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DEVELOPMENT AND IN VITRO EVALUATION OF ORAL DISSOLVABLE FILMS OF DICLOFENAC SODIUM USING ALBIZIA AND KHAYA GUMS AS FILM FORMING POLYMERS

By

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DECLARATION

"I, Martina Aduenimaa Bonsu, declare that I have fully undertaken the study reported herein under the supervision of Prof. K. Ofori-Kwakye and that except portions where references have been duly cited, this dissertation is the result of my research".

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DEDICATION

This work is dedicated to Mr. Hubert Osei-Wusuansa, his wife, Mrs. Catherine OseiWusuansa and their lovely daughter, Maria Osei-Wusuansa.

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O my God, what shall I render
To thy name still the same
Gracious God to thee I raise
My song of praise
Great is thy faithfulness
Great is your love for me
I will always sing thy praise
Great is thy faithfulness

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ABSTRACT

An oral dissolvable film (ODF) is a novel drug delivery system that offers a fast and accurate dosing without the need for water or any measuring device. Also, dysphagic, schizophrenic and dementia patients can use ODFs with little or no difficulty. The study sought to develop and evaluate ODFs of diclofenac sodium using albizia and khaya gums as film forming polymers. Authenticated crude albizia and khaya gums were taken through a purification process and both the crude and purified gums were evaluated for their physicochemical properties. Purified albizia and khaya gums were used to formulate seven different batches of ODFs of diclofenac sodium (~ 50 mg/4 cm^2 film) using varying proportions of the gums and HPMC as a reference film forming polymer. The possible drug-excipient interaction of the formulated films was determined using FTIR spectroscopy. In vitro evaluation tests, namely: uniformity of weight, disintegration, assay, tensile strength, percentage elongation, elastic modulus and folding endurance were used to assess the physicochemical and mechanical properties of the formulated ODFs. In vitro dissolution testing was conducted in phosphate buffer pH 6.8 and the dissolution data was analysed using dissolution efficiency, difference (f1) and similarity (f2) factors, kinetic models and ANOVA-based methods. The yields of albizia and khaya gums were 39.38 % and 67.50 % respectively. The gums were free from objectionable microorganisms and had no intrinsic antimicrobial activity against nine test microorganisms. Elemental analysis showed the absence of toxic heavy chemicals like arsenic, lead, cyanide and mercury, suggesting the safety of the gums as pharmaceutical excipients. All the batches passed the assay and dissolution tests for immediate release dosage forms. All the films, except F7, showed over 80 % drug release within 14 min. Dissolution of diclofenac sodium in the film batches followed the Higuchi kinetic model with batch F1 (reference batch) having the highest dissolution efficiency. The dissolution profiles of formulations

F2, F3, F4, F5 and F6 were similar to F1 ($p>0.05$; $f1 <15$ and $f2 \geq 50$) while F7 differed markedly from F1 ($p<0.001$; $f1 >15$ and $f2 <50$).

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CHAPTER 1

INTRODUCTION

1.1 General Introduction

In the last decades, innovative research and development has pointed the way to the discovery of many novel drug delivery systems in a bid to improve upon lapses of conventional dosage forms like tablets, syrups, suspensions among others. Concerns of drug efficacy, safety and patient compliance have been major factors that hinder successful therapeutic outcomes. More so, the complex nature of the drug development process, right from the identification of potential drug targets, through preclinical testing and clinical trials to preformulation testing is cost intensive and very time consuming. Thus, attention has shifted to developing and designing cutting-edge drug delivery systems for already existing drugs. In all these, the specific target groups that come up are paediatrics and geriatrics (ElSetouchy and Abd El-Malak, 2010).

Over the years, the oral route of drug administration has been the most accepted as a result of the simplicity of administration, non-invasiveness, acceptability and patient compliance. As a result, continuous research is being carried out to develop technologies for those target groups who find it difficult using this route. These technologies focus on eliminating the disadvantages that exist with conventional oral dosage forms. Bioadhesive mucosal dosage forms including ODFs are scores of technological advancements (Irfan et al., 2015).

ODFs are one of the novelties that has enjoyed massive acceptance and patronage from patients because it offers a rapid and definite dose in a safe and effective presentation that is acceptable, portable and does not require the use of water or any measuring device (Arya et al., 2010; Frey, 2006). Also, dysphagic, schizophrenic and dementia patients can use ODFs, with little or no difficulty.

ODFs come as flexible solid dosage forms, the dimensions of a postage stamp that rapidly break up or dissolve on a patient‟s tongue (upon placing in the mouth) releasing the active ingredient in the mouth for oromucosal absorption (Barnhart and Vondrak, 2008).

The application of these films began in the confectionery industry where they were mostly used as breath freshening products containing menthol and other fruity flavours. Johnson & Johnson (New Brunswick, NJ), Wrigley (Chicago) and Boots (Nottingham, UK) have breath freshening films available on the market. Also, Zengen (Woodland Hills, CA) manufactures chloraseptic strips that contains a local anaesthetic called benzocaine, for the treatment of sore throat.

ODFs may be composed of hydrophilic film formers like hydroxypropylmethyl cellulose (HPMC), hydroxypropyl cellulose (HPC), carboxymethyl cellulose (CMC), pullulan, eudragit, polyvinyl acetate (PVA) as well as natural polymers like pectin, starch, acacia, xanthan, albizia, khaya, etc. Other ingredients that may be included in the formulation of ODFs include plasticizers, flavouring agents, sweetening agents, colouring agents, surfactants, saliva-stimulating agents among others (Mishra and Amin, 2009).

Most drugs can be conveniently formulated as ODFs; these include NSAIDs, antihistamines, antiulcer, nicotine replacement, antipsychotics, antialzheimers, antiepileptics, drugs for sleeping disorders etc.

In the manufacturing process, the following methods are used; solvent casting, semisolid casting, hot-melt extrusion (HME), solid-dispersion extrusion, spray technique and rolling methods (Mishra and Amin, 2007).

The adoption of polymeric films for delivery of medication into the buccal cavity has great prospects because the lining of the oral cavity is richly supplied with blood vessels thus medicaments can be taken in directly into systemic circulation evading first-pass hepatic

metabolism. With this, the daily doses can be scaled down, eventually reducing the doserelated side effects of some drugs.

1.2 Justification

The quest for alternative excipients that are acquired from renewable sources has stepped up considerably in modern years. And if a country can find a way of locally producing most of the basic natural excipients that support its local pharmaceutical industries rather than importing, it will yield tremendous results.

For instance, synthetic hydrocolloids like HPMC, eudragit, pullulan, etc. are very expensive to import thus they increase the cost of production of the medicines they are used in. Patients who cannot afford these may resort to cheaper ones regardless of their quality. Therefore, the need for cheaper alternatives that possess the same or even better properties cannot be over emphasized.

In addition to the above, certain incompatibilities have been documented to occur between some synthetic polymers and certain active pharmaceutical ingredients (APIs). These incompatibilities, both physical and chemical affect the stability and efficacy of the APIs. For instance, the acidic nature of eudragit polymers have been shown to chemically interact with NSAIDs like diflunisal, flurbiprofen, ibuprofen and piroxicam. Again some interaction with ranitidine, an antiulcer medicine has been documented (Bharate et al., 2010). Also, Avicel® PH-101 (microcrystalline cellulose, MCC) has shown interactions with enalapril (antihypertensive), isosorbide mononitrate (antiangina) and clenbuterol (bronchodilator). Cellulose acetate is also known to interact with isosorbide mononitrate. Lastly, hydroxypropyl methylcellulose acetate succinate (HPMCAS) and hydroxypropyl cellulose show interactions with dyphilline (bronchodilator) and trichlormethiazide (diuretic) respectively (Bharate et al., 2010).

What this means is that, all the above mentioned APIs, though they fall within the class of medicines that are good candidates for ODFs, may not be formulated as such because of their known incompatibilities with the commonly used film formers employed in ODFs.

More so, natural polymers find vast application in the pharmaceutical manufacturing sector, where they are used as thickening agents, emulsifying agents, binding agents, disintegrating agents, gelling agents, sustaining agents in matrix tablets, film forming agents, humidifying and stabilizing agents. Examples of dosage forms that include these polymers are emulsions, suspensions, jellies, tablets, pastes, ODFs etc. (Aulton, 1990).

For the above reasons, investigating the suitability of albizia and khaya gums as possible film formers in ODFs is essential. This is because, if they show good outcomes, they may be used to formulate APIs like ibuprofen, piroxicam, enalapril, isosorbide mononitrate, ranitidine, etc. as ODFs. Also, because they are readily available locally in large quantities, their continuous supply is assured. Again, the fact that they are cheaper than their synthetic congeners means that, cost of production and subsequently the cost of medicines can be cut down.

The characterisation and evaluation of albizia and khaya gums as possible film-forming polymers in ODFs will add to the current literature on both gums and encourage their commercial cultivation and use in the pharmaceutical industries.

1.3 Aim of study

The aim of the study was to develop and evaluate oral dissolvable films of diclofenac sodium using albizia and khaya gums as film forming polymers.

1.4 Specific objectives

- To extract and purify crude albizia and khaya gum exudates
- To evaluate the physicochemical properties of albizia and khaya gums

- To determine the swelling index and water retention capacity of albizia and khaya gums
- To determine the true densities of albizia and khaya gums
- To determine the flow properties of albizia and khaya mucilages
- To determine the microbial quality and antimicrobial properties of albizia and khaya gums
- To determine the elemental content and ash values of albizia and khaya gums
- To formulate ODFs using diclofenac sodium as the API
- To assess the physicochemical, mechanical and in vitro dissolution properties of formulated films
- To statistically analyse dissolution data employing one-way ANOVA followed by Dunnett‟s plot
- To determine the kinetics and mechanism of drug release by fitting the dissolution data into various kinetic models

CHAPTER 2

LITERATURE REVIEW

2.1 Oral dissolvable films (ODFs)

Fast-dissolving drug-delivery systems originally came about in the late 1970s as a recourse to tablets, capsules and syrups for pediatric and geriatric patients who have difficulties taking conventional oral solid-dosage forms. In acknowledgement of this need, different orally disintegrating tablet (ODT) formats were marketed. Most ODTs were developed to disperse barely a minute upon exposure to saliva to form a solution that can be easily swallowed. Oral dissolvable films (ODFs) emerged in recent years from the confectionery and oral care department in the form of breath strips and soon grew into a contemporary and widely accepted form by end users for delivering vitamins and personal care products.

Manufacturing companies with knowledge in the formulation of polymer coatings containing APIs for transdermal drug delivery cashed in on this opportunity to transform this technology to ODF formats. Presently, ODFs are an established and accepted technology for the systemic delivery of APIs for over-the-counter (OTC) medications and are in the primary developmental stages for prescription drugs (Arya et al., 2010). Current developments in this area have introduced appropriate dosage alternatives via the oral route for pediatric, geriatric, bedridden, nauseous or refractory patients.

Of late, oral drug delivery has become an essential route of drug administration (Malke et al., 2007). ODFs, an advanced drug delivery system for the delivery of the drugs through the mouth, were elaborated based on the technology of the transdermal patch. The delivery system comprises of a very delicate oral strip, which is just placed on the patient‟s tongue or any oral mucosal tissue. The film is straight away wet by saliva, promptly hydrates and attaches to the site of application. It then quickly breaks apart, mix with saliva and release the medication for oromucosal absorption. The formula may be revised to ensure that, the film maintains its fast-

dissolving property, allowing for gastrointestinal assimilation to be attained upon ingestion. Unlike other current rapid dissolving dosage forms that are made up of liophylisates, the smart films can be developed with a manufacturing procedure that matches with the production costs of ordinary tablets (Vollmer and Galfetti, 2006).

Pharmaceutical manufacturers and users alike have adopted ODFs as a very useful and recognized option to traditional OTC dosage forms such as syrups, suspensions, tablets, and capsules. ODFs presents a quick, precise dose in an innocuous and potent form that is handy and doesn‟t require the use of water or measuring devices (Borsadia et al., 2003).

ODFs are usually the same dimensions as a postage stamp and disintegrate on the tongue immediately for the quick release of active agents (Barnhart and Vondrak, 2008).

2.1.1 Characteristics of ODFs

• Thin stately film

• Comes in all manner of sizes and formats

• No choking risks

• Great adherence to oral mucosa

• Quick disintegration

• Accelerated release (Borsadia et al., 2003)

2.1.2 Advantages of orally dissolving films

- User-friendly in terms of dosing
- Water is not required for administration
- No danger of choking
- Possibility of adequately masking unpleasant taste and smell
- Improved stability
- Better patient acceptability and compliance

Oral dissolvable films (ODFs) further have obvious benefits over the orally dissolving tablets (ODTs):

• ODTs are at times challenging to move about, store and handle because they are very delicate.

• Many ODTs are manufactured by using sophisticated and costly lyophilisation process (Vollmer and Galfetti, 2006)

Several drugs can be formulated as oral ODFs. For target groups like paediatrics and geriatrics and for certain conditions, creative brands may enhance the therapeutic outcomes (Mashru et al, 2005).

- Children (antitussives, expectorants, antiasthmatics)
- Elderly (antiepileptic, expectorants)
- Gastrointestinal conditions
- Nausea as a result of chemotherapy
- Painful crisis as occurs in migraine and cramps
- Drugs that affect the CNS for example antiparkinsonism medicines

2.1.3 Composition of films

ODFs appear as flexible films of about 5- 20 cm^2 surface area (Vollmer and Galfetti, 2006) with active ingredient incorporated. They usually consist of water-soluble polymers that form a special grid through which water or saliva pass to dissolve the API. Active agents can be included up to a single dose of 15mg. To ensure adequate mechanical properties of the films, plasticizers are added to the formulation. Plasticizers have been reported to significantly improve mechanical properties of the films by reducing the glass transition temperature (Vollmer and Galfetti, 2006).

A classical ODF consists of the following ingredients;

API	1-25 %
Water-soluble polymer	40-50 %
Plasticizers	0-20 %
Colours, sweeteners, etc.	0-40 %

2.1.3.1 Drugs

Various drugs can be good candidates for use as APIs in ODFs. They include drugs for ulcer management (proton pump inhibitors e.g. esomeprazole), antiasthmatics (β2 agonist e.g. salbutamol sulphate; cough suppressant e.g. dextromethorphan hydrobromide; mucolytics e.g. guaifenesin; antihistamines e.g. cetirizine), NSAID‟s (e.g. diclofenac) (Mashru et al. 2005; Sharma et al. 2007).

2.1.3.2 Water-soluble film-formers

The films are formed from water-soluble polymers. The adoption of film formers in ODF technology has drawn substantial attention in pharmaceutical and nutraceutical applications. The water-soluble polymers impart instant disintegration, good texture and mechanical strength to the ODFs. The rate of disintegration of the polymers is indirectly proportional to the molecular weight of polymer base. Examples of water soluble polymers employed as film formers are hydroxypropyl methylcellulose (HPMC), methylcellulose, pullulan, carboxymethylcellulose (CMC), polyvinyl pyrrolidone (PVP), polyvinyl alcohol, maltodextrins, pectin, gelatin, sodium alginate, hydroxypropylcellulose, and Eudragit® RD (Chien et al. 2007; Sharma et al. 2007). Also, polymerized rosin has been used as a film forming polymer in recent studies (Fulzele et al. 2002).

Ideal hydrophilic polymers must possess these characteristics;

- They should not be harmful
- They should not cause any irritation to delicate membranes
- They should not retard the disintegration of the formulation

- They should be affordable and available
- They should maintain their stability in aqueous solutions
- They should exhibit good spreadability
- They should possess sufficient tensile strength and good mechanical properties.

2.1.3.3 Plasticizers

Plasticizers have been shown to improve the mechanical properties of films. These mechanical properties include tensile strength, elastic modulus, percentage elongation and folding endurance of the films. Therefore, alteration of plasticizer concentration may affect these properties. Examples of plasticizers include glycerol, polyethylene glycols, dibutylpthallate, etc. (Chien et al. 2006).

2.1.3.4 Surface active agents

Surfactants may be used either as solubilizing or wetting agents to ensure that the film dissolves instantly to release the API. Examples of the mostly used ones include tweens, sodium lauryl sulphate, benzalkonium chloride, benzethonium chloride, etc. Poloxamer 407 has also been used as a surface active agent (Wade and Weller, 1994).

2.1.3.5 Flavour

The obnoxious smell of certain APIs can be appropriately masked by adding any flavour, for example, fruit flavours, intense mints, or sweet confectionery flavours (Arya et al, 2010).

2.1.3.6 Colour

An array of colours, e.g. natural colours, FD&C colours, EU colours, and custom pantonematched colours may be used to enhance the appearance of ODFs (Bhyan et al., 2011). Colours are usually chosen to match with flavours for enhanced acceptability (Panda et al., 2012).

2.1.3.7 Saliva stimulating agents

Agents that stimulate the production of saliva may also be included to enhance the disintegration and subsequent release of the API. These include malic acid, citric acid, tartaric acid, ascorbic acid and succinic acid (Chapdelaine et al. 2003).

2.1.4 Manufacturing procedures

The following processes may be used singly or in combination to manufacture ODFs (Mishra and Amin, 2007).

- Solvent casting
- Semisolid casting
- Hot melt extrusion
- Solid dispersion extrusion
- Rolling

2.1.4.1 Solvent casting method

In this method, a polymeric solution is formed by dissolving the water soluble polymer in water. The drug and the other excipients are also dissolved in an appropriate solvent and then the two are put together and stirred till homogenous. The mixture is finally cast into glass petri dishes and dried in an oven.

2.1.4.2 Semi-solid casting

In the semi-solid casting method, an aqueous solution of film forming polymer is made. This is then added to a solution of acid insoluble polymer (e.g. cellulose acetate phthalate, cellulose acetate butyrate), which has been dissolved in ammonium or sodium hydroxide. The required quantity of plasticizer is then added to obtain a gel-like mass. Lastly the gel-like mass is cast into films or ribbons by the help of thermally controlled drums. The thickness of the ODFs

formulated using this method is usually about 0.015-0.05 inches. To ensure this, the ratio of the acid insoluble polymer to film former should be 1: 4.

2.1.4.3 Hot melt extrusion

In this method, the active agent is mixed with carriers in a dried state. The mixture is then heated till it melts. Then the melted mixture is formed into films by the help of special dies (Arya et al., 2010). Hot melt extrusion is associated with some advantages over the other methods (Coppens et al., 2006). - Less operation units

- Excellent uniformity of content
- Does not require the use of water

2.1.4.4 Solid dispersion extrusion

With solid dispersion extrusion, all the excipients are mixed with the API to form a solid dispersion. The solid dispersions are then moulded into thin films by means of dies (Arya et al., 2010).

2.1.4.5 Rolling Method

In this method, all the excipients and the API are dissolved in a suitable solvent to form a solution. If a true solution cannot be formed, a suspension containing drug may be used. This is then rolled onto a carrier. The most common solvent used is water or mixture of water and alcohol. The film is dried on the carriers and cut into desired shapes and sizes (Arya et al., 2010).

2.1.5 New technologies in the formulation of ODFs.

SOLULEAVES™ technology has been employed in an array of oral films that contain active agents and other excipients that mask unpleasant taste, odour and appearance. This technology ensures that the films dissolve immediately in the mouth, delivering the API along with other excipients. This characteristic feature makes this technology a superior way to deliver assorted

brands that are expected to be quickly released into the buccal cavity. For therapeutic purposes, SOLULEAVES™ films have shown tremendous results in dysphagic infants and old people. Among suitable candidates for this technology are medicines for pain, cough, cold, gastrointestinal conditions as well as certain nutraceuticals. With some modifications, SOLULEAVES™ films can stick to mucous membranes and slowly release the API over some minutes (Arya et al., 2010).

WAFERTAB™ technology is used to present API in the form of edible filmstrip. On contact with saliva, the system affords express release of drugs in the mouth. The WAFERTAB™ filmstrip can be sweetened, flavoured and coloured. This technology ensures accuracy of dose and protection from exposure to high temperatures and humidity that can possibly affect product stability. The WAFERTAB™ system has excellent prospects in terms of innovative product design for instance multiple films containing different active agents can be fused together. WAFERTAB™ films come in a range of colours, shapes and sizes (Arya et al., 2010).

FOAMBURST™ is a modification of the SOLULEAVES™ technology. Here, an unreactive gas is allowed into the film during the manufacturing stage. This ensures that the film comes out with a honeycomb-like structure. This structure enhances rapid dissolution and great mouth feel. FOAMBURST™ technology is used mainly in the food and confectionery industry.

XGEL™ film technology was patented by Meldex International. The technology is applied in all the film systems produced by the company. The ingredients used in producing XGEL™ films are not gleaned from animal sources therefore it is appropriate for vegetarians. It is also free from genetically modified organisms. XGEL™ film technology allows the possibility for taste masking, colouring and layering. This technology can also produce films that possess enteric features. The XGEL™ film technology may also be applied in the encapsulation of most oral dosage forms and the resultant capsules maintain excellent dissolution properties in

both hot and cold water. All the excipients used in this technology are optimized to suit their proposed use and are generally regarded as safe (GRAS) (Arya et al., 2010).

2.1.6 Evaluating parameters

2.1.6.1 Mechanical properties

The mechanical properties of films can be assessed using a texture analyzer equipment fitted with a 5kg load cell. The films are held in between two clamps placed 3cm apart. Then the strips are drawn apart at a rate of 2mm/sec. The force required to cause the films to break is recorded as well as the elongation at break. Among the mechanical properties investigated are tensile strength, elastic modulus and percentage elongation (Peh and Wong, 1999).

Tensile strength is defined as the maximum stress applied to a point at which the film specimen breaks and can be computed from the exerted force at break (as an average of triplicate measurements) and the cross-sectional area of the ruptured film as shown in the equation :

$$\text{Tensile strength} = \frac{\text{Force at break (N)}}{\text{Initial cross-sectional area of the film (mm2)}}$$ (Mishra and Amin, 2009).

Elastic modulus is the ratio of applied stress and corresponding strain (force in N) in the region of approximately linear proportion of elastic deformation on the load displacement profile and is calculated using the equation below :

$$\text{Elastic modulus} = \frac{\text{Force at corresponding strain}}{\text{cross-sectional area of film}} \times \frac{1}{\text{corresponding strain}}$$ (Mishra and Amin, 2009).

Percentage elongation is also calculated using the equation below:

$$\text{Percentage elongation} = \frac{\text{Increase in length}}{\text{Original length}} \times 100\%$$ (Mishra and Amin, 2009)

Folding endurance is established by folding the films of uniform cross-sectional area and consistency at the same point till it cracks (Cilurzo et al., 2008).

