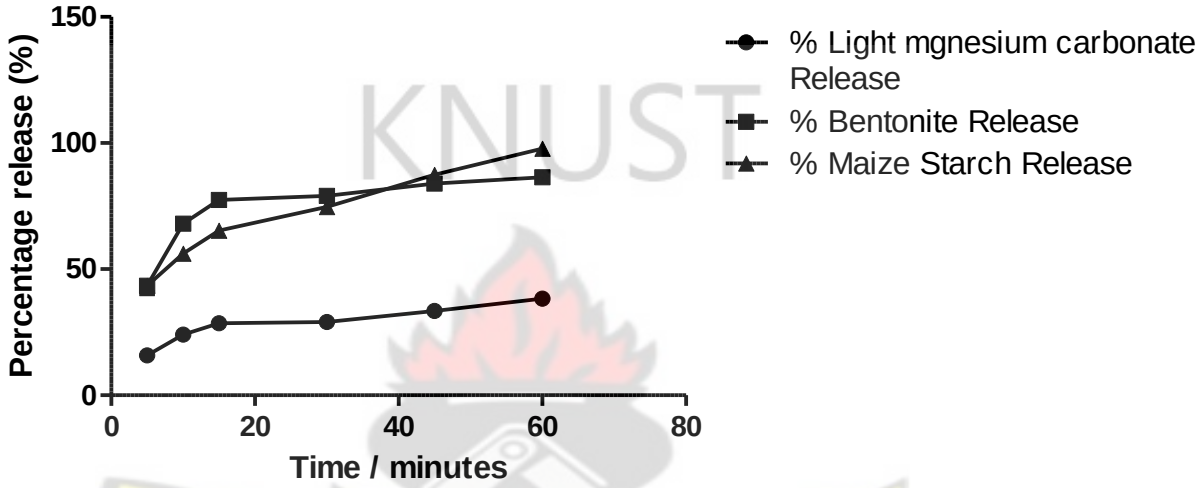


Figure 3.1.8: Dissolution profile of Asena granules using 180mg per dose of different adsorbents – Appendix A (Table A-7.5 Dissolution data of Asena granules using 180mg per dose of different adsorbents)

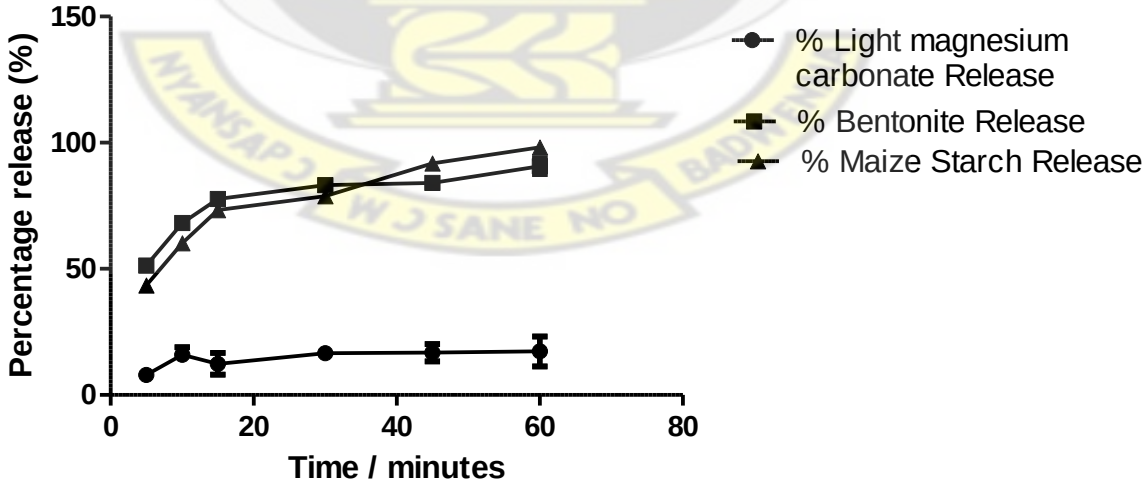


Figure 3.1.9: Dissolution profile of Asena granules using 200mg per dose of different adsorbents – Appendix A (Table A-7.6 Dissolution data of Asena granules using 200mg per dose of different adsorbents)

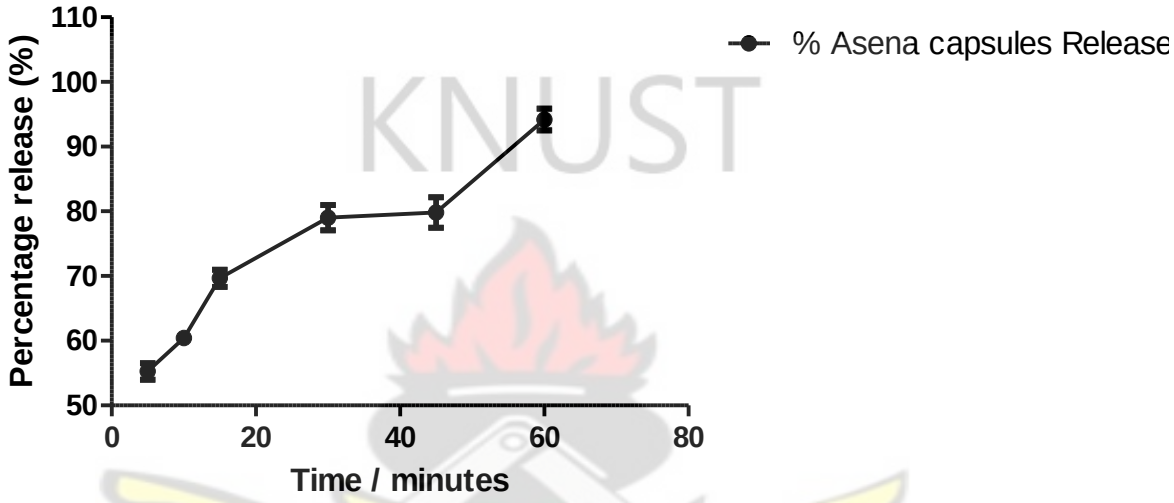


Figure 3.1.10: Dissolution profile of capsules of Asena formulated using 200mg per dose of maize starch as adsorbent– Appendix A (Table A-8.2 Dissolution profile of Asena capsules)

Table 3.1.9 Uniformity of weight of Asena capsules

Capsule	Weight (g) = y	Deviation ($\bar{y}$ - y)	% Deviation
1	0.411	-0.0072	-1.79
2	0.4108	-0.0070	-1.74
3	0.4009	0.0029	0.71
4	0.400	0.0038	0.94
5	0.400	0.0038	0.94
6	0.4020	0.0018	0.44
7	0.4100	-0.0062	-1.54
8	0.4000	0.0038	0.94
9	0.4000	0.0038	0.94
10	0.4001	0.0037	0.91
11	0.4012	0.0026	0.64
12	0.4000	0.0038	0.94
13	0.4010	0.0028	0.69
14	0.4005	0.0033	0.81

15	0.4010	0.0028	0.69
16	0.4160	-0.0122	-3.03
17	0.4000	0.0038	0.94
18	0.3990	0.0048	1.18
19	0.4015	0.0023	0.57
20	0.4207	-0.0169	-4.19

Key

Net mass of capsule contents	**Deviation %**	**Number of capsules**
300 mg and over	± 7.5	minimum 18
	± 15.0	maximum 2

Calculation

Weight of 20 capsules = 8.0757g

Mean weight ($\tilde{y}$) = 8.0757/20 = 0.4038g

Table 3.1.10 Disintegration test of Asena capsules

Test	**1**	**2**	**3**
Time (minutes)	8.04	8.15	7.90

Mean time = 8.03

Standard deviation = 0.1253

Disintegration time = 8.03± 0.13

3.2 Formulation of Enterica Preparations

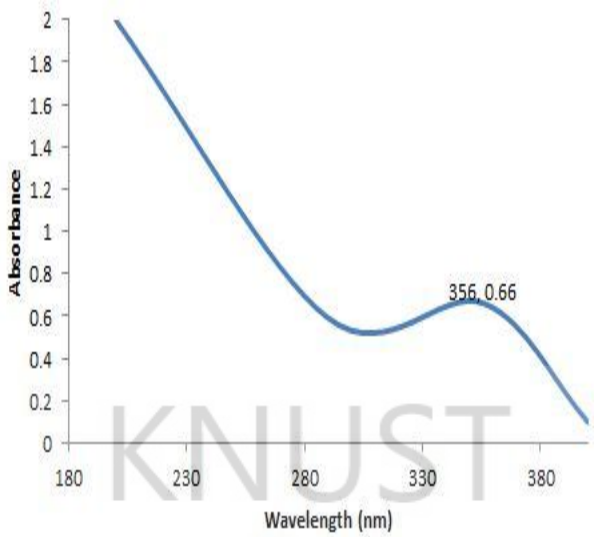
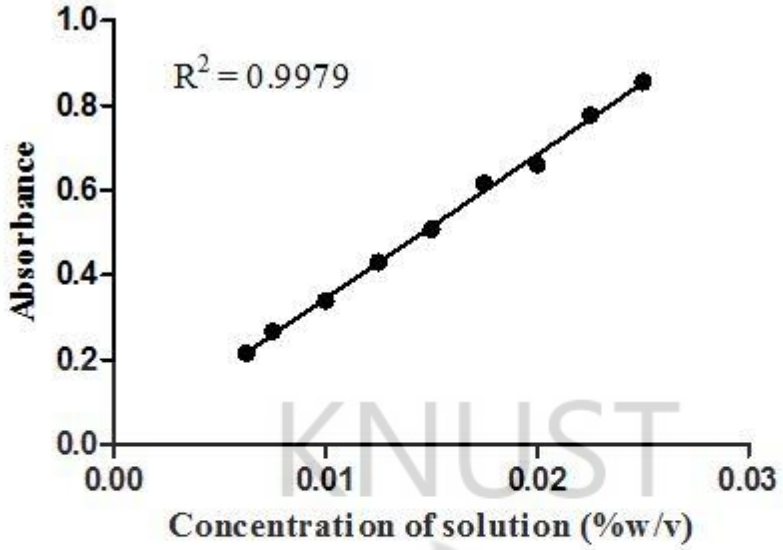


Figure 3.2.1 UV spectrum graph of Enterica Extract

Comment

A solution of native extract of Enterica in distilled water of concentration 0.020%w/v gave an observable peak at a wavelength of 356nm. This peak was used as a marker for quantification in all dissolution studies of Enterica preparations.



Best-fit values	
Slope	33.91 ± 0.6550
Y-intercept when X=0.0	0.005198 ± 0.01073
X-intercept when Y=0.0	-0.0001533
1/slope	0.02949

Figure 3.2.2 calibration curve for solution of Enterica extract

Comment

Equation of curve is Y= 33.91X + 0.0005

The absorbances used for the calibration curve were obtained at a wavelength of 356nm which was the maximum wavelength of absorption of the extract. The equation of the calibration curve was used to determine the concentration of all Enterica dissolution samples. The calibration data is shown in Appendix B (Table B-5 Calibration data of Enterica extract without Adsorbent).