2.1.6.2 Evaluation of taste

A taste panel with a drug load of 50 mg is used for the taste assessment. Afterwards, a film containing 50 mg of drug is retained in the mouth of volunteers for about 5-10 seconds and then spewed. The level of bitterness is then noted (Agarwal et al., 2000). In between administration of active agent and the film sample, volunteers are made to rinse out their mouth with distilled water. The scale below is used :

- + = very bitter
- ++ = moderate to bitter
- +++ = slightly bitter
- ++++ = tasteless or taste masked.

2.1.6.3 Surface morphology

A scanning electron microscope (SEM) of clearly defined magnification is employed in the studies of the surface morphology of the films (Mashru et al., 2005).

2.1.6.4 Swelling property of films

Investigation into the swelling ability of the film is carried out in simulated saliva solution. The film is initially weighed and put in a stainless steel wire mesh of known weight. The mesh holding the film is immersed into a container filled with about 15 ml of simulated saliva solution. Change in weight of film sample as a result of absorption of the medium is determined at definite time intervals till a consistent weight is seen (Peh and Wong, 1999;

Jyothi et al., 2013).

The swelling index is evaluated using the equation:

$$\frac{wt - wo}{wo}$$

wt is final weight of film at time t, and wo

is initial weight of film at time zero.

2.1.6.5 Contact angle of films

Determination of the contact angle of the formulated films is carried out at room temperature using a goniometer (AB Lorentzen and Wettre, Germany). During the determination, a drop of distilled water is carefully put on the surface of the dry film. Digital images of the water droplet are taken in a period of 10 seconds of placement of the water drop using a camera. These digital images are examined using imageJ 1.28v software (NIH, USA) to determine the contact angle. About five readings are taken at different points of the film (on both sides of the drop) and the average is recorded. (Bettini et al. 2008).

2.1.6.6 In vitro disintegration time of films

The disintegration time is established by placing a film of uniform dimensions in a petri dish containing 10 ml distilled water with intermittent swirling. The time taken for disintegration to occur is noted when the film starts to rupture (Chien et al., 2006).

2.1.6.7 In vitro dissolution studies

The dissolution studies is conducted using simulated saliva or phosphate buffer of pH 6.4. The apparatus used is the USP paddle apparatus. A film is placed in each of the compartments of the apparatus. The experimental temperature is maintained at 37 ± 0.5°C. 10 ml aliquots are taken out at constant time intervals and analysed using UV-visible spectrophotometer (Mashru et al., 2005).

2.1.6.8 Determination of dissolution rate by conductivity method

Most of the oral films available on the market these days contain active ingredients that are ionisable. As a result, the conductivity of the dissolution medium can be used to monitor the dissolution profile of such ODFs (Jayjock et al., 2005).

2.1.7 Packaging

Different packaging alternatives exist for ODFs. As a general rule, therapeutic ODFs are supposed to be packed singly. An aluminium pouch is often used. The Rapid Card is an innovative packaging system, patented by APR-Labtec expressly for their Rapid film. It comes in the dimensions of a credit card carrying three films on each side. A single dose can be conveniently taken out for use (Vollmer and Galfetti, 2006).

Manufacturers may choose to emboss logos and other related product facts plainly onto the ODF before packing for branding purposes. However, industry regulations must be kept in mind, for instance; the requirement of packing each dose singly, Universal Product Code labelling, adequate instructional material, childproof seals and convenient packaging for use by the aged.

The packaging material chosen must possess these properties:

- They should offer optimum primary protection of the dosage form from external factors; moisture, temperature, light etc.
- They should have approval from the FDA.
- They should be in accordance with appropriate specifications for tamperproof formats.
- They should not be toxic.
- They should be physically and chemically inert.

Examples of suitable packaging units include:

- Single pouch made of aluminium foil, plastic or paper
- Blister card with several compartments

- Barrier films e.g. polychlorotrifluoroethylene (PCTFE) film, polypropylene film etc. (Lachman et al., 1986; Verma et al., 2013)

Table 2.1 List of marketed fast dissolving films

No.	Product	Manufactured by
1	Diphenhydramine citrate (cough and cold) Dextromethorphan hydrobromide (cough) Breath strips	MonoSolRx (www.monosolrx.com)
2	Donepezil rapid dissolving films Ondansetron rapid dissolving films	Labtec Pharma
3	Rotavirus vaccine for infants	Undergraduate Biomedical engineering students of John Hopkins (www.physorg.com)
4	Methylcobalamin fast dissolving films Folic Acid 1mg fast dissolving films Caffeine fast dissolving films	Hughes Medical Corporation (www.hughes-medical.com)
5	Altoid cinnamon strips Boots vitamin c strips Benzocaine films	Dow Chemical Company
6	Listerine pocket pack	Pfizer's Warner-Lambert Consumer Healthcare Div.
7	Acetylsalicylic acid ODFs Dexamethasone ODFs Nitroglycerine ODFs Risperidone ODFs	ODF Technologies Inc. (www.odftechnologies.com)

2.2 Gums and mucilages

2.2.1 The formation and classification of gums.

Gums are thought to be pathological compounds produced as a result of mechanical injury to certain plants or undesirable climatic conditions, such as drought. Gums are formed via an extracellular process known as gummosis, and it is characterized by the breakdown of cell walls. Mucilages are mainly physiological products of cellular metabolism that occur within the cells and can be created in the absence of injury to the plant (Shah et al., 2008).

Generally, gums easily disperse in water, whereas, mucilage assume slimy forms (Jani et al., 2009). Examples of gums include acacia, tragacanth, albizia, khaya, xanthan and guar gum.

Mucilages, however can occur in specific sections of the plant. Mucilage can be formed in the epidermal cells of leaves as occurs in senna, in the coatings of seeds as seen in linseed and psyllium and also in the roots for example marshmallow. In slippery elm plant and aloe, mucilages occur in the bark and middle lamella respectively (Evans, 2009).

Several theories have been proposed to elucidate the essence and motive behind the formation and exudation of gums. One of the theory purports that the generation of gums is a defensive mechanism used by plants to seal off mechanical injury and disease conditions. Another proposed theory propounds that gums occur as a result of normal physiological activities of plants. Some evidence on acacia trees favours the former theory. Disease-free acacia trees growing under suitable requirements such as moisture, temperature and soil produces no gum. Rather, under undesirable conditions such as drought and elevated temperatures, gum secretion is promoted (Blunt, 1926).

Another school of thought believes that gums are synthesised in response to microbial invasion of the plant by pathogens. In a bid to localize the infection, gums are produced to prevent the spread of infection to other surrounding sections of the plant (Evans, 2009). Also, gums are thought to be formed as a result of fungal invasion. These fungi discharge enzymes that infiltrate the plant tissue converting the cellulose cell wall to gum. For example, the parasite *Stereum purpureum*, which causes lead disease, induces plum trees to produce a considerable amount of gum at the site where the parasite grows. Yet another theory postulates that gums occur in response to bacterial infection of the plants. Different types of gums are produced by characteristic bacteria (Evans, 2009).

Comparatively, the theory that postulates that gums are produced by certain plants during injury to seal of the affected part thus preventing the spread of infections remains the most rational summary of gum formation. This idea is buttressed by evidence that gums are produced

immediately by gum producing plants once they are injured deliberately. Gums and mucilages are said by some, to arise from starch, whilst others suggest that they are produced at the expense of cellulose or hydrocellulose (Thaysen and Bunker, 1927; Smith and Montgomery, 1959).

Some similarities exist between gums and mucilages. They are both hydrocolloids obtained from plant sources. They appear as semi-opaque and irregular exudates. They are mainly composed of monosaccharide or mixed monosaccharides fused with uronic acids. Upon hydrolysis, gums and mucilages yield same components, sugars and uronic acids that occur as an admixture. Both hydrocolloids are composed of hydrophilic molecules that readily produce viscid solutions or gel-like masses in water. The physical and chemical properties of gums is generally influenced by the constituents of the gum. For instance, straight-chain polysaccharides take up more volume and form more viscous solutions in water than the notably branched polysaccharides of similar molecular weight. The branched ones are more stable than the straight-chain polysaccharides because there is little or no interaction due to their structural conformation (Jani et al., 2009).

Classification of gums is based on the origin;

(a) Gums from marine sources: agar, alginic acid, carrageenan and laminarin

(b) Gums from plant sources: i.) Exudates from trees: gum arabic, gum ghatti, gum karaya, tragacanth, khaya and albizia gums ii.) Gums obtained from seeds: guar gum, locust bean gum iii.) Extracts: pectin, larch gum iv.) Tuber and roots: potato starch

(c) Gums obtained from animal sources: chitin, chitosan, hyaluronic acid and chondroitin sulphate

(d) Gums obtained from microbial sources: xanthan, dextran, curdian, pullulan, zanflo, emulsan, Baker‟s yeast glycan, schizophyllan, lentinan (Choudhary and Pawar, 2014).

Gums may also be classified as acidic, neutral or basic. Natural gums are either acidic or neutral. No basic gum occurs in nature. Examples of acidic gums are acacia, tragacanth and albizia gums. Examples of neutral gums are asparagus gum and plantago seed gums (Smith and Montgomery, 1959).

Also, gums may be classified on the basis of the charge they carry. Gums that possess no charges are referred to as non-ionic gums and they include guar gum, locust bean gum, tamarind gum, xanthan gum, amylose, arabinans, cellulose and galactomanans. Gums that carry negative charges are termed as anionic gums. Examples include gum arabic, karaya, tragacanth, agar, algin, carrageenan and pectic acid (Jani et al., 2009).

Gums may also be classified on the basis of their structure. Straight-chain: cellulose, algins, amylose, and pectin. Branched: i) Short branches − galactomanan, xanthan, xylan; ii) Branchon-branch – tragacanth, amylopectin, gum arabic (Jani et al., 2009).

In addition to the above, gums may be classified into natural (examples are: acacia, tragacanth and xanthan), modified or semi- synthetic (examples are: carboxymethylcellulose, and microcrystalline cellulose) and synthetic (examples are: carboxypolymethylene and colloidal silicon dioxide).

Lastly, gums are sometimes classified as either water swellable (example albizia) or water soluble (example acacia).

2.2.2 Physical properties of gums

One factor that influences the commercial use of gums is their appearance. It affects their pricing and their end usage. High grade gums are more expensive. Depending on the biological

source of gums, they may vary in terms of appearance. There is a significant difference between gums collected from trees of the same species but are cultivated in different climatic situation or even from the same tree at different collection times. Also, the length of time that the gums remain at the exudation site as well as the mode of harvesting and how the gums are treated after collection all affect the appearance and physical properties of the gums (Glicksman, 1969).

2.2.2.1 Colour

The colour of an object is perceived when light of a suitable wavelength falls on it. It is a typical feature of light and can be quantified by means of intensity and wavelength (Nussinovitch, 2009). Colour assessment is very key in the commercial sale of gums where gums with little or no pigments are rated higher than pigmented ones. Several factors may influence the colouration of natural gums; for instance, the age of the trees, the type of soil the trees are cultivated on, the species of the trees, the prevailing climatic conditions etc. When exudate gums harden, they assume various colours ranging from plain white, reddishyellow to yellowish-brown. Others appear pinkish or in a shade of green. Pigmentation almost always connotes the presence of foreign materials in the exudates as they remain longer on the sites where they flow out from. The longer they remain, the more extraneous materials find themselves in them. Some phytochemicals produced by the trees, like tannins may be responsible for gums appearing darker than they usually are. Again the abounding conditions during harvesting also affects the colour of gums for instance, smoke from bush fires may deepen pigmentation of gums (Howes, 1949).

2.2.2.2 Size and structure

Natural gums appear in various states and conformations (Howes, 1949). Freshly produced gums are unwrinkled and uniform but as they become exposed, they roughen and fracture due

to contraction. These cracks, depending on how deep-seated they are can rupture into variable fragments. The fragments usually come in different shapes; some ball-shaped, cylindrical and others amorphous. For instance, cashew gum appears as uneven cylindrical fragments whiles tragacanth comes in the form of arched narrow strips (Verbeken et al., 2003).

2.2.2.3 Taste and odour

Most natural gums may be bland (Lewis, 1791), however some may have distinctive taste. This may be due in part to the botanical source of the plant. Gums that are meant for pharmaceutical use or for use in the food industry must be free of any characteristic taste or aftertaste (Howes, 1949).

Generally, natural gums are without any distinctive odour (Howes, 1949). Nonetheless, some plant exudates have characteristic smell e.g. myrrh. Myrrh belongs to the class of compounds called gum resins. These are composed primarily of gums and resins and possess distinct odour and sour taste (Bache and Thomas, 1819). Gum resins occur in most parts of certain plants for instance, stem, roots, leaves, fruits and flowers. On hardening, they become fragile (Loudon, 1825).

2.2.2.4 Hardness and density

Hardness tests are conducted on materials to evaluate mechanical strength (Mohsenin, 1986). Technically, hardness is a primary function of plasticity and a secondary function of elasticity (Mohsenin, 1986). A variety of scales are used in the engineering sector to measure hardness e.g. the Mohs scale, Vickers scale, Rockwell scale etc. Comparison of readings obtained from any of the scales can be done using systematic conversion charts (Idris et al., 1998).

Unlike minerals, efforts to categorize gums on the basis of hardness has proven futile (Howes, 1949). Investigations has however confirmed that, the hardness of gums may be dependent on the moisture content of the gum sample (Howes, 1949).

Facts about the density of gums as a basis for identification is not well established, because it varies even within the same species of gum. However, the density of gums may be partly determined by the amount of air that infiltrates tissues during the production of the gums (Nussinovitch, 2009).

2.2.2.5 pH

Natural gums are mostly acidic with a few being neutral. No basic gums exist in nature. The acidity is due to the presence of uronic acid units in addition to the sugar polymers. Generally, the pH of gum solutions ranges from 3-6, with albizia gum between 3.5 and 5 and that of tragacanth mucilage between 5 and 6. (Ashton et al., 1975).

2.2.2.6 Polarisation

Optical rotation can be defined as the ability of a molecule to rotate the plane of polarized light. These molecules mostly consist of a central carbon to which four entirely different substituents are attached. This position is referred to as a chiral centre and it is responsible for optical activity. Examples of optically active compounds include sucrose, ascorbic acid, amino acids, etc. (Ashutosh, 2005).

Gums may exist as either levorotatory or dextrorotatory in aqueous solvents. Several studies have been conducted on various gums in this regard. For example, samples of *Pereskia guamacho* (Cactaceae) gum are dextrorotatory in aqueous solution (de Pinto et al., 1994). *Hymenaea courbaril* (Caesalpiniaceae) gum samples are also dextrorotatory when dissolved in water (Anez et al., 2007). The composition of gums is chiefly responsible for optical rotation. Most components of gums have chiral centres e.g. sugar, amino acid etc. and therefore determines the optical rotation of such gums. Also, the proportion of these constituents present in the gum also counts. *Acacia seyal* and *Acacia senegal* gum have very similar composition

of sugar and amino acids but they occur in different proportions. As a result, *Acacia seyal* shows dextrorotatory activity in aqueous solutions whiles *Acacia senegal* is levorotatory (Flindt et al., 2005).

2.2.2.7 Solubility

Several factors affect the solubility of gums in aqueous solvents. They include the age of the plant from which the gum is collected, the duration of time that the gum remained at the exudation site, pre-treatment of gums etc. Generally, insolubility of gums is due to the presence of foreign materials that permeate the gum during their formation and exudation for instance, local karaya trees produce gums that solely absorbs water and swell but never dissolves, due to acetyl groups found in the gum (Imeson, 1992). Also, high grade gums yield a lesser quantity of residual material when mixed with water than low grade ones (Howes, 1949). To improve solubility in water, particle size reduction of gums may be used. Also proper collection and purification methods can exclude most of the foreign materials (Verbeken et al., 2003).

Nearly all naturally occurring gums can associate with water molecules. Some imbibe water, swell and form mucilaginous mixtures e.g. tragacanth, albizia, khaya, etc. These gums mostly have a soluble portion and insoluble portion in aqueous medium. Other gums are completely soluble in water and form true solutions e.g. acacia. At concentrations above 5 % most gums are unable to pass into aqueous solutions as a result of the degree of thickness. However, gum arabic is soluble in water up to 50 $\%^{w}/_{v}$. Gums are generally not soluble in oil and in solvents from organic sources. Above ethanol concentration of 60 $\%\ ^{v}/_{v}$, gums show practically no solubility (Nussinovitch, 2009).

2.2.2.8 Viscosity

Viscosity is defined as the resistance offered by liquid molecules to flow (Bourne, 2002). Hydrocolloids are able to increase the viscosity of aqueous solutions because, they absorb

water and swell, with the ultimate effect of increasing the consistency of the solvent phase (Glicksman, 1969). This quality accounts for their use in suspensions, emulsions, etc. Making the right choice of hydrocolloids for use in a formulation is very important as it automatically affects the quality and stability of the final product. A lot of factors influence the viscosity of a colloidal set-up. These include i) concentration of the system ii) temperature iii) previous mechanical deformation iv) degree of solvation v) presence of electrical charges vi) presence of electrolytes vii) pH (Ostwald, 1917).

Albizia zygia mucilage shows Newtonian behaviour at concentrations lower than 1 % but above 2 % it exhibits non-Newtonian flow. In the presence of electrolytes, specifically NaCl and $MgCl_2$, the viscosity decreases (Szczesniak and Farkas, 1962).

2.2.3 Chemical properties of gums

Gums consists primarily of carbon, hydrogen and oxygen. Minute amounts of minerals and occasionally, nitrogen and tannins may be present in some gums (Howes, 1949). Different gums vary in their composition and this variation may be due to the type, age and source of the gum. Because of this, it becomes difficult to define the structure of these natural polymers (Verbeken et al., 2003).

The physical properties manifested by the various gums and for that matter their use in the pharmaceutical and other industries depends on the structural and chemical composition of the gum. The chemical properties involve the constituent sugars and the sugar acids of the polysaccharides, the presence of other organic and functional groups and also the presence of inorganic ions (Avachat et al., 2011).

Gums and mucilage have been found to be made up of polysaccharides which are composed of sugars in a bound or free state, sugar acids in a bound or free state and also derivatives of sugar and sugar acids. These groups of compounds found in gums are generally classified into aldopentoses (arabinose and D-xylose), aldohexoses (D-glucose, D-mannose, Dgalactose, L-

galactose, 3,6-anhydro-D-galactose, 3,6-anhydro-L-galactose, etc.), ketohexoses (D-fructose and D-tagatose), 6-deoxyhexoses (L-fructose and L-rhamnose), uronic acids (Dgalactouronic acid, D-gluconic acid, etc.) and hexitols (D-mannitol) (Dror et al.,2006) .

Gums and mucilage have been found to contain some ester groups such as methoxyl and acetyl residues. Alkyl groups such as methyl groups are also present in some gums as derivatives of the sugar and sugar acids. Other functional groups are the anhydro- and deoxy- groups. The acetyl and methyl groups occur in gums such as karaya and khaya respectively (Smith and Montgomery, 1959).

Inorganic ions particularly metallic ions are also present in most gums as salts. Examples of such gums are albizia, acacia and tragacanth. These ions include sodium, potassium, calcium and magnesium, the commonly occurring being calcium followed by magnesium or potassium, with sodium being in traces. The calcium and magnesium contributes to the insolubility of some gums (Nussinovitch, 2009).

2.2.4 Collection, extraction and purification of gums and mucilages

Naturally, gums are exuded from incisions made on the stems. Gums are especially common in plants of arid regions. The gums are collected from the trees that produce them during the dry season, within the months of February and May. For gum arabic, good harvest is obtained when the fruits are ripe. But in general, plant gum exudates give better yields in unfavourable conditions such as drought, bush fires, etc. Horizontal grooves are made in the stem of the plant using a sharp knife and thin sections of the bark are ripped off. The molten gum gradually flows out as a thick fluid in a characteristic pattern. Upon exposure to air, the exudates solidify. After they are hard enough, the exudates can be harvested (www.faculty.ucr.edu).

Gums for analysis are first oven-dried at a temperature of 50˚C till sufficiently friable and then comminuted in a mill. The pulverized gum are then soaked in double strength chloroform water

for 72 hours with occasional stirring. The mixture is filtered using calico strainer. A considerable volume of ethyl alcohol, 96 % is added to precipitate the gum. Repeated precipitation from acidified aqueous solutions with ethanol serves to remove inorganic ions and proteinaceous impurities. Elimination of inorganic ions may also be effected by electro dialysis or by passing an aqueous solution of the material through cation exchange resin. The set-up is left to settle and the gum is filtered. Diethyl ether is used to wash the residue so as to remove all fat components. The residue is oven-dried at 40˚C. The anhydrous gum is milled into fine powder and kept in a sealed pouch for subsequent use (Femi-Oyewo et al., 2004).

2.2.5 Characterization of gums and mucilages

In the characterization of gums, an appropriate approach is used to conserve resources. Care must be taken in order to avoid over-characterization since it is wasteful and may even delay the discovery of a novel excipient. A collective approach may be employed (Jani et al., 2009). Gums and mucilages may be distinguished on the basis of their structure. Gums are mainly constituted of sugars. Therefore, investigating the different sugars present by chromatographic techniques may be carried out (Eddy et al., 2013). Elucidation of the molecular structure of the gums may be done using nuclear magnetic resonance and mass spectroscopy (Evans, 2009; Dror et al., 2006; Drummond and Percival, 1961; Flindt et al., 2005).

The purity of gums and mucilage is affected by the presence of extraneous plant chemicals, e.g. alkaloids, glycosides, carbohydrates, flavonoids, steroids, amino acids, terpenes, saponins, oils and fats, tannins, phenols, etc. Determining the presence or otherwise of these phytochemicals is important in the characterization of these gums (Evans, 2009; Shah et al., 2008).

Gums may also be distinguished on the basis of their physicochemical features. These features are characteristic for each gum and are important identification tools. They include colour, odour, form, taste, texture, solubility in different solvents, pH, swelling index, moisture

content, angle of repose, bulk and true densities, etc. (Mhinzi, 2002). Flow properties, level of microbial contamination and ash values may also be determined (Nussinovitch, 2009; Kumasah and Oghene, 1981).

Because gums find wide application in the food and pharmaceutical industry, toxicity studies is very essential to rule out any adverse effects on consumers. Acute toxicity studies may be carried out on experimental animals. The OECD guidelines can be employed to determine toxicity parameters like LD_{50} (Mazumder et al., 2010).

Lastly, gums and mucilages are used together with API in formulations. Investigating the compatibility of these gums with the API is key to ensure the quality, safety and efficacy of the dosage form. Compatibility studies can be conducted using several analytical techniques including FTIR, DSC, etc. (Jani et al., 2009).

Table 2.2 presents a list of specifications definitive for each gum as stated in the various pharmacopoeias.