Table 3.2.1 Phytochemical test of Enterica

Test	Observation	Inference
Phenolics	Dark green colouration of solution	Phenolics may be present
Saponins	Froth formed did not break readily on standing	Saponins may be present
Reducing sugars	A brick red precipitate was formed	Reducing sugars may be present

Table 3.2.2 Determination of weight of extract per dose of Enterica Decoction

Dish	A	B	C
Weight of dish +extract (g)	43.6902	43.4650	28.8151
Weight of empty dish (g)	42.5000	43.2750	28.6251
Weight of extract (g)	0.1902	0.1900	0.1900

Calculation

$$\text{Mean weight} = \frac{0.1902 + 0.1900 + 0.1900}{3}$$

Mean weight = 0.1901 ± 0.00012 g

Comment

30 mls decoction of Enterica was used for this procedure because that was the dose stated on the label of Enterica decoction produced by CSRPM. The weight of extract per dose used for subsequent calculations was 190mg.

Table 3.2.3 Determination of quantity of decoction of Enterica per unit area which dries completely

Container	Diameter of base (m) of container	Area of base(m^2) = πr^2	Quantity of decoction (L)	Nature of extract after 48 hours of drying
A	0.16m	0.0201	0.5	Dried
B			1.0	Dried
C			1.5	Dried
D	0.30m	0.0707	0.5	Dried
E			1.0	Dried
F			1.5	Dried
G			2.0	Gummy

Calculation

Quantity (volume) of decoction per unit area = 1.5/ 0.0707 = 21.21L/m^2

Comment

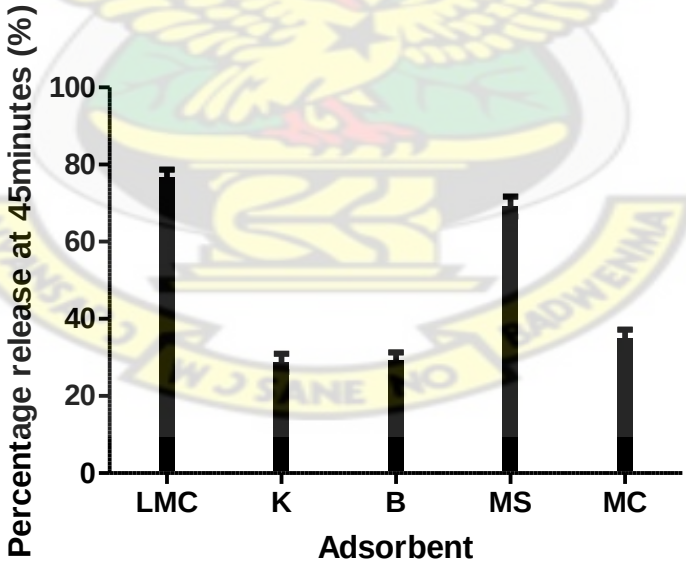
This procedure was done to determine how completely dried extract of the decoction could be obtained with an optimum moisture content for analytical work and to establish the need for the use of adsorbents to enhance processing of extract into granules for encapsulation. It was observed that the decoction of Enterica was lighter compared to that of Asena hence a dried extract could be obtained more easily with the volumes used with the corresponding containers.

Table 3.2.4 Ease of scrapping and processing of extract Adsorbent mix of Enterica after drying using different adsorbents

Adsorbent	Weight of adsorbent per dose				
	22mg	44mg	66mg	88mg	110mg
Magnesium carbonate	Very easy	Very easy	Very easy	Very easy	Very easy
Maize starch	Very easy	Very easy	Very easy	Very easy	Very easy

Comment

These two adsorbents were chosen for processing of Enterica after initial screening of five adsorbents with relation to ease of scrapping and processing as well as dissolution characteristics of the five adsorbents - Appendix B (Table B-1Ease of scrapping and processing of five different adsorbents).



LMC – Light magnesium carbonate, K – Kaolin, B – Bentonite, MS – Maize starch, MC – Microcrystalline cellulose

Figure 3.2.3: Dissolution (percentage release) of Enterica adsorbents mix using five different adsorbents at 110mg per dose.

Comment

This was done in order to enable the selection of appropriate adsorbents for the formulation of Enterica granules for encapsulation. The dissolution data is shown in Appendix B(Table B-6 Dissolution data of granules of Enterica using 110mg per dose of five different adsorbents). Kaolin, Bentonite and microcrystalline cellulose were eliminated from further study due to poor dissolution characteristics.

Table 3.2.5 Percentage loss in weight of Enterica granules formulated using different adsorbents

Adsorbent	**Weight of adsorbent per dose (mg) / percentage loss in weight (%)**				
	22mg	**44mg**	**66mg**	**88mg**	**110mg**
Light magnesium carbonate	3.02	7.31	6.41	5.04	2.07
Maize starch	5.00	5.85	7.81	4.89	2.73

Comment

The percentage loss in weight was established based on the formulation of granules of Enterica using light magnesium carbonate and maize starch for a hundred doses of product. The detailed results are shown in Appendix B (Table B-2 Percentage loss in weight of Enterica granules using different adsorbents).

Table 3.2.6 Size distribution of Enterica granules formulated using different adsorbents

Adsorbent		Weight of adsorbent per dose				
	Percentages (%)	**22mg**	**44mg**	**66mg**	**88mg**	**110mg**
Light Magnesium carbonate	**Coarse**	44.75	46.93	42.99	43.56	45.13
	Fines	55.25	53.07	57.01	56.44	54.87
Maize starch	**Coarse**	55.66	54.02	62.63	56.54	56.27
	Fines	44.34	45.98	37.37	43.46	43.73

Granules were formulated using hundred doses of decoction

Comment

The detailed results are shown in Appendix B (Table B-3 Size distribution of Enterica granules using different adsorbents).

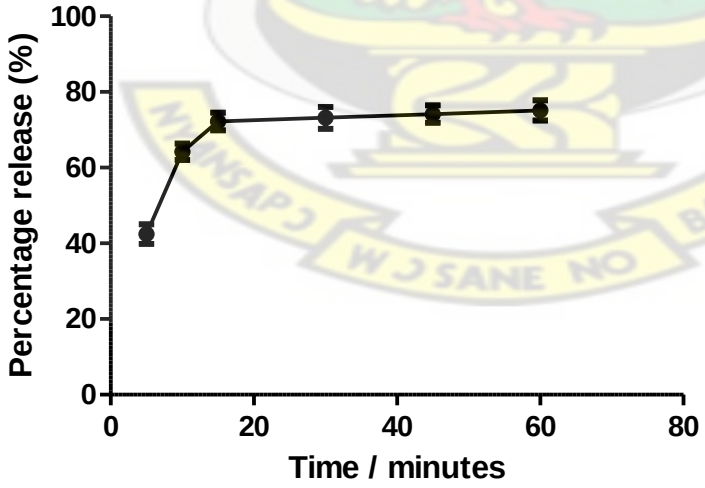


Figure 3.2.4: Dissolution profile of native extract of Enterica without adsorbent – Appendix B (Table B-7.1 Dissolution data of native extract of Enterica without adsorbent)

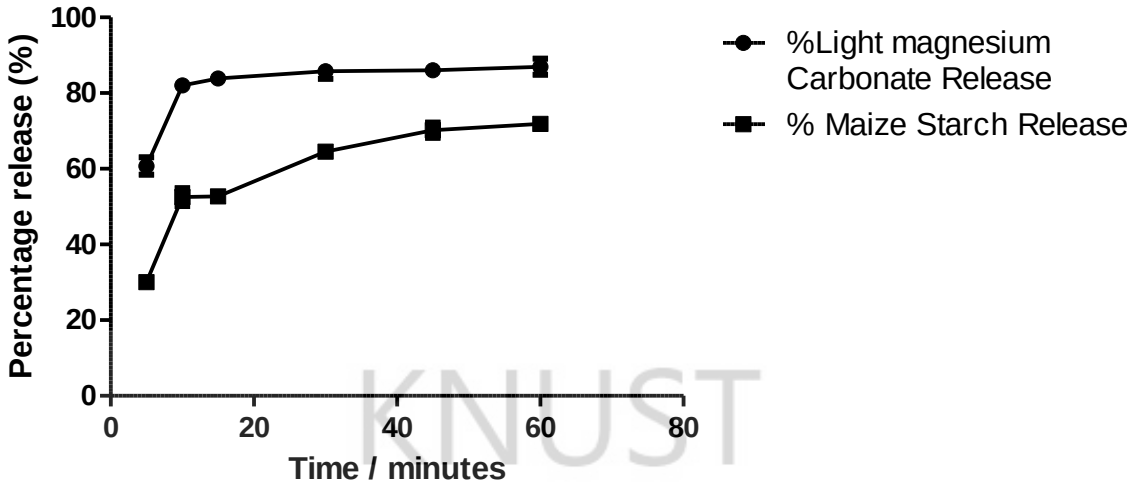


Figure 3.2.5: Dissolution profile of Enterica granules using 22mg per dose of different adsorbents – Appendix B (Table B-7.2 Dissolution data of Enterica granules using 22mg per dose of different adsorbents)

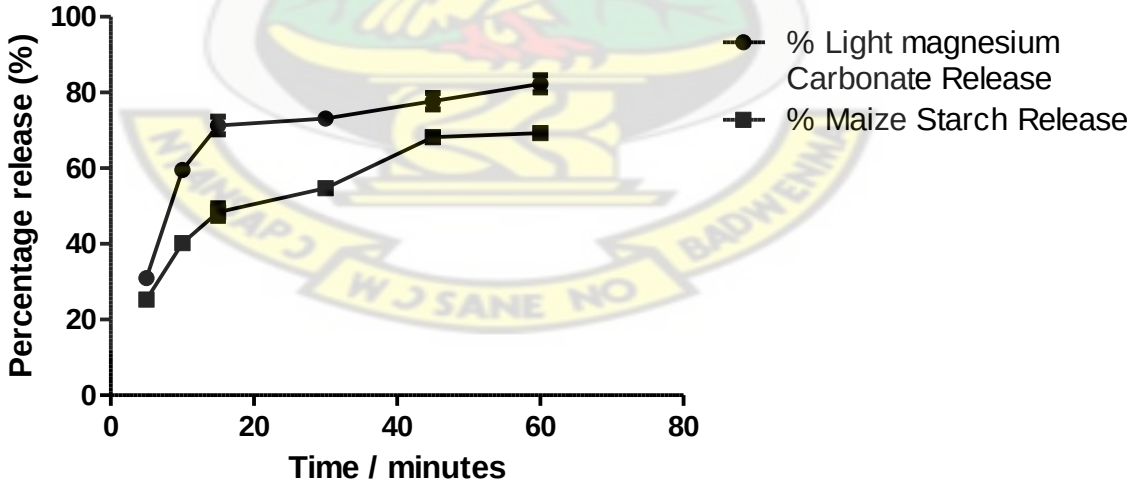


Figure 3.2.6: Dissolution profile of Enterica granules using 44mg per dose of different adsorbents – Appendix B (Table B-7.3 Dissolution data of Enterica granules using 44mg per dose of different adsorbents)