2.2.6 Advantages of natural gums and mucilages in the pharmaceutical industry

These naturally occurring polymers are biodegradable. They pose no danger to the environment because they are easily decomposed by microorganisms.

Gums and mucilages are biocompatible and therefore do not cause any harm to living tissues. This is because, they are made up of long chains of sugar molecules (Jani et al., 2009). Naturally occurring polymers are relatively cheaper than the synthetic ones. This is because no sophisticated synthetic procedures are involved in their production and purification.

Environmental conservation is maintained with gum production and collection because, the trees are not destroyed during the process. The seasonal gums are only tapped in ways that allow the perennial trees to continue growing.

Natural gums and mucilages are widely available especially in areas that are highly agriculture-dependent. Commercial plantations can be cultivated to ensure regular supply of large quantities of gums.

Because gums and mucilages are of natural origin, they are free from any artificial chemicals that can have adverse reactions on users. As a result, they are widely accepted.

Gums and mucilages are obtained from comestible sources therefore they can be safely used in the food and pharmaceutical industry.

2.2.7 Disadvantages of natural gums and mucilages

Most gums contain residual water molecules even after purification. Also, gums have the tendency to absorb moisture from the environment, and being polysaccharides, may become rich substrates for microbial contamination. This can however be prevented by hygienic processing and storage as well as the addition of antimicrobial agents.

The physical and chemical characteristics of gums are chiefly dependent on geographical location, prevailing climatic conditions and time of harvesting. As a result there may be significant variations in gums collected from the same tree at different times. This inter-batch difference becomes a challenge since certain variables cannot be adequately controlled (Kottke and Rudnic, 2002).

Gums dissolve in water to form viscid solutions. Because of the complexity of natural polymers and their derivatives, there seems to be a constant decrease in the viscosity of mucilages of these gums upon storage, irrespective of how conducive the storage conditions

are.

Table 2.2 Pharmacopoeial specifications for gums (Jani et al., 2009).

Gum	Test	Pharmacopoeia
Acacia	Microbial limit, ash values	USP, JP, PhEur

Carrageenan	Solubility limit, pH, loss on drying, ash value	USP, PhEur
Guar gum	pH, microbial contamination, apparent viscosity, loss on drying, ash galactomannans, organic volatile impurities	USP, PhEur
Tragacanth	Microbial limits, flow time, lead, acacia, and other soluble gums, heavy metals	USP, JP, PhEur
Xanthan gum	pH, viscosity, microbial limits, loss on drying, ash, heavy metals, organic volatile impurities	USP, PhEur
Alginic acid	Microbial limit, pH, loss on drying	USP. PhEur
Dextrin	Loss on drying, residue on ignition, reducing sugars	USP, BP, JP

2.2.8 Applications and uses of gums and mucilage

Gums and mucilages find wide application in the food industry primarily because they are wholesome for consumption (Stephen and Churms, 2006). Guar and locust bean gums are both used as stabilizing and humidifying agents in some foods. Carrageenan also enjoys patronage by manufacturers of ice cream and meat as an effective stabilizer. Agar may be included in products like milk, cheese, sweets and meat for their stabilizing effect (Jani et al., 2009).

The functions of gums and mucilages in the pharmaceutical industry is enormous. They are used as viscosity enhancing agents in some suspensions, as emulsifying agents in stabilizing emulsions, as binding agents in tablets and capsules, etc. They have also shown tremendous prospects as gelling agents, film forming agents in ODF technology, coating agents in tablet cores targeted to only disintegrate in the colon (Odeku and Fell, 2005; Jain et al., 2007) and also in modified release dosage for sustaining the API as it is gradually released with time (Jani et al., 2009).

In therapeutics, some gums and mucilages are prescribed as bulk forming laxatives. For dry irritating coughs, remedies that contain gums and mucilages are used for their demulcent

properties. Also, most of the adhesives used in dentistry are incorporated with gums to impart stickiness.

In addition to the above, gums are employed in the cosmetic industry as stabilizers, gelling agents and humectants.

Finally in the paint and art industry, gums are used in the production of paints, resins and pigments and in the manufacture of paper and its related products. Most adhesives used in this sector are made from gums. In the production of creative pictures, drawings and writings, the oils, pigments and grease that are used contain gums (Jani et al., 2009).

2.2.9 Albizia zygia

Albizia zygia is widely distributed in tropical Africa, where they occur from Senegal through to Kenya, northern Angola and Tanzania (Fern, 2014).

Local name: albizia (English), nongo (Swahili), red nongo (Uganda), okuro (Ghana), nyie avu (Igbo), ayin rela (Yoruba), angoyen, kulo, mtanga, tumbum etc. (www.world agroforestry.org).

Parts used: Leaf, bark, roots

Substitutes: *Albizia gummifera*, *Albizia glaberrima* (www.tropicalferns.com).

Description: *Albizia zygia* is a medium-sized deciduous tree that grows up to 30m tall (www.database.prota.org).

2.2.9.1 Herbal medicinal uses

In traditional medicine, the bark is seen as antidote, aphrodisiac, analgesic, purgative, stomachic and vermifuge. A decoction of it is used as a remedy in lung diseases, fever due to malaria and barrenness. The ground bark is instilled in sores, lesions and wounds to improve healing. The sap in the bark is used to alleviate toothache and also placed in the eyes to cure

inflammatory eye conditions. It is also used in pregnancy as an anti-abortifacient. The bark as well as the leaves have analgesic and antioxidant properties (www.plants.jstor.org).

The ground roots are used as an expectorant and are added to food to treat coughs. It is used as a laxative as well as a vermifuge. It is also employed as a home remedy in pulmonary troubles. Decoction of the leaf is prepared and used to treat fever and diarrhoea. The leaf has been used as a painkiller and has also been shown to possess molluscicidal properties.

2.2.9.2 Plant chemicals

Extracts of *Albizia zygia* dried stem bark using methanol (polar) and hexane (non-polar) solvents have been shown to contain alkaloids, tannins, saponins, glycosides, steroids, resins and reducing sugars. Flavonoids and cardio active glycosides are only observed in the hexane extract (Abdalla et al., 2010).

In addition to lupeol, chondrillasterol and *p*-hydroxybenzoic acid, three flavones have been discovered from the bark of *Albizia zygia* collected from Sudan. These new agents have been identified as 3‟, 4‟, 7-trihydroxyflavone, 3-O-methoxyfisetin and 3‟, 7-dihydroxyflavonone. Their structures have been determined by nuclear magnetic resonance and mass spectroscopic techniques and have been confirmed by analysis in relation to what is in literature (Abdalla et al., 2010).

2.2.9.3 Biological activity and clinical research

Methanolic extract of *Albizia zygia* stem bark shows pronounce effect on *Plasmodium falciparum* K1 strain and *Trypanosoma brucei rhodesiense* with IC_{50} values of 1.0 µg/ml and 0.2 µg/ml respectively (Ndjakou Lenta et al, 2007). Also, the methanolic extract has activity against *Trypanosoma cruzi.*

The antimicrobial activities of the methanolic stem extract of *Albizia zygia* against

Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella typhi, Candida albicans, Rhizopus stolon, Aspergillus niger and *Penicillum notatum* is greater than the n-hexane extract at 50 mg/ml (Oloyede and Ogunlade, 2013).

Antioxidant screening of methanolic extract of *Albizia zygia* by scavenging effect on 2, 2diphenyl-1-picryl hydrazyl (DPPH) and hydroxyl radical produced from hydrogen peroxide methods shows 93.28 % inhibition at 1.0 mg/ml in the DPPH method and was superior to that of ascorbic acid and α-tocopherol (Oloyede and Ogunlade, 2013).

2.2.10 Khaya grandifoliola

Khaya grandifoliola is sighted in Benin, D.R. Congo, Cote D"Ivoire, Ghana, Togo, Nigeria, Sudan, Guinea, and Uganda (Hawthorne, 1998). Khaya also occurs in the transitional zones between savannah and closed forests.

Other names: African mahogany, Benin mahogany, Large-leaved mahogany, Senegal mahogany.

Local names: kissi diala (Guinea), baule lukru (Ivory Coast), mende bafili (Sierra Leone), manding-bambara (Upper Volta), munyama (Uganda) (www.plants.jstor.org).

Parts used: Stem bark, fruit pericarp, seeds, gum and resins.

Substitutes: *Khaya ivoriensis, Khaya anthotheca*

Description**:** *Khaya grandifoliola* is usually a deciduous, monoecious, and medium-sized to grand tree that can grow to a height of 40m (www.database.prota.org).

2.2.10.1 Herbal medicinal uses

Concoctions made from the bark of *Khaya grandifoliola* is used as a remedy for high fevers, cough, stomach problems, arthritis, fungal skin infections, malaria fever and also in the prevention of miscarriage in local Nigerian settings (Odugbemi et al, 2007; Olowokudejo et al, 2008). The stem bark has also been found to possess anti-ulcer property (Njikam and Njikam, 2006), anti-anaemic (Adeyemi and Gbolade, 2006), hypoglycaemic, hypoproteinaemic and hypocholesterolaemic effects (Bumah et al., 2005). The aqueous extract of *K. grandifoliola* is used in traditional Nigeria setting as a remedy against cough, *Mycobacterium tuberculosis* and other bacterial infections.

2.2.10.2 Plant chemicals

Phytochemical analysis was conducted on the powdered crude sample of *Khaya grandifoliola* and was confirmed that khaya contains alkaloids, tannins, saponins and flavonoids (Stephen et al. 2009). Some of the chemical constituents reported also include limonoids (Zhang et al. 2008).

These phytochemicals are responsible for the activity of the plant (Musa et al., 2013). Flavonoids are known to have antimicrobial properties (Tsuchiya et al., 1996). Alkaloids have shown impressive physiological activity especially on central nervous system. Sofowora (1993); Levetin and McMahon (2007) and Ya et al. (1989) also confirmed antimicrobial and antioxidant activities of tannins. However flavonoids, as reported to be present in the stem extract of *K. grandifoliola* conflicts with findings of Ibrahim et al. (2006) and Ojokuku et al. (2010) who confirmed their absence.

2.2.10.3 Biological activity and clinical research

In vitro biological activity has been demonstrated by the crude plant extracts of the bark and seeds of *Khaya grandifoliola* against *Plasmodium falciparum* with K_{50} value of 13.23 µg/ml.

Seven (7) lemonoids were collected after purification. They are methylangolensate (1), 6methylhydroxyangolensate (2), acetylswietenolide (8) and one flavonoid, catechin (4). Five out of the seven limonoids (1, 3, 5, 6, and 8) showed activity with IC_{50} values within concentrations of 1.25 and 9.63 µg/ml. No activity was observed in catechin. Gedunin, the most potent of the seven showed an additive effect with chloroquine (Bickii et al., 2000).

Methanolic extracts of *Khaya grandifoliola* stem bark was examined for antimicrobial action against some bacteria; MRSA, *Bacillus subtilis, Klebsiella pneumonia and Proteus mirabilis* at different test doses. The isolates showed a minimum inhibitory concentration (MIC) of 0.4 µg/ml except for *B. subtilis* where the MIC was 0.002 g/ml (Stephen et al., 2009).

2.3 Diclofenac in pain and inflammation management

Osteoarthritis is the third leading diagnosis in the aged (Felson et al. 2000) causing substantial amount of pain that may result in impairment and reduced quality of life in patients above the age of 65 years (Mili et al., 2003). Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to alleviate the pain of osteoarthritis. NSAIDs relieve inflammatory pain and also slows down the inflammatory process and its associated tenderness and joint constraints. In spite of the adverse effects associated with NSAIDs, they are still preferred. Examples of NSAIDs include diclofenac, aceclofenac, ibuprofen,

celecoxib, etc.

Diclofenac sodium is a poorly water soluble drug of pKa 4 with low solubility and high permeability. It belongs to the Biopharmaceutics Classification System (BCS) Class II. (British Pharmacopoeia 2013)

The mechanism of action of diclofenac is by interfering with the action of cyclo-oxygenase (COX) enzyme. COX enzymes aid in the synthesis of specific inflammatory mediators called prostaglandins. Prostaglandins are mostly released at the injured site resulting in pain and

inflammation. Antagonizing the action of COX enzymes therefore reduces the levels of prostaglandins released at the damaged site and ultimately the discomfort and inflammation is alleviated (Allen, 2012).

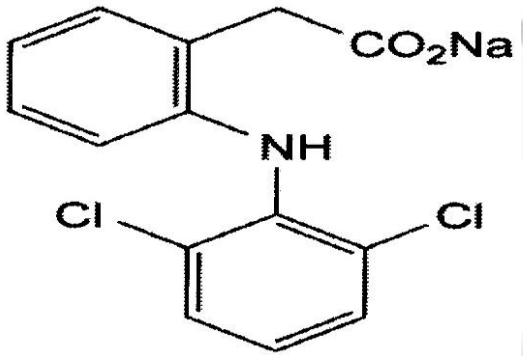

Figure 2.1 Structure of diclofenac sodium (British Pharmacopoeia, 2013)

The fact that majority of geriatric patients have difficulties swallowing conventional tablets has necessitated the formulation of drugs originally existing as tablets and capsules into oral dissolvable films (ODFs) to improve patient acceptability and compliance (Mishra and Amin, 2009).

2.4 Drug release kinetics

Drug release is the mechanism by which a drug moves out of a dosage form and is exposed for absorption, distribution, metabolism, and excretion (ADME), with the ultimate intent of enhancing pharmacological activity.

Drug release can be outlined in several ways. Immediate release dosage forms permits drugs to dissolve in physiological fluids within the shortest possible time. Modified release dosage forms involve both delayed and extended release dosage forms. Delayed release is described as the release of a drug at any latter time apart from instantly after administration. Extended release drugs are specially developed to prolong the duration of action after administration. Finally, controlled release includes extended and repeated release dosage forms. In repeated

release, the API is delivered in definite quantities at specific time intervals (Singhvi and Singh, 2011).

In vitro dissolution is regarded as very essential in preformulation studies. A number of theoretical models seek to explain the mechanism of drug dissolution from both immediate and modified release products. The specific release kinetics of a dosage form is influenced by several factors including type of API, its polymorphic form, crystallinity, particle size, solubility and quantity of API included in the dosage form. For instance, in formulating a water soluble drug in a sustained release format, the API can be integrated into a matrix and by the process of diffusion, the drug will be released. For drugs of low water solubility however, auto–eroding matrix may be the appropriate release method.

Three main approaches are used to investigate the kinetics of drug release from controlled release formulations. They include

- Statistical methods: exploratory data analysis, repeated measures design, ANOVA, MANOVA, etc.
- Model dependent methods: zero order, first order, Higuchi, Hixson-Crowell, etc.
- Model independent methods: difference factor (f1), similarity factor (f2) (Dash et al., 2010)

2.4.1 Mathematical models

2.4.1.1 Zero order release kinetics

Zero order release kinetics pertains to the mechanism of uniform drug release from a dosage form for instance, oral osmotic tablets, transdermal systems, matrix tablets with lowsolubility drugs etc. Zero order kinetics may be simplified as:

$$\mathbf{Q = Q_0 + K_0 t}$$

Where Q is the quantity of drug released or dissolved,

Q_0 is the initial quantity of drug in solution,

K_0 is the zero order release constant (Singhvi and Singh, 2011).

2.4.1.2 First order release kinetics

Noyes and Whitney propounded the first equation relating dissolution rate quantitatively as:

dC/dt = k (Cs - Ct)

Where dC/dt is the rate of change in concentration with respect to time, and k is the rate constant. Integrating the above equation, **ln [Cs/ (Cs - Ct)] = kt**

Log C = Log C_0 - kt / 2.303

Where, C_0 is the initial concentration of drug and k is first order constant.

Comparing the equation to the other rate equations, it can be seen that first order is dependent on the concentration difference i.e. (Cs - Ct) between the static liquid layer next to the solid surface and the bulk liquid (Singhvi and Singh, 2011).

2.4.1.3 Hixson-Crowell cube root model

The Hixson-Crowell cube root law depicts the release from systems where there is an alteration in surface area and diameter of particles or tablets. For a powdered drug consisting of particles of uniform size, it is possible to deduce an equation that relates the rate of dissolution to the cube root of the particle size.

$Q_{01/3} - Q_{t1/3} = K_{HC}t$

Where, Q_t is the amount of drug released at time t, Q_0 is the initial amount of the drug in tablet and K_{HC} is the rate constant for Hixson-Crowell rate equation (Singhvi and Singh, 2011).

2.4.1.4 Higuchi model

Most controlled-release products are modelled on the principle of embedding the API in a porous matrix. Water molecules imbibe the matrix and causes dissolution of the drug, which then diffuses into the external medium. Higuchi attempted to relate the rate of drug release to

the physical constants based on the laws of diffusion. Higuchi originally derived an equation to express drug release from an insoluble matrix as the square root of a time-dependent process derived from Fickian diffusion.

$$\mathbf{Q_t = [2DS\varepsilon\ (A - 0.5S\varepsilon)]^{0.5} * t^{0.5}}$$

In simple terms,

$$\mathbf{Q_t = k_H\ (t)^{0.5}}$$

Where, Q_t is the quantity of API released at time t, D is the diffusion coefficient, S is the solubility of API in the dissolution medium, ε is the porosity, A is the amount of drug per cubic centimetre of matrix tablet, and k_H is the rate constant for the Higuchi model (Singhvi and Singh, 2011).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Crude albizia and khaya gums were obtained from forest areas around Kwahu in the Eastern Region of Ghana, as natural exudates from the incised trunks of the trees *Albizia zygia,* (DC.) J. F. Macbr. family Leguminosae and *Khaya grandifoliola* C.DC. family Meliaceae respectively. Mr Asare, a technician at the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST authenticated, collected and supplied the crude gums. Other materials used include diclofenac sodium powder (Trade Winds Chemists, Kumasi), hydroxypropyl methylcellulose, HPMC E15 (UK Chemicals, Kumasi), pineapple flavour and titanium dioxide (Kinapharma Ltd, Accra), aspartame (Aspee Pharmaceutical Ltd, Kumasi), glycerol, tween 80 and citric acid were obtained from the chemical stores of the Departments of Pharmaceutics and Pharmaceutical Chemistry, KNUST, Kumasi.

3.1.1 Chemicals and reagents

All the reagents used for the research were of analytical grade. Chloroform, 96 % ethanol, concentrated hydrochloric acid, diethyl ether, potassium dihydrogen orthophosphate (Fisons Laboratory), sodium hydroxide pellets and propylhydroxy benzoate were obtained from the chemical store of the Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi. Distilled water was freshly prepared and used.

3.1.2 Equipment and apparatus

Analytical balance (SN: AE 436647 Adam Equipment, UK), Eutech pH meter (pH 510, pH/mV/°C meter, SN: 2025520, Singapore), Digital calliper (POWERFIX, Milomex Ltd, UK), UV spectrophotometer (T90 UV/VIS spectrometer, PG Instruments Ltd, UK), Bruker

Fourier Transform Infra-Red spectrophotometer (Alpha, Platinum ATR, Jos Hansen & Soehne GmbH, Hamburg-Germany), Brookfield Viscometer (Brookfield Engineering Lab. Inc., Middleboro, MA 02346, USA), Brookfield Texture Analyzer (Type CT3-100, with TexturePro CT Software. Probe type: TA-DE and TA-DGA with accessory fixtures; Brookfield Engineering Lab. Inc., Middleboro, MA 02346, USA), Erweka Dissolution Apparatus (Type DT6, GmbH Heusenstamm, Germany), Stuart melting point apparatus (SN: R000105350, Bibby Scientific Ltd., UK), Retsch test sieves, hot air oven, mortar and pestle, Number 4 sintered glass filter among others were used.

3.2 Methods

3.2.1 Purification of gums

Albizia and khaya gums were prepared by sorting out the bark, soil and any foreign matter. The gums were oven−dried at a temperature of 50°C for 48 hours. The dried gums were sorted out into two grades, light coloured and dark coloured ones. The light coloured grades were milled into powder. The powdered gums were used in some subsequent tests as crude albizia and crude khaya gums. A quantity of 800 g each of albizia and khaya gums were hydrated in 2 L of double strength chloroform water for 48 hours with occasional stirring. Impurities and debris were filtered using a piece of calico. Both filtrates were precipitated with three times their volumes of 96 % ethanol to obtain the purified gums which were then filtered and washed with diethyl ether. The gums were subsequently dried in a hot air oven at 40°C for 24 hours (Femi-Oyewo et al., 2004).

The dried purified gums of albizia and khaya were triturated again using a porcelain mortar and pestle and screened using sieve of aperture size 250 µm. Both gums were packed separately into air tight plastic pouches and stored in a desiccator pending subsequent tests and analysis as purified albizia and khaya gums.

3.2.1.1 Determination of percentage yield

The weight of dry powdered crude gum before purification, W_1 and that of the dry powdered purified gum, W_2 were determined using an analytical balance (SN: AE 436647 Adam Equipment, UK). The percentage yield was evaluated as;

$$\text{Percentage yield} = \frac{W_2}{W_1} \times 100\ \%$$

3.2.2 Examination of physicochemical properties of gums

3.2.2.1 Macroscopic properties of crude albizia and khaya gums

The macroscopic properties of albizia and khaya gums, namely: colour, odour, taste, surface appearance, size, form, shape and fracture were observed.

3.2.2.2 Moisture content of albizia and khaya gums

Two (2) grams each of powdered crude and purified albizia and khaya gums were weighed accurately into porcelain crucibles which had previously been dried to constant weight. The gums were placed in a hot air oven and maintained at a temperature of 105°C. After 5 hours, the gums were removed and cooled after which they were placed in a desiccator for 30 minutes. The weights of both crucibles and gums were recorded. The determination was done in triplicates. The moisture content was expressed as a percentage of both gum samples (Shehzadi, 2014).

3.2.2.3 Insoluble matter of crude and purified albizia and khaya gums

Two (2) grams each of powdered crude and purified albizia and khaya gums were weighed separately into a 250 ml round bottomed flask. To each was added 100 ml of water followed by the addition of 14 ml of 2 M HCl. The mixtures were boiled gently for 15 minutes while shaking frequently. The resultant mixtures were filtered whilst hot through number 4 sintered

glass filters. The residues were then washed with hot water and dried at 105°C to constant weight. The weight of the insoluble matter was expressed as a percentage (Shah et al., 2008).

3.2.2.4 Solubility of purified albizia and khaya gums in various solvents

The solubility of albizia and khaya gums were investigated in cold and hot distilled water, acetone, chloroform and ethanol (96 %). One (1) gram each of the gums was placed in 50 ml of solvents and allowed to stand overnight. A volume of 25 ml of the supernatants were placed in pre-weighed petri dishes and evaporated to dryness over a constant water bath. The mass of the residues with reference to the volume of the solutions were measured using an analytical balance (SN: AE 436647 Adam Equipment, UK) and expressed as the percentage solubility of each of the gums in the respective solvents (Carter, 2005).

3.2.2.5 Swelling index of purified albizia and khaya gums

The swelling index of albizia and khaya gums was determined by weighing one (1) gram each of the gums into a 10 ml measuring cylinder. The initial volumes of both gums were noted. Distilled water was added to the 10 ml mark. The cylinders were stoppered, mixed lightly and allowed to stand for 24 hours. The volume occupied by each of the gum sediments were noted after 24 hours (Jaiyeoba et al., 2013).

Swelling Index, SI was calculated using the equation below

$$\text{Swelling Index, SI} = \frac{Xt - Xo}{Xo} \times 100\ \%$$

Where X_t is the volume of gum after 24 hours

X_o is the initial volume of gum

3.2.2.6 Water retention capacity of purified albizia and khaya gums

The contents of the measuring cylinders from the previous experiment was filtered through a calico strainer. The water was completely drained into a dry 10 ml measuring cylinder. The volumes of water drained from the respective gums were recorded and the difference between

the initial volumes of mucilage and the volumes of water drained was recorded as the water absorbed by the gum samples.

3.2.2.7 Determination of true density of purified albizia and khaya gums

The true densities of both albizia and khaya gums were determined by liquid displacement method at 25°C. It is calculated as the weight of the solid material divided by the weight of the liquid it displaces. The material whose density is to be determined should be insoluble in the liquid. The weight, W_1 of a clean, dry 50 ml density bottle and top was determined using an analytical balance (SN: AE 436647 Adam Equipment, UK). The relative density bottle was filled with water and the top inserted into the mouth of the bottle. The excess water around the bottle was blotted with a filter paper and weighed as W_2. The procedure was repeated using chloroform to obtain the weight of the bottle and Chloroform, W_3. Chloroform was used as the displacement liquid. A quantity of three (3) grams of the gum was transferred into a dried density bottle and weighed as W_4. The bottle already containing the gum was filled with chloroform and the weight, W_5 was measured (Ghosh and Jasti, 2005). The density of the chloroform used was calculated using the formula;

Density of chloroform ρ, $= \frac{(W_3 - W_1)\ 0.9971}{(W_2 - W_1)}$, density of water at 25°C = 0.9971 g/ml

The true density of gum was obtained from the formula below;

$$\text{Density of sample} = \frac{(W_4 - W_1)}{[(W_3 - W_1)/\rho] - [(W_5 - W_4)/\rho]}$$

3.2.2.8 Determination of temperature of charring of purified albizia and khaya gums The charring temperature of purified albizia and khaya gums were established by open capillary method using Stuart melting point apparatus (SN: R000105350, Bibby Scientific Ltd., UK). An open capillary tube was sealed using a Bunsen burner. The tube was packed by pressing

the open end gently into a sample of the dried powdered gums. The gum was transferred from the open end to the bottom of the tube by gently tapping the bottom on the bench. The sample tube was then inserted into the apparatus and the temperature at which the sample changed colour was determined.

3.2.2.9 Effect of preservation on pH of 1 % mucilage of purified albizia and khaya gums

The pH values of 1 % mucilage of purified albizia and khaya gums were determined using a Eutech pH meter (pH 510, pH/mV/°C meter, SN: 2025520, Singapore). The pH values of the samples were determined weekly for five (5) weeks. A test batch of 1 % mucilage of both gums was preserved with 0.1 % propylhydroxy benzoate. The pH of the test samples was measured every week for five (5) weeks.

3.2.2.10 Effect of storage temperature on the pH of 1% mucilage of purified albizia and khaya gums

Samples of 1 % mucilage of purified albizia and khaya gums were prepared with distilled water. The samples were then kept at room temperature (25°C), 50°C and in a fridge of temperature 4°C. The pH of the above samples was measured weekly for five (5) weeks using a standardised Eutech pH meter (pH 510, pH/mV/°C meter, SN: 2025520, Singapore).

3.2.2.11 Effect of concentration on viscosity of mucilage of albizia and khaya gums

Samples of albizia and khaya gum mucilages (5, 10, 20, 30 and 40 $\%^{w}/_{v}$) were prepared using distilled water. The viscosity of the prepared samples was measured at a shear rate of 30 rpm using a Brookfield Viscometer (Brookfield Engineering Lab. Inc., Middleboro, MA 02346, USA) at 25°C.

3.2.2.12 Effect of temperature on viscosity of mucilage of albizia and khaya gums A concentration of 40 $\%^{w}/_{v}$ mucilage of purified albizia and khaya gums was prepared using distilled water. The viscosity of the prepared samples was determined at a shear rate of 30 rpm

using a Brookfield Viscometer (Brookfield Engineering Lab. Inc., Middleboro, MA 02346, USA) at temperatures of 25, 50, 65, 75 and 85°C.

3.2.2.13 Determination of microbial quality of purified albizia and khaya gums Purified albizia and khaya gums, 0.1g each, was dissolved separately in 10 ml of sterile water. 1 ml of the resultant mucilage was inoculated into already stabilized bismuth sulphite agar, cetrimide agar, MacConkey agar, mannitol agar, nutrient agar and sabouraud agar. The inoculated agar was allowed to stand for about 30 minutes, inverted and incubated at 37°C (25°C in sabouraud agar) for 48 hours. 10 ml of the various stabilized growth media were plated and incubated under similar conditions to serve as control. The growth of characteristic organisms based on the type of selective media was observed as present or not present.

3.2.2.14 Determination of antimicrobial properties of purified albizia and khaya gums Different concentrations (3, 4, 5 $\%^{w/}_{v}$) of mucilage of purified albizia and khaya gums were prepared and the antimicrobial activity determined against *Bacillus subtilis* (NTCC 10073), *Candida albicans* (clinical strain), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (clinical strain), *Pseudomonas aeruginosa* (ATCC 4853), *Salmonella typhi* (clinical strain), *Staphylococcus aureus* (ATCC 25923), and *Streptococcus pyrogenes* (clinical strain) in nutrient agar using the cup plate method. The petri dishes were left to stand for about 30 minutes for the mucilage to diffuse into the agar. A control was set up by plating only the seeded agar. Both groups were then kept in an incubator at 37°C for 48 hours. The petri dishes were observed for any zones of growth inhibition.

3.2.2.15 Determination of organic carbon

The organic carbon content of albizia and khaya gums was determined by the modified

Walkley-Black Wet oxidation method. A quantity of gum sample weighing 0.50 g was placed into a 500 ml conical flask. Ten millilitres of 1.0 N $K_2Cr_2O_7$ solution was added followed by 20 ml concentrated H_2SO_4 . The mixture was allowed to cool on an asbestos material for 30 minutes. 200 ml of distilled water was then added, followed by 10 ml of H_3PO_4 and then 1.0 ml of diphenylamine indicator. The resultant mixture was titrated with 1.0 N ferrous sulphate solution until the colour changed from a blue-black colour to a persistent green colour. A blank determination was carried out in a similar way for both gum samples analysed.

Calculation:

$$\%C = \frac{N \times (V_{bl} - V_s) \times 0.003 \times 1.33 \times 100}{g}$$

Where, N =Normality of $FeSO_4$ solution

V_{bl} = volume (in ml) of $FeSO_4$ used for blank titration

V_s = volume (in ml) of $FeSO_4$ used for sample titration

g = mass of plant sample taken in gram

0.003 = milliequivalent weight of C in grams (12/4000)

1.33 = correction factor used to convert the wet combustion C value to the true C value since the wet combustion method is about 75 % efficient in estimating C value , (i.e. 100/75 = 1.33).

3.2.2.16 Preparation and dry ash digestion of purified gums for elemental analysis One (1) gram of gum was placed in a ceramic crucible. The sample was placed in a cool muffle furnace and the temperature ramped to 500°C over a period of two (2) hours. The temperature was maintained further for two hours. The sample was then left to cool in the furnace. After it

was sufficiently cooled, it was taken out of the furnace avoiding any external air. The ashen sample was first placed into previously labelled 50 ml centrifuge tubes. The crucible was washed with 10 ml of distilled water into the centrifuge tube. The crucible was further washed with 10 ml of aqua regia. The sample was agitated for about 5 minutes on a mechanical reciprocating shaker to ensure effective mixing. The sample was then centrifuged for 10 minutes at 3000 rpm and then transferred into a 100 ml volumetric flask. The volume was made up to the 100 ml mark with deionized water. The supernatant digest was poured into a reagent bottle for the elemental analysis of P, Ca, Mg, K and Na (Okalebo et al., 1993).

3.2.2.17 Determination of iron (Fe), copper (Cu), zinc (Zn) and Manganese (Mn)

The atomic absorption spectrophotometer (AAS) was set up at conditions of air pressure 5060 psi, acetylene pressure 10-15 psi and voltage 208-240 V. The file for the analysis and hollow cathode lamps were also set at appropriate wavelengths, Fe at 248.3 nm, Cu at 324.8 nm, Zn at 213.9 nm and Mn at 279.5nm. A calibration curve was plotted for each of the elements to be analyzed from the stock standards. The prepared sample digests were analyzed for the respective elements.

Calculation:

$$\text{Conc. (Cu, Fe, Mn, Zn) (mg/kg)} = \frac{\text{Concentration recorded from AAS X Nominal volume}}{\text{Sample weight (g)}}$$

where nominal volume =100 ml

sample weight = 1.00 g

3.2.2.18 Determination of calcium (Ca) and magnesium (Mg)

A volume of 5.0 ml of sample digest was placed in a 100 ml Erlenmeyer flask. 10 ml of 10 % KOH solution was added followed by 1 ml of 30 % triethanolamine, then five drops of 2 % KCN and one drop of Erichrome Black T indicator solution was also added. The mixture was

shaken to ensure homogeneity. The mixture was titrated with 0.02 N EDTA solution from a red to blue end point.

Calcium in mg = titre value of EDTA x 0.40

$$\% \text{ Calcium} = \frac{\text{Calcium (mg)}}{\text{sample weight}} \times 100$$

For magnesium a 5.0 ml sample digest was emptied into a 100 ml Erlenmeyer flask. 5 ml of ammonium chloride – ammonium hydroxide buffer solution was added followed by 1 ml 30% triethanolamine. To the resultant solution was added three drops of 10 % KCN and a few drops of Erichrome Black T indicator solution. The mixture was shaken to ensure homogeneity. The mixture was titrated with 0.02 N EDTA solution from a red to blue endpoint.

Magnesium in mg = Titre value of EDTA x 0.24

$$\% \text{ Mg} = \frac{\text{Magnesium (mg)}}{\text{sample weight}} \times 100$$

3.2.2.19 Determination of Phosphorus

A vanadomolybdate reagent was prepared by dissolving 22.5 g of ammonium molybdate in 400 ml of distilled water and 1.25 g of ammonium vanadate in 300 ml of boiling distilled water. The vanadate solution was added to the molybdate solution and cooled to room temperature. 250 ml of analytical grade HNO_3 was added to the solution mixture and diluted to one litre with deionized water. The standard phosphate solution was also prepared by dissolving 0.2195 g of analytical grade KH_2PO_4 in 1000 ml distilled water. This solution contains 50 µg/ml elemental phosphorus. Different concentrations of the standard solution was prepared by pipetting 1, 2, 3, 4, 5 and 10 ml of standard solution in 50 ml volumetric flasks. 10 ml of vanadomolybdate reagent was added to each flask and the volume made up to 50 ml to produce solutions of concentration 1, 2, 3, 4, 5, and 10 µg/ml elemental phosphorus. These

concentrations were measured on the Jenway 6051 colorimeter to give absorbance measurements at a wavelength of 430 nm. A plot of absorbance against concentration (calibration curve) was drawn.

Five (5) ml of the sample solution from the digest was put into a 50 ml volumetric flask. 10 ml of vanadomolybdate reagent was added and volume made up to 50 ml with deionized water. The sample was allowed to stand for 30 minutes undisturbed for colour development.

A stable yellow colour was developed. The sample was viewed on the colorimeter at 430 nm. The observed absorbance was used to determine the phosphorus content from the standard curve. The % phosphorus was calculated as:

Phosphorus content (g) in 100 g sample (%) $= \frac{C\ x\ df\ x\ 100}{1\ 000\ 000} = \frac{C\ x\ 1000\ x\ 100}{1\ 000\ 000} = \frac{C}{10}$

Where C = concentration of phosphorus (µg /ml) as read from the standard curve;

df = dilution factor, which is 100 × 10 = 1000, as calculated below:

1 g of sample made to 100 ml (100 times);

5 ml of sample made to 50 ml (10 times)

1 000 000 = factor for converting µg to g

3.2.2.20 Determination of potassium (K) and sodium (Na)

Analytical grade KCl and NaCl weighing 1.908 g and 2.542 g respectively (previously dried in an oven for 4 hours at 105ºC) were each dissolved in 200 ml of deionized water. The two solutions were mixed together and volume made up to 1000 ml. This gave a combined standard of 1000 ppm. For K, a calibration curve of 200, 400, 600 and 800 ppm was prepared. Similarly, a calibration curve of 20, 40, 60 and 80 ppm was prepared for sodium. All the absorbance readings were taken using the flame photometer. The sample solution from the $HClO_4$ and HNO_3 was read on the flame photometer. From the calibration curve, the concentration of K and Na were calculated using the particular absorbance observed for the sample.

Calculation:

K content (µg) in 1.0 g of plant sample = C × df

K content (g) in 100 g plant sample, (% K) = $\frac{C \times df \times 100}{1000000} = \frac{C \times 100 \times 100}{1000000}$

$= \frac{C}{100}$

where,

C = concentration of K (µg / ml) as read from the standard curve

df = dilution factor, which is 100 x1 = 100 calculated as:

1.0 g of sample made up to 100 ml (100 times) 1000

000 = factor for converting µg to g.

3.2.2.21 Determination of cadmium (Cd), lead (Pb), Mercury (Hg), Arsenic (Ar)

The atomic absorption spectrophotometer (AAS) was set up at conditions of air pressure 5060 psi, acetylene pressure 10-15 psi and voltage 208-240 V. The file for the analysis and hollow cathode lamps were set at appropriate wavelengths, Cd at 228.9 nm, Pb at 283.3 nm, Hg at 253.7 nm and Ar at 193.7 nm. A calibration curve was plotted for each of the elements to be analyzed from the stock standards. The prepared sample digests were analyzed for the respective elements.

Calculation:

Conc. (Cd, Pb, Hg, Ar) (mg/kg) = $\frac{\text{Concentration recorded from AAS X Nominal volume}}{\text{Sample weight (g)}}$

where nominal volume =100 ml

sample weight = 1.00 g

3.2.2.22 Determination of total ash

A clean crucible was placed in an oven until it dried to a constant weight. It was then taken out of the oven and left in a desiccator to cool. The weight of the empty crucible was recorded as

A. 2.0 g of gum sample (B) was placed in the crucible. The sample was then placed into a muffle furnace for 4 hours at a temperature of 550°C. It was allowed to cool below 200°C and maintained for another 20 minutes. The sample was put in a desiccator to cool off completely. The weight of the ashen sample was determined as C.

Using the formula below, the total ash was calculated;

(A + B) − A = B

(A + C) – A = C % total ash = C/B × 100 where A = weight of crucible, B = weight of sample, C = weight of ash.

3.2.2.23 Determination of acid insoluble ash

A volume of distilled water was heated to near boiling point. 2+5 HCl was prepared by mixing 2 volumes of concentrated HCl with 5 volumes of distilled water. A volume of 25 ml 2+5HCl was used to transfer the total ash residue from the crucible to a beaker. The contents of the beaker was stirred with a glass rod and covered with a watch glass. It was then heated gently for about five minutes in a hood. The bottom of the watch glass was rinsed with hot water into the beaker. The acid solution was filtered through an ashless filter paper. It was ensured that all traces of the acid and ash were completely rinsed (with hot water) from the beaker and crucible onto the filter. More washing of the filter paper was done until the washings were acid free to litmus.

The filtrate was allowed to drain and the residue was carefully transferred into a weighed crucible. It was then dried in an oven and ignited in the muffle furnace at 600°C, cooled and weighed (Okalebo et al., 1993).

The acid insoluble ash was evaluated using the equation below;

$$\% \text{ Acid insoluble ash} = \frac{A - B}{C - B} \times 100$$

where A = weight of crucible and ash

B = weight of empty crucible

C = weight of crucible and original sample weight.

3.2.3 Formulation of diclofenac sodium oral dissolvable films (ODFs)

ODFs of diclofenac sodium were formulated using the solvent-casting method. Table 3.1 shows the composition of the seven formulations prepared. The water soluble polymers were immersed in half the volume of distilled water overnight to obtain a homogeneous dispersion. The specified amount of glycerol (as plasticizer) was added to the aqueous dispersion and mixed until homogeneous. The aqueous solution was allowed to stand for an hour to take out all entrapped air bubbles. Another aqueous solution was made by dissolving diclofenac sodium, aspartame, titanium dioxide, flavour, tween 80 and citric acid in their specific proportions in the remainder of distilled water. Both aqueous solutions were put together, stirred and sonicated for 30 minutes. Twenty millilitre (20 ml) portions of the resultant mixture were cast onto glass petri dishes of diameter 90 mm and oven−dried at 60°C for 24 hours (Mishra and Amin, 2009). Films containing HPMC were also formulated to serve as a control. The dried films were meticulously taken out of the petri dishes, inspected for any imperfections and cut into the required size (2 cm × 2 cm). They were then packaged singly in aluminium foils and kept in a desiccator pending assessment.

Table 3.1 Composition of formulations

Code	Drug (g)	HPMC (g)	Albizia (g)	Khaya (g)	Gly. (g)	Tw. (g)	Fla. (g)	Asp. (g)	Tit. (g)	C.A. (g)	Water to (ml)
F1	4.0	3.0			5.0	1.0	5.0	1.0	1.0	1.0	100.0
F2	4.0	2.0	2.0		5.0	1.0	5.0	1.0	1.0	1.0	100.0
F3	4.0	2.0		5.0	5.0	1.0	5.0	1.0	1.0	1.0	100.0
F4	4.0		5.0		5.0	1.0	5.0	1.0	1.0	1.0	100.0
F5	4.0		2.0	5.0	5.0	1.0	5.0	1.0	1.0	1.0	100.0
F6	4.0			10	5.0	1.0	5.0	1.0	1.0	1.0	100.0
F7	4.0	1.0	2.0	5.0	5.0	1.0	5.0	1.0	1.0	1.0	100.0

**Each 4 cm^2 film contains 50 mg diclofenac sodium*

Gly. – Glycerol, Tw. – Tween, Fla. – Pineapple flavour, Asp. – Aspartame, Tit. – Titanium dioxide and C.A. – Citric acid.

3.2.4 Evaluation of formulated diclofenac sodium ODFs

3.2.4.1 Appearance

All prepared films were checked for their appearances, whether uniform, presence or absence of air bubbles among others (Arya et al., 2010).

3.2.4.2 Uniformity of thickness

The thickness of five randomly selected 2 cm × 2 cm films was determined using a digital calliper (POWERFIX, Milomex Ltd, UK). The measurements were taken along various planes of the film and the mean and standard deviation was calculated (Bhyan et al., 2011).

3.2.4.3 Determination of mean weight

The individual weights of ten (10) randomly selected 2 cm × 2 cm films were determined using an analytical balance (SN: AE 436647 Adam Equipment, UK). The average weight and standard deviations were calculated (Mishra and Amin, 2009).

3.2.4.4 pH of film

The pH of the films was determined by dissolving a 2 cm × 2 cm film in 10 ml of distilled water. The pH of the resulting solution was measured using a standardized Eutech pH meter (pH 510, pH/mV/°C meter, SN: 2025520, Singapore). The mean of five (5) determinations of each film was calculated (Arya et al., 2010).

3.2.4.5 Investigation of possible drug-excipient interaction using FTIR spectroscopy The Bruker FTIR spectrophotometer run by the Opus software (7.2 Build 7.2. 139. 1294) was set to baseline to format previous entries that may interfere with the determination. About 0.1 g of diclofenac sodium was loaded onto the stage directly on top of the platinum. The force gauge was pulled closer to the sample to compress the sample. When the setup was ready, the

Opus software generated the spectrum of the loaded sample on the monitor of a computer. The IR spectra of both albizia and khaya films (containing diclofenac sodium as API) were also determined (using the same process) to assess any possible interaction between diclofenac sodium and the other excipients in the formulation (Patil and Shrivastava, 2014). The spectra of all three samples were superimposed using the Opus software to make comparison of the spectra easier. The spectra were compared on the basis of whether or not the principal bands present in the pure diclofenac sodium were still present in the formulated diclofenac sodium ODFs.

3.2.4.6 Disintegration time of films

For the determination of the disintegration time of the film, the petri dish method was employed. Five (5) randomly selected 2 cm × 2 cm films were placed into 25 ml distilled water in a petri dish at temperature 37 ± 0.5°C. The petri dish and its contents were swirled gently every 10 seconds till the film started to break up. The time taken by the film to break up completely was recorded as the disintegration time (Chien et al., 2006).

3.2.4

.7 Determination of drug content in films

3.2.4.7.1 Calibration curve

A stock solution of diclofenac sodium of concentration 0.1 $\%^{w}/_{v}$ was prepared by dissolving 0.1 g of pure diclofenac sodium powder in a small volume of 0.1 M NaOH and then made up to 100 ml with 0.1M NaOH. The following concentrations: 0.00075, 0.00100, 0.00125, 0.00150 and 0.00250 $\%^{w}/_{v}$ were then prepared from the stock solution. The absorbance of these solutions were determined spectrophotometrically at λ-max of 276 nm and 0.1 M NaOH was used as blank. A calibration curve showing the relationship between concentration and absorbance was plotted and the equation and correlation values of the curve was generated from the scatter plot.

3.2.4.7.2 Assay

Five randomly selected 2 cm × 2 cm films of diclofenac sodium containing approximately 50 mg diclofenac sodium were placed in separate conical flasks containing 70 ml 0.1 M sodium hydroxide (NaOH). The flasks were shaken for fifteen (15) minutes using a shaker. Sufficient quantities of 0.1 M NaOH were added to produce 100 ml in each flask. The resultant solutions were filtered and 2 ml each of the filtrate was diluted to 100 ml with 0.1 M NaOH. The drug concentrations were then evaluated spectrophotometrically at a temperature of 25°C and λ-max of 276 nm by using the equation of the calibration curve (Bansal et al., 2013).

3.2.4.8 Evaluation of mechanical properties

Mechanical properties of the formulated 2 cm × 2 cm films were evaluated using Brookfield Texture Analyzer CT3-100 (with TexturePro CT Software), fitted with TA-DE and TA-DGA probes and equipped with a 10 kg load cell. The required test parameters were entered into the Texture Loader Software and the appropriate mode was chosen. A randomly selected film was placed beneath the probe and clamped between the fixture bed using low pressure clips.