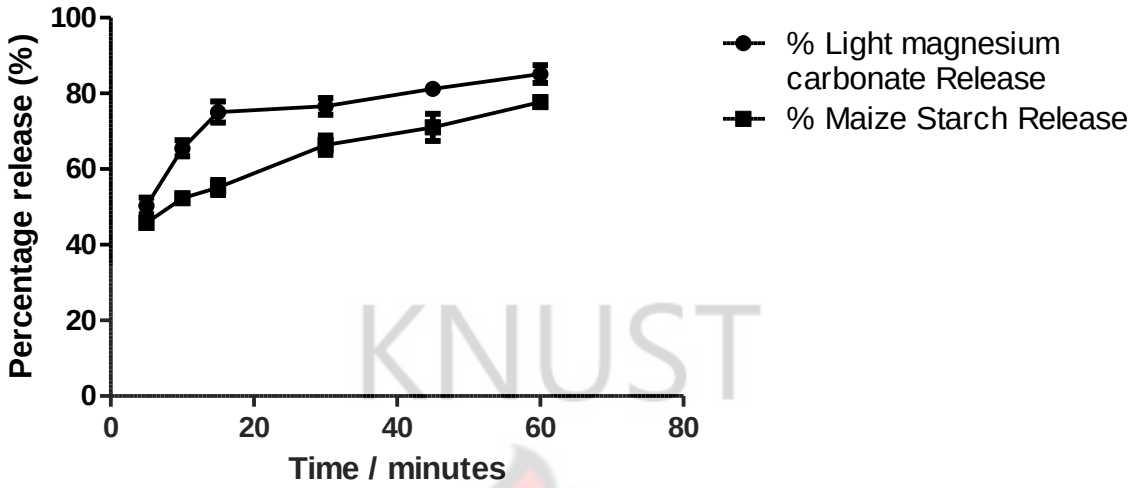


Figure 3.2.7: Dissolution profile of Enterica granules using 66mg per dose of different adsorbents – Appendix B (Table B-7.4 Dissolution data of Enterica granules using 66mg of different adsorbents)

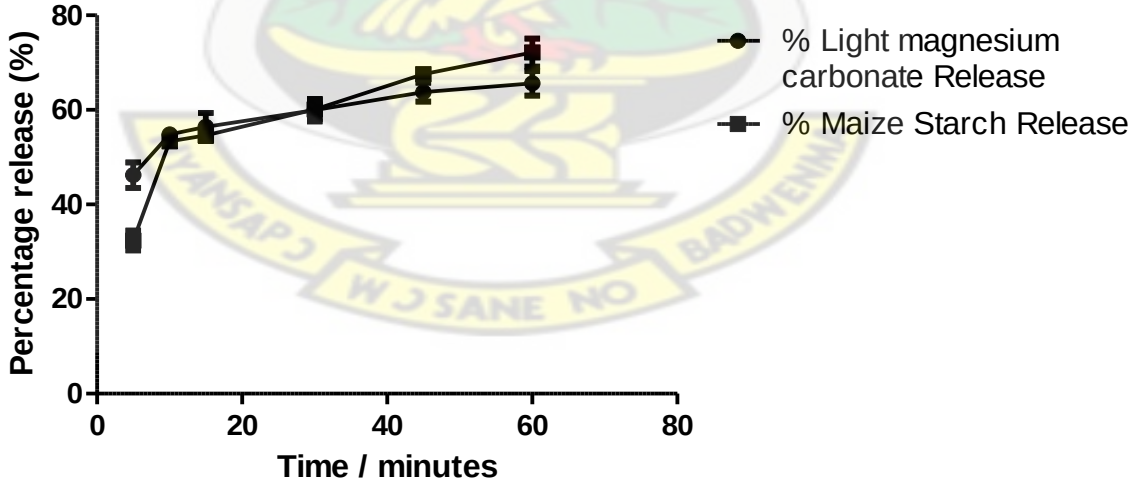


Figure 3.2.8: Dissolution profile of Enterica granules using 88mg of different adsorbents – Appendix B (Table B- 7.5 Dissolution data of Enterica granules using 88mg per dose of different adsorbents)

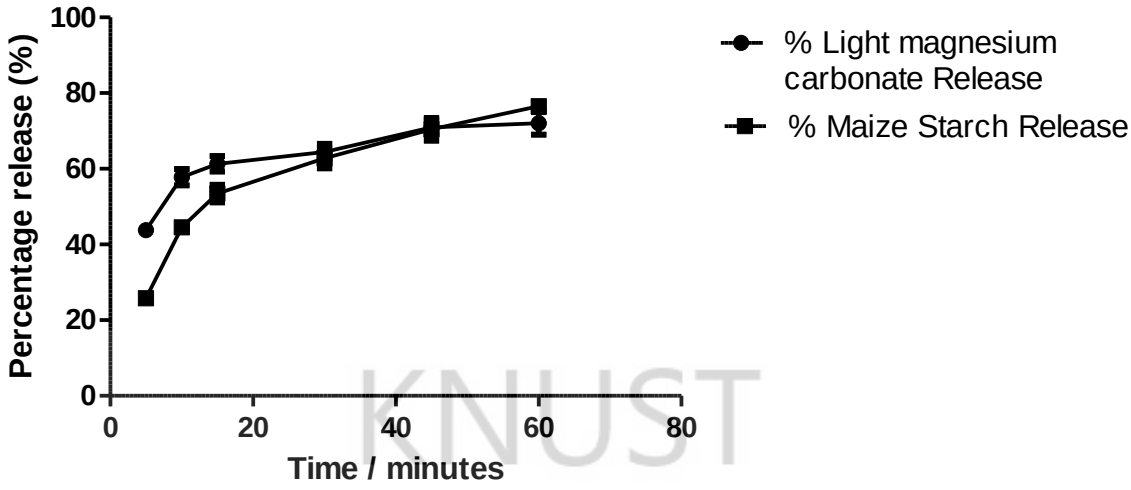


Figure 3.2.9: Dissolution profile of Enterica granules using 110mg per dose of different adsorbents – Appendix B (Table B-7.6 Dissolution data of Enterica granules using 110mg per dose of different adsorbents)

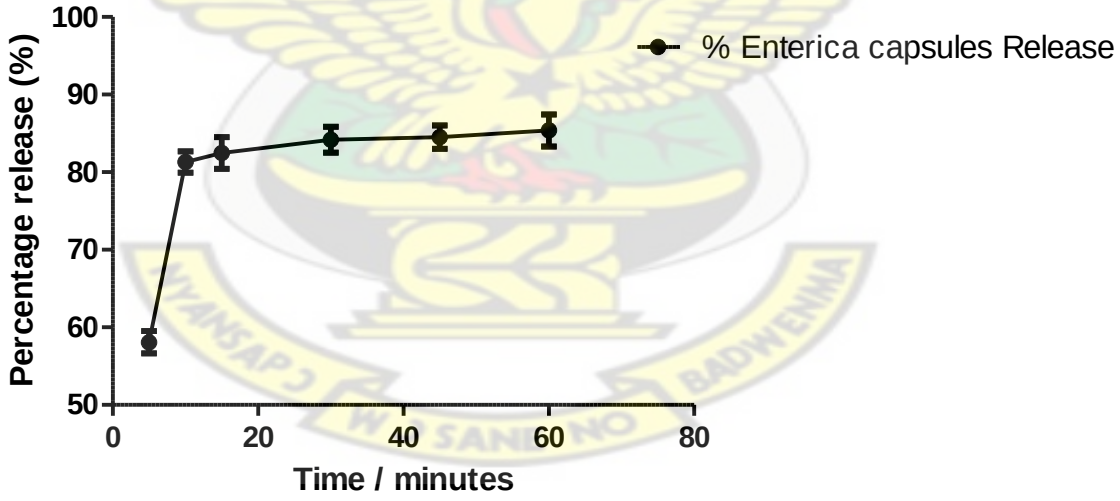


Figure 3.2.10: Dissolution profile of Enterica capsules formulated using 22mg per dose of light magnesium carbonate as adsorbent – Appendix B (Table B-8.3 Dissolution profile of Enterica capsules)

Table 3.2.7 Uniformity of weight of Enterica capsules

Capsule	Weight (g) = y	Deviation (ỹ - y)	% Deviation
1	0.3350	0.0170	4.21
2	0.3500	0.0020	0.49
3	0.3502	0.0018	0.44
4	0.3500	0.0020	0.49
5	0.3408	0.0112	2.77
6	0.3511	0.0009	0.22
7	0.3498	0.0022	0.54
8	0.3500	0.0020	0.49
9	0.3490	0.0030	0.74
10	0.3601	-0.0081	-2.01
11	0.3412	0.0108	2.67
12	0.3500	0.0020	0.49
13	0.3511	0.0009	0.22
14	0.3855	-0.0335	-8.30
15	0.3490	0.0030	0.74
16	0.3600	-0.0080	-1.99
17	0.3511	0.0009	0.22
18	0.3600	-0.0080	-1.99
19	0.3550	-0.0030	-0.75
20	0.3507	0.0013	0.32

Key

Net mass of capsule contents	Deviation %	Number of capsules
300 mg and over	± 7.5	minimum 18
	± 15.0	maximum 2

Calculation

Weight of 20 capsules = 7.0396g

Mean weight (ỹ) = 7.0396/20 = 0.3520g

Table 3.2.8 Disintegration test of Enterica capsules

Test	**1**	**2**	**3**
Time (minutes)	4.14	4.65	4.00

Mean time = 4.26

Standard deviation = 0.34

Disintegration time = 4.26 ± 0.34

CHAPTER FOUR: DISCUSSION, CONCLUSION AND RECOMMENDATION

4.1DISCUSSION

According to the World Health Organization (WHO), 80 per cent of the developing world's rural population depends on traditional medicines for its primary healthcare needs. This underlies the urgent need for investing in the standardisation and development of traditional medicine. Such an investment could ensure that local communities benefit from traditional methods of healing for centuries to come and it would contribute substantially to the Millennium Development Goals (MDGs)[2].

The acceptance of traditional medicines into the health care delivery systems in most countries in the world is increasing. Alongside this growing interest, there have been concerns over the quality and standardization of traditional medicines. Although production of traditional medicines has been scaled-up from a local to a global level, international standardization of how such medicines are produced and used has yet to catch up. The challenge is to develop modern, international standards for medicines and practitioners that have originated in varied cultural settings within a framework that can be universally understood. [1, 3]

Substances extracted from plants and animals remain among the most potent and widely used drugs. Raw extracts from plants and animals often contain a cocktail of active ingredients.

Early drug development focused on identifying the most important ingredients and purifying them. However, traditional practitioners and herbalists consider the effect of herbal preparation to be related to the whole product and not single molecules. [8, 9]

Drug development from animals and plants is continuing by two approaches; the investigation of traditional remedies by seeking evidence of drug effect and screening of extracts against batteries of biological and genomic test systems by seeking a drug action. Quality standardisation of traditional medicines covers the quality of raw materials, the process and finished products. The starting point is to establish the identity of the raw material by consulting a reliable traditional source such as texts as well as traditional knowledge holders[9]. This was the reason for allowing all plant materials to be collected and processed by the CSRPM.

Most of the herbal preparations produced by the CSRPM come in the form of decoctions. These are preserved to prevent spoilage during use.

Asena and Enterica were chosen as liquid products to be formulated into solid dosage forms due to their regular use at the clinic, and evidence of efficacy from the CSRPM. The dose sizes needed are in large volumes and many patients are uncomfortable with the taste of the products. Compliance is compromised since the dosage regime for the required effect may not be adhered to by patients. The two products which are produced as decoctions by the CSRPM are used in the treatment of generalized Body pains and typhoid fever respectively. These two conditions are common in Ghana. Though made up of several plants materials, the products are known to exhibit the required therapeutic effects.