It was essential that a sufficient distance of 3 cm was left between the sample surface and the base of the probe. During measurement the film was pulled at a rate of 2 mm/sec. The force at break and elongation was shown on the CT3 display when the film broke. With an attached computer and TexturePro CT software modulus, the mechanical parameters namely tensile strength, elastic modulus and percentage elongation were obtained.

The experiment was carried out in triplicate for each batch of formulation and the average and standard deviations were calculated. (Peh and Wong, 1999)

3.2.4.8.1 Folding endurance

The folding endurance of the 2 cm × 2 cm film was determined by folding the film of uniform cross-sectional area and thickness in the same place until it broke. The number of times the film was folded in the same plane (before breaking) was recorded as the folding endurance of that film. Three (3) randomly sampled 2 cm × 2 cm films of uniform thickness (per batch) were used in this experiment. An average of the three determinations was recorded as the folding endurance of that batch of films. (Arya et al., 2010)

3.2.4.9 In vitro dissolution studies

3.2.4.9.1 Calibration Curve

A stock solution of diclofenac sodium of concentration 0.1 $\%^{w}/_{v}$ was prepared by dissolving 0.1 g of pure diclofenac sodium powder in a small volume of phosphate buffer (pH 6.8) and made up to the 100 ml mark with phosphate buffer (pH 6.8). The following concentrations: 0.00075, 0.00100, 0.00125, 0.00150 and 0.00250 $\%^{w}/_{v}$ were prepared from the stock solution. The absorbance of these solutions were determined spectrophotometrically at λ-max of 276 nm and phosphate buffer (pH 6.8) was used as blank. A calibration curve showing the relationship between concentration and absorbance was plotted and the equation and correlation values of the curve was generated from the scatter plot.

3.2.4

.9.2 Dissolution Testing

Dissolution testing was conducted using Erweka dissolution apparatus (Type DT6, GmbH Heusenstamm, Germany) at 100 rpm. Six (6) dosage units of each batch were evaluated.

For in vitro dissolution studies, a 2 cm × 2 cm film was placed with the help of forceps into a vessel containing 300 ml phosphate buffer (pH 6.8) as dissolution medium, maintained at 37 ± 0.5°C. The medium was stirred at 100 rpm. 10 ml aliquots of the medium were withdrawn from a zone midway between the surface of the dissolution medium and the top of the rotating paddle blade, not less than 1 cm from the vessel wall, at 0, 7, 14, 21 and 28 minutes. 10 ml of fresh dissolution medium was added to the vessel from which the aliquots were taken. The 10 ml aliquots were filtered using Whatman filter paper (No. 5) and 5ml of the filtrate was diluted ten (10) times with phosphate buffer (pH 6.8). The absorbance of the diluted filtrates were determined by a UV-visible spectrophotometer (T90 UV/VIS spectrometer, PG Instruments Ltd, UK) at 276 nm. Using the equation obtained from the calibration curve, the concentration of diclofenac sodium in samples withdrawn at times 0, 7, 14, 21 and 28 minutes were calculated. The percentage of diclofenac sodium released with reference to the expected content in each film was then calculated (Arya et al., 2010).

3.2.4.10 Dissolution Data Comparison

A graph of cumulative mean percentage content of drug dissolved against respective time points was plotted for each batch to obtain the release profiles using Graphpad Prism (Version 5). The profiles were compared to that of the reference formulation (F1) using simple model independent approach namely; dissolution efficiency, (Anderson et al., 1998), similarity factor, (f2) and difference factor (f1), (US FDA, 1995) and one-way ANOVA.

3.2.4.10.1 Dissolution efficiency (DE)

The dissolution efficiency was calculated using the equation below;

DE= {$(0\int^{t} Y.dt) / Y100. (t_2 - t_1)$}×100

$(0\int^{t} Y.dt)$ = area under the dissolution curve

(AUC) Y= the percentage dissolved at t_2 t_2= time

for all active ingredient to dissolve $t_{1=}$ time at

which first sample was withdrawn.

The dissolution efficiency (DE) was then calculated for each batch of formulation.

3.2.4.10.2 Difference and similarity factors

Dissolution data obtained from the dissolution profiles were fitted into equations to determine the difference and similarity factors of the various brands compared to the reference formulation.

f1= {[$S_{t=1}^{n} |R_t - T_t|$] / [$S_{t=1}^{n} R_t$]} x100..... (1) f2 = 50

x log {[1+ (1/n) $S_{t=1}^{n} (R_t - T_t)^2$] $^{-0.5}$ x 100}..... (2) where f

1 = difference factor f2 = similarity factor n

= time points

R_t = cumulative percentage of drug dissolved at time t for the reference

T_t = cumulative percentage of drug dissolved at time t for the test

.10.3 One-way ANOVA analysis

The area under dissolution curve (AUC) were determined from the cumulative percentage drug released versus time curves and were compared by one-way analysis of variance (oneway

3.2.4

ANOVA) followed by Dunnett‟s test using Graphpad prism (version 5). „P‟ values were obtained after performing ANOVA test. „P‟ value gives an idea of whether the means of the paired samples are actually significantly different.

Table 3.2 Interpretation of „P‟ values

P value	Meaning
< 0.001	Extremely significant
0.001 to 0.01	Very significant
0.01 to 0.05	Significant
>0.05	Not significant

3.2.4.11 Kinetics and mechanism of drug release

The drug release data recorded were fitted into the zero order, first order, Higuchi and Hixson-Crowell equation models to determine the release mechanism. The model that produced good linearity by a high R^2 value was considered the best to describe the release kinetics of the formulated films (Singhvi and Singh, 2011).

CHAPTER 4

RESULTS

After carrying out the necessary tests on both crude and purified albizia and khaya gums as well as the seven formulated batches of diclofenac sodium ODFs, the following results were obtained;

4.1 Purification of albizia and khaya gums

The crude gums were taken through a purification procedure to get rid of extraneous materials. After the purification, the yield was calculated from the equation below. The yield of khaya was satisfactory but that for albizia was lower than expected.

4.1.1 Percentage yield of purification

$$\frac{Final\ weight\ of\ gum\ after\ purification}{Initial\ weight\ of\ gum\ before\ purification} \times 100$$

%

Hence for albizia gum,

Percentage yield = $\frac{315\ g}{800\ g}$ x 100 %

= **39.38 %**

For khaya gum,

Percentage yield = $\frac{540\ g}{800\ g}$ x 100 %

= **67.50 %** **4.2 Physicochemical tests on albizia and khaya gums**

Physicochemical tests were conducted on albizia and khaya gums to obtain in-depth characterization details on both gums. This will add onto the literature already gathered on both gums.

4.2.1 Physical appearance of albizia and khaya crude gums

The physical appearance of both albizia and khaya gums are outlined in the table below. These parameters play very important roles in the identification, characterization and evaluation of both albizia and khaya gums.

Table 4.1 Macroscopic properties of crude albizia and khaya gums

Characteristics	Albizia	Khaya
Colour	Yellow to dark brown	Colourless to reddish brown
Odour	Odourless	Odourless
Taste	Bland	Tasteless
Surface appearance	Smooth	Not too smooth
Size	4-7 cm long, 3 mm wide	2-5 cm long, 1.5 mm wide
Form	Round elongated tears	Slender translucent tears
Fracture	Thin ones fracture easily	Fractures easily

4.2.2 Moisture content and insoluble matter of gums

Moisture content determination helps to quantify the amount of water contained in a powdered sample. The moisture content in crude albizia was higher than that of the purified gum, but in khaya, the purified gum showed a slightly higher moisture content than the crude gum.

Generally, purified gums of albizia and khaya contained a lower amount of insoluble matter compared to the crude gums.

Table 4.2 Moisture content and insoluble matter of albizia and khaya gums, n= 3

Gum	Moisture content (%)	Insoluble matter (%)
Crude albizia	13.11 ± 0.535	0.530 ± 0.304
Purified albizia	12.42 ± 2.098	0.275 ± 0.041
Crude khaya	13.63 ± 2.586	0.450 ± 0.578
Purified khaya	13.92 ± 1.507	0.282 ± 0.013

4.2.3 Solubility of gums in various solvents

The results for the solubility of the purified gums of both albizia and khaya in cold water, warm water, acetone, chloroform and ethanol is shown in Table 4.3 below. In all instances, albizia showed higher solubility than khaya under the same conditions.

Table 4.3 Solubility of purified albizia and khaya gums in various solvents, n= 3

Solvent	Albizia (%)	Khaya (%)
Cold water	1.273 ± 0.064	0.380 ± 0.020
Warm water	1.747 ± 0.083	0.527 ± 0.064
Acetone	0.110 ± 0.044	0.127 ± 0.031
Chloroform	0.070 ± 0.062	0.020 ± 0.020
Ethanol (96 %)	0.093 ± 0.023	0.087 ± 0.042

4.2.4 Swelling index and water retention capacity of gums

As natural hydrocolloids, gums absorb water and dissolve or swell in it, the degree of which depends on the type of gum and the prevailing conditions. Albizia shows a higher swelling index and also retains slightly more water than khaya under similar conditions.

Table 4.4 Swelling index and water retention capacity of purified gums, n= 3

Gum	Swelling Index (%)	Water retention/10 ml
Albizia	611.290 ± 4.072	8.467 ± 0.153
Khaya	477.973 ± 8.675	7.667 ± 0.208

4.2.5 True density and temperature of charring of gums

All matter has mass and can occupy volume. Density gives an understanding of this property of matter. The true density of albizia is slightly lower than khaya. Albizia however, chars at a slightly higher temperature than khaya gum.

Table 4.5 True density and temperature of charring of purified albizia and khaya gums, n= 3

Gum	True Density (g/ml)	Temperature of C (
Albizia	1.363 ± 0.012	258.33 ± 2.887
Khaya	1.412 ± 0.073	242.67 ± 2.517

4.2.6 pH studies on 1 % mucilage of albizia and khaya gums, n= 3 pH studies of albizia and khaya gums was conducted over a period of five weeks under different conditions to know how those conditions affect the pH of the gums. The graphs shown below in Figures 4.1, 4.2 and 4.3 summarize the observations made over the fiveweek period.

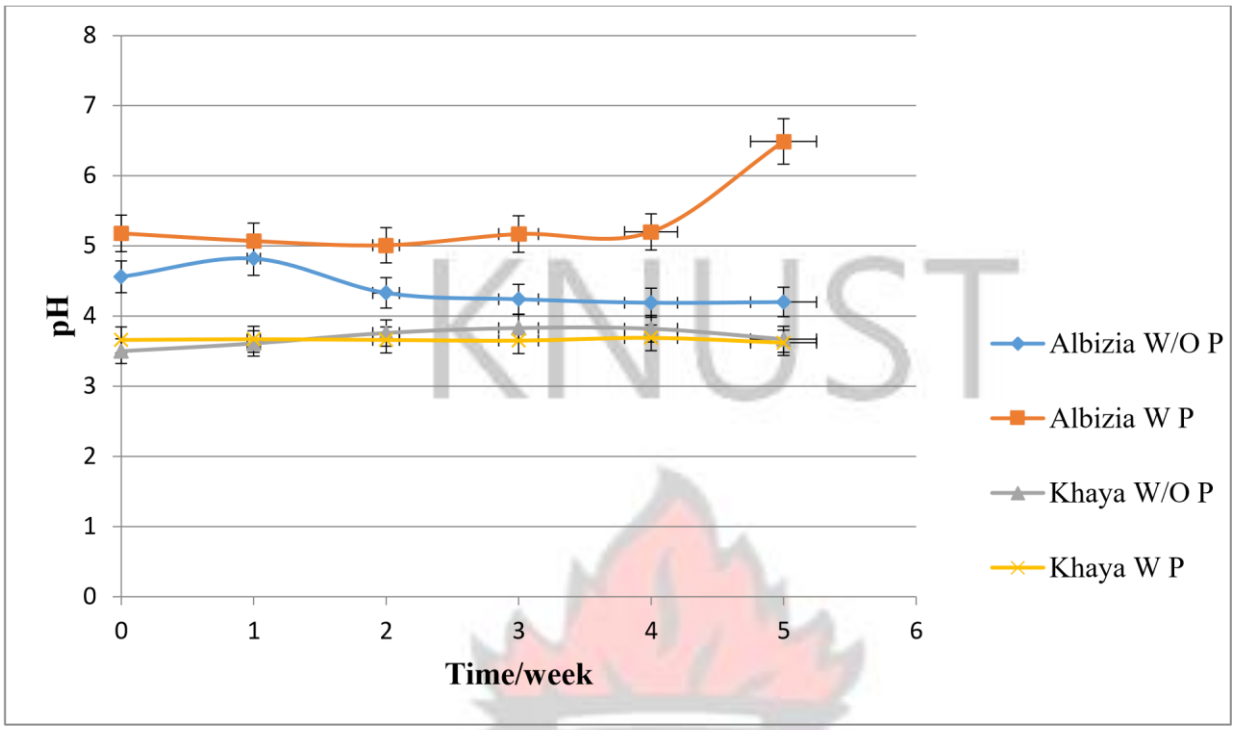

Figure 4.1 Effect of preservation on pH of 1 % albizia and khaya mucilage

Key: W/O P – without preservative
W P – with preservative

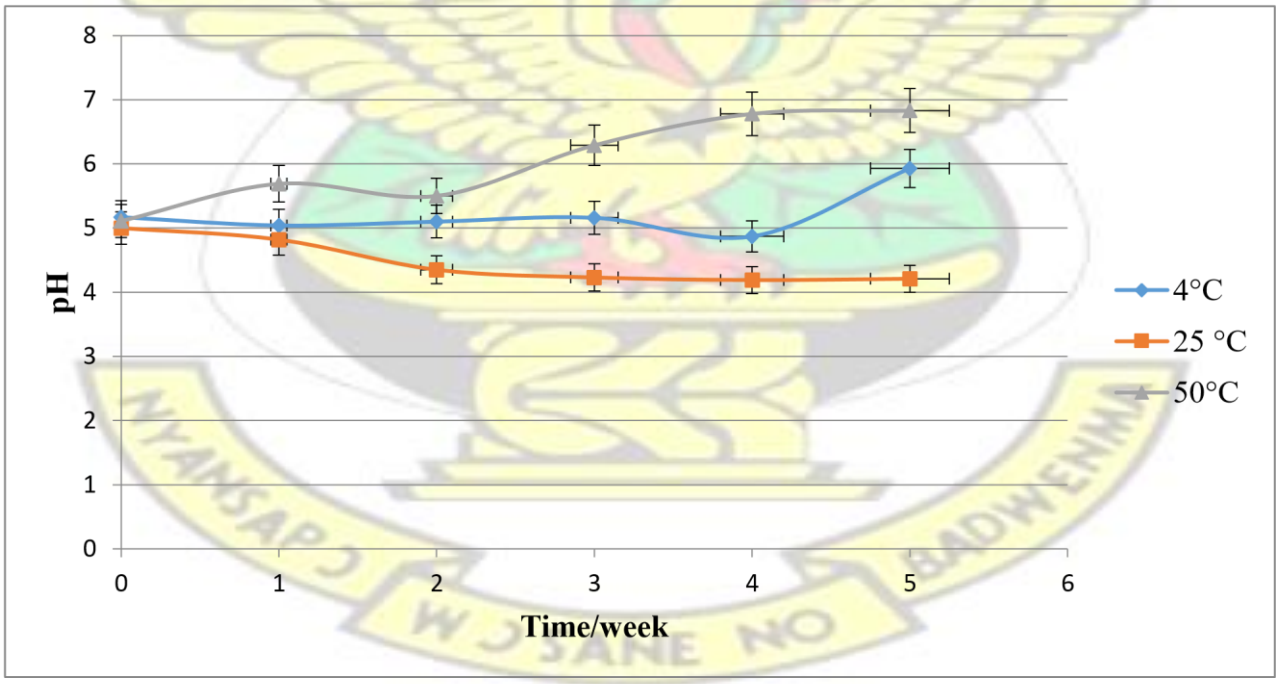

Figure 4.2 Effect of storage temperature on pH of 1 % albizia mucilage

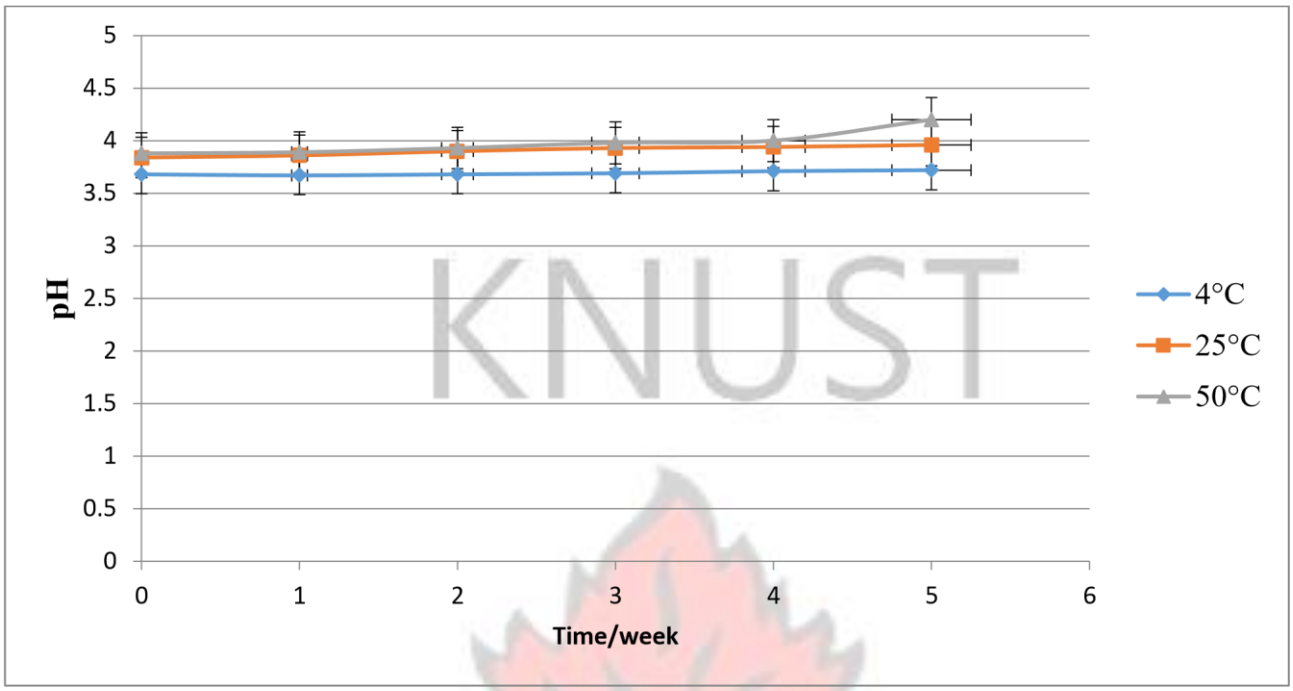

Figure 4.3 Effect of storage temperature on pH of 1 % khaya mucilage

4.2.7 Flow properties of gums

The rate of flow of mucilages prepared from both albizia and khaya gums were studied under conditions of applied stress and temperature. The graphs shown in the Figures 4.4 and 4.5 below describes the flow properties of both gums under varying conditions.

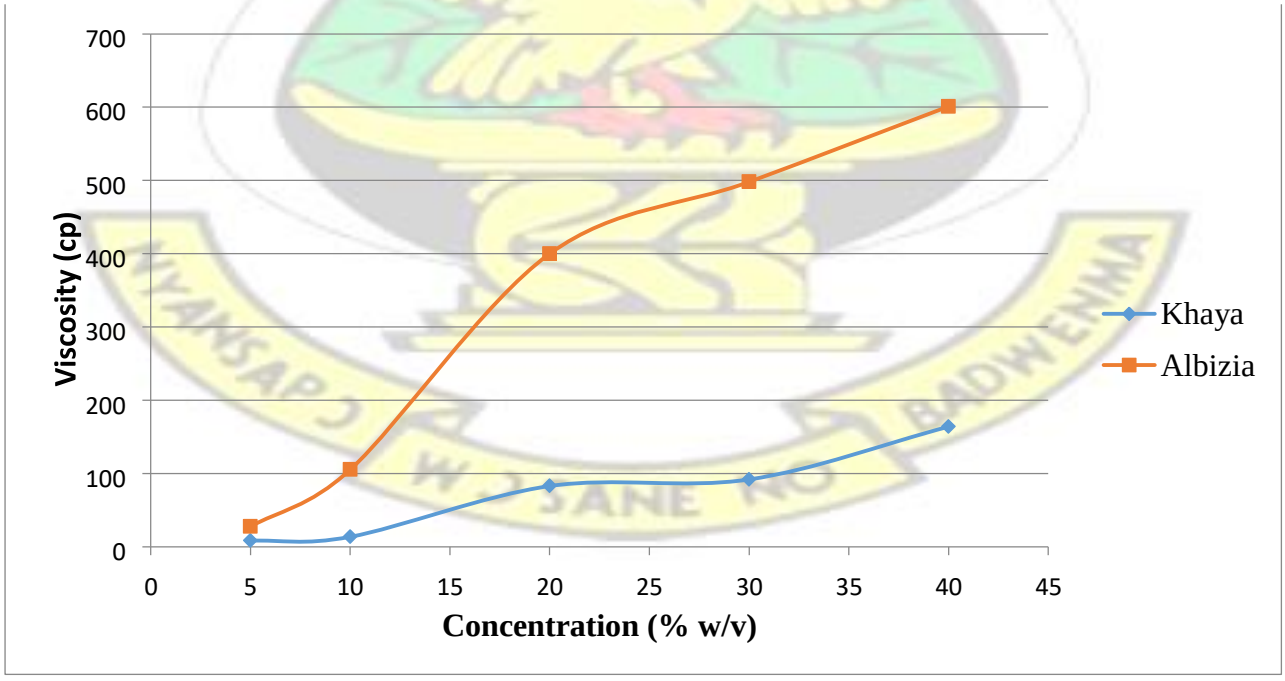

Figure 4.4 Effect of concentration on viscosity of mucilage

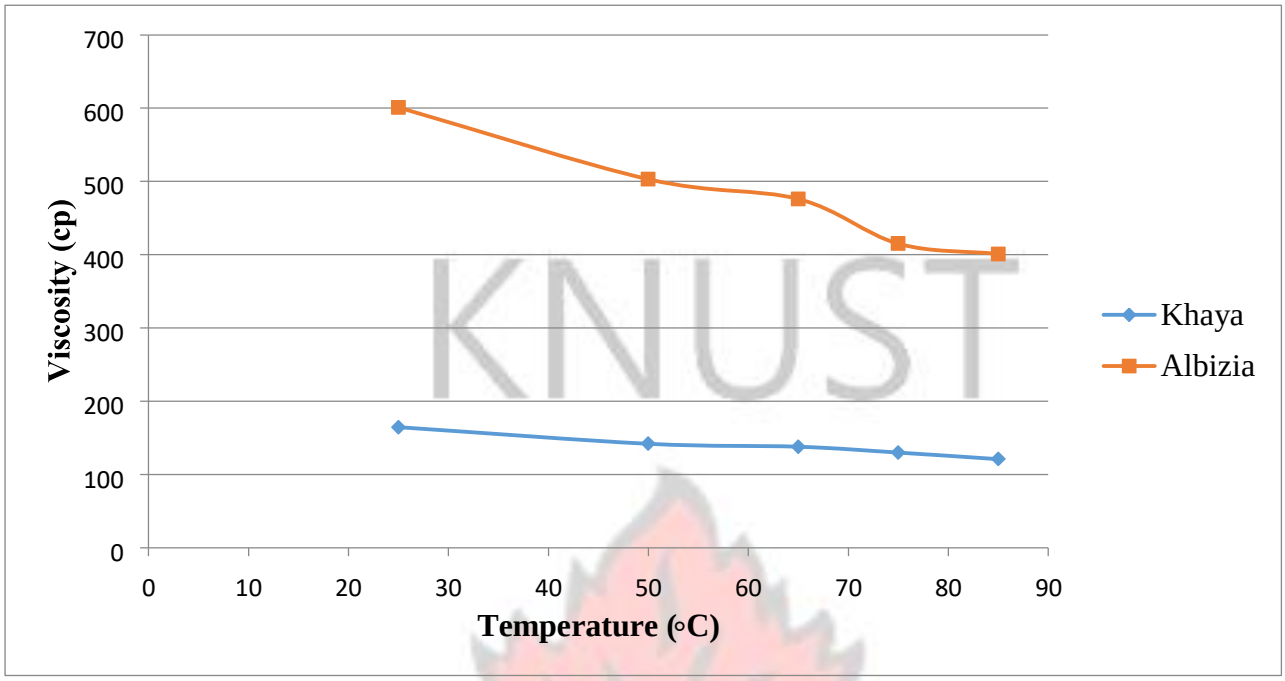

Figure 4.5 Effect of temperature on viscosity of mucilage

4.2.8 Microbial quality of purified gums

All pharmaceutical raw materials are expected to be microbiologically wholesome for safe use. The tables below summarize the observations made when 1 % mucilage of albizia and khaya gums were inoculated into different sterile media. Both gums were free from potentially pathogenic microorganisms.