The work sought to identify means of efficient production of capsules of Asena and Enterica with the optimum therapeutic effect. The decoctions of the plant material were used to facilitate the extraction of the active ingredients. Formulation of suitable granules for encapsulation from the decoction using the simplest, cost effective and efficient method was the aim in this project. The method of analyses chosen was the UV visible Spectroscopy due to its accessibility and ability to identify markers of extracts for further quantification. The UV spectra of Asena and Enterica showed clear markers at maximum wavelengths of absorption of 278nm and 356nm respectively(Figure 3.1.1 UV spectrum graph of Asena extract and Figure 3.2.1 UV spectrum graph of Enterica extract). The calibration curve of Asena with R^2 of 0.9979(Figure 3.1.2, Table A-5)shows a good relationship between the concentration of the marker and the absorbances recorded. The calibration curve of Enterica also had an R^2 of 0.9979 (Figure 3.2.2,Table B-5)

Asena and Enterica decoctions contained saponins, phenolics and reducing sugars as shown in Tables 3.1.1and Table 3.2.1. These constituents may be responsible for their therapeutic effect.

The mean weight of Asena extract per dose of Asena decoction was 0.4023 ± 0.0025 g (Table 3.1.3). This shows that a dose of Asena could be put into one five hundred milligram capsule or into two 250mg capsules.

The mean weight of Enterica extract per dose of Enterica decoction was 0.1901 ± 0.0001g (Table 3.2.2). This shows that a dose of Enterica could be put into a 250mg capsule.

The drying of the decoctions was done in the oven because this is the readily available method of drying. It is also a convenient method for drying of extracts whose change in

properties cannot be ascertained since a constant temperature can be maintained and product checked from time to time. The oven is also the method of drying normally used for drying when working with development samples. The temperature of 60^{o}C used for the drying of preparations was optimum. As stated in literature, the optimum temperature for the drying of plant extracts in the oven could be between 50 – 80^{o}C.

Table 3.1.4: Determination of quantity of decoction of Asena per unit area which dries completely, demonstrates difficulty in obtaining dry extracts after drying of large volumes of the decoction of Asena. The extract formed becomes gummy and difficult to process. However the nature of the extract could be related to the size of the container used for the drying process. The larger the base of the container used, the easier the drying process. This occurs because a thin layer dries easily. It could be observed that if dried extract is required, then the volume of decoction per unit area before drying should be 23.05 ± 1.30L/m^2(Table 3.4.1).This demonstrates that to obtain dried extracts of Asena, very large containers are required. Photograph of Asena extract is shown in Appendix C (Figure C-1).

Table 3.2.3: Determination of quantity of decoction of Enterica per unit area which dries, shows a similar result for decoction of Enterica with a quantity (volume) of decoction per unit area required to obtain dry extracts as 21.21L/m^2(Table 3.2.3). Photographs of Enterica extract and concentrated miscella of Enterica decoction are shown in Appendix C (Figures C-5 and C-6).When dried extracts are formed, they have to be scrapped for further work. The scrapping is difficult and time consuming. The addition of a substance which will aid in the processing of the extract into granules after drying by forming flakes or cakes was the method of choice. This is the rational for the use of adsorbents

which are capable of adsorbing the moisture from the preparation. The addition of the adsorbents enhances drying, decreases drying time and enhances scrapping and processing. The major challenge with the use of an adsorbent is adequate release of the extract for its therapeutic effect when taken orally. The importance of the dissolution characteristics of the adsorbent extract mix is therefore key to the selection for use in the formulation.

The dissolution profile of Asena extract without adsorbent as shown in Figure 3.1.4(Table A-7.1) demonstrates that Asena extract has good dissolution characteristics(101.05 ± 2.20% at 45 minutes). Optimum release means that 75% of the extract is dissolved within 45 minutes. [71] Asena extract is not considered for encapsulation due to difficulty in processing and its hygroscopic nature when dried in large quantities. The dissolution profile of Enterica extract without adsorbent as shown in Figure 3.2.4(Table B-7.1) demonstrates that the amount of Enterica extract released by 45minutes was 74.15 ± 2.28%. There is a need to improve upon its dissolution characteristics.

To determine the appropriate adsorbents required for the formulations of Asena and Enterica, five adsorbents (bentonite, microcrystalline cellulose, maize starch, kaolin and light magnesium carbonate) were initially analysed for ease of processing with extracts. The dissolution characteristics within 45 minutes were also determined. The ease of processing for Asena using 200mg per dose of the five adsorbents is shown in Table A-1.

The release of the extract from the adsorbent extract mix was the most important factor considered for selecting the appropriate adsorbent. The percentage release of formulations of granules for encapsulation and capsules should be at least 75% at

45minutes[71]. The percentage release within 45 minutes of Asena adsorbents mix using five different adsorbents is shown in Figure 3.1.3 and Table A-6. It could be observed from Figure 3.1.3 that the most suitable adsorbents for the formulation of Asena granules for further investigation were bentonite and maize starch. The adsorbents used for further work were Light magnesium carbonate, bentonite and maize starch. Bentonite and maize starch demonstrated good ease of processing. Ease of scrapping and processing of formulations of Asena containing five different adsorbents are shown in Table A-1. Dissolution Data at 45 minutes revealed that light magnesium carbonate did not have good dissolution characteristics (Figure 3.1.3 and Table A-6) Further work was done to ascertain whether a decrease in the amount of adsorbent used could cause a significant increase in percentage release(Figures 3.1.5, 3.1.6, 3.1.7, 3.1.8 and 3.1.9; Tables A-7.2, A-7.3, A-7.4, A-7.5 A-7.6). Variation in concentrations of light magnesium carbonate did not show any linear relationship; the highest release obtained with light magnesium carbonate was 23.68 ± 2.79 % (Table A-7.2) in five minutes.

Figure 3.2.3 and Table B-6 shows the release of Enterica extract from Enterica adsorbent mix using five different adsorbents. Percentage release of Enterica granules with maize starch and light magnesium carbonate at 45minutes were 69.12 ± 2.60% and 76.52 ± 2.17% respectively. This demonstrates that the adsorbents to be considered for further work in the formulation of Enterica should be maize starch and light magnesium carbonate. Table B-1 also shows that these two adsorbents had good processing effects.

The results show that different preparations may require different adsorbents to provide optimum release.

The percentage loss in weight of Asena granules using different adsorbents is shown in Table 3.1.6. Results from the table indicate that the loss in weight of granules was independent of the amount of adsorbent used for granulation. However, granules formulated with light magnesium carbonate as adsorbent had a lower loss. The highest loss in weight was 5.88% when 200mg of light magnesium carbonate per dose was used. Granules of Asena formulated using maize starch also had varied loss in weight with the highest loss of 12.42% when 80mg of maize starch per dose (Table 3.1.6 and Table A-2). Photographs of granules of Asena with light magnesium carbonate, maize starch and bentonite are shown in Appendix C (Figures C-2, C-3 and C-4).

The percentage loss in weight of Enterica granules can be observed from Table 3.2.5. The lowest loss recorded was 2.07% for light magnesium carbonate at 110mg per dose. The highest loss recorded was 7.81% for maize starch at 66mg per dose. Photographs of granules of Enterica with light magnesium carbonate and maize starch are shown in Appendix C (Figures C-7 and C-8).

The percentage loss in weight could be attributed to difficulty in processing. Ease of granule processing should be considered when production is done on a much larger scale.

The size distribution of formulated granules of Asena is recorded in Table 3.1.7.Size distribution of Asena granules using different adsorbents demonstrates that the amount of coarse granules were higher with all the adsorbents. The size distribution of formulated granules did not also depend on the amount being used. An increase in weight of adsorbent per dose did not necessarily cause an increase in percentage fines or coarse produced (Table 3.1.7 and Table A-3). However, in the case of light magnesium

carbonate, an increase in the amount of adsorbent per dose caused a decrease in the percentage of coarse granules produced on processing (Table 3.1.7 and Table A-3). In the formulation of granules for encapsulation, the size distribution of the granules is of much importance. [33]Granules containing more coarse particles may cause a decrease in release. Formation of large particle size granules may be one of the reasons explain why formulation using light magnesium carbonate exhibited poor release with Asena.

In the formulation of Enterica granules using maize starch and light magnesium carbonate, it was observed that the size distribution of granules did not depend on the amount of adsorbent being used (Table 3.2.6). Granules formulated using light magnesium carbonate had more fines. However, granules formulated using maize starch had coarser than fine particles. The better release profiles of granules formulated using light magnesium carbonate could be attributed to the high percentage of small particle size granules.

The flow properties of Asena granules were generally good according to Carr's index, Hausner ratio and Angle of repose (Table 3.1.8). Good flow properties enhance uniform filling of capsules [33]. Hence the uniformity of weight of formulated capsules may indicate good or poor flow properties of the formulated granules for encapsulation. The formulated capsules of Asena passed the uniformity of weight test, Appendix A (Table 3.1.9).

For the formulation of Enterica granules, the flow properties were determined before encapsulation. The granules exhibited good flow properties. Carr's index and Hausner

ratio values are indicated in Appendix B (Table B-4.1 and B-4.2). The Enterica capsules passed the uniformity of weight test, Appendix B (Table 3.2.7).

The dissolution profiles of Asena granules formulated using different quantities of light magnesium carbonate, Bentonite and maize starch, revealed that bentonite and maize starch were suitable for the formulation of granules of Asena for encapsulation (Figures 3.1.4, 3.1.5, 3.1.6, 3.1.7, 3.1.8 and 3.1.9; Tables A-7.2, A-7.3, A-7.4, A-7.5 A-7.6). In the formulation of Asena granules for encapsulation, maize starch was chosen as adsorbent at a weight of 200mg per dose (Figure 3.1.10 and Table A-8.2). Maize starch was chosen over bentonite though they both exhibited similar release characteristics (Figure 3.1.9 and Table A-7.6) due to availability and lower cost. From Table A-8.2, it can be observed that at 45 minutes, the amount of Asena extract released was 79.82 ± 2.36 %. Thus the formulated Asena capsules exhibited desired in vitro dissolution characteristics. Photograph of Asena capsules is shown in Appendix C (Figure C-9).

The dissolution profiles of Enterica granules using light magnesium carbonate and maize starch, revealed that light magnesium carbonate at a weight of 22mg per dose was the best option (Figures 3.2.5, 3.2.6, 3.2.7, 3.2.8 and 3.2.9; Tables B-7.2, B-7.3, B-7.4, B-7.5 and B-7.6.). The percentage release from the 22mg light magnesium carbonate at 45 minutes was 86.08 ± 1.64% (Figure 3.2.5 and table 7.2). Thus 22mg of light magnesium carbonate per dose was used in the formulation of granules of Enterica for encapsulation. The dissolution of Enterica capsules showed a percentage release of 84.51 ± 1.51 % at 45 minutes(Figure 3.2.10 and Table B-8.3).Photograph of Enterica capsules is shown in Appendix C (Figure C-10).

The equations of plots (Tables A-7.1, A-7.2, A-7.3, A-7.4, A-7.5and A-7.6; Tables B-7.1, B-7.2, B-7.3, B-7.4, B-7.5, B-7.6) are polynomials to the fourth order which are equations of best fit curves for the dissolution profiles of Asena and Enterica respectively (Figures 3.1.4, 3.1.5, 3.1.6, 3.1.7, 3.1.8 and 3.1.9; Figures 3.2.4, 3.2.5, 3.2.6, 3.2.7, 3.2.8 and 3.2.9). These equations enable the percentage release of the extracts of Asena and Enterica to be determined at any particular time. X in the equation represents time and Y represents the percentage release.

Both Asena and Enterica capsules also showed good disintegration properties with a time of 8.03 ± 0.13 minutes for Asena (Table 3.1.10) and 4.26 ± 0.34 minutes for Enterica (Table 3.2.8).

The patient diagnosed with acute pain of severity six on the numbered scale reported a decrease of severity to one by the seventh day during the case study involving Asena capsules. The patient with osteoarthritis also reported a decrease in the severity of pain by the seventh day from a numbered scale of seven to three. This indicates that Asena capsules may be effective when used in place of the decoction. However, detailed clinical studies have to be done to ascertain the in vivo bioavailability of the capsules and other pharmacokinetic parameters. This will help to predict the accurate dose size and frequency to ensure optimum therapy.

Administration of one capsule of Enterica three times daily for a period of two weeks during the case study involving Enterica capsules, the abdominal pains, fever, headaches and other symptoms in the patient were absent. The widal test still showed a reading of TO: 1/160 and TH: 1/160. Further clinical studies on Enterica capsules will help ascertain

the pharmacokinetic profile and in vivo antimicrobial effect. This will help to predict the appropriate dosage regime to ensure optimum therapeutic effect is achieved.

4.2 CONCLUSION

The weight of extract per dose of Asena was 400mg (Table 3.1.3). Capsules of Asena were formulated using maize starch as adsorbent at a weight of 200mg per dose(Table A-8.1). Maize starch was also used as diluent. A dose of Asena was filled into two 250mg capsules (Table A-8.1). The capsules passed the dissolution and disintegration test with dissolution (release) at 45minutes being 79.82 ± 2.36% (Table A-8.2)and disintegration time of 8.03 ± 0.13 minutes(Table A-8.4).Asena capsules passed the uniformity of weight test (Table A-8.3). This implies that maize starch may be the adsorbent of choice at a quantity of 200mg per dose.

The weight of extract per dose of Enterica was 190mg (Table 3.2.3). Capsules of Enterica were formulated using light magnesium carbonate as adsorbent at a weight of 22mg per dose (Table-B-8.1). Lactose was used as diluent. A dose was filled into one 250mg capsule shell (Table B-8.1). The percentage release of Enterica capsules in the dissolution experiment at 45minutes was84.51 ± 1.51% (Figure 3.2.10 and Table B-8.2). The disintegration time of the capsules was4.26 ± 0.34 minutes (Table B-8.4). Enterica capsules also passed the uniformity of weight test (Table B-8.3). Light magnesium carbonate may be used as adsorbent at a weight of 22mg per dose in the formulation of Enterica granules for encapsulation.

From the results obtained in the various determinations, it can be concluded that different preparations may require different adsorbents to ensure optimum release of extract. The release of extract from adsorbent does not necessarily depend on the amount of adsorbent used in the formulation.

The patient suffering from acute pain reported severity of six on the numbered scale during the case study. After taking two capsules of Asena three times daily for seven days, the pain had decreased to one on the numbered scale. The patient diagnosed with osteoarthritis reported a decrease in severity of pain from seven to three on the numbered scale by the seventh day using the same dosage of Asena capsules.

After administration of one capsule of Enterica three times daily for a period of two weeks during the case study involving Enterica capsules, the abdominal pains, fever, headaches and other symptoms in the patient were absent. The widal test still showed a reading of TO: 1/160 and TH: 1/160.

4.3 RECOMMENDATION

A recommended quality control method for the determination of the active content of capsules may involve use of dried powdered extracts as standards and capsules as test samples. The UV method of analyses can then be used to ascertain the active content of capsules by recording absorbances at the particular wavelength of absorption.

Other advanced methods used in the formulation of granules such as spray drying and fluidized bed granulation can be tried to ascertain their usefulness in the preparation of capsules of Asena and Enterica. Other methods of analyses such as HPLC can also be tried.

Capsules of Asena may be formulated using the 500mg capsule shell or size since the amount of extract per dose of Asena is 400mg. this will ensure that instead of taking two capsules, one capsule will be taken as a dose.

The stability and pharmacokinetic profiles of capsules of Asena and Enterica should be determined. A detailed clinical study of the effectiveness of both liquid preparations and capsules of Asena and Enterica should be conducted. A more specific method of diagnosis of typhoid fever such as blood culture investigations should be used to enhance specificity when clinical studies are conducted on a larger scale.

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APPENDIX

APPENDIX A: ASENA FORMULATIONS

Table A-1Ease of scrapping and processing of formulations of Asena containing five different adsorbents

Adsorbent (200mg/dose)	Ease of scrapping	Ease of processing
Maize starch	Very easy	Very easy
Light magnesium carbonate	Very easy	Very easy
Kaolin	Very Difficult	Very Difficult
Bentonite	Easy	Easy
Microcrystalline cellulose	Difficult	Difficult

Table A-2Percentage loss in weight of Asena granules using different adsorbents

Absorbent	Weight of adsorbent per dose (mg)	Expected weight of granules (g)	Actual weight of granules (g)	Percentage loss (%)
Light magnesium carbonate	40	44.00	42.54	3.32
	80	48.00	46.30	3.54
	160	56.00	54.56	2.57
	180	58.00	56.98	1.76
	200	60.00	56.47	5.88
Bentonite	40	44.00	40.45	8.07
	80	48.00	43.65	9.06
	160	56.00	55.01	1.61
	180	58.00	54.56	5.93
	200	60.00	54.85	8.58
Maize starch	40	44.00	39.88	9.36
	80	48.00	42.04	12.42
	160	56.00	50.98	8.96
	180	58.00	55.88	3.66
	200	60.00	58.57	2.17

Granules were made using 100 doses of decoction and adsorbents

Table A-3Size distribution of Asena granules using different adsorbents

Adsorbent	Weight of adsorbent per dose (mg)	Total weight of granules (g)	Weight of coarse granules (g)	Weight of fine granules (g)	Percentage of coarse (%)	Percentage of fines (%)
Light magnesium carbonate	40	42.54	30.87	11.67	72.57	27.43
	80	46.30	32.53	13.80	70.26	29.74
	160	54.56	31.58	22.98	57.88	42.12
	180	56.98	31.90	25.08	55.98	44.02
	200	56.47	30.77	25.70	54.49	45.51
Bentonite	40	40.45	24.85	15.60	61.43	38.57
	80	43.65	28.31	15.34	64.86	35.14
	160	55.01	37.14	17.87	67.51	32.49
	180	54.56	35.73	18.83	65.49	34.51
	200	54.85	33.96	20.89	61.91	38.09
Maize starch	40	39.88	25.68	14.20	64.39	35.61
	80	42.04	21.08	20.96	50.14	49.86
	160	50.98	28.08	22.90	55.08	44.92
	180	55.88	30.02	25.86	53.72	46.28
	200	56.28	37.80	18.48	67.16	32.84

Granules were made using 100 doses of decoction and adsorbents

Table A-4.1 Flow properties of Asena granules formulated from different adsorbents using Hausner ratio and Carr's index

Adsorbent	Weight of adsorbent per dose (mg)	Weight of granules (g)	Vo (ml)	Vf (ml)	Do (g/ml)	Df (g/ml)	H.R	C.I (%)
Light magnesium carbonate	40	42.54	63	55	0.6752	0.7735	1.15	12.71
	80	46.30	76	64	0.6092	0.7234	1.19	15.79
	160	54.56	115	94	0.4744	0.5804	1.22	18.26
	180	56.98	118	100	0.4828	0.5698	1.18	15.27
	200	56.47	120	108	0.4706	0.5229	1.11	10.00
Bentonite	40	40.45	54	47	0.7491	0.8606	1.15	12.96
	80	43.65	56	48	0.7795	0.9094	1.17	14.29
	160	55.01	68	60	0.8090	0.9168	1.13	11.76
	180	54.56	62	56	0.8800	0.9743	1.11	9.68
	200	54.85	68	62	0.8066	0.8847	1.10	8.82
Maize starch	40	39.88	55	52	0.7251	0.7669	1.06	5.45
	80	42.04	62	52	0.6781	0.8085	1.19	16.13
	160	50.98	69	61	0.7388	0.8357	1.13	11.59
	180	55.88	80	67	0.6985	0.8340	1.19	16.25
	200	56.28	82	73	0.6863	0.7710	1.12	10.98

Granules were made using 100 doses of decoction and adsorbents

Calculations

Initial volume = V_o ; Final volume = V_f ; Weight of granules = W

$$\text{Initial density}(D_o) = \frac{W}{V_o}$$

$$\text{Final density} = D_f = \frac{W}{V_f}$$

$$\text{Hausner ratio (H. R)} = \frac{D_f}{D_o}$$

$$\text{Carr's index (C. I)} = \left(\frac{D_f - D_o}{D_f}\right) \times 100$$

For 4.00g of light magnesium carbonate as absorbent,

W= 42.54g, V_o = 63ml, V_f = 55ml

$$D_o = \frac{42.54}{63} = 0.6752\text{g/ml}$$

$$D_f = \frac{42.54}{55} = 0.7735\text{g/ml}$$

$$\text{H. R} = \frac{0.7735}{0.6752} = 1.15$$

$$\text{Carr}'\text{s Index} = \left(\frac{0.7735 - 0.6752}{0.7735}\right) \times 100 = 12.71\%$$

Table A-4.2 Flow properties of Asena granules by the fixed height cone method

Adsorbent	Weight of adsorbent per dose (mg)	Base of cone formed (cm)	Height of cone (cm)	Angle of repose (Ɵ)
Light magnesium carbonate	40	7.0	3.5	45
	80	8.0	3.5	41
	160	9.0	4.0	42
	180	8.0	3.0	37
	200	8.5	3.0	35
Bentonite	40	7.5	2.0	28
	80	8.0	3.0	37
	160	7.0	2.0	30
	180	7.0	2.0	30
	200	7.0	1.5	23
Maize starch	40	7.0	2.8	39
	80	8.0	3.0	37
	160	7.0	3.0	41
	180	6.5	3.0	43
	200	6.5	3.0	43

Granules were made using 100 doses of decoction and adsorbents

Calculations

$$\text{Angle of repose } (\theta) = tan^{-1}\left(\frac{H}{R}\right)$$

H = height of cone formed and R= radius of base = $\frac{\text{base length}}{2}$

For 4.00g of light magnesium carbonate;

$$\text{H} = 3.5\text{cm}, R = \left(\frac{7.0}{2}\right) = 3.5$$

$$\theta = tan^{-1}\left(\frac{3.5}{3.5}\right) = 45^{o}$$

Table A-5 Calibration data for solution of Asena extract without Adsorbent

Concentration of solution (%w/v)	**Absorbance**
0.0175	1.105
0.0150	0.959
0.0125	0.806
0.0100	0.689
0.0075	0.504
0.0050	0.349
0.0025	0.183

Table A-6 Dissolution data of granules of Asena formulated using 200mg per dose of five different adsorbents at 45minutes

Adsorbent	**Absorbance**± SD	**% Release** ± SD
Light magnesium carbonate	0.150 ± 0.009	15.79 ± 6.00
Kaolin	0.340 ± 0.008	47.37± 2.35
Bentonite	0.601 ± 0.010	90.79± 1.66
Maize starch	0.594 ± 0.013	86.84± 2.19
Microcrystalline cellulose	0.285 ± 0.005	39.47± 1.75

Selection of adsorbents was done considering both ease of scrapping and processing as well as dissolution at 45 minutes

Table A-7.1 Dissolution data of Asena extract without adsorbent

Time (minutes)	Mean A ± SD	% Release ± SD
5	0.514 ± 0.012	75.00 ± 2.33
10	0.590 ± 0.010	88.51 ± 1.69
15	0.631 ± 0.013	98.33 ± 2.06
30	0.632 ± 0.010	100.43 ± 1.58
45	0.635 ± 0.014	101.05 ± 2.20
60	0.639 ± 0.015	104.64 ± 2.35
Equation of plot : $Y=-2.9e\text{-}5x^4 + 4.6e\text{-}3x^3 - 0.3x^2 + 6.1x + 50$		

Calculation of % Release

Weight per dose of Asena extract = 400mg (0.4g)

Volume of dissolution medium = 900ml

Concentration of solution if all 400mg dissolves= $\left(\frac{0.4 \times 100}{900}\right) = 0.044\%\ w/v$

To make the concentration as near as possible to 0.01%w/v in order to obtain accurate absorbance readings, 5.70ml of the filtrate is made up to 25 ml in a volumetric flask.