Table 4.6 Microbial quality of 1 % albizia mucilage

Selective medium	**Results**	**Inference**
Bismuth sulphite agar	No black colonies observed	Salmonella absent
Cetrimide agar	No growth observed	Pseudomonas absent
MacConkey agar	Whitish colonies present.	Non - lactose fermenting enterobacteria present
Mannitol agar	No growth observed	Halophiles absent, therefore *Staph. aureus* absent
Nutrient agar	Whitish colonies observed	Aerobic non - fastidious microorganisms present
Sabouraud agar	Creamish colonies observed	Fungi may present

Table 4.7 Microbial quality of 1 % khaya mucilage

Selective media	Results	Inference
Bismuth sulphite agar	No black colonies observed	Salmonella absent
Cetrimide agar	No growth observed	Pseudomonas absent
MacConkey agar	No visible growth observed.	Non - lactose fermenting enterobacteria absent
Mannitol agar	No growth observed	Halophiles absent, therefore *Staph. aureus* absent
Nutrient agar	Whitish colonies observed	Aerobic non – fastidious microorganisms present
Sabouraud agar	Creamish colonies observed	Fungi may present

4.2.9 Antimicrobial activity of purified gums

Concentrations of 3, 4 and 5 $\%^{w}/_{v}$ of both albizia and khaya did not exhibit any antimicrobial activity against *Bacillus subtilis* (NTCC 10073), *Candida albicans* (clinical strain), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (clinical strain), *Pseudomonas aeruginosa* (ATCC 4853), *Salmonella typhi* (clinical strain), *Staphylococcus aureus* (ATCC 25923), and *Streptococcus pyrogenes* (clinical strain). This is because no zones of growth inhibition were observed against the test organisms.

4.2.10 Mineral content of purified albizia and khaya gums

In the mineral analysis of the two gums, it was observed that both were free from toxic heavy minerals like mercury etc. Figure 4.6 below compares the mineral content of albizia and khaya.

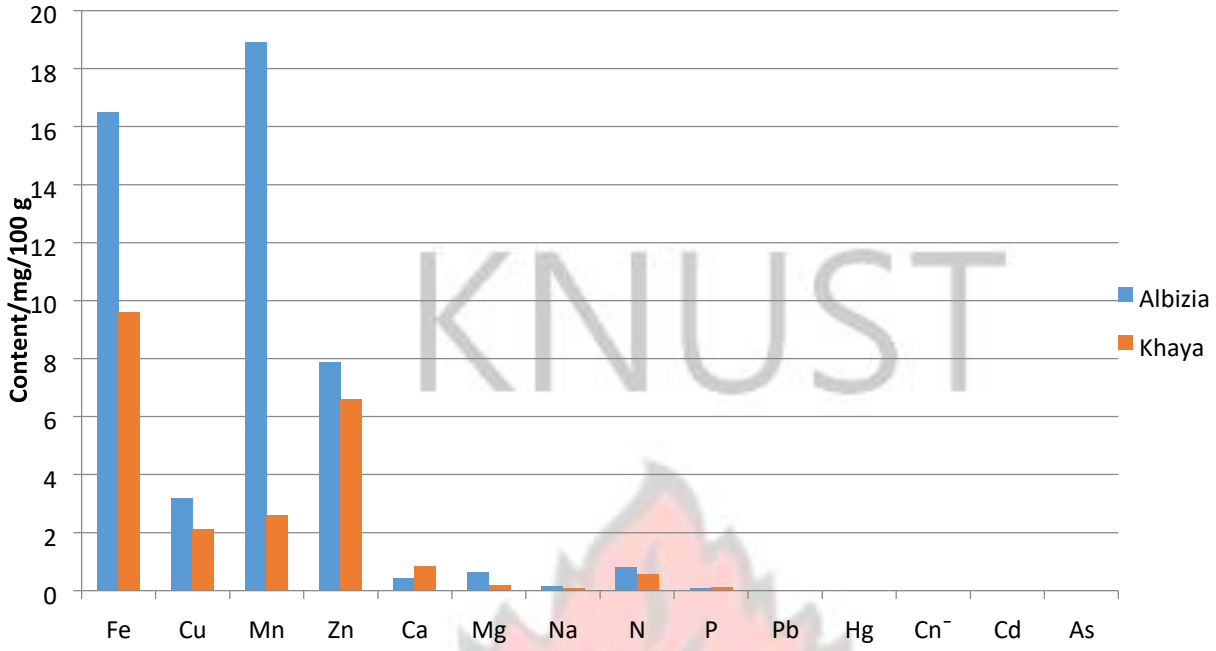

Figure 4.6 Mineral content of purified albizia and khaya gums, n= 2

4.2.11 Ash values of purified albizia and khaya gums

Ash value determination is key to detecting any adulteration in crude samples. The results of ash values of both albizia and khaya is outlined in the table below.

Table 4.8 Ash values of purified albizia and khaya gums, n= 3

Ash value	Albizia	Khaya
Total ash (%w/w)	7.883 ± 0.021	5.603 ± 0.015
Water soluble ash (%w/w) Acid insoluble ash (%w/w)	1.290 ± 0.020 0.613 ± 0.025	1.203 ± 0.023 0.597 ± 0.015

4.3 Formulation of films

The films were formulated using the solvent casting method. Plates 4.1 and 4.2 show snapshots of the cast films and 2 cm × 2 cm sized films respectively.

Plate 4.1 Snapshot of formulated films

Plate 4.2 Snapshot of 2 cm × 2 cm films

4.4 Evaluation of various formulations of diclofenac sodium ODFs

The assessment of the formulated batches of diclofenac sodium ODFs is important to ensure that the films are within specifications in terms of quality, safety and efficacy.

4.4.1 Physical characteristics of formulated ODFs

The formulated films were evaluated for physical defects and inconsistencies. All seven batches of formulations were opaque with uniform intra-batch thickness and pH. The details of the various parameters are shown in the table below.

Table 4.9 Appearance, thickness, (n= 5) and pH, (n= 3) of formulations

Code	Appearance	Thickness (mm)	pH
F1	Opaque	0.128 ± 0.013	4.66 ± 0.452
F2	Opaque	0.128 ± 0.015	5.08 ± 0.181
F3	Opaque	0.142 ± 0.008	4.82 ± 0.084
F4	Opaque	0.138 ± 0.023	3.80 ± 0.035
F5	Opaque	0.126 ± 0.013	3.09 ± 0.026
F6	Opaque	0.108 ± 0.008	3.62 ± 0.060
F7	Opaque	0.174 ± 0.011	5.23 ± 0.043

4.4.2 Weight uniformity and disintegration time of formulations

As with all pharmaceutical dosage forms, the uniformity of weight and the time taken for the films to disintegrate was determined for each batch. All ten randomly selected 2 cm × 2 cm films showed minimal variation in weight judging from the insignificant standard deviations recorded. The disintegration time of each of the batches is also shown in the table below.

Table 4.10 Mean weight, (n= 10) and disintegration time (n= 5) of formulations

Code	Mean weight/g	Disintegration time/s
F1	0.186 ± 0.019	43.19 ± 0.077
F2	0.250 ± 0.016	37.16 ± 0.043
F3	0.194 ± 0.023	41.23 ± 0.070
F4	0.208 ± 0.019	40.32 ± 0.081
F5	0.254 ± 0.011	38.33 ± 0.053
F6	0.116 ± 0.011	43.13 ± 0.058
F7	0.298 ± 0.095	47.45 ± 0.050

4.4.3 Investigation of possible drug-excipient interaction using FTIR spectroscopy All the principal bands present in the spectrum for pure diclofenac sodium were still present after it was formulated into ODFs using albizia and khaya gums as film forming polymers.

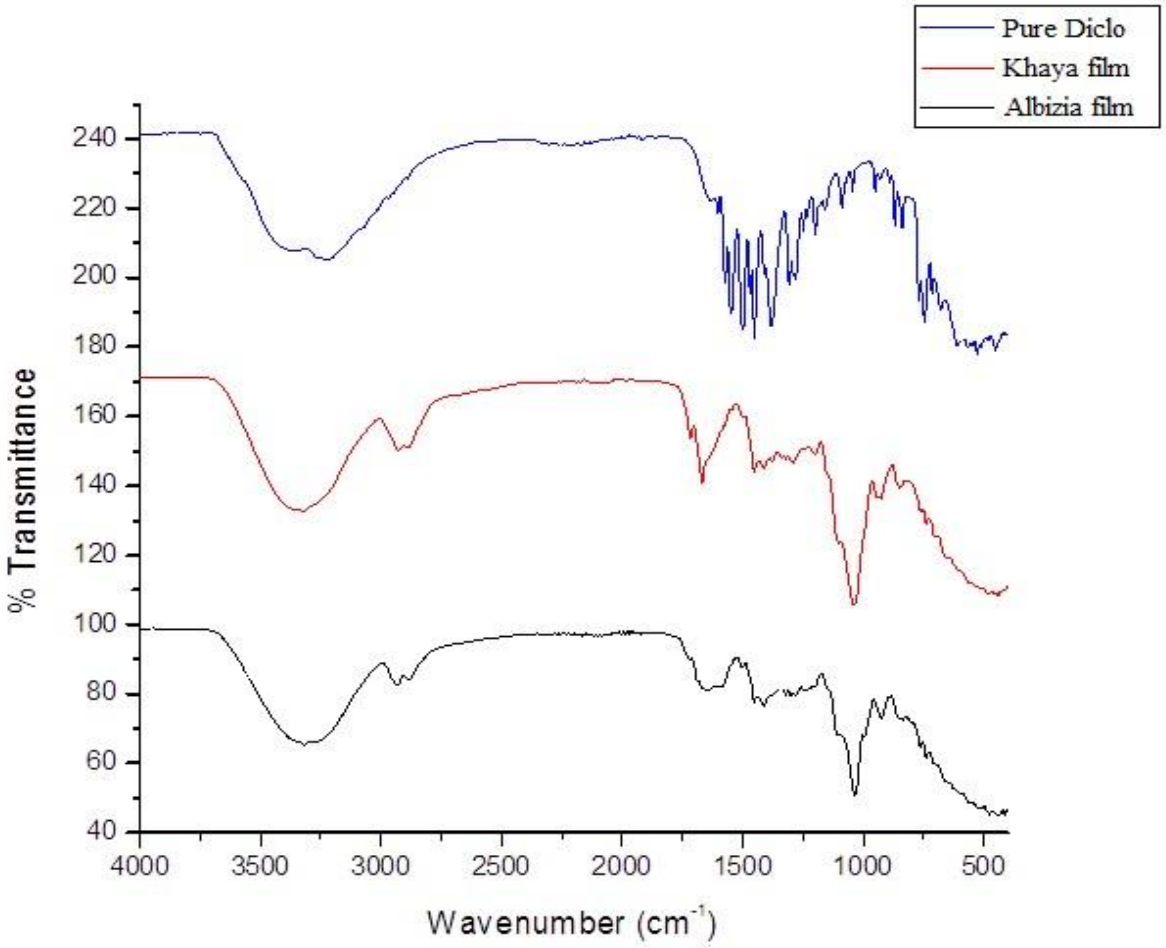

Figure 4.7 FTIR spectra of diclofenac sodium, khaya film and albizia film

4.4.4 Drug content of formulations

For optimum quality, safety and efficacy of a pharmaceutical dosage form, the content of API is very crucial. Too high or too low drug content is undesirable. The BP (2013) provides assay specifications for most APIs. UV analysis was used in this study to quantify the amount of diclofenac sodium contained in the various formulations. All the batches of formulations passed this test.

Table 4.11 Calibration of diclofenac sodium in 0.1 M NaOH

Concentration (%w/v)	**Absorbance**

0.0025	0.764
0.0015	0.442
0.00125	0.356
0.001	0.276
0.00075	0.163

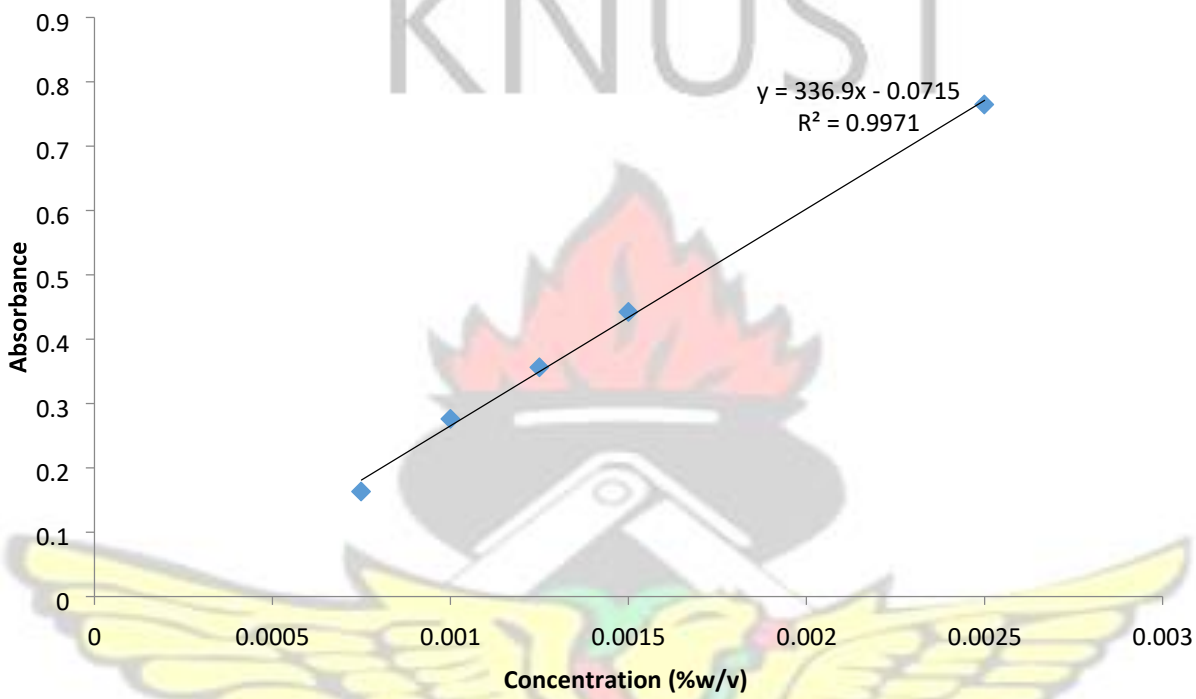

Figure 4.8 Calibration curve of diclofenac sodium in 0.1 M NaOH

Table 4.12 Assay of formulations, n= 5

Code	Assay (%)
F1	98.74 ± 0.448
F2	99.46 ± 0.337
F3	99.94 ± 0.388
F4	100.41 ± 0.212
F5	99.68 ± 0.542
F6	97.58 ± 0.231
F7	98.82 ± 0.734

4.4.5 Mechanical properties of various formulations

For easy handling and durability, ODFs are expected to possess optimum mechanical strength, good stretch and flexibility. Tensile strength, elastic modulus, percentage elongation and

folding endurance are mechanical parameters that were evaluated. The details are shown in the tables below.

Table 4.13 Tensile strength, elastic modulus and percentage elongation of formulations, n= 5

Code	Tensile strength (MPa)	Elastic modulus (MPa)	Percentage elongation (%)
F1	5.95 ± 0.976	4.20 ± 0.786	17.87 ± 0.432
F2	6.32 ± 0.542	3.89 ± 0.321	17.64 ± 0.156
F3	6.51 ± 0.985	4.39 ± 0.465	13.49 ± 0.768
F4	5.67 ± 0.231	4.05 ± 0.654	15.91 ± 0.563
F5	6.14 ± 0.331	4.46 ± 0.943	10.73 ± 0.105
F6	7.32 ± 0.432	4.86 ± 0.543	8.20 ± 0.445
F7	7.19 ± 0.652	5.11 ± 0.213	7.65 ± 0.154

Table 4.14 Folding endurance of formulations, n= 3

Code	Folding endurance
F1	85 ± 1.567
F2	103 ± 2.056
F3	54 ± 1.564
F4	67 ± 1.452
F5	79 ± 2.152
F6	39 ± 0.976
F7	46 ± 1.568

4.4.5 In vitro dissolution studies of formulations

Drug dissolution is an important step in drug delivery and related technology because until a drug dissolves in physiological fluid, it cannot be absorbed. The in vitro dissolution testing was carried out using the paddle method with Erweka dissolution apparatus and the analysis was done using UV. A calibration curve was drawn for diclofenac sodium in phosphate buffer, pH 6.8.

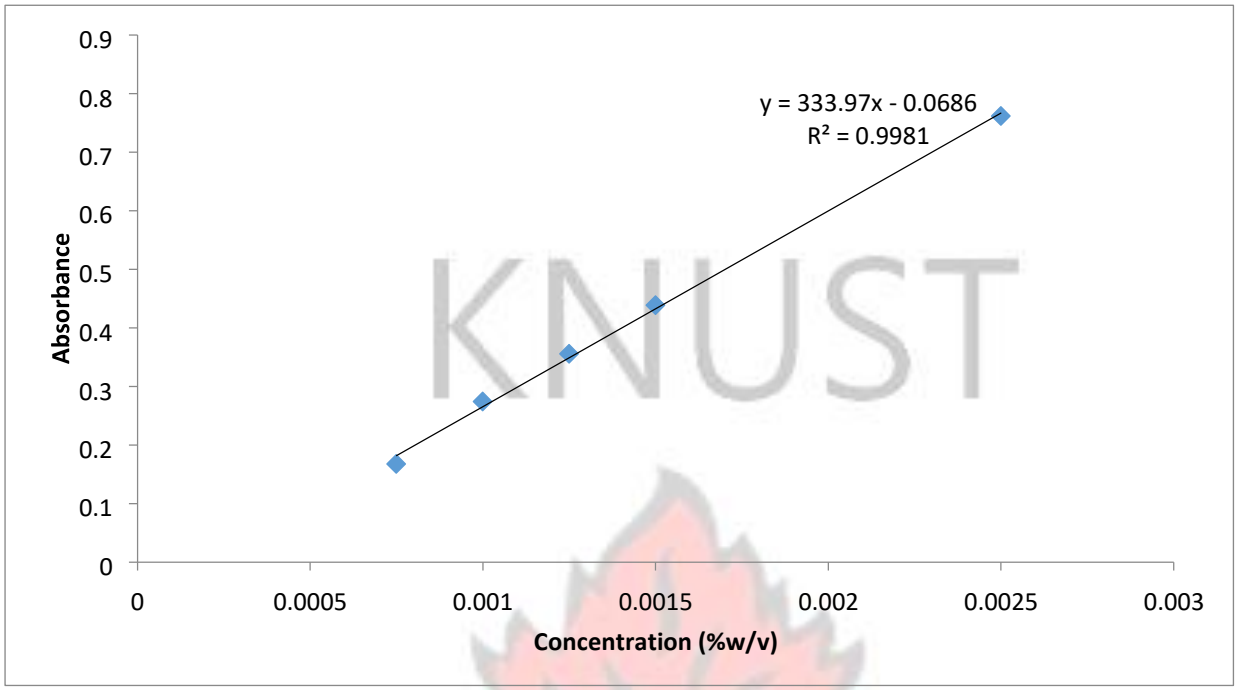

Figure 4.9 Calibration curve of diclofenac sodium in phosphate buffer (pH 6.8)

Table 4.15 Percentage drug released at various times

	Percentage drug released at various time intervals (%)			
Code	**7 min**	**14 min**	**21 min**	**28 min**
F1	86.22 ± 0.208	94.26 ± 0.472	96.39 ± 0.271	98.42 ± 0.185
F2	75.74 ± 0.649	84.69 ± 0.484	92.23 ± 0.180	96.61 ± 0.360
F3	69.05 ± 0.442	85.69 ± 0.474	96.32 ± 0.289	99.44 ± 0.163
F4	89.28 ± 0.453	93.38 ± 1.931	97.21 ± 2.315	99.32 ± 1.417
F5	77.84 ± 0.632	86.20 ± 0.618	94.74 ± 0.442	97.48 ± 0.270
F6	79.04 ± 0.208	88.26 ± 0.632	95.52 ± 0.586	97.48 ± 0.191
F7	51.07 ± 0.208	63.48 ± 0.360	75.72 ± 0.459	82.94 ± 0.288

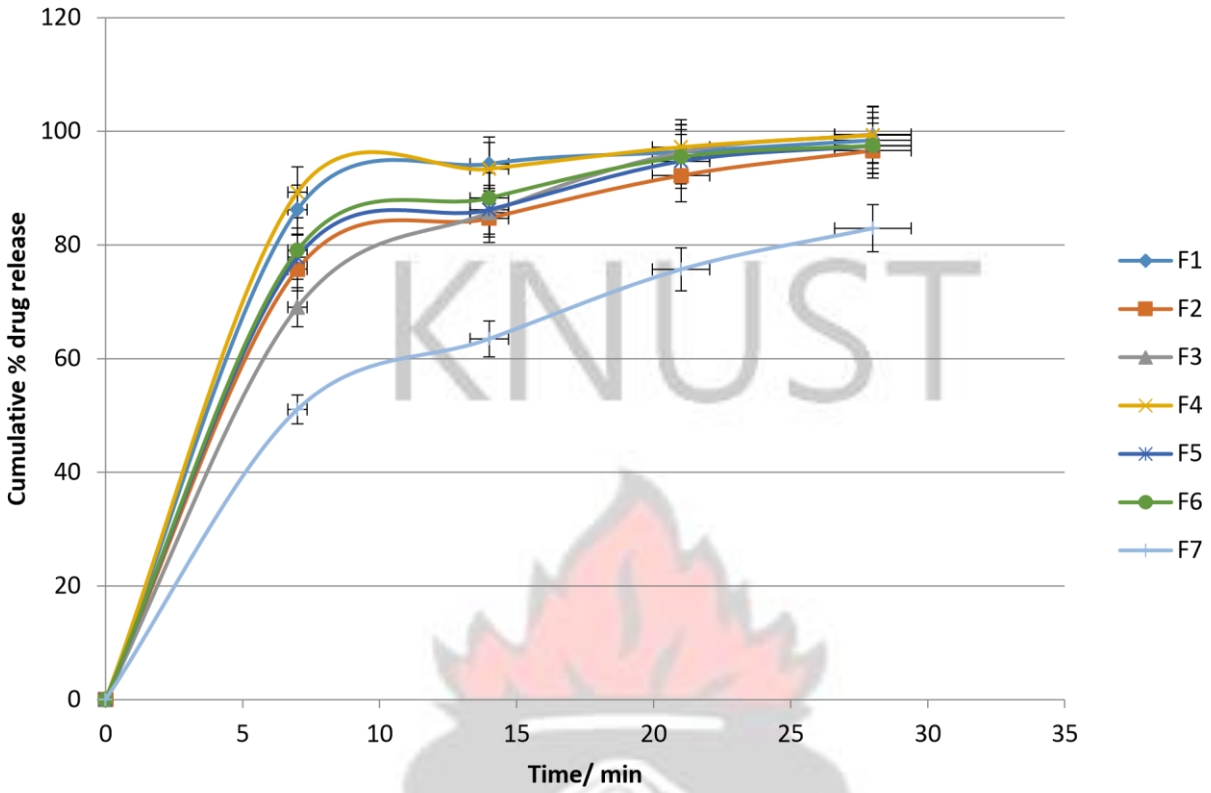

Figure 4.10 Dissolution profiles of formulations in phosphate buffer (pH 6.8)

4.4.6 Dissolution data comparison using F1 as reference formulation

The data obtained from the in vitro dissolution testing was compared to a reference formulation, F1, on the basis of their dissolution efficiencies, difference and similarity factors and the P values obtained from one-way ANOVA followed by Dunnett‟s plot, the details of which are summarized in the tables below.

Table 4.16 Dissolution efficiencies of the formulations

Code	Dissolution efficiency (%)
F1	95.85
F2	91.88
F3	89.27
F4	89.37
F5	91.84
F6	93.01
F7	82.85

Table 4.17 Difference factor, (f1) and similarity factor, (f2) of the formulations

Code	Difference factor (f1)	Similarity factor (f2)
F1	- 7	-
F2	7	61
F3	2	55
F4	5	89
F5	4	66
F6	27	70
F7		33

Table 4.18 One-way ANOVA followed by Dunnett‟s test

One-way ANOVA followed by Dunnett's multiple comparisons test summary	
F1 vs F2	NS
F1 vs F3	NS
F1 vs F4	NS
F1 vs F5	NS
F1 vs F6	NS
F1 vs F7	SD *

ANOVA summary: SD - significantly different (P value $< 0.001)$ and NS - not significant ($P > 0.05$)

4.4.7 Kinetics and mechanism of drug release

Several mathematical models have been used to describe the release kinetics of pharmaceutical dosage forms. In the current study, the dissolution data obtained were fitted into these models to see which of them best elucidates the release kinetics of the individual formulations. It was seen that, all the formulations fitted Higuchi‟s model of drug release.

The R^2 values and release constants for the various models are shown in the table below.

Table 4.19 Drug release kinetic models of the various formulations

Code	Release kinetics models							
	Zero order		**First order**		**Higuchi**		**Hixson-Crowell**	
	K_0	**R^2**	**K_1**	**R^2**	**K_H**	**R^2**	**K_{HC}**	**R^2**
F1	32.54	0.8685	0.163	0.8580	34.46	0.9250	0.505	0.8615
F2	60.09	0.9834	0.979	0.9736	63.04	0.9989	0.865	0.9651
F3	87.18	0.9097	0.428	0.8860	85.29	0.9575	1.497	0.8942
F4	29.08	0.9793	0.146	0.9755	30.11	0.9938	0.976	0.9770
F5	57.79	0.9464	0.946	0.9400	58.80	0.9722	0.955	0.9601

F6	53.6	0.9144	0.266	0.9059	55.71	0.9554	0.893	0.9089
F7	92.4	0.9853	0.874	0.9704	96.24	0.9941	1.896	0.9753

CHAPTER 5

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Purification of albizia and khaya gums

Crude exudate gums are obtained from incisions made on the stem of the trees that produce them. Mostly they are left to harden on the bark before they are collected. As they exude, foreign materials collect on them; these include dust, dirt, smoke, debris, pigments, and phytochemicals in the tree bark, among others. These sometimes make the gum appear darker than those that have not aged on the stem of the tree (Nussinovitch, 2009). The crude exudates are therefore taken through a purification process to remove all these impurities so as to improve upon their appearance and the quality of the gums.

The percentage yields obtained from the purification of albizia and khaya gums were 39.38 % and 67.50 % respectively. From the results, it can be inferred that the yield, especially for albizia, was less satisfactory. This may be due to the extraction procedure not being too well suited to the species of the gums. It was however realised that, slight modification of the purification process produced better results. For instance, milling the dried crude gum into coarser particles (instead of fine) and soaking it in four times its volume of double strength chloroform water improved the yield. Also, serial precipitation of filtered gum solution with 96 % ethanol further increased the yield. For gums with very poor yield, salting out method can be employed to obtain better yield (Molla et al., 2014).

5.1.2 Evaluation and characterization of natural products

The evaluation of natural products used in the pharmaceutical industry is important because it helps in the identification of biochemical variations in natural products. This may be due to collection

from different plants of the same species growing at different geographic location or the same plant at different times. Also, it helps in the assessment of the extent of breakdown due to purification, treatment and storage. But most importantly, it helps to detect adulteration and/or substitution (Shehzadi, 2014). In general, evaluation ensures accurate identification as well as the safety and quality of natural products.

5.1.2.1 Macroscopic properties of albizia and khaya gums

Table 4.1 shows the macroscopic features of crude albizia and khaya gums. These characteristic features serve as identification tools employed in the authentication of these gums. The physical form of the exudates mostly depends on how the molten gum exudes from the incision site. As it flows out, it hardens upon contact with air and assumes characteristic forms.

The colour partly depends on extraneous materials that come into contact with the gum, especially those that age on the tree. Smith and Montgomery (1959) reported that, gums that remain on the bark for longer periods accumulates lots of tannins that imparts darker colouration to them. Also, how the incisions on the stem are made as well as conditions under which they are made also play a role. For instance, if fire was set beneath the tree to facilitate exudation of the gum, the smoke from the fire can affect the colour of the exudates.

The age of the exudates on the bark of the tree influences how the tears fracture. Generally, exudates that remain on the bark for longer periods tend to be dry and brittle, hence they fracture easily. Conversely, not too dry masses are gummy, tough and do not fracture easily.

Gums are generally odourless or nearly so (Howes, 1949) and may be tasteless or generally devoid of any distinctive taste (Lewis, 1791). Both albizia and khaya exudates were bland and odourless, properties that make them well suited for use as pharmaceutical excipients.

5.1.2.2 Moisture content

Moisture content of natural products may be responsible for decomposition of crude material due to chemical change and/or microbial invasion. Moisture content determination is very important in the pharmaceutical industry because it affects the flow properties and microbiological stability of powders. High moisture content may encourage microbial growth and cause some powders to clump together forming hard aggregates. Also, in economic terms, one may not get value for money because using raw weights of powders can be elusive, such that, if a powder has a high moisture content, it appears heavier than it actually is.

The moisture contents of both purified and crude albizia and khaya gums are shown in Table 4.2. For albizia, the moisture content of the purified gum was lower than the crude gum. This may be as a result of the extraction and purification procedure; since it involves drying of the purified gums before milling into fine powder. Also, 96 % ethanol that is used in the precipitation of the pure gum has dehydrating properties and so may have partly influenced the moisture content of the purified gum.

For khaya, however, the moisture content was marginally higher in the purified gum. Generally, purified gums absorb water molecules more readily than crude gums. This is why after purification, the gums should be placed in air-tight plastic pouches and stored in desiccator containing silica gel.

The British Pharmacopoeia (2013) sets the standard for moisture content at not exceeding 15 %w/w. Therefore, it can be inferred that, both crude and purified gums complied with the specifications of the BP (2013).

5.1.2.3 Insoluble matter

Most insoluble matter present in gums are due to either foreign matter that find themselves in the exudates as they remain on the bark (Howes, 1949), or due to the mode of collection. It is therefore not surprising that the percentage of insoluble matter in the crude gum is higher than the purified gum, as seen in Table 4.2. It can therefore be inferred that, the purification process removes most of the insoluble extraneous matter from the gum.

According to the BP (2013), a limit of 0.5 %w/w insoluble matter is allowed for gums sourced from nature. From the results, all except crude albizia passed the test. The deviation, though insignificant can be resolved by adopting proper and hygienic harvesting techniques.

5.1.2.4 Solubility of purified albizia and khaya gums in various solvents

Gums are generally soluble in water at low concentrations; at high concentrations however, they tend to absorb water, swell and form very viscous solutions. They are however insoluble in oil and most organic solvents. Gums may be soluble in aqueous ethanol up to a limit of about 60 % because of the water component (Nussinovitch, 2009).

The results for the solubility of both purified gums of albizia and khaya gums in various solvents is shown in Table 4.3. A slight increase was observed in the solubility of both gums in warm water compared to cold water. This can be explained that, increase in temperature facilitates solubility because solute molecules acquire more kinetic energy at such elevated temperatures making them more mobile to interact with solvent molecules.

The purified gums showed slight solubility in both acetone and chloroform. Both solvents are organic and slightly polar with polarity indices of 5.1 and 4.1 respectively (Burdick & Jackson‟s Solvents Database). Their polarity is responsible for the slight solubility of both gums in them.

Gums are practically insoluble in 96 % ethanol, a characteristic that is exploited during the precipitation of pure gum from aqueous solutions. This explains why both albizia and khaya showed insignificant solubility in 96 % ethanol.

5.1.2.5 Swelling index and water retention capacity of purified albizia and khaya gums

Gums are hydrocolloids and in aqueous media, thicken due to liquid absorption and resultant swelling of the gums. This informs their use as suspending, gelling and thickening agents in the pharmaceutical industry (Jani et al, 2009).

Swelling index is an essential characteristic of a hydrophilic polymer intended for use in ODFs because it is directly associated with the rate at which the active pharmaceutical ingredient in the film will be released. This is because, the ODF will have to absorb water, swell and then break up to release the drug for dissolution and oromucosal absorption (Arya et al., 2010).

The results for the swelling index and water retention capacity determination is shown in Table 4.4. It can be said that, both albizia and khaya have good swelling and water retention capacities because they can swell up to six and four times their original size respectively. It can however be inferred that, albizia is more hydrophilic and retains more water than khaya under similar conditions.

5.1.2.6 True density and temperature of charring of purified albizia and khaya gums True density of a solid is the mass per unit volume exclusive of all voids that are not constitutive parts of the molecular packing arrangement (Ghosh and Jasti, 2005). True density determination plays an essential part in the uniformity of content of solid dosage forms, drug dissolution and their production. Also, in completely characterizing both albizia and khaya gums, their densities are key to their identification. From the results in Table 4.5, the densities of purified albizia and khaya gums are 1.363 and 1.412 g/ml respectively.

An attempt was made to determine the melting points of both purified albizia and khaya gums. It was however realized that, for both albizia and khaya, beyond 258 and 242°C respectively, the gums changed appearance (charred), the change becoming more pronounce as the temperature increased. They however failed to melt. The temperature at which the gums changed appearance was referred to as "temperature of charring". Charring is a chemical process of incomplete combustion of certain solids when subjected to high heat. Charring removes hydrogen and oxygen from solid substances by the action of heat so that the remaining char is primarily carbon. The temperature of charring is partly affected by density and moisture content and can be effectively applied for instance, in product development to set limits for certain internal and external parameters that can affect the appearance of the gum. For instance knowledge of the temperature of charring of gums will inform the choice of formulation procedures (internal parameter) that will avoid such high temperatures that can cause charring of the gum. Also, limits may be set for external parameters like storage conditions of the gums before formulation, during formulation as well as the final products to ensure that the gums do not change in appearance.

Also the absence of a sharp melting points indicates that both gum samples are not pure compounds but a mixture of components, buttressing findings of several studies already conducted in this regard (Howes, 1949; Verbeken et al., 2003; Dror et al., 2006).

5.1.2.7 pH studies pH is a numeric scale used to identify the acidity or alkalinity of an aqueous solution. It is the negative logarithm (to base 10) of the activity of the hydrogen ion. Solutions with pH less than 7 are acidic and those with pH greater than 7 are alkaline or basic.

pH studies on gums and mucilages is important because, beyond a certain pH, the viscosity of some mucilages change. For instance, Calvo et al. (1998) reported that, the viscosity of gums tend to

increase with increase in pH indicating that, the pharmaceutical applications of gums (suspending agents, thickening agents, etc.) that are dependent on their viscosity are in turn pH-dependent.

In this research, the pH was studied over a period of five (5) weeks and certain conditions (storage temperature, use of antimicrobial preservative or not) were varied to establish their effect or otherwise on pH of the aqueous gums. Different storage temperatures of 4, 25 and 50°C were chosen to investigate their effect on the pH of the mucilage (formed from the gums) with time. 0.1 % propylhydroxy benzoate was used to preserve one group of the aqueous gums. The optimum pH for enhanced activity of parabens is in the range of 4-8 (Johnson and Steer, 2006). From the results, the pH of the freshly prepared mucilage of both gums were within the working range of the paraben, a reason for the choice of that preservative. Preservation is important for aqueous pharmaceutical products and even solid ones that have great affinity for water because the presence of moisture facilitates microbial growth. Therefore, products that are supposed to remain awhile on the shelf must have antimicrobial preservatives to maintain stability over the required shelf life. In general, microbial thrive is optimal within pH range of 6-8. Outside this range, the rate of growth significantly dwindles.

From Figures 4.1, 4.2 and 4.3, it can be deduced that both albizia and khaya are acidic gums, confirming what is stated in literature (Nussinovitch 2009). From Figure 4.1, the pH of preserved albizia mucilage was fairly constant for the first four (4) weeks after which a sharp rise was realized. The increase may be due to hydrolysis of the sugar and uronic acid units constituting the gum. For the unpreserved freshly prepared mucilage of albizia, the pH was lower than that of the preserved, implying that, the preservative increased the pH of the mucilage. Also, a slight increase in pH was observed within the first week after which a slight decrease was seen and then, the pH remained fairly steady in subsequent weeks. The slight increase in pH may be attributed to microbial activity because the mucilage was unpreserved. The decrease thereafter may be because the pH was no longer conducive for the

microorganisms, thus, killing or stopping their growth. Hence, without their activity, the pH declined to the initial and then remained constant through the study period.

For khaya, both the preserved and unpreserved mucilages showed no significant change over the weeks of study as seen in Figure 4.1. Just like albizia, the pH of the unpreserved freshly prepared mucilage was slightly lower than the preserved.

Again, the storage temperature was varied to see its effect on the pH of both albizia and khaya mucilage. From Figure 4.2, there was a gradual increase in pH of albizia mucilage stored at 50°C in the first two (2) weeks, then a consistent increase in subsequent weeks. At 25°C, a moderate decline in pH was observed in the first two (2) weeks, but levelled off in the rest of the weeks. At 4°C, the pH was fairly constant in the first four (4) weeks until it increased steadily within the fifth week. Changes in pH of the mucilage over the period of study may be as a result of microbial decomposition and/or hydrolysis of the sugar and uronic acid moieties in the gum.

For khaya, as seen in Figure 4.3, the pH of 1% mucilage stored at 4°C was constant throughout the study, suggesting that, refrigeration may have had preservative effect on the mucilage (due to reduced or no microbial activity). The mucilage stored at 50°C and 25°C similarly did not show any significant changes in pH over the five (5) weeks. In all, it can be deduced that, khaya gum is more stable in aqueous solution than albizia gum.

5.1.2.8 Rheological properties of purified albizia and khaya gums

An in-depth comprehension of the rheological properties of pharmaceutical materials is important in the preparation, development, evaluation and efficacy of pharmaceutical dosage forms (Aulton, 1990). Moreover, advancements in the evaluation methods of the viscoelastic characteristics of semi solids and biological materials have shown beneficial relationship with bioavailability and function.

Fig. 4.4 presents the variation of viscosities of both gums with concentration. It is evident that the viscosities of both albizia and khaya increased with increasing concentration. OforiKwakye et al. (2010) have also reported that the viscosity of cashew gum increased with increasing concentration. The high viscosity recorded at higher concentrations of both gums may be as a result of the surge in the power of molecule-molecule interaction and the parallel reduction in the molecule-solvent interaction. Again, it was observed that, the viscosity of albizia mucilage was higher than khaya at all concentrations indicating that, albizia gum forms thicker and viscid dispersion in water than khaya.

In Fig. 4.5, the change in viscosity with temperature for albizia and khaya gums are shown. It can be seen that, the viscosity of both gums decreased with increasing temperature. This is so because, at higher temperatures, the increased kinetic energy facilitates the cleavage of intermolecular bonds between contiguous layers, the resultant effect being a decrease in the viscosity of the mucilage. Also, it may also be explained that, heating the mucilage results in breakdown or structural changes that is responsible for the observed trend.

5.1.2.9 Microbial quality of purified gums

According to WHO Technical Report (1992), all starting materials (including excipients) should be comprehensively assessed or otherwise authenticated prior to use. They are mostly subjected to identification tests, microbiological tests etc. to ascertain that they meet the required specifications.

The microbial quality of pharmaceutical excipients employed in production can significantly affect the results of individual manufacturing stages and the microbiological properties of the end product. Therefore, albizia and khaya gums intended for use as film forming polymers in the formulation of ODFs were assessed to know how appropriate they are for the said purpose.

Tables 4.6 and 4.7 shows the observations made when 1% mucilage of albizia and khaya respectively, were inoculated into various selective media. It can be inferred that, the gums were free from objectionable microorganisms, including specified microorganisms, frank pathogens and opportunistic pathogens that are known from clinical literature to cause infections. These organisms are mostly associated with major recalls for microbial contamination e.g. *Salmonella typhi*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa, Escherichia coli* etc. If these microorganisms are present in a drug product even below the microbial limit, the batch would be rejected. The absence of these microorganisms in both gum samples studied may be due in part to hygienic harvesting, efficient purification procedure and proper storage.

5.1.2.10 Antimicrobial activity of purified gums

Clark et al. (1993) reported some growth inhibitory activity of gum arabic on certain periodontal disease-causing microorganisms such as *Prophyromonas gingivalis and Prevotella intermedia* that cause tooth decay and gingivitis. Based on this, the study sought to ascertain the presence or otherwise of any intrinsic antimicrobial activity of the two gums. Different concentrations of both albizia and khaya mucilage were prepared and tested against various clinical and typed strains of selected microorganisms using the agar diffusion method. The presence or absence of any zones of inhibition was observed to assess the susceptibility of the organisms to the various concentrations of both albizia and khaya mucilage used.

From the observations made for albizia and khaya gums, it can be inferred that, at the stated concentrations, both gums have no intrinsic antimicrobial activity against the selected microorganisms or the selected microorganisms show no susceptibility to the concentrations of the two gums used.

5.1.2.11 Mineral content of albizia and khaya gums

In the characterisation of both albizia and khaya gums, the elemental composition and heavy metal content of the gums were determined. Both qualitative and quantitative analysis were done to know the presence, if any, and the percentages of these elements in the two gums under study. Figure 4.6 shows the mineral composition of albizia and khaya gums. The elements present include iron, copper, manganese, zinc, calcium, magnesium, sodium, nitrogen, phosphorus and carbon, with their respective percentages shown.

The presence of nitrogen and sulphur may indicate the presence of proteins in the gums. Sodium ions are important ions in the human body and they partake in several metabolic activities in the body. Deficiency or excess of these ions in the body can be attributed to several conditions including arrhythmias (BNF 65, 2013). Therefore, quantifying these ions in both albizia and khaya is very essential especially since both gums are intended for use as pharmaceutical excipients. Sodium ions were only present in minute quantities confirming what has been stated in literature (Nussinovitch, 2009). Therefore, both albizia and khaya are appropriate excipients for use in medicines meant for people on sodium restricted diet.

It must however be pointed out that, the elemental analysis showed the absence of toxic heavy chemicals like arsenic, lead, cadmium, cyanide and mercury. This suggests the safety of both gums for use as pharmaceutical excipients.

5.1.2.12 Ash values of purified albizia and khaya gums

Ash values mostly designate the inorganic remnants present in natural products and other pharmaceutical substances (Ashutosh, 2005). Total ash typically represents the presence of inorganic salts e.g. calcium oxalate, carbonates, silicates, phosphates and other inorganic materials from external sources. It aids in detecting and checking adulteration with extraneous materials that may have been unintentionally included during harvesting and treatment e.g. soil,

sand, etc. The results for total ash present in both albizia and khaya gums is shown in Table 4.8. The total ash of natural products may alter within wide limits even for unadulterated specimen because majority of natural products contain calcium oxalate which varies often times. Thus, the total ash values of both albizia and khaya, though low, is not conclusive as to whether there is adulteration or not.