Equation of calibration curve is $y = 61.26x + 0.0439$

x = concentration

y = Average absorbance at specific time

At 5minutes for pure Asena extract

y= 0.514

Therefore;

x at 5 minutes = $\left(\frac{0.514 - 0.0439}{61.26}\right) = 0.0077\%\ w/v$

Therefore; 100ml 0f solution = 0.0077g of extract

25ml $= \left(\frac{0.0077 \times 25}{100}\right) = 0.0019g$

But 5.70ml of solution = 0.0019g extract

900ml $= \left(\frac{0.0019 \times 900}{5.70}\right) = 0.30g$

Weight of extract released = 0.3000g

% Release $= \left(\frac{\text{weight of extract released} \times 100}{\text{weight of extract used}}\right)$

Hence % release $= \left(\frac{0.30 \times 100}{0.40}\right) = 75.0\ \%$

At 10 minutes,

Y = 0.590

Therefore;

X at 10 minutes $= \left(\frac{0.590 - 0.0439}{61.26}\right) = 0.0089\%\ w/v$

Therefore; 100ml 0f solution = 0.0089g of extract

25ml $= \left(\frac{0.0089 \times 25}{100}\right) = 0.0022g$

But 5.70ml solution = 0.0022g of extract

Hence; 900ml $= \left(\frac{0.0022 \times 900}{5.70}\right) = 0.3473g$

Weight of extract released = 0.3473g

Weight of extract in 20ml aliquot pipetted at 5 minutes ;

900ml = 0.3029 g

$20ml = \left(\frac{0.3029 \times 20}{900}\right) = 0.0067g$

Hence Total weight of extract released at 10minutes = 0.3473g + 0.0067g

= 0.3540g

$$\% \text{ Release } = \left(\frac{\text{weight of extract released} \times 100}{\text{weight of extract used}}\right)$$

$$\text{Hence \% release } = \left(\frac{0.3540 \times 100}{0.40}\right) = 88.51\ \%$$

The calculations were repeated for other percentages released at various times for different adsorbents

Table A-7.2 Dissolution data of Asena granules using 40mg per dose of different adsorbents

Time / minutes	Light magnesium carbonate		Bentonite		Maize starch	
	Mean A ± SD	**% Release ± SD**	**Mean A ± SD**	**%Release ± SD**	**Mean A ± SD**	**% Release ± SD**
5	0.179 ± 0.005	23.68 ± 2.79	0.477 ±0.002	71.05 ± 0.42	0.398 ± 0.009	55.26 ± 2.26
10	0.181 ± 0.003	24.21 ± 1.66	0.530 ±0.010	80.52 ± 1.89	0.480 ± 0.008	72.28 ± 1.67
15	0.217 ± 0.004	28.68 ± 1.84	0.602 ±0.012	94.12 ± 1.99	0.548 ± 0.009	85.70 ± 1.64
30	0.228 ± 0.004	33.24 ± 1.75	0.615 ±0.010	96.14 ± 1.63	0.582 ± 0.009	91.49 ± 1.55
45	0.259 ± 0.004	37.89 ± 1.54	0.611 ±0.012	98.15 ± 1.96	0.609 ± 0.013	96.49 ± 2.13
60	0.252 ± 0.003	38.68 ± 1.19	0.601±0.015	100.17 ± 2.50	0.598 ± 0.01	99.38 ± 1.67

Mean A represents the mean absorbance of six samples, SD represents the standard deviation

Adsorbent	Equation of plot
Light magnesium carbonate	$Y=-1.3e-4x^4 - 1.2e-2x^3 -0.4x^2 – 3.4x + 33.3$
Bentonite	$Y= 4.0e-4x^4 - 2.7e-2x^3 + 0.7x^2 – 4.4x + 78.9$
Maize starch	$Y= 1.2e-4x^4 - 9.1e-3x^3 + 0.1x^2 + 2.9x + 38.7$

Table A-7.3 Dissolution data of Asena granules using 80mg per dose of different adsorbents

Time / minutes	Light magnesium carbonate		Bentonite		Maize starch	
	Mean A ± SD	**% Release ± SD**	**Mean A ± SD**	**%Release ± SD**	**Mean A ± SD**	**% Release ± SD**
5	0.132 ± 0.005	15.80 ± 3.79	0.445 ±0.005	63.15 ± 1.12	0.380 ± 0.009	55.26 ± 2.37
10	0.181 ± 0.004	24.03 ± 2.21	0.493 ±0.008	72.45 ± 1.62	0.421 ± 0.008	60.44 ± 1.90
15	0.198 ± 0.002	24.56 ± 1.01	0.523 ±0.005	81.93 ± 0.96	0.558 ± 0.005	85.44 ± 0.90
30	0.219 ± 0.003	29.03 ± 1.37	0.556 ±0.005	87.63 ± 0.90	0.607 ± 0.008	95.17 ± 1.32
45	0.227 ± 0.005	29.65 ± 2.20	0.560 ±0.004	88.33 ± 0.71	0.618 ± 0.009	97.19 ± 1.46
60	0.242 ± 0.002	34.21 ± 0.83	0.597 ±0.009	99.20 ± 1.51	0.604 ± 0.012	99.20 ± 1.99

Mean A represents the mean absorbance of six samples, SD represents the standard deviation

Adsorbent	Equation of plot
Light magnesium carbonate	$Y= -8.6e-6x^4 + 1.5e-3x^3 - 9.1e-2x^2 + 2.3x + 6.7$
Bentonite	$Y= - 1.8e-6x^4 - 1.1e-3x^3 - 0.1x^2 + 3.4x + 47.3$
Maize starch	$Y= 1.2e-5x^4 - 6.6e-4x^3 - 4.5e-2x^2 + 3.7x + 35.7$

Table A-7.4 Dissolution data of Asena granules using 160mg per dose of different adsorbents

Time / minutes	Light magnesium carbonate		Bentonite		Maize starch	
	Mean A ± SD	**% Release ± SD**	**Mean A ± SD**	**%Release ± SD**	**Mean A ± SD**	**% Release ± SD**
5	0.147 ±0.005	15.79 ± 3.40	0.302 ±0.005	43.42 ± 1.66	0.396 ±0.006	55.26 ± 1.52
10	0.186 ±0.002	24.03 ± 1.08	0.421 ±0.006	60.17 ± 1.43	0.426 ±0.005	64.38 ± 1.17
15	0.213 ±0.005	28.51 ± 2.35	0.511 ±0.012	77.28 ± 2.35	0.484 ±0.009	73.68 ± 1.86
30	0.237 ±0.005	33.07 ± 2.11	0.581 ±0.013	90.79 ± 2.24	0.531± 0.012	83.15 ± 2.26
45	0.236 ±0.005	33.59 ± 2.12	0.604 ±0.014	96.31 ± 2.32	0.570 ±0.009	87.98 ± 1.58
60	0.267 ±0.006	38.42 ± 2.25	0.600 ±0.013	98.68 ± 2.17	0.597 ±0.013	98.59 ± 2.2

Mean A represents the mean absorbance of six samples, SD represents the standard deviation

Adsorbent	**Equation of plot**
Light magnesium carbonate	$Y= - 7.3e-6x^4 + 1.5e-3x^3 - 0.1x^2 + 2.9x + 3.9$
Bentonite	$Y= - 2.0e-5x^4 + 3.5e-3x^3 - 0.2x^2 + 6.8x + 14.2$
Maize starch	$Y= - 2.4e-6x^4 + 9.7e-4x^3 - 8.7e-2x^2 + 3.2x + 40.9$

Table A-7.5 Dissolution data of Asena granules using 180mg per dose of different adsorbents

Time / minutes	Light magnesium carbonate		Bentonite		Maize starch	
	Mean A ± SD	**% Release ± SD**	**Mean A ± SD**	**%Release ± SD**	**Mean A ± SD**	**% Release ± SD**
5	0.149 ±0.003	15.79 ± 2.01	0.321 ±0.005	43.42 ± 1.56	0.307 ±0.009	43.42 ± 2.93
10	0.198 ±0.002	24.03 ± 1.01	0.453 ±0.006	68.07 ± 1.32	0.398 ±0.009	56.23 ± 2.26
15	0.209 ±0.005	28.51 ± 2.39	0.502 ±0.005	77.45 ± 1.00	0.442 ±0.010	65.35 ± 2.26
30	0.219 ±0.002	29.12 ± 0.91	0.520 ±0.010	79.12 ± 1.92	0.497 ±0.012	74.65 ± 2.41
45	0.243 ±0.002	33.42 ± 0.82	0.532 ±0.009	83.94 ± 1.69	0.550 ±0.010	87.45 ± 1.82
60	0.255 ±0.002	38.33 ± 0.78	0.542 ±0.008	86.48 ± 1.48	0.602 ±0.010	97.80 ± 1.66

Mean A represents the mean absorbance of six samples, SD represents the standard deviation

Adsorbent	**Equation of plot**
Light magnesium carbonate	$Y= - 2.7e\text{-}5x^4 + 3.9e\text{-}3x^3 - 0.2x^2 + 4.0x - 6.3e\text{-}3$
Bentonite	$Y= - 7.2e\text{-}5x^4 + 1.0e\text{-}2x^3 - 0.5x^2 + 11.0x + 0.8$
Maize starch	$Y= - 3.4e\text{-}5x^4 + 4.8e\text{-}3x^3 - 0.2x^2 + 5.5x + 20.9$

Table A-7.6 Dissolution data of Asena granules using 200mg per dose of different adsorbents

Time / minutes	Light magnesium carbonate		Bentonite		Maize starch	
	Mean A ± SD	**% Release ± SD**	**Mean A ± SD**	**%Release ± SD**	**Mean A ± SD**	**% Release ± SD**
5	0.095 ± 0.002	7.89 ± 2.11	0.370 ±0.005	51.32 ± 1.35	0.313 ±0.009	43.42 ± 2.88
10	0.135 ± 0.004	15.96 ± 2.96	0.456 ±0.006	68.24 ± 1.32	0.422 ±0.006	60.17 ± 1.42
15	0.116 ± 0.005	12.37 ± 4.31	0.511 ±0.005	77.63 ± 0.98	0.473 ±0.007	73.33 ± 1.48
30	0.154 ± 0.004	16.58 ± 2.60	0.526 ±0.012	83.24 ± 2.28	0.521 ±0.008	78.86 ± 1.54
45	0.147 ± 0.005	16.75 ± 3.40	0.530 ±0.015	84.12 ± 2.83	0.579 ±0.010	91.75 ± 1.73
60	0.151 ± 0.009	17.28 ± 5.96	0.551 ±0.017	90.69 ± 3.09	0.602 ±0.012	98.24 ± 1.99

Mean A represents the mean absorbance of six samples, SD represents the standard deviation

Adsorbent	**Equation of plot**
Light magnesium carbonate	$Y= - 1.2e\text{-}5x^4 + 1.7e\text{-}3x^3 - 8.6e\text{-}2x^2 + 1.8x + 1.6$
Bentonite	$Y= - 2.7e\text{-}5x^4 + 4.6e\text{-}3x^3 - 0.3x^2 + 6.7x + 24.2$
Maize starch	$Y= - 5.7e\text{-}5x^4 + 8.1e\text{-}3x^3 - 0.4x^2 + 8.5x + 9.5$

Table A-8.1 Formula for preparation of Asena capsules

Ingredient	Weight per capsule/mg	(× 500) Scaled quantities (g)
Asena extract	200	100
Maize starch	196	98.00
Talc (1%w/w)	4	2.00

Calculations

A 250mg capsule was used and from initial experimentation a maximum weight of 400mg of Asena granules filled each capsule. This implies that a dose of Asena (400mg) will be filled into two capsules each containing 200mg of Asena extract. Maize starch was used as both adsorbent (100mg per capsule) and diluent (96mg per capsule).

Weight of extract per capsule = 200mg

Talc = 1% of 200mg = 2mg

Weight of maize starch used = 400mg – (200 + 4)

= 196mg

Table A-8.2 Dissolution profile of Asena capsules with maize starch used as adsorbent and diluent

Time (minutes)	Mean Absorbance ± SD	% Release ± SD
5	0. 390 ± 0.005	55.26± 1.28
10	0.412 ± 0.003	60.44± 0.73
15	0.461 ± 0.006	69.65± 1.30
30	0.511 ± 0.010	79.03± 1.96
45	0.508 ± 0.012	79.82± 2.36
60	0.591 ± 0.010	94.20± 1.69
Equation of plot	$Y= 2.2e\text{-}5x^4 - 2.0e\text{-}3x^3 + 0.04x^2 + 1.2x + 48.3$	

Table A-8.3 Disintegration test of Asena capsules

Test	**1**	**2**	**3**
Time (minutes)	8.04	8.15	7.90

Mean time = 8.03

Standard deviation = 0.1253

Disintegration time = 8.03± 0.13

Table A- 8.4 Uniformity of weight of Asena capsules

Capsule	**Weight (g) = y**	**Deviation (ȳ - y)**	**% Deviation**
1	0.411	-0.0072	-1.79
2	0.4108	-0.0070	-1.74
3	0.4009	0.0029	0.71
4	0.400	0.0038	0.94
5	0.400	0.0038	0.94
6	0.4020	0.0018	0.44
7	0.4100	-0.0062	-1.54
8	0.4000	0.0038	0.94
9	0.4000	0.0038	0.94
10	0.4001	0.0037	0.91
11	0.4012	0.0026	0.64
12	0.4000	0.0038	0.94
13	0.4010	0.0028	0.69
14	0.4005	0.0033	0.81
15	0.4010	0.0028	0.69
16	0.4160	-0.0122	-3.03
17	0.4000	0.0038	0.94
18	0.3990	0.0048	1.18
19	0.4015	0.0023	0.57
20	0.4207	-0.0169	-4.19

Key

Net mass of capsule contents	**Deviation %**	**Number of capsules**
300 mg and over	± 7.5	minimum 18
	± 15.0	maximum 2

Calculation

Weight of 20 capsules = 8.0757g

Mean weight ($\tilde{y}$) = 8.0757/20 = 0.4038g

APPENDIX B: ENTERICA FORMULATIONS

Table B-1Ease of scrapping and processing of five different adsorbents

Adsorbent (110mg/dose)	Ease of scrapping	Ease of processing
Maize starch	Very easy	Very easy
Light magnesium carbonate	Very easy	Very easy
Kaolin	Very Difficult	Very Difficult
Bentonite	Easy	Easy
Microcrystalline cellulose	Easy	Easy

NB: maize starch and light magnesium carbonate (ease of processing- VERY EASY) were selected for further work.

Table B-2 Percentage loss in weight of Enterica granules using light magnesium carbonate and maize starch as adsorbents

Adsorbent	Weight of adsorbent per dose (mg)	Expected weight of granules (g)	Actual weight of granules (g)	Percentage loss (%)
Light magnesium carbonate	22	21.20	20.56	3.02
	44	23.40	21.69	7.31
	66	25.60	23.96	6.41
	88	27.80	26.40	5.04
	110	30.00	29.38	2.07
Maize starch	22	21.20	20.14	5.00
	44	23.40	22.03	5.85
	66	25.60	23.60	7.81
	88	27.80	26.44	4.89
	110	30.00	29.18	2.73

All the calculations were made for a hundred doses of the product.

$$\%\text{loss in weight} = \left(\frac{\text{expected weight} - \text{actual weight}}{\text{expected weight}}\right) \times 100$$

Table B-3 Size distribution of Enterica granules using light magnesium carbonate and maize starch as adsorbents

Adsorbent	weight of adsorbent per dose (mg)	Total weight of granules (g)	Weight of coarse granules (g)	Weight of fine granules (g)	Percentage of coarse (%)	Percentage of fines (%)
Light magnesium carbonate	22	20.56	9.20	11.36	44.75	55.25
	44	21.69	10.18	11.51	46.93	53.07
	66	23.96	10.30	13.66	42.99	57.01
	88	26.40	11.50	14.9	43.56	56.44
	110	29.38	13.26	16.12	45.13	54.87
Maize starch	22	20.14	11.21	8.93	55.66	44.34
	44	22.03	11.90	10.13	54.02	45.98
	66	23.60	14.78	8.82	62.63	37.37
	88	26.44	14.95	11.49	56.54	43.46
	110	29.18	16.42	12.76	56.27	43.73

Table B-4.1 Flow properites of Enterica granules using Angle of repose of formulated granules

Granules	Base of cone formed	Height of cone	Angle of repose (Ɵ)
Granules formulated for encapsulation with 22mg per dose of light magnesium carbonate	8.50	3.00	35

Table B-4.2 Flow properites of Enterica granules using Carr's index and Hausner's ratio

Granules	Weight	V_o	V_f	D_o	D_f	Hausner's ratio	Carr's index
Enterica granules formulated for encapsulation	50.00	105	85	0.4762	0.5882	1.24	19.04

Table B-5 Calibration data for Enterica extract without Adsorbent

Concentration of solution (%w/v)	Absorbance
0.0250	0.855
0.0225	0.777
0.0200	0.660
0.0175	0.616
0.0150	0.508
0.0125	0.430
0.0100	0.339
0.0075	0.267
0.00625	0.215

Table B-6 Dissolution data of granules of Enterica using 110mg per dose of five different adsorbents

Adsorbent	**Absorbance** ± SD	**% Release** ± SD at 45 minutes
Light magnesium carbonate	0.553 ± 0.012	76.52 ± 2.17
Kaolin	0.210 ± 0.005	28.61 ± 2.38
Bentonite	0.215 ± 0.005	29.31 ± 1.96
Maize starch	0.500 ± 0.013	69.12 ± 2.60
Microcrystalline cellulose	0.255 ± 0.005	34.89 ± 2.33

Selection of adsorbents was done considering both ease of scrapping and processing as well as dissolution at 45 minutes

Table B-7.1 Dissolution data of Enterica extract without adsorbent

Time (minutes)	Mean A ± SD	% Release ± SD
5	0.309 ± 0.008	42.44 ± 2.59
10	0.463 ± 0.010	64.19 ± 2.16
15	0.518 ± 0.012	72.23 ± 2.32
30	0.522 ± 0.015	73.19 ± 2.87
45	0.526 ± 0.012	74.15 ± 2.28
60	0.530 ± 0.014	75.12 ± 2.64
$Y=-5.75e\text{-}5x^4 + 8.54e\text{-}3x^3 - 0.44x^2 + 9.4x + 5.76$		

Mean A represents the mean absorbance of six samples, SD represents the standard deviation

Calculation of % Release

Weight per dose of Enterica extract = 190mg (0.19g)

Volume of dissolution medium = 900ml

Concentration of solution if all 190mg dissolves $= \left(\frac{0.19 \times 100}{900}\right) = 0.0211\ \%\ w/v$

Equation of calibration curve is y = 33.91x + 0.0052

x = concentration

y = Average absorbance at a specific time

At 5minutes for pure Enterica extract

y = 0.309

Therefore;

x at 5 minutes $= \left(\frac{0.309-0.0052}{33.91}\right) = 0.00895\ \%\ w/v$

Therefore; 100ml of solution = 0.009g of extract

$$900ml = \left(\frac{0.00895\ \times\ 900}{100}\right) = 0.0806\ g$$

Weight of extract released = 0.0806g

% Release $= \left(\frac{\text{weight of extract released} \times 100}{\text{weight of extract used}}\right)$

Hence % release = $\left(\frac{0.0806 \times 100}{0.190}\right) = 42.43\%$

At 10 minutes,

Y = 0.463

Therefore;

X at 10 minutes = $\left(\frac{0.463\ -0.0052}{33.91}\right) = 0.0135\%\text{w/v}$

Therefore; 100ml of solution = 0.013g of extract

Hence; $900\ ml = \left(\frac{0.0135 \times 900}{100}\right) = 0.1215g$

Weight of extract released = 0.1215g

Weight of extract in 5ml aliquot pipetted at 5 minutes ;

900ml = 0.081g

$$5\,ml = \left(\frac{0.0806\ \times\ 5}{900}\right) = 0.0005g$$

Hence Total weight of extract released at 10minutes = 0.1215g + 0.0005g

= 0.1220g

Hence % release = $\left(\frac{0.122 \times 100}{0.190}\right) = 64.19\%$

The calculations were repeated for other percentages released at various times for different adsorbents

Table B-7.2 Dissolution data of Enterica granules using 22mg per dose of different adsorbents

Time / minutes	**Light magnesium carbonate**		**Maize starch**	
	Mean A ± SD	**% Release ± SD**	**Mean A ± SD**	**% Release ± SD**
5	0.440 ± 0.010	60.74 ± 2.27	0.220 ± 0.004	30.01 ± 1.82
10	0.590 ± 0.009	82.03 ± 1.53	0.380 ± 0.002	52.52 ± 2.37
15	0.600 ± 0.010	83.88 ± 1.67	0.379 ± 0.004	52.68 ± 1.06
30	0.610 ± 0.012	85.75 ± 1.97	0.462 ± 0.005	64.56 ± 1.08
45	0.609 ± 0.010	86.08 ± 1.64	0.500 ± 0.010	70.23 ± 2.00
60	0.612 ± 0.013	86.97 ± 2.12	0.509 ± 0.009	71.87 ± 0.39
Adsorbent		**Equation of plot**		
Light magnesium carbonate		$Y=-5.3e\text{-}5x^4 + 7.8e\text{-}3x^3 - 0.4x^2 + 8.1x + 30$		
Maize starch		$Y=-4.4e\text{-}5x^4 + 6.3e\text{-}3x^3 - 0.3x^2 + 7.0x + 3.5$		

Mean A represents the mean absorbance of six samples, SD represents the standard deviation

Table B-7.3 Dissolution data of Enterica granules using 44mg per dose of different adsorbents

Time / minutes	Light magnesium carbonate		Maize starch	
	Mean A ± SD	% Release ± SD	Mean A ± SD	% Release ± SD
5	0.227 ± 0.002	30.98 ± 0.88	0.186 ± 0.002	25.26 ± 1.08
10	0.430 ± 0.008	59.51 ± 1.86	0.292 ± 0.004	40.20 ± 1.37
15	0.512 ± 0.012	71.30 ± 2.34	0.349 ± 0.008	48.39 ± 2.29
30	0.522 ± 0.010	73.09 ± 1.92	0.392 ± 0.006	54.67 ± 1.53
45	0.552 ± 0.012	77.69 ± 2.17	0.487 ± 0.009	68.24 ± 1.85
60	0.582 ± 0.013	82.31 ± 2.23	0.492 ± 0.008	69.31 ± 1.63
Adsorbent		**Equation of plot**		
Light magnesium carbonate		$Y=-7.9e\text{-}5x^4 + 1.2e\text{-}2x^3 - 0.6x^2 + 12.7x - 18.6$		
Maize starch		$Y=-5.8e\text{-}5x^4 + 7.6e\text{-}3x^3 - 0.4x^2 + 7.0x - 2.7$		

Mean A represents the mean absorbance of six samples, SD represents the standard deviation

Table B-7.4 Dissolution data of Enterica granules using 66mg of different adsorbents

Time / minutes	Light magnesium carbonate		Maize starch	
	Mean A ± SD	% Release ± SD	Mean A ± SD	% Release ± SD
5	0.365 ± 0.008	50.26 ± 2.19	0.333 ± 0.003	45.79 ± 0.90
10	0.472 ± 0.010	65.49 ± 2.12	0.378 ± 0.005	52.33 ±1.32
15	0.538 ± 0.015	75.07 ± 2.79	0.396 ± 0.008	55.14 ± 2.02
30	0.546 ± 0.012	76.61 ± 2.20	0.474 ± 0.012	66.34 ± 2.53
45	0.576 ± 0.010	81.22 ± 1.74	0.505 ± 0.018	71.04 ± 3.56
60	0.601 ± 0.014	85.16 ± 2.33	0.550 ± 0.010	77.71 ± 1.82
Adsorbent		**Equation of line**		
Light magnesium carbonate		$Y=-4.5e\text{-}5x^4 + 6.6e\text{-}3x^3 - 0.4x^2 + 7.3x + 21.1$		
Maize starch		$Y=-5.1e\text{-}6x^4 + 4.4e\text{-}4x^3 - 6.4e\text{-}6x^2 + 1.1x + 40.6$		

Mean A represents the mean absorbance of six samples, SD represents the standard deviation

Table B- 7.5 Dissolution data of Enterica granules using 88mg per dose of different adsorbents

Time / minutes	Light magnesium carbonate		Maize starch	
	Mean A ± SD	% Release ± SD	Mean A ± SD	% Release ± SD
5	0.336 ± 0.009	46.21 ± 2.68	0.237 ± 0.005	32.38 ± 2.11
10	0.396 ± 0.006	54.85 ± 1.52	0.386 ± 0.006	53.37 ± 1.55
15	0.405 ± 0.012	56.41 ± 2.96	0.393 ± 0.004	54.65 ± 1.02
30	0.428 ± 0.010	59.94 ± 2.34	0.430 ± 0.009	60.12 ± 2.09
45	0.453 ± 0.009	63.76 ± 1.99	0.481 ± 0.006	67.58 ± 1.25
60	0.464 ± 0.012	65.65 ± 2.59	0.511 ± 0.015	72.14 ± 2.94
Adsorbent		**Equation of plot**		
Light magnesium carbonate		$Y=-2.3e\text{-}5x^4 + 3.2e\text{-}3x^3 - 0.2x^2 + 3.2x + 33.4$		
Maize starch		$Y=-5.9e\text{-}5x^4 + 8.2e\text{-}3x^3 - 0.4x^2 + 7.9x + 2.6$		

Mean A represents the mean absorbance of six samples, SD represents the standard deviation

Table B-7.6 Dissolution data of Enterica granules using 110mg per dose of different adsorbents

Time / minutes	Light magnesium carbonate		Maize starch	
	Mean A ± SD	% Release ± SD	Mean A ± SD	% Release ± SD
5	0.319 ± 0.006	43.83 ± 1.88	0.190 ± 0.002	25.81 ± 1.05
10	0.417 ± 0.009	57.77 ± 2.16	0.323 ± 0.005	44.54 ± 1.55
15	0.440 ± 0.009	61.30 ± 2.05	0.385 ± 0.009	53.45 ± 2.34
30	0.460 ± 0.010	64.44 ± 2.17	0.450 ± 0.012	62.82 ± 2.67
45	0.504 ± 0.010	70.94 ± 1.98	0.502 ± 0.015	70.44 ± 2.99
60	0.509 ± 0.015	72.03 ± 2.95	0.543 ± 0.010	76.55 ± 1.84
Adsorbent		**Equation of plot**		
Light magnesium carbonate		$Y=-4.5e\text{-}5x^4 + 6.3e\text{-}3x^3 - 0.3x^2 + 6x + 21.1$		
Maize starch		$Y=-4.0e\text{-}5x^4 + 6.0e\text{-}3x^3 - 0.3x^2 + 7.3x - 3.6$		

Mean A represents the mean absorbance of six samples, SD represents the standard deviation

Table B-8.1 Formula for preparation of Enterica capsules

Ingredient	Weight per dose/mg	(× 500) Scaled quantities (g)
Enterica extract	190	95.00
Light magnesium carbonate	22	11.00
Talc (1%w/w)	3.5	1.75
Lactose	134.5	67.25

Calculations

A 250mg capsule was used and from initial experimentation of capsule fill a maximum weight of 350mg of Enterica granules filled each capsule;

Weight of extract = 190mg

Talc = 1% of 350mg = 3.5mg

Weight of lactose to be used = 350mg – (190 +22+ 3.5) mg

=134.5 mg

Table B-8.2 Uniformity of weight of Enterica capsules

Capsule	Weight (g) = y	Deviation (ỹ - y)	% Deviation
1	0.3350	0.0170	4.21
2	0.3500	0.0020	0.49
3	0.3502	0.0018	0.44
4	0.3500	0.0020	0.49
5	0.3408	0.0112	2.77
6	0.3511	0.0009	0.22
7	0.3498	0.0022	0.54
8	0.3500	0.0020	0.49
9	0.3490	0.0030	0.74
10	0.3601	-0.0081	-2.01
11	0.3412	0.0108	2.67
12	0.3500	0.0020	0.49
13	0.3511	0.0009	0.22
14	0.3855	-0.0335	-8.30
15	0.3490	0.0030	0.74
16	0.3600	-0.0080	-1.99
17	0.3511	0.0009	0.22
18	0.3600	-0.0080	-1.99
19	0.3550	-0.0030	-0.75
20	0.3507	0.0013	0.32

Key

Net mass of capsule contents	Deviation %	Number of capsules
300 mg and over	± 7.5	minimum 18
	± 15.0	maximum 2

Calculation

Weight of 20 capsules = 7.0396g

Mean weight (ỹ) = 7.0396/20 = 0.3520g

Table B-8.3 Dissolution profile of Enterica capsules

Time (minutes)	Mean Absorbance ± SD	% Release ± SD
5	0.421 ± 0.006	58.08± 1.43
10	0.585 ± 0.008	81.32± 1.37
15	0.590 ± 0.012	82.47± 2.03
30	0.599 ± 0.010	84.18± 1.67
45	0.598 ± 0.009	84.51± 1.51
60	0.601 ± 0.013	85.39± 2.06
Equation of plot	$Y=-5.9e-5x^4 + 8.6e-3x^3 -0.4x^2 + 8.8x + 24.9$	

Table B-8.4 Disintegration test of Enterica capsules

Test	1	2	3
Time (minutes)	4.14	4.65	4.00

Mean time = 4.26

Standard deviation = 0.34

Disintegration time = 4.26 ± 0.34

APPENDIX C: PHOTOGRAPHS

Figure C-1 Native Extract of Asena

Figure C-2Asena Granules with Light Magnesium carbonate

Figure C-3 Asena Granules with Bentonite

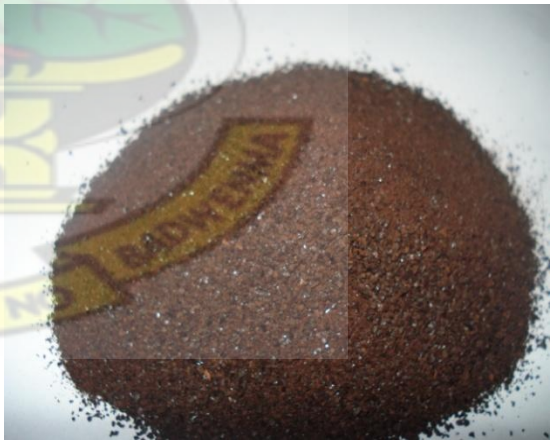

Figure C-4 Asena Granules with Maize Starch

Figure C-5 Native extract of Enterica

Figure C-6 concentrated miscella of Enterica

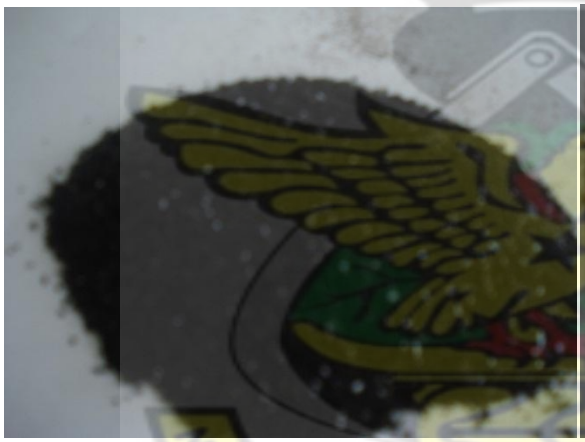

Figure C-7 Granules of Enterica with maize Starch

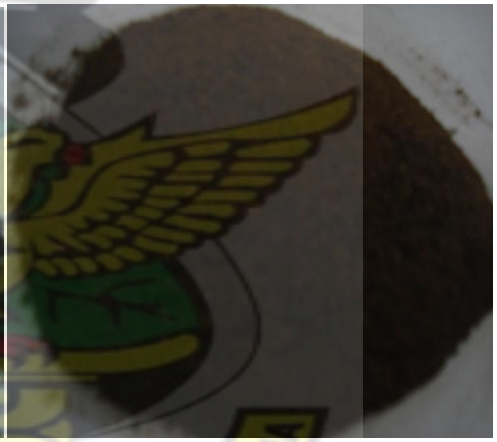

Figure C-8 Enterica granules with light magnesium carbonate

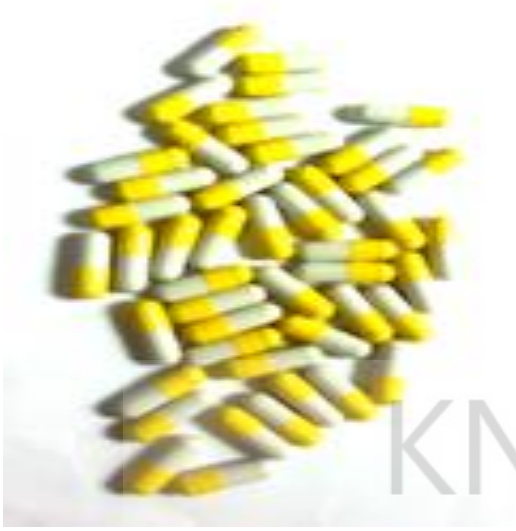

Figure C-9: Asena capsules

Figure C-10: Enterica capsules

Figure C-11: Package for capsules