A more specific and accurate way to determine adulteration and the presence of earthly matter will be to determine the acid-insoluble ash because, the calcium oxide and carbonates yielded by the ignition of oxalate is soluble in HCl when the ash is treated with HCl. The remaining ash (acid-insoluble) is an accurate representation of the earthly matter present (Evans, 2009). The results for the acid-insoluble ash determination is also shown in Table 4.8. The low figures suggest that, both albizia and khaya contain insignificant amounts of earthly materials. This may be attributed to proper harvesting, handling and cleaning of the crude exudates.

Water-soluble ash is particularly key in identifying components which have been extracted with water. The results for both gums under study is shown in Table 4.8.

5.1.3 Formulation of diclofenac sodium oral dissolvable films, ODFs

In the preparation of the diclofenac films, albizia gum, khaya gum and HPMC (reference polymer) were used as film forming polymers. Glycerol was included as plasticizer to improve the flexibility of the films. Aspartame and citric acid were used as sweetening and saliva stimulating agents respectively. 1 % titanium dioxide was used to mask the unpleasant appearance of the natural gums. Tween 80 was also incorporated as a wetting agent. The films were formulated using the solvent casting method. The process is simple and economical. Also, the ingredients are safe and commonly used in the pharmaceutical

industry.

5.1.4 Evaluation of formulated diclofenac sodium oral dissolvable films, ODFs

5.1.4.1 Appearance, thickness and pH of films

The formulated diclofenac films were opaque and whitish because of the colouring agent that was used in the formulation. Titanium dioxide imparts a whitish colouration to formulations they are used in. The films were thin, soft and flexible (with the exception of few batches). They had a stately smooth and glossy surface because of the glycerol that was added. Plates 4.1 and 4.2 show snapshots of formulated films (as removed from petri dish) and 2 cm × 2 cm sized films respectively.

The average thickness of the formulated films ranged from 0.108 ± 0.008 to 0.174 ± 0.011 mm. The order of thickness of the various formulations was as follows; F6< F5< F2< F1< F4< F3< F7. Uniformity of thickness is a very key parameter to consider in the evaluation of ODFs because it has direct correlation with the precision of dose of the films. From the results in Table 4.9, it can be seen that, the standard deviations of the thickness for each of the batches are low meaning that, the thickness of the individual films in the same batch is fairly uniform. The inter-batch differences in thickness can be attributed to the difference in the viscosities of the polymeric solutions and their various combinations. The thickness increased steadily as the number of polymers in a combination increased, supporting findings of Karthikeyan et al. (2013).

The pH of the films was determined to know how it will affect the mucous membrane in the mouth. The pH of a product is reflective of the API and the excipients used in the formulation to enhance solubility, stability, palatability or microbial stability (Elder and Crowley, 2012). Generally, acidic and basic oral formulations have an adverse effect of causing inflammation of oral mucosa (Patel and Poddar, 2009). From the results in Table 4.9, the average pH of the formulations ranged from 3.09 ± 0.026 to 5.23 ± 0.043. All the formulations were in the acidic range. This may

be due to the contributory effect of the API (diclofenac) and the excipients, especially albizia, khaya and citric acid. The above observation means that, the final pH of the polymeric solution will have to be adjusted to an almost neutral pH (before casting into films) to prevent any irritation to the oral mucosal lining.

5.1.4.2 Uniformity of weight and disintegration time

A customary prerequisite of all pharmaceutical dosage forms is the constancy in dose of different dosage units of the same batch. In actuality, slight dissimilarity in weight within a single batch is satisfactory but they should be within specified limits (BP, 2013). Generally, fluctuations in the amount of active ingredient is determined by both uniformity of weight and content because, if the API makes up the most part of the dosage unit, any alteration in the weight undoubtedly pinpoints a variation in the API.

From the results in Table 4.10, it can be seen that, the individual weights of films within the same batch vary only slightly because of the low standard deviations. The average weight of the films ranged from 0.116 ± 0.011-0.298 ± 0.095 mg. It can be inferred that, the average weight of the films increased as the number of polymers were increased, as seen in the order; F1, F4, F6 (only one polymer) < F2, F3, F5 (two polymers) < F7 (three polymers).

For acceptable ODFs, the API should be released in a controlled and reproducible way. The primary step towards drug release (dissolution) is mostly the breaking up of the dosage unit through disintegration. In disintegration, the administered film adheres to mucosal surface, absorbs saliva, swell and disperse releasing the medicament for dissolution and absorption. Thus, disintegration test is a very important procedure to undertake in the quality control of ODFs.

Generally, the disintegration time of ODFs should be in the range of 5-30 seconds (Bala et al., 2013) though this may alter depending on the formulation. For instance, Liew et al. (2012) reported a time of 43 seconds for orally disintegrating films composed of HPMC, corn starch,

polyethylene glycol and lactose monohydrate with donepezil as API. From Table 4.10, it can be seen that the average disintegration time for the formulations was in the range of 37.16 ± 0.043 seconds - 47.45 ± 0.05 seconds. None of the formulations fell within the range stated by Bala et al. (2013). It must however be emphasized that there are no authoritative specifications and guidelines for determining the disintegration time of ODFs (Bhyan et al., 2011). But from the results, it can be seen that, F2 had the least disintegration time of 37.16 seconds whiles F7 took the longest time to disintegrate in 47.45 seconds. It is realised that combining the polymers in the formulation slightly prolonged the disintegration time. This may be partly due to crosslinking of the individual polymer molecules making them less penetrable to solvent molecules.

5.1.4.3 Investigation of possible drug-excipient interaction using FTIR spectroscopy

Figure 4.7 shows the FTIR spectra of pure diclofenac sodium, albizia film and khaya film. The spectrum for pure diclofenac sodium shows a very broad band at 3225.26 cm^{-1} sloping into the aliphatic –CH region around 3000 cm^{-1}. This indicates the presence of a carboxylic acid –OH which overlaps causing the disappearance of the –CH group in the aromatic system. Medium intensity bands at 1603.42 cm^{-1}, 1572.03 cm^{-1} and 1547.76 cm^{-1} indicates the presence of aromatic groups, and this is confirmed by bending vibrations between 700900 cm^{-1}.

All the above principal bands are present in the spectra generated for the films made with albizia and khaya as seen in Figure 4.7. Therefore, it can be inferred that, there is no possible interaction between the excipients used in the formulation and the active diclofenac sodium because all the prominent bands are still present in the ODF after formulation.

5.1.4.4 Assay

Assays have become a routine part of pharmaceutical drug analysis where the API in a dosage unit is quantitatively determined. It helps to identify substandard drugs. UV analysis was

carried out on the films to determine the content of diclofenac present in them. But first, a calibration curve for diclofenac in 0.1 M NaOH was drawn (Figure 4.8). The correlation coefficient obtained from the equation of the line depicts the linearity of the analytical procedure.

According to the BP (2013), an immediate release diclofenac dosage unit must contain not less than 95 % and not more than 105 % of pure diclofenac sodium. From Table 4.12, the average content of diclofenac in the formulations ranged from 97.58 ± 0.231 %-100.41 ± 0.212 %, meaning, all the batches passed the assay. The low standard deviation calculated for each of them shows that, the content of diclofenac sodium in the films is fairly uniform.

5.1.4.5 Mechanical properties of formulations

Pharmaceutical ODFs are supposed to possess very good mechanical properties so as to maintain their integrity even with handling, packaging and transportation. The mechanical properties solely rely on the excipients and techniques used in the formulation. These excipients should be compatible with the API, protect the API and make the film easy to handle. Mechanical properties may be influenced by the film forming polymer, the technique employed in producing the films as well as the type and amount of plasticizer used. Plasticizers, especially, confer pliability to films and greatly improve their mechanical properties (Irfan et al., 2015).

In the present study, the tensile strength, elastic modulus and the percentage elongation were determined for all batches of formulations. The use of varying combinations of the film forming polymers was to optimize the mechanical properties of the films. These mechanical parameters have direct influence on the physicochemical properties and the intended use of the films. For example, a soft and weak film shows very low tensile strength and low percentage elongation whereas a hard and brittle film is characterised by moderate tensile strength and low percentage elongation. Again, a soft and tough film exhibits moderate tensile strength and high percentage

elongation whiles a hard and tough film shows high tensile strength and percentage elongation (Lever and Rhys, 1962).

Tensile strength provides a hint of the mechanical strength and hardness of films (OforiKwakye and Adom, 2007). High tensile strength values are required to ensure that, the films will not break or crack during handling, packaging and transportation. The tensile strength of the formulations ranged from 5.67 ± 0.231-7.32 ± 0.432 MPa (Table 4.13). Tensile strength may vary considerably as a result of differences in techniques used in manufacturing the films, the excipients used and even the testing procedure. Thus comparing films on the basis of their tensile strength may be cumbersome and elusive (Macleod et al., 1997). But from the results in Table 4.13, and the observed physical nature of the films, it was seen that khaya alone (F6) produced hard films and albizia alone (F4) produced soft films, as seen from their respective tensile strengths. Addition of HPMC to albizia increased the tensile strength of albizia films (as seen in F2). Addition of HPMC to khaya however reduced the hardness of khaya films (as seen in F3).

Elastic modulus describes the stiffness of the films (Irfan et al., 2015). It also informs about how well the film can resist mechanical deformation. Ofori-Kwakye and Adom (2007), reported that, films that have low elastic modulus have low rigidity which translates into a high elasticity and vice versa. In simple terms, elastic modulus is inversely proportional to elasticity.

From Table 4.13, the range of elastic modulus recorded for the formulations was 3.89 ± 0.321-5.11 ± 0.213 MPa. Generally, a film with high tensile strength and elasticity (low elastic modulus) is preferred because it can withstand stress better and can also resist changes due to mechanical deformation. It can be seen that, adding HPMC to both albizia and khaya increased their elasticity by decreasing their elastic modulus as seen in F2 and F3 respectively. Therefore, it can be inferred that, HPMC confers flexibility to albizia and khaya films. Khaya again, increased the rigidity of albizia films as seen in F5. The highest elastic modulus was observed

in the combination of all three polymers (F7) and this may be due to the effect of khaya in the combination because albizia/HPMC combination (F2) showed moderate elastic modulus.

Upon exertion of stress on a film, the film stretches. This effect is called strain and it expresses the change in length of a film divided by its initial length before the applied strain (Arya et al., 2010). It describes the pliability of films. A low elongation at break signifies a low capacity of the film to resist deformation and hence, the film will be easily breakable. A high elongation at break means that, the film has a high capacity to withstand mechanical strain and thus, is very resilient.

From Table 4.13, the percentage elongation of the formulations ranged from 7.65 ± 0.154-17.87 ± 0.432 %. F7 showed the least flexibility and confirms the results and deductions made from the elastic modulus determinations in Table 4.13. It can be seen that, HPMC increased the flexibility of both albizia (as seen in F2) and khaya (F3) by increasing their respective percentage elongations. A combination of albizia and khaya (F5) showed a decrease in flexibility compared to albizia films alone (F4). Therefore, it can be deduced that, khaya produces very brittle films and its combination with other polymers increases their inelasticity.

Folding endurance expresses the capacity of the film to resist breaking when folded repeatedly along the same plane. High folding endurance values portray considerable mechanical strength of the film. It is directly regulated by the type and amount of plasticizer used in the formulation. However, in the present study, the same plasticizer, glycerol, was used in all the formulations in the same amount, meaning that, any effect on the mechanical properties of the films were solely due to the intrinsic nature of the polymers used.

From Table 4.14, the folding endurance of the films were in the order, F6< F7< F3< F4< F5< F1< F2, with the highest being 103 ± 2.056 and the lowest being 39 ± 0.976. This trend further confirms the results and deductions made from the mechanical properties investigated in Table

4.13. Nafee et al. (2003), stated that, a folding endurance of 300 is considered satisfactory for oral films. And since that is influenced by the concentration of plasticizer, glycerol can be increased from the initial 5 % used so as to improve the folding endurance of the films.

5.1.4.6 In vitro dissolution studies

The oral route remains one of the most effective means of drug administration in the treatment of diseases. The efficacy of oral dosage forms depends on disintegration and dissolution of the dosage unit in G.I fluids and the subsequent absorption into systemic circulation. The rate at which this occurs is therefore very vital and worth studying. However, a big obstacle the industry now faces is how to optimise the amount of drug available to the body (bioavailability). This is because, any insufficiency in the bioavailability of a dosage form connotes ineptness of treatment and in the worst scenario, toxic overdose (www.tabletdissolution.com).

According to Singhvi and Singh (2011), dissolution is the rate of mass transfer from a solid surface into the dissolution medium or solvent under standardized conditions of liquid/solid interface, temperature and solvent composition. Immediate release drug products must allow drugs to dissolve without retarding dissolution and subsequent drug absorption.

Panda et al. (2012), reports that, generally for ODFs, the dissolution time is defined as the time at which not less than 80 % of the API under testing dissolves in aqueous media. From the results in Table 4.15, F1 and F4 had over 80 % dissolution within the first 7 minutes. With the exception of F7, the rest of the formulations showed over 80 % release in the next 7 minutes. F7 only showed 82.94 % release within 28 minutes. Though, still faster than traditional tablets, it is not too desirable for ODFs. This may be attributed to the combination of three different polymers in that batch of formulation. The release of diclofenac sodium from the formulation

was slowed down due to possible polymer crosslinking. Generally, increase in the number of polymers increased the dissolution time.

5.1.4.7 Dissolution data comparison using F1 as reference

5.1.4.7.1 Dissolution efficiencies of formulations

According to Anderson et al. (1998), dissolution efficiency assists in confirming how competent a dosage form is in terms of releasing its API for pharmacological effect. The higher the dissolution efficiency, the more capable the dosage form is, at releasing its deepseated API.

From Table 4.16, the order of dissolution efficiencies of the formulations was F7< F3< F4< F5< F2< F6< F1. From this, F1 can be said to be the superior batch in terms of drug release. This trend supports the findings from the drug dissolution studies in Section 5.1.4.6.

5.1.4.7.2 Difference, (f1) and similarity, (f2) factors

Comparing two drugs containing the same API in terms of therapeutic efficacy is an important way of determining the suitability of one being substituted for the other. The US Food and Drugs Administration, FDA guidance on immediate release medicines approves the difference factor (f1) and similarity factor (f2) in comparing the dissolution profiles of reference and test drug samples.

The difference factor estimates the percentage difference between the two profiles at every point and is a measure of the relative error between the dissolution profiles of both test and reference drug. Usually, f1 values in the range of 0-15 indicates insignificant difference between the two batches (FDA 1995). The difference factor also shows the magnitude of dissolution of a particular drug in relation to the reference. The greater the difference, the smaller the cumulative amount of test drug dissolved across all time points.

From Table 4.17, all batches, with the exception of F7, had f1 values within the stated range of 0-15. This means that, in terms of dissolution, F2, F3, F4, F5 and F6 differ only marginally

from F1, the reference formulation. F7 however can be said to differ markedly from F1 because of its f1 value of 27.

In terms of similarity, a test formulation is considered similar to the reference if the f2 value is in the range of 50-100 (Mukesh et al., 2005). In comparing identical dosage forms, f2 must be equal to 100. According to the results on Table 4.17, all the batches except F7 had f2 values within the range. Thus, it can be inferred that, F2, F3, F4, F5 and F6 are similar to F1 (reference) in terms of dissolution characteristics and can be used as alternatives in terms of release performance and bioequivalence. F7 on the other hand cannot be said to be similar to the reference.

5.1.4.7.3 One-way ANOVA analysis

Using one-way ANOVA followed by Dunnet‟s test, the dissolution rates of the formulations were compared. The superiority of ANOVA to other statistical methods of comparison stems from the fact that, it is not bound to any preconditions as in the case of similarity factor (f2) and also, it does not rely on fitting into any specific kinetic model.

From the results in Table 4.18, no significant difference (p value > 0.05) was seen in the comparisons of the reference formulation, F1 and test formulations F2, F3, F4, F5 and F6. However, there was a significant difference (p value < 0.001) between F1 and F7. This buttresses the initial outcomes of the FDA guidelines in Section 5.1.4.7.2.

5.1.4.8 Kinetics and mechanism of drug release

The role of dissolution testing has now extended to drug release studies at various levels, where mathematical models are employed to better understand the mechanism of drug release from dosage forms. Classical drug delivery should follow zero order kinetics, where serum levels of drug remains unchanged throughout the delivery period. This ideal release is essential in certain classes of medicines like antibiotics, heart and blood pressure regulating medicines, medicines

for pain management and antidepressants. Advancement in this area has made it possible to predict the release kinetics of drugs based on which mathematical model the dissolution data best fits (Singhvi and Singh, 2011).

The kinetic models employed in the present study were zero order, first order, Higuchi and Hixson-Crowell models. The data from the dissolution studies conducted were put into the individual kinetic models to determine their linearity, using the coefficient of regressions. Table 4.19, shows the summary of the results obtained. The higher the coefficient of regression, the more linear the curve and the more suited the formulation is, for that particular kinetic model.

From the results in Table 4.19, the highest R^2 values were recorded with the Higuchi model. Higuchi (1963) elucidated drug release mechanism based on the hypotheses that; i) initial drug concentration in a matrix is much greater than drug solubility ii) drug diffusion occurs only in one dimension iii) drug particles are smaller compared to thickness of the system iv) matrix swelling and dissolution is insignificant v) drug diffusivity is continual and vi) absolute sink conditions are always achieved in the release medium (Dash et al., 2010). The concept holds true at all times except when there is complete exhaustion of the drug in therapeutic system. It is mostly applied in describing the drug dissolution from various modified release pharmaceutical dosage forms especially in transdermal systems and matrix tablets with water soluble drugs (Higuchi, 1963).

5.2 CONCLUSION

From the experiments conducted and the deductions made in the discussion, the following conclusions can be made:

- Both albizia and khaya gums can be purified to improve upon their appearance and quality. The percentage yields obtained from the purification procedure of albizia and khaya gums were 39.38 % and 67.50 % respectively.
- The gums are free from objectionable microorganisms and both gums have no intrinsic antimicrobial activity against the selected microorganisms.
- The elemental analysis showed the absence of toxic heavy chemicals like arsenic, lead, cadmium, cyanide and mercury. This suggests the safety of both gums for use as pharmaceutical excipients.
- Seven different batches of ODFs were formulated using the polymers alone and in various combinations, using HPMC as the reference polymer. The formulated diclofenac ODFs were evaluated for their physicochemical properties.
- All the batches passed the assay and dissolution test for conventional immediate release dosage forms. With the exception of F7, all the formulations showed over 80% drug release within 14 minutes.
- Drug release of diclofenac in the batches followed Higuchi drug release kinetic model.
- Batch F1 (reference batch) had the highest dissolution efficiency and therefore could be considered to be superior among other batches in terms of drug release.
- All batches, with the exception of F7, had f1 and f2 values within the stated range of 0-15 and 50-100 respectively. This means that, in terms of dissolution, F2, F3, F4, F5 and F6 are similar to F1, the reference formulation. F7 however can be said to differ markedly from F1.
- From the ANOVA analysis, no significant difference was seen in the dissolution profiles of reference formulation, F1 and test formulations F2, F3, F4, F5 and F6. However, there was a significant difference between F1 and F7.

5.3 RECOMMENDATIONS

- Scanning electron microscopy should be used to study the surface morphology of the films.
- In vivo work should be done on the films to assess their efficacy and acceptability (in terms of taste and feel).

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APPENDICES

Appendix I: **Preparation of solutions**

- Phosphate buffer (pH 6.8) - A solution of 0.2 M potassium dihydrogen orthophosphate of volume 250 ml was mixed with 118.3 ml of 0.2 M sodium hydroxide solution and then diluted with distilled water to produce 1000 ml.
- Sodium hydroxide (0.1 M) – 4 g of NaOH pellets was weighed into a beaker and was completely dissolved with a small amount of distilled water. It was then quantitatively transferred into a 1000 ml volumetric flask and made up to volume with distilled water.

Appendix II: **Sample calculations Calculation of cumulative percentage drug released**

Strength of diclofenac sodium = 50 mg

Volume of dissolution medium (phosphate buffer, pH = 6.8) = 300 ml

From the calibration curve, the equation of the graph of pure diclofenac sodium powder dissolved in phosphate buffer (pH 6.8) y = 333.97x - 0.0686

Where, y= mean absorbance at time t, and x = concentration

Hence x = y + 0.0686/333.97

Thus for F1, at 7 minutes, the mean absorbance was 0.412, therefore,

x = (0.412 + 0.0686/ 333.97) x = 0.0014391 %w/v

Multiplying „x" by the dilution factor of 10 gives the concentration of drug dissolved.
x = 0.014391 %w/v

Thus, 100 ml of solution = 0.014391 g of diclofenac sodium

300 ml = (0.014391 × 300/100) = 0.0432 g

Amount of drug released at the 7^{th} minute = 0.0432 g

% drug release = (amount of drug released/ amount of drug expected) × 100

Hence, % drug release = (0.0432/0.05) × 100 = **86.34 %**

At 14th minute, y = 0.449, therefore,

„x" at 14th minute = (0.449 + 0.0686/333.97) = 0.0015498 %w/v

Multiplying „x" by the dilution factor of 10 gives the concentration of drug dissolved x

= 0.015498 %w/v

Thus, 100 ml of solution = 0.015498 g of diclofenac sodium

300 ml = (0.015498 × 300/100) = 0.0465 g

Amount of drug released at the 14th minute = 0.0465 g

But weight of diclofenac sodium in 10 ml aliquot pipetted at the 7th minute;

100 ml = 0.01439 g

Therefore, 10 ml = (0.01439 × 5)/100 = 0.001439 g

Thus, total amount of diclofenac sodium released at the 14th minute = (0.0465 + 0.001439) g

= 0.0479 g

Hence, % drug release = (0.0479/0.05) × 100 = **95.88 %**

The calculations were repeated for the percentage drug released at various times for other formulations.

Appendix III: Calculation of dissolution efficiency (DE)

For F1,

AUC = 1981

AUC was determined from the mean percentage drug release using Graphpad prism.

Y = 98.42 % (total amount of drug dissolved within 28 minutes)

Using the equation**,** Dissolution efficiency = $\{(0\int^{t} Y.dt) / Y100.\ (t_2 - t_1)\} \times 100$

$(0\int^{t} Y.dt)$ = area under the dissolution curve

(AUC) Y= the percentage dissolved at t_2 t_2 =

time for all active ingredient to dissolve t_1 = time

at which first sample was withdrawn

$$DE = \frac{1981 \times 100}{98.42\ (28 - 7)}$$

= **95.85 %